Sodium/Calcium Exchange: Its Physiological Implications

MORDECAI P. BLAUSTEIN AND W. JONATHAN LEDERER

Departments of Physiology and Medicine and the Center for Vascular Biology and Hypertension, University of Maryland School of Medicine, and Department of Molecular Biology and Biophysics, The Medical Biotechnology Center of the Maryland Biotechnology Institute, Baltimore, Maryland

I. Introduction 764
II. Historical Background 766
III. Properties of the Sodium/Calcium Exchanger 769
   A. Modes of operation of the Na\(^+\)/Ca\(^{2+}\) exchanger 769
   B. Energetics and kinetics of Na\(^+\)/Ca\(^{2+}\) exchange 771
   C. Controversial kinetic and mechanistic topics 781
   D. Electrogenic (or rheogenic) properties and voltage sensitivity of the Na\(^+\)/Ca\(^{2+}\) exchanger 785
   E. Regulation of the Na\(^+\)/Ca\(^{2+}\) exchanger by catalytic modulation 789
   F. Inhibitors of the Na\(^+\)/Ca\(^{2+}\) exchanger 796
IV. Molecular Biology of the Sodium/Calcium Exchanger 799
   A. Purification, cloning, and reconstitution of the exchanger protein 799
   B. Structure/function of the Na\(^+\)/Ca\(^{2+}\) exchanger 801
   C. Alternative splicing 803
   D. Gene structure (multiple genes) 803
   E. Isoforms 803
   F. Development 805
   G. Modulation 805
   H. The Drosophila exchanger 805
      I. The Na\(^+\)/(Ca\(^{2+}\) + K\(^+\)) exchanger; NCKX1 and NCKX2 806
V. Physiological Roles and Pathophysiological Consequences of Sodium/Calcium Exchanger Activity in Cells and Tissues: General Principles 807
   A. Localization of the Na\(^+\)/Ca\(^{2+}\) exchanger: possible clues to function 807
   B. Ca\(^{2+}\) extrusion versus Ca\(^{2+}\) entry 809
   C. Control of SR/ER Ca\(^{2+}\) content 810
VI. Physiological Roles of the Sodium/Calcium Exchanger in Various Tissues 810
   A. Heart muscle 811
   B. Smooth muscle 820
   C. Vertebrate skeletal muscle 822
   D. Invertebrate skeletal muscle 822
   E. Nervous system 823
   F. Na\(^+\)/Ca\(^{2+}\) exchange in blood cells: physiology and pathophysiology 825
   G. Kidney 826
   H. Intestinal absorption of Ca\(^{2+}\) 827
      I. Eye 828
      J. Secretory cells 829
      K. Na\(^+\)/Ca\(^{2+}\) exchanger in miscellaneous other types of cells 830
VII. Epilogue 830

Blaustein, Mordecai P., and W. Jonathan Lederer. Sodium/Calcium Exchange: Its Physiological Implications. Physiol. Rev. 79: 763–854, 1999.—The Na\(^+\)/Ca\(^{2+}\) exchanger, an ion transport protein, is expressed in the plasma membrane (PM) of virtually all animal cells. It extrudes Ca\(^{2+}\) in parallel with the PM ATP-driven Ca\(^{2+}\) pump. As a reversible transporter, it also mediates Ca\(^{2+}\) entry in parallel with various ion channels. The energy for net Ca\(^{2+}\) transport by the Na\(^+\)/Ca\(^{2+}\) exchanger and its direction depend on the Na\(^+\), Ca\(^{2+}\), and K\(^+\) gradients across the PM, the membrane potential, and the transport stoichiometry. In most cells, three Na\(^+\) are exchanged for one Ca\(^{2+}\). In vertebrate photoreceptors, some neurons, and certain other cells, K\(^+\) is transported in the same direction as Ca\(^{2+}\), with a coupling ratio of four Na\(^+\) to one Ca\(^{2+}\) plus one K\(^+\). The exchanger kinetics are affected by nontransported...
Ca\(^{2+}\), Na\(^{+}\), protons, ATP, and diverse other modulators. Five genes that code for the exchangers have been identified in mammals: three in the Na\(^{+}/Ca\(^{2+}\) exchanger family (NCX1, NCX2, and NCX3) and two in the Na\(^{+}/Ca\(^{2+}\) plus K\(^{+}\) family (NCKX1 and NCKX2). Genes homologous to NCX1 have been identified in frog, squid, lobster, and Drosophila. In mammals, alternatively spliced variants of NCX1 have been identified; dominant expression of these variants is cell type specific, which suggests that the variations are involved in targeting and/or functional differences. In cardiac myocytes, and probably other cell types, the exchanger serves a housekeeping role by maintaining a low intracellular Ca\(^{2+}\) concentration; its possible role in cardiac excitation-contraction coupling is controversial. Cellular increases in Na\(^{+}\) concentration lead to increases in Ca\(^{2+}\) concentration mediated by the Na\(^{+}/Ca\(^{2+}\) exchanger; this is important in the therapeutic action of cardiotoxic steroids like digitalis. Similarly, alterations of Na\(^{+}\) and Ca\(^{2+}\), apparently modulate basolateral K\(^{+}\) conductance in some epithelia, signaling in some special sense organs (e.g., photoreceptors and olfactory receptors) and Ca\(^{2+}\)-dependent secretion in neurons and in many secretory cells. The juxtaposition of PM and sarco(endo)plasmic reticulum membranes may permit the PM Na\(^{+}/Ca\(^{2+}\) exchanger to regulate sarco(endo)plasmic reticulum Ca\(^{2+}\) stores and influence cellular Ca\(^{2+}\) signaling.

I. INTRODUCTION

Cytosolic Ca\(^{2+}\) plays a key role in intracellular signaling in virtually all types of animal cells. In many instances, as exemplified by cardiac muscle contraction and the transient release of neurotransmitter substances, the elevated cytosolic free Ca\(^{2+}\) concentration ([Ca\(^{2+}\)]\(_i\)) may be required for only brief periods of time.\(^1\) The Ca\(^{2+}\) must then be rapidly removed from the cytosol. In other situations, such as during the maintenance of tonic tension in smooth muscle, elevated [Ca\(^{2+}\)]\(_i\), may be required for long periods of time. Consequently, [Ca\(^{2+}\)]\(_i\), \(I\) must be precisely controlled, 2) must be able to be increased and rapidly decreased to provide transient signals, and 3) must be modulated so that steady-state [Ca\(^{2+}\)]\(_i\) can be altered to fit cell needs. Moreover, in at least some cells (neurons are a good example), there must be spatial as well as temporal variation in [Ca\(^{2+}\)]\(_i\) so that multiple Ca\(^{2+}\)-regulated processes within these cells are not all indiscriminately activated simultaneously. Furthermore, it follows that abnormal cell Ca\(^{2+}\) regulation is likely to have profound pathophysiological consequences. For these several reasons, much interest has focused on the regulation of [Ca\(^{2+}\)]\(_i\) in all types of cells.

Calcium is uniquely suited for these signaling functions, due, in part, to its abundance (Ca\(^{2+}\) is the most prevalent cation in the body) as well as to its unusual chemistry (the combination of ionic radius, charge density, and coordination number) (981). Furthermore, the resting (or “background”) intracellular free Ca\(^{2+}\) concentration ([Ca\(^{2+}\)]\(_{i\text{(rest)}}\)) is sufficiently low (on the order of \(10^{-7}\) M; Refs. 89, 109, 156, 939) so that a large signal-to-background ratio \(\Delta[Ca^{2+}]_{i}/[Ca^{2+}]_{i\text{(rest)}}\) can be generated with only a relatively small increment in the absolute amount of Ca\(^{2+}\) added to the cytosol (89). This “signal Ca\(^{2+}\)” \(\Delta[Ca^{2+}]\) can come either from the extracellular fluid, via voltage-gated or receptor-operated Ca\(^{2+}\)-selective channels located in the plasma membrane (PM) or from intracellular stores [especially endoplasmic reticulum (ER) or, in muscle, the sarcoplasmic reticulum (SR)]. Calcium is released from these intracellular stores by Ca\(^{2+}\)-activated or inositol trisphosphate-activated mechanisms (63, 64, 112, 883). Moreover, the amount of Ca\(^{2+}\) sequestered in the stores, and thus potentially available for release during cell activation, is largely dependent on [Ca\(^{2+}\)]\(_{i\text{(rest)}}\). An additional critical feature of Ca\(^{2+}\) metabolism is its slow diffusion in the cytosol (102, 419, 543). This is largely a consequence of Ca\(^{2+}\) buffering by cytoplasmic proteins such as parvalbumin and vitamin D-dependent Ca\(^{2+}\)-binding protein (92, 93, 156, 994) and Ca\(^{2+}\) sequestration in intracellular organelles such as the SR/ER (105, 106, 647, 739) and mitochondria (277, 404, 459, but see Refs. 157, 882). Therefore, it is possible to generate local increases in [Ca\(^{2+}\)]\(_i\), and local responses, without having to raise [Ca\(^{2+}\)] throughout the cell. This is energetically important because only limited energy expenditure is then required to remove this “local” Ca\(^{2+}\) from the cytosol.

Calcium-dependent signaling is mediated by specific receptors for Ca\(^{2+}\) such as calmodulin, troponin C, protein kinase C (PKC), and synaptotagmin. Cells must also have mechanisms to remove Ca\(^{2+}\) from the cytosol. Two important mechanisms are the ATP-driven Ca\(^{2+}\) pumps in the ER (or SR) for resequestering Ca\(^{2+}\), and in the PM, for extruding Ca\(^{2+}\) and helping to maintain steady Ca\(^{2+}\) balance. These ATP-driven Ca\(^{2+}\) pumps bind Ca\(^{2+}\) with high affinity but have relatively low turnover rates (i.e., the number of Ca\(^{2+}\) transported per carrier per unit time), typically \(~100\) s\(^{-1}\) (155, 156, 160, 240, 242). Their capacity to transport Ca\(^{2+}\) depends on both the turnover rate and the density of transport molecules in the membrane.

The PM of most animal cells also contain another Ca\(^{2+}\) transport system, the Na\(^{+}/Ca\(^{2+}\) exchanger, that operates in parallel with the Ca\(^{2+}\)-selective channels and the ATP-driven Ca\(^{2+}\) pump. The Na\(^{+}/Ca\(^{2+}\) exchanger can move Ca\(^{2+}\) either into or out of cells, depending on the net electrochemical driving force on the exchanger. Interestingly, this driving force, and thus the net Ca\(^{2+}\) move-

---

\(^1\) Cation (M\(^{+}\)) concentrations are shown in square brackets, where M\(^{+}\) = Na\(^{+}\), K\(^{+}\), Ca\(^{2+}\), or Mg\(^{2+}\). Subscripts \(o\) and \(i\) refer to extracellular and intracellular concentrations, respectively.
ment mediated by the exchanger, may change direction during a cell’s activity cycle, when the membrane potential ($V_m$) varies (e.g., during an action potential) and/or when the cytosolic Na$^+$ concentration ([Na$^+]_i$) or [Ca$^{2+}$]$_i$ is altered. As described in section miB6, the Na$^+$/Ca$^{2+}$ exchanger has about a 10-fold lower affinity for Ca$^{2+}$ but 10- to 50-fold higher turnover rate than the ATP-driven Ca$^{2+}$ pumps.

Sodium-dependent Ca$^{2+}$ transport has also been described in other organelles, including mitochondria (156, 214, 372) and secretory vesicles (451, 538, 539, 724, 804). This review is limited, however, to an examination of the properties of the PM Na$^+$/Ca$^{2+}$ exchanger.

A puzzling feature of cell Ca$^{2+}$ regulation concerns the apparent redundancy of the PM Ca$^{2+}$ transport systems, since the Na$^+$/Ca$^{2+}$ exchanger operates in parallel with both the Ca$^{2+}$-selective channels and the ATP-driven pump. As we shall see, however, the exchanger has several unique features that may help to explain the need for this redundancy as well as the exchanger’s utility. For example, the high maximal turnover rate of the Na$^+$/Ca$^{2+}$ exchanger may be important in cells that need to extrude a lot of Ca$^{2+}$ rapidly (as do cardiac muscle cells; see sect. vA). Indeed, the rate of Ca$^{2+}$ mediated by the exchanger increases and decreases as [Ca$^{2+}$]$_i$ is raised and lowered, respectively, during the cell activity cycle so that its activity is modulated to meet cellular demands for rapid transport of Ca$^{2+}$ and regulation of [Ca$^{2+}$]$_i$. Also, the electrochemical driving force on the exchanger in excitable cells is usually altered markedly during the cell cycle. Therefore, in some instances (such as during the plateau of the cardiac action potential), the decrease in the driving force will reduce the net rate of Ca$^{2+}$ extrusion compared with the rate expected at −80 mV. Thus the exchanger may help to maintain Ca$^{2+}$ at a relatively elevated level for an extended period of time. In addition, the efflux of Ca$^{2+}$ via the exchanger is associated with an inward current (see sect. miD) that will tend to prolong the cardiac action potential (367). The time course of these effects will depend on the time course of the action potential and on that of the Ca$^{2+}$ transient. During quiescent periods, the electrochemical driving force ($\Delta\mu_{Na/Ca}$, see below) on the exchanger can be modulated by altering $V_m$ or [Na$^+$]. Changes in these parameters may therefore be used to modulate quasi-steady-state [Ca$^{2+}$]$_i$ as well as the amount of Ca$^{2+}$ sequestered in the intracellular stores. This may be particularly important in cells that must sustain Ca$^{2+}$-dependent activities (such as tonic tension in certain smooth muscle cells). One feature of the exchanger that has recently come to light and that may contribute in important ways to its physiological role, is its localization in restricted portions of the PM of at least some types of cells. As discussed in section vA, certain transport proteins are not universally distributed in the PM but are localized in particular domains of the PM.

The Na$^+$/Ca$^{2+}$ exchanger was first described in the late 1960s. Since then, it has been identified in most types of cells in higher animals in which assessment has been carried out; the human erythrocyte is a noteworthy exception. The properties of the exchanger appear to be very similar in most of these cells. The exchanger in vertebrate photoreceptors has some unusual ion coupling features, however, and is the product of a separate gene (see sect. iv). Therefore, after a brief historical note, we review the general properties of the exchangers found in most cells. The various modes of transport mediated by the Na$^+$/Ca$^{2+}$ exchanger are described, and a summary of the exchanger’s thermodynamic and kinetic properties is presented in the context of overall cell Ca$^{2+}$ homeostasis. We then discuss the molecular structure of the exchanger and its pharmacology. The final portion of this article is devoted to a discussion of the physiological roles of the Na$^+$/Ca$^{2+}$ exchanger (and possible pathophysiological implications) in various tissues in which the exchanger has been identified.

A number of reviews published during the past several years have covered selected aspects of the Na$^+$/Ca$^{2+}$ exchanger and its physiological role in specific tissues. Some of the more extensive reviews published within the last decade include those by DiPolo and Beauge (250, 254) on excitable cells, by Philipson and Nicoll (721, 722) on the molecular and kinetic properties of the exchanger, by Reeves and co-workers (759–761) and Khananshvili (489) on the cardiac exchanger, by Sheu and Blaustein (845) on the cardiac and vascular smooth muscle exchangers, and by Schnetkamp (832) and Lagnado and McNaughton (547) on vertebrate photoreceptor exchanger. In addition, many aspects have been summarized in the proceedings of three international conferences on the Na$^+$/Ca$^{2+}$ exchanger (13, 97, 415). The literature on the Na$^+$/Ca$^{2+}$ exchanger has exploded in the last few years. Major advances have been made in exploring the molecular biology of the exchanger. Moreover, there has been renewed emphasis on exchanger kinetics, mechanism, and regulation. A new comprehensive review now seems warranted to summarize and integrate the most recent literature and latest views on the properties, physiological roles, and possible pathophysiological significance of the Na$^+$/Ca$^{2+}$ exchanger. In many instances, it is not yet clear how the various observations fit together, and there are still numerous controversies. Nevertheless, we have endeavored to present a full spectrum of new concepts and observa-

---

2 We shall define the "turnover rate" of the Na$^+$/Ca$^{2+}$ exchanger as the rate of exchanger cycling, or its equivalent, i.e., the rate of net Ca$^{2+}$ transport mediated by the exchanger. The turnover rate is not a fixed value, however, but is dependent on the specific conditions. As discussed in section miB6, the turnover rate is modulated by a variety of ligands including nontransported (intracellular) Ca$^{2+}$, protons, and voltage.
tions in the context of earlier work that established the basic framework. A number of areas that are in need of further investigation are identified. We have tried to provide the reader with a current perspective that we hope will not be misleading. Perhaps our review will encourage others to prepare reviews on more specialized aspects of Na\(^+\)/Ca\(^{2+}\) exchange that have been touched on only superficially in this very broad review.

Our review of the literature was completed in 1998. Because of the continually expanding literature on the subject of Na\(^+\)/Ca\(^{2+}\) exchange, we are able to provide only selected citations to some of the vast literature (especially on Na\(^+\)/Ca\(^{2+}\) exchange in the heart, about which much has been written). We regret omissions of specific citations, including some “first reports,” and the citation to several reviews instead. Nevertheless, we hope that we have provided a useful perspective of the current status of many aspects of Na\(^+\)/Ca\(^{2+}\) exchange.

II. HISTORICAL BACKGROUND

In the 1880s, Ringer (785, 786) demonstrated that Ca\(^{2+}\) in the bathing medium were required for cardiac muscle contraction. Nearly 40 years later, Daly and Clark (221) drew attention to the fact that the effect of extracellular Na\(^+\) removal “shows a striking resemblance to the effect of strophanthidin” in its ability to enhance cardiac muscle contraction. Then, in 1948, these two observations were brought together, when Wilbrandt and Koller (980) demonstrated an interaction between Na\(^+\) and Ca\(^{2+}\) at the PM. They found that the strength of contraction of the frog’s heart was directly related to the ratio \([\text{Ca}^{2+}]_o/(\text{Na}^+)_o\)^2, where subscript o refers to extracellular. Ten years later, Luttgau and Niedergerke (611) showed that, even in depolarized frog cardiac muscle, removal of external Na\(^+\) induced contractures, and in 1963, Niedergerke (690) demonstrated that frog ventricles gained Ca\(^{2+}\) when exposed to Na\(^+\)-depleted solutions. These ventricles promptly lost Ca\(^{2+}\) and relaxed when they were returned to control (Na\(^+\)-rich) Ringer solution. On the basis of these observations, Niedergerke (690) postulated that Ca\(^{2+}\) enters the muscle fibers via a carrier mechanism for which external Na\(^+\) and Ca\(^{2+}\) compete, presumably in the ratio 2 Na\(^+\):1 Ca\(^{2+}\).

Also in the mid 1960s, attention was directed toward the possible role of cell Na\(^+\) accumulation to explain the positive inotropic responses associated with cardiotonic steroids (775) and the staircase phenomenon (treppe) in the heart (550); however, no specific mechanisms were proposed. Subsequently, Langer (551) suggested that Na\(^+\) and Ca\(^{2+}\) might compete at intracellular membrane sites (on the sarcotubular system).

Competition between Na\(^+\) and Ca\(^{2+}\) at the PM was also observed in both twitch and slow skeletal muscle in the frog. In the rectus abdominis, a slow muscle, reduction of [Na\(^+\)]\(_o\) caused sustained contractures (818). Cosmos and Harris (207) found that frog twitch muscle fibers gained Ca\(^{2+}\) when incubated in Na\(^+\)-depleted media. This led them to conclude that in this tissue, too, Na\(^+\) and Ca\(^{2+}\) compete for entry sites. With hindsight, it is also possible to explain some of their other observations in terms of an Na\(^+\)/Ca\(^{2+}\) exchange mechanism, namely, conditions that induced net Na\(^+\) gain (removal of external K\(^+\), cooling, or treatment with ouabain) also promoted net Ca\(^{2+}\) gain in frog muscle. They used radioactive tracer labeled Sr\(^{89}\) (\(^{89}\)Sr) as a substitute for Ca\(^{2+}\) in efflux experiments; after \(^{89}\)Sr loading, external Na\(^+\) promoted (net) \(^{89}\)Sr efflux from this tissue.

Similarly, reports of apparent competition between Na\(^+\) and Ca\(^{2+}\) in mammalian smooth muscles were published in the 1960s. The key evidence was that the Ca\(^{2+}\) content of guinea pig tenia coli (362) and rabbit aorta (136) increased when [Na\(^+\)]\(_o\) was lowered. Even earlier, Friedman and co-workers (323, 324) had suggested that blood pressure was, in part, dependent on “sodium transfer systems” that govern the influx and efflux of Na\(^+\) in vascular smooth muscle cells, but the importance of Ca\(^{2+}\) was not yet recognized.

These findings were reinterpreted and extended in the late 1960s by investigators in three different laboratories: Reuter and co-workers in Germany and Switzerland (783, 352); Baker, Blaustein, and Hodgkin and their co-workers in England (34, 35, 102); and Martin and DeLuca in the United States (625). While studying different tissues, they independently and simultaneously reached the conclusion that a coupled countertransport mechanism, involving the exchange of Na\(^+\) for Ca\(^{2+}\) (i.e., “Na\(^+\)/Ca\(^{2+}\) exchange”), rather than just a competition between Ca\(^{2+}\) and Na\(^+\) for binding sites, may be involved in Ca\(^{2+}\) transport across the PM in mammalian cardiac muscle (352, 783), in invertebrate neurons (34, 35, 102), and in mammalian small intestinal epithelium (625). It is noteworthy that during the 1950s and 1960s experiments were also being carried out on the transport of small solutes (sugars and amino acids) that revealed a coupling between the fluxes of these other solutes and of Na\(^+\) (181, 182, 211, 784, 836). In these latter cases, Na\(^+\) was cotransported with the sugars or amino acids. This coupling of the Na\(^+\) electrochemical gradient (145) to the transport of other solutes (see sect. methods) is often referred to as “secondary active transport.” It is widely recognized as a general mechanism that has been adapted in a variety of ways for the transport, against a concentration or electrochemical gradient (see sect. methods) of numerous ions and other solutes in virtually all types of animals cells.

In studies on guinea pig, sheep, and calf heart, Reuter and Seitz (782, 783) showed that Ca\(^{2+}\) efflux from \(^{45}\)Ca-loaded auricular and ventricular muscle was largely dependent on external Na\(^+\) and Ca\(^{2+}\). They interpreted
that in control Tyrode solution which contained 1.8 mM Ca$^{2+}$ from a $^{45}$Ca-loaded guinea pig auricle (top) and a sheep ventricular trabeculum (bottom). Ordinate shows $^{45}$Ca efflux rate constant. Concentrations of Na$^{+}$ and Ca$^{2+}$ present in various external solutions at various times are indicated between these two curves; in Na$^{+}$-free solutions, NaCl was replaced by isosmotic sucrose. Note that most of Ca$^{2+}$ efflux into Ca$^{2+}$-free Tyrode is dependent on external Na$^{+}$. [From Reuter and Seitz (783).]

these observations as evidence for a Na$^{+}$/Ca$^{2+}$ exchange mechanism (see Fig. 1). Subsequently, Glitsch et al. (352) found that Ca$^{2+}$ influx in mammalian cardiac muscle was proportional to ([Na$^{+}$])$^{2}$ over a wide range of [Na$^{+}$]$_{o}$ (see Ref. 85).

At about the same time, Baker et al. (35) observed an external Ca$^{2+}$-dependent, ouabain-insensitive Na$^{+}$ efflux from squid axons (Figs. 2 and 3). In addition, Ca$^{2+}$ influx appeared to be a function of the [Na$^{+}$]/[Na$^{+}$]$_{i}$ ratio; increasing [Na$^{+}$], and/or decreasing [Na$^{+}$]$_{o}$ (Fig. 3) promoted Ca$^{2+}$ influx in this preparation (444). Similar observations were also made on crab nerve (34). The fact that the Ca$^{2+}$ efflux from $^{45}$Ca-injected squid axons was, in part, dependent on external Na$^{+}$ (102) fostered the idea that Ca$^{2+}$ extrusion involved a Na$^{+}$/Ca$^{2+}$ exchange mechanism. In sum, these Ca$^{2+}$ influx and efflux data seemed consistent with a transport mechanism that could move Ca$^{2+}$ in either direction across the PM, in exchange for Na$^{+}$; net Ca$^{2+}$ movement (and the Ca$^{2+}$ gradient) appeared to depend on the prevailing Na$^{+}$ electrochemical gradient $\Delta \mu$$_{Na}$ (35). The Na$^{+}$/Ca$^{2+}$ exchange systems in squid axons and mammalian cardiac muscle appeared to be similar, if not identical. Therefore, these findings led directly to the suggestion that the positive inotropic action of cardiac glycosides was due to a Na$^{+}$/Ca$^{2+}$ exchanger-mediated rise in cell Ca$^{2+}$, as a result of Na$^{+}$ pump inhibition and the consequent reduction in $\Delta \mu$$_{Na}$ across the sarcolemma (35 and see sect. VI A7). One feature that has challenged this hypothesis is the question of whether the very limited Na$^{+}$ pump inhibition produced by low (nanomolar) concentrations of cardiotonic steroids is sufficient to account for the observed enhancement of contraction (see sect. VI A7).

Martin and DeLuca (625) examined the effect of Na$^{+}$ in the mucosal and serosal fluids on the transepithelial transport of Ca$^{2+}$ in rat duodenum. They found that Na$^{+}$ was required in the serosal fluid to effect the net transfer of Ca$^{2+}$ from mucosa to serosa. They therefore suggested that the $\Delta \mu$$_{Na}$ across the basolateral membrane of the columnar epithelial cells powers the extrusion of Ca$^{2+}$ from the cytoplasm into the serosal medium via a Na$^{+}$/Ca$^{2+}$ exchange mechanism.

The regulation of the exchanger by ATP-dependent phosphorylation and by internal Ca$^{2+}$ was first hinted at by the observations of Baker and Glitsch (36). Subsequently, DiPolo (237, 238) clearly demonstrated the activation of exchanger-mediated Ca$^{2+}$ efflux by ATP, and Blaustein (88) showed that the effect was due to a shift in the cation activation kinetics. DiPolo (239, 244; see also Ref. 37) also made the unanticipated (and counterintuitive) observation that the Ca$^{2+}$ entry (and Na$^{+}$ exit) mode of exchange exhibited an absolute requirement for internal Ca$^{2+}$. This observation presages the finding that intracellular Ca$^{2+}$ catalyzes the turnover rate of the the Na$^{+}$/Ca$^{2+}$ exchanger (see sect. II A4).

Early observations that the Ca$^{2+}$ efflux was activated by the cooperative action of more than two Na$^{+}$ provided a clue that the coupling ratio of the exchanger was $>$2 Na$^{+}$:1 Ca$^{2+}$ (85). This led to the view that the exchange
was voltage sensitive (108, 670). Subsequently, Hume and Uehara (438, 439) observed “creep currents” in cardiac muscle, which they attributed to a rheogenic Na\(^+\)/Ca\(^{2+}\) exchange. Kimura, Noma, and co-workers (514, 515) then clearly identified a current attributable to the Na\(^+\)/Ca\(^{2+}\) exchanger in patch-clamped cardiac myocytes (Fig. 4). With the development of the giant membrane patch method by Hilgemann and co-workers (190, 405), rapid and accurate measurement of a current attributable to Na\(^+\)/Ca\(^{2+}\) exchange in cardiac myocyte membranes could be made.

The apparent stoichiometry (coupling ratio) of the exchanger was established on the basis of a large variety of tracer flux and electrophysiological experiments in various tissues. These included cardiac muscle (285, 514, 726, 762, 765), squid axons (249), and barnacle muscle (757, 754). In all of these tissues, a coupling ratio of 3 Na\(^+\):1 Ca\(^{2+}\) was observed.

Additional properties of the exchanger were elucidated as a result of the application of other novel methods: ion-selective Ca\(^{2+}\) and Na\(^+\) electrodes (846, 847), release of caged Ca\(^{2+}\) (695), and electrical measurements of transport in reconstituted proteoliposomes fused to a planar lipid bilayer (289).

Experiments on vertebrate photoreceptors by Yau and Nakatani (997) demonstrated that these cells, too, also contain a PM Na\(^+\)/Ca\(^{2+}\) exchanger. The studies of Schnetkamp et al. (830) and Cervetto, McNaughton, and Nakatani (997) demonstrated that these cells, too, also contain a PM Na\(^+\)/Ca\(^{2+}\) exchange. The studies of Schnetkamp et al. (830) and Cervetto, McNaughton, and Nakatani (997) demonstrated that these cells, too, also contain a PM Na\(^+\)/Ca\(^{2+}\) exchange. The studies of Schnetkamp et al. (830) and Cervetto, McNaughton, and Nakatani (997) demonstrated that these cells, too, also contain a PM Na\(^+\)/Ca\(^{2+}\) exchange. The studies of Schnetkamp et al. (830) and Cervetto, McNaughton, and Nakatani (997) demonstrated that these cells, too, also contain a PM Na\(^+\)/Ca\(^{2+}\) exchange. The studies of Schnetkamp et al. (830) and Cervetto, McNaughton, and Nakatani (997) demonstrated that these cells, too, also contain a PM Na\(^+\)/Ca\(^{2+}\) exchange. The studies of Schnetkamp et al. (830) and Cervetto, McNaughton, and Nakatani (997) demonstrated that these cells, too, also contain a PM Na\(^+\)/Ca\(^{2+}\) exchange. The studies of Schnetkamp et al. (830) and Cervetto, McNaughton, and Nakatani (997) demonstrated that these cells, too, also contain a PM Na\(^+\)/Ca\(^{2+}\) exchange. The studies of Schnetkamp et al. (830) and Cervetto, McNaughton, and Nakatani (997) demonstrated that these cells, too, also contain a PM Na\(^+\)/Ca\(^{2+}\) exchange. 

Twenty years ago, Reeves and Sutko (764) launched the biochemical approach to the exchanger with their seminal studies of Na\(^+\)/Ca\(^{2+}\) exchange in cardiac sarcoplasmic vesicles. This led to the partial purification of the cardiac exchanger by Philipson et al. (719) and to the eventual cloning of this exchanger by Nicoll et al. (688). The exchanger from bovine rod outer segments was purified by Cook and co-workers (206, 767) (and see also Ref. 575), and subsequently cloned by Reilander et al. (768). The photoreceptor exchanger and the cardiac/neuronal exchanger have very different primary sequences, and it seems clear that these two types of exchangers evolved independently. This encourages speculation about the physiological need for such exchangers and the process of evolution, a topic that is discussed in section IVII.

Very recently, Tsoi et al. (940) cloned a novel ex-

![Graph](http://physrev.physiology.org/)

**Fig. 3.** Ca\(^{2+}\) influx (left) and external Ca\(^{2+}\) (Ca\(_o\))-dependent Na\(^+\) efflux (right) in squid giant axons, graphed as a function of [Na\(^+_o\)]. At reduced [Na\(^+_o\)], Na\(^+\) was replaced by isosmotic dextrose (dextrose-Na curves, •) or Li\(^+\) (Li-Na curves, ▼). All external solutions were K\(^+\)-free and contained 10^-5 M ouabain. Na\(^+\) influx data (right) are shown as either efflux rate constants (left ordinate scale) or calculated Ca\(_o\)-dependent effuxes (right ordinate scale). Na\(^+\) efflux curves were calculated from a model described in original article (35). Both Ca\(^{2+}\) influx (left panel) and ouabain-insensitive, Ca\(_o\)-dependent Na\(^+\) efflux (right) are activated by low [Na\(^+_o\)], and inhibited by high [Na\(^+_o\)]. [From Baker et al. (35).]

**Fig. 4.** Activation of Ca\(_o\)-dependent outward (Na\(^+\)/Ca\(^{2+}\) exchange) current by Ca\(_o\) in a single patch-clamped guinea pig cardiac myocyte. A; voltage-clamp records showing voltage (top traces) and current (bottom traces). Ramp pulses from a holding potential of -30 to +60 mV, and then to -120 mV, at a ramp speed of 0.2 V/s, were given every 10–15 s. In first panel, patch pipette (internal solution) contained 30 mM Na\(^+\) and 2 mM CsCl to block K\(^+\) channels. Pipette solutions all contained 126 mM CsCl to reduce current through K\(^+\) channels. B and C, current-voltage (I-V) curves were obtained from descending portions (+60 to -120 mV) of ramp pulses. In B, curves were obtained before (a) and during (b) superfusion with solution containing 1 mM Ca\(^{2+}\). In C, I-V relations were obtained with internal Ca\(^{2+}\) (Ca\(_i\)) equal to 75 mM before (c) and during (d) superfusion with solution containing 1 mM Ca\(^{2+}\). In this case, there is a Ca\(_i\)-activated outward current (d minus c) that is also dependent on internal Ca\(^{2+}\); current also depended on internal Na\(^+\). [From Kimura et al. (515).]
The exchanger from rat brain. The exchanger expressed from this clone is very homologous to the photoreceptor exchanger, and Ca\(^{2+}\) transport mediated by this brain exchanger, like the photoreceptor exchanger, also requires K\(^+\) (see also Ref. 219). Surprisingly, as discussed in sections viA and viE, both the K\(^-\)-independent (cardiac-type) and K\(^+\)-dependent (vertebrate photoreceptor-type) exchangers appear to be coexpressed in some of the same neurons (465, 940). These observations open important new avenues for exploration, just as they raise critical questions about our previously conceived notions about the physiological role(s) of Na\(^+/Ca^{2+}\) exchange.

### III. Properties of the Sodium/Calcium Exchanger

The observations on Na\(^+/Ca^{2+}\) exchange in nerve, muscle, and epithelia made during the late 1960s provided a starting point and a paradigm for numerous studies on Ca\(^{2+}\) transport that have been carried out during the past three decades. A Na\(^+/Ca^{2+}\) exchanger has now been identified in most types of tissues, in both vertebrates and invertebrates. Here we describe the properties of the very widely distributed “cardiac/neuronal” type of exchanger that is found in most tissues. Some of the unusual properties of the vertebrate photoreceptor exchanger, already alluded to above, are also discussed.

#### A. Modes of Operation of the Na\(^+/Ca^{2+}\) Exchanger

Initial studies in several preparations indicated that the Na\(^+/Ca^{2+}\) exchanger could move net Ca\(^{2+}\) either out of or into cells depending on the prevailing electrochemical driving forces (i.e., the Na\(^+\) and Ca\(^{2+}\) concentration gradients and the V\(_{m}\)). These two modes of Na\(^+/Ca^{2+}\) exchange (see Figs. 1 and 3) will be referred to, respectively, as “Ca\(^{2+}\) exit mode” and “Ca\(^{2+}\) entry mode” exchange. The often used terms forward mode and reverse mode will be avoided because the latter may be construed, incorrectly, as an abnormal mode of exchange. Indeed, a very important part of the exchanger’s function may be to promote Ca\(^{2+}\) entry (and to reduce Ca\(^{2+}\) exit) under some normal physiological conditions. A clear-cut example is in the erythrocytes of certain mammals such as dogs and ferrets (see sect. viF1), where the Na\(^+/Ca^{2+}\) exchanger plays an important role in the extrusion of Na\(^+\).

1. **Ca\(^{2+}\) entry mode exchange**

The Ca\(^{2+}\) entry mode of exchange is usually identified as an internal Na\(^+\) (Na\(_i\))-dependent Ca\(^{2+}\) influx and an external Ca\(^{2+}\) (Ca\(_o\))-dependent, ouabain-insensitive Na\(^+\) efflux. A critical feature of the (net) Ca\(^{2+}\) influx mode of operation (and also the Ca\(^{2+}\) efflux mode) is that it is dependent on (nontransported) intracellular Ca\(^{2+}\) (37, 239, 244, 246, 258, 406, 514, 515, 757). The [Ca\(^{2+}\)]\(_i\), required for half-maximal activation (i.e., K\(_{50}\)) for intracellular Ca\(^{2+}\) of Ca\(^{2+}\) influx mode exchange in squid axons (239, 244, 254) and cardiac myocyte giant patches (406, 412; but see Ref. 514) is on the order of 1 \(\mu\)M under physiological conditions. Consequently, only a small fraction of the exchangers are active at the normal resting [Ca\(^{2+}\)]\(_i\) (\(\sim\)100 nM) in most cells. In contrast, the exchanger is fully activated when [Ca\(^{2+}\)]\(_i\) is in the low micromolar range (i.e., at about the [Ca\(^{2+}\)]\(_i\) expected during peak activity in many types of excitable and secretory cells). The kinetic and physiological consequences of this regulation by internal Ca\(^{2+}\) are discussed in section viE4.

Another surprising feature of Ca\(^{2+}\) influx mode exchange (see Figs. 2 and 3) is its activation by low concentrations of external alkali metal ions, including Na\(^+\) (35, 98, 314, 336); the activating monovalent cations are not transported (57). High [Na\(^+\)]\(_o\) reduces the exchanger-mediated Ca\(^{2+}\) influx, however. The kinetics of this activation by alkali metal ions and the biphasic actions of external Na\(^+\) are also described in section viE4.

2. **Ca\(^{2+}\) exit mode exchange**

This mode of exchange is defined operationally as an external Na\(^+\) (Na\(_o\))-dependent Ca\(^{2+}\) efflux and an internal Ca\(^{2+}\) (Ca\(_i\))-dependent ouabain- and tetrodotoxin (TTX)-insensitive Na\(^+\) influx. Like the Na\(_i\)-dependent Ca\(^{2+}\) influx, the Na\(_o\)-dependent Ca\(^{2+}\) efflux is activated by internal alkali metal ions and is markedly inhibited by high concentrations of internal Na\(^+\) (88) (but see Refs. 249, 254). The Ca\(^{2+}\) efflux mode of exchange also appears to be activated by nontransported intracellular Ca\(^{2+}\) (412) (and see sect. viE4), even though the K\(_{50}\) for regulation and for transport may be similar. In contrast to Ca\(^{2+}\) entry mode exchange, however, this Ca\(^{2+}\) efflux does not require extracellular Ca\(^{2+}\) for activation (88, 249); this is clear functional evidence that the exchanger is asymmetric.

The hydrolysis of ATP is not required to power net Ca\(^{2+}\) extrusion mediated by the Na\(^+/Ca^{2+}\) exchanger, and Na\(^+/Ca^{2+}\) exchange can be demonstrated in ATP-depleted cells (88, 102, 244). Nevertheless, as discussed in section viE1, cytosolic ATP does substantially alter the exchanger kinetics (88), presumably by phosphorylating critical sites on the exchanger molecules (237, 238). This is further evidence that the exchanger is asymmetric. The asymmetry apparently enables the exchanger to function optimally because of the differences in the affinities for Ca\(^{2+}\) on the two sides of the membrane and the modulation of the transport kinetics and turnover by various ligands.
3. Ca\textsuperscript{2+}/Ca\textsuperscript{2+} exchange mediated by the Na\textsuperscript{+}/Ca\textsuperscript{2+} exchanger

The identification of both Ca\textsuperscript{2+} entry and Ca\textsuperscript{2+} exit modes of exchange demonstrated that the exchanger can mediate the bidirectional movements of both Na\textsuperscript{+} and Ca\textsuperscript{2+} across the PM. This raised the question of whether the exchanger could also mediate the coupled exchange of internal Ca\textsuperscript{2+} for external Ca\textsuperscript{2+} (i.e., Ca\textsuperscript{2+}/Ca\textsuperscript{2+} exchange) and/or internal Na\textsuperscript{+} for external Na\textsuperscript{+} (Na\textsuperscript{+}/Na\textsuperscript{+} exchange) (see Fig. 5). The presence of these two modes of exchange, while perhaps not functionally important in terms of net ion translocation, could provide critical insight into the transport mechanism.

The Ca\textsuperscript{2+}/Ca\textsuperscript{2+} exchange is usually identified as a trans-Ca\textsuperscript{2+}-dependent Ca\textsuperscript{2+} flux (i.e., Ca\textsubscript{in} dependent Ca\textsuperscript{2+} efflux and Ca\textsubscript{out} dependent Ca\textsuperscript{2+} influx), where trans refers to ions acting on opposite sides of the PM. On the basis of this criterion, a Ca\textsuperscript{2+}/Ca\textsuperscript{2+} exchange that appears to be mediated by the Na\textsuperscript{+}/Ca\textsuperscript{2+} exchanger has been identified in various types of cells. A few examples are squid axons (35, 88, 107, 249, 254), barnacle muscle (686, 754, 757, 800), mammalian neurons (98), and cardiac muscle (763, 863).

Two lines of evidence indicate that these Ca\textsuperscript{2+} fluxes are mediated by the Na\textsuperscript{+}/Ca\textsuperscript{2+} exchanger. One stems from the observations that the Ca\textsubscript{i}-dependent Ca\textsuperscript{2+} influx is activated by external (i.e., on the same side of the PM, or cis to Ca\textsuperscript{2+}) alkali metal ions and is inhibited by external Na\textsuperscript{+} in much the same way as is Ca\textsuperscript{2+} entry mode exchange (35, 107, 108). In this case, however, internal alkali metal ions (e.g., K\textsuperscript{+} or Li\textsuperscript{+}) are also required for activation of the exchange, and high [Na\textsuperscript{+}]\textsubscript{i} is also markedly inhibitory (88). Second, the Na\textsuperscript{+}/Ca\textsuperscript{2+} exchange and Ca\textsuperscript{2+}/Ca\textsuperscript{2+} exchange do not appear to be independent because they cannot both operate simultaneously at maximal rates. Inhibition of Ca\textsuperscript{2+}/Ca\textsuperscript{2+} exchange by external Na\textsuperscript{+} is associated with a shift from Ca\textsuperscript{2+}/Ca\textsuperscript{2+} exchange to Ca\textsuperscript{2+} exit mode exchange (88). Conversely, inhibition by internal Na\textsuperscript{+} (107, 138) is likely due to a shift in the operation from Ca\textsuperscript{2+}/Ca\textsuperscript{2+} exchange to Ca\textsuperscript{2+} entry mode exchange.

The Ca\textsuperscript{2+}/Ca\textsuperscript{2+} exchange mode exhibits a coupling ratio of 1 Ca\textsuperscript{2+}:1 Ca\textsuperscript{2+} (107) yet is (surprisingly) voltage sensitive (249) but electroneutral (562). There is no net transfer of charge during Ca\textsuperscript{2+}/Ca\textsuperscript{2+} exchange, in contrast to the situation during Na\textsuperscript{+}/Ca\textsuperscript{2+} exchange (see below). Whether or not the activating alkali metal ions are also transported during Ca\textsuperscript{2+}/Ca\textsuperscript{2+} exchange is not known. However, the fact that cis-activating alkali metal ions (other than Na\textsuperscript{+}) do not appear to be transported during Na\textsuperscript{+}/Ca\textsuperscript{2+} exchange (57, 996) (and see below) suggests that they may not be transported during Ca\textsuperscript{2+}/Ca\textsuperscript{2+} exchange.

Adenosine 5`-triphosphate also modulates the kinetics of Ca\textsuperscript{2+}/Ca\textsuperscript{2+} exchange (686, 249). The Ca\textsuperscript{2+}/Ca\textsuperscript{2+} mode of exchange can proceed at a very high rate in ATP-depleted cells (686). Adenosine 5`-triphosphate appears to inhibit the Ca\textsuperscript{2+}/Ca\textsuperscript{2+} exchange mode, probably by increasing the affinity of the exchanger for Na\textsuperscript{+} (88).

In sum, all of these studies are consistent with the view that the Na\textsuperscript{+}/Ca\textsuperscript{2+} exchanger can also mediate Ca\textsuperscript{2+}/Ca\textsuperscript{2+} exchange.

4. Na\textsuperscript{+}/Na\textsuperscript{+} exchange mediated by the Na\textsuperscript{+}/Ca\textsuperscript{2+} exchanger

Evidence for coupled Na\textsuperscript{+} influx and Na\textsuperscript{+} efflux mediated by the Na\textsuperscript{+}/Ca\textsuperscript{2+} exchanger was first obtained in cardiac sarcolemmal preparations by Reeves and Sutko (764, 759). They described a Na\textsuperscript{+} efflux that was dependent on external Na\textsuperscript{+}; activation was sigmoid, with a K\textsubscript{0.5} of 15 mM. DiPolo and Beaugé (246) subsequently identified, in squid axons, a ouabain-insensitive component of Na\textsubscript{in}-dependent Na\textsuperscript{+} efflux that corresponds to a Na\textsuperscript{+}/Na\textsuperscript{+} exchange (see Fig. 5) mediated by the Na\textsuperscript{+}/Ca\textsuperscript{2+} exchanger. This view is based on the evidence that the Na\textsubscript{in}-dependent Na\textsuperscript{+} efflux, like the Ca\textsuperscript{2+} entry mode of exchange, is 1) dependent on internal Ca\textsuperscript{2+} for activation, as is the Ca\textsuperscript{2+} entry mode of exchange; 2) activated by...
internal alkalization; 3) inhibited by internal Mg$^{2+}$; 4) activated by internal ATP and by adenosine 5'-O-(3-thiotriphosphate) which is a substrate for kinases but not ATPases (370, 793); and 5) inhibited by external Ca$^{2+}$ under conditions that promote Ca$^{2+}$ entry mode exchange (i.e., external Ca$^{2+}$ shifts the exchanger from Na$^+$/Na$^+$ exchange mode to Ca$^{2+}$/entry mode). A Ca$^-$$^-$ independent component of Na$$^-$$-dependent Na$^+$ efflux was also identified; this flux had different properties and did not appear to be mediated by the Na$^+$/Ca$^{2+}$ exchanger (246).

5. “Uncoupled” transport of Na$^+$ or Ca$^{2+}$ mediated by the Na$^+$/Ca$^{2+}$ exchanger

A few attempts have been made to study half-cycles or single complete cycles of the Na$^+$/Ca$^{2+}$ exchanger to identify and characterize intermediate steps in the exchange cycle (407, 414, 693, 694). Nevertheless, there is no evidence that the exchanger can mediate significant net efflux of Ca$^{2+}$ (or Na$^+$) in the absence of external Na$^+$ or Ca$^{2+}$, or net influx of Ca$^{2+}$ (or Na$^+$) in the absence of internal Na$^+$ or Ca$^{2+}$. Thus it appears that the coupled countertransport is an obligatory feature of the mechanism: the exchanger does not mediate “uncoupled” transport of either Na$^+$ or Ca$^{2+}$. In other words, unloaded forms of the exchanger molecules apparently do not cycle or cycle very slowly.

B. Energetics and Kinetics of Na$^+$/Ca$^{2+}$ Exchange

1. Thermodynamic considerations

In the resting cells of higher animals, [Na$^+$]$_i$ and [Ca$^{2+}$]$_i$ are on the order of 10 mM and 0.1 μM (for present purposes, we refer to concentrations rather than activities; see Ref. 85). The extracellular Na$^+$ and free Ca$^{2+}$ concentrations ([Na$^+$]$_o$ and [Ca$^{2+}$]$_o$, respectively) are usually on the order of 450 mM and 3–4 mM in marine invertebrates (85) and 120–145 mM and 1–2 mM in most vertebrates and other invertebrates, respectively. Thus, with $V_M$ values on the order of −80 to −60 mV, there is a large, inwardly directed electrochemical gradient for Na$^+$ ($\Delta \mu_{Na}$) and a very large, inwardly directed electrochemical gradient for Ca$^{2+}$ ($\Delta \mu_{Ca}$). Both ions tend to move into the cell passively and must be extruded by energy-dependent mechanisms. The two electrochemical gradients are defined, respectively, as

$$\Delta \mu_{Na} = F(E_{Na} - V_M)$$

(1)

$$= RT \ln \left( \frac{[Na^+]_o}{[Na^-]_i} \right) - V_M F$$

(1A)

and

$$\Delta \mu_{Ca} = 2F(E_{Ca} - V_M)$$

(1')

$$= RT \ln \left( \frac{[Ca^{2+}]_o}{[Ca^{2-}]_i} \right) - 2V_M F$$

(1A')

where $E_{Na}$ and $E_{Ca}$ are, respectively, the equilibrium potentials for Na$^+$ and Ca$^{2+}$, and $R$, $T$, and $F$ are the gas constant, absolute temperature and Faraday’s number, respectively.

For a transport system in which the flux of one Ca$^{2+}$ is coupled to the counterflow of n Na$^+$, the equilibrium relationship between the two electrochemical gradients is

$$\Delta \mu_{Ca} = n \Delta \mu_{Na}$$

(2)

If the fluxes of one Ca$^{2+}$ and one K$^+$ are coupled to the counterflow of n Na$^+$, as in vertebrate photoreceptors, the equilibrium relationship is given by

$$\Delta \mu_{Ca} = n \Delta \mu_{Na} - \Delta \mu_{K}$$

(2A)

where $\Delta \mu_{K}$ is the electrochemical gradient for K$^+$, defined as

$$\Delta \mu_{K} = F(E_{K} - V_M)$$

$$= RT \ln \left( \frac{[K^+]_o}{[K^-]_i} \right) - V_M F$$

From the definitions of the electrochemical gradients, Equations 2 and 2A can be expanded to, respectively

$$\frac{[Ca^{2+}]_o}{[Ca^{2-}]_i} = \left( \frac{[Na^+]_o}{[Na^-]_i} \right)^n \times \exp \left[ -\frac{(n - 2)V_M F}{RT} \right]$$

(3)

and

$$\frac{[Ca^{2+}]_o}{[Ca^{2-}]_i} = \left( \frac{[Na^+]_o}{[Na^-]_i} \right)^n \times \left( \frac{[K^+]_o}{[K^-]_i} \right) \times \exp \left[ -\frac{(n - 3)V_M F}{RT} \right]$$

(3A)

2. The coupling ratio of the cardiac/neuronal-type Na$^+$/Ca$^{2+}$ exchanger

The coupling ratio ($n$) is obviously a key parameter in Equation 3. We shall adopt the terminology of Läuger (559) and shall refer to the ratio of the number of transported Na$^+$ to the number of transported Ca$^{2+}$ as the “coupling ratio” ($n_T$). This may be different from the ratio of the number of binding sites for transported Na$^+$ and transported Ca$^{2+}$ on the exchanger which, following Läuger (559), we shall refer to as the “stoichiometry” ($n_B$).
Numerous attempts have been made to determine the value of $n_T$ or of the stoichiometry, including several direct measurements of the coupled fluxes. The critical problem in direct measurements of the coupling ratio is to account for all of the exchanger-mediated fluxes of $Na^+$ and $Ca^{2+}$ and to exclude all other, parallel movements of these two ions (i.e., those not mediated by the exchanger). Without a specific inhibitor of the $Na^+/Ca^{2+}$ exchanger, it is difficult to ascertain that all fluxes through other pathways are excluded.

The Hill coefficient corresponding to the cooperativity between external $Na^+$ in activating $Ca^{2+}$ efflux was used initially to estimate the coupling between $Na^+$ and $Ca^{2+}$ (35, 85, 800). The first direct measurement of the coupling ratio (i.e., measurement of the coupled fluxes of $Na^+$ and $Ca^{2+}$) was made in internally dialyzed, ATP-depleted squid axons. The ratio of the $Ca_{i}$-dependent $Na^+$ influx to the $Na_{i}$-dependent $Ca^{2+}$ influx (i.e., “$Ca^{2+}$ influx mode exchange”) was $3.1:1$ (107). In internally perfused, ATP-fueled barnacle muscle cells, the ratio of the $Ca_{i}$-dependent ($Ca_{i}$-activated) $Na^+$ influx to the $Na_{i}$-dependent ($Ca_{i}$-activated) $Ca^{2+}$ influx, over a wide range of $[Na^+]_{o}$ was $2.8–3.2:1$, with an average of $3.1:1$, as shown in Figure 6 (754, 757). In these barnacle muscle experiments, the exchanger-mediated fluxes were defined not only by the counter-ion dependence, but also, independently, by the activation by nontransported internal $Ca^{2+}$ (Figs. 5 and 6, and see sect. iiE); thus ion movements mediated by other transport mechanisms could be excluded.

Measurements of coupled $Na^+$ and $Ca^{2+}$ fluxes, from “initial flux rates,” in cardiac sarcolemmal vesicles also yielded a coupling ratio of $3 Na^+:1 Ca^{2+}$ (101), whereas $n_T$ was reported to have a value of 4 in dog heart sarcolemmal vesicles (568) and in rat brain plasmalemmal vesicles (47, 48). In cultured rat heart cells, the ratio of the $Ca_{s}$-dependent $Na^+$ efflux to the $Na_{s}$-dependent $Ca^{2+}$ uptake was $3.1:1$ (965, 966). Measurements of net $Ca_{s}$-dependent $Na^+$ efflux and $Ca^{2+}$ uptake in cardiotonic steroid-treated rabbit ventricles also yielded a $Na^+$-to-$Ca^{2+}$ flux ratio of $3:1$ (131), although this was not based on initial rate data.

These direct flux measurements only address the coupling ratio between $Na^+$ and $Ca^{2+}$; they ignore possible coupled ($Na^+/Ca^{2+}$ exchanger-mediated) movements of other ions such as $H^+$, $K^+$, or $Cl^-$. However, if more than two $Na^+$ are exchanged for one $Ca^{2+}$, and the net charge is not counterbalanced by the coupled movements of other ions, then the exchange should be voltage sensitive and should involve net charge movement that ought to be detectable as an exchanger-generated current (i.e., the flux should be rheogenic).

Indirect methods for determining the coupling ratio of the $Na^+/Ca^{2+}$ exchanger are generally model dependent (290). Several elegant experiments were designed to determine the exchanger coupling ratio on the assumption that the only ions transported by the exchanger are $Na^+$ and $Ca^{2+}$ and that the $Ca^{2+}$ is not accompanied by a proton, for example, to compensate for the net charge carried by $Na^+$ if more than two $Na^+$ are exchanged for one $Ca^{2+}$. Reeves and Hale (762) used a null-point method (based on Eq. 2) to establish the $Na^+$ and $Ca^{2+}$ concentration gradient and $V_M$ under conditions in which there was no net exchanger-mediated flux of $Ca^{2+}$ into bovine cardiac sarcolemmal vesicles; their results yielded a coupling ratio of $3:1$ (Fig. 7).

Kimura et al. (514) determined the reversal potential ($E_{Na/Ca}$) see Eqs. 5 and 6 for the exchanger-mediated inward and outward currents in single, internally perfused guinea pig cardiac myocytes. They found that $E_{Na/Ca}$ was very close to the value expected for a coupling ratio of 3 and very different from that predicted for $n_T = 4$ (Fig. 8). This result was confirmed and extended by Ehrara et al. (285).

Bridge et al. (132) compared the integral of the inward, nifedipine-sensitive $Ca^{2+}$ current evoked by depolarization in cardiac myocytes (i.e., net entering $Ca^{2+}$, which should equal the amount of $Ca^{2+}$ that must be
and components of the thermodynamic driving force for exchanger (see lead to the same conclusion: the cardiac/neuronal type the coupling ratio, using several different methods, all
possibility that $K_1$ coupling ratio.

I the integral of $Na$ extruded during recovery) with the integral of the inward $Na^+/Ca^{2+}$ exchange current after repolarization (Fig. 9). The integral of $Ca^{2+}$ current ($I_{Ca}$) was twice as large as the integral of $I_{Na/Ca}$. This, too, is consistent with a 3:1 coupling ratio.

Yasui and Kimura (996) (and see Ref. 283) tested the possibility that $K^+$ might be transported by the cardiac exchanger, as in the vertebrate photoreceptor (see below). But, again, $E_{Na/Ca}$ fit a coupling ratio of 3 $Na^+:1 Ca^{2+}$, irrespective of the external $K^+$ concentration.

The exchanger in squid axons also has a reversal potential close to that predicted for a 3 $Na^+:1 Ca^{2+}$ coupling ratio (249). Furthermore, neither the squid axon exchanger (204, 249) nor the barnacle muscle exchanger (755) requires external $K^+$.

Taken together, these numerous measurements of the coupling ratio, using several different methods, all lead to the same conclusion: the cardiac/neuronal type $Na^+/Ca^{2+}$ exchanger has a coupling ratio of 3 $Na^+:1 Ca^{2+}$ when operating in either the $Ca^{2+}$ influx or $Ca^{2+}$ efflux mode.

3. Coupling ratios in the vertebrate photoreceptor

Two groups (420, 546, 997) measured the net charge carried by the $Na^+/Ca^{2+}$ exchanger in amphibian rod photoreceptor outer segments (ROS) during net extrusion of $Ca^{2+}$ when $V_M$ and the internal and external ion concentrations were varied. In both studies, the charge movement associated with the $Na_+$-dependent efflux of $Ca^{2+}$ suggested that one of the $Na^+$ entered as an uncompensated positive charge. They concluded that $Na^+/Ca^{2+}$ exchange in ROS was consistent with a fixed coupling ratio of 3 $Na^+:1 Ca^{2+}$. Schnetkamp et al. (830) (see also Refs. 320, 575, 687, 831), however, observed that $Na^+/Ca^{2+}$ exchange in ROS was halted in the absence of $K^+$, and they concluded that the exchanger in ROS also transports $K^+$. Cervetto et al. (165) (see also Ref. 548) then showed that $E_{Na/Ca}$ in the amphibian photoreceptor ROS is consistent with a coupling ratio of 4 $Na^+$ to 1 $Ca^{2+}$ and 1 $K^+$ (Fig. 10). With hindsight, this difference in coupling ratio between the exchanger in ROS and that present in many extruded during recovery) with the integral of the inward $Na^+/Ca^{2+}$ exchange current after repolarization (Fig. 9).

The integral of $Ca^{2+}$ current ($I_{Ca}$) was twice as large as the integral of $I_{Na/Ca}$. This, too, is consistent with a 3:1 coupling ratio.

Yasui and Kimura (996) (and see Ref. 283) tested the possibility that $K^+$ might be transported by the cardiac exchanger, as in the vertebrate photoreceptor (see below). But, again, $E_{Na/Ca}$ fit a coupling ratio of 3 $Na^+:1 Ca^{2+}$, irrespective of the external $K^+$ concentration.

The exchanger in squid axons also has a reversal potential close to that predicted for a 3 $Na^+:1 Ca^{2+}$ coupling ratio (249). Furthermore, neither the squid axon exchanger (204, 249) nor the barnacle muscle exchanger (755) requires external $K^+$.

Taken together, these numerous measurements of the coupling ratio, using several different methods, all lead to the same conclusion: the cardiac/neuronal type $Na^+/Ca^{2+}$ exchanger has a coupling ratio of 3 $Na^+:1 Ca^{2+}$ when operating in either the $Ca^{2+}$ influx or $Ca^{2+}$ efflux mode.

3. Coupling ratios in the vertebrate photoreceptor

Two groups (420, 546, 997) measured the net charge carried by the $Na^+/Ca^{2+}$ exchanger in amphibian rod photoreceptor outer segments (ROS) during net extrusion of $Ca^{2+}$ when $V_M$ and the internal and external ion concentrations were varied. In both studies, the charge movement associated with the $Na_+$-dependent efflux of $Ca^{2+}$ suggested that one of the $Na^+$ entered as an uncompensated positive charge. They concluded that $Na^+/Ca^{2+}$ exchange in ROS was consistent with a fixed coupling ratio of 3 $Na^+:1 Ca^{2+}$. Schnetkamp et al. (830) (see also Refs. 320, 575, 687, 831), however, observed that $Na^+/Ca^{2+}$ exchange in ROS was halted in the absence of $K^+$, and they concluded that the exchanger in ROS also transports $K^+$. Cervetto et al. (165) (see also Ref. 548) then showed that $E_{Na/Ca}$ in the amphibian photoreceptor ROS is consistent with a coupling ratio of 4 $Na^+$ to 1 $Ca^{2+}$ and 1 $K^+$ (Fig. 10). With hindsight, this difference in coupling ratio between the exchanger in ROS and that present in many
FIG. 10. Na\(^+/\)Ca\(^{2+}\) exchanger currents in salamander rod outer segments. A: currents measured during exposure to a solution containing 0.5 mM K\(^+\), 1 mM Ca\(^{2+}\), and indicated Na\(^+\) concentrations; after 20 s, solution was switched to one containing 110 mM Na\(^+\) and 5 mM K\(^+\). B: relation between charge flow on return to second solution and \([\text{Na}^+]_o\) during first 20-s period. Intersection of the horizontal broken line and the continuous diagonal line extrapolated from the data indicates that there is no (net) charge transfer at \([\text{Na}^+]_o = 62\) mM. Arrows show predicted crossing points for exchange stoichiometries of \(n\) Na\(^+\):1 K\(^+\), where \(n = 3, 4,\) or 5. C: data from a similar experiment in which \([\text{Ca}^{2+}]_o\) and \([\text{Na}^+]_o\) were changed simultaneously. In this case, rod outer segment (ROS) was maintained in a solution containing 110 mM Na\(^+\), 5 mM Ca\(^{2+}\), and 5 mM K\(^+\); \([\text{Ca}^{2+}]_o\) was reduced to 1 mM, and \([\text{Na}^+]_o\) was changed to concentration shown on abscissa for 100 s. Ordinate shows charge transferred upon return to original solution. Continuous line, which was drawn by eye, crosses dashed line (no net charge transfer) at \([\text{Na}^+]_o = 72\) mM. Arrows show expected crossing points for exchange stoichiometries of \(n\) Na\(^+\):1 Ca\(^{2+}\), where \(n = 3, 4,\) or 5. D: effect of a simultaneous change in \(V_M\) and \([\text{K}^+]_o\) on charge transfer by ROS. ROS was maintained in a solution containing 110 mM Na\(^+\), 1 mM Ca\(^{2+}\), and 5 mM K\(^+\); \([\text{K}^+]_o\) was reduced to 2 mM for 40 s; depolarizing pulses from a holding potential of −14 mV coincided with these solution changes. Graph shows charge transfer (ordinate) as a function of pulse amplitude (abscissa). Arrow indicates change in \(V_M\) required to compensate for a 2.5-fold change in \([\text{K}^+]_o\) if one charge exchanges for 1 K\(^+\). The only coupling ratio consistent with all of these data is 4 Na\(^+\):(1 Ca\(^{2+}\) + 1 K\(^+\)). [From Cervetto et al. (165).]
other tissues does not seem surprising. The [Na+]i in vertebrate photoreceptors is much higher than in many other cells, being on the order of 40 mM. This high [Na+]i is due to the “dark current” (inward Na- current observed in the absence of light). Thus there is insufficient energy in ROS \( \Delta \mu_{Na} \) for an exchanger with a coupling ratio of 3 Na+:1 Ca2+ to be able to extrude Ca2+ against the large Ca2+ gradient. In contrast, invertebrate photoreceptors employ a different type of phototransduction mechanism, and they do not have a dark current or a relatively high [Na+]i. The exchanger in invertebrate photoreceptors therefore does not require K+ and appears to be more like the cardiac/neuronal type exchanger, with a coupling ratio of 3 Na+:1 Ca2+ (22, 225, 657, 659) (and see sect. vi). 

Recently, a K+-dependent Na+/Ca2+ exchanger was identified in human platelets (517). Whether this exchanger mediates a 4 Na+:(1 Ca2++ 1 K+) exchange (i.e., 4 Na+ are exchanged for 1 Ca2+ + 1 Na+), and whether it has sequence homology to the vertebrate photoreceptor exchanger is not yet known. Also, transcripts of a clone that is homologous to the bovine ROS exchanger (940). The expressed protein mediates K+-dependent Na+/Ca2+ exchange.

As discussed in section nI, there is little amino acid sequence homology between the vertebrate ROS exchanger and the cardiac/neuronal type Na+/Ca2+ exchanger. Thus the ROS Na+/Ca2+ exchanger probably evolved de novo, and it appears that there are two different molecules with very similar functions. This implies that there was evolutionary (and physiological) pressure to develop a transport system of this type (i.e., an Na+/Ca2+ exchanger, in contrast to an ATP-driven Ca2+ pump) to fit the needs of the vertebrate photoreceptor. Detailed structural comparison of the cardiac-type and ROS-type Na+/Ca2+ exchangers should provide novel information about the nature of the Na+ and Ca2+ binding sites on these exchangers.

4. Equilibrium conditions

The sum of these varied types of observations, carried out on numerous different tissues and species under a wide variety of conditions, indicates that the Na+/Ca2+ exchanger in most tissues (but not ROS) 1) likely has a fixed coupling ratio of 3 Na+:1 Ca2+ and 2) does not normally transport other ions along with the Na+ and Ca2+. Thus Equation 3 can be rewritten as

\[
\frac{[\text{Ca}^{2+}]_o}{[\text{Ca}^{2+}]_i} = \left( \frac{[\text{Na}^+]_o}{[\text{Na}^+]_i} \right)^3 \times \exp \left( \frac{-V_M F}{RT} \right) \tag{4}
\]

We may then ask how well the equilibrium conditions defined by Equations 3 and 4 fit with experimental observations of Na+ and Ca2+ distribution and \( V_M \). In other words, if \([\text{Ca}^{2+}]_o\), \([\text{Na}^+]_o\), \([\text{Na}^+]_i\), and \( V_M \) are all known, can Equation 4 be used to predict \([\text{Ca}^{2+}]_i\)? Alternatively, if \([\text{Ca}^{2+}]_i\) is known, as well, does the calculated coupling ratio \( n \) (Eq. 3) equal 3 (i.e., can we confirm Eq. 4)?

A good illustration of the application of these equations comes from the work of Sheu and Fozzard (846, 847) (see also Ref. 32). When the ion concentration and \( V_M \) values obtained from their ion-selective electrode studies of resting (quiescent) cardiac muscle cells were inserted into Equation 3, Sheu and Fozzard (846) calculated a value of \( n = 2.5 \), i.e., their measured \([\text{Ca}^{2+}]_i\) was higher than the value predicted if \( n \) is actually equal to 3 (Eq. 4). This means that the Na+/Ca2+ exchanger was not driving \([\text{Ca}^{2+}]_i\), down to the value expected from the energy available in \( \Delta \mu_{Na} \), that is, entry of Ca2+ via other pathways and relatively slow extrusion via the Na+/Ca2+ exchanger (because of the low \([\text{Ca}^{2+}]_i\); see below) were sufficient to maintain \([\text{Ca}^{2+}]_i\) slightly above the level predicted from \( E_{NaCa} \). When the Na+ pump was inhibited with ouabain, however, causing \([\text{Na}^+]_i\), and \([\text{Ca}^{2+}]_i\), to rise (presumably thereby saturating more of the exchanger’s intracellular binding sites; see next section), insertion of the new, elevated \([\text{Na}^+]_i\), and \([\text{Ca}^{2+}]_i\) values into Equation 3 fit with a value of \( n = 3 \) (Fig. 11). These observations are, nevertheless, consistent with the idea that the Na+/Ca2+ exchanger has a fixed coupling ratio of 3. When \([\text{Na}^+]_i\) and \([\text{Ca}^{2+}]_i\) are low, and the exchanger’s ion binding sites are

\[ \text{FIG. 11.} \text{ Interrelationship between Na}^+ \text{ and Ca}^{2+} \text{ electrochemical gradients (} \mu_{Na} \text{ and } \mu_{Ca} \text{) in cardiac ventricular muscle. Graph shows relationship between electrochemical gradients for Ca}^{2+} \text{ 2} \left( E_{Na} - V_M \right) \text{ and Na}^+ \text{ 1} \left( E_{Na} - V_M \right) \text{ when ventricular myocytes are treated with 5 } \mu \text{M ouabain. In all cases, control data (in absence of ouabain) are at top right ends of graph; each symbol represents a different cell. After Na}^+ \text{ pump inhibitor, values for both electrochemical gradients declined progressively, as indicated by each of lines connecting symbols. Broken lines represent predicted coupling ratio (} n \text{) relationships between } \mu_{Na} \text{ and } \mu_{Ca} \text{ for values of } n = 2, 3, \text{ and 4. Data best fit relationship for a 3 } \text{Na}^+:1 \text{Ca}^{2+} \text{ exchange. [From Sheu and Fozzard (847).]} \]
unsaturated so that exchanger net transport is low, other mechanisms will play an important, but not exclusive, role in the control of $[\text{Ca}^{2+}]$. Indeed, the exchanger may even drive $\text{Ca}^{2+}$ into the cells, depending on the polarity of the term $V_M - E_{\text{Na/Ca}}$ (see Eq. 5). Alternatively, we must consider the possibility that some of the exchanger molecules may be operating in the $\text{Ca}^{2+}$ efflux mode while others are mediating $\text{Ca}^{2+}/\text{Ca}^{2+}$ exchange. Under these circumstances, the equilibrium conditions required for the application of Equation 4 will not prevail. The apparent coupling ratio calculated from Equation 3 will then be lower than the exchanger’s true coupling ratio [see Heinz and Geck (398) regarding coupling efficiency]. However, when $[\text{Na}^+]$ and $[\text{Ca}^{2+}]$ are high enough to saturate many of the binding sites on the exchanger so that turnover is high, the exchanger may play a dominant role in controlling $[\text{Ca}^{2+}]$ in cardiac myocytes. This leads us into a discussion of the kinetics of $\text{Na}^+/\text{Ca}^{2+}$ exchange.

5. Kinetics of $\text{Na}^+/\text{Ca}^{2+}$ exchange

As mentioned above, $\text{Ca}^{2+}$ can be transported both inwardly and outwardly via the $\text{Na}^+/\text{Ca}^{2+}$ exchanger. Net $\text{Ca}^{2+}$ flux mediated by the exchanger $[J_{\text{Ca}(\text{Na/Ca})}]$ is expressed in units of moles/(area $\times$ time). The flux is governed by the difference between $V_M$ and the reversal potential of the exchanger, $E_{\text{Na/Ca}}$, a thermodynamic term, as well as by the kinetic parameters that control the rate of exchange

$$J_{\text{Ca}(\text{Na/Ca})} = k_{\text{Na/Ca}}(V_M - E_{\text{Na/Ca}})$$

(5)

where

$$E_{\text{Na/Ca}} = n_E E_{\text{Na}} - 2E_{\text{Ca}}$$

(6)

and the equilibrium potentials for $\text{Na}^+$ and $\text{Ca}^{2+}$, $E_{\text{Na}}$ and $E_{\text{Ca}}$ respectively, are

$$E_{\text{Na}} = (RT/F) \times \ln ([\text{Na}^+]/[\text{Na}^+])$$

(6A)

and

$$E_{\text{Ca}} = (RT/2F) \times \ln ([\text{Ca}^{2+}]_{\text{o}}/[\text{Ca}^{2+}])$$

(6B)

Likewise, the net $\text{Na}^+$ flux mediated by the exchanger $[J_{\text{Na}(\text{Na/Ca})}]$ is given by

$$J_{\text{Na}(\text{Na/Ca})} = -n_E k_{\text{Na/Ca}}(V_M - E_{\text{Na/Ca}})$$

(5')

The variable $k_{\text{Na/Ca}}$ in Equations 5 and 5’ is a complex kinetic parameter that is analogous to a conductance density (i.e., a conductance per unit area). This variable depends on 1) the number of carriers, 2) the fractional saturation of the carrier binding sites by the activating ions and transported ions, 3) $V_M$, 4) ATP, and 5) other factors that may modulate the exchanger including phosphorylation and dephosphorylation (see sect. wD).

With a coupling ratio of 3, the reversal potential of the exchanger, $E_{\text{Na/Ca}}$ is given by (from Eq. 3)

$$E_{\text{Na/Ca}} = 3E_{\text{Na}} - 2E_{\text{Ca}}$$

(6')

Moreover, with this coupling ratio, the $\text{Na}^+/\text{Ca}^{2+}$ exchanger mediates the transfer of net charge, and thereby generates an ionic current. $\text{Na}^+/\text{Ca}^{2+}$ exchange is therefore a rheogenic (current-generating) process, and its activity is now often measured as a current (e.g., see sect. wD). Thus it is appropriate to write an equation equivalent to the flux equations (i.e., Eqs. 5 and 5’) to describe the net current density mediated by the exchanger ($I_{\text{Na/Ca}}$)

$$I_{\text{Na/Ca}} = g_{\text{Na/Ca}}(V_M - E_{\text{Na/Ca}})$$

(7)

$I_{\text{Na/Ca}}$ is the current per unit area (or current density); it corresponds to the net charge transfer that results from the sum of the $\text{Ca}^{2+}$ and $\text{Na}^+$ counterfluxes [i.e., $J_{\text{Ca}(\text{Na/Ca})} + J_{\text{Na}(\text{Na/Ca})}$]. The kinetic term $g_{\text{Na/Ca}}$ is therefore equivalent to a conductance per unit area (or conductance density). It corresponds to the slope of the relationship between the electrical driving force ($V_M - E_{\text{Na/Ca}}$) and the current density $I_{\text{Na/Ca}}$. Like $k_{\text{Na/Ca}}$ in Equations 5 and 5’, $g_{\text{Na/Ca}}$ is a nonlinear, complex term, and both are influenced by the same kinetic and regulatory parameters.

In contrast to its role in primary active transport systems such as the PM or SR/ER $\text{Ca}^{2+}$ pumps, ATP does not directly fuel the $\text{Na}^+/\text{Ca}^{2+}$ exchanger. ATP may, however, provide the energy for the phosphorylation of the exchanger. This phosphorylation apparently alters the kinetics of binding and translocation of the transported ions in cardiac muscle (410, 412) and in squid axons (88, 245), the two tissues that have been studied most extensively. Under normal physiological conditions, in ATP-replete cells, the exchanger molecules appear to be at least partially phosphorylated; this phosphorylated form exhibits a high affinity for external $\text{Na}^+$ and internal $\text{Ca}^{2+}$ (88, 243, 245).

A particularly important and paradoxical finding is the activation of the $\text{Ca}^{2+}$ entry mode of exchange by internal $\text{Ca}^{2+}$ (243, 244, 246, 757). As illustrated for barnacle muscle (Fig. 6) and cardiac muscle (Fig. 4), there is an absolute requirement for internal $\text{Ca}^{2+}$. Indeed, no internal $\text{Na}^+$-dependent, external $\text{Ca}^{2+}$-activated outward current is observed in the absence of internal $\text{Ca}^{2+}$. The apparent half-maximal activation of the $\text{Na}^+/\text{Ca}^{2+}$ exchanger by internal $\text{Ca}^{2+}$ is observed with a $[\text{Ca}^{2+}]_{\text{o}}$ in the dynamic physiological range [between 0.1 and 1.0 $\mu$M, for example, in squid axons (246), barnacle muscle (757), and...
TABLE 1. Na\textsuperscript{+}/Ca\textsuperscript{2+} exchanger ion binding sites for Na\textsuperscript{+} and Ca\textsuperscript{2+}

<table>
<thead>
<tr>
<th>Site</th>
<th>Ions Bound</th>
<th>Role</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Cytoplasmic face</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>C\textsubscript{1}</td>
<td>1 Ca\textsuperscript{2+}</td>
<td>Catalysis</td>
</tr>
<tr>
<td>C\textsubscript{2}</td>
<td>1 Ca\textsuperscript{2+}</td>
<td>Transport</td>
</tr>
<tr>
<td>N\textsubscript{1}</td>
<td>(&quot;Na&quot;), Li\textsuperscript{+}, K\textsuperscript{+}, (&quot;Rb&quot; , Cs&quot;)*</td>
<td>Catalysis (&quot;transport of Na&quot;)†</td>
</tr>
<tr>
<td>N\textsubscript{2}</td>
<td>2 (or 3) Na\textsuperscript{+}</td>
<td>Transport</td>
</tr>
</tbody>
</table>

| **Extracellular face** | | |
| C\textsubscript{1\text{o}} | No equivalent site to C\textsubscript{1} | Transport |
| C\textsubscript{2\text{o}} | 1 Ca\textsuperscript{2+} | Transport |
| N\textsubscript{1\text{o}} | 1 Na\textsuperscript{+}, Li\textsuperscript{+}, K\textsuperscript{+}, Rb\textsuperscript{+}, or Cs\textsuperscript{+} | Catalysis ("transport of Na")† |
| N\textsubscript{2\text{o}} | 2 (or 3) Na\textsuperscript{+} | Transport |

* Only Li\textsuperscript{+} and K\textsuperscript{+} have been tested. † Although cardiac/neuronal-type Na\textsuperscript{+}/Ca\textsuperscript{2+} exchanger mediates exchange of 3 Na\textsuperscript{+} for 1 Ca\textsuperscript{2+}, it is not clear whether there is a separate binding site for 3rd transported Na\textsuperscript{+}. We speculate that the Na\textsuperscript{+} that binds to site N\textsubscript{1\text{o}} or N\textsubscript{1} can be transported when site N\textsubscript{2\text{o}} or N\textsubscript{2} is loaded with 2 Na\textsuperscript{+} (see text). Subscripts i and o refer to intracellular and extracellular, respectively.

There is no a priori reason to expect that all Na\textsuperscript{+}/Ca\textsuperscript{2+} exchangers have identical kinetics; indeed, as we shall see, the exchangers from different species may have some surprisingly different kinetic or mechanistic properties. Furthermore, it is important to note that some of the kinetic parameters are modulated by ATP and membrane potential (244, 246, 254, 631) as well as by a variety of other factors that will be discussed in more detail in section III, D and E. The kinetic constants (ion \( K_{0.5} \) values = ion concentrations at half-maximal activation or inhibition) listed here are all based on transport studies (measurements of ionic fluxes or ionic currents). These \( K_{0.5} \) values include the effects of catalytic modulation that prevailed during the measurements. The maximum flux (\( J_{\text{max}} \)) values are not listed because they may vary greatly as a result of exchanger modulation. It is beyond the scope of this review to list all of the measured kinetic constants under all conditions that have thusfar been studied; the interested reader should consult the review by DiPolo and Beaugé (254) as well as the original literature cited in the text, tables, and figures.

A) Ca\textsuperscript{2+} entry mode exchange. External Ca\textsuperscript{2+} activation of the exchanger functioning in the Ca\textsuperscript{2+} entry mode is hyperbolic. The half-maximum activation (\( K_{0.5} \) at site C\textsubscript{2\text{o}}; see Table 1) is observed at about \([Ca^{2+}]_o\) of 2–7 mM in marine invertebrates incubated in Na\textsuperscript{+}-free media (88, 107). In standard Na\textsuperscript{+}-containing media, the apparent affinity for (transported) Ca\textsubscript{o} is greatly reduced (\( K_{0.5} \) = 50–100 mM); this presumably is a reflection of the antagonism between Na\textsubscript{o} and Ca\textsubscript{o} (e.g., see Fig. 3B). Mammalian heart (631) and brain (314, 809) exhibit a much higher affinity for Ca\textsubscript{o} (\( K_{0.5} \) = 0.1–0.3 mM). Antagonism between Na\textsubscript{o} and Ca\textsubscript{o} is also observed in these mammalian preparations (Table 2).

As noted above, the Ca\textsuperscript{2+} entry mode of operation of the exchanger is also totally dependent on internal Ca\textsuperscript{2+} (239, 246, 757). No Ca\textsubscript{o}-dependent Na\textsuperscript{+} efflux or Na\textsubscript{i}-dependent Ca\textsuperscript{2+} influx is observed when \([Ca^{2+}]_i\) < 10\textsuperscript{-8} M (e.g., see Figs. 4 and 6), and the rate of transport therefore depends directly on this activating Ca\textsubscript{i}. The \( K_{0.5} \) at this Ca\textsuperscript{2+} binding site (C\textsubscript{1}) is ~0.6–2 \( \mu \)M internal Ca\textsuperscript{2+} in ATP-fueled cells. This internal Ca\textsuperscript{2+} is not transported by the exchanger, however, because little or no Ca\textsubscript{o}-dependent Ca\textsuperscript{2+} efflux (Ca\textsuperscript{2+}/Ca\textsuperscript{2+} exchange) is observed when external Na\textsuperscript{+} is replaced by a monovalent cation that is not an alkali metal ion and [Na\textsuperscript{+}] is high (i.e., under the conditions favoring Ca\textsuperscript{2+} entry mode exchange). The affinity for internal activating Ca\textsuperscript{2+} is reduced by a factor of ~10 in ATP-depleted cells (88, 246); this may indicate that the binding of Ca\textsuperscript{2+} to site C\textsubscript{1} is modulated by phosphorylation. It is important to note that there is no evidence for activation by Ca\textsubscript{i} (i.e., there is no “C\textsubscript{1} site” in our notation) analogous to the activation by Ca\textsubscript{o} (at site C\textsubscript{1}; see Table 1).

The activation of Ca\textsuperscript{2+} influx by Na\textsubscript{i} is a sigmoid...
function of \([\text{Na}^+]_i\); the \(K_{0.5}\) (at site \(N_2^{-}\)) is \([\text{Na}^+]_i\) equals 15–25 mM in mammalian cardiac muscle and 30–60 mM in squid axons and barnacle muscle fibers (see Table 2 for references). The apparent affinity for \(\text{Na}^+\) is increased by the activating \(\text{Ca}^{2+}\) (239). As shown in Figure 6, the \([\text{Na}^+]_i\) activation curve for \(\text{Ca}^{2+}\) influx in barnacle muscle has a Hill coefficient of 3. This implies that \(\text{Ca}^{2+}\) efflux depends on the cooperative action of at least 3 \(\text{Na}^+\), which is consistent with the coupling ratio of 3 \(\text{Na}^+:1\) \(\text{Ca}^{2+}\). Here, and in the ensuing discussion, we present mean \(K_{0.5}\) values for \(\text{Na}^+\) that correspond to the half-maximal activation (or inhibition) by the several \(\text{Na}^+\) acting cooperatively, but the situation is probably much more complex, as discussed below. It is not clear whether all three activating \(\text{Na}^+\) bind to site \(N_2^{-}\) in this case, while only two \(\text{Na}^+\) bind to the site when internal \(\text{Na}^+\) inhibits \(\text{Ca}^{2+}\) exit.

### Table 2. \(\text{Na}^+/\text{Ca}^{2+}\) exchanger ion binding “constants” (apparent \(K_{0.5}\) values)

<table>
<thead>
<tr>
<th>Site</th>
<th>Ion</th>
<th>Role</th>
<th>(K_{0.5}) Values</th>
<th>Mammals</th>
<th>Reference No.</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td>(+\text{ATP})</td>
<td>(-\text{ATP})</td>
<td></td>
</tr>
<tr>
<td>(C_1^{-})</td>
<td>(\text{Ca}^+)</td>
<td>Activation</td>
<td>0.6–2 (\mu)M</td>
<td>10–15 (\mu)M</td>
<td>0.02–0.05 (\mu)M</td>
</tr>
<tr>
<td>(C_2^{-})</td>
<td>(\text{Ca}^+)</td>
<td>Inhibition</td>
<td>(&gt;800) (\mu)M</td>
<td></td>
<td></td>
</tr>
<tr>
<td>(N_1^{-})</td>
<td>(\text{Na}^+)</td>
<td>Activation?</td>
<td>?</td>
<td>15–25 mM</td>
<td></td>
</tr>
<tr>
<td>(N_2^{-})</td>
<td>(\text{Na}^+)</td>
<td>Transport</td>
<td>30–60 mM</td>
<td>15–25 mM</td>
<td></td>
</tr>
<tr>
<td>(C_2^{-})</td>
<td>(\text{Ca}^+)</td>
<td>Transport</td>
<td>2–50 mM</td>
<td>0.1–1.4 mM</td>
<td>11, 34, 35, 314, 314, 314</td>
</tr>
<tr>
<td>(N_1^{-})</td>
<td>(\text{Na}^+)</td>
<td>Activation</td>
<td>50–70 mM</td>
<td>2 mM</td>
<td>35, 314</td>
</tr>
<tr>
<td>(L_1^{-})</td>
<td>(\text{Li}^+)</td>
<td>Activation</td>
<td>70 mM</td>
<td>2 mM</td>
<td>35</td>
</tr>
<tr>
<td>(N_2^{-})</td>
<td>(\text{Na}^+)</td>
<td>Inhibition</td>
<td>50–100 mM</td>
<td>45–70 mM</td>
<td>34, 35, 314, 425, 661</td>
</tr>
</tbody>
</table>

### \(\text{Ca}^{2+}\) entry mode exchange

<table>
<thead>
<tr>
<th>Site</th>
<th>Ion</th>
<th>Role</th>
<th>(K_{0.5}) Values</th>
<th>Mammals</th>
<th>Reference No.</th>
</tr>
</thead>
<tbody>
<tr>
<td>(C_1^{-})</td>
<td>(\text{Ca}^+)</td>
<td>Activation</td>
<td>?</td>
<td>?</td>
<td>412</td>
</tr>
<tr>
<td>(N_1^{-})</td>
<td>(\text{Na}^+)</td>
<td>Activation?</td>
<td>?</td>
<td>?</td>
<td>88, 240, 631, 661, 686, 801</td>
</tr>
<tr>
<td>(N_2^{-})</td>
<td>(\text{Ca}^+)</td>
<td>Inhibition</td>
<td>30 mM</td>
<td>?</td>
<td>88, 107</td>
</tr>
<tr>
<td>(C_2^{-})</td>
<td>(\text{Ca}^+)</td>
<td>Transport</td>
<td>7.5–15 (\mu)M</td>
<td>110–140 mM</td>
<td>88, 240, 631, 661, 686, 801</td>
</tr>
<tr>
<td>(N_1^{-})</td>
<td>(\text{Na}^+)</td>
<td>Activation?</td>
<td>?</td>
<td>?</td>
<td>29, 809</td>
</tr>
<tr>
<td>(N_2^{-})</td>
<td>(\text{Na}^+)</td>
<td>Transport</td>
<td>50–80 mM</td>
<td>30 mM</td>
<td>88, 98, 514, 631, 801, 809</td>
</tr>
</tbody>
</table>

### \(\text{Ca}^{2+}/\text{Ca}^{2+}\) (self) exchange mode

<table>
<thead>
<tr>
<th>Site</th>
<th>Ion</th>
<th>Role</th>
<th>(K_{0.5}) Values</th>
<th>Mammals</th>
<th>Reference No.</th>
</tr>
</thead>
<tbody>
<tr>
<td>(C_1^{-})</td>
<td>(\text{Ca}^+)</td>
<td>Activation</td>
<td>?</td>
<td>?</td>
<td>412</td>
</tr>
<tr>
<td>(N_1^{-})</td>
<td>(\text{Na}^+)</td>
<td>Activation?</td>
<td>?</td>
<td>?</td>
<td>88, 107</td>
</tr>
<tr>
<td>(C_2^{-})</td>
<td>(\text{Ca}^+)</td>
<td>Inhibition</td>
<td>?</td>
<td>?</td>
<td>88, 107</td>
</tr>
<tr>
<td>(N_1^{-})</td>
<td>(\text{Na}^+)</td>
<td>Inhibition</td>
<td>50 mM</td>
<td>15–25 mM</td>
<td>254</td>
</tr>
<tr>
<td>(N_2^{-})</td>
<td>(\text{Na}^+)</td>
<td>Activation?</td>
<td>?</td>
<td>?</td>
<td>246</td>
</tr>
<tr>
<td>(L_1^{-})</td>
<td>(\text{Li}^+)</td>
<td>Activation</td>
<td>?</td>
<td>?</td>
<td>88, 108</td>
</tr>
</tbody>
</table>

### \(\text{Na}^+/\text{Na}^+\) (self) exchange mode

<table>
<thead>
<tr>
<th>Site</th>
<th>Ion</th>
<th>Role</th>
<th>(K_{0.5}) Values</th>
<th>Mammals</th>
<th>Reference No.</th>
</tr>
</thead>
<tbody>
<tr>
<td>(C_1^{-})</td>
<td>(\text{Ca}^+)</td>
<td>Activation</td>
<td>?</td>
<td>?</td>
<td>246</td>
</tr>
<tr>
<td>(N_1^{-})</td>
<td>(\text{Na}^+)</td>
<td>Activation?</td>
<td>?</td>
<td>?</td>
<td>631</td>
</tr>
<tr>
<td>(C_2^{-})</td>
<td>(\text{Ca}^+)</td>
<td>Inhibition</td>
<td>?</td>
<td>?</td>
<td>246</td>
</tr>
<tr>
<td>(N_1^{-})</td>
<td>(\text{Na}^+)</td>
<td>Inhibition</td>
<td>?</td>
<td>?</td>
<td>246</td>
</tr>
<tr>
<td>(N_2^{-})</td>
<td>(\text{Na}^+)</td>
<td>Transport</td>
<td>?</td>
<td>?</td>
<td>246</td>
</tr>
</tbody>
</table>

\(K_{0.5}\), mean affinity constant. Subscripts i and o refer to intracellular and extracellular, respectively.
mode exchange (see below). For simplicity (and in the absence of relevant data), we will assume that N1 binds a single alkali metal ion (see below), that N2 can bind only two Na⁺, and that exchanger can cycle when both sites are loaded with (a total of three) Na⁺.

Raising [Ca^{2+}]ᵢ would be expected to inhibit the Ca^{2+} entry mode of exchange by shifting the exchanger to the Ca^{2+} efflux mode or to Ca^{2+}/Ca^{2+} (self) exchange (see below). This effect of Ca₃ has not been studied explicitly, however.

Calcium entry mode exchange is activated (at site N1ᵢ) by nontransported external alkali metal ions (57) including Na⁺ (K_{0.5} ~50–75 mM in squid axons and crab nerves and ~2 mM in synaptosomes). Monovalent cations that are not alkali metal ions, such as choline and tetramethylammonium (TMA), are ineffective at activating that are not alkali metal ions, such as choline and tetra-

motic invertebrates as well as in mammals (Table 2). In

entry (35), presumably because they cannot bind to site N1ᵢ. High [Na⁺]ₒ, however, inhibits the exchange; the K_{0.5} (or IC_{50}) for Naᵢ, is 100–200 mM in squid axons and 45–70 mM in mammalian tissue (35, 314, 336). The inhibi-
tion is directly proportional to ([Na⁺]₀)², which indicates that two Na⁺ act cooperatively to effect the inhibition (314), presumambly at site N2ₒ. The biphasic effect of external Na⁺, viz. activation by low [Na⁺]₀ and inhibition by high [Na⁺]₀, hints at the very complex kinetics. The available data (see Ref. 314) indicate that a single Na⁺ or other alkali metal ion binds to the activating (relatively nonselective) site, N1ᵢ, whereas two Na⁺ interfere with Ca^{2+} binding by, themselves, binding to the second (Na⁺-selective) site, N2ₒ. The latter site presumably acco-
adates only two Na⁺. How this site relates to the external site that binds Ca^{2+} (C₂ₒ) is unclear. One possibility is that the same site can bind either two Na⁺ and, perhaps with some conformational rearrangement (to account for noncompetitive inhibition), one Ca^{2+}; in other words, sites N2ₒ and C₂ₒ may simply be slightly different confor-
mations of the same ion binding site. Thus inhibition of Ca^{2+} entry by Naᵢ may reflect a shift in exchanger mode to Ca^{2+} exit mode or to Na⁺/Na⁺ self-exchange (see below).

b) Ca^{2+} exit mode exchange. In ATP-fueled cells, the Ca^{2+} exit mode of exchange is activated by internal Ca^{2+} with a K_{0.5} in the range of [Ca^{2+}]ᵢ equal to 0.6–6 μM in marine invertebrates as well as in mammals (Table 2). In ATP-depleted squid axons, the K_{0.5} is increased to 8–15 μM. Molecular studies indicate that when the Ca₉ regulatory site (C₁ᵢ) is mutated, the affinity for Caᵢ at the transport site (C₂ᵢ) is greatly reduced (634, 635). Thus it seems likely that two Ca^{2+} may act cooperatively to promote Ca^{2+} efflux and that one of these Ca^{2+} (at the lower affinity site, C₂ᵢ) is transported. The other Ca^{2+} (at the higher affinity site, C₁ᵢ) may simply serve in a catalytic role, similar to the role played by internal Ca^{2+} during Ca^{2+} entry mode exchange; in other words, the C₁ᵢ sites for Ca^{2+} influx and efflux may be one and the same.

The low affinity of the Na⁺/Ca^{2+} exchanger for Caᵢ, relative to the resting [Ca^{2+}]ᵢ, which in most cells is on the order of 100 nM, raises an important question about how the exchanger can extrude net Ca^{2+} (see Ref. 722). Indeed, the PM Ca^{2+} pump (PMCA pump) has about a 10-fold higher affinity for Caᵢ than does the exchanger (239). Several factors enter into the answer. 1) The exchanger has at least a 10-fold higher turnover rate than the PMCA pump (where turnover relates to the cycling rate of the exchanger molecules). 2) In many tissues (e.g., cardiac muscle), the exchanger has a larger “capacity” or maximum velocity of Ca^{2+} transport than the PMCA pump (where capacity corresponds to the product of the pump or exchanger density per unit membrane area and the turnover rate). 3) In many tissues (e.g., cardiac muscle), the exchanger plays a major role in the extrusion of Ca^{2+} following cell activation and the substantial elevation of [Ca^{2+}]ᵢ. Under these circumstances the PMCA pump may be saturated with Ca^{2+}, and its capacity to extrude Ca^{2+} rapidly may be limited, while the exchanger may still have a large reserve capacity. One other possibility is that local conditions may maintain [Ca^{2+}]ᵢ at a somewhat elevated level in the vicinity of the exchanger molecules; this will be discussed further in section VI A7.

The activation of the exchanger by ATP, and the apparent increase in affinity for Ca^{2+} at site C₂ᵢ (and perhaps also at site C₁ᵢ) involves a phosphorylation reaction because only MgATP and hydrolyzable analogs of ATP are effective (237, 243, 252, 253). CrATP, which is an excellent inhibitor of most protein kinases, blocks the stimulation of the exchanger by MgATP. Nevertheless, CrATP does not affect the maximum velocity of the exchanger that is achieved at saturating [Ca^{2+}]ᵢ (252).

Internal alkali metal ions (acting at site N₁ᵢ) activate this mode of exchange (88). As is the case for Naᵢ at the extracellular surface, however, internal Na⁺ has a biphasic effect and, at high concentration, also inhibits (by binding at site N2ₒ) the Ca^{2+} exit mode of exchange (88). The Naᵢ apparently interferes, in a noncompetitive manner, with the action of internal Ca^{2+} (107, 357, 776) but (see below) and shifts the exchanger into the Ca^{2+} entry mode or Na⁺/Na⁺ self-exchange mode. Thus raising [Na⁺]ᵢ causes the apparent affinity for internal Ca^{2+} to decrease (107, 138, 776). These interactions between Naᵢ and Caᵢ occur at the transport site (sites N2ₒ and C₂ₒ). As is true also for the external ion binding sites during Ca^{2+} entry mode exchange (see above), the precise mechanism of the interaction between internal Na⁺ and Ca^{2+} is unclear. Here, too, we may speculate that the two ionic species bind to different conformations (i.e., N2ₒ and C₂ₒ) of the same site.

In contrast to these interactions between Na⁺ and Ca^{2+} at the intracellular transport site(s), there is evidence that high [Na⁺]ᵢ does not have an inhibitory effect on the exchanger kinetics. Although Ca^{2+} exit mode exchange and Ca^{2+}/Ca^{2+} exchange are both completely in-
hibited at [Na\(^+\)]\(_i\) of 140 mM, increasing [Na\(^+\)]\(_i\) even to 350 mM continues to increase Ca\(^{2+}\) entry mode exchange (251, 253).

The Ca\(^{2+}\) exit mode of exchange obviously depends on Na\(_o\); the Na\(^+\) activation curve is sigmoid with a Hill coefficient of ~3 in most instances. Half-maximal activation occurs with [Na\(^+\)]\(_o\) equal to 50–80 mM in ATP-fueled squid axons and with substantially higher [Na\(^+\)]\(_o\) (~110–140 mM) in ATP-depleted axons; in other words, internal ATP also increases the affinity of the exchanger for external Na\(^+\) (88). The nature of the sites to which the external three Na\(^+\) bind is also unclear. We speculate, as above, that one Na\(^+\) binds to N1\(_o\) and two Na\(^+\) bind to N2\(_o\), then when all three ions are bound, the exchanger is able to cycle.

The interaction between ATP and Na\(_o\) is also noteworthy. At very low (nominal “0”) [Na\(^+\)]\(_i\), ATP has little or no activating effect on the Ca\(^{2+}\) efflux, whereas at [Na\(^+\)]\(_i\) = 40–60 mM, ATP activates the Ca\(^{2+}\) efflux (243). Whether ATP activates the Ca\(^{2+}\) efflux mode by directly or indirectly increasing the affinity for Ca\(_i\) and/or reducing the affinity for Na\(_i\) is not yet clear.

As expected from the aforementioned kinetic properties of an exchanger that can operate to either extrude Ca\(^{2+}\) or mediate Ca\(^{2+}\) entry in exchange for Na\(^+\), the concentrations of both Na\(^+\) and Ca\(^{2+}\) on both sides of the PM play key roles in determining whether there is net exit or net entry of Ca\(^{2+}\). Thus it is not surprising that external Ca\(^{2+}\) inhibits the Ca\(^{2+}\) efflux mode of exchange because binding of external Ca\(^{2+}\) at site C2\(_o\) promotes either Ca\(^{2+}\) entry mode exchange or Ca\(^{2+}\)/Ca\(^{2+}\) (self) exchange. The K\(_{0.5}\) (or IC\(_{50}\)) for Ca\(_o\) (at site C2\(_o\)) is not known precisely, but is >1 mM in barnacle muscle fibers (29) and <1 mM in synaptosomes (809).

Before leaving this discussion of the kinetics of Ca\(^{2+}\) entry and Ca\(^{2+}\) exit mode exchanges, it is important to mention, briefly, the topic of voltage sensitivity, although this will be extensively discussed in section mD. Both of these net Ca\(^{2+}\) transport modes are voltage sensitive and rheogenic. The Ca\(^{2+}\) entry mode is associated with an outward current and is augmented by depolarization and attenuated by hyperpolarization (661, 631). Conversely, the Ca\(^{2+}\) exit mode of exchange is associated with an inward current and is augmented by hyperpolarization and attenuated by depolarization (514, 515, 631). Furthermore, several of the ion dissociation constants (or K\(_{0.5}\) values), such as those for Ca\(_i\), are influenced by the membrane potential (631).

c) Ca\(^{2+}\)/Ca\(^{2+}\) Exchange. Ca\(^{2+}\)/Ca\(^{2+}\) exchange obviously requires both external and internal Ca\(^{2+}\). This mode of operation of the exchanger also has an absolute requirement for internal as well as external activating alkaline metal ions (88). In general, the kinetics of the ion interactions at the two surfaces of the exchanger are comparable to those observed for the net Ca\(^{2+}\) transport modes (Table 2). Thus, in squid axons and giant barnacle muscle fibers, the K\(_{0.5}\) value for Ca\(_i\) ranges between 1 and 3 mM in the presence of activating alkaline metal ions (88, 107, 108, 686). The K\(_{0.5}\) value for Ca\(_i\) is somewhat lower in mammalian preparations, 0.2–0.8 mM (98; and Blaustein, unpublished data), and is comparable to the K\(_{0.5}\) for Ca\(_o\) in Ca\(^{2+}\) influx mode exchange (see above). Both Sr\(^{2+}\) and Ba\(^{2+}\) can substitute for external Ca\(^{2+}\) in activating the exchanger-mediated Ca\(^{2+}\) efflux (88, 203).

The K\(_{0.5}\) values for Ca\(_i\) are similar those for Ca\(^{2+}\) exit mode exchange, ~2.5 μM in ATP-replete squid axons and 10–15 μM in ATP-depleted axons. These values likely reflect Ca\(^{2+}\) binding to site C2\(_o\). Whether or not Ca\(_i\) also binds to site C1\(_i\) (the regulatory site) is unknown and is difficult to measure. Nevertheless, it seems likely that occupation of site C1\(_i\) is required for Ca\(^{2+}\)/Ca\(^{2+}\) exchange because it is necessary for all the other modes of operation of the exchanger.

The kinetics of activation of Ca\(^{2+}/Ca\(^{2+}\) exchange by external Li\(^+\) (Li\(_o\)) is similar to those of activation of the Ca\(^{2+}\) entry mode of exchange. The K\(_{0.5}\) value for Li\(_o\) is 50–100 mM in squid axons and ~30 mM in synaptosomes (98, 108). The kinetics of activation by internal alkaline metal ions have not been studied.

Ca\(^{2+}/Ca\(^{2+}\) (self) exchange is electroneutral and involves the transfer of equal numbers of Ca\(^{2+}\) in both directions across the PM. Nevertheless, studies on marine invertebrate preparations (but not mammalian preparations; see below) demonstrate that it is voltage sensitive (12, 249, 254, 756; D. W. Hilgemann, personal communications). Depolarization reduces Ca\(^{2+}/Ca\(^{2+}\) exchange, whereas hyperpolarization augments it. The mechanism that underlies this voltage sensitivity is not clear, but these observations suggest that at least one of the Ca\(^{2+}\) binding or Ca\(^{2+}\) translocation steps is rate limiting. In this context, it may be relevant that the K\(_{0.5}\) for Ca\(_i\) is influenced by the V\(_M\) (631).

d) Na\(^+\)/Na\(^+\) exchange. The kinetics of Na\(^+\)/Na\(^+\) exchange have been less extensively studied. In the Na\(^+\)/Na\(^+\) self-exchange mode, the squid axon exchanger K\(_{0.5}\) for Na\(_o\) is comparable to the K\(_{0.5}\) for Na\(_o\) activation in the Ca\(^{2+}\) exit mode, 120–130 mM for Na\(_o\). Although the K\(_{0.5}\) for Na\(_o\) in this (Na\(^+\)/Na\(^+\) exchange) mode has not been determined, a value on the order of 10–20 mM is expected because the Na\(_o\) sites are saturated by 100 M Na\(^+\) (246). Like all other modes of operation of the exchanger, including the Ca\(^{2+}\) influx mode, Na\(^+\)/Na\(^+\) self-exchange exhibits an absolute requirement for Ca\(_i\) (at site Cl); see Fig. 5), with a K\(_{0.5}\) of ~10 μM Ca\(^{2+}\) in the absence of ATP (244, 246). Also, like the other modes of exchange, Na\(^+\)/Na\(^+\) exchange is inhibited by Mg\(_i\) with a K\(_{0.5}\) (IC\(_{50}\)) of ~1 mM Mg\(^{2+}\).

Na\(^+\)/Na\(^+\) exchange, like Ca\(^{2+}/Ca\(^{2+}\) exchange, is electroneutral and presumably involves the transfer of equal numbers of Na\(^+\) in the two directions across the PM.
Unlike Ca\textsuperscript{2+}/Ca\textsuperscript{2+} exchange, however, Na\textsuperscript{+}/Na\textsuperscript{+} exchange in marine invertebrate preparations is not voltage sensitive (249, 756). The significance of this observation is not yet clear. It may indicate that the Na\textsuperscript{+}-loaded form of the exchanger does not bear a net charge. This, however, conflicts with kinetic models of the exchanger operation that are based on data from mammalian cardiac muscle (631) because the data indicate that the Na\textsuperscript{+}-bound form of the cardiac Na\textsuperscript{+}/Ca\textsuperscript{2+} exchanger is voltage sensitive (631, 693). As discussed in section D, there may be some surprising species differences.

In sum, the aforementioned kinetic properties of the Na\textsuperscript{+}/Ca\textsuperscript{2+} exchanger indicate that the mode of operation of the exchanger depends critically on these kinetic properties and on the prevailing ion concentrations in the cytosol and the extracellular fluid (ECF). Thus, whether the exchanger, at the cytoplasmic surface, loads with Na\textsuperscript{+} or Ca\textsuperscript{2+} depends primarily on [Na\textsuperscript{+}]\textsubscript{i}, the K\textsubscript{0.5} for Na\textsuperscript{+}, [Ca\textsuperscript{2+}]\textsubscript{i}, and the K\textsubscript{0.5} for Ca\textsuperscript{2+} at site C\textsubscript{2}. Likewise, whether the exchanger, at the extracellular surface, loads with Na\textsuperscript{+} or Ca\textsuperscript{2+} depends primarily on [Na\textsuperscript{+}]\textsubscript{o}, the K\textsubscript{0.5} for Na\textsuperscript{+} (at sites N\textsubscript{1}\textsubscript{o} and N\textsubscript{2}\textsubscript{o}), [Ca\textsuperscript{2+}]\textsubscript{o}, and the K\textsubscript{0.5} for Ca\textsuperscript{2+} at site C\textsubscript{2}.o. These K\textsubscript{0.5} values, as well as the J\textsubscript{max} values, depend, in part, on “catalytic” modulation by a variety of factors, including Ca\textsuperscript{2+} and ATP, that will be discussed in section E. In addition, Ca\textsuperscript{2+}/Ca\textsuperscript{2+} exchange requires activation by both internal and external alkali metal ions and thus depends on both the alkali metal ion concentrations and the K\textsubscript{0.5} values for these ions at the the two faces of the membrane (sites N\textsubscript{1}\textsubscript{i} and N\textsubscript{1}\textsubscript{o}). Whether the exchanger cycles, in any of its modes of operation, depends critically on activation by Ca\textsuperscript{2+} at site C\textsubscript{1}.

C. Controversial Kinetic and Mechanistic Topics

In this section we cover four critical controversial and unresolved issues: 1) the mechanism of exchange (consecutive, or Ping-Pong, versus simultaneous transport), 2) the cis-interactions between Ca\textsuperscript{2+} and Na\textsuperscript{+} (competitive versus noncompetitive), 3) asymmetrical binding of Ca\textsuperscript{2+} to the exchanger at the internal and external surfaces of the plasma membrane, and 4) the role of activating alkali metal ions in both Na\textsuperscript{+}/Ca\textsuperscript{2+} exchange and Ca\textsuperscript{2+}/Ca\textsuperscript{2+} exchange. A fifth key kinetic issue, modulation of the exchanger kinetics by phosphorylation, cytosolic Ca\textsuperscript{2+}, and pH, is covered in section E. Regrettably, it appears that additional structural information about the exchanger protein will be needed to resolve many of the controversies. Nevertheless, the features elucidated to date provide a framework of very useful information to help us understand the physiological role(s) of the exchanger.

1. Mechanism of ion translocation

Several possible models of Na\textsuperscript{+}/Ca\textsuperscript{2+} exchange can be envisioned [as discussed by Milanick and Frame (655), we use the term mechanism to refer to the physical model and the term kinetics to refer to the mathematical behavior]. 1) One simple model, analogous to that for the Na\textsuperscript{+}/pump (803), is that the exchanger may bind only one species of transported ion at a time. For example, a Ca\textsuperscript{2+} may bind to the exchanger at the cytoplasmic face, be transported to the ECF, and may dissociate before Na\textsuperscript{+} (or another Ca\textsuperscript{2+}) is bound (Fig. 12). This is the so-called “consecutive” or “Ping-Pong” mechanism (see Ref. 494). In this case, there is no intermediate conformation of the exchanger in which transported Ca\textsuperscript{2+} and Na\textsuperscript{+} are bound simultaneously. 2) A second possibility, the “simultaneous” exchange mechanism, involves the simultaneous binding of, for example, 3 Na\textsuperscript{+} at the external site and 1 Ca\textsuperscript{2+} at the cytoplasmic face. Then, with all sites occupied, the exchanger could undergo a conformational change so that the Ca\textsuperscript{2+} is discharged to the ECF and the Na\textsuperscript{+} are released to the cytosol. 3) A variant of the consecutive type of mechanism [termed “sequential” by Milanick and Frame (655)] involves an unusual binding sequence. For example, cytosolic Ca\textsuperscript{2+} may be bound at the internal face and be translocated to the external face of the membrane by a conformational change, but external Na\textsuperscript{+} (or perhaps another alkali metal ion) may need to be bound to promote the dissociation of the Ca\textsuperscript{2+} into the extracellular fluid (655). (We might speculate that this could be the role of the activating alkali metal ion binding; however, such a possibility has not, to our knowledge, been tested.) Thus this sequential mechanism differs from the consecutive mechanism in that at least one conformation of the exchanger has both Na\textsuperscript{+} (i.e., at least 1 Na\textsuperscript{+}) and Ca\textsuperscript{2+} bound simultaneously, even though only one species is translocated at a time.

An early suggestion was that Na\textsuperscript{+}/Ca\textsuperscript{2+} exchange proceeded via a simultaneous countertransport of Na\textsuperscript{+} and Ca\textsuperscript{2+} (88; see also Refs. 580, 581). This view was based on two key observations. First, the kinetics of activation of Ca\textsuperscript{2+} efflux by external Na\textsuperscript{+} in squid axons appeared to be unaffected by the fractional saturation of the internal site with Ca\textsuperscript{2+}. Second, because the same exchanger mediates both Na\textsuperscript{+}/Ca\textsuperscript{2+} exchange and alkali metal ion-activated Ca\textsuperscript{2+}/Ca\textsuperscript{2+} exchange (see above), it appeared that both the internal and external sites must be loaded simultaneously to effect any ion translocation. Otherwise, we might expect a large component of uncoupled (unidirectional) exchanger-mediated transport, which is not observed. However, this rather narrow view did not take into account the possibilities that either unloaded exchanger molecules cannot cycle (a necessary element of a consecutive or sequential mechanism) or that dissociation of one species (e.g., Ca\textsuperscript{2+}) requires the
binding of the second ion species (Na\textsuperscript{+}, in this example, or another Ca\textsuperscript{2+} in the case of Ca\textsuperscript{2+}/Ca\textsuperscript{2+} exchange). The latter is the type of sequential mechanism proposed by Milanick and co-workers (653–656) to explain their data from ferret red blood cells.

Subsequently, Læuger (558) discussed kinetic methods to distinguish between simultaneous and consecutive mechanisms of exchange. Khananshvili (488) then applied the principles enunciated by Læuger to a study of Na\textsuperscript{+}/Ca\textsuperscript{2+} exchange in cardiac sarcolemmal vesicles. In particular, Khananshvili applied Læuger’s “zero-trans” conditions in which Ca\textsuperscript{2+} was absent on one side of the membrane and Na\textsuperscript{+} was absent on the other side. He showed that the $J_{\text{max}}$-to-Michaelis constant ($K_m$) ratio did not change over a wide range of [Ca\textsuperscript{2+}][o] and [Na\textsuperscript{+}]i values, when $J_{\text{max}}$ and $K_m$ both varied markedly. Accordingly, he concluded that these results fit Læuger’s conditions for a consecutive exchange mechanism.

Milanick and co-workers (653–656) examined the kinetics of Na\textsuperscript{+}/Ca\textsuperscript{2+} exchange in ferret erythrocytes. They estimated the $J_{\text{max}}/K_m$ directly from Ca\textsuperscript{2+} influx measurements (as a function of [Na\textsuperscript{+}][i]) at very low [Ca\textsuperscript{2+}][o] (<$K_m$), where the Michaelis-Menten equation reduces to $J = (J_{\text{max}} \times [\text{Ca}^{2+}][o])/K_m$. In contrast to Khananshvili’s results, these data showed that $J$ was dependent on [Na\textsuperscript{+}]i, which seems inconsistent with a consecutive mechanism. This led to the conclusion that the exchanger mechanism involves Ca\textsuperscript{2+} binding and translocation but that at least one Na\textsuperscript{+} must then bind before the Ca\textsuperscript{2+} dissociates; in other words, a sequential mechanism. It is possible that a model such as this may resolve some of the controversy between consecutive and simultaneous exchange mechanisms. Some of the controversy may, however, be the result of differences in the experimental methods used to determine the transport mechanism. Alternatively, the precise kinetic mechanism may depend on the particular exchanger gene or splice variant (see sect. IV, D and E), as exemplified by the Cl\textsuperscript{−}/HCO\textsubscript{3}\textsuperscript{−} exchanger, in which one isoform apparently operates by a consecutive mechanism (327, 327a) and another by a simultaneous mechanism (778).

2. Cis-interactions between Na\textsuperscript{+} and Ca\textsuperscript{2+}

Several investigators have examined the kinetic behavior of Na\textsuperscript{+} and Ca\textsuperscript{2+} in terms of their mutual antago-
Cations bind to two types of sites on the exchanger. One possibility is that the sites are relatively “rigid” and so that it can accommodate, preferentially, either two Na\(^{+}\) (in one conformation) or one Ca\(^{2+}\) (in the alternative conformation). These structural features might, depending on circumstances, give rise to either competitive or noncompetitive kinetics. Another possibility, mentioned in section IV, C, 1, is that different Na\(^{+}\)/Ca\(^{2+}\) exchanger gene products or splice variants (see sect. IV, D and E) might exhibit different kinetic behaviors.

3. Asymmetrical binding of Ca\(^{2+}\) at the internal and external sites on the exchanger

We have attempted to avoid some of the ambiguities of sidedness referred to, above, with respect to partially purified PM vesicle experiments. Therefore, this discussion is limited to a review of studies in which sidedness is unambiguous. Furthermore, ion-ion interactions, \(V_M\) and various ligands (e.g., ATP and pH; see sect. III, C) influence binding. Therefore, this discussion is limited primarily to studies on squid giant axons under a limited set of conditions that illustrate the basic principles.

The exchanger, like most integral membrane proteins, is asymmetric (also see sect. IV, A). Nevertheless, one could imagine that the exchanger’s ion binding sites at the internal and external faces of the PM might be comparable in terms of affinity. Indeed, this appears to be the case for the Na\(^{+}\) binding sites under “normal” physiological conditions (see Table 2). In squid axons [reviewed and summarized by DiPolo and Beaugé (254)], the internal Na\(^{+}\) activates Ca\(^{2+}\) influx with a \(K_{m(Na)}\) of 40–60 mM, and external Na\(^{+}\) activates Ca\(^{2+}\) influx with a \(K_{m(Na)}\) of 50–80 mM. Furthermore, the \(K_{i(Na)}\) values for inhibition of Ca\(^{2+}\) influx and influx by, respectively, internal and external Na\(^{+}\) are both ~30 mM. Comparable values for these parameters are also obtained in vertebrate tissues. For
example, in cardiac myocytes, $K_{m(Na)}$ on both sides is $\sim$30 mM (717, 661), and $K_{i(Na)}$ at the external surface is $\sim$44 mM (661). In rat brain synaptosomes, the $K_{m(Na)}$ values for activation by internal and external Na$^+$ are, respectively, $\sim$20 mM (314) and 34 mM (809), and $K_{i(Na)}$ at the external surface is $\sim$50–60 mM (314). In contrast, the $K_{m(Ca)}$ values for transported Ca$^{2+}$ at the two surfaces of the PM are very different. In the squid, $K_{m(Ca)}$ at the internal surface is $\sim$1 $\mu$M in fueled axons but ranges between 2 and 50 $\mu$M at the external surface (88, 254). In guinea pig cardiac myocytes, the comparable values are 0.6 $\mu$M and 1.4 $\mu$M (661). Also, in rat brain synaptosomes, the $K_{m(Ca)}$ at the external surface is 0.23 mM, while Ca$^{2+}$ is extruded via the exchanger when [Ca$^{2+}$]$_{i}$ is $<0.5$ $\mu$M (314). Thus the apparent affinities for Ca$^{2+}$ at the inward and outward facing Ca$^{2+}$ binding sites differ by a factor of $\sim$10$^3$.

An important, unresolved question is, How can we explain this asymmetrical binding of transported Ca$^{2+}$ at the two sides of the PM if we assume that there is only a single binding site for transported Ca$^{2+}$? One possibility is that the exchanger undergoes a spontaneous conformational change that alters the affinity of the binding site when the Ca$^{2+}$ is translocated across the membrane.

4. Role of nontransported monovalent cations

A) MONOVALENT CATIONS: LI, CHOLINE, TMA, AND K$^+$. Another interesting feature of the Na$^+/Ca^{2+}$ exchanger is the action of small monovalent cations. The initial experiments that were carried out about 20–30 years ago have raised questions about transport kinetics that are still unexplained. The basic observations focus on the self-exchange modes of the Na$^+/Ca^{2+}$ exchanger, i.e., Ca$^{2+}$/Ca$^{2+}$ exchange or Na$^+$/Na$^+$ exchange. The experiments on Ca$^{2+}$/Ca$^{2+}$ exchange are carried out in the absence of Na$^+$ to block net Na$^+/Ca^{2+}$ transport via the exchanger. In the absence of Na$^+$, Ca$^{2+}$ isotope can be transported across the membrane in which the Na$^+/Ca^{2+}$ exchanger resides even though there is no net Ca$^{2+}$ transported. Thus the unidirectional Ca$^{2+}$ flux reflects the components of the exchanger transport reaction that involve Ca$^{2+}$ binding to the Na$^+/Ca^{2+}$ exchanger on one side of the membrane, Ca$^{2+}$ translocation by the Na$^+/Ca^{2+}$ exchanger across the membrane, and Ca$^{2+}$ unbinding (dissociation) from the Na$^+/Ca^{2+}$ exchanger on the other side of the membrane. The unidirectional flux of Ca$^{2+}$ can be followed because $^{45}$Ca$^{2+}$ is added to one side of the membrane only; one can then follow the appearance of $^{45}$Ca$^{2+}$ on the other side of the membrane. The model in Figure 12 illustrates the elements of the Na$^+/Ca^{2+}$ exchanger transport reactions noted here. To the extent that monovalent cations can alter this isotopic flux of Ca$^{2+}$, as noted above, we have ignored this property in the model of the transport reactions shown in Figure 12. Although it is easy to change our model, the problems presented by the experiments that illustrate the action of nontransported monovalent cations described below indicate that it is not yet clear how they relate to the other data.

b) $Ca^{2+}$/Ca$^{2+}$ EXCHANGE AND THE ACTION OF cis- AND trans-MONOVALENT CATIONS THAT ARE NOT TRANSPORTED. In squid axons, $^{45}$Ca$^{2+}$ efflux was at the “background” level when all of the extracellular Na$^+$ was replaced by choline (88). When the extracellular Na$^+$ was replaced by Li$^+$, the $^{45}$Ca$^{2+}$ efflux was greatly increased, an effect that was also observed in barnacle muscle (686). Neither Li$^+$ nor choline$^+$ is transported by the Na$^+/Ca^{2+}$ exchanger (35, 88, 251, 422, 423). The problem presented by these results is, How does the identity of the nontransported monovalent cation (Li$^+$ or choline$^+$) act to alter Ca$^{2+}$/Ca$^{2+}$ exchange? At which step in the hypothesized reaction sequence do these nontransported monovalent cations act? They work from the trans-side, i.e., the side opposite to the side from which the Ca$^{2+}$ is transported. Thus the effects described above focus on $^{45}$Ca$^{2+}$ efflux and the effects that extracellular Li$^+$ and extracellular choline$^+$ have. In addition, these $^{45}$Ca$^{2+}$ efflux experiments demonstrate the requirement for nontransported monovalent cation on the inside or cis-side of the membrane (i.e., cis with respect to the $^{45}$Ca$^{2+}$). The aforementioned experiments were carried out with intracellular K$^+$ as the cis-monovalent cation. When the cis-monovalent cation was changed to Li$^+$, the $^{45}$Ca$^{2+}$ efflux was significantly reduced (by $\sim$30%). When the cis-monovalent cation was changed to TMA (a nonalkali metal cation), the $^{45}$Ca$^{2+}$ efflux was reduced by $\sim$80% (compared with K$^+$ as the cis-monovalent cation).

Clearly, our understanding of the actions of the nontransported cis- and trans-monovalent cations is limited. Knowledge of the mechanism(s) of action of these monovalent ions would undoubtedly help us to understand how the Na$^+/Ca^{2+}$ exchanger works at the molecular level. The problem is even more complicated, however. Effects of the internal monovalent cations were also observed when the Na$^+/Ca^{2+}$ exchanger operated in either the Ca$^{2+}$ influx or Ca$^{2+}$ efflux mode, but the effects were qualitatively quite different (88). When cis (internal) Li$^+$ was present, the Na$^+$-dependent Ca$^{2+}$ efflux was largest, being about twice that observed when cis-K$^+$ was present, and almost four times that observed when cis-TMA$^+$ was present. Recent experiments, although not as complete as the earlier results (88), are consistent with these basic observations insofar as the results overlap (99, 314, 335). We are thus left with a modeling dilemma. How can one explain the actions of the cis- and trans-monovalent cations that are not transported? One model of such interactions was given by Doering and Lederer (267), who discussed
the way nontransported protons could inhibit the Na\(^+\)/Ca\(^{2+}\) exchanger (see sect. mE4). In this model, intracellular protons inhibit transport by altering the exchanger kinetics: intracellular protons were hypothesized to bind to the Na\(^+\)/Ca\(^{2+}\) exchanger only when Na\(^+\) are already bound. Then the Na\(^+\) cannot unbind until the protons first dissociate. Thus intracellular protons are believed to “trap” the exchanger in a largely nonfunctional conformation.

D. Electrogenic (or Rheogenic) Properties and Voltage Sensitivity of the Na\(^+\)/Ca\(^{2+}\) Exchanger

Although all rheogenic (current generating) transport mechanisms must be sensitive to voltage, not all voltage-sensitive processes must be rheogenic or electrogenic (i.e., many nonelectrogenic transport processes are voltage sensitive). Many early reports did not seem to take this simple notion into account and thus tended to infer rheogenic (or electrogenic) properties of the Na\(^+\)/Ca\(^{2+}\) exchanger from the observed voltage-dependent properties [reviewed in Eisner and Lederer (290)]. From the beginning, two experimental issues have continued to confound investigations of both the rheogenic and the voltage-sensitive properties of the Na\(^+\)/Ca\(^{2+}\) exchanger. The first problem, which persists even today, is the absence of a specific high-affinity blocker of Na\(^+\)/Ca\(^{2+}\) exchanger function. This means that simple voltage-dependent difference currents (sensitive to this as yet unidentified blocker) cannot be used to describe, unambiguously, current or flux through the Na\(^+\)/Ca\(^{2+}\) exchanger in any of its modes of operations (i.e., Ca\(^{2+}\)/Ca\(^{2+}\) or Na\(^+\)/Na\(^+\) self-exchange, or Ca\(^{2+}\) exit mode or entry mode Na\(^+\)/Ca\(^{2+}\) exchange). The second problem is the confusing and multifaceted overlapping Na\(^+\) and Ca\(^{2+}\) fluxes in biological tissues. For example, changes in [Ca\(^{2+}\)]\textsubscript{i} can affect not only the Na\(^+\)/Ca\(^{2+}\) exchanger, but also Ca\(^{2+}\) channels, Ca\(^{2+}\)-activated channels (including channels that conduct Ca\(^{2+}\)), and enzymes that modify channels and transporters (including the Na\(^+\)/Ca\(^{2+}\) exchanger). Sodium also has diverse direct and indirect effects on transporters, ion channels, enzymes and, of course, several effects on the Na\(^+\)/Ca\(^{2+}\) exchanger.

1. Rheogenic (“electrogenic”) transport

The Na\(^+\)/Ca\(^{2+}\) exchanger was first identified in tracer flux experiments on squid giant axons (35, 102) and guinea pig and sheep heart muscle (783). In those experiments, isotopes of Ca\(^{2+}\) and Na\(^+\) were used to monitor the unidirectional movement of these ions (Figs. 1–3). By measuring the Na\(_\text{out}\)-dependent Ca\(^{2+}\) efflux and the Ca\(^{2+}\)-dependent Na\(^+\) influx, Baker et al. (35) estimated that the coupling ratio of the transport was about 3 Na\(^+\) to 1 Ca\(^{2+}\). This early estimate of the coupling ratio was uncertain, in part because there was considerable scatter in the data [see sect. mB2 and the review by Eisner and Lederer (290), regarding the determination of the coupling ratio]. Nevertheless, these early experiments provided the first suggestion that the exchanger might be rheogenic, and further determinations of the coupling ratio (see sect. mB2) helped to substantiate this view.

The first clear measurement of the Na\(^+\)/Ca\(^{2+}\) exchanger current was made by Kimura et al. (515). These experiments (Figs. 4 and 13), carried out in guinea pig cardiac myocytes, provided evidence that the Na\(^+\)/Ca\(^{2+}\) exchanger actually produced measurable membrane current. Although such results were predicted by a 3:1 stoichiometry (35) or a 4:1 stoichiometry (666, 669), they would not be consistent with a 2:1 coupling ratio (783). The measured Na\(^+\)/Ca\(^{2+}\) exchanger current thus helped to resolve an important issue and provided direct evidence that the Na\(^+\)/Ca\(^{2+}\) exchanger coupling ratio was >2:1 (see also Refs. 844, 846, 847). The data of Kimura et al. (515) were also consistent with the Ca\(_\text{in}\)-activated inward current in cardiac muscle that had been attributed to the Na\(^+\)/Ca\(^{2+}\) exchanger (189, 193, 436, 438, 439, 594, 645, 650). The presence of Ca\(_\text{in}\)-activated inward current in heart cells due to a Ca\(_\text{in}\)-activated cation channel (146, 286, 285, 477, 691), however, complicated many experiments (292, 294, 566, 594). Independent measurements on the cardiac Na\(^+\)/Ca\(^{2+}\) exchanger, using an equilibrium (null point) method (Fig. 7) (762), however, provided compelling evidence for a 3:1 coupling ratio (290, 844), as noted above (also see Figs. 6, 8, 9, and 11).

Quantitative measurements of the current carried by the Na\(^+\)/Ca\(^{2+}\) exchanger became more manageable with the development of the “giant patch” technique (Fig. 14) (199, 405, 406). This technique enabled investigators to measure the Na\(^+\)/Ca\(^{2+}\) exchanger current (and the current from other rheogenic or electrogenic transporters) in many cell types including heart cells, transfected cells, and frog oocytes. Because the maximum turnover rate of the Na\(^+\)/Ca\(^{2+}\) exchanger is much lower than that of single channels (by a factor of ~10\(^3\)), the electrical signal (i.e., the current generated by the exchanger) is much smaller and, thus, very difficult to measure with standard patch-clamp techniques. The giant patch has a surface area >100 times greater than that of the standard patch but retains the giga-ohm seal. Thus it is the increase in surface area in the giant patch that provides the increased signal needed to measure Na\(^+\)/Ca\(^{2+}\) exchanger current with a patch clamp. The giant patch-clamp technique has enabled electrophysiological investigations of native, cloned, mutated, and chimeric Na\(^+\)/Ca\(^{2+}\) exchanger proteins. It is now the preferred method for many types of studies of Na\(^+\)/Ca\(^{2+}\) exchanger electrophysiology. Electroneutral fluxes of the kind associated with Na\(^+\)/Ca\(^{2+}\) exchanger “self-exchange” (i.e., Ca\(^{2+}\)/Ca\(^{2+}\) exchange or Na\(^+\)/Na\(^+\) exchange) cannot, however, be measured with...
this method. Such self-exchange reactions represent reversible partial reactions of the Na\(^+\)/Ca\(^{2+}\) exchanger protein; they can be observed in the absence of the counter-ion (i.e., Ca\(^{2+}\)/Ca\(^{2+}\) exchange can be measured as the unidirectional isotopic Ca\(^{2+}\) flux that is observed in the absence of intracellular and extracellular Na\(^+\)). Without additional criteria, however (such as activation by internal Ca\(^{2+}\), or inhibition by cis-Na\(^+\)) on the side from which the Ca\(^{2+}\) is flowing or, even better, by an as yet unidentified specific inhibitor), one cannot be certain that all of the unidirectional Ca\(^{2+}\) flux is mediated by the exchanger (see below).

\section*{2. Voltage-dependent currents and fluxes: theoretical considerations}

The voltage dependence of a current or ion flux through a channel or a transporter represents a “kinetic” property of the protein. Traditionally, one expects the flux to be predicted by the Nernst-Planck current equation.
[see Hille (416)] which describes the voltage dependence of current through a resistive pore with assumptions of a constant electrical field. Such assumptions are, however, frequently inadequate to describe the current flow through single channels. Electrogenic or rheogenic transporters do not even have such a simple model, and it is therefore useful to discuss some simple “expectations” before reviewing the experimental findings.

A) TRAPPING OF THE EXCHANGER. At extremes of voltage, electrically charged exchangers and pumps will tend to be trapped in specific conformations as a consequence of energetic constraints. Suppose the “naked” exchanger (i.e., with neither Na$^+$ nor Ca$^{2+}$ bound) carries an average net charge of $-2.5$ charges (693, 695). Under these circumstances, and with the assumption of a consecutive transport mechanism (see sect. mCI and Fig. 12), as suggested by electrical evidence (411, 414, 693, 694) and flux evidence (488), the conformation of the Na$^+$/Ca$^{2+}$ exchanger ("X"; see Fig. 12) with 3 Na$^+$ bound, Na$_3$X, would have a net charge of +0.5, and the conformation with 1 Ca$^{2+}$ bound, CaX, would have a net charge of $-0.5$. An assumption frequently made for consecutive transport schemes (see Fig. 12) is that only the fully occupied conformations cross the membrane; thus a charged conformation must cross the voltage field whenever Ca$^{2+}$ or 3 Na$^+$ are transported by the Na$^+$/Ca$^{2+}$ exchanger (and see Ref. 631). Although there is quantitative uncertainty about the apparent charge associated with each of these hypothesized entities, CaX and Na$_3$X, it is believed that they have a net negative charge and a net positive charge, respectively (Hilgemann, personal communication). With these assumptions about the charge on CaX and Na$_3$X, it is useful to discuss the voltage-dependent self-exchange data.

B) Na$^+$/Na$^+$ EXCHANGE. At extremely positive potentials, the positively charged 3NaX$^+$ conformation should be trapped in the extracellular conformation because, for energetic reasons, it cannot cross the voltage field. At extremely negative potentials, it should be trapped in the intracellular conformation because, again, it cannot cross the voltage field for energetic reasons. Thus, with the consideration of Na$^+$/Na$^+$ exchange only, the kinetics of isotopic transport should increase from zero at the two extremes of potential (see Fig. 15). The shape of the curve describing the voltage dependence of unidirectional Na$^+$/Na$^+$ exchange transport of Na$^+$ should presumably look like a trapezoid. The curve should rise from zero at very negative potentials, display a (possibly sloped) plateau region in the mid-voltage range, and then decrease to zero at positive potentials. The fact that Na$^+$/Na$^+$ exchange in marine invertebrate tissues is voltage insensitive (249, 756) raises the possibility that the Na$^+$-loaded exchanger in these preparations does not bear a net charge and that these considerations therefore do not apply here. This is obviously different from the vertebrate cardiac exchanger, in which the Na$^+$-bound form of the exchanger is clearly voltage sensitive (414, 693).

c) Ca$^{2+}$/Ca$^{2+}$ EXCHANGE. Similar considerations should apply to the negatively charged CaX$^-$ conformation. At extremely positive potentials, the CaX conformation should be trapped in the intracellular conformation, and at extremely negative potentials, CaX should be trapped in the extracellular conformation because, in either situation, it cannot cross the voltage field for energetic reasons. Thus the curve that describes the voltage dependence of unidirectional Ca$^{2+}$/Ca$^{2+}$ exchange transport of
Ca\(^{2+}\) should presumably also look like a trapezoid (as for Na\(^+\)/Na\(^+\) exchange). It should rise from zero at very negative potentials, display a flat region, and then decrease at more positive potentials (see Fig. 15).

D) SHAPE OF THE "FLAT REGION" OF THE CURVES THAT ILLUSTRATE THE VOLTAGE DEPENDENCE OF SELF-EXCHANGE. There is no a priori reason to favor any particular shape for the region of the curve that describes the voltage dependence of Ca\(^{2+}\)/Ca\(^{2+}\) or Na\(^+\)/Na\(^+\) exchange. If the various conformations of the Na\(^+\)/Ca\(^{2+}\) exchanger protein do not change with voltage over a range of potentials, then the transport would be voltage independent in this voltage range. For the purposes of this discussion, however, we will assume that the CaX conformation carries a net negative charge; the electrical field will then tend to favor the location of CaX at one side of the membrane or the other. Under these circumstances, the exchanger-mediated current will exhibit a maximal plateau region in the mid-voltage range and will slope downward at the negative and positive ends of the curve. Nevertheless, parts of the protein other than the ion binding sites may also carry net charge. If these portions of the protein are located in the voltage field, or if they move into the field, they may thereby be influenced by this field. Should these regions affect the kinetics of ion translocation, they may influence the voltage dependence of self-exchange.

E) VOLTAGE-DEPENDENT ION BINDING. In the simple models described here, we assumed that binding of transported cations to the Na\(^+\)/Ca\(^{2+}\) exchanger occurs outside the voltage field. Under these circumstances, no voltage dependence of the ion binding steps should affect the kinetics of self-exchange. Should binding occur within the voltage field, however, the availability of ions will be sensitive to voltage. This, too, should impart some voltage dependence to the transport (337, 559, 748). If a cation binds to the unloaded or empty (X) conformation of the Na\(^+\)/Ca\(^{2+}\) exchanger within the voltage field on the intracellular side, cation binding should be favored as the potential becomes more positive. Conversely, if a cation binds to the X conformation within the voltage field on the extracellular side, cation binding would be favored as the potential becomes more negative.

F) A PROBLEM FOR STUDIES OF SELF-EXCHANGE (Na\(^+\)/Na\(^+\) OR Ca\(^{2+}\)/Ca\(^{2+}\) EXCHANGE). Because self-exchange produces no net transport, it is assumed that the unidirectional Na\(^+\)/Na\(^+\) exchange fluxes (i.e., Na\(^+\) influx and Na\(^+\) efflux) are identical for any set of ionic conditions. From a theoretical point of view this presents no problem. From an experimental perspective, however, one never knows for sure whether the unidirectional flux that is measured in an experiment is contaminated by Na\(^+\) flux from a source other than the Na\(^+\)/Ca\(^{2+}\) exchanger operating in the Na\(^+\)/Na\(^+\) (self) exchange mode. Influx and efflux are rarely measured under identical conditions, and there are no good inhibitors of the Na\(^+\)/Ca\(^{2+}\) exchanger. Thus uncertainty about whether the two unidirectional fluxes are truly equal in magnitude continues to cloud the interpretation of many fine experiments.

G) MEASURED VOLTAGE DEPENDENCE OF Ca\(^{2+}\)/Ca\(^{2+}\) EXCHANGE. Measurements of Ca\(_o\)-dependent Ca\(^{2+}\) efflux have been used to examine the voltage dependence of Ca\(^{2+}\)/Ca\(^{2+}\) exchange. As an electroneutral transport process, voltage-clamp and patch-clamp methods offer little, except under special circumstances (see Refs. 693, 694). Thus virtually all of the relevant work has been carried out using tracer flux measurements. In squid axons (249), Ca\(_o\)-dependent Ca\(^{2+}\) efflux decreased with depolarization; the decline with voltage was linear over the range of potentials from −40 to +40 mV. The Ca\(^{2+}\) efflux was compared with the baseline efflux at 0 mV. This appearance of a voltage-dependent component of Ca\(^{2+}\) efflux that decreases with depolarization might be consistent with increased trapping, in the extracellular conformation, of the negatively charged Ca\(^{2+}\)-bound form of the Na\(^+\)/Ca\(^{2+}\) exchanger (CaX) at positive potentials. But, as noted above, other explanations are also available. At the very least, we can conclude that there is a charge on the protein that slows the kinetics of Ca\(^{2+}\) transport out of the cell as the membrane potential gets more positive. This voltage dependence is consistent with experimental observations in guinea pig cardiac ventricular myocytes (693) and in barnacle muscle (756).

It is interesting that the voltage dependence of the Na\(_o\)-dependent Ca\(^{2+}\) efflux in squid axon was similar to that of the Ca\(_o\)-dependent Ca\(^{2+}\) efflux (i.e., the Ca\(^{2+}\) efflux declined with depolarization; Ref. 249). The mechanism that underlies this result is incompletely understood. Depolarization might increase trapping of CaX in the intracellular conformation, increase trapping of Na\(_o\)X in the extracellular conformation, and/or reduce binding of extracellular Na\(^+\) (if there is a high resistance access channel; Refs. 337, 559, 748).

H) MEASURED VOLTAGE DEPENDENCE OF Na\(^+\)/Na\(^+\) EXCHANGE. According to the "consensus models" of Na\(^+\)/Ca\(^{2+}\) exchanger function, the exchanger molecules with 3 Na\(^+\) bound (i.e., Na\(_o\)X) bear a net positive charge. Thus trapping of the Na\(_o\)X conformation would be expected at extremely negative and extremely positive potentials. Over a potential range from −60 to +40 mV, however, no change in the Na\(_o\) dependence of Na\(^+\) efflux was observed in either squid axons (249) or barnacle muscle fibers (756). This could simply mean that the voltage range in which trapping or voltage-dependent binding occurs was not tested in these experiments or that the voltage-dependent properties of the invertebrate and vertebrate exchangers are different, or that some other aspect of the Na\(_o\)-dependent Na\(^+\) efflux is rate limiting. At first consideration, these results do not seem consistent with the "ES" model (a model with 8 kinetically defined states) of Matsuoka and Hilgemann (631) or other transport models.
that attribute at least some of the voltage dependence of the Na\(^+/\)Ca\(^{2+}\) exchanger to a voltage-dependent Na\(^+\) translocation step (see, for example, Refs. 414, 693). It would therefore be helpful to understand why this Na\(^+/\)Na\(^+\) exchange, apparently mediated by the Na\(^+/\)Ca\(^{2+}\) exchanger, is voltage insensitive, when we would expect to find a voltage-sensitive step associated with either Na\(^+\) translocation or Na\(^+\) binding.

1) Action of α-Chymotrypsin on Self-Exchange Mediated by the Na\(^+/\)Ca\(^{2+}\) Exchanger. Hilgemann and co-workers (634, 635) used the giant patch technique to examine the kinetics of the current carried by the Na\(^+/\)Ca\(^{2+}\) exchanger (\(I_{\text{Na/Ca}}\)) in patches from mammalian cardiac myocytes (Fig. 14). They observed that \(I_{\text{Na/Ca}}\) was modulated by [Na\(^+\)], and [ATP], and that this modulation could be removed by exposing the intracellular aspect of the giant patch to a low concentration of the proteolytic enzyme α-chymotrypsin. This mild proteolytic treatment did not appear to alter the transport capabilities of the Na\(^+/\)Ca\(^{2+}\) exchanger but did appear to “deregulate” this protein (406, 723). It is noteworthy that removal of most of the large intracellular loop of the Na\(^+/\)Ca\(^{2+}\) exchanger by molecular biological techniques produced a similarly deregulated Na\(^+/\)Ca\(^{2+}\) exchanger (634, 635). Therefore, it seems logical to suggest that α-chymotrypsin may act by chemically removing the intracellular loop (or a critical portion of this loop) as well. With the assumption that this explanation is correct, however, it is not entirely clear why the remaining halves of the Na\(^+/\)Ca\(^{2+}\) exchanger protein continue to function (perhaps the 2 halves are so well attracted to one another, and so well fitted together that they do not drift apart). Thus we are left with an enigma about the actual tertiary structure of the Na\(^+/\)Ca\(^{2+}\) exchanger protein.

The self-exchange modes of operation of the Na\(^+/\)Ca\(^{2+}\) exchanger have also been examined after treatment with α-chymotrypsin. Rasgado-Flores and colleagues (756, 925) reported that Na\(^+/\)Na\(^+\) exchange was unaltered by this protease but that the voltage dependence of Ca\(^{2+}\)/Ca\(^{2+}\) exchange was dramatically changed in barnacle muscle fibers. Instead of declining with depolarization (i.e., at more positive internal potentials), as observed in the mammalian heart (see above), following α-chymotrypsin exposure, the Ca\(_o\)-dependent Ca\(^{2+}\) efflux in barnacle muscle increased with depolarization. This finding suggests that α-chymotrypsin alters a part of the protein that controls/influences a voltage-dependent process. Because there is no evidence that this treatment alters any thermodynamic process, and because Ca\(^{2+}\)/Ca\(^{2+}\) exchange is electroneutral, the results imply that Ca\(^{2+}\) binding or translocation step kinetics are altered. The questions that this raises are as follows: Where is this part of the Na\(^+/\)Ca\(^{2+}\) exchanger protein? What does this region of the exchanger normally do? Perhaps comparison of the molecular structures of the vertebrate and invertebrate exchangers will give us some clues.

2) Question for Future Study. Examination of the Na\(^+/\)Ca\(^{2+}\) exchanger by examining self-exchange has raised an exciting new question. How is it possible for Na\(^+/\)Na\(^+\) self-exchange to be voltage independent when it has been argued that this same membrane-crossing transition by Na\(_iX\) is one of the most significant voltage-sensitive transport reactions of the Na\(^+/\)Ca\(^{2+}\) exchanger? One possibility is that the Na\(^+\)-dependent transition is not voltage dependent. Should this explanation be correct, we would need to reexamine our current models of Na\(^+/\)Ca\(^{2+}\) exchanger behavior. Another possibility is that, during Na\(^+/\)Na\(^+\) exchange, the Na\(^+\) translocation step is no longer rate limiting (as may occur, for example, if the Na\(^+\) binding/unbinding step becomes rate limiting and takes place outside the electric field). In any event, it will be interesting and instructive to elucidate the molecular basis for the observed phylogenetic differences in the voltage sensitivity of the Na\(^+\) - and Ca\(^{2+}\)-bound forms of the Na\(^+/\)Ca\(^{2+}\) exchanger.

E. Regulation of the Na\(^{2+}\)/Ca\(^{2+}\) Exchanger by Catalytic Modulation

Calcium plays such a central role in so many cellular processes that it is obviously essential for cells to control cytoplasmic and stored Ca\(^{2+}\) very precisely, both temporally and spatially. Because Na\(^+/\)Ca\(^{2+}\) exchange is a major route of Ca\(^{2+}\) exit and/or Ca\(^{2+}\) entry in many types of cells, it is not surprising that the activity of the exchanger is regulated in many different ways. Here, we describe a variety of such regulatory mechanisms.

The Na\(^+/\)Ca\(^{2+}\) exchanger uses the electrochemical ion gradients of Na\(^+\) and Ca\(^{2+}\) to provide energy to move three Na\(^+\) in one direction, in exchange for each Ca\(^{2+}\) moved in the opposite direction. To investigate the modulation of the Na\(^+/\)Ca\(^{2+}\) exchanger by a number of “factors” that include the ions Na\(^+\) and Ca\(^{2+}\), it is important to clarify the normal transport process and to distinguish it from what we will call the “catalytic” modulation of the transport process. This distinction is necessary because the transported ions (Na\(^+\) and Ca\(^{2+}\)) can also modulate transport. In this section, we focus on mechanisms that alter the activity of the exchanger by influencing the activation kinetics (i.e., the catalytic modulators), in contrast to those effects that are dependent on changes in the thermodynamic parameters \(\Delta \mu_{\text{Na}}\), \(\Delta \mu_{\text{Ca}}\), or \(V_{\text{Na}} E_{\text{Na/Ca}}\). It is apparent that multiple mechanisms must operate simultaneously on the exchanger in situ. Nevertheless, it is not yet clear how the various regulatory processes are coordinated; as the limitations of the results described below suggest, this remains a major problem for future investigation.
1. ATP, phosphoarginine, and acidic phospholipids

In an early study, Baker and Glitsch (36) provided the first evidence that the exchanger might be activated by ATP. This was further documented by DiPolo (236–238) who, in a series of elegant experiments on dialyzed squid axons, demonstrated that ATP and analogs with a hydrolyzable terminal phosphate promoted Na⁺/Ca²⁺ exchange. This ATP-dependent mechanism was subsequently shown to be mediated by a Ca²⁺-dependent phosphorylation (245). This activation by ATP appears to involve an increase in the affinity for internal Ca²⁺ and external Na⁺ (88) and a decrease in inhibition by internal Na⁺ (238); indeed, in nominally Na⁺-free conditions, ATP does not enhance exchanger-mediated Ca²⁺ efflux. CrATP, which serves as a “substrate” for most types of kinases, and inhibits them, blocks the activation of Na⁺/Ca²⁺ exchange by MgATP; CrATP does not, however, interfere with the Na⁺ transport activity observed in the absence of ATP (252). Recently, DiPolo et al. (259) reported that a novel (but as yet, unsequenced) 13-kDa peptide is required for the modulation of the squid axon Na⁺/Ca²⁺ exchanger by MgATP. Whether this ATP-dependent modulation of the exchanger is mediated by PKC or protein kinase A (PKA), or is affected by a change in phosphatase activity (see sect. III E2 and E3), is unknown. Microinjection of exogenous alkaline phosphatase into squid axons, however, reverses the activation by the exchanger by ATP (257).

Phosphoarginine (Pa), but not phosphocreatine, was also found to activate Na⁺/Ca²⁺ exchange in squid axons by a mechanism that depends on intracellular Mg²⁺ and Ca²⁺ (256). The activation by Pa is mediated by a different mechanism than that of ATP (257). Activation by Pa does not affect the affinities for the transported ions. Phosphoarginine promotes Na⁺/Ca²⁺ exchange, but not Ca²⁺/Ca²⁺ exchange. The action of Pa is not blocked by CrATP, but it is reversed by microinjected alkaline phosphatase; thus the activation by ATP and Pa both appear to involve phosphorylation-dephosphorylation reactions (257).

Using giant membrane patches from cardiac myocytes, Hilgemann et al. (413) showed that the exchanger is inactivated by intracellular Na⁺ (Fig. 14A). This inactivation could be relieved by adding MgATP to the solution bathing the cytoplasmic face of the membrane. Indeed, this may be the explanation for DiPolo’s (238) observation that that ATP has no effect on Ca²⁺ efflux in Na⁺-free media.

Despite the evidence that some type of phosphorylation is required for activation by ATP, it should be clear that, in contrast to the PMCA pump, the exchanger does not use energy directly from the hydrolysis of ATP to transport Ca²⁺ (i.e., a phosphorylation is not required for each cycle of the exchanger). Indeed, the Na⁺/Ca²⁺ exchanger can operate in the virtual absence of ATP (88, 238, 686). Recent observations have now begun to clarify the mechanism of this regulation.

Several investigators (198, 410, 412, 608, 957) have shown that acidic lipids, including phosphatidylserine and some inositol phosphates [phosphatidylinositol 4-phosphate and phosphatidylinositol 4,5-bisphosphate (PIP₂)], promote Na⁺/Ca²⁺ exchange. Furthermore, Mene et al. (648) suggested that activation of phospholipase C by vasoconstrictors stimulates Na⁺/Ca²⁺ exchange (presumably via the phosphoinositide cascade) in human mesangial cells. Recently, Hilgemann and Ball (409) examined the effects of some of these lipids in greater detail in cardiac myocyte patches. They showed that the effect of ATP was mediated by PIP₂ and was inhibited by a PIP₂-specific phospholipase C (PLC); high [Ca²⁺], reversed the effect of ATP, perhaps by activating an endogenous Ca²⁺-dependent PLC. Thus a high [Ca²⁺], might tend to inhibit Ca²⁺ extrusion by promoting inactivation of the exchanger (a positive feedback effect on [Ca²⁺]). These results illustrate the complexity of regulation of the exchanger by phospholipids and Ca²⁺.

2. Effects of PKC activation

The effects of phorbol esters (which activate PKC) and cyclic nucleotides on Na⁺/Ca²⁺ exchange vary from tissue to tissue, and there are some conflicting results even in similar tissues. For example, the exchanger in cultured rat aortic myocytes was augmented by brief exposure to phorbol esters such as phorbol 12-myristate 13-acetate (PMA), presumably via activation of PKC (959, 960), since it was associated with phosphorylation of the exchanger (448). Furthermore, the activating effect of platelet-derived growth factor BB (PDGF-BB) was associated with a phosphorylation of the exchanger; this effect of PDGF-BB was prevented by prior prolonged incubation with PMA (which downregulates PKC). Also, in rabbit renal arterioles, the exchanger was activated by PMA, and this effect was blocked by PKC inhibition (315).

Along a similar line, Khoyi et al. (497, 498) observed that the exchanger in aortic myocytes was augmented by phorbol 12,13-dibutyrate (PDBu), but not by the inactive analog 4α-phorbol 12,13-didecanoate (4α-PDD). They also found that the exchanger was stimulated by norepinephrine, and this effect was inhibited by 1-(5-isouquinolinesulfonyl)-2-methylpiperazaine (H-7), a relatively nonselective inhibitor of PKC. They therefore suggested that the action of norepinephrine, like that of PDBu, was mediated by PKC, even though norepinephrine usually acts through PKA-dependent mechanisms. Their data do not, however, rule out the possibility that the exchanger in these cells was modulated by both PKC (PDBu) and PKA (norepinephrine; see sect. mE3).

Studies in cardiac muscle indicate that Na⁺/Ca²⁺ exchange is activated by a pathway involving PKC. Bal-
laid and colleagues (41, 42) found that PMA and the agonists phenylephrine, angiotensin II, and endothelin-1, which augment PKC activity via a G protein (Gq)-mediated mechanism, enhanced Na\(^{+}\)-dependent Ca\(^{2+}\) uptake into cardiac sarcolemmal vesicles. This transport activity was also augmented by the nonhydrolyzable GTP analog 5′-guanylyl amidophosphate.

In the central nervous system, too, there is evidence that stimulation of PKC increases the activity of the exchanger. Externally Na\(^{+}\)-dependent Ca\(^{2+}\) efflux and Na\(^{+}\)-dependent Ca\(^{2+}\) influx in rat brain synaptosomes were both greatly augmented by PDBu, but not by 4α-PDD (99). Also, PDBu, but not 4α-PDD, promoted phosphorylation of the exchanger. Furthermore, this phosphorylation, as well as the augmentation of the Ca\(^{2+}\) fluxes by PDBu, were blocked by H-7. Pharmacological studies on adrenal medullary cells also suggest that the exchanger is modulated by PKC, but not PKA (446).

In two other studies, however, activation of PKC by phorbol esters was found to decrease exchanger activity. Phorbol 12-myristate 13-acetate inhibited both basal and vasoconstrictor-stimulated, exchanger-mediated Ca\(^{2+}\) influx in rat brain synaptosomes were both greatly augmented by PDBu, but not by 4α-PDD (99). Also, PDBu, but not 4α-PDD, promoted phosphorylation of the exchanger. Furthermore, this phosphorylation, as well as the augmentation of the Ca\(^{2+}\) fluxes by PDBu, were blocked by H-7. Pharmacological studies on adrenal medullary cells also suggest that the exchanger is modulated by PKC, but not PKA (446).

In two other studies, however, activation of PKC by phorbol esters was found to decrease exchanger activity. Phorbol 12-myristate 13-acetate inhibited both basal and vasoconstrictor-stimulated, exchanger-mediated Ca\(^{2+}\) influx in rat brain synaptosomes were both greatly augmented by PDBu, but not by 4α-PDD (99). Also, PDBu, but not 4α-PDD, promoted phosphorylation of the exchanger. Furthermore, this phosphorylation, as well as the augmentation of the Ca\(^{2+}\) fluxes by PDBu, were blocked by H-7. Pharmacological studies on adrenal medullary cells also suggest that the exchanger is modulated by PKC, but not PKA (446).

In two other studies, however, activation of PKC by phorbol esters was found to decrease exchanger activity. Phorbol 12-myristate 13-acetate inhibited both basal and vasoconstrictor-stimulated, exchanger-mediated Ca\(^{2+}\) influx in rat brain synaptosomes were both greatly augmented by PDBu, but not by 4α-PDD (99). Also, PDBu, but not 4α-PDD, promoted phosphorylation of the exchanger. Furthermore, this phosphorylation, as well as the augmentation of the Ca\(^{2+}\) fluxes by PDBu, were blocked by H-7. Pharmacological studies on adrenal medullary cells also suggest that the exchanger is modulated by PKC, but not PKA (446).

In two other studies, however, activation of PKC by phorbol esters was found to decrease exchanger activity. Phorbol 12-myristate 13-acetate inhibited both basal and vasoconstrictor-stimulated, exchanger-mediated Ca\(^{2+}\) influx in rat brain synaptosomes were both greatly augmented by PDBu, but not by 4α-PDD (99). Also, PDBu, but not 4α-PDD, promoted phosphorylation of the exchanger. Furthermore, this phosphorylation, as well as the augmentation of the Ca\(^{2+}\) fluxes by PDBu, were blocked by H-7. Pharmacological studies on adrenal medullary cells also suggest that the exchanger is modulated by PKC, but not PKA (446).

In two other studies, however, activation of PKC by phorbol esters was found to decrease exchanger activity. Phorbol 12-myristate 13-acetate inhibited both basal and vasoconstrictor-stimulated, exchanger-mediated Ca\(^{2+}\) influx in rat brain synaptosomes were both greatly augmented by PDBu, but not by 4α-PDD (99). Also, PDBu, but not 4α-PDD, promoted phosphorylation of the exchanger. Furthermore, this phosphorylation, as well as the augmentation of the Ca\(^{2+}\) fluxes by PDBu, were blocked by H-7. Pharmacological studies on adrenal medullary cells also suggest that the exchanger is modulated by PKC, but not PKA (446).

In two other studies, however, activation of PKC by phorbol esters was found to decrease exchanger activity. Phorbol 12-myristate 13-acetate inhibited both basal and vasoconstrictor-stimulated, exchanger-mediated Ca\(^{2+}\) influx in rat brain synaptosomes were both greatly augmented by PDBu, but not by 4α-PDD (99). Also, PDBu, but not 4α-PDD, promoted phosphorylation of the exchanger. Furthermore, this phosphorylation, as well as the augmentation of the Ca\(^{2+}\) fluxes by PDBu, were blocked by H-7. Pharmacological studies on adrenal medullary cells also suggest that the exchanger is modulated by PKC, but not PKA (446).

In two other studies, however, activation of PKC by phorbol esters was found to decrease exchanger activity. Phorbol 12-myristate 13-acetate inhibited both basal and vasoconstrictor-stimulated, exchanger-mediated Ca\(^{2+}\) influx in rat brain synaptosomes were both greatly augmented by PDBu, but not by 4α-PDD (99). Also, PDBu, but not 4α-PDD, promoted phosphorylation of the exchanger. Furthermore, this phosphorylation, as well as the augmentation of the Ca\(^{2+}\) fluxes by PDBu, were blocked by H-7. Pharmacological studies on adrenal medullary cells also suggest that the exchanger is modulated by PKC, but not PKA (446).
important to emphasize the fact that $\Delta P_{Na}$, which drives the exchanger, also may be influenced by PKA- and PKC-mediated phosphorylations that affect the activity of the Na$^+$ pump (58, 72, 114). Thus it is also essential to know how much the exchanger (in isolation) is directly affected by these various regulatory mechanisms.

4. Modulation of the Na$^+$/Ca$^{2+}$ exchanger by nontransported ions and other ligands

The consecutive transport model shown in Figure 12 (as well as other models) implies that the kinetics of transport or the turnover rate of the Na$^+$/Ca$^{2+}$ exchanger will be affected by the availability of Na$^+$ and Ca$^{2+}$ on both sides of the plasma membrane. In more concrete terms, even the "unidirectional" translocation of an ion will be affected by the concentration of that ion. If Ca$^{2+}$, for example, is to be translocated from inside to outside, the initial requirement must be that the Na$^+$/Ca$^{2+}$ exchanger be available so that intracellular Ca$^{2+}$ can bind to the exchanger. A translocation step is then required to move the bound Ca$^{2+}$ from inside to outside, followed by a Ca$^{2+}$ unbinding step at the outside. This "vectorial" translocation involves a minimum of four kinetic steps. The rate of this unidirectional movement clearly depends on 1) the rate at which an "available-for-binding" state of the Na$^+$/Ca$^{2+}$ exchanger protein appears at an intracellular site; 2) the rate of Ca$^{2+}$ binding to the Na$^+$/Ca$^{2+}$ exchanger protein "X" at the intracellular site (C2), which depends on [Ca$^{2+}$]; 3) the rate of translocation of the bound Ca$^{2+}$ (as CaX); and 4) the rate of Ca$^{2+}$ dissociation at the extracellular surface (which should not depend on [Ca$^{2+}$]). There is a similar unidirectional movement of Ca$^{2+}$ into the cell that depends on the four kinetic steps acting in reverse (see Fig. 12). If these unidirectional fluxes of Ca$^{2+}$ are equal, then there is no net (exchanger-mediated) transport of Ca$^{2+}$.

A similar and symmetric consideration applies to the unidirectional movements of Na$^+$ into and out of the cell. The unidirectional movements of Ca$^{2+}$ or of Na$^+$ reflect the kinetic features of the Na$^+$/Ca$^{2+}$ exchanger, while the net movements of Ca$^{2+}$ and of Na$^+$ are dependent on thermodynamic considerations. The two are clearly and necessarily interconnected. Net transport of Na$^+$ and Ca$^{2+}$ requires unequal, exchanger-mediated unidirectional fluxes of each ion species; the ratio of the inequalities of Na$^+$ and Ca$^{2+}$ transport (i.e., the ratio of the net Na$^+$ and net Ca$^{2+}$ fluxes) then provides a measure of the average coupling ratio. Because available evidence suggests that the transporter cannot "translocate" the ion binding sites in an empty state, and that the transport coupling ratio is fixed, the unidirectional transport inequality provides information on the net movement of ions by the Na$^+$/Ca$^{2+}$ exchanger.

Here, we use the term catalytic to refer to factors (other than the concentration dependence of the binding of transported ions) that affect the kinetics of the Na$^+$/Ca$^{2+}$ exchanger. Thus, for example, virtually all phosphorylation of the exchanger by PKA, PKC, or calmodulin kinases affect transport by catalytic mechanisms. Clearly, it should be possible to determine how these catalytic factors affect the Na$^+$/Ca$^{2+}$ exchanger-mediated net transport of ions by measuring the unidirectional ion movements. Particularly noteworthy (and unusual) features of catalytic regulation of the exchanger are the regulatory roles of Na$^+$ and Ca$^{2+}$, the transported ion species.

A) Catalytic modulation following $\alpha$-chymotrypsin exposure to the intracellular side of the Na$^+$/Ca$^{2+}$ exchanger protein ("deregulation"). A surprising observation was reported by Hilgemann et al. in 1992 (413), shortly after he first described the "giant" patch-clamp method (405, 406). They found that there was a Na$^+$- and time-dependent reduction in outward Na$^+$/Ca$^{2+}$ exchanger current (i.e., the $I_{Na/Ca}$ associated with the Ca$^{2+}$ entry mode). This reduction (Fig. 14A), called Na$^+$-dependent inactivation (see below), appeared to arise from an alteration in the kinetics of Na$^+$/Ca$^{2+}$ exchange, and not from a change in the thermodynamic "driving force" on the exchanger. Both the proton-dependent inhibition of Na$^+$/Ca$^{2+}$ exchange (see below) and the Ca$^{2+}$-dependent activation of Na$^+$/Ca$^{2+}$ exchange (see below) also result from changes in the kinetics of the Na$^+$/Ca$^{2+}$ exchanger, rather than from changes in the energetics of transport. Thermodynamic considerations alone would suggest that an increase in [Na$^+$] should increase rather than decrease the Na$^+$/Ca$^{2+}$ exchanger-mediated outward current. Likewise, thermodynamic considerations alone would also suggest that increasing [Ca$^{2+}$] should decrease the Na$^+$/Ca$^{2+}$ exchanger outward current, rather than increase it. Thermodynamically, protons should not affect the Na$^+$/Ca$^{2+}$ exchanger net transport because protons are not transported by this exchanger. Consequently, alterations in the Na$^+$/Ca$^{2+}$ exchanger turnover rate induced by changes in the intracellular concentrations of Na$^+$, Ca$^{2+}$, and H$^+$ appear to depend on kinetic modulation of Na$^+$/Ca$^{2+}$ exchanger function and thus have been referred to as catalytic actions. This terminology is particularly appropriate for both Na$^+$ and Ca$^{2+}$ because these ions obviously also contribute to the energetics of transport.

Several observations suggest that the putative intracellular loop region of the Na$^+$/Ca$^{2+}$ exchanger is responsible, at least in part, for kinetic modulation of the Na$^+$/Ca$^{2+}$ exchanger transport by Na$^+$, Ca$^{2+}$, and protons. Following the initial report of activation of the Na$^+$/Ca$^{2+}$ exchanger by $\alpha$-chymotrypsin in cardiac vesicles (406, 635), Matsuoka and Hilgemann (632) showed that exposure of the intracellular surface of the giant patch to the protease, $\alpha$-chymotrypsin, removed the modulatory action of Ca$^{2+}$. A similar result was obtained in internally
perfused giant barnacle muscle fibers (299, 755). Subsequent studies revealed that α-chymotrypsin also removed the modulatory action of H⁺ (266, 267). It has not yet been proven that treatment with α-chymotrypsin removes the intracellular loop (or a large portion of it) from the remaining part of the Na⁺/Ca²⁺ exchanger protein. Nevertheless, several sites in the loop are candidate cleavage sites for proteolysis induced by the introduction of α-chymotrypsin into the cytosol. Furthermore, Philipson and co-workers (634, 635) showed that removal or modification of the intracellular loop by mutagenesis also removed the catalytic modulation of the Na⁺/Ca²⁺ exchanger by Ca²⁺. Thus there is strong evidence that the intracellular loop plays an important role in regulating the kinetics of the Na⁺/Ca²⁺ exchanger.

The aforementioned studies focus primarily on the activity of the Na⁺/Ca²⁺ exchanger while it is operating in the Ca²⁺ entry mode and while it is extruding net Na⁺ rather than net Ca²⁺ (i.e., when it is producing a net outward current). Rasgado-Flores and colleagues (756, 299) have shown, however, that α-chymotrypsin deregulation affects all modes of transport mediated by the exchanger in giant barnacle muscle fibers. This includes the “normal” (Ca²⁺ exit) mode of operation, in which net Ca²⁺ is transported out of the intracellular compartment, as well as both self-exchange modes.

b) CATALYTIC MODULATION BY [Na⁺], (NAI-DEPENDENT INACTIVATION). A large patch of cardiac sarcolemma can be used with electrophysiological methods to investigate ion transport mediated by the Na⁺/Ca²⁺ exchanger (i.e., the giant patch-clamp method) (405, 406). A tight seal is made between a wide-tipped (e.g., 10 μm diameter) glass pipette and a bleb of sarcolemma. By pulling the pipette away from the cell, the membrane sticking to the glass is ripped away from the cell. This exposes the intracellular surface to the bathing solution while the extracellular surface of the membrane is exposed to the contents of the pipette.

In this configuration, when the bath [Na⁺] (= “cytoplasmic” Na⁺, or “Naᵢ”) was elevated, “outward” current carried by the Na⁺/Ca²⁺ exchanger increased. Surprisingly, however, when [Na⁺] in the bathing medium was increased in a stepwise manner, outward current increased almost instantly, but the increase was only transient. Following the peak, the current declined in magnitude over time, suggesting that there was a time-dependent inactivation (Fig. 14A). This time-dependent inactivation of outward I_{Na/Ca} was termed Naᵢ-dependent inactivation because it was produced by elevating [Na⁺]ᵢ (413, 632). Inactivation of I_{Na/Ca} is analogous to inactivation of ionic current flowing through channels and may depend on the rearrangement of a protein or part of a protein. For example, voltage-dependent inactivation of Na⁺ or Ca²⁺ channels is believed to depend on part of the channel protein simultaneously rearranging itself spatially to plug the channel pore (i.e., the ball and chain model; see Hille, Ref. 416). As suggested above, the putative intracellular loop region of the Na⁺/Ca²⁺ exchanger is a good candidate to play a key role in underlying Naᵢ-dependent inactivation. Indeed, intracellular application of α-chymotrypsin, which may clip off all or parts of the cytoplasmic loop of the exchanger, removes Naᵢ-dependent inactivation (632). The molecular mechanisms that underlie ion transport and exchange are not established, however, and we do not know how the intracellular loop participates in the Naᵢ-dependent inactivation. We speculate that two independent processes take place simultaneously, but at different rates. One process (the more rapid) involves Na⁺ binding to its transport site on the Na⁺/Ca²⁺ exchanger to participate in Na⁺/Ca²⁺ exchange. The other (slower) process involves Na⁺ binding to a region of the intracellular loop, thereby causing the loop to interact with the part of the protein that controls transport kinetics. In the case of Na⁺ binding to the exchanger inhibitory peptide (XIP) region of the intracellular loop (633), the effect is to reduce the outward I_{Na/Ca}.

The implication is that a rise in [Ca²⁺]ᵢ has both a kinetic effect, viz. a decrease in Ca²⁺ influx, and an opposite thermodynamic effect, viz. a rise in [Ca²⁺]ᵢ, as a result of the change in the Na⁺ gradient; the former (kinetic) effect should only be observed with very high [Na⁺]ᵢ.

c) CATALYTIC MODULATION BY [Ca²⁺], DiPolo (239) made the counterintuitive observation that the squid axon exchanger has an absolute requirement for intracellular Ca²⁺ while operating in the Ca²⁺ entry mode. This modulatory effect of intracellular Ca²⁺ could explain the inhibition of exchanger-mediated Ca²⁺ entry by intracellular Ca²⁺ chelators such as quin 2 (10) and EGTA (859). Also, removal of intravesicular Ca²⁺ by preincubating cardiac sarcloemnal vesicles with EGTA also reduces Naᵢ-dependent Ca²⁺ uptake (763). This catalytic effect of internal Ca²⁺ does not involve PKC because specific calmodulin inhibitors have little effect on the exchanger-mediated current in cardiac myocytes (512).

The giant patch-clamp method enables the study of the Ca²⁺ entry mode of exchange with electrophysiological methods. This permits one to investigate the modulation of transport function of the Na⁺/Ca²⁺ exchanger by catalytic amounts of [Ca²⁺],. If intracellular Ca²⁺ activates this Ca²⁺ entry mode of exchange, the kinetic and thermodynamic effects of a rise in [Ca²⁺], should be exerted in opposite directions. Thus activation of net influx of Ca²⁺ or net extrusion of Na⁺ by the Na⁺/Ca²⁺ exchanger, induced by elevation of [Ca²⁺], must be brought about by a modulatory or catalytic action of the Ca²⁺. With the use of this method of investigation, the Na⁺/Ca²⁺ exchanger in the heart is half-maximally activated at a [Ca²⁺], level of ~1 μM (412). In squid axons (243, 244) and barnacle muscle (753, 757), tracer flux studies yielded similar results (see Table 2). In fact, the
Ca$^{2+}$ entry mode of exchange was abolished when [Ca$^{2+}$]$_i$ was reduced to $<0.1$ M.

There also is strong evidence that intracellular Ca$^{2+}$ can augment the turnover rate of the Na$^+/Ca^{2+}$ exchanger when it is operating in the Ca$^{2+}$ exit mode (412, 661). This catalytic effect of intracellular Ca$^{2+}$ could, because it is activated by a rise in [Ca$^{2+}$]$_i$, be confused with an increase in the thermodynamic driving force favoring the extrusion of Ca$^{2+}$. It is difficult to separate the two effects experimentally, and there continues to be uncertainty about the exact level of [Ca$^{2+}$]$_i$ needed to produce the catalytic activation of Na$^+/Ca^{2+}$ exchanger transport. Reported $K_{0.5}$ values range from $\sim 10–50$ nM (661) to on the order of 1 $\mu$M (247, 248, 258, 412).

With the use of a Ca$^{2+}$-overlay analysis of the Na$^+/Ca^{2+}$ exchanger protein on a Western blot, it has been possible to identify part of the intracellular loop in NCX1 as the site of the Ca$^{2+}$-protein interaction (579). The conclusion from these experiments has been supported by deletion mutagenesis studies which suggest that residues 371 to 508 are necessary for Ca$^{2+}$ binding. Two highly acidic regions (residues 446–454 and 499–510), each containing three consecutive aspartic acid residues, appear to be particularly important. When Ca$^{2+}$ binds to this region, the rate of Na$^+/Ca^{2+}$ exchange is accelerated.

Because the steady-state activities of cytosolic Na$^+$ and Ca$^{2+}$ have opposite catalytic effects on the Na$^+/Ca^{2+}$ exchanger, with Na$^+$ decreasing transport rate and Ca$^{2+}$ increasing it, one may speculate that one of two basic mechanisms is operating. One possibility is that the baseline function of the Na$^+/Ca^{2+}$ exchanger involves tonic but incomplete autoinhibition in which some or all of the intracellular loop participates. If this is the case, then increasing [Na$^+$]$_i$ augments that autoinhibition to produce the Na$^+$-dependent inactivation. Conversely, increasing [Ca$^{2+}$]$_i$ should decrease the autoinhibition and thereby increase the rate of Na$^+/Ca^{2+}$ exchange. The other possibility is that the intracellular loop is involved in autoinhibition of the exchanger. If this is the case, then increasing [Ca$^{2+}$]$_i$ should lead to the reduction of the autoinhibition of Na$^+/Ca^{2+}$ exchange. Conversely, increasing [Ca$^{2+}$]$_i$ should augment the autoinhibition and thereby increase Na$^+/Ca^{2+}$ exchange. Because treatment with $\alpha$-chymotrypsin increases the Na$^+/Ca^{2+}$ exchanger current, and because we presume that this proteolysis removes the intracellular loop, we favor the autoinhibition hypothesis. The alternative hypothesis would have been supported if $\alpha$-chymotrypsin treatment had decreased the rate of Na$^+/Ca^{2+}$ exchange.

The functional significance of the different splice variants of the NCX1 Na$^+/Ca^{2+}$ exchanger (527) is only now beginning to be studied. Recent preliminary experiments suggest that the cardiac and the kidney isoforms of the Na$^+/Ca^{2+}$ exchanger behave differently (795). The differences noted include different responses to PKA-dependent phosphorylation, to [Ca$^{2+}$]$_i$, and to V$\text{M}$. How these reported differences in exon A-containing isoforms (e.g., heart) and exon B-containing isoforms (e.g., kidney) affect cellular physiology remains unknown. The main structural differences in these splice variants occur in the large putatively cytoplasmic loop. No clear mechanistic model has emerged that allows one to understand how the slice variants could bring about these reported differences. Nevertheless, distant relatives of the mammalian Na$^+/Ca^{2+}$ exchanger such as the Drosophila Na$^+/Ca^{2+}$ exchanger appear to have splice variants in the same intracellular loop region (796). Furthermore, recent findings (431, 796) suggest that intracellular Ca$^{2+}$ regulation by the Drosophila Na$^+/Ca^{2+}$ exchanger differs from that observed in the mammalian Na$^+/Ca^{2+}$ exchanger. Distinctive regulation of Drosophila isoforms (796) by diverse modulators has not been demonstrated.

The speed at which the catalytic actions of Na$^+$ and Ca$^{2+}$ occur may have important physiological consequences. When [Na$^+$]$_i$ increases, net transport by the Na$^+/Ca^{2+}$ exchanger (Ca$^{2+}$ entry and Na$^+$ exit) normally increases rapidly (see Fig. 14A). This occurs because the net transport of Na$^+$ out of the intracellular compartment is then favored thermodynamically and is no longer substrate limited. Following this increased net outward Na$^+$ transport, however, the exchanger spontaneously inactivates (Na$^+$-dependent inactivation; $t_{1/2} = 1.5–2$ s at 37°C; see Fig. 14A and Refs. 413, 632). Catalytic activation by Ca$^{2+}$ is fairly rapid; the component that is independent of Na$^+$ activates with a $t_{1/2}$ of 100 ms at 37°C (412; Hilgemann, personal communication). Even during a single cardiac [Ca$^{2+}$] transient lasting $\sim 100–200$ ms or more, catalytic activation by [Ca$^{2+}$]$_i$ will occur. Importantly, Ca$^{2+}$-dependent activation decays slowly upon removal of Ca$^{2+}$ (Fig. 14A; $t_{1/2}$ is on the order of seconds under physiological conditions; see Ref. 412). These catalytic actions of [Ca$^{2+}$]$_i$ on the Na$^+/Ca^{2+}$ exchanger turnover rate mean that as the time-averaged [Ca$^{2+}$]$_i$ rises, the Na$^+/Ca^{2+}$ exchanger becomes more responsive to changes in [Ca$^{2+}$]$_i$.

The functional importance of catalytic activation of the Na$^+/Ca^{2+}$ exchanger transport by increased [Ca$^{2+}$]$_i$, appears to make sense in cells where Ca$^{2+}$ signaling depends on the frequency and amplitude of [Ca$^{2+}$]$_i$, transients rather than on the mean averaged [Ca$^{2+}$]$_i$, such as heart cells and neurons [see discussions on local and global [Ca$^{2+}$]$_i$, signaling and on the frequency and amplitude modulation of this signaling (65, 113)]. In these cells a premium is placed on the restoration of ion balance even when Ca$^{2+}$ entry is increased. The catalytic activation of the Na$^+/Ca^{2+}$ exchanger will support this process, since this is a means of increasing the turnover rate of the Na$^+/Ca^{2+}$ exchanger when it operates in the Ca$^{2+}$ exit mode. The Na$^+/Ca^{2+}$ exchanger under physiological conditions works in the Ca$^{2+}$ efflux mode almost all of the time in most cells. The slow net catalytic effect of the Na$^+/Ca^{2+}$ exchanger to increased [Ca$^{2+}$]$_i$, serves the pur-
pose of integrating the effects of $[Ca^{2+}]_i$ transients on the Na$^+/Ca^{2+}$ exchanger. The Na$_3$-dependent inactivation may reflect molecular and biophysical processes of the Na$^+/Ca^{2+}$ exchanger; it may not play an important functional role because normal $[Na^+]_i$ is low (5–10 mM) and is not likely to effect much inactivation. Thus the Na$_3$-dependent inactivation may be a valuable tool for use when exploring the molecular operation of the Na$^+/Ca^{2+}$ exchanger but may not be an important physiological modulator. Nevertheless, insofar as Na$_3$-dependent inactivation is turned on, it will tend to reduce the turnover rate of the Na$^+/Ca^{2+}$ exchanger and thus will tend to increase global averaged $[Ca^{2+}]_i$. Interestingly, under the conditions of elevated $[Na^+]_i$, the $Ca^{2+}$-dependent and Na$_3$-dependent catalytic processes would appear to work against each other.

A further complication is the evidence that, in some cells, the Na$^+/Ca^{2+}$ exchanger may operate on Na$^+$ and Ca$^{2+}$ in a tiny restricted subplasmalemmal volume of cytosol where the concentrations of these ions may be quite different from those in “bulk” cytosol (103, 328, 618, 895a; see sect. viA7). Under these circumstances, the kinetic modulation of the Na$^+/Ca^{2+}$ exchanger could not be predicted from the dynamic changes in bulk $[Na^+]_i$ and $[Ca^{2+}]_i$.

D) CATALYTIC MODULATION OF THE Na$^+/Ca^{2+}$ EXCHANGER BY PROTONS. The giant patch-clamp method was also used to examine the modulation of the Na$^+/Ca^{2+}$ exchanger by intracellular pH (266, 267). The basic protocol was to produce a large outward Na$^+/Ca^{2+}$ exchanger current ($I_{Na/Ca}$), similar to those described above, by changing the bath [Na$^+$] (i.e., $[Na^+]_o$) from a low level (e.g., 0 mM) to a high level (e.g., 60 mM Na$^+$). Intracellular pH was changed at various times, under these conditions. The data (Fig. 16) confirmed earlier reports (241, 718) that protons inhibited the Na$^+/Ca^{2+}$ exchanger, but there were two unexpected results. The first was that the normal intracellular pH ($\sim$7.2) was in the middle of the curve that relates exchanger activity to pH$_i$ (Fig. 16B). This means that any variation of intracellular pH may have a great impact on Na$^+/Ca^{2+}$ exchanger by either inhibiting transport (at more acidic pH) or augmenting Na$^+/Ca^{2+}$ exchanger transport (at more basic pH). The maximum rate of Na$^+/Ca^{2+}$ exchange could thereby range between zero and double the rate at normal pH. Because $\alpha$-chymotrypsin did not augment Na$^+/Ca^{2+}$ exchange at high (basic) intracellular pH (Fig. 16A), these results suggest that protons may promote autoinhibition of the Na$^+/Ca^{2+}$ exchanger by interacting with one or more regions of the intracellular loop. As noted above, the Ca$_3$ and Na$_3$ catalytic effects also appear to depend on interactions of the intracellular loop with the transmembrane regions of the Na$^+/Ca^{2+}$ exchanger.

The second surprising result was that the proton-dependent modulation of the Na$^+/Ca^{2+}$ exchanger involves Na$_3$-dependent modulation of the Na$^+/Ca^{2+}$ exchanger. In the absence of intracellular Na$^+$, protons had little effect, whereas, at high (basic) pH, intracellular Na$^+$ did not produce inactivation. These findings suggest that both Na$^+$ and protons are needed to produce the autoinhibition of the exchanger by the intracellular loop. This second result thus further reinforces the working hypothesis that has evolved from the work of several groups (266, 267, 413, 590). According to this hypothesis, the intracellular loop region of the Na$^+/Ca^{2+}$ exchanger is critically involved with modulation of the Na$^+/Ca^{2+}$ exchanger function.
E) Modulation of the Na⁺/Ca²⁺ Exchanger by Temperature. The isoform of the Na⁺/Ca²⁺ exchanger that is expressed in the heart is very sensitive to temperature at temperatures near body temperature, with a Q₁₀ between 3 and 4 in guinea pig cardiac myocytes (693). Using the giant patch method, Kappl and Hartung (475) obtained a slightly lower value (2.6) for a temperature of 21–38°C and a very high Q₁₀ (6.7) for temperatures below 21°C (475). This wide range of values for the measured Q₁₀ is expected since at lower temperatures, the Na⁺/Ca²⁺ exchanger function falls to zero (see Ref. 693). Clearly, this zero-function temperature (TZF) represents a discontinuity for Q₁₀ measurements and varies with species and condition. Measuring Q₁₀ close to TZF may account for the wide variations in measured values. The observations noted above have generally been supported by recent investigations by others (386, 495, 924, 969) in heart muscle, and Khananshvili et al. (495) found a decrease in the Q₁₀ at temperatures above 30°C (495). Again, this is not an unexpected result, given the proximity to TZF. The Q₁₀ for Na⁺/Ca²⁺ exchange in rat brain is −3.0 at 30–35°C (606). As suggested above, it is likely that some of this marked temperature sensitivity depends on which gene encodes for the expressed Na⁺/Ca²⁺ exchanger, or on which splice variant of the Na⁺/Ca²⁺ exchanger is expressed as well as on the physiological body temperature. This likelihood is supported by the fact that, in the giant axon of the squid and muscle of the lobster, cold water invertebrates, the Na⁺/Ca²⁺ exchanger is used to extrude Ca²⁺ at temperatures at which the guinea pig heart Na⁺/Ca²⁺ exchanger activity is largely inhibited (693). Moreover, most studies on Na⁺/Ca²⁺ exchangers from invertebrates and fishes reveal Q₁₀ values on the order of 1.2–1.8 (289, 464, 921, 924); the frog heart exchanger also has a lower Q₁₀ than those from dogs and rabbits (71). Ruscak et al. (799), however, obtained a Q₁₀ of 3.2 for the exchanger from crayfish muscle. An understanding of the temperature dependence of the different gene products may provide valuable information on the relationship between the structure of the Na⁺/Ca²⁺ exchanger and its function.

F. Inhibitors of the Na⁺/Ca²⁺ Exchanger

A convenient as well as important way to examine the physiological role of a transport system is to block the system with a highly selective inhibitor. Good examples are the use of ouabain to inhibit the Na⁺ pump and TTX to inhibit the voltage-gated Na⁺ channel in excitable cells. Even somewhat less selective inhibitors have been used effectively to identify and characterize some transport systems when the cell type being studied contains, almost exclusively, just one of the transport proteins affected by the inhibitor. A case in point is amiloride, which has been used to identify and characterize the Na⁺ channel present in high-resistance (“tight”) epithelia such as the apical membranes of mammalian renal cortical collecting tubules (59).

The situation is not so simple for the Na⁺/Ca²⁺ exchanger. Regrettably, there are no inhibitors of the Na⁺/Ca²⁺ exchanger that are sufficiently selective that they can be used routinely to characterize the exchanger in intact cells and tissues unless the exchanger is more-or-less isolated from other Na⁺ or Ca²⁺ transport systems or current carriers. Another complicating factor is that the exchanger mediates both Ca²⁺ influx and Ca²⁺ efflux. It is possible that the kinetics of these two modes of exchange may be differently affected by inorganic cations. For example, some divalent cations, when added to the extracellular fluid, may interfere with the binding of Ca²⁺ at the extracellular surface of the PM, but may also, themselves, be transported by the exchanger so that they promote Ca²⁺ extrusion (see sect. mF5).

The subject of Na⁺/Ca²⁺ exchanger inhibitors was extensively reviewed a decade ago (474). Several new developments have taken place since then. The XIP was introduced in 1991 (587). A new nonpeptidic inhibitor, an isothiourea derivative, was also described recently (449, 970). Using another approach that followed the cloning of the exchanger, several laboratories employed antisense oligodeoxynucleotides (AS-oligos) to knock out the exchanger by selectively inhibiting expression of the protein (83, 596, 865–867, 907).

1. Amiloride analogs

Numerous structurally distinct organic molecules have been tested for their ability to inhibit the Na⁺/Ca²⁺ exchanger. Some of the most effective and potent compounds identified to date are certain amiloride analogs (472, 474, 520, 854, 861). Amiloride, an acylguanidine diuretic, is, itself, a weak inhibitor of the exchanger (IC₅₀ ~1 mM), but it is a much more potent blocker of certain epithelial Na⁺ channels (59). In contrast, hydrophobic substitutions at the acylguanidinium nitrogen (e.g., with a benzyl group) or at the 5-position amino group of the pyrazine ring, substantially increase the potency for block of the exchanger. Two examples are “benzamil,” with an IC₅₀ of 100 µM, and N²-(2,4-dimethylbenzyl)amiloride, with a Kᵢ of 10 µM. Unfortunately, many of these compounds have very limited solubility in aqueous solutions. The major problem with them, however, is their lack of specificity. For example, N²-substituted analogs are also potent inhibitors of Na⁺/H⁺ exchange, while guanidino N-substituted analogs are potent blockers of epithelial Na⁺ channels. A further problem with using amiloride analogs to study Ca²⁺ homeostasis is that some of the more effective inhibitors of Na⁺/Ca²⁺ exchange, such as 3,4-dichlorobenzamil, also block voltage-gated Ca²⁺ chan-
channels even more potently than they block the exchanger, at least in some preparations (75, 304, 505, 506). Consequently, interpretation of physiological/pharmacological studies on the action of amiloride analogs in intact cells or tissues (142, 223, 224, 342, 504, 609, 727, 869) or even in vesicles (825) must be viewed with considerable caution. Nevertheless, a number of investigators have used amiloride analogs such as 3,4-dichlorobenamid to identify Na\(^+\)/Ca\(^{2+}\) exchanger-mediated currents when other ion channels were already blocked (593, 693).

The mechanism of block of the exchanger by amiloride analogs is complex (861). At low concentrations they bind preferentially to an Na\(^+\) binding site, whereas, at higher concentrations, they also interact at another site to which Na\(^+\), Ca\(^{2+}\), and K\(^+\) also bind.

### 2. Antiarrhythmic agents

Several antiarrhythmic agents such as quinacrine (797, 75) and bepridil (a substituted pyrroolidine ethanamine) (474) also inhibit the Na\(^+\)/Ca\(^{2+}\) exchanger. These molecules, too, are nonselective; they inhibit other transport processes as well, at concentrations similar to those needed inhibit the exchanger.

### 3. Other organic molecules

Various local anesthetics (e.g., tetracaine and dibucaine; Ref. 651), general anesthetics (e.g., halothane; Refs. 53, 391, 392), and Ca\(^{2+}\) entry blockers (457) have also been shown to inhibit the Na\(^+\)/Ca\(^{2+}\) exchanger. They do so, however, at concentrations that may be considerably higher than the concentrations at which they exert their primary actions. For example, the Ca\(^{2+}\) channel blocker verapamil inhibits the Na\(^+\)/Ca\(^{2+}\) exchanger (297), but the effect is a very weak one, compared with its ability to block L-type Ca\(^{2+}\) channels (457). Thus the main problem is the overzealous use of this compound in experimental situations where interpretation of its action as a relatively selective block of Ca\(^{2+}\) channels may be compromised because of secondary effects such as block of the Na\(^+\)/Ca\(^{2+}\) exchanger.

A number of other molecules such as ascorbic acid (629, 909), chlorpromazine (161) and other anti-depressants (560), quinacrine (which also activates the exchanger under some circumstances) (228), quinidine (230), neomycin (152), polymyxin B (723), and the amino oxidase inhibitor harmaline (898), also are weak inhibitors of the Na\(^+\)/Ca\(^{2+}\) exchanger. Again, however, these molecules all have other actions that can be expected to overshadow their effects on the Na\(^+\)/Ca\(^{2+}\) exchanger except under very selective circumstances.

Recently, a new isothiourea derivative was reported to inhibit the Ca\(^{2+}\) entry mode of the exchanger preferentially (449, 970). This agent, 2-[2-4-(4-nitrobenzoyloxy)phenyl]ethyl]isothiourea methanesulfonate (compound number 7943), inhibited, with high affinity (IC\(_{50}\) = 1–3 \(\mu\)M), Na\(^+\)-dependent \(\text{Ca}^{2+}\) influx (and the concomitant rise in [Ca\(^{2+}\)]\(_i\)) in vascular smooth muscle, cardiac sarcolemmal vesicles, and fibroblasts transfected with Na\(^+\)/Ca\(^{2+}\) exchanger cDNA (449). In contrast, the IC\(_{50}\) for inhibition of the Na\(_o\)-dependent Ca\(^{2+}\) efflux and Na\(_o\)-dependent decline in [Ca\(^{2+}\)]\(_i\)] was \(\sim 30 \mu\)M. Similarly, compound number 7943 inhibited outward \(I_{\text{Na/Ca}}\) (corresponding to Ca\(^{2+}\) entry mode exchange) with an IC\(_{50}\) of \(\sim 0.3 \mu\)M, but inward \(I_{\text{Na/Ca}}\) (corresponding to Ca\(^{2+}\) exit mode exchange) with an IC\(_{50}\) of \(\sim 17 \mu\)M (970). In other words, it is apparently \(\sim 50\)-fold less effective in blocking the Ca\(^{2+}\) influx mode of exchange than the efflux mode. The significance of this preferential block of one mode of net Ca\(^{2+}\) transport is difficult to understand based on current models of the Na\(^+\)/Ca\(^{2+}\) exchanger mechanism. Furthermore, this agent is not very selective, in that it also blocks Na\(^+\) channels, Ca\(^{2+}\) channels, and certain K\(^+\) channels with IC\(_{50}\) values of 7–14 \(\mu\)M (970).

### 4. Peptide inhibitors of the Na\(^+\)/Ca\(^{2+}\) exchanger

When they cloned and sequenced the cardiac Na\(^+\)/Ca\(^{2+}\) exchanger, Philipson and colleagues (587, 687) observed that the large intracellular loop of the exchanger contained a segment of interspersed hydrophobic and basic residues reminiscent of a calmodulin binding site. To examine the possibility that this site might be autoinhibitory, they synthesized a peptide with this sequence ("exchanger inhibitory peptide," XIP) and showed that it did, indeed, inhibit Na\(^+\)/Ca\(^{2+}\) exchange (587). Other studies, on various preparations (179, 255, 519, 635), have confirmed this effect. Use of XIP as an exchanger inhibitor in appropriate isolated systems may provide insight into the modulatory role of the intracellular loop (377, 396). The physiological consequences of XIP action may be difficult to interpret, however, because XIP can be expected to bind indiscriminately to other calmodulin binding proteins and to interfere with the actions of endogenous calmodulin in intact cells.

An opiate-like binding site has also been functionally identified in the cardiac Na\(^+\)/Ca\(^{2+}\) exchanger. The molluscan peptide Phe-Met-Arg-Phe-amide (FMRF-amide) is known to bind to sites with an opiate binding site sequence. FMRF-amide has now been shown to inhibit the exchanger in cardiac sarcolemmal vesicles (492, 493), in internally dialyzed squid giant axons (255), and in pancreatic β-cells (951).

Recently, Khananshvili et al. (494) synthesized a series of positively charge hexapeptides (Arg\(_5\), Phe\(_2\), Cys\(_2\), containing an internal disulfide bond) with a terminal Arg-Phe-amide to target the internal opiate-like binding site on the exchanger. A subsequent study revealed that one of these molecules, FRRCRF-amide, when dialyzed into cardiac myocytes at a concentration of 1 \(\mu\)M or more,
completely and selectively inhibited \( \frac{I_{Na}}{I_{Ca}} \) [418; and see Khananshvili and colleagues (490, 491)]. This agent had no effect on L-type \( Ca^{2+} \) current or on \( K^+ \) currents.

5. Inorganic cations

A) LANTHANIDES. A variety of divalent and trivalent cations have long been employed as substitutes or inhibitors for various \( Ca^{2+} \)-dependent processes because these ions, with a high density of positive charge, often bind to sites that also bind \( Ca^{2+} \). Therefore, it is not surprising that \( La^{3+} \) was tested for its effects on \( Na^+/Ca^{2+} \) exchange beginning with some of the earliest studies on this transporter (35). Numerous reports indicate that \( La^{3+} \) inhibits \( Na^+/Ca^{2+} \) exchange in a large variety of cell types, including nerve and various kinds of muscle (35, 139, 153, 289, 347, 443, 514, 544, 868, 936). With the exception of a few studies on isolated or reconstituted vesicles (289, 347, 936), the \( La^{3+} \) was applied only in the extracellular fluid. However, \( La^{3+} \) can apparently enter cells, e.g., as a surrogate for \( Ca^{2+} \) on the \( Na^+/Ca^{2+} \) exchanger (712, 849).

Thus, in at least some instances, it is possible that \( La^{3+} \) may be acting at the inside of the plasma membrane. More importantly, Katzung et al. (481) first showed that external \( La^{3+} \) had a relatively low affinity for the cardiac \( Na^+/Ca^{2+} \) exchanger. This has also been observed in vascular smooth muscle, where the \( K_{50} \) exceeds 0.5 mM (333, 849). Because external \( La^{3+} \) is known to inhibit the plasmaemmal \( Ca^{2+} \) pump (and voltage-gated \( Ca^{2+} \) channels) with much higher affinity (744, 814), it is possible to use low-dose \( La^{3+} \) (<0.5 mM) to inhibit these other \( Ca^{2+} \) transport systems selectively (333, 849, 866) so that the function of the exchanger can be examined in relative isolation. Nevertheless, several investigators have used \( La^{3+} \) to block (and identify) exchanger-mediated ionic currents in electrophysiological experiments (153, 514).

Trosper and Philipson (936) studied the effects of several other lanthanides (\( Nd^{3+} \), \( Sm^{3+} \), and \( Y^{3+} \)) on \( Na^+/Ca^{2+} \) exchange in cardiac sarcosomal vesicles; these ions were all less-effective inhibitors than \( La^{3+} \). Also, \( Gd^{3+} \) does not inhibit \( Na^+/Ca^{2+} \) exchange, although it blocks the \( Ca^{2+} \) channels in nerve terminals.

B) \( Ni^{2+} \) AND OTHER DIVALENT CATIONS. Many divalent cations bind to \( Ca^{2+} \) binding sites on proteins and have thus been employed as either surrogate ions for \( Ca^{2+} \) or as inhibitors of \( Ca^{2+} \)-dependent processes. Indeed, some ions may be transported by the \( Na^+/Ca^{2+} \) exchanger and may also inhibit the exchanger, as in the case of high \( La^{3+} \) concentrations (849).

Among the alkaline earth ions, \( Mg^{2+} \) is reported to be a very weak inhibitor (243, 513, 868) that does not appear to be transported by the exchanger (227, 936). Indeed, in at least some cell types, \( Mg^{2+} \) is transported by an independent, \( Na^+ \)-coupled countertransport (\( Na^+/Mg^{2+} \) exchange) system (361, 247, 918).

In the heart, for example, external \( Mg^{2+} \) inhibits the exchanger with an \( IC_{50} \) of ~12.5 mM (513). Physiological levels of intracellular \( Mg^{2+} \) (~4 mM in squid axons) inhibit both the \( Ca^{2+} \) influx and \( Ca^{2+} \) efflux modes of the squid axon exchanger by ~50% (138, 243, 246). Other evidence, however, indicates that external \( Mg^{2+} \) does not inhibit the squid axon exchanger (254).

In cultured arterial smooth muscle, it has been reported that \( Mg^{2+} \) inhibits \( Ca^{2+} \) entry with a \( K_{i} \) of 93 \( \mu \)M (868). In contrast, preliminary results indicate that the \( Na_{ox} \)-dependent extrusion of \( Ca^{2+} \) in these cells is negligibly affected by 10 mM extracellular \( Mg^{2+} \) (A. Arnon and M. P. Blaustein, unpublished data; see Refs. 849, 866).

Unlike \( Mg^{2+} \), \( Sr^{2+} \) and, to a lesser extent, \( Ba^{2+} \) are both transported by the \( Na^+/Ca^{2+} \) exchanger (88, 203, 800; see also Refs. 85, 207); however, the maximal rate of transport of these ions by the exchanger is much slower than the rate of transport of \( Ca^{2+} \) (88). Furthermore, both \( Sr^{2+} \) and \( Ba^{2+} \) also interfere with \( Ca^{2+} \) transport mediated by the exchanger (88, 800, 936).

\( Cd^{2+} \) (417, 936), \( Co^{2+} \) (405), \( Mn^{2+} \) (9, 800, 936), \( Zn^{2+} \) (200), and \( Ni^{2+} \) (514) all inhibit \( Na^+/Ca^{2+} \) exchange, but none of these ions is a very specific inhibitor. Moreover, at least \( Ni^{2+} \), \( Mn^{2+} \), and \( Cd^{2+} \) may be transported (as surrogates for \( Ca^{2+} \)) by the exchanger (316, 655).

\( Ni^{2+} \) is an inhibitor of \( Na^+/Ca^{2+} \) exchange that has often been used to block, and thereby identify, \( Na^+/Ca^{2+} \) exchanger-mediated currents in electrophysiological experiments (328, 514, 618, 693, 695, 699). The concentrations of \( Ni^{2+} \) required are on the order of 2–5 mM so that the \( Ni^{2+} \) might be expected to have other effects unless the exchanger current is examined in relative “isolation” (i.e., after other \( Ni^{2+} \)-sensitive ionic currents are inhibited by different agents).

6. Antibodies

It should be possible to inhibit \( Na^+/Ca^{2+} \) exchange with appropriate antibodies raised against the \( Na^+/Ca^{2+} \) exchanger. Unfortunately, however, almost all of the readily accessible epitopes are located in the large cytoplasmic domains of the exchanger; polyclonal antibodies appear to bind relatively poorly to the extracellular domains of the exchanger (460, 610). One monoclonal antibody was originally reported to block \( Na^+/Ca^{2+} \) exchanger function, but this could not be confirmed (474), and there have been no further reports concerning block of \( Na^+/Ca^{2+} \) exchanger function by antibodies.

7. Antisense oligodeoxynucleotides

One other way to interfere with the function of the \( Na^+/Ca^{2+} \) exchanger is to inhibit the expression of the exchanger protein. Recently, several groups employed AS-oligos directed against the exchanger mRNA to this end. Lipp et al. (596) targeted a single 19-mer nonchimeric
phosphorothioated oligo to the conserved 3’-untranslated region of the NCX1 gene mRNA. They reported that their AS-oligo, at a rather high concentration (3 μM), knocked down 80% of exchanger function (measured as a reduction of [Ca^{2+}]; after release of “caged” Ca^{2+}) in cardiac myocytes (reversibly) within 24 h. Takahashi and colleagues (83, 907) also targeted a single nonchimeric phosphorothioated 20-mer AS-oligo to a region near the untranslated 3’-end of the NCX1 exchanger mRNA. They treated cardiac myocytes with 10 μM AS-oligo in the presence of lipofectamine (used to promote AS-oligo uptake) and reported that the Ca^{2+} influx from Na^{+}-free medium was reduced by 20–30%, and beat frequency was increased in 30% of treated cells. The integrity of other physiological properties was not shown in either study, nor was knockdown of the exchanger protein demonstrated biochemically. The specificity of the knock down (as opposed to a nonspecific effect of these AS-oligos) may be questioned in these cases because both groups used relatively high AS-oligo concentrations and reported substantial knock down within 24 h; this would imply an exchanger protein half-life of <12 h, which seems very short for an integral membrane transport protein.

Slodzinski and colleagues (865–867) targeted a tandem pair of short (15- and 17-mer) chimeric phosphorothioated AS-oligos to the region around the start codon of the NCX1 gene mRNA. Low concentrations (0.5 μM) of these AS-oligos markedly and reversibly knocked down Na^{+}/Ca^{2+} exchanger function (measured as Na_{i}-dependent Ca^{2+} entry and Na_{o}-dependent Ca^{2+} exit) in cultured arterial smooth muscle cells, while sparing other membrane transport functions (866, 867). During fast (every 3 min) but not slow (every 15 min) repetitive stimulation with serotonin, the recovery of [Ca^{2+}]_{i} to control levels was greatly slowed in the AS-oligo-treated arterial myocytes. These AS-oligos did not, however, interfere with the growth and division of the arterial myocytes. These same chimeric oligos also knocked down exchanger expression and function in ~60% of treated neonatal rat cardiac myocytes, but the effect was apparent only after 4 days of incubation with the AS-oligos, which is consistent with the measured exchanger half-life of ~33 h in these cells (865). In contrast to the results reported by Takahashi et al. (907), the spontaneous activity was inhibited in the 60% of the AS-oligo-treated cells in which the exchanger function was knocked down (865).

In a study on cultured rat astrocytes, Takuma and colleagues (630) employed antisense strategy to knock down exchanger function. This attenuated reperfusion injury (manifested by elevated cytosolic [Ca^{2+}]) and reduced cell toxicity. The implication is that the cells gained Na^{+} when external Ca^{2+} was withdrawn; then, upon reintroduction of external Ca^{2+}, the usual rapid Ca^{2+} entry was apparently mediated by the Na^{+}/Ca^{2+} exchanger, since it was reduced in AS-oligo-treated cells.

The effects of AS-oligos have also been studied in rat pancreatic β-cells (951a). The results of Na^{+}/Ca^{2+} exchanger knock-down with the AS-oligos indicate that the exchanger mediates Ca^{2+} entry in response to depolarization and is responsible for up to 70% of Ca^{2+} removal following cell activity.

IV. MOLECULAR BIOLOGY OF THE SODIUM/CALCIUM EXCHANGER

In 1990, the Na^{+}/Ca^{2+} exchanger gene was first cloned from dog heart muscle by Nicoll et al. (688). This work was a turning point in Na^{+}/Ca^{2+} exchanger research. All of the molecular biology research work described in the present review was influenced by this work. As indicated below, this seminal study has made possible a wide range of physiological studies, including investigations into issues of structure and function.

The Na^{+}/Ca^{2+} exchangers comprise a family of three genes (to date): NCX1 (21), NCX2 (85) and NCX3 (689). These genes have similar hydropathy patterns, suggesting similar overall structure and moderate amounts of sequence identity. Genes homologous to NCX1 have been characterized in diverse mammalian species including human (525, 530), rabbit (527), cattle (4), guinea pig (943), mouse (507), and rat (331, 606) as well as other species. Interestingly, genes similar to the Na^{+}/Ca^{2+} exchanger gene family have also been cloned from nonmammalian species such as Drosophila (796, 840), Xenopus (450), Caenorhabditis elegans (nematode; genebank accession number Z70312) (982), and Loligo (squid; genebank accession number U93214) (397).

The successful cloning of the Na^{+}/Ca^{2+} exchanger has stimulated numerous investigations and has spawned specific findings that bear on diverse biochemical, physiological, biophysical, and molecular aspects of the exchanger’s properties. Because of this recent explosion of studies, this section of the review is necessarily limited to a brief overview of the work on the molecular biology of the Na^{+}/Ca^{2+} exchanger. Additional relevant discussion is distributed throughout the other sections.

A. Purification, Cloning, and Reconstitution of the Exchanger Protein

Finding a starting point for the cloning of the Na^{+}/Ca^{2+} exchanger was key to the eventual success of Philipson’s group. The process involved functional tests, antibody development and use, and expression cloning. Investigation of Na^{+}/Ca^{2+} exchanger function in cardiac sarclemmal vesicles laid the foundation for the work.

1. Na^{+}/Ca^{2+} exchanger in vesicles

Although one of the two initial identifications and descriptions of the Na^{+}/Ca^{2+} exchanger was carried out
in mammalian heart (783), the physiological importance and multiple roles of the Na⁺/Ca²⁺ exchanger have only been realized more recently. Much of that appreciation arises from the now classic transport studies of the Na⁺/Ca²⁺ exchanger in cardiac sarcolemmal vesicles. These early studies demonstrated that, in the heart, there was rapid and massive Na⁺-dependent Ca²⁺ movement, that the coupling ratio was 3 Na⁺:1 Ca²⁺, and that the transport was probably rheogenic (electrogenic) (70, 161, 440, 726, 762, 764, 765). This work laid the foundation for the protein purification (719) that made the cloning of the Na⁺/Ca²⁺ exchanger possible.

Preparation of cardiac sarcolemmal vesicles, which led to the eventual protein purification, was carried out as follows (264, 720). Heart tissue was homogenized and purified to obtain sarcolemmal vesicles. These vesicles were purified with respect to the Na⁺/K⁺-ATPase (726, 764, 765). Because the Na⁺/Ca²⁺ exchanger is also predominantly a PM protein (172, 199, 318, 501), this purification strategy was valuable. The first strong indication of the rheogenicity (726) and the coupling ratio (765) of the cardiac Na⁺/Ca²⁺ exchanger was based on vesicle experiments using this purification method. That the Na⁺/Ca²⁺ exchanger was present in the cardiac PM vesicle preparations was demonstrated by examining the Na⁺-dependent Ca²⁺ influx in the vesicles that was stimulated by lowering [Na⁺]o (70, 726, 764, 765).

2. Strategy for cloning the Na⁺/Ca²⁺ exchanger

Cloning of the Na⁺/Ca²⁺ exchanger was testament to both thoughtful and careful work combined with persistence and the clever application of traditional and novel methods. The strategy used by Nicoll et al. (688) involved purification of the protein. This key step led to the identification of polypeptides with molecular masses 70, 120, and 160 kDa (719). Polyclonal antibodies were raised against these exchanger proteins and were used to screen a cardiac expression library. A clone that coded for a protein fragment that cross-reacted with these antibodies was initially identified, and this clone was used for further library screenings. A full-length clone was identified and used to produce cRNA that, when injected into Xenopus oocytes, induced the expression of Na⁺/Ca²⁺ exchanger activity (688).

3. Demonstration that the protein derived from the Na⁺/Ca²⁺ exchanger clone is functional

The canine cardiac Na⁺/Ca²⁺ exchanger clone (of the NCX1 gene) was shown to express a functional protein by injecting cRNA into Xenopus oocytes and measuring transport function in the injected oocytes. Nicoll et al. (688) showed that Na⁺-dependent (low [Na⁺]o stimulated) Ca²⁺ influx could be measured in oocytes injected with the cRNA for the Na⁺/Ca²⁺ exchanger but not in water-injected controls (688). In those experiments, the Xenopus oocytes were first preloaded with Na⁺ by incubating the oocytes with a cardiotonic steroid to inhibit the Na⁺-K⁺-ATPase before changing extracellular Na⁺. In contrast, when the human Na⁺/Ca²⁺ exchanger was cloned (525, 530), function was first demonstrated using an electrophysiologic measurement of Na⁺/Ca²⁺ exchanger current in HEK 293 cells and in COS cells transfected with a vector containing the cDNA encoding the Na⁺/Ca²⁺ exchanger (525). These same cells were also shown to contain Na⁺/Ca²⁺ exchanger protein on the plasmalemmal membrane by examining net Na⁺,sensitive Ca²⁺ movement in these cells by confocal [Ca²⁺]ı imaging (525).
Importantly, the giant patch-clamp method has been used effectively to examine the functional expression of the Na⁺/Ca²⁺ exchanger genes by electrophysiological methods in Xenopus oocytes that had been injected with cRNA (199, 408, 414, 587, 636).

**B. Structure/Function of the Na⁺/Ca²⁺ Exchanger**

The canine NCX1 cDNA open reading frame is composed of a sequence of 2,910 nucleotides from the full-length canine cardiac Na⁺/Ca²⁺ exchanger clone. This provides the primary information from which to deduce the 970 amino acids that make up the protein (688). This protein (Fig. 17) has a deduced (calculated) molecular mass of 108 kDa. Although there are six possible N-linked glycosylation sites, a single site is glycosylated in the native dog protein (433).

The intracellular loop is thought to be involved in modulation of the Na⁺/Ca²⁺ exchanger function not only by intracellular kinases, but also by intracellular ions such as Ca²⁺, Na⁺, and H⁺, and perhaps other factors. The entire intracellular loop can be deleted, leaving only sufficient residual amino acids to permit a connection between the region of five transmembrane crossings of the protein (at the amino-terminal end) and the region of six transmembrane crossings (at the carboxy-terminal end). This shortened protein is, nevertheless, a functional Na⁺/Ca²⁺ exchanger, as assessed by the giant membrane patch method (Fig. 14B; see Ref. 635). This result supports the notion that the intracellular loop region does not play a direct role in ion translocation. The treatment of giant patches taken from native cardiac myocyte preparations with the protease α-chymotrypsin, which "derepresses" the Na⁺/Ca²⁺ exchanger (406, 632), is thought to operate much like the loop-deletion mutation described above. These findings have led to speculation that modulation of the Na⁺/Ca²⁺ exchanger by intracellular protons (266, 267), and Ca²⁺ and Na⁺ (632), may depend on the intracellular loop.

The Ca⁺-dependent modulation of the Na⁺/Ca²⁺ exchanger has been investigated at the molecular level (578, 579). Using a ⁴⁵Ca²⁺ "overlay" method, Levitsky et al. (579) identified the region of high-affinity Ca²⁺ binding as the segment between amino acids 371 and 508; this is in the middle of the intracellular loop region of the protein. Within this span there are two highly acidic sequences, each containing three consecutive aspartic acid residues that, when mutated, lead to a markedly reduced Ca²⁺ affinity. This suggests that the Ca⁺-dependent modulation of the Na⁺/Ca²⁺ exchanger kinetics (88, 96, 258, 265, 406, 412, 661) may arise from the binding of Ca²⁺ to this domain of the exchanger protein.

At the 5'-end of the cDNA, a Kosak-like consensus sequence marks the initiation site of the open reading frame. There appears to be an initial leader peptide (M0), followed by five transmembrane regions (M1-M5), a large intracellular loop region, and six transmembrane regions (M6-M11) at the carboxy-terminal end (525, 688, 807). These distinct regions, suggested by hydrophobicity analysis, provide a putative model for the protein that places the amino terminus in the extracellular space and the large loop and the carboxy terminus in the intracellular domain (Fig. 18).

It may be less surprising that in similar tissues but in different species, there are no clearly important differences in the gene structure for the relevant isoform of NCX1. This
can be best appreciated in the heart, where the primary cardiac isoforms of NCX1 from a variety of mammalian species are almost identical, as shown in Figure 19A. In contrast, the signal sequences are only 60–75% identical (Fig. 19B; and see Refs. 284, 570, 839). The significance of the signal sequences is, however, unclear; several groups have shown that the signal peptide is not required to target the exchanger to the PM (330, 605, 807).

The NCX1 gene from which this general structure was first identified (688) appears to be the model for all expressed Na\(^+/\)Ca\(^{2+}\) exchanger genes except that of the vertebrate rod (NCKX1) and possibly the platelet (see above). The human NCX1 gene is found on chromosome 2p22-p23 (641, 848), but the locations of NCX2 and NCX3 are not yet known.

Consensus phosphorylation sites have been identified that suggest the Na\(^+/\)Ca\(^{2+}\) exchanger may be a target for PKA and/or one of the members of the PKC family (D. Schulze, personal communication). Growing evidence of this expected modulation of the Na\(^+/\)Ca\(^{2+}\) exchanger by phosphorylation/dephosphorylation is available (99, 447, 448, 873, 874; see sect. III E). However, a variety of seemingly inconsistent and conflicting physiological results have been obtained following PKA or PKC activation (for references, see sect. III E). The site(s) of this putative phosphorylation remains to be identified but is expected to be on the intracellular loop.

### C. Alternative Splicing

Calcium metabolism in different tissues is remarkably disparate. In the heart, for example, [Ca\(^{2+}\)]\(_i\) transiently rises to a peak of 1–3 μM in 10–20 ms and declines with a \(t_{1/2}\) of \(~150\) ms (146, 812, 977). In contrast, the [Ca\(^{2+}\)]\(_i\) transients in synaptic boutons may be larger and faster than those in the heart; during a nerve action potential, [Ca\(^{2+}\)]\(_i\) may rise to a peak of \(~100\) μM or more within a few hundred microseconds and may then decline with a \(t_{1/2}\) in the range of a few milliseconds (877). A
greater contrast is found in the connecting tubule of the nephron, where \([Ca^{2+}]_i\) is presumed to be quite constant over time.

These three tissues (heart, nerve, and kidney) are distinctly different in their \(Ca^{2+}\) signaling and in the time courses of their \([Ca^{2+}]_i\) transients, but they are similar in that each possesses a great deal of \(Na^{+}/Ca^{2+}\) exchanger protein (318, 465, 501, 610, 613, 719, 746) and RNA from the \(NCX1\) gene (525, 526). Because the \(Na^{+}/Ca^{2+}\) exchanger is so well expressed in these tissues, and yet, \(Ca^{2+}\) signaling is so different, the exchanger itself might be expected to display large differences in the primary cDNA sequence as well as functional differences. This is not the case, however. Instead, there are tissue-specific isoforms (see below) that, with respect to the coded sequence, express at least 32 distinctive isoforms of \(NCX1\) (838). The alternatively spliced \(NCX1\) variants arise from diversity in a limited region of the intracellular loop (527). Thusfar, however, no distinctive functions of the different isoforms have been observed, although a brief report has suggested that the cardiac isoform, but not the renal isoform, is activated by PKA (394). Perhaps the alternatively spliced isoforms are important because of spatial targeting that permits the \(Na^{+}/Ca^{2+}\) exchanger to be placed at an appropriate subcellular site (see sect. vA, below, showing the immunofluorescent localization). These possibilities still need to be examined experimentally.

D. Gene Structure (Multiple Genes)

The intron/exon structure of the gene \(NCX1\) is presented in Figure 20 (527). The entire gene spans more than 200 kb (527, 532, 533, 826, 838) and includes a 5'-end alternatively spliced area before the start of the open reading frame (570). This region presumably plays a role in regulating expression of the different isoforms, although the mechanism of this hypothesized action is unknown. The major alternatively spliced region in the open reading frame is found in the putative intracellular loop region of the protein (see below and Fig. 18). Following the stop codon there are conserved sequences across isoforms and species (532, 533). This conservation of sequence beyond the stop signal presumably influences and regulates expression, but the mechanism of action is not known.

Two other related genes that code for \(Na^{+}/Ca^{2+}\) exchangers [in contrast to the vertebrate photoreceptor \(Na^{+}/(Ca^{2+}+K^{+})\) exchanger] have also been identified in mammals. These have been designated \(NCX2\) (586, 590, 743) and \(NCX3\) (590, 689, 743). The \(NCX1\) gene product is widely distributed in tissues and cells (including cardiac, skeletal, and smooth muscles), neurons, astrocytes, kidney, lung, and spleen (743). In contrast, the \(NCX2\) and \(NCX3\) gene products have thusfar been found only in brain and skeletal muscle (586, 689). Moreover, there do not appear to be large functional differences among the three \(NCX\) gene products (590).

The abundance of these gene products in diverse tissues has been explored; the available evidence indicates that \(NCX1\) apparently is the dominant gene in mammals. To date, at least 32 alternatively spliced isoforms of the \(NCX1\) gene product have been identified. The roles of each of these genes and the importance of their gene products are topics still under active examination.

The level of tissue-specific expression of \(NCX1\) is also quite broad and varies over 100-fold at the mRNA level (525). Furthermore, multiple alternatively spliced isoforms of the \(NCX1\) gene product have been identified. The roles of each of these genes and the importance of their gene products are topics still under active examination.

The open reading frame of \(NCX1\) is alternatively spliced to produce a number of isoforms that are found in diverse tissue (394, 525–527, 530, 770, 838). A mutually

E. Isoforms

FIG. 20. Sequence of rabbit genomic structure of alternatively spliced region of intracellular loop. Exon identification by letters (A–F) are noted by nucleotides in capital letters, and flanking sequences of nucleotides (introns) are identified by lower case letters. **Exons A and B** are alternative (only 1 can be used), whereas **exons C–F** are cassette exons (any 1 or more can be used). [From Kofuji et al. (527).]
FIG. 21. Organization of alternatively spliced region of NCX1 Na\(^+\)/Ca\(^{2+}\) exchanger. A: comparison of alternatively spliced isoforms of rabbit Na\(^+\)/Ca\(^{2+}\) exchanger from heart, brain, and kidney. There is 1 isoform from heart (NACA1), whereas there are 2 from brain (NACA4 and NACA6) and 2 from kidney (NACA3 and NACA2). [From Kofuji et al. (527).] B: genomic organization from Fig. 19 is shown at top. Below are diagramatic representations of exon organization of 7 isoforms of Na\(^+\)/Ca\(^{2+}\) exchanger. [Modified from Kofuji et al. (527).]

FIG. 22. Localization of plasma membrane (PM) Ca\(^{2+}\) pump (PMCA) and Na\(^+\)/Ca\(^{2+}\) exchanger (NCX) in primary cultured rat mesenteric artery myocytes. Aa and Ba: low-magnification images of myocytes labeled with antibodies raised against PMCA (Aa) and NCX (Ba) show uniform distribution of PMCA and reticular distribution of NCX in PM. Ab and Bb: restored high-magnification images of portions of 2 myocytes labeled with antibodies raised against PMCA (Ab) and NCX (Bb) show: cells were later stained with DiOC to visualize sarcoplasmic reticum (SR, double arrowheads) and mitochondria (arrows) (Ac and Bc). PMCA distribution is uniform (Ab); in contrast, NCX distribution (Bb) parallels underlying SR structure (Ac). [Modified from Juhaszova and Blaustein (461).]
exclusive pair of exons (called A or B) combined with four cassette exons (called C, D, E, and F) are organized to produce up to 32 distinct isoforms (Figs. 21 and 22). These exons are placed sequentially in the open reading frame in a position toward the end of the intracellular loop. In heart, the dominant isoform is composed of an alternatively spliced region composed of the exons A, C, D, E, and F. One unanswered question is, Why are there different isoforms? Does this region of alternative splicing underlie alterations in the kinetics of the Na$^+$/Ca$^{2+}$ exchanger such as Ca$^{2+}$-dependent modulation of transport, Na$^+$-dependent inactivation, ATP-dependent modulation, or phosphorylation? Or, does the region subserve some other important function? Does it affect other proteins that interact with the Na$^+$/Ca$^{2+}$ exchanger, perhaps altering protein-protein interactions?

**F. Development**

The regulation of [Ca$^{2+}$]$_i$, which is crucial for cellular function, also varies during development. In rats at birth, for example, heart cells lack transverse (t) tubules as well as the tight coupling between sarcolemmal L-type Ca$^{2+}$ channels (that are present in the sarcolemmal membrane including the t-tubule membrane) and the SR Ca$^{2+}$-release channels (or ryanodine receptors). In these neonatal rat heart cells, the Ca$^{2+}$ current density ($I_{Ca}$) is higher than it is in adult heart cells (196). Thus it makes sense that the Na$^+$/Ca$^{2+}$ exchanger may also change with development and that this change may be linked to other changes in Ca$^{2+}$ handling by the cell during development. Artman and colleagues (25, 110) found that the Na$^+$/Ca$^{2+}$ exchanger current density peaked in the early neonatal period (days 1–4) and then declined over the following 3 wk. This is consistent with their investigation of mRNA levels in both rabbit and rat showing a rise in fetal levels that peaked a few days after birth. At that time, the Na$^+$/Ca$^{2+}$ exchanger mRNA levels were approximately eight times those found in the adult.

The role of the Na$^+$/Ca$^{2+}$ exchanger during development is still under investigation. It is not clear whether the exchanger acts as a “follower” or a “leader” of cellular change and to what extent the role played by the exchanger is tissue dependent. There appears to be a reciprocal relationship between the expression of the exchanger and certain isoforms of the Na$^+$ pump in the heart. During early postnatal development, and in the transition from a hypothyroid to a hyperthyroid condition, expression of the Na$^+$ pump high ouabain-affinity $\alpha_2$-isoform increases markedly, while expression of the Na$^+$/Ca$^{2+}$ exchanger protein reportedly decreases (617). In contrast, however, Hojo et al. (421) report that 3,3',5-triiodothyronine increases Na$^+$/Ca$^{2+}$ exchanger expression markedly, and morphological evidence suggests that there is increased expression of the exchanger during the development of t tubules (172).

**G. Modulation**

Specific regions of the exchanger protein appear to be involved in the modulation of Na$^+$/Ca$^{2+}$ exchange (see sect. ivB). The Ca$^{2+}$ overlay and mutagenesis studies (578, 579) indicate that specific parts of the protein are involved in the binding of Ca$^{2+}$. Furthermore, consensus sequences have been hypothesized to be targets for phosphorylation or for the binding of ATP (Schulze, personal communication). Functional studies have been involved in the identification, however crudely, of regions of the Na$^+$/Ca$^{2+}$ exchanger protein that play a role in Na$^+$-dependent modulation or in the inhibition by H$^+$ (268). Figure 18 shows where these postulated regions involved in modulation are located with respect to structures in the protein.

**H. The Drosophila Exchanger**

The Drosophila melanogaster Na$^+$/Ca$^{2+}$ exchanger gene was recently cloned and sequenced and identified as DRONCX (genebank accession number L39835) (796, 838, 840). Hydrophobicity analysis of the deduced amino acid sequence indicates that the protein structure is similar to that suggested for NCX1 (796, 840; Schulze, personal communication). Interestingly, the Drosophila cDNA sequence appears to be equally divergent from NCX1 and NCX2 (Fig. 19A) and may represent a common progenitor to both (367). Recent physiological studies have shown that Ca$^{2+}$ effects on the Drosophila Na$^+$/Ca$^{2+}$ exchanger are quite different than they are on NCX1 isoforms (432). In the cardiac isoform of NCXI, increasing [Ca$^{2+}$]$_i$ activates the Na$^+$/Ca$^{2+}$ exchanger as a “regulator” over the range of 0–10 $\mu$M. In Drosophila, [Ca$^{2+}$]$_i$ elevation serves as a regulator by inhibiting Na$^+$/Ca$^{2+}$ exchanger activity. Furthermore, in Drosophila, Na$^+$/Ca$^{2+}$ exchange mediated by the Na$^+$/Ca$^{2+}$ exchanger is greatly reduced when compared with human heart Ca$^{2+}$/Ca$^{2+}$ exchange (796). Functional distinctions between NCX1 and the Drosophila Na$^+$/Ca$^{2+}$ exchanger should help us to understand how each functions, as well as structure-function relationships. Interpretation of these findings will certainly be aided by future studies that are expected to examine how NCX2 and NCX3 work.

Schwarz and Benzer (840) examined DRONCX and identified two important motifs: an $\alpha$-motif and a $\beta$-motif (840). The $\alpha$-motif is a tandem repeat comprising two pairs of transmembrane segments: 2M and 3M and also 8M and 9M. This motif is found on all NCX exchangers (i.e., NCX1, NCX2, and NCX3 as well as DRONCX) and is also present on the NCKX exchangers (see below). This
has been linked to the anionic amino acids that may constitute part of the negative charge of the “naked” (without transported ions bound) Na⁺/Ca²⁺ exchanger (414, 693). As such, the two motifs may contribute to the parts of the Na⁺/Ca²⁺ exchanger that contribute to ion translocation. In contrast, the two β-motifs are found on the putative intracellular loop region and may contribute to catalytic regulation of the Na⁺/Ca²⁺ exchanger by Ca²⁺; they are found on the NCX series of Na⁺/Ca²⁺ exchanger but not on the NCKX exchangers (see below).

I. The Na⁺/(Ca²⁺ + K⁺) Exchanger: NCKX1 and NCKX2

1. Vertebrate rod

Physiological studies showed that the “cardiac” Na⁺/Ca²⁺ exchanger was distinct from the Na⁺/(Ca²⁺ + K⁺) exchanger that was found in the vertebrate eye (165). Functionally, the retinal rod exchanger was found to have a coupling ratio of 4 Na⁺ to 1 Ca²⁺ plus 1 K⁺. This contrasts with the 3 Na⁺ to 1 Ca²⁺ coupling ratio of the cardiac/neuronal/renal Na⁺/Ca²⁺ exchanger that is typical of the transport modalities found in the different isoforms of the NCX1 gene. This functional difference is reflected in the different thermodynamic constraints of the two types of Na⁺/Ca²⁺ exchangers (see below). The gene (NCKX1) that codes for the rod Na⁺/(Ca²⁺ + K⁺) exchanger was identified and was found to be virtually unrelated to the cardiac-type Na⁺/Ca²⁺ exchanger, except for two features (5, 769). First, both proteins exhibited Na⁺-dependent Ca²⁺ transport. Second, the general topologies of the deduced proteins were similar (see below). The open reading frame of NCKX1 contained 3,597 bp; this exchanger has a deduced sequence containing 1,199 amino acids and a calculated molecular mass of ~130 kDa.

One could rationalize the idea that a gene related to the NCX1 gene coded for the rod Na⁺/(Ca²⁺ + K⁺) exchanger. Nevertheless, it also seems reasonable for a novel photoreceptor gene (NCKX1) to have evolved largely independently (as appears to be the case from the minimal sequence homology). The two regions of homology are located within regions of putative transmembrane spans and may thus represent a critical feature in the protein, possibly an ion binding or translocation motif (769). This appears to be an example of evolutionary convergence.

The identity of the gene (and protein) responsible for Na⁺,Ca²⁺ extrusion in the invertebrate eye is still unknown, but the absence of a maintained dark current may indicate that a traditional cardiac type of Na⁺/Ca²⁺ exchanger (with a coupling ratio of 3 Na⁺:1 Ca²⁺) may be involved (see sect. vii). A surprising recent finding is the evidence that the platelet Na⁺,Ca²⁺ transport is mediated by a Na⁺/(Ca²⁺ + K⁺) exchange (517). It would be particularly valuable to identify the gene responsible for this function and compare it with that for the vertebrate eye Na⁺/(Ca²⁺ + K⁺) exchanger.

Furthermore, understanding why the platelet uses a different Ca²⁺ extrusion mechanism than NCX1 found in diverse other tissues may help us understand platelet Na⁺ and Ca²⁺ metabolism. Do platelets have a background Na⁺ influx analogous to the dark current of the vertebrate rod? Is [Na⁺]i elevated in platelets? Is a second, cardiac-like, Na⁺/Ca²⁺ exchanger present in platelets? Are the two homologous regions on the rod Na⁺/(Ca²⁺ + K⁺) exchanger and the Na⁺/Ca²⁺ exchanger, that have been identified in NCKX1 and NCKX1, also found in the platelet Na⁺/(Ca²⁺ + K⁺) exchanger? The answers to these questions may help us to understand the role(s) of Na⁺/Ca²⁺ exchange in Ca²⁺ homeostasis in cells.

2. Brain Na⁺/(Ca²⁺ + K⁺) exchanger

Recently, a second K⁺-dependent Na⁺/Ca²⁺ exchanger, NCKX2, was cloned from rat brain (940). The cDNA encodes a protein of 670 amino acids with a predicted size of 75 kDa. The hydropathy plot suggests 12 transmembrane (M) regions, and the first is thought to be cleaved signal peptide. An intracellular loop region separates the last six transmembrane regions from the first five. The overall structure is thus very similar to both NCX1 and NCKX1. An initial calmodulin binding domain, like the XIP domain of NCX1, is also present. Other features suggest functional and structural similarities to NCX1: a single extracellular glycosylation site just before M11, an alternatively spliced region in the intracellular loop, and consensus sites for protein kinase phosphorylation. Within hydrophobic clusters, NCKX2 shows 91% similarity and 80% identity with NCKX1; overall, NCKX2 and NCKX1 have 67% similarity and 55% identity. Interestingly, there is nearly 100% identity in the repeat regions first recognized by Schwarz and Benzer (840).

NCKX2 is found in diverse brain tissues including striatum, parietal cortex, cerebellum, hippocampus, and the medial geniculate body of the thalamus. By in situ hybridization, expression was restricted to neuronal cell bodies except in the neocortex. It is speculated that the maximum turnover rate of NCKX2 may be as low as that thought to be found with NCX1 of between 2 and 115 s⁻¹ (787, 320) and thus may subserve a very different function than the NCX1 with maximum turnover rates in the range of 1,000 to 5,000 (176, 414, 693). Understanding the functional role of NCKX2 starts with the K⁺ requirement but must include consideration of the possible low maximum turnover rate and also its genealogy. It was initially speculated that NCKX1 was a specific adaptation for the vertebrate eye (see above) because the rod operates with a high background dark current leading to a high [Na⁺]i and [Ca²⁺]i. The K⁺ requirement would conse-
SODIUM/CALCIUM EXCHANGE

July 1999

subsequently permit the Na\(^+/\)Ca\(^{2+} + K^+\) exchanger, using both the K\(^+\) and Na\(^+\) electrochemical gradients to power the extrusion of Ca\(^{2+}\). Lytton and colleagues (940) speculate, however, that the identification of NCKX2 in rat brain, as well as the discovery of highly related sequences in other nonvertebrate species (e.g., C. elegans, Drosophila, and Arabidopsis), indicate that NCKX2 origins predate the development of the vertebrate rod.

V. PHYSIOLOGICAL ROLES AND PATHOPHYSIOLOGICAL CONSEQUENCES OF SODIUM/CALCIUM EXCHANGER ACTIVITY IN CELLS AND TISSUES: GENERAL PRINCIPLES

The PM Na\(^+/\)Ca\(^{2+}\) exchanger operates in parallel with several Ca\(^{2+}\) transport systems (e.g., the PMCA pump and Ca\(^{2+}\) channels) and in series with other Ca\(^{2+}\) transport systems that are located in organellar membranes (e.g., SR/ER Ca\(^{2+}\) pumps, mitochondrial Ca\(^{2+}\) transport). In exploring Na\(^+/\)Ca\(^{2+}\) exchanger function, a critical question that must be addressed is, How does the activity of the PM Na\(^+/\)Ca\(^{2+}\) exchanger relate to these other parallel and series Ca\(^{2+}\) transport systems, in terms of their relative contributions to cell Ca\(^{2+}\) homeostasis? To address this very complex question, it is important to consider the demands. How much Ca\(^{2+}\) must be moved (both at rest, and during and following activity)? What are the relative capabilities of the various transport systems (i.e., what are their “turnover” numbers, and what are their maximum capacities to move Ca\(^{2+}\)?) For example, the transport or turnover numbers for these systems are very different. A single open Ca\(^{2+}\) channel has a turnover rate of up to \(10^7\) ions/s (416), a single exchanger can transport up to \(\sim 5 \times 10^7\) Ca\(^{2+}\)/s (see sect. mB), and a PM or SR/ER Ca\(^{2+}\) pump or Na\(^+\) pump can transport \(\sim 10^2\) Ca\(^{2+}\)/s (891). These numbers must, of course, be considered in relation to the relative densities of the various transport systems, i.e., how many exchanger or Ca\(^{2+}\) pump molecules are located near each Ca\(^{2+}\) channel? Although we do not have definitive answers to these questions, using these questions as a guide may enable us to better understand the physiological role(s) of the Na\(^+/\)Ca\(^{2+}\) exchanger.

A. Localization of the Na\(^+/\)Ca\(^{2+}\) Exchanger: Possible Clues to Function

Immunocytochemical studies have revealed an unexpected feature of the Na\(^+/\)Ca\(^{2+}\) exchanger, namely, its very specific localization in at least some types of cells. In smooth muscle cells (both freshly isolated and cultured), and in cultured astroglial cells and neurons, the exchanger appears to be confined to regions of the PM that are closely apposed to underlying junctional SR or ER (see Fig. 22D) (96, 359, 460, 465, 663). This focal distribution of the exchanger contrasts with the uniform distribution of the PM ATP-driven Ca\(^{2+}\) pump in some of the same cells, as illustrated in Figure 22A (465). The different distributions of the two Ca\(^{2+}\) transport systems in the same PM suggest that the distributions have a functional basis. For example, the PMCA pump may, at least in some cells, have primarily a “housekeeping role” in helping to keep bulk cytoplasmic [Ca\(^{2+}\)] low, while the exchanger may play an indirect role in helping to modulate Ca\(^{2+}\) stores. This is discussed further in section VI A.7.

There is some disagreement about the distribution of the exchanger in cardiac myocytes (Fig. 23). Frank et al. (318) have suggested that in adult cardiac myocytes the

![FIG. 23. Confocal immunofluorescent images of distribution of Na\(^+/\)Ca\(^{2+}\) exchanger in guinea pig and rabbit heart cells. A: Na\(^+/\)Ca\(^{2+}\) exchanger protein is found on all guinea pig cardiac myocyte membranes exposed to extracellular solution including intercalated disks and transverse tubules and exterior surface membranes. Bar = 10 μm. [From Kieval et al. (501).] B: similar results from adult rabbit cardiac myocytes. In this case, there appears to be more exchanger in t-tubule membranes than in peripheral sarcolemma. [From Frank and Garfinkel (317).] C: guinea pig myocyte t tubules are shown in cross-section, and Na\(^+/\)Ca\(^{2+}\) exchanger protein (large bright spots) is seen in t tubules. D: surface membrane at junction with t tubules. Figure reveals presence of Na\(^+/\)Ca\(^{2+}\) exchanger in both membranes. Bars in C and D = 2 μm. [C and D from Kieval et al. (501).]
exchanger is found predominantly in the t tubules, whereas Kieval et al. (501) (and see Chen et al., Ref. 172) have reported that the exchanger is uniformly distributed in the plasma membrane of cardiac myocytes. This is discussed further in section VI A.

In neurons, the very abundant Na\(^+\)/Ca\(^{2+}\) exchanger is expressed at high concentrations in presynaptic nerve terminals, relative to other parts of the cells (465, 610, 781). Here it may play a role in the modulation of Ca\(^{2+}\)-dependent neurotransmitter release as well as in Ca\(^{2+}\) homeostasis. The exchanger is also abundant in neuronal processes (spines) and in growth cones (610). Thus it appears to be particularly prevalent in regions of neurons where relatively large amounts of Ca\(^{2+}\) must be transported. In the neuron cell body, the exchanger appears to be distributed over the surface in a reticular pattern that resembles the pattern of the underlying reticulum (Fig. 24); this is analogous to the distribution in vascular smooth muscle (Fig. 22B) and in astrocytes (359).

At nerve terminals, the exchanger molecules and PMCA pumps are differently distributed. Preliminary evidence suggests that the PMCA pumps are located close to the synaptic vesicle docking sites, whereas the exchangers are located at some distance from the release sites (463; M. Juhaszova, P. Church, M. P. Blaustein, and E. F. Stanley, unpublished data). The clear implication is that these two transport systems have different functions at the terminals.

Another surprise arises from the recent discovery of a K\(^+\)-dependent exchanger in rat brain (219, 940). Lytton and colleagues (940) have obtained in situ hybridization data showing that the K\(^+\)-dependent exchanger message is extensively expressed in hippocampal pyramidal cells and granule cells. However, the NCX1 exchanger message is also strongly expressed in these same cells (465, 624). The significance of these observations is unknown. Because these two types of exchangers have different energetics, it is highly likely that these exchangers have different functions, but it is not known whether they are expressed in the same PM domains. The latter information might help to elucidate their respective roles in the physiology of the neurons.

In skeletal muscle, the exchanger may be absent from the peripheral (surface) PM and may be confined to t-tubule membrane (see sect. IV C). Indeed, Sacchetto et al. (802) have suggested that the Na\(^+\)/Ca\(^{2+}\) exchanger, PMCA pump isoform 1 (PMCA1), and the dyhydropyridine receptor (i.e., voltage-gated Ca\(^{2+}\) channel) may all be confined to junctional regions of the transverse tubule membrane system. The ouabain-sensitive \(\alpha_2\)-isoform of the Na\(^+\) pump also appears to be located primarily in t tubules (980a).

The kidney presents a different problem in transporter localization for two reasons. In the first place, net transcellular transport must occur sequentially across apical and basolateral membranes in series; Ca\(^{2+}\) moves down a steep electrochemical gradient, from kidney tubule lumen to cytosol, across the apical membrane, and up a large gradient across the basolateral membrane in cells all along the nephron. Second, each segment along the nephron has a different function. All segments of the rabbit kidney contain a PM Ca\(^{2+}\)-ATPase or PMCA pump.
proximal tubules are at top left; cross-section of a connecting tubule is shown in middle, and portions of proximal tubules are at top left and bottom right. Study was carried out with polyclonal antibodies raised in guinea pigs against a synthetic fragment of rabbit cardiac Na\(^+/\)Ca\(^{2+}\) exchanger. Antiserum was diluted 1:1,000; secondary goat anti-guinea pig IgG was diluted 1:100. Note that staining is confined primarily to basal (antiluminal) and lateral membranes of connecting tubular cells (ct); little staining is observed in apical membranes (facing tubule lumen) of these cells, or in proximal tubular cells (pt). Magnification, ×400. (Figure courtesy of Dr. Robert Reilly, unpublished experiment.)

(274), but the capacity of this PMCA pump is apparently insufficient to keep pace with the absorptive flux of Ca\(^{2+}\) in all segments of the renal tubule except for the medullary thick ascending limb (321). Thus there is a clear need for another Ca\(^{2+}\) transport system. Attempts to localize the Na\(^+/\)Ca\(^{2+}\) exchangers in the kidneys have yielded some seemingly paradoxical data. Early functional studies were controversial; some investigators (356, 917, 919, 947, 948, 983) found evidence for a basolateral membrane Na\(^+/\)Ca\(^{2+}\) exchanger in rat, rabbit, and mudpuppy (Necturus) proximal tubules, whereas others did not (620, 749, 771, 834). Functional evidence for the presence of exchangers has also been obtained for the distal convoluted tubule, connecting tubule, and cortical collecting duct (919, 983). The basolateral membrane exchanger helps to export Ca\(^{2+}\) into the interstitial fluid. Molecular biological and immunological methods have been used to demonstrate localization of the exchanger in the basolateral membrane of cells in the proximal tubule (269–271), distal convoluted tubule (1002), and connecting tubule (33, 701, 771). Reilly et al. (771) concluded that the full-length (120-kDa) exchanger was expressed primarily in the connecting tubule basolateral membrane (Fig. 25) and that the absence of significant levels of exchanger expression in other nephron segments (Fig. 25) may have been due to expression of different isoforms. This particular localization of the exchanger in the basolateral membrane of the connecting tubule cells (Fig. 25) raises the possibility that it may play a role in the hormonally regulated fine control of Ca\(^{2+}\) reabsorption (see sect. VI-G). The presence of exchanger on the basolateral membrane, and its apparent absence from the apical membrane (Fig. 25), parallels the distribution of Na\(^+\) pump molecules in renal tubule cells. This distribution likely reflects the fact that Ca\(^{2+}\) transport across the apical membrane is downhill, and across the basolateral membrane is uphill.

B. Ca\(^{2+}\) Extrusion Versus Ca\(^{2+}\) Entry

The fact that the exchanger can mediate both net Ca\(^{2+}\) entry and net Ca\(^{2+}\) exit raises the obvious question of whether the exchanger actually functions in both modes during normal physiological activity. This question has been very difficult to answer, in part because of the lack of selective inhibitors. Nevertheless, there is broad agreement with the view that the exchanger plays an important role in the extrusion of Ca\(^{2+}\) in many types of cells, especially after periods of activity, when cell Ca\(^{2+}\) is elevated (i.e., when \(V_M\) is more negative than \(E_{\text{Na/Ca}}\); see Eq. 5). This is usually demonstrated experimentally by showing that recovery is inhibited (i.e., the decline in [Ca\(^{2+}\)], to resting levels is slowed) when Na\(^+\)-dependent Ca\(^{2+}\) efflux is blocked. The exchanger is particularly well suited for this role because its >10-fold higher turnover rate than that of the PMCA pump means that the exchanger may have the capacity to extrude a relatively large amount of Ca\(^{2+}\) rapidly when [Ca\(^{2+}\)] is elevated and the driving force (difference between \(V_M\) and \(E_{\text{Na/Ca}}\); see Eq. 5) is large.

One situation in which the exchanger clearly operates primarily in the Ca\(^{2+}\) influx mode is in the erythrocytes of dogs, and some rodents and other carnivores (655, 705). The PM of these cells contains both a Ca\(^{2+}\) pump and an Na\(^+/\)Ca\(^{2+}\) exchanger but no Na\(^+\) pump. Thus, in these cells, the Na\(^+/\)Ca\(^{2+}\) exchanger is driven by the energy stored in the Ca\(^{2+}\) gradient (generated by the PMCA pump); the maximum velocity of the Ca\(^{2+}\) pump may be 10 times greater than that of the exchanger in these cells (655). Here, the job of the exchanger is, paradoxically, to regulate [Na\(^+\)], and cell volume (see sect. VI-F).

Another type of cell in which Ca\(^{2+}\) influx mediated by...
the exchanger may play an important physiological role is the cardiac myocyte. Several investigators have suggested that this flux may be critically involved in excitation-contraction coupling (437, 561). Recent evidence, however, has raised some questions about this issue, which is discussed further in section V A3. It is conceivable that the high density of exchanger molecules at presynaptic nerve terminals may likewise be involved in excitation-secretion coupling, but critical data are lacking. A key difference between cardiac myocytes and neurons is the duration of the action potential; it is much shorter in neurons than in cardiomyocytes. Thus the peak rise in \([\text{Ca}^{2+}]_i\) occurs after the membrane potential has substantially repolarized, whereas in many cardiac cells, the peak \([\text{Ca}^{2+}]_i\) occurs during the action potential plateau. As a result, the thermodynamic driving force on the exchanger, \(V_m-E_{\text{Na/Ca}}\) (see Eq. 5), may favor \(\text{Ca}^{2+}\) entry mode exchange during the early part of the action potential plateau (94), and this \(\text{Ca}^{2+}\) may contribute to excitation-contraction coupling (but see sect. V A3). Nevertheless, resting \([\text{Ca}^{2+}]_i\) in most cells is only \(\sim 0.1\ \mu\text{M}\). Thus the exchanger kinetics do not favor rapid cycling under these conditions, and the exchanger may not transport much \(\text{Ca}^{2+}\) in resting cells despite the large \(\Delta\mu_{\text{Na}}\).

For small exchanger-mediated increases in cell \(\text{Ca}^{2+}\), it may be difficult, if not impossible, to decide whether the changes are due to increased \(\text{Ca}^{2+}\) entry or decreased exit. For example, partial inhibition of the \(\text{Na}^+\) pump has been shown to increase ER/SR stores of \(\text{Ca}^{2+}\) in a variety of cell types (95). Unless it can be shown that the \(\text{Ca}^{2+}\) influx under these circumstances is greater than under control conditions, one cannot exclude the possibility that the rise in stored \(\text{Ca}^{2+}\) is due to reduction of \(\text{Ca}^{2+}\) efflux via the exchanger. Unfortunately, selective inhibition of the exchanger would not resolve this dilemma because inhibition would normally be expected to block \(\text{Ca}^{2+}\) influx as well as efflux.

C. Control of SR/ER \(\text{Ca}^{2+}\) Content

The localization of the \(\text{Na}^+/{\text{Ca}}^{2+}\) exchanger, at least in some cells (Figs. 22–25), in PM that overlies junctional SR/ER may, as noted above, provide a clue about one of the functions of the exchanger. Because the SR/ER is a \(\text{Ca}^{2+}\) storage organelle, this localization of the exchanger implies that it probably plays a role in loading and unloading the \(\text{Ca}^{2+}\) stores, or at least in modulating the fractional saturation of the stores. Furthermore, because release and resequestration of \(\text{Ca}^{2+}\) in the SR/ER play a central role in intracellular \(\text{Ca}^{2+}\) signaling and cell activation (63, 64), the obvious implication is that the activity of the \(\text{Na}^++/{\text{Ca}}^{2+}\) exchanger influences overall cell activity. In this way, the exchanger can be expected to influence many \(\text{Ca}^{2+}\)-dependent physiological processes in most types of cells (95).

Clearly, changes in \(\Delta\mu_{\text{Na}}\) will, by affecting the \(\text{Na}^+/\text{Ca}^{2+}\) exchanger, alter \([\text{Ca}^{2+}]_i\) and modulate activities that are dependent on internal \(\text{Ca}^{2+}\). In this regard, it is noteworthy that, in some cells, \(\text{Na}^+\) pump isoforms (\(\alpha_2\) and \(\alpha_3\)) with a high affinity for ouabain (i.e., \(K_{0.5} = 10–20\) and 20–500 nM, respectively) are also localized to PM regions that overlie junctional SR/ER (461, 462) and thus must be colocalized with the exchanger. It is intriguing to speculate on the possibility that these particular (high ouabain affinity) \(\text{Na}^+\) pumps are modulated by a circulating endogenous ouabainlike compound (EOLC) (364, 365, 381, 382). In turn, these \(\text{Na}^+\) pumps may influence, indirectly (via the \(\text{Na}^+/\text{Ca}^{2+}\) exchanger), the \([\text{Ca}^{2+}]_i\), in a postulated “restricted cytosolic space” between the PM and SR/ER (“fuzzy space”) (564) and, consequently, the amount of \(\text{Ca}^{2+}\) stored in the SR/ER (95, 103, 115) (and see sect. V A7).

Direct regulation of the \(\text{Na}^+/\text{Ca}^{2+}\) exchanger by nontransported ions (see sect. VII E4) can be expected to alter the kinetics of exchange by either increasing or decreasing both \(\text{Ca}^{2+}\) influx and efflux. In contrast, modulation of the \(\text{Na}^+\) pump can be expected to promote either \(\text{Ca}^{2+}\) influx or efflux, by altering the electrochemical driving force on the exchanger. Thus one can envision the need for upregulation of the exchanger in neurons following a burst of activity, when simply increasing the \(J_{\text{max}}\) of the exchanger will help to extrude \(\text{Ca}^{2+}\) faster (see sect. VII E4). Conversely, when increased cellular responsiveness is required, increasing plasma EOLC levels may reduce \(\Delta\mu_{\text{Na}}\) in the vicinity of the exchanger molecules (see sect. V A7) and thus increase the amount of “signal \(\text{Ca}^{2+}\)” stored in the SR/ER.

VI. PHYSIOLOGICAL ROLES OF THE SODIUM/CALCIUM EXCHANGER IN VARIOUS TISSUES

The \(\text{Na}^+/\text{Ca}^{2+}\) exchanger is found on the PM of cells. Messenger RNA for the \(\text{Na}^+/\text{Ca}^{2+}\) exchanger has been identified in virtually every cell type examined (530, 570, 743), suggesting that this transport protein is omnipresent. The average density of exchanger molecules per unit PM area, however, varies greatly but appears to be greatest in the heart, brain, and kidney (176, 414, 525, 545, 570, 693). Although the \(\text{Na}^+/\text{Ca}^{2+}\) exchanger is located on the PM of these cells, there are also reports of \(\text{Na}^+/\text{Ca}^{2+}\) exchanger activity in mitochondria (125, 135, 209, 214, 215, 467, 584, 644, 684, 725, 984) and secretory vesicles (451, 538, 539, 724, 804, 805). To the extent that these reports accurately reflect \(\text{Na}^+/\text{Ca}^{2+}\) exchanger activity, it must be argued that the \(\text{Na}^+/\text{Ca}^{2+}\) exchanger proteins in these organelle membranes are quite different from the PM \(\text{Na}^+/\text{Ca}^{2+}\) exchanger. The exchanger proteins in these organelar membranes are not recognized by polyclonal
or monoclonal antibodies raised against the PM NCX1 Na\(^+\)/Ca\(^{2+}\) exchanger, nor by cDNA screens for the three related genes (NCX1, NCX2, and NCX3). It should be noted, however, that these screens also did not detect the retinal rod outer segment Na\(^+\)/Ca\(^{2+}\) exchanger (732) that is encoded by an unrelated gene (769).

A. Heart Muscle

The existence of a Na\(^+\)/Ca\(^{2+}\) exchanger was first discovered 30 years ago in mammalian heart (783) and squid giant axon (35, 102). The function of the Na\(^+\)/Ca\(^{2+}\) exchanger in heart muscle, arguably, has been determined much more completely than in any other tissue (97). Part of this may be due to its high density of 250–400 exchanger molecules/\(\mu\)m\(^2\) of PM (176, 414, 693), and its central role in normal cardiac cell function (Figs. 26–28) (132, 213). Additionally, it was from canine heart that the first Na\(^+\)/Ca\(^{2+}\) exchanger genes were cloned (688) and from heart protein that the first effective anti-Na\(^+\)/Ca\(^{2+}\) exchanger antibodies were produced (15, 16, 501, 610, 688, 956). Thus it is not surprising that significant advances have been made in understanding the cardiac Na\(^+\)/Ca\(^{2+}\) exchanger. These studies have led to a profound appreciation of the central importance of this transport system in the heart. The role of the Na\(^+\)/Ca\(^{2+}\) exchanger as a primary Ca\(^{2+}\) extrusion mechanism in the heart is widely accepted (154a, 213, 366, 367). Many other functions have also been attributed to the Na\(^+\)/Ca\(^{2+}\) exchanger; some of these are based on incomplete data or conflicting results, however, and remain controversial.

1. Location of the Na\(^+\)/Ca\(^{2+}\) exchanger in heart

Confocal microscopy of adult guinea pig and rat heart cells reveals that the Na\(^+\)/Ca\(^{2+}\) exchanger is found in all membranes of the myocytes that face the extracellular space. There has been some controversy regarding the spatial distribution of the Na\(^+\)/Ca\(^{2+}\) exchanger. The data of Kieval et al. (501) suggest that all of the extracellular-facing membranes of the cell [including the transverse (t) tubular membrane, the external surface membrane, and the intercalated disk membranes] have about the same number of Na\(^+\)/Ca\(^{2+}\) exchangers per unit surface area [but see the studies of Frank and colleagues (318, 406, 501) for an alternative interpretation]. The variable appearance of the immunofluorescence images ob-
served when viewing single heart cells, particularly adult rat and guinea pig (267, 317, 318, 501), may reflect the folding of the membranes and or their shape with respect to the confocal image plane. Thus the intercalated disk regions appear particularly bright because there is much infolding of the membranes. The t tubules may appear quite bright relative to the external surface membrane because the xy-resolution of the confocal microscope is about 0.4 µm and the tubule may have a diameter of 0.2 µm or more. It thus has a “brightness factor” of about \( \pi D/(0.4) \) or about 0.8 for a 0.1-µm tubule, 1.6 for a 0.2-µm tubule, and 2.4 for a 0.3-µm tubule. The brightness factor assumes that the surface membrane is normal to the image plane. If, however, it is not, then the relative brightness of the t tubules increases further because of the dim surface membrane. On the other hand, Frank et al. (318) have suggested the Na\(^+/Ca\(^{2+} \) exchanger protein is located preferentially in cardiac myocyte t tubules based on immunofluorescence intensity measurements. Earlier, this group had reported that the exchanger binds to the cytoskeletal protein ankyrin and had raised the possibility that the ankyrin might confine the Na\(^+/Ca\(^{2+} \) exchanger to triad junctional regions of the sarcolemma (585). More recent work from the Frank laboratory (172), on rabbit heart, indicates that the exchanger is present in all of the “external” membranes, although it may be more prevalent in the t-tubule membrane. They have also shown that, during early development, the Na\(^+/Ca\(^{2+} \) exchanger is found first on the surface membrane and later appears along the Z-lines of heart cells as the t tubules develop (172).

Resolution of the distribution of the Na\(^+/Ca\(^{2+} \) exchanger in the heart is not adequate to make compelling quantitative arguments, perhaps because the exchanger is so prevalent in this tissue. No obvious patch-wise variation in Na\(^+/Ca\(^{2+} \) exchanger concentration along the surface PM and the t-tubule membrane has been demonstrated in this tissue, however. Giant patch-clamp experiments indicate that there is a great deal of Na\(^+/Ca\(^{2+} \) exchanger activity in blebs formed from the extracellular facing surface member (199), although it is not prevalent when the blebs first form (Hilgemann, personal communication). This might fit with the view (318) that the exchanger is more prevalent in the t tubules than in the surface (peripheral) sarcolemma. Nevertheless, it seems possible (or, perhaps, likely) that different types of cells may have different distributions of the exchanger molecules, to fit with specific cellular functions. With the exception of the possible triggering of SR Ca\(^{2+} \) release or the control of SR Ca\(^{2+} \) stores (see below), there is no clear physiological imperative to have local collections of Na\(^+/Ca\(^{2+} \) exchanger molecules. The very high average density of Na\(^+/Ca\(^{2+} \) exchanger molecules in the heart is used to control global [Ca\(^{2+} \)]; thus there might be an advantage for a fairly uniform distribution of exchanger in this tissue. As noted by Kieval and colleagues (501), the particularly high concentration of the Na\(^+/Ca\(^{2+} \) exchanger noted at the intercalated disk region can be accounted for by the significant infolding of the PM in those areas. In contrast to the punctate distribution of the exchanger immunofluorescent label on the extracellular surface (which may correspond to clusters of exchanger molecules) in astrocytes, neurons, and arterial myocytes (see Figs. 22 and 24) (460, 465), the label seems to have a more uniform distribution in cardiac myocyte PM (Fig. 23). Spatial organization of the Na\(^+/Ca\(^{2+} \) exchanger at a resolution greater than that available with the wide-field light microscope may still occur in heart muscle, but such conclusions require quantitative immunoelectron microscopy. As noted above, the punctuate appearance of Na\(^+/Ca\(^{2+} \) exchanger label along the t tubules (e.g., see Fig. 23C) appears to correspond to regions where the t tubules extend through the sections perpendicular to the plane of focus (501). The occasional longitudinal strips of labeling

---

**Figure 28.** Time course of rewarming (relaxation) phase, normalized to peak height, after rapid cooling contracture of rabbit ventricular muscle (data from traces B-E in Fig. 26). Removal of external Na\(^+ \) (0Na) only very slightly slowed rate of relaxation, relative to that in normal Tyrode solution (NT). Caffeine (Caff), which releases Ca\(^{2+} \) from SR, slowed relaxation rate by a factor of −2; however, removal of external Na\(^+ \) in presence of caffeine markedly slowed relaxation rate. [From Bers and Bridge (67).]
may correspond to the connections between two t tubules within the plane of focus (174, 501). The aforementioned divergence of opinion about the immunofluorescent localization of the Na\(^+\)/Ca\(^{2+}\) exchanger in adult heart cells needs to be resolved.

2. Primary role of the Na\(^+\)/Ca\(^{2+}\) exchanger in heart

The Na\(^+\)/Ca\(^{2+}\) exchanger is a rheogenic transporter with a \(E_{Na/Ca}\) between \(-10\) and \(-50\) mV under resting, diastolic conditions in heart muscle. This estimate of \(E_{Na/Ca}\) (Eq. 5) assumes a transport coupling ratio of 3 Na\(^+\):1 Ca\(^{2+}\), intracellular Na\(^+\) between 8 and 14 mM with [Na\(^+\)]\(_o\) at 145 mM, and [Ca\(^{2+}\)]\(_i\) around 100 nM with [Ca\(^{2+}\)]\(_o\) at 1 mM. Thus the estimate of \(E_{Na/Ca}\) is clearly positive to the maximum diastolic potential (\(-80\) mV for ventricular muscle) and indicates that the exchanger will tend to extrude Ca\(^{2+}\) during diastole. When the cardiac cell is initially depolarized during an action potential, however, the membrane potential may reach +60 mV or more. In some mammalian species (e.g., guinea pig), a very positive membrane potential is maintained for hundreds of milliseconds. Thus there is a brief period during which \(E_{Na/Ca}\) is more negative than the membrane potential and thermodynamics favor the net entry of Ca\(^{2+}\) via the Na\(^+\)/Ca\(^{2+}\) exchanger (Eq. 5).

The amount of Ca\(^{2+}\) that is actually transported by the Na\(^+\)/Ca\(^{2+}\) exchanger under any condition always depends on the kinetics of the Na\(^+\)/Ca\(^{2+}\) exchanger as well as the "thermodynamic driving force" (\(V_M - E_{Na/Ca}\)), as indicated in Equation 5. Although the thermodynamic driving force is very approachable and can be defined by measuring the ion gradients (since the coupling ratio is fixed at 3 Na\(^+\):1 Ca\(^{2+}\)), the kinetics are almost unpenetrable. [One other caveat, however, is the possibility that the subplasmalemmal Na\(^+\) and Ca\(^{2+}\) concentrations may differ from those in bulk solution (328, 618, 895a, 974).] The measured kinetics must include knowledge of unidirectional fluxes, net fluxes, and specific fluxes of Ca\(^{2+}\) and Na\(^+\). Each of these measurements is difficult to make and, in the absence of a specific high-affinity inhibitor of the Na\(^+\)/Ca\(^{2+}\) exchanger, is almost always controversial. As discussed in section u, B and E, the kinetics of the exchanger can be modulated by many factors. We suspect that as \(V_M\) changes during the cardiac action potential, the change in the thermodynamic driving force of the Na\(^+\)/Ca\(^{2+}\) exchanger will lead to changes in Ca\(^{2+}\) flux via the Na\(^+\)/Ca\(^{2+}\) exchanger and hence will affect [Ca\(^{2+}\)]\(_i\). There is, however, also much recent evidence that depolarization rapidly activates Na\(^+\) channels (<1 ms) and Ca\(^{2+}\) channels (1 ms), and the transfer of ions through these channels will alter local (subsarcolemmal) [Na\(^+\)] and [Ca\(^{2+}\)] on this time scale (151, 437, 554, 561, 813, 879). These local concentrations have not been measured; nevertheless, they will profoundly affect the Na\(^+\)/Ca\(^{2+}\) exchanger which also resides in the PM. Thus the net effect of depolarization of the PM on the \((V_M - E_{Na/Ca})\) term (Eq. 5) cannot be defined. Because the ionic manifestations of these thermodynamic changes also depend on the kinetics of the Na\(^+\)/Ca\(^{2+}\) exchanger at the time of depolarization (e.g., activation by internal Ca\(^{2+}\) and inactivation by internal Na\(^+\); see sect. mE), the observed experimental results, like so many other aspects of Na\(^+\)/Ca\(^{2+}\) exchanger function, remain controversial.

One of the kinetic constraints that has been estimated, the maximum Na\(^+\)/Ca\(^{2+}\) exchanger turnover rate, depends on the estimated density of Na\(^+\)/Ca\(^{2+}\) exchanger molecules in the PM. It is possible to measure the Na\(^+\)-dependent counterflux of Ca\(^{2+}\) or the Ca\(_{\text{a}}\)-activated Na\(^+\)-dependent membrane current. These experiments establish a boundary limit for the product of the exchanger density and the maximum turnover rate of the Na\(^+\)/Ca\(^{2+}\) exchanger. The maximum turnover rate of the Na\(^+\)/Ca\(^{2+}\) exchanger protein has been estimated by several groups to be 1,000–5,000 s\(^{-1}\) (176, 414, 475, 693). One group, however, using a method broadly similar to that of Niggli and Lederer (693) and Kappl and Hartung (475), suggested that the turnover rate of the exchanger in heart is 10- to 100-fold slower but with a 10- to 100-fold greater density (737). The weight of the evidence, however, favors the higher turnover rates of 1,000–5,000 s\(^{-1}\), with the lower (but still very high) density of 200–400 exchanger molecules/\(\mu\)m\(^2\).

The kinetics of Na\(^+\)/Ca\(^{2+}\) exchanger net transport of Na\(^+\) and Ca\(^{2+}\) depend on many factors including the specific isoform of the Na\(^+\)/Ca\(^{2+}\) exchanger, [Ca\(^{2+}\)]\(_i\), [Na\(^+\)]\(_o\), [H\(^+\)]\(_o\), and phosphorylation state. In healthy adult heart muscle, only the cardiac isoform of the Na\(^+\)/Ca\(^{2+}\) exchanger is expressed to any significant extent (525). Depending on the kinetics of the Na\(^+\)/Ca\(^{2+}\) exchanger transport, the effects of the thermodynamic driving force may have a more or less dramatic effect. During the plateau phase of the cardiac action potential, the membrane potential is close to 0 mV but can also be much more positive in some species (e.g., in the guinea pig). With many variations that depend on the species, [Ca\(^{2+}\)]\(_i\) rises dramatically from the diastolic level of \(-100\) nM to between 1 and 10 \(\mu\)M over 10–20 ms, before returning to the diastolic level with a \(t_{1/2}\) of decay of \(-150\) ms (68, 69, 146, 175, 562). This change in [Ca\(^{2+}\)]\(_i\) dramatically alters the \(E_{Na/Ca}\) at the peak of the [Ca\(^{2+}\)]\(_o\) transient, from a "resting" level that is more positive than the diastolic membrane potential to one that is still positive to \(V_M\) at between +10 and +85 mV. Because \(V_M\) changes more rapidly than [Ca\(^{2+}\)] and \(E_{Na/Ca}\), however, there is a brief period during which \(E_{Na/Ca}\) is more negative than \(V_M\) (666–668). From a thermodynamic point of view, Ca\(^{2+}\) entry is then favored, but the actual rate of Ca\(^{2+}\) entry depends on the kinetics (including the time dependence of activation by Ca\(_{\text{a}}\)). After this period, the membrane
begins to hyperpolarize, and $V_M$ becomes more negative than $E_{Na/Ca}$, and thermodynamics again favors Ca$^{2+}$ extrusion. This dynamic modulation of the Na$^+$/Ca$^{2+}$ exchanger has recently been examined by Cannell's group (213) as well as by others (45, 300, 367, 452, 453, 978), who find that the overall contribution of the Na$^+$/Ca$^{2+}$ exchanger to the action potential shape is greater than previously anticipated. Presumably, one important reason is that the catalytic action of intracellular Ca$^{2+}$ is minimal when the driving force, $V_M - E_{Na/Ca}$, most favors Ca$^{2+}$ entry. When [Ca$^{2+}$]$_i$ is in the 1 μM range (close to the half-maximal concentration for catalytic activation of the exchanger), $V_M$ is close to $E_{Na/Ca}$ so that the driving force is small (see Eq. 5).

A number of careful experiments have been carried out to determine how the Na$^+$/Ca$^{2+}$ exchanger contributes to cardiac Ca$^{2+}$ metabolism. Some of the most compelling experiments were carried out by Crespo et al. (213), in which they examined how [Ca$^{2+}$]$_i$ depended on the steady-state $V_M$ in voltage-clamped ventricular myocytes. They provide evidence that the Na$^+$/Ca$^{2+}$ exchanger is the dominant transporter in regulating steady-state [Ca$^{2+}$]$_i$ as the membrane potential slowly changes. They identify a small fraction of the Ca$^{2+}$ transport that depends on some other transport system that they speculate is a sarcolemmal Ca$^{2+}$-ATPase. Bridge and co-workers (46, 133, 154a) carried out independent experiments that used a different method but that came to the same overall conclusion. Specifically, they investigated how the Ca$^{2+}$ influx through Ca$^{2+}$ channels (needed to activate contraction) was extruded from the intracellular compartment. Using a rapid sample changer, and measuring $I_{Ca}$ and $I_{Na/Ca}$ with appropriate blockers, they were able to show that virtually all of the Ca$^{2+}$ influx via $I_{Ca}$ was extruded by the Na$^+$/Ca$^{2+}$ exchanger (Fig. 9). Complementing these investigations, Bers and co-workers (49, 51, 61, 66, 68, 452) also determined that ~7% (rat) to 25–30% (rabbit, ferret, cat, and guinea pig) of the decay of the [Ca$^{2+}$]$_i$ transient was due to extrusion by the Na$^+$/Ca$^{2+}$ exchanger (Figs. 27 and 28).

There is now a consensus that the primary role of the Na$^+$/Ca$^{2+}$ exchanger in ventricular heart muscle is to extrude Ca$^{2+}$ and that the primary extrusion mechanism is the Na$^+$/Ca$^{2+}$ exchanger. In no other tissue is the result so clean and complete. As indicated below, however, there may be reason to believe that the Na$^+$/Ca$^{2+}$ exchanger may play other important roles in ventricular muscle and in other cells within the heart.

### 3. Role in excitation-contraction coupling

In addition to the role it plays in extruding Ca$^{2+}$ brought in predominantly by $I_{Ca}$, the Na$^+$/Ca$^{2+}$ exchanger may play other roles in excitation-contraction coupling. It can influence the amount of Ca$^{2+}$ within the myocyte, and it can, in principle, trigger SR Ca$^{2+}$ release by bringing Ca$^{2+}$ into the cell under certain conditions. Because of the ability of the SR to accumulate Ca$^{2+}$, small changes in Ca$^{2+}$ transport by the Na$^+$/Ca$^{2+}$ exchanger, and hence small changes in resting [Ca$^{2+}$]$_i$, may have profound effects on SR Ca$^{2+}$ load. This role of the Na$^+$/Ca$^{2+}$ exchanger is central to its indirect control of Ca$^{2+}$ stores, a role that it plays to some extent in many tissues (95). In the heart, this function is particularly prominent (66, 290, 292, 293) because the exchanger is the dominant Ca$^{2+}$ extrusion mechanism, responsible for virtually all of the Ca$^{2+}$ transport out of the cell (46, 133, 154a, 213).

A. Ca$^{2+}$ Load in the SR. In a simple pump-leak model for the control of [Ca$^{2+}$]$_i$, the only factors that control steady-state [Ca$^{2+}$]$_i$ are the pump and the leak. The intracellular stores are themselves unable to affect the steady-state [Ca$^{2+}$]$_i$ because they have a limited capacity to take up or release Ca$^{2+}$. Nevertheless, they have a profound effect on the total amount of Ca$^{2+}$ in the intracellular compartment. The cardiac SR is a high-capacity, dynamic Ca$^{2+}$ store that contains (concentrates) ~10–100 times the total amount of Ca$^{2+}$ in the cell that can be estimated from the cell volume, [Ca$^{2+}$]$_s$, and the non-SR and nonmitochondrial Ca$^{2+}$ stores. Therefore, its content will have a large influence on the Ca$^{2+}$-induced Ca$^{2+}$ release (CICR)-dependent [Ca$^{2+}$]$_s$, transient. The SR content is determined by its own pump-leak balance and the buffering capacity of the SR. One of the important factors that can influence the SR content is [Ca$^{2+}$]$_i$, itself (see above). It is because the Na$^+$/Ca$^{2+}$ exchanger controls [Ca$^{2+}$]$_i$ that the Na$^+$/Ca$^{2+}$ exchanger controls the SR Ca$^{2+}$ content.

A number of recent experimental results suggest that the relationship between SR Ca$^{2+}$ content and the amount of Ca$^{2+}$ released during a Ca$^{2+}$ transient is highly nonlinear. As the SR Ca$^{2+}$ content increases, a larger fraction of the SR Ca$^{2+}$ content is released (50, 60, 397, 642, 816, 888). This result is consistent with the idea that the elementary SR Ca$^{2+}$ release events (e.g., Ca$^{2+}$ sparks; Refs. 173–175) are not very sensitive to [Ca$^{2+}$]$_s$ (148, 173, 175, 692) under normal conditions but become very sensitive as SR Ca$^{2+}$ load increases (173). It is this sensitivity of SR Ca$^{2+}$ release to SR Ca$^{2+}$ load and the central role played by the Na$^+$/Ca$^{2+}$ exchanger in controlling SR Ca$^{2+}$ load through its influence on [Ca$^{2+}$]$_i$ that make inotropic agents work. In particular, the positive inotropic actions of the cardiotonic steroids appear to arise largely as a result of their action on the Na$^+$ pump, which should lead to a slight rise in [Na$^+$]$_i$ (but see sect. VI A7). As a result, [Ca$^{2+}$]$_i$ is increased slightly via the Na$^+$/Ca$^{2+}$ exchanger and this, in turn, increases the SR Ca$^{2+}$ content, the [Ca$^{2+}$]$_i$, transient, and the strength of contraction of the heart. Other possible actions of the cardiotonic steroids are not, however, ruled out. Two provocative, but unconfirmed, examples are 1) cardiotonic steroids (CTS) such as digoxin may directly alter the sensitivity of the SR Ca$^{2+}$...
release channels (i.e., ryanodine receptors) to activation by $[\text{Ca}^{2+}]_i$ transient is abolished when inward current is carried by Li$^+$ (B). These data have been interpreted as an indication that $I_{\text{Na}}$ raises $[\text{Na}^+]_i$, in a subsarcolemmal ("fuzzy") space and that this initiates $\text{Ca}^{2+}$ entry via $\text{Na}^+/\text{Ca}^{2+}$ exchange, thereby elevating $[\text{Ca}^{2+}]_i$ and triggering release of $\text{Ca}^{2+}$ from SR. [From Hume et al. (437).]

B) TRIGGERING SR $\text{Ca}^{2+}$ RELEASE. The idea that the $\text{Na}^+/\text{Ca}^{2+}$ exchanger may act to bring net $\text{Ca}^{2+}$ into the cardiac myocyte during an action potential was developed 20 years ago (665–668) in theoretical examinations of the $\text{Na}^+/\text{Ca}^{2+}$ exchanger. The discussions about the reversibility of the $\text{Na}^+/\text{Ca}^{2+}$ exchanger began with the first presentations of the energetics of $\text{Na}^+/\text{Ca}^{2+}$ exchanger behavior (35). A number of investigators have speculated about this feature since then, but it was not until a relatively recent observation (Fig. 29) (561) that the issue was seriously considered. Nevertheless, theories based only on thermodynamic parameters (i.e., the ion concentrations and electrochemical driving forces) have limited applicability to this issue (290); furthermore, kinetic considerations and exchanger modulation greatly complicate the situation.

Leblanc and Hume (561) showed that a significant fraction of the $[\text{Ca}^{2+}]_i$ transient could be triggered when $I_{\text{Ca}}$ was blocked. The triggering of CICR required the presence of $I_{\text{Na}}$ (Fig. 29), however, and it could be blocked by the $\text{Na}^+$ channel blocker TTX. This finding suggested that the $\text{Na}^+/\text{Ca}^{2+}$ exchanger might be involved

**FIG. 29.** Does $\text{Na}^+/\text{Ca}^{2+}$ exchanger directly activate excitation-contraction coupling in heart? Top panel shows that a $[\text{Ca}^{2+}]_i$ transient can be measured in guinea pig cardiac muscle cells when there is an $I_{\text{Na}}$ but no $I_{\text{Ca}}$ (A). $[\text{Ca}^{2+}]_i$ transient is abolished when inward current is carried by Li$^+$. [From Hume et al. (437).]

**FIG. 30.** Does $\text{Na}^+/\text{Ca}^{2+}$ exchanger directly activate excitation-contraction coupling in heart? Guinea pig ventricular myocytes were incubated at 35°C with 10 $\mu$M verapamil to block $I_{\text{Ca}}$, and with the SR loaded with $\text{Ca}^{2+}$. A: from a conditioning prepulse of $-40$ mV (to inactivate $I_{\text{Na}}$), depolarization to $+5$ mV induced a small inward current (middle) and small $\text{Ca}^{2+}$ transient (bottom). B: activation of $I_{\text{Na}}$ (recovered during a 50-ms prepulse to $-90$ mV) by depolarization to $+5$ mV. The $I_{\text{Na}}$ (middle) now induced a $\text{Ca}^{2+}$ transient (bottom) that was no greater than with $I_{\text{Na}}$ inactivated (in A). These data imply that SR $\text{Ca}^{2+}$ release is activated primarily by $I_{\text{Ca}}$ and that stimulation of $\text{Na}^+/\text{Ca}^{2+}$ exchanger-mediated $\text{Ca}^{2+}$ entry plays little role in mobilization of SR $\text{Ca}^{2+}$. [From Evans and Cannell (300).]
in triggering CICR by bringing Ca\(^{2+}\) into the cell in a voltage-dependent manner as a result of the [Na\(^{+}\)]\(_i\) elevation that resulted from the Na\(^{+}\) influx via \(I_{Na}\). Kinetic considerations (661) suggested, however, that for the Na\(^{+}/Ca\(^{2+}\) exchanger to work in this manner, [Na\(^{+}\)]\(_i\) would have to be elevated locally (437, 458, 564, 565; and see Ref. 974).

The findings of Leblanc and Hume (561) have been supported by a number of other groups carrying out experiments on guinea pig heart (528, 561, 577, 592). Furthermore, there is some direct evidence for microheterogeneity of subplasmalemmal [Na\(^{+}\)]\(_i\) (974). On the other hand, the results of experiments on rat heart indicate that the Na\(^{+}/Ca\(^{2+}\) exchanger does not significantly activate normal excitation-contraction coupling in this species (563, 810, 843). Thus it is possible that the mechanisms involved in triggering SR Ca\(^{2+}\) release in different species may be quantitatively, if not qualitatively, different so that all of these observations and interpretations may be incorrect. It has been suggested, however, that the aforementioned experiments in guinea pig are flawed by poor voltage control (149, 300, 842). Indeed, recent experiments in guinea pig imply that there is little or no Na\(^{+}/Ca\(^{2+}\) exchanger-activated CICR (Fig. 30) (149, 300, 367). It is apparent that the kinetic properties of the Na\(^{+}/Ca\(^{2+}\) exchanger on which the resolution of this issue turns are poorly understood. The low [Ca\(^{2+}\)]\(_i\) at the time depolarization is initiated might not favor a triggering role for the Na\(^{+}/Ca\(^{2+}\) exchanger because the catalytic activation of the exchanger by intracellular Ca\(^{2+}\) is slow (see Fig. 14). Nevertheless, if there is any change in local [Ca\(^{2+}\)]\(_i\), the coupling between triggering Ca\(^{2+}\) influx and SR Ca\(^{2+}\) release will be changed. This would come about because the local resting [Ca\(^{2+}\)] or the “[Ca\(^{2+}\)] pedestal” is altered transiently.

C) Reciprocity of [Ca\(^{2+}\)]\(_i\) pedestal. Local [Ca\(^{2+}\)] may influence excitation-contraction coupling by making it easier or more likely that a subthreshold trigger can release Ca\(^{2+}\) from the SR (812). Although the opening of a single L-type Ca\(^{2+}\) channel may be sufficient to trigger the opening of ryanodine receptors to produce a Ca\(^{2+}\) spark, at more positive potentials, a larger number of L-type Ca\(^{2+}\) channels may need to open. Each of the channel openings may contribute Ca\(^{2+}\) to elevate local [Ca\(^{2+}\)] but only one opening may be the “trigger.” In a similar manner, the Na\(^{+}/Ca\(^{2+}\) exchanger could contribute Ca\(^{2+}\) to elevate local [Ca\(^{2+}\)] so that the opening of an L-type Ca\(^{2+}\) channel that otherwise would not have triggered a Ca\(^{2+}\) spark now can (601). Thus the Na\(^{+}/Ca\(^{2+}\) exchanger can elevate the [Ca\(^{2+}\)] pedestal, at least locally. There is no reason why Ca\(^{2+}\) flux through Na\(^{+}\) channels, which has been reported to trigger Ca\(^{2+}\) sparks and SR Ca\(^{2+}\) release directly (813), would not also be more effective at triggering SR Ca\(^{2+}\) release if the Na\(^{+}/Ca\(^{2+}\) exchanger first increased the [Ca\(^{2+}\)] pedestal. T-type Ca\(^{2+}\) channels could also more readily trigger Ca\(^{2+}\) sparks and SR Ca\(^{2+}\) release (858) if the Na\(^{+}/Ca\(^{2+}\) exchanger first increased the [Ca\(^{2+}\)] pedestal. By reciprocity, it could have been the Ca\(^{2+}\) influx through L-type or T-type Ca\(^{2+}\) channels (or even through Na\(^{+}\) channels; Ref. 813) that was responsible for elevating the [Ca\(^{2+}\)] pedestal, and it could have been the Na\(^{+}/Ca\(^{2+}\) exchanger that was the “proximate cause” or trigger. In any case the “credit” for triggering excitation-contraction coupling could be shared.

Hilgemann’s giant patch-clamp method has made investigations of the Na\(^{+}/Ca\(^{2+}\) exchanger kinetics possible with greater resolution than ever before (405, 406). This method has been used to examine the roles of Na\(^{+}\), Ca\(^{2+}\), and ATP (406, 409–412, 414, 471, 632, 635, 636, 905) and protons (266, 267). As discussed in section \(\mu E\), the complexities of modulation of the kinetics by monovalent and divalent cations are only now beginning to be appreciated. In any case, resolution of the question of whether the exchanger plays an important role in triggering SR Ca\(^{2+}\) release will undoubtedly depend on 1) careful examination of the kinetics of the Na\(^{+}/Ca\(^{2+}\) exchanger isoforms (see sect. \(\nu E\)), 2) the abundance and localization of the exchanger proteins in different tissues, and 3) their modulation by diverse ions, proteins, and other factors. The most recent investigations provide important new data but do not resolve the issue.

Sipido et al. (858) and Cannell and co-workers (300, 367) place limits on the fractional activation of CICR by the Na\(^{+}/Ca\(^{2+}\) exchanger in guinea pig to (our estimate) <10%. In contrast, recently Levi’s and Wressner’s groups (383, 590, 961, 962, 969) have suggested that the use of Cs\(^{+}\) (instead of K\(^{+}\)) and room temperature (instead of core body temperature) may distort the findings. Using guinea pig and cat cardiac myocytes, respectively, they reported that Na\(^{+}/Ca\(^{2+}\) exchanger activation of CICR is inhibited by Cs\(^{+}\) and by a (room) temperature substantially below body temperature. In view of the physiological importance of excitation-contraction coupling in cardiac muscle, these findings clearly merit more extensive investigation. Interpretation of these findings is complicated by observations in two areas. First, Ferrier’s group (427, 428) and Levi’s (660) groups have reported that SR Ca\(^{2+}\) release may be controlled directly by PM depolarization. A necessary precondition for these results is either the activation of PKA or cells that are not dialyzed by a patch-clamp pipette. Thus some of the experimental conditions for these experiments are similar to those needed to investigate a role of the Na\(^{+}/Ca\(^{2+}\) exchanger to trigger SR Ca\(^{2+}\) release. This ambiguity will need to be resolved in future experiments. Second, under conditions of either PKA activation or exposure to CTS, Ca\(^{2+}\) may permeate Na\(^{+}\) channels (813). This
result also indicates that there are experimental complexities that need to be resolved.

4. Role in cardiac arrhythmias

Ideas about the role of the Na+/Ca2+ exchanger in the genesis of cardiac arrhythmias began with the first speculations by Glynn (353–355) and by Baker et al. (35) about how the Na+ pump works and how CTS act (35, 144, 343–345, 355). Baker et al. (35) and Langer (552) suggested that the positive inotropic action of the CTS depended on two factors, the inhibition of the Na+ pump and the presence of the Na+/Ca2+ exchanger (35, 552, 553, 555). As noted above, the tendency of the SR to amplify the effect of any change in [Ca2+]i by significantly changing SR Ca2+ stores provides a large gain in signal (95, 291–293, 955). It is this same mechanism, however, that can increase “gain” so much that the SR becomes spontaneously active. This condition, known as SR Ca2+ overload (77, 477), depends on the Na+/Ca2+ exchanger-mediated increase in Ca2+ entry and/or decrease in Ca2+ exit, as a result of an elevated [Na+]i.

When the SR spontaneously releases its Ca2+, the release is a local, subcellular event (147, 173, 175, 205). This local release can trigger a global response that involves propagating waves of elevated [Ca2+]i. The spontaneous SR Ca2+ release is associated with a novel arrhythmogenic transient inward current (IT) (477, 567) that is dependent on Ca2+-activated, nonselective channels (150, 286, 511, 691) and on the Na+/Ca2+ exchanger (188–193, 195, 188, 591, 593, 594, 691, 734). These inward currents are responsible for depolarizing diverse cells, including cardiomyocytes and cardiac Purkinje fiber cells, which can then produce extrasystoles (137, 212, 222, 305, 306, 477, 524, 567, 789, 790, 986). The IT contributes to the pacemaker current to early afterdepolarizations (EAD) and delayed afterdepolarizations (DAD) that underlie diverse triggered arrhythmias. The EAD and DAD may also be involved in peri-infarction cardiac arrhythmias and in diverse, but less common, arrhythmias such as those associated with the long QT syndrome (280, 476, 740, 810, 811, 875, 999, 1010). The consistent finding is that with the prolonged depolarization of the long QT syndrome, more Ca2+ enters via Ca2+ channels and less is extruded by the Na+/Ca2+ exchanger. Together these processes lead to increased Ca2+ in the SR, which leads to Ca2+ overload and instability of SR Ca2+ release. The resultant Ca2+ sparks and Ca2+ waves lead to elevated [Ca2+]i, which can activate both Ca2+-activated channels and the Na+/Ca2+ exchanger itself. In both cases, the resulting inward current produces extrasystoles and arrhythmias.

The Na+/Ca2+ exchanger therefore can play two crucial roles in cardiac arrhythmogenesis. The first is that the exchanger can significantly influence SR Ca2+ load and therefore contribute to the production of SR Ca2+ overload. The second role that the Na+/Ca2+ exchanger can play is in the inward (depolarizing) current that it can generate when it is activated (along with Ca2+-activated nonselective cation channels) by elevations of [Ca2+]i. With a normal Ca2+ load, this inward current tends to prolong the plateau phase of the cardiac action potential. This action promotes EAD directly and may augment DAD through an increasing Ca2+ load (secondary to the prolonged depolarization) to produce a transient Ca2+ overload. Under conditions of sustained Ca2+ overload, when SR Ca2+ release may occur spontaneously, the Ca2+-activated Na+/Ca2+ exchanger can contribute inward current that depolarizes the cells to produce extrasystoles directly via EAD or DAD.

5. Developmental changes and changes associated with pathology

To estimate the changes in Na+/Ca2+ exchanger availability under different conditions, mRNA levels have been used. Although this is clearly the easiest method to use, it is generally recognized to be faulty. It assumes that the mRNA level is proportional to the level of functional Na+/Ca2+ exchanger protein. This would be true only if the lifetime of the protein is constant under the conditions compared, if the degradation rate of the Na+/Ca2+ exchanger mRNA is unchanging, if the efficacy of translation is constant, and if the function of the Na+/Ca2+ exchanger protein is equivalent. These weaknesses notwithstanding, it has been shown that the mRNA level in cardiac myocytes peaks in the late embryonic period. No distinctive variations in mRNA level have been reported under other developmental conditions or during disease states except in congestive heart failure, where the mRNA level of the Na+/Ca2+ exchanger is elevated (310, 311, 389, 390, 500, 892, 1001).

A particularly interesting observation is the reciprocal relationship between the level of ouabain-sensitive (α2) Na+ pump (catalytic subunit) protein and the level of the Na+/Ca2+ exchanger protein under several physiological and pathophysiological conditions. McDonough and colleagues (617) have observed that the α2 levels are increased and Na+/Ca2+ exchanger levels are reduced in rat heart during preweanling development and during the transition from a hypothyroid to a hyperthyroid state. [However, Hojo et al. (421) report that thyroid hormone increases Na+/Ca2+ exchanger expression in 48 h, whereas McDonough and colleagues (617) studied the expression after 8 days of hormone treatment.] Conversely, the α2 levels are decreased, and the exchanger protein levels are increased during the development of renovascular hypertension and during treatment with the antiarrhythmic agent amiodarone (617). Also, in guinea pig heart, the levels of α2-mRNA and protein are increased, and Na+/Ca2+ exchanger mRNA is decreased in...
the hypertension induced by aldosterone and salt (749a). During all of these transitions, the level of the ouabain-insensitive (α1) Na⁺ pump levels are but little changed. In view of the apparent tight coupling between the Na⁺ pump and the Na⁺/Ca²⁺ exchanger in the heart (328, 618, 895a), and the observed colocalization of α₁β₁γNa⁺ pumps and the exchanger in the PM microdomains of several other cell types (461, 462; and see Refs. 317, 318 and sect. viA), these findings may have relevance to the mechanism of action of CTS (see sect. viA7).

Although these early reports encourage more work to be done, many questions are raised by these findings. 1) Is the Na⁺/Ca²⁺ exchanger cardiac isoform unchanged in development and in response to pathology? 2) Is there any variation in the sarcolemmal Ca²⁺-ATPase? 3) Does the protein or mRNA degradation rate change? 4) Are there alterations in the factors that modulate the Na⁺/Ca²⁺ exchanger such as phosphorylation, pH, and temperature? 5) Does cellular localization of the protein change? Investigations now in progress should begin to answer these questions.

6. Reperfusion injury

Much has been written about ischemia, reperfusion injury, and the “calcium paradox.” Many authors have invoked Na⁺/Ca²⁺ exchange-mediated Ca²⁺ entry to help explain the resulting rise in [Ca²⁺]ᵢ when the heart is reperfused after a period of hypoxia or a period in which perfusion is halted (18, 166, 167, 210, 502, 619, 683, 774, 908, 916, 944, 946). Unfortunately, the situation is very complex, and many alterations, including changes in ATP levels, pHᵢ, intracellular ion concentrations, ion conductances, and membrane potential, may all contribute to the reperfusion-induced rise in [Ca²⁺]ᵢ (18, 952). Thorough discussion of reperfusion injury is therefore beyond the scope of the present review.

Nevertheless, one clinically relevant situation seems worthy of comment in the present context. Jynge and colleagues (468–470) have noted that bolus infusions of contrast media during coronary angiography may be expected to induce rapid ionic and osmolar shifts in the vascular, interstitial, and intracellular compartments. These shifts may be responsible for the rare side effects of coronary angiography such as cardiac arrhythmias and coronary spasms. Jynge and colleagues raise the possibility that contrast media with a low Na⁺ concentration and/or “Na⁺/Ca²⁺ imbalance” may acutely alter Ca²⁺ distribution in the coronary arteries and the heart and may therefore contribute to the induction of the aforementioned side effects. These authors suggest that careful attention to the composition of contrast media, especially to the ionic composition and osmolality, may help to minimize these side effects.

7. Mechanism of action of CTS

Cardiotonic steroids were employed therapeutically as diuretic agents by the Chinese and Japanese for centuries, and by the ancient Egyptians, Greeks and Romans (95) long before Withering’s carefully documented case studies published in his seminal Account of the Foxglove in 1785 (987). Nevertheless, use of these agents had greatly declined in Europe before Withering’s report (991). During the past two centuries, however, CTS have been a mainstay in the physician’s armamentarium for the therapy of congestive heart failure (487), and a recent, large-scale study has documented their efficacy in relieving the symptoms of heart failure (703a, 921a).

The chemistry of CTS was explored extensively in the 19th century (95, 991); however, major advances in understanding the mode of action of these agents have been made primarily in the past 60 years (576, 991). Milestones in this story include Cattell and Gold’s (164) observation that CTS increase the force of contraction of cardiac muscle and Wood and Moe’s (989) finding that this was associated with a loss of intracellular K⁺ concentration ([K⁺]ᵢ). In 1953, Schatzmann (821) discovered that CTS were selective and potent inhibitors of the PM Na⁺ pump, and in 1960, Skou (860) showed that these agents specifically inhibited the PM Na⁺-K⁺-ATPase. Some investigators have maintained skepticism that this inhibitory effect is central to the cardiotonic action because low (nanomolar) doses of CTS sometimes stimulate the Na⁺ pump slightly (38, 295), even though higher doses invariably inhibit the Na⁺ pump (576, 991). Nevertheless, it is noteworthy that reduction of [K⁺]ᵢ, a normal “substrate” for the Na⁺ pump, induces cardiotonic and toxic effects similar to those evoked by the CTS (576). Although reduced [K⁺]ᵢ also affects E₉ and V₉, the data support the view that Na⁺ pump inhibition (with either CTS or reduced [K⁺]ᵢ) is responsible for the positive inotropic effect.

Numerous early studies had implicated cell Ca²⁺ in the cardiotonic effect (90, 576, 775, 991), and these ideas were greatly strengthened when it became clear that contraction was triggered by a rise in cytosolic Ca²⁺. Indeed, Repke (775) postulated that Na⁺ pump inhibition might somehow increase the delivery of Ca²⁺ to the contraction machinery in cardiac muscle. Then, with the discovery of the PM Na⁺/Ca²⁺ exchanger (35, 95, 783), this transport system appeared to provide the missing link between Na⁺ pump inhibition and the cardiotonic effect (35, 90, 555, 576, 991). According to this currently popular view (878), CTS partially inhibit the Na⁺ pump, thereby raising [Na⁺]. Then, as a result of Na⁺/Ca²⁺ exchanger activity, [Ca²⁺], also rises slightly. Most of the intracellular Ca²⁺ is sequestered in ER or (in muscle) SR, where much of it is buffered; the concentration gradient between the ER or SR free Ca²⁺ and cytosolic Ca²⁺, [Ca²⁺]ᵢ, is
FIG. 31. Diagram of a PLasmERosome region. A: relationship between PM [unlabeled dark horizontal thick line above extracellular fluid (ECF)] and “restricted” cytosolic space (12–15 nm high × 100–200 nm diameter in vascular smooth muscle (231, 882, 884) and junctional SR/junction endoplasmic reticulum. B: enlarged region of PLasmERosome and adjacent PM, showing key Na\(^+\) and Ca\(^{2+}\) transporters present in these regions. [From Blaustein et al. (103).]

>1,000:1. Thus a small rise in resting [Ca\(^{2+}\)]\(_{i}\) will be reflected by a substantial rise in SR/ER Ca\(^{2+}\) (i.e., the “amplification effect” or “gain” mentioned in sect. vi, A3\(_a\) and A4). The additional stores of Ca\(^{2+}\) can then be mobilized and can cause augmented responses (e.g., enhanced cardiac contraction) whenever the cells are activated (90, 95, 576, 991) (and see sect. viA3\(_a\)).

The central role of the Na\(^+\)/Ca\(^{2+}\) exchanger in the positive inotropic response associated with increased delivery of Na\(^+\) may be illustrated by the properties of cardiomyocytes from transgenic mice with overexpressed exchanger (54). In the electrically driven cardiomyocytes from these animals, the sodium channel agonist BDF-9148 augmented systolic tension to a much greater extent than it did in cardiomyocytes from normal mice. No such effect was seen with isoprenaline, which acts by a different mechanism. The results from the transgenic mice seem more comparable to the greater cardiotoxic effect of BDF-9148 in the failing human myocardium, which exhibits increased expression of the Na\(^+\)/Ca\(^{2+}\) exchanger, than to normal human myocardium (311).

The key features of the aforementioned sequence of events have all been experimentally verified, i.e., CTS cause SR/ER Ca\(^{2+}\) stores to increase (95, 103, 115, 977). Nevertheless, this theory poses a dilemma. It has been difficult to understand how very low (nanomolar) concentrations of CTS can induce large cardiotonic (and vasotonic; Refs. 733, 973) effects if the steroids block only the high ouabain affinity (IC\(_{50}\) values <100 nM) isoforms, \(\alpha_2\) and \(\alpha_3\), which also have relatively low affinity for [Na\(^+\)]\(_{i}\) (\(K_d = 24–33\) mM; Ref. 1006), are confined to PM domains that overlie neighboring (junctional) ER or SR (jER or jSR) (462). Thus these isoforms (\(\alpha_2\) or \(\alpha_3\)) appear to be clustered in close proximity to the Na\(^+\)/Ca\(^{2+}\) exchanger molecules that are also located in PM microdomains adjacent to the jSR or jER (460). This functional unit, which consists of the PM domain containing the high ouabain affinity Na\(^+\) pump isoforms and the Na\(^+\)-Ca\(^{2+}\) exchanger, the adjacent jSR or jER, and the intervening “restricted” cytoplasmic space, has been named the “PLasmERosome” (103, 461). This complex (Fig. 31) appears to be identical to the jSR regions in cardiac and skeletal muscle where surface couplings occur (319); similar surface couplings between jER and junctional PM domains (jPM) have been observed in neurons (401). In contrast, the low ouabain affinity (IC\(_{50}\) >1,000 nM in the rat) and high [Na\(^+\)]\(_{i}\), affinity (\(K_d = 12\) mM) Na\(^+\) pump isoform \(\alpha_4\) appears to be distributed more uniformly in the PM (776) but perhaps excluded from these microdomains; this may be the “housekeeping” isoform that serves to maintain a low [Na\(^+\)]\(_{i}\) in the “bulk” cytoplasm.

The morphology of the PM-jSR (PLasmERosome) regions has been elucidated in skeletal and cardiac muscles, where SR terminal cisternae form diads and triads with t tubules (319); the triads consist of pairs of terminal cisternae, one on each side of the t tubule cross-section. In smooth muscles, single elements of the jSR form only “diads” with the jPM (232, 883, 884) because there are no t tubules; a similar diadic arrangement occurs in other cell types as well (e.g., neurons; Ref. 401). In these regions, the 10- to 15-nm clefts that separate the jPM from the jSR contain a very thin layer of cytoplasm that is traversed by numerous electron-dense “foot” processes (identified as...
ryanodine receptors in skeletal and cardiac muscle) running between the two membranes (319). The high density of negatively charged phospholipid head groups in both the PM and SR membranes may be expected to promote locally increased concentrations of cations acting as counterions. Without detailed information about local topology, membrane constituents, and other cellular factors, we cannot estimate either the local surface charge profile or the local concentrations of cations. Nevertheless, it seems possible (if not probable) that the structure and properties of the aqueous phase in this tiny restricted cytosolic volume (<$10^{-18}$ l) may differ from those in bulk cytosol (974). It is conceivable, therefore, that the phospholipid negative charges will have an effect on the local cation concentrations, and this may affect the ion channels and transporters in the PlasmERosome. Several groups have drawn attention to either the special properties or the dynamic characteristics including restricted diffusion of the constituents within these tiny subplasmalemmal regions of cytoplasm (i.e., fuzzy space) in the cardiac diadic junctions (148, 151, 328, 554, 564, 565, 618, 692, 812, 874, 879, 895a, 974). Its implications for the cardiac diadic junctions (148, 151, 328, 554, 564, 565, 618, 692, 812, 874, 879, 895a, 974). Its implications for the action of CTS have, however, only recently been considered (103, 461, 462).

To the extent that local [Na$^+$]$_i$ (within the PlasmERosome) may be elevated (974), we can speculate that the $\alpha_2$ and $\alpha_3$-isoforms of the Na$^+$ pump may have a special function, at least in some tissues (including the heart; see Ref. 450a). The $\alpha_2$ and $\alpha_3$-isoforms may regulate not only [Na$^+$]$_i$ in the restricted (PlasmERosome) region of the cytoplasm, but also, indirectly, [Ca$^{2+}$]$_i$ in this region (via Na$^+$/Ca$^{2+}$ exchange) and, thus, the Ca$^{2+}$ stores in the jSR/jER. In this way, the $\alpha_2$ and $\alpha_3$-isoforms of the Na$^+$ pump may help to regulate cell responsiveness. This hypothesis may provide an explanation for the cardiotonic effects of low-dose CTS (103). This also indicates how an endogenous ouabainlike compound (364, 381, 628, 1009), an adrenal cortical hormone (556, 557), could function physiologically when it acts only on the $\alpha_2$ and $\alpha_3$-isoforms of the Na$^+$ pump. The problem now is to determine experimentally whether restricted subplasmalemmal cytosolic regions, with the properties described here, actually do exist in cells (328, 618, 895a, 974).

As noted above, there is an additional way in which the restricted junctional spaces could affect excitation-contraction coupling in heart. The Na$^+$/Ca$^{2+}$ exchanger can contribute to the local [Ca$^{2+}$]$_i$ level, and if there is additional trigger Ca$^{2+}$ entry, then it is possible for the Na$^+$/Ca$^{2+}$ exchanger to bias the triggering. If CTS also activate Ca$^{2+}$ flux through TTX-sensitive Na$^+$ channels (813), this, too, might contribute to the influence of the restricted junctional spaces. Santana et al. (813) postulated that this was due to a protein-protein interaction between the Na$^+$ pumps and the TTX-sensitive Na$^+$ channels. This novel hypothesis will, of course, have to await replication. The hypothesis cannot, however, explain the effects of low-dose CTS on other tissues, for example, the vasotonic effect on arterial myocytes (103) that apparently do not express voltage-gated Na$^+$ channels (1005). Moreover, left ventricular performance is enhanced in rats with genetically reduced expression of $\alpha_2$ (but not $\alpha_1$) Na$^+$ pumps (450a), in accord with the model of Figure 31.

B. Smooth Muscle

The contraction of smooth muscles is normally initiated by a rise in [Ca$^{2+}$]$_i$, as in all other types of muscle. This trigger Ca$^{2+}$ may enter the cytosol either via voltage-gated and/or agonist receptor-operated Ca$^{2+}$ channels in the PM, or via mobilization of Ca$^{2+}$ from the SR stores. Indeed, the mobilization of stored Ca$^{2+}$ is an important aspect of the function of many smooth muscle agonists. It is therefore important to understand the factors and mechanisms that control Ca$^{2+}$ homeostasis in smooth muscles, including those that, as in cardiac muscle (see sect. viA) modulate the Ca$^{2+}$ content of the SR stores. Nonetheless, it is also important to remain cognizant of the fact that tonic smooth muscles can maintain Ca$^{2+}$-dependent tension for very long periods of time. Thus the PM mechanisms may be responsible for the prolonged maintenance of suprathereshold [Ca$^{2+}$]$_i$ in these tissues.

1. Vascular smooth muscle

Direct functional evidence for Na$^+$/Ca$^{2+}$ exchange in vascular smooth muscle was first described 25 years ago (780). Beginning with that report, numerous studies on vascular smooth muscle and other types of smooth muscles have demonstrated that reducing the PM Na$^+$ gradient promotes Ca$^{2+}$ entry and/or decreases Ca$^{2+}$ exit, raises [Ca$^{2+}$]$_i$, augments agonist-evoked contractions, and even induces tonic contractions in unstimulated smooth muscles (14, 111, 499, 664, 741, 815, 870, 990, 993; for reviews, see Refs. 91, 94, 844, 845). Furthermore, Ca$^{2+}$ extrusion is inhibited, and evoked Ca$^{2+}$ transients are prolonged, as are evoked contractions, when [Na$^+$]$_o$ is markedly reduced or the Na$^+$ pump is inhibited (2, 27, 74, 118, 123, 127, 589, 595, 664, 703, 716, 862, 871, 960). Nevertheless, the presence and physiological role of Na$^+$/Ca$^{2+}$ exchange in vascular smooth muscle was challenged by a number of investigators (124, 162, 671, 672, 716, 881). Many of these negative views could be explained by the particular experimental paradigms these investigators used to test for Na$^+$/Ca$^{2+}$ exchange, and by the types of questions asked (94, 100, 845). For example, the fact that reduction of [Na$^+$]$_o$ or elevation of [Na$^+$]$_i$, often does not significantly elevate [Ca$^{2+}$]$_i$ or induce contractions is not evidence that the Na$^+$/Ca$^{2+}$ exchanger is not present, or is even “latent” (872), or nonfunctional.
These results ignore the fact that the SR sequestration mechanism serves as a powerful and efficient Ca$^{2+}$ buffer; SR Ca$^{2+}$ content and evoked contractions are substantially augmented by these manipulations despite the minimal rise in resting (unstimulated) [Ca$^{2+}$]. (27, 100, 115, 123). Nevertheless, it is important to note that Na$^+$ pump inhibition or other manipulations that reduce $\Delta \mu_{\text{Na}}$ are likely to induce contractions by promoting neurotransmitter release from nerve terminals (or varicosities) in the vessel wall (1).

The controversy was subsequently addressed by the direct demonstration that the Na$^+$/Ca$^{2+}$ exchanger RNA and protein are expressed in primary cultured vascular smooth muscle cells (96, 460); this smooth muscle exchanger was also cloned (676). Immunocytochemical studies then revealed that the exchanger in smooth muscles appears to be restricted primarily to PM regions that are adjacent to junctional SR (96, 460, 663). In contrast, the PMCA pump is distributed much more uniformly over the surface of the vascular smooth muscle cells, and therefore presumably has a different function (461). This localization of the Na$^+$/Ca$^{2+}$ exchanger may imply that a major role of the exchanger in smooth muscles is to modulate, indirectly, the Ca$^{2+}$ content of the SR stores (95, 123, 640) and thereby influence Ca$^{2+}$ signaling and tension development. Because Na$^+$ pump inhibition tends to drive Ca$^{2+}$ into the cells (and stores) via Na$^+/$/Ca$^{2+}$ exchange (see sect. viA7), the localization of different Na$^+$ pump isoforms is worth mentioning in the present context. Most cells express both low ouabain affinity ($\alpha_1$) and high ouabain affinity ($\alpha_2$ and/or $\alpha_3$) isoforms of the Na$^+$ pump catalytic ($\alpha$) subunit, but only the latter ($\alpha_2$ and/or $\alpha_3$) isoforms are localized to PM overlying junctional SR in smooth muscle and some other cells (461, 663). The colocalization of the Na$^+$ pump $\alpha_2$-isoform and the Na$^+$/Ca$^{2+}$ exchanger is particularly noteworthy. This finding suggests that modulation of the local Na$^+$ gradient, either as a result of cell activity, or the action of an EOLC (381) which, at low (nanomolar) plasma levels, would be expected to inhibit only the Na$^+$ pump $\alpha_2$- and $\alpha_3$-isoforms, may thereby modulate SR Ca$^{2+}$ store content (103).

Although the precise role(s) of the Na$^+$/Ca$^{2+}$ exchanger in vascular smooth muscle is unresolved, extrusion of Ca$^{2+}$ via the exchanger dominates over extrusion via the PMCA pump, at least under some circumstances. This is illustrated by data from arteries that are constricted in K$^+$-rich media, in which the SR Ca$^{2+}$ stores are presumably overloaded (27) (also see Ref. 979). Under these circumstances, relaxation is markedly slowed by removing extracellular Na$^+$, and greatly accelerated by adding back the Na$^+$.

Recent data involving the application of AS-oligos targeted to the NCX1 mRNA in primary cultured arterial myocytes (865, 867) are consistent with this view. The results indicate that Na$^+$/Ca$^{2+}$ exchanger knockdown prolongs agonist responses and delays the return of [Ca$^{2+}$]$_{i}$ to the original (resting) level when the arterial myocytes are repetitively activated at short intervals (e.g., every 3 min).

2. Other (nonvascular) smooth muscles

Functional evidence for Na$^+$/Ca$^{2+}$ exchange has also been obtained in a variety of other (nonvascular) smooth muscles, including tracheal/bronchial (941, 942), intestinal (424, 640, 745, 823, 824), uterine (614, 637, 927), and urinary tract (ureter and vas deferens) (8) smooth muscles. For other relevant references, the reader should consult a review of this subject (94). The precise physiological roles of the exchanger in these tissues are unknown. Nevertheless, it seems obvious that the exchanger must influence Ca$^{2+}$ homeostasis and, thus, contractile activation. For example, the exchanger may contribute to the delivery of Ca$^{2+}$ to the contractile apparatus of uterine smooth muscle during the delivery of the fetus. Indeed, the early uterine contractions and premature delivery in women with preeclampsia might be the result of excessive levels of the EOLC (see sect. viA7) that would be expected to augment SR Ca$^{2+}$ stores in this smooth muscle (95).

3. Pathophysiology of smooth muscles: the role of Na$^+$/Ca$^{2+}$ exchange

A) HYPERTENSION. The initial report of Na$^+$/Ca$^{2+}$ exchange activity in vascular smooth muscle (780) was followed by suggestions that this transport system might be important for the regulation of blood pressure and might thereby play a role in the pathogenesis of (salt-dependent) essential hypertension (86, 87). The hypothesis put forth was that salt retention and plasma volume expansion augment secretion of a natriuretic hormone that is also hypertensinogenic; this hypothetical hormone appeared to have the properties of a ouabainlike substance (87, 233, 374). The subsequent discovery of such a substance (EOLC, ouabain itself, or a closely related isomer) in the blood plasma of humans (381, 382) and other mammals (913, 1009), the finding of elevated levels in human subjects (382, 791) and other mammals (913) with hypertension, the demonstration that chronic low-dose ouabain administration induces hypertension in normal rats (542, 621, 1004), and the identification of the adrenal cortical glomerulosa cells as a major source of this hormone (556, 557), have all lent credence to this hypothesis. The large literature on this subject is beyond the scope of the present review; interested readers should consult some of the recent reviews on this subject (95, 365, 382, 583).

B) BRONCHOSPASM AND ASTHMA. The fact that the Na$^+$/Ca$^{2+}$ exchanger is well expressed in tracheal/bronchial smooth muscles (143, 178, 482, 864) suggests that the
exchanger may play a role in the control of bronchomotor tone (178). Cardiotonic steroids increase pulmonary airway resistance in vivo by a direct effect on the bronchial smooth muscle (623), although an additional effect, due to their action on neurons, has not been excluded. Indeed, there is now evidence that a circulating factor, perhaps EOLC, may contribute to the bronchospasm of asthma (934).

C. Vertebrate Skeletal Muscle

There are two general types of vertebrate skeletal muscle fibers, namely, those that, when activated, respond with a phasic contraction (twitch) and those that respond with a tonic contraction (e.g., the rectus abdominis). Moreover, the phasic fibers can be subdivided into two types, those that respond with a fast twitch (e.g., the extensor digitorum longus) and those that give a slower twitch (e.g., the psoas and soleus muscles). A rise in $[Ca^{2+}]_{i}$ is the immediate trigger for contraction in all of these types of muscle fibers. There are important differences in the morphology of the SR in these fiber types. The t tubules and SR are well developed in the fast-twitch fibers, but the SR is relatively sparse in the tonic fibers (711). Thus it seems that differences in $Ca^{2+}$ metabolism are a critical factor in the differences in excitation-contraction coupling in these various fiber types. In fast-twitch fibers, almost all of the activator $Ca^{2+}$ is mobilized from the SR during a twitch and is resequestered in the SR during relaxation (22a). In contrast, most of the activator $Ca^{2+}$ in tonic muscle fibers must come from the extracellular fluid. Consequently, there must be a large traffic of $Ca^{2+}$ across the PM in tonic muscle fibers during activation and relaxation, whereas there is only a small flux of $Ca^{2+}$ in fast-twitch muscle fibers (217).

The $Na^{+}/Ca^{2+}$ exchanger has been identified, functionally, in the PM of all three types of vertebrate skeletal muscle fibers, on the basis of contraction experiments and $Ca^{2+}$ flux studies, for example, in tonic fibers (818, 435) and in fast-twitch (163, 216, 218, 340, 429, 484, 758) and slow-twitch (154, 163, 207, 360, 434, 483, 574, 698, 1007, 1008) fibers. Studies on isolated sarcolemmal vesicles indicate that there is a $Na^{+}$-dependent $Ca^{2+}$ flux in these preparations (273, 347, 652), and Donoso and Hidalgo (273) showed, in studies on membrane vesicles, that the exchanger in frog skeletal muscle is located primarily in the t tubules (802). The latter observation now needs to be verified in immunocytochemical studies.

There is also one electrophysiological study of the exchanger in skeletal muscle. Gonzales-Serratos et al. (360) patch-clamped membrane blebs from mouse psoas fibers. The data indicate that there is a readily measurable $Na^{+}$- and $Ca^{2+}$-dependent current in these giant membrane patches that is blocked by Ni$^{2+}$ and by 3,4-

dichlorobenzamil. As in other preparations, the normal spontaneous inactivation of this current is abolished by $\alpha$-chymotrypsin. The magnitude of the current density is $\sim$10- to 20-fold smaller than in cardiac muscle giant patches (405) (Fig. 14); this implies that the role of the exchanger in skeletal muscle is probably different from that in cardiac muscle.

Indeed, the physiological role of the $Na^{+}/Ca^{2+}$ exchanger is likely to be different in the different types of skeletal muscle fibers. For example, removal of external $Na^{+}$ induces contractions in slow-twitch but not in fast-twitch muscle fibers (574); this might imply that the exchanger is more prevalent in the slow-twitch fibers. While comparative physiological and biochemical studies might yield clues to physiological function, this has not been systematically investigated in the different fiber types. We speculate that the exchanger may be involved in $Ca^{2+}$ extrusion in tonic fibers and, perhaps, in slow-twitch fibers, but this is not likely to be the case in fast-twitch fibers. By virtue of its location in phasic muscle fiber t-tubule membranes (273), perhaps the exchanger plays a role in the long-term regulation of $Ca^{2+}$ store content and, thus, in the modulation of contractile tension.

D. Invertebrate Skeletal Muscle

The depolarizing current in invertebrate “skeletal” muscle is carried by $Ca^{2+}$, and not by $Na^{+}$ (375, 927). In addition, however, and unlike tonic vertebrate skeletal muscle, invertebrate muscle also has an extensive SR network. However, invertebrate muscle does not have a t-tubule system; instead, it has deep branching clefts and invaginations (430) so that most of the myoplasm is not far from sarcolemma. It is not clear whether most of the activator $Ca^{2+}$ for these cells comes from the extracellular fluid or whether the SR is a source of activator $Ca^{2+}$ as well as a sink for $Ca^{2+}$ during relaxation. A number of tracer flux studies (28, 29, 100, 754, 757, 800), contraction experiments (86, 100, 615, 896), and membrane vesicle studies (798, 799) have shown that the exchanger is prevalent in the PM of invertebrate muscle cells and that it has properties similar to those of the vertebrate (cardiac) exchanger. A further difference between invertebrate and vertebrate skeletal muscles, however, is the high level of $Na^{+}/Ca^{2+}$ exchanger expression in the invertebrate muscles (280, 798, 799). The invertebrate exchanger, like the vertebrate NCX1, has a molecular mass of $\sim$120 kDa, and it cross-reacts with antibodies raised against the vertebrate cardiac exchanger (289); thus the invertebrate exchanger is clearly homologous to the vertebrate (NCX1) $Na^{+}/Ca^{2+}$ exchanger. Because the depolarization and contraction of invertebrate muscle fibers is slow and graded (375), it is possible that the $Na^{+}/Ca^{2+}$ exchanger can participate in excitation-contraction coupling in these...
cells by carrying some of the entering Ca\(^{2+}\). Also, because relaxation is generally slow, extrusion of Ca\(^{2+}\) via the exchanger may contribute directly to relaxation. Nevertheless, the physiological role of the exchanger in invertebrate muscles has not yet been systematically investigated.

E. Nervous System

1. Peripheral and central neurons

The Na\(^{+}/Ca^{2+}\) exchanger protein is expressed in both neurons (331, 465, 1000) and glial (359, 910, 911). Although NCX1, the “cardiac type” of 3 Na\(^{+}/1\) Ca\(^{2+}\) exchanger, is the dominant exchanger gene expressed in the brain (351, 1003), Philipson and colleagues (586, 590, 689) have identified two additional exchanger genes (NCX2 and NCX3) that are also expressed in the central nervous system. In addition, a “photoreceptor type” 4 Na\(^{+}/(1\) Ca\(^{2+} + 1\) K\(^{+}\)) exchanger is also apparently extensively expressed in the brain (219, 940), but the full significance of the coexpression of NCX1 and NCKX-type exchangers in the brain (and probably in the same neurons) has not yet been explored.

Exchanger expression levels in neurons are very high, especially in regions where there is a large traffic of Ca\(^{2+}\) across the PM: at synapses and at growth cones (93, 465, 610, 781). This prevalence and localization of the Na\(^{+}/Ca^{2+}\) exchanger in neurons implies that they play an important role here, but the precise function(s) is not yet clearly established. As in so many other instances, the availability of a selective inhibitor of the exchanger would go far toward sorting out the specific role(s) of the exchanger. Indeed, recent application of AS-oligos to knock down exchanger function in neurons (83) might be useful; however, AS-oligos have not yet been used to test, critically, the role of the exchanger in such specific functions as transmitter release. The recent observation of an \(I_{Na/Ca}\) in neurons (351, 1003) suggests that electrophysiological studies may also help to elucidate the role of the exchanger in these cells.

Elevation of \([Ca^{2+}]_i\) is the immediate trigger for neurotransmitter release at presynaptic nerve terminals (33, 478, 479, 693, 889). Release is activated, in large part, as a result of voltage-gated Ca\(^{2+}\) channel-mediated Ca\(^{2+}\) entry (750, 779). Calcium channels are found at high density at presynaptic terminals (479, 480, 675; see also review by Reuter, Ref. 779), and inhibition of these channels interferes with neurotransmitter release (275, 281). Thus it seems likely that Ca\(^{2+}\) extrusion via the exchanger, following neuronal activation, may contribute in an important way to the maintenance of Ca\(^{2+}\) balance in neurons (93, 348, 829). Indeed, after depolarization-induced Ca\(^{2+}\) entry, Ca\(^{2+}\) efflux from isolated nerve terminals (synaptosomes) is markedly slowed by the removal of extracellular Na\(^{+}\) (314, 809). The situation is therefore analogous to that in the heart (see sect. vA), where Ca\(^{2+}\) entry plays a critical role in triggering contraction, and the exchanger is involved primarily in the subsequent removal of Ca\(^{2+}\) and restoration of Ca\(^{2+}\) balance.

\([Ca^{2+}]_i\) is on the order of \(10^{-7}\) M in resting neurons, and the half-maximal activation of the exchanger by Ca\(^{2+}\) at the internal catalytic site is \(\sim 10^{-6}\) M. Moreover, depolarization-evoked Ca\(^{2+}\) transients at nerve terminals last less than a millisecond (116, 603) and are thus much shorter than in the heart. Consequently, it is unlikely that Ca\(^{2+}\) entry via the exchanger contributes significantly to the triggering of transmitter release, even in depolarized neurons. Thus it seems that the primary role of the exchanger in the neurons is in Ca\(^{2+}\) extrusion following activity; this “recovery” may be a relatively slow process (i.e., it may occur over many milliseconds to a few seconds; Ref. 93).

In addition to this seemingly straightforward role of the exchanger in Ca\(^{2+}\) removal, however, there appear to be very important, albeit more subtle, effects of the exchanger on transmitter release, per se. This follows from the fact that the exchanger helps regulate resting \([Ca^{2+}]_i\), in quiescent cells) and Ca\(^{2+}\) store content (since the ER stores are a source of Ca\(^{2+}\) for modulation of transmitter release; Ref. 713). Moreover, especially after repetitive firing, when \([Na^{+}]_i\) and \([Ca^{2+}]_i\) may both be elevated, the subsequent release of transmitter may be altered depending on how fast the various Ca\(^{2+}\) removal systems (Ca\(^{2+}\) buffering by cytosolic proteins, extrusion via the exchanger and PMCA pump, and sequestration in the ER) can restore the normal resting \([Ca^{2+}]_i\) and Ca\(^{2+}\) balance. Many observations indicate that release is modulated by the Na\(^{+}\) gradient.

2. Transmitter release: influence of the Na\(^{+}/Ca^{2+}\) exchanger

Over the years, numerous studies have demonstrated that reduction of the Na\(^{+}\) gradient across the plasma membrane augments neurotransmitter release, whether by removal of external Na\(^{+}\) or by treatment with ouabain, K\(^{+}\)-free media, monensin, or veratridine to load the terminals with Na\(^{+}\) (e.g., see the review by Torock, Ref. 931). This has been observed at, for example, invertebrate synapses (30, 31, 168, 169, 902), the frog neuromuscular junction (80, 81, 486, 646, 662, 747), and at mammalian peripheral synapses (84, 518, 906) and central synapses (122, 197, 781, 903, 904). The manifestations include increases in the frequencies of miniature excitatory currents (i.e., the frequency of spontaneous vesicular release of transmitters) and increases in facilitation and posttetanic potentiation. These effects may be observed despite only very modest increases in \([Na^{+}]_i\) and \([Ca^{2+}]_i\) (781).

This wealth of data demonstrates the presence of the
Na⁺/Ca²⁺ exchanger in nerve terminals and its impact on transmitter release. Nevertheless, the precise role of the exchanger in transmitter release is unknown, even though it is clear that modulation of cell Ca²⁺ plays critical roles in long-term potentiation and long-term depression (346) as well as in short-term processes such as facilitation (31). Does the exchanger mediate some of the Ca²⁺ entry that directly triggers transmitter release? This seems unlikely for very rapid release, which occurs within ~1 ms of the time that an action potential invades a synapse (116, 604). This release is triggered primarily by Ca²⁺ entry via voltage-gated Ca²⁺ channels and perhaps Ca²⁺ mobilization from ryanodine-sensitive ER stores (713). Alternatively, does inhibition of exchanger-mediated Ca²⁺ efflux (as a consequence of the depolarization that reduces the driving force, E_{Na/Ca}) directly influence the availability of Ca²⁺ for triggering release? Again, this seems unlikely for very brief presynaptic action potentials with durations of only about a millisecond. However, the membrane potential may influence the fraction of Ca²⁺ that is extruded during and immediately after an action potential, i.e., slow or incomplete repolarization should slow Ca²⁺ extrusion and increase Ca²⁺ storage in the ER. Moreover, Gleason et al. (350, 351) have shown that, in chick retinal amacrine cells, where transmitter release may continue after Ca²⁺ channel closure, prolonged depolarization induces a second (slow) phase of [Ca²⁺]ᵢ elevation and transmitter release. This slow transmitter release closely parallels a presynaptic outward current that is mediated by the Na⁺/Ca²⁺ exchanger. Furthermore, repolarization of these cells is associated with a slow inward, Na⁺/Ca²⁺-mediated current; this exchanger-mediated Ca²⁺ exit accounts for ~60% of the Ca²⁺ removed from the cytosol during the recovery phase after transmitter release.

If ER Ca²⁺ mobilization does contribute to the Ca²⁺ transients evoked by nerve terminal depolarization (713), then the exchanger could contribute to synaptic modulation in the short term (from pulse to pulse) as well as over the long term. Trains of action potentials may substantially elevate [Na⁺]ᵢ in small nerve terminals, and this would be expected to alter global or local (subplasmalemmal) [Ca²⁺]ᵢ, as well as the ER stores of Ca²⁺. Also, the cytosolic acidification that results from chronic activation would promote Na⁺ entry (via Na⁺/H⁺ exchange) and inhibit Ca²⁺ extrusion via Na⁺/Ca²⁺ exchange (see sect. 3.4). In any case, the net effect will be a change in the background [Ca²⁺]ᵢ, and the amplitudes of the evoked Ca²⁺ transients and, thus, in the efficacy of transmitter release.

Another consideration relates to the distribution of Ca²⁺ transport proteins (the PMCA pump and the Na⁺/Ca²⁺ exchanger) at nerve terminals. In a chick calyx-type synapse, both transporters were found at the terminals (463, 889). The PMCA pump was, however, present close to the release sites, where it may help to maintain a very low resting [Ca²⁺]ᵢ, to help prime the release sites following activity. In contrast, the Na⁺/Ca²⁺ exchanger was located at more distant sites in the terminals, where it may be involved in helping to extrude Ca²⁺ after it has diffused away from the release sites and has been sequestered in the ER (91, 463).

3. Na⁺/Ca²⁺ exchange in glia

There is strong molecular biological and immunocytochemical evidence as well as functional evidence that the Na⁺/Ca²⁺ exchanger is expressed in glial cells (307, 359, 423, 910). Nevertheless, here again, the precise role of the exchanger is unknown, although it surely must play a role in Ca²⁺ homeostasis. In a recent attempt to address this issue, Takuma et al. (911) employed sodium nitroprusside (SNP) to generate NO and activate the Na⁺/Ca²⁺ exchanger (26) and AS-oligos to inhibit the exchanger in glia. The Ca²⁺ transients evoked by a variety of agents (norepinephrine, l-glutamate, ATP, and ionomycin) were attenuated by SNP and augmented by the AS-oligos and by the exchange inhibitor 3,4-dichlorobenzamil (911, 630). These results suggest that the exchanger plays a role in controlling the amplitude of physiologically evoked Ca²⁺ transients.

Some glial cells exhibit Ca²⁺ waves that slowly propagate from cell to cell via gap junctions; these waves are largely dependent on mobilization of Ca²⁺ from intracellular stores (958). It is possible that the frequency, speed, and amplitudes of the waves may be modulated by Na⁺/Ca²⁺ exchange (890) as a result of the functional localization of the exchanger molecules in PM adjacent to underlying (junctional) ER (359, 465).

Another possible glial cell function, which has not been generally discussed, is that glia may maintain the ionic environment (including extracellular Ca²⁺ levels) in the restricted interstitial spaces between neurons and glia. Thus the exchanger may play a role analogous to the role of K⁺ permeability which, in glia, is involved in regulating the extracellular K⁺ concentration (509, 540, 541).

4. Role of Na⁺/Ca²⁺ exchange in neuronal and glial pathophysiology

Calcium overload has been associated with cell injury and cell death in a large variety of cell types including neurons (180) and glial cells (630, 751, 752, 894, 895, 971, 972, 895). In neurons, glutamate-induced neurotoxicity is associated with a prolonged increase in [Ca²⁺]ᵢ (485), whereas elevated external K⁺ produces only a transient rise in [Ca²⁺]ᵢ, which does not lead to delayed cell death (500). Inhibition of the Na⁺/Ca²⁺ exchanger (e.g., with 3,4-dichlorobenzamil), elevation of [Na⁺]ᵢ (by inhibition of the Na⁺ pump with ouabain), or reduction of [Na⁺]₀
potentiates excitotoxic cell death (19, 485, 496, 572, 573, 639). In addition to effects that are due directly to inhibition of Na⁺/Ca²⁺ exchanger-mediated Ca²⁺ extrusion because of the decline in Δ\(\mu\)ex, excitotoxin-induced cell acidification may also inhibit the exchanger (522) (see sect. II E4).

Increased Na⁺/Ca²⁺ exchanger activity has been reported in brain tissue from Alzheimer’s disease subjects (201, 201). It has even been suggested that the efficacy of treatments for manic symptoms in patients with affective disorders may be related to inhibition of the exchanger (296).

Inhibition of the Na⁺ pump by low (nonlethal) concentrations of ouabain induces epileptiform bursts of neuronal action potentials in the hippocampus (40, 76). Whether or not this, too, is a consequence of exchanger-mediated Ca²⁺ entry into the neurons, and/or decreased exit from neurons, has not yet been determined. If the Na⁺/Ca²⁺ exchanger does play a role in this phenomenon, it might be “treatable,” at least in the experimental animal, by inhibiting Ca²⁺ entry via the exchanger (e.g., with 3,4-dichlorobenzanil).

In glial cells, too, anoxia and hypoxia, and other similar insults, promote Na⁺/Ca²⁺ exchanger-mediated Ca²⁺ gain and delayed cell death (510, 630, 895). The underlying mechanism(s) is uncertain but might involve a reduction in the ATP/ADP and inhibition of the Na⁺ pump, with a secondary rise in [Ca²⁺]. Indeed, Matsuda et al. (630) have demonstrated that this rise in [Ca²⁺] and cell death can be attenuated by growing astrocytes with AS-oligos directed against the Na⁺/Ca²⁺ exchanger. A curious finding is that astrocytes seem to be less sensitive than neurons to insults such as hypoxia (358). Whether this relates to a higher level of Na⁺/Ca²⁺ exchanger expression in neurons (465; M. Juhaszova, personal communication) or a better ability of neurons to buffer Ca²⁺ loads, or some other factor, remains to be determined.

F. Na⁺/Ca²⁺ Exchange in Blood Cells: Physiology and Pathophysiology

1. Red blood cells

Erythrocytes are unusual cells with respect to their expression and utilization of the Na⁺/Ca²⁺ exchanger. In some species, such as humans and chickens (examples of species with anucleate and nucleate red blood cells, respectively), the mature erythrocytes have no functionally detectable Na⁺/Ca²⁺ exchanger (822; Blaustein, unpublished data). In these cells, the PMCA pump appears to be the sole mechanism for extruding Ca²⁺. In other species, exemplified by dogs and ferrets, the erythrocytes usually have no detectable Na⁺ pump (for exceptions, see Refs. 329, 616) but do have an Na⁺²⁺/Ca²⁺ exchanger. Here, the exchanger serves the unusual role of extruding Na⁺ in exchange for entering Ca²⁺; this Ca²⁺ is then extruded by the PMCA pump that operates in parallel with the exchanger (653, 705, 708, 983). These erythrocytes also have an Na⁺/H⁺ exchanger (708), and the Na⁺/Ca²⁺ and Na⁺/H⁺ exchangers operate in parallel to help regulate cell volume: the former functions to remove Na⁺ to prevent cell swelling, whereas the latter promotes Na⁺ entry in shrunken cells (329, 707, 709). The kinetics of the erythrocyte Na⁺/Ca²⁺ exchanger were discussed in section IV B.

The Na⁺/Ca²⁺ exchanger has also been identified in Friend virus-infected murine erythroleukemia cells (876). Inhibition of the Na⁺ pump in these cells promotes terminal erythroid differentiation; the rate-limiting step in this process appears to be the stimulation of Ca²⁺ uptake via the Na⁺/Ca²⁺ exchanger.

2. White blood cells

Sodium/calcium exchange activity has been demonstrated in lymphocytes (39, 945, 971, but see Ref. 272), neutrophils (220, 534, 851, 857), macrophages (272, 880), and probably in mast cells (456). Kimball and Sell (508) suggest that cyclosporin-induced immunosuppression in lymphocytes is the result of a tonic rise in [Ca²⁺], that results from shutdown of Na⁺/Ca²⁺ exchanger-mediated extrusion of Ca²⁺. [Note, however, that FK-506, another immunosuppressant, has no effect on the cardiac Na⁺/Ca²⁺ exchanger (279).] In neutrophils, elevation of [Na⁺] and inhibitors of Ca²⁺ extrusion via Na⁺/Ca²⁺ exchange augments cytokinin-mediated cytolsis, which implies that the exchanger serves as a “counterlytic mechanism” (534). Also, chemotactic factor-induced superoxide radical production and phagocytosis by neutrophils, but not chemotaxis, presumably involve Na⁺/Ca²⁺ exchange-mediated Ca²⁺ entry, since they are blocked by benzamil (220, 856). In mast cells, ouabain augments the secretory response (456), and in macrophages, cell activation induces a Ca²⁺ transient that is attenuated by Ca²⁺ extrusion via the exchanger (272).

3. Platelets

Tracer flux measurements and Ca²⁺-sensitive dye data have provided strong functional evidence for an Na⁺/Ca²⁺ exchanger in platelets (126, 516, 571, 773, 819, 949). Inhibition of the Na⁺ pump elevates [Ca²⁺], (819) and increases the Ca²⁺ content of the intracellular stores, and thereby augments the Ca²⁺ transients evoked by platelet activation (445, 788). Indeed, it was long ago established that cardiotonic steroids, in vivo as well as in vitro, augment blood coagulation by a thromboplastin mechanism (231, 527). The latter observations imply that the exchanger normally functions to extrude Ca²⁺ and attenuate these Ca²⁺ transients.

Recently, Kimura et al. (517) reported that Ca²⁺ entry
via the Na+/Ca2+ exchanger in platelets was stimulated by, but not absolutely dependent on, external K+. This raised the possibility that the platelet exchanger may be the photoreceptor type [4 Na+/(1 Ca2++ + 1 K+)], but this remains to be verified by molecular biological methods.

The subject of Ca2+ metabolism in the platelets of patients with essential hypertension has received considerable attention in recent years. It has been well documented that the platelets of hypertensives exhibit enhanced aggregation and responsiveness (226, 380, 700). Numerous studies now indicate that these platelets have elevated resting [Ca2+]i (298, 806, 933) and augmented Ca2+ transients (729, 933). This has been attributed to the presence of elevated levels of a circulating EOLC in the plasma of these patients (95, 791).

G. Kidney

In section vA we discussed some special features that relate to the location and regulation of the Na+/Ca2+ exchanger in the kidney. Here, we consider the physiological role(s) of the Na+/Ca2+ exchanger in relation to renal cell function and the kidney’s handling of Ca2+ excretion and Ca2+ balance (cf. Ref. 523). In this discussion (see also sect. vA), we make some general statements about renal Ca2+ handling and the Na+/Ca2+ exchanger; the reader should be cognizant of the fact that there may be some important species differences [e.g., compare Bachmann et al. (33) with Obermuller et al. (701)] that are ignored here.

The total plasma Ca2+ concentration in humans is ~2.5 mM, of which ~45% is bound to plasma proteins; the remainder is subject to glomerular filtration. At a filtration rate of 120 ml/min, ~240 mmol Ca2+ appear in the glomerular filtrate each day. Because only ~5 mmol Ca2+ are absorbed by the gastrointestinal tract each day, only ~5 mmol can be excreted in the urine to maintain Ca2+ balance. Thus ~98% of the filtered Ca2+ load must be reabsorbed by the kidneys. Of course, even small differences between the amount absorbed in the gastrointestinal tract and the amount excreted by the kidneys will, if continued over many days, have a profound impact on Ca2+ balance; precise control of renal Ca2+ excretion is therefore crucial for Ca2+ homeostasis.

1. Proximal tubule

Approximately 70% of the total filtered Ca2+ load is reabsorbed in the proximal convoluted tubules. About four-fifths of this amount is reabsorbed by bulk flow (and, when the lumen becomes positive, in the latter half of the proximal tubule, by electrodiffusion) through paracellular pathways. The remaining one-fifth of the Ca2+ reabsorbed in this segment of the nephron passes through the proximal tubular cells (i.e., transcellular transport).

Entry of Ca2+ into these epithelial cells across the apical membrane is passive, down a steep electrochemical gradient. Conversely, Ca2+ extrusion across the basolateral membrane occurs against a steep gradient and is thus an energy-dependent process. The proximal tubule cells contain both an Na+/Ca2+ exchanger (995) and a PMCA pump in their basolateral membranes, but the relative roles of these two mechanisms are not known. Some of the Ca2+ that exits from the proximal tubules cells across the basolateral membrane undoubtedly does so via the Na+/Ca2+ exchanger, although much of the net Ca2+ extrusion across the basolateral membrane may be mediated by the PMCA pump (356, 953). An important question for functional studies on isolated tubule segments is whether cortical proximal tubule could always be distinguished from cortical distal tubule.

A major role of the exchanger in the proximal tubule cells may be to help regulate [Ca2+]i, or, at least, to keep it from rising excessively. Another possibility (see sect. vA C) is that the exchanger may help to regulate the ER Ca2+ content and may thereby play a role in Ca2+-mediated cell signaling of various processes, perhaps including the control of apical membrane permeability to Na+ in urinary tract epithelia (128, 170, 171, 208, 322, 325, 326). An intriguing and important dilemma in this regard is the discrepancy between the strong functional evidence for a high level of Na+/Ca2+ exchanger activity in proximal tubule cells (269–271, 995) that contrasts with the relative difficulty encountered in detecting the exchanger in this segment by molecular biological methods (976, 1002, and contrast Ref. 270) and immunocytochemical methods (771; see sect. vA). One possibility is that a different Na+/Ca2+ exchanger (perhaps even the K+-dependent exchanger) operates in this segment, but there is not yet any information on this point.

2. Loop of Henle and distal tubule

About 20% of the filtered Ca2+ load is reabsorbed in the thick ascending limb (TAL) of the loop of Henle. Because this segment is impermeable to water, passive paracellular Ca2+ reabsorption is driven by the lumennegative potential across the epithelium. Transcellular Ca2+ reabsorption in the TAL is mediated by processes similar to those in the proximal tubule. The paracellular Ca2+ reabsorption mechanisms in the proximal tubule and TAL (bulk flow and electrodiffusion) are secondary to Na+ transport (including Na+ pump-mediated Na+ extrusion across the basolateral membrane). Changes in Na+ reabsorption in these segments will therefore directly influence Ca2+ reabsorption independent of effects on the Na+/Ca2+ exchanger. For example, inhibition of the Na+ pump will inhibit paracellular Ca2+ transport as well as the transcellular Ca2+ transport that is mediated by the Na+/Ca2+ exchanger. Furthermore, there is some evi-
dence that, at least in some species, parathyroid hormone (PTH) promotes Ca^{2+} reabsorption across the TAL (119, 897). As discussed below, this “active” reabsorption of Ca^{2+}, that is regulated by PTH, may involve Na^{+}/Ca^{2+} exchange.

Calcium reabsorption in the distal tubule, connecting tubule (see also sect. νA) and cortical collecting duct is entirely transcellular. The extent of Ca^{2+} reabsorption in these segments (~8–9% of filtered load) is crucial for overall Ca^{2+} balance. Fine control of urinary Ca^{2+} excretion occurs in the distal segments of the nephron. Here, Ca^{2+} excretion is regulated primarily by PTH, although the hormones calcitonin and calcitriol also play a role.

The exchanger is expressed at a high level in these segments of the nephron (78, 121, 749, 771, 917, 1002). As noted in section νG1, however, a major dilemma is the discrepancy between the apparently high level of Na^{+}/Ca^{2+} exchanger expression in the proximal nephron and the immunocytochemical data showing that most of the detectable exchanger is confined to the basolateral membrane of the connecting tubule cells. Functional data demonstrate that PTH greatly stimulates distal nephron Ca^{2+} reabsorption, primarily by augmenting Na^{+}/Ca^{2+} activity via a cAMP-mediated mechanism (see sect. μE3).

[Some evidence suggests that the cloned and expressed kidney isoform of NCX1 is poorly activated by PKA (394, 395). It seems possible, however, that cloned and natively expressed exchangers may behave differently perhaps because of differences in splicing (see sect. νC).] The implication is that a major role of the exchanger in the distal nephron is the extrusion of Ca^{2+} across the basolateral membrane.

By virtue of its effect on [Ca^{2+}], the exchanger also appears to play a central role in regulating K^{+} transport in cortical collecting duct principal cells. The distal nephron is intimately involved in K^{+} homeostasis; the cells in this portion of the nephron can either secrete K^{+} into or reabsorb K^{+} from the tubular fluid, depending on bodily needs. When K^{+} is secreted, it moves down its electrochemical gradient through K^{+} channels in the apical membrane of the principal cells. These apical channels are inhibited by elevated [Ca^{2+}], presumably acting through a PKC-dependent mechanism (967). In addition, K^{+} must be recycled across the basolateral membrane of the principal cells to avoid an excessive rise of [K^{+}], or excessive secretion of K^{+} across the apical membrane as a result of K^{+} transport into the cells via the basolateral Na^{+} pump. This return of K^{+} to the interstitial fluid occurs via K^{+} channels that are directly inhibited by cytosolic Ca^{2+} (828). Inhibition of the Na^{+} pump in these cells raises [Na^{+}], and, via Na^{+}/Ca^{2+} exchange, [Ca^{2+}]; the latter increase then inhibits both K^{+} secretion across the apical membrane (967) and K^{+} recycling across the basolateral membrane (828). Thus the Na^{+}/Ca^{2+} exchanger not only influences net Ca^{2+} transport but also plays a key role in coupling Na^{+} transport to K^{+} transport in the renal cortical collecting duct, an idea first hinted at by Schultz and Hudson more than a decade ago (835, 837).

The role of the Na^{+}/Ca^{2+} exchanger in regulating Na^{+} transport in the distal nephron is also interesting. Evidence suggests that the stimulation of Ca^{2+} entry (at least in part via Na^{+}/Ca^{2+} exchange) will elevate [Ca^{2+}], and that this will, in turn, reduce the rate of Na^{+} entry across the apical membrane by feedback inhibition (128, 129, 326).

The precise mechanism by which PTH activates the exchanger is controversial. Some investigators have suggested that PTH does not affect [Ca^{2+}] (735) so that the activation of the Na^{+}/Ca^{2+} exchanger cannot be explained by increased “substrate” availability. Indeed, studies on isolated basolateral membrane vesicles, which demonstrate PTH-induced stimulation of Na^{+} gradient-dependent Ca^{2+} transport (454, 841), imply that a rise in [Ca^{2+}] is not required for this effect. Calcitonin, on the other hand, raises [Ca^{2+}] and appears to promote Ca^{2+} reabsorption by increasing apical Ca^{2+} permeability via a phospholipase C-mediated pathway (674, 735). Thus calcitonin and PTH may act in concert on distal tubule cells to reduce urinary Ca^{2+} loss via the simultaneous enhancement of apical Ca^{2+} entry and basolateral extrusion (to the interstitial fluid) via Na^{+}/Ca^{2+} exchange (735). This simple view must, however, be tempered by recognition of the fact that these hormones act via second messenger systems that exert numerous other effects on these cells. Also, others have reported that PTH, itself, as well as 8-bromo-cAMP, induce a sustained increase in [Ca^{2+}], in connecting tubule cells, primarily as a result of Ca^{2+} entry across the apical membrane (120, 121). How the cells could tolerate such an increase in [Ca^{2+}], and why the vitamin D-dependent Ca^{2+} binding protein that is expressed at high levels in the distal nephron (920) would not adequately buffer this Ca^{2+}, is unclear (see Ref. 321). Moreover, we have ignored possible dynamic changes in [Ca^{2+}], or even in [Na^{+}], and intracellular pH (whether local or global), that may markedly influence the operation of the exchanger (see sect. μE4). To resolve at least some of these issues, we need direct information about whether PTH actually influences exchanger phosphorylation and kinetics. Although there have already been a number of studies on this subject, the results are controversial. For example, two groups have suggested, on the basis of studies in isolated renal brush-border membrane vesicles, that PTH increases the $J_{\text{max}}$ of the exchanger, but one group suggests that a cAMP-dependent mechanism is involved (117), whereas the other disagrees (841).

H. Intestinal Absorption of Ca^{2+}

The main site of intestinal Ca^{2+} absorption is in the small intestine. Calcium is transported against an electro-
chemical gradient from the intestinal lumen into the serosal fluid/blood plasma. As in the kidneys, some of the Ca\(^{2+}\) is transported by a paracellular route and some by the transcellular route; the transcellular transport of Ca\(^{2+}\) is coupled to transcellular Na\(^{+}\) transport (673). Transcellular Ca\(^{2+}\) transport occurs in several steps. Calcium enters the enterocytes across the apical (brush-border) membrane down a steep electrochemical gradient. In the cells, the Ca\(^{2+}\) is bound to a vitamin D-dependent Ca\(^{2+}\) binding protein that helps to keep the cytosolic free Ca\(^{2+}\) concentration low. Calcium is then transported out of the enterocytes across the basolateral membrane against a very large electrochemical gradient. The Na\(^{+}/\text{Ca}^{2+}\) exchanger and the PMCA pump are both expressed in the basolateral membrane of mammalian small intestine enterocytes (269, 503, 625, 673, 954). In fish enterocytes, the Na\(^{+}/\text{Ca}^{2+}\) exchanger appears to be the main mechanism by which transcellular fluxes of Ca\(^{2+}\) are extruded from the cells at the serosal surface (312, 833). Whether this is also the case in mammals is less clear (673, 954).

I. Eye

1. Photoreceptors

A) VERTEBRATE PHOTORECEPTORS. Calcium ions play a critical role in signal transduction in vertebrate photoreceptor rods and cones (742). In the dark, high levels of cGMP maintain a substantial influx of Na\(^{+}\) and Ca\(^{2+}\) in the rod and cone outer segments (accounting for the "dark current") to keep the photoreceptors partially depolarized. The Na\(^{+}\) and Ca\(^{2+}\) balances are then maintained by the Na\(^{+}\) pumps located in the inner segments of the photoreceptors and the Na\(^{+}/\text{Ca}^{2+}\) exchangers located in the inner segment PM (52, 373). Phototransduction begins when light is turned on; this activates the rhodopsin in the disks, and this, in turn, activates a cascade of biochemical reactions that leads to hydrolysis of cGMP and lowering of the cGMP levels in the outer segments (521). As a result, the cGMP-activated channels close, and the dark current carried by Na\(^{+}\) and Ca\(^{2+}\) is turned off, causing the photoreceptors to hyperpolarize. The Na\(^{+}/\text{Ca}^{2+}\) exchanger can now extrude more Ca\(^{2+}\) because the electrical driving force is increased (see Eq. 3A). The lowering of [Ca\(^{2+}\)]\(_{i}\), which continues even after the light is turned off, stimulates guanylate cyclase activity (521), and the rise in the cGMP level then reactivates the dark current.

The photoreceptors can respond over a very large range of light levels, at least in part because their sensitivity to transient illumination decreases as the background light intensity increases, a phenomenon called "light adaptation" (56). When the Na\(^{+}/\text{Ca}^{2+}\) exchange is inhibited, by removing extracellular Na\(^{+}\), light adaptation is abolished (302, 638, 677, 678). Thus extrusion of Ca\(^{2+}\) via the Na\(^{+}/\text{Ca}^{2+}\) exchanger appears to be responsible for light adaptation in vertebrate photoreceptor rods and cones.

B) INVERTEBRATE PHOTORECEPTORS. In contrast to the vertebrates, the photoreceptors of invertebrates are normally much more polarized in the dark, and the resting potential is maintained primarily by the K\(^{+}\) electrochemical gradient across the PM. Light activates rhodopsin, and this leads to a rise in [Ca\(^{2+}\)]\(_{i}\); most of the signal Ca\(^{2+}\) comes from the ER Ca\(^{2+}\) stores (24, 385, 658). The photoreceptors are depolarized by light, and there is activation of Na\(^{+}\) and Ca\(^{2+}\) entry; indeed, the inward current, which may be mediated by store-operated ion channels that are permeable to both Na\(^{+}\) and Ca\(^{2+}\) (349, 992), may make a major contribution to the depolarizing current. Much of the light-induced rise in [Ca\(^{2+}\)]\(_{i}\) in the horseshoe crab (Limulus) ventral photoreceptor results from mobilization of intracellular Ca\(^{2+}\) stores (225). The initial fast phase of recovery is apparently due to release of Ca\(^{2+}\) from the ER, whereas the slower phase is the result of Ca\(^{2+}\) extrusion via a Na\(^{+}/\text{Ca}^{2+}\) exchanger that has a coupling ratio greater than 2 Na\(^{+}\):1 Ca\(^{2+}\). The exchanger may be the "predominant link" between [Ca\(^{2+}\)]\(_{i}\) and [Ca\(^{2+}\)]\(_{o}\) (225). In mutant Drosophila, with photoreceptors that do not exhibit a light response, release of caged Ca\(^{2+}\) by flash photolysis induces a small inward current that is abolished when external Na\(^{+}\) is replaced by Li\(^{+}\) (388). Because this current is unaltered when internal K\(^{+}\) is replaced by Cs\(^{+}\), it is presumably mediated by a cardiac/neuronal-type Na\(^{+}/\text{Ca}^{2+}\) exchanger, and not by a vertebrate photoreceptor-type Na\(^{+}/(\text{Ca}^{2+} + \text{K}^{+})\) exchanger.

The light-induced rise in [Ca\(^{2+}\)]\(_{i}\) promotes light adaptation in the invertebrate photoreceptor (597, 598) by mechanisms that are still controversial (23). This adaptation (i.e., the degree of desensitization of the photoreceptors to bright light flashes) is reduced by lowering [Na\(^{+}\)]\(_{o}\) and inhibiting Ca\(^{2+}\) extrusion via the Na\(^{+}/\text{Ca}^{2+}\) exchanger (702). Indeed, because of the central role of Ca\(^{2+}\) in invertebrate photoreceptor transduction, O’Day et al. (702) have suggested that the Na\(^{+}/\text{Ca}^{2+}\) exchanger helps to regulate the resting sensitivity to light as well as the initial response to excitation, and the recovery of light sensitivity (dark adaptation) after adapting illumination, as well as light adaptation.

2. Lens of the eye

The anterior surface of the lens consists of a single layer of epithelial cells from which the underlying fiber cells are derived. Although the epithelial cells bear the major responsibility for ion transport, fiber cells may also have some of these functions (998). Calcium metabolism in the lens is especially important because accumulation of Ca\(^{2+}\) appears to play a central role in the formation of at least some types of cataracts (622).
Calcium efflux from the intact rat lens, in vitro, is dependent on an appropriate Na\(^+\) gradient; efflux is reduced by ~55% when external Na\(^+\) is removed (928). It is difficult, however, to envision a simple model of Ca\(^{2+}\) transport and homeostasis in the lens because both the Na\(^+\) pump (363) and the Na\(^+\)/Ca\(^{2+}\) exchanger (998) are expressed in the apical membrane of the lens epithelial cells that borders the fiber cells. Furthermore, PM vesicles prepared from lens fiber cells also exhibit Na\(^+\)/Ca\(^{2+}\) exchange activity (339, 998).

Removal of external Na\(^+\) not only inhibits Ca\(^{2+}\) efflux but also augments Ca\(^{2+}\) influx, as does 0.1 mM ouabain (928). The net gain of Ca\(^{2+}\) associated with these treatment results in cataract formation, and this can be prevented if the Na\(^+\)-free medium is also Ca\(^{2+}\) free (928). In preweaning rats, subcutaneous injection of sodium selenite induces mature nuclear cataracts that are associated with a selective reduction in Na\(^+\)/Ca\(^{2+}\) exchange activity (968). Moreover, unusually high levels of an endogenous Na\(^+\) pump inhibitor (the bufodienolide, norbubalin) have been found in human cataractous lenses (588, 808). Thus the Na\(^+\)/Ca\(^{2+}\) exchanger may play a key role in the regulation of Ca\(^{2+}\) homeostasis in the lens, and inhibition may lead to cataract formation. In any event, the aforementioned observations clearly indicate that the exchanger is intimately involved in Ca\(^{2+}\) extrusion from the lens.

J. Secretory Cells

1. Adrenal chromaffin cells

The Na\(^+\)/Ca\(^{2+}\) exchanger has been very extensively studied in adrenal chromaffin cells [e.g., see the reviews by Phyllis and Wu (725) and Torok (931)]. The earliest study along these lines was that of Banks (43), who showed that ouabain augmented ACh-evoked catecholamine release from the isolated adrenal gland. Since then, numerous investigators have identified Na\(^+\)/Ca\(^{2+}\) exchanger-mediated (Na\(^+\)-dependent) Ca\(^{2+}\) fluxes in adrenal medullary cells (44, 177, 426, 602, 696, 730, 731, 738, 887, 963, 964). Additionally, many investigators have shown that Na\(^+\) pump inhibition or lowering [Na\(^+\)]\(_o\) increases basal catecholamine release and/or augments release evoked by a large variety of agents (3, 278, 679–682, 725, 730, 886, 915, 932, 964). These data do not necessarily directly implicate the exchanger in the sequence of events involved in triggering evoked catecholamine release. They do, however, imply that the exchanger may very likely influence the amplitude and/or time course of the evoked Ca\(^{2+}\) transients that trigger catechol secretion.

The data cited in the preceding paragraph are all consistent with a Na\(^+\)/Ca\(^{2+}\) exchanger that is located in the adrenal chromaffin cell PM, and that mediates the transfer of Ca\(^{2+}\) between the extracellular fluid and the cytosol (or some restricted portion thereof; see sect. viA7). Some investigators, however, have reported that a Na\(^+\)/Ca\(^{2+}\) exchanger is also located in the secretory vesicles of chromaffin cells (538, 539, 724, 804) and also in coated vesicles from the neurohypophysis (804). It has been suggested that the Na\(^+\)/Ca\(^{2+}\) exchanger in the vesicle membrane participates in Ca\(^{2+}\) buffering (768) and in the extrusion of Ca\(^{2+}\) from the cytosol following fusion of the vesicles with the PM (451). Recent biochemical studies indicate, however, that the NCX1-like protein in brain membranes comigrates with PM markers, and not with synaptic vesicle markers (M. Juhaszova, unpublished data).

2. Pancreatic β-cells

There is also a long history of studies on the possible role of the Na\(^+\)/Ca\(^{2+}\) exchanger in influencing insulin secretion by pancreatic β-cells, beginning with the studies of Hales and Milner (378, 379). These workers provided the key evidence that reducing the Na\(^+\) gradient augments external Ca\(^{2+}\)-dependent basal insulin release. A number of subsequent studies have expanded on these findings (371, 549, 950) and have demonstrated that glucose-evoked insulin secretion is enhanced by Na\(^+\) gradient reduction (282, 399, 853). Indeed, in the intact dog, infusion of ouabain into the hepatic portal vein induces hypoglycemia by enhancing insulin secretion (935). Other studies have provided direct evidence that some of the Ca\(^{2+}\) fluxes across the PM of β-cells are mediated by a Na\(^+\)/Ca\(^{2+}\) exchanger (229, 368, 400, 402, 403, 728, 852, 988). Here, again, the exchanger obviously must play a role in the physiological response of β-cells to glucose stimulation, even though this role has not yet been defined. At the very least, the exchanger must, via its effect on stored Ca\(^{2+}\), influence the shape of the glucose-evoked Ca\(^{2+}\) transient (368) and must thereby modulate modulate evoked insulin release.

3. Miscellaneous endocrine cells

Sodium/calcium exchange activity has been detected in adrenal glomerulosa cells, where it has been postulated to play a role in aldosterone production (441, 529); however, ouabain is reported to both stimulate (376, 827, 914) and inhibit (20) aldosterone secretion. An Na\(^+\)/Ca\(^{2+}\) exchange activity has also been observed in thyroid follicular cells (17, 929), where its role (if any) in secretion is unknown.

Beginning with the studies of Dicker (234) and Drefuss et al. (276) on the neurohypophysis and Fleischer and colleagues (308, 309) on the adenohipophysis, several groups have reported that Na\(^+\) gradient reduction augments Ca\(^{2+}\) gain (473, 531) and the release of a variety of pituitary hormones including oxytocin, vasopressin, ACTH, growth hormone, prolactin, and thyrotropin-re-
leaching hormone (263, 885, 926, 930). Evidence for an Na⁺/Ca²⁺ exchanger has also been obtained in insect neuroendocrine cells (704). Finally, follicle-stimulating hormone is reported to regulate Ca²⁺ transport in Sertoli cells by inhibiting Na⁺/Ca²⁺ exchange (369, 854).

4. Unusual endocrine cells (in which a rise in cell Ca²⁺ inhibits secretion)

A) JUXTAGLOMERULAR CELLS AND RENIN SECRETION. Whereas a rise in the [Ca²⁺]i triggers the secretion of most hormones (see preceding paragraphs), the secretion of renin by the juxtaglomerular cells of the kidney is reduced by a rise in [Ca²⁺]i (185). Furthermore, Na⁺ pump inhibition with ouabain (187, 612) or reduced external K⁺ (186) inhibits renin secretion by a Ca²⁺-dependent mechanism. This mechanism is, presumably, a Na⁺/Ca²⁺ exchange-mediated increase in intracellular Ca²⁺ (183, 184). Reduction of [Na⁺]o also inhibits renin secretion, possibly by promoting Ca²⁺ influx and reducing Ca²⁺ efflux (185).

B) PARATHYROID CELLS AND PTH SECRETION. The secretion of parathyroid hormone is controlled by the concentration of ionized Ca²⁺ in the blood plasma; an increase in [Ca²⁺]o inhibits PTH secretion. In parathyroid tissue, inhibition of the Na⁺ pump with ouabain or reduced external K⁺ (140, 141, 341, 792) and removal of external Na⁺ (792) suppress PTH secretion, as would be expected if the parathyroid cells possess an Na⁺/Ca²⁺ exchanger. It seems that in both the juxtaglomerular cells and the parathyroid cells, the Na⁺/Ca²⁺ exchanger simply operates as a Ca²⁺ transport system and that inhibition of Ca²⁺ extrusion or stimulation of Ca²⁺ entry via this exchanger influences intracellular Ca²⁺ and thereby modulates hormone release, i.e., the exchanger has no special or integral role in the secretion process in these cells.

5. Exocrine secretion

In various exocrine cells, a rise in [Ca²⁺]i regulates the secretory process (715, 923). There is evidence that several types of exocrine cells possess an Na⁺/Ca²⁺ exchanger, e.g., pancreatic acinar cells (55, 442), parotid cells (912), and submandibular cells (383, 626). Thus, in these cells too, the Na⁺/Ca²⁺ exchanger presumably plays an indirect role in modulating the secretory responses to a large variety of secretagogues.

K. Na⁺/Ca²⁺ Exchanger in Miscellaneous Other Types of Cells

Sodium/calcium exchanger activity has also been described in a large variety of other types of cells where this transport system most likely plays a role in Ca²⁺ homeostasis and, thus, in Ca²⁺-regulated activities. Indeed, it should be readily apparent that if the exchanger is present and influences Ca²⁺ homeostasis in any cell, it is certain to play a role in a variety of physiological processes in that cell. Nevertheless, the precise role(s) of the exchanger in the physiological activities of many of these cells remains rather vague. Thus there is considerable opportunity to elucidate precisely how the exchanger contributes to the physiology of various types of cells. For example, hormonally activated bone mineral resorption appears to involve Na⁺/Ca²⁺ exchange (535–537, 850), although there are few details regarding the precise locus and action of the exchanger. Likewise, exchanger-mediated transport of Ca²⁺ by the gills and hepatopancreas of marine invertebrates may play an important role especially during the molt cycle, when the mineral in the exoskeleton is resorbed and then redeposited (6, 7, 313, 975). Sodium/calcium exchange has also been observed in the gills of euryhaline fishes, where it may even play vastly different roles in the freshwater and seawater environments. The exchanger is also present in mammalian hepatocytes (62, 158, 159, 820, 893) as well as in adipocytes (714). The exchanger contributes in important ways to the function of special sensory cells such as primary photoreceptor cells of both vertebrates and invertebrates (see sects. II.B3 and II.I2) and to the physiology of retinal amacrine cells (350, 351) and horizontal cells (393, 650a), olfactory receptors (235, 466, 772), cochlear outer hair cells (443), and carotid body cells (82). The exchanger is present not only in vascular smooth muscle and cardiac myocytes, but also in the underlying endothelial cells (387, 582, 806, 985). Finally, the exchanger has been implicated in the regulation of Ca²⁺ in a variety of cells directly involved in reproduction, including Sertoli cells (369), the follicle cells of the vertebrate oocyte (899), and both invertebrate (817) and vertebrate (794) spermatozoa, including human spermatozoa (855). This, of course, is not an exhaustive or all-inclusive list of cells in which the exchanger may play a physiological role. Perhaps most important is the wealth of evidence that dispels the early notion that the Na⁺/Ca²⁺ exchanger was expressed only in “excitable” cells; it is now clear the the exchanger is present in most cells of higher animals and that it plays a physiological role in Ca²⁺ homeostasis in these cells, even if the precise role has not yet been fully characterized.

VII. EPILOGUE

The cloning of the Na⁺/Ca²⁺ exchanger(s) and the development of the giant patch method for studying exchanger-mediated currents with patch-clamp methods are two of the seminal advances of the past decade in this field. In addition, the widespread use of new ion-selective fluorescent dyes and fluorescent antibody probes with digital imaging methods (both wide-field and confocal) have provided exciting new opportunities to study Na⁺/
Ca\textsuperscript{2+} exchanger distribution and function. These new methods have greatly increased interest in studying the exchanger from both biochemical/molecular biological and physiological/biophysical vantage points, and the literature on Na\textsuperscript{+}/Ca\textsuperscript{2+} exchange has therefore expanded almost exponentially during recent years. The daunting task of sorting through this enormous literature (not all of which could be cited here) has challenged the authors. Nevertheless, we have endeavored to place the many new developments in perspective and to summarize the way(s) in which they have helped to elucidate the physiological roles of the Na\textsuperscript{+}/Ca\textsuperscript{2+} exchanger. In addition to the rather straightforward view that the exchanger plays an important role in Ca\textsuperscript{2+} extrusion and the maintenance of overall Ca\textsuperscript{2+} balance in some cells, some novel concepts have emerged. One such idea is that in some cells, the Na\textsuperscript{+}/Ca\textsuperscript{2+} exchanger operates on a special, restricted subplasmalemmal portion of the cytoplasm, where it helps to control primarily the “signal Ca\textsuperscript{2+}” stored in the SR/ER. And, of course, in some cells, the exchanger plays an important role in mediating Ca\textsuperscript{2+} entry rather than exit.

Probably the most important lesson that has emerged, however, is the recognition of the large gaps in our knowledge. We have made an effort to pinpoint several of these gaps to foster research in these areas. The discovery of multiple Na\textsuperscript{+}/Ca\textsuperscript{2+} exchanger genes and gene products, and multiple splice variants, as well as exchangers with different coupling ratios [i.e., the 3 Na\textsuperscript{+}:1 Ca\textsuperscript{2+} exchanger a Na\textsuperscript{+}/Ca\textsuperscript{2+} exchanger and the 4 Na\textsuperscript{+}:1 (Ca\textsuperscript{2+} + K\textsuperscript{+}) exchanger] has added to the complexity. We must even consider the possibility that some cells may express more than one Na\textsuperscript{+}/Ca\textsuperscript{2+} exchanger [or Na\textsuperscript{+}/(Ca\textsuperscript{2+} + K\textsuperscript{+}) gene product or alternatively spliced isoform] and that these exchangers may perform different functions. Suffice it to say that we are barely at the threshold in terms of beginning to understand the physiological implications of this diversity of exchangers. Rather than simply cataloguing results, we have tried to provide a perspective that, however biased, gives some focus to often contradictory results. Undoubtedly, much of this perspective may prove to be wrong. Nevertheless, we hope that our readers have not been led too far astray and that critical analysis of our views may enable future investigators to clarify issues on which we may have been led astray. There is no doubt, however, that the time is ripe for major new advances in this field. Indeed, we anticipate that the next decade will be a period of greatly improved insight into the precise roles of the Na\textsuperscript{+}/Ca\textsuperscript{2+} exchanger along with novel insight into its mechanism of operation based on its three-dimensional molecular structure.

Yet it seems not unreasonable on the other hand, tho’ not far to indulge, yet to carry our Reasonings a little farther than the plain Evidence of Experiments will warrant; for since at the utmost Boundaries of those Things which we clearly know, there is a kind of Twilight cast from what we know, on the adjoining Borders of Terra incognita, it seems therefore reasonable in some degree to indulge Conjecture there; otherwise we should make but very slow Advances in future Discoveries.

Stephen Hales, Statical Essays: containing Haemastatics, London, 1733

We thank the many colleagues who read sections of a preliminary version of this article and provided critical comments, especially Drs. Reinaldo DiPolo, Kenneth D. Philipson, John P. Reeves, Robert F. Reilly, and Dan H. Schulze. We also thank Becky Saunders for invaluable secretarial assistance, Robert S. Rogowski, and Dr. Magdalena Juhaszova for help with the figures.

This work was supported by National Institutes of Health Grants HL-45215 (to M. P. Blaustein), HL-25675 (to W. J. Le- derer), and NS-16106 (to M. P. Blaustein).

REFERENCES


70. BERS, D. M., K. D. PHILIPSON, AND A. Y. NISHIMOTO. Sodium-

71. BERSOHN, M. M., R. VEMURI, D. W. SCHUIL, R. S. WEISS, AND

72. BERTORELLI, A. M., A. APERIA, S. I. WALASS, A. C. NAIRN, AND

74. BERS, D. M., AND J. H. BRIDGE. Relaxation of rabbit ventricular

75. BERS, D. M., L. LI, H. SATOH, AND EM CCALL. Factors that control


77. BIHLER, I., P. C. SAWH, AND P. CHARLES. Stimulation of glucose

78. BINDELS, R. J. M., P. L. M. RAMAKERS, J. A. DEMPSTER, A.

79. BENOS, D. J., S. CUNNINGHAM, R. R. BAKER, K. B. BEASON, Y.


100. BLAUSTEIN, M. P., W. F. GOLDMAN, G. FONTANA, B. K. KRUEGER, E. M. SANTIAGO, AND T. D. STEELE. Physiological

---


---

For the full text, please refer to the original source.


252. DiPOLO, R., AND L. BEAUGE. In squid axons the Ca$^{2+}$-dependent regulatory site of the Na$^{+}$/Ca$^{2+}$ exchanger is drastically modified by sulfhydryl blocking agents. Evidences that intracellular Ca$^{2+}$ regulatory and transport sites are different. Biochim. Biophys. Acta 1145: 75–84, 1993.


295. FERRIER, G. R., J. H. SAUNDERS, and C. MENDEZ. A cellular mechanism for the generation of ventricular arrhythmias by ace


311. FRINDT, G., C. O. LEE, J. M. YANG, and E. E. WINDHAGER.


378. GRASSO, P. M. JOSEPH, AND L. E. REICHERT, Jr. A new role for follicle-stimulating hormone in the regulation of calcium flux...


July 1999

SODIUM/CALCIUM EXCHANGE


490. KHANANSHVILI, D., D. BAZAZOV, E. WEIL-MASLANSKY, G. SHALOUL, and B. MESTER. Rapid interaction of FRCCRFa with the cytosolic side of the cardiac sarcolemmal Na\(^+\)/Ca\(^{2+}\) exchanger blocks the ion transport without preventing the binding of either sodium or calcium. Biochemistry 35: 15933–15940, 1996.
496. KHODOROV, B., V. PINELIS, V. GOLOVINA, D. FAJUK, N. ANDREEVA, and T. UVAROVA. On the origin of a sustained increase


MORDECAI P. BLAUSTEIN AND W. JONATHAN LEDERER Volume 79

486


MURER, H., AND B. HILDAMANN. Transcellular transport of calcium and inorganic phosphate in the small intestinal epithelium.


REEVES, J. P., AND J. L. SUTKO. Competitive interactions of sodium and calcium with the sodium-calcium exchange system of...


875. STYS, P. K., S. G. WAXMAN, AND B. R. RANSON. Ionic mechanisms of anoxic injury in mammalian CNS white matter: role of...
Na\textsuperscript{+} channels and Na\textsuperscript{+}/Ca\textsuperscript{2+} exchanger. J. Neurosci. 12: 430–439, 1992.


TAGLIALATELA, M., G. DIRENZO, AND L. ANNUNZIATO. Na\textsuperscript{+}/Ca\textsuperscript{2+} exchange activity in central nerve endings. I. Ionic conditions that discriminate Na\textsuperscript{+}/Ca\textsuperscript{2+} uptake through the exchanger from that occurring through voltage-operated Ca\textsuperscript{2+} channels. Mol. Pharmacol. 38: 385–392, 1990.


935. TRINER, L., P. KILLIAN, AND G. G. NAHAS. Ouabain hypoglyce-


936. TROSPER, T. L., AND K. D. PHILIPSON. Effects of divalent and


937. TRUESDELL, A. H., AND C. L. CHRIST. Glass electrodes for cal-

cium and other divalent cations. In: Glass Electrodes for Hydro-
gen and Other Cations, edited by G. Eisenman. New York: Dek-


938. VAN OS, C. H. Transcellular calcium transport in intestinal and


