Electrophysiology of the Sodium-Potassium-ATPase in Cardiac Cells

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Glitsch, Helfried Günther. Electrophysiology of the Sodium-Potassium-ATPase in Cardiac Cells. Physiol Rev 81: 1791–1826, 2001.—Like several other ion transporters, the Na⁺-K⁺ pump of animal cells is electrogenic. The pump generates the pump current $I_p$. Under physiological conditions, $I_p$ is an outward current. It can be measured by electrophysiological methods. These methods permit the study of characteristics of the Na⁺-K⁺ pump in its physiological environment, i.e., in the cell membrane. The cell membrane, across which a potential gradient exists, separates the cytosol and extracellular medium, which have distinctly different ionic compositions. The introduction of the patch-clamp techniques and the enzymatic isolation of cells have facilitated the investigation of $I_p$ in single cardiac myocytes. This review summarizes and discusses the results obtained from $I_p$ measurements in isolated cardiac cells. These results offer new exciting insights into the voltage and ionic dependence of the Na⁺-K⁺ pump activity, its effect on membrane potential, and its modulation by hormones, transmitters, and drugs. They are fundamental for our current understanding of Na⁺-K⁺ pumping in electrically excitable cells.

I. INTRODUCTION

A. Definition of Electrogenic Na⁺-K⁺ Pumping and Historical Background

The Mg²⁺-dependent, Na⁺-, and K⁺-activated ATPase (EC 3.6.1.37; Ref. 165) is the molecular basis of the Na⁺-K⁺ pump in animal cell membranes. The elucidation of the amino acid sequence of the Na⁺-K⁺-ATPase α-subunit (105, 163) and of the β-subunit (106, 162) of various species (180) has prompted numerous studies on ATPase molecules modified by mutagenesis and heterologously expressed in various cells to investigate the relationship between structure and function of the Na⁺-K⁺-ATPase (98, 117, 180). Furthermore, the existence of Na⁺/K⁺ isozymes has been studied in a large variety of species and tissues (19, 174). The Na⁺-K⁺-ATPase consists of at least two subunit proteins in stoichiometric amounts, the α- and β-subunit. The α-subunit exhibits a molecular mass of ~110 kDa and probably spans 10 times the cell membrane. Both NH₂ and COOH termini face the cytoplasm. The α-subunit contains the binding sites for ATP, Na⁺, K⁺, cardiac glycosides, specific inhibitors of the enzyme, and the phosphorylation site. Thus the α-subunit is largely responsible for the catalytic, transport, and pharmacological characteristics of the ATPase. The smaller β-unit, with a molecular mass of ~50 kDa (depending on the degree of glycosylation), has only one transmembrane domain. The COOH terminus is located at the large ectodomain of the subunit, whereas the NH₂ terminus is exposed to the cytoplasm. The activity of the Na⁺-K⁺-ATPase requires the β-subunit. The subunit modulates the transport characteristics of the ATPase and plays an important role in the maturation and proper membrane insertion of the Na⁺-K⁺-ATPase (19). It is still unclear whether the enzyme in vivo works as an(αβ)-monomer or an (αβ)₂-diprotomer (154). A small, hydrophobic protein of ~12 kDa, termed the γ-subunit, copurifies with the α- and β-subunits of the Na⁺-K⁺-ATPase. It has been found in various tissues of different species (124). The physiological function of the γ-subunit is not yet known. Like other cellular proteins, α- and β-subunits are expressed in various isoforms. At present four α-subunits (α₁-α₄) and three β-subunits (β₁-β₃) have been identified. The α₁, α₂, and α₃ are expressed in a variety of tissues, whereas the α₄-protein has been detected so far only in the rat testis (186). Both the α- and β-isomers of the Na⁺-K⁺-ATPase are expressed in a tissue-specific pattern (reviewed in Ref. 19). As to the α-subunits, the α₁-isofom is expressed ubiquitously, whereas the α₂-expression is predominant in cardiac and skeletal muscle, brain, and adipocytes. The α₃-isofom is abundant in neural tissues and in the ovary (19, 186). The tissue-specific expression of ATPase isoforms can be altered during development and by hormones. Any combination between one of the α-subunits α₁-α₃ and one of the β-subunits β₁-β₃ may result in an active Na⁺-K⁺-ATPase isoform (27). The isoforms differ in their kinetic characteristics with regard to activation by Na⁺, K⁺, ATP, and inhibition by cardiac glycosides. For example, the rat α₁β₁-isofom expressed in Sf9 insect cells shows a higher Na⁺ and K⁺ affinity but a lower affinity for ATP and a lower sensitivity toward cardiac glycosides than the α₂β₁-isofom of the Na⁺-K⁺-ATPase (19). Because of their different kinetic characteristics and tissue-specific expression, the various Na⁺-K⁺-ATPase isoforms probably meet different physiological demands. On the one hand, the α₁β₁-isofom may be a general, housekeeping enzyme, since it is ubiquitously expressed and exhibits suitable kinetic properties with relatively high Na⁺ and K⁺ affinities. On the other hand, α₂,α₃,isoforms are especially suited to restore the Na⁺ and K⁺ gradients across the cell membrane of electrically excitable cells due to their lower Na⁺ and K⁺ affinities and higher ATP affinity. With regard to the heart, the expression of the Na⁺-K⁺-ATPase isoforms is species specific. There is a marked variation of α₂- and α₃-expression among the species, whereas the α₁-isofom of the ATPase is present in cardiac tissue of all species studied. For instance, ventricular cells from adult human or macaque hearts express three α-isofoms, ventricular myocytes from the adult rat heart contain mainly α₁- and α₂-isofoms of the Na⁺-K⁺-ATPase, whereas the sheep heart expresses only the α₁-isofom (175). Whether guinea pig ventricular cells exclusively contains the α₁-isofom (175) or additional α₂-isoforms is not yet known. Like other cellular proteins, α- and β-subunits are expressed in various isoforms.
Electrogenicity denotes the characteristic of a biological transport mechanism to produce electrical current. The concept of electrogenic Na\textsuperscript{+}-K\textsuperscript{+} pumping slowly emerged during the 1950s (176). However, a most influential paper on active Na\textsuperscript{+}-K\textsuperscript{+} transport in axons of Sepia and Loligo (94) lent little support to this idea. Connelly (26) was the first to conclude on the basis of experimental evidence that the Na\textsuperscript{+}-K\textsuperscript{+} pump in nerve fibres is electrogenic. “... since 1960 more and more evidence has accumulated showing that the pump is probably always at least partly electrogenic, with more sodium being extruded than potassium taken up” (176). Today, it is generally accepted that the Na\textsuperscript{+}-K\textsuperscript{+} pump of animal cells is electrogenic and generates the pump current \(I_p\). Since, under physiological conditions, three Na\textsuperscript{+} are removed from the cell but only two K\textsuperscript{+} are taken up per pump cycle, \(I_p\) is an outward current. The existence of an electrogenic Na\textsuperscript{+}-K\textsuperscript{+} pump in cardiac cells was first suggested to explain the high temperature sensitivity of the cardiac resting potential (29). The early experimental data demonstrating electrogenic Na\textsuperscript{+} pumping in nerve and muscle have been reviewed in detail several years ago (119, 176). The experimental evidence for the electrocity of the pump is still controversial. Because the \(\alpha\)-subunits are specific cardiac steroid receptors exhibiting different sensitivity for these drugs, this controversy echoes in contradictory reports on the number of different glycoside receptors present in guinea pig cardiac ventricular myocytes (see below). For further information about the isoforms of the Na\textsuperscript{+}-K\textsuperscript{+}-ATPase, the reader is referred to pertinent reviews (19, 174).

The Na\textsuperscript{+}-K\textsuperscript{+} pump maintains the Na\textsuperscript{+} and K\textsuperscript{+} gradients between the cytosol and extracellular medium. The maintenance of the gradients is a prerequisite for the ionic homeostasis of the cells, for cell volume regulation, and for secondary active transports of amino acids, sugars, bile acids, neurotransmitters, ions, and other solutes across the cell boundary. Furthermore, in electrically excitable cells, creation and maintenance of Na\textsuperscript{+} and K\textsuperscript{+} gradients across the membrane are required for the generation of the resting potential and the generation and propagation of action potentials. It is clear that Na\textsuperscript{+}-K\textsuperscript{+} pumping is of prime functional significance in cells displaying relatively frequent electrical discharges over a long period of time, like cardiac cells. Our present understanding of Na\textsuperscript{+}-K\textsuperscript{+} pumping is outlined in the Post-Albers cycle (3, 137–139). For detailed information about the experimental basis and the scientific elaboration of the concept, the reader is referred to excellent, introductory reviews (72, 111). The simplified scheme of the pump cycle, shown in Figure 1, may facilitate the appreciation of the findings and ideas discussed below. According to the Post-Albers cycle, the Na\textsuperscript{+}-K\textsuperscript{+} pump essentially exists in two conformations, \(E_1\) and \(E_2\), which may be phosphorylated \((E_1\cdot P; E_2\cdot P)\) in the Fig. 1) or dephosphorylated. In the \(E_1\) ATP conformation, the cation-binding sites of the pump face the cytoplasm and preferentially bind Na\textsuperscript{+}, whereas in the P-\(E_2\) confirmation the binding sites face the extracellular space and preferably bind extracellular K\textsuperscript{+} \((K_o)\) (or its congeners). Some binding sites are located at the bottom of an “access channel” and not just at the surface of the pump molecule. During the Na\textsuperscript{+} or K\textsuperscript{+} translocation across the cell membrane carried out by the pump, there are states \([\text{Na}_3]E_1\cdot P\) and \(E_2(K_2)\) in Fig. 1 in which the transported cations are “occluded” in the pump molecule and unable to interchange with ions in the surrounding media.

\[\text{Na}_3E_1 \cdot \text{ATP} \rightarrow (\text{Na}_3)E_1 \cdot \text{P} \rightarrow \text{P} \cdot E_2 \cdot \text{Na}_2 \]

\[\text{K}_2E_1 \rightarrow E_1(K_1) \rightarrow \text{P} \cdot E_2K_1 \]

**FIG. 1.** An electrostatic model of the Na\textsuperscript{+}-K\textsuperscript{+} pump. The conformation \(E_2\) of the pump faces the cytosol and exhibits at the surface two negatively charged binding sites for which intracellular Na\textsuperscript{+} and K\textsuperscript{+} compete with different affinities. A third Na\textsuperscript{+}-specific neutral binding site is located inside the pump molecule. To reach the binding site the ion has to migrate through a narrow “access channel.” Phosphorylation of the pump by ATP induces Na\textsuperscript{+} occlusion \([\text{Na}_3]E_1\cdot P\). Transition to the \(E_2\) conformation opens the access to the extracellular medium via a narrow access channel. Binding of two K\textsuperscript{+} probably occurs at the bottom of the channel. It causes dephosphorylation and K\textsuperscript{+} occlusion \([\text{E}_2(K_2)]\). Release of two K\textsuperscript{+} into the cytoplasm completes the pump cycle. [From Heyse et al. (80), by copyright permission of The Rockefeller University Press.]
cardiac Na\textsuperscript{+}-K\textsuperscript{+} pump from multicellular preparations has been presented in various reviews (45, 47, 63, 65, 181).

The introduction of new methods and techniques into the electrophysiology of the Na\textsuperscript{+}-K\textsuperscript{+}-ATPase during the last two decades has markedly improved our knowledge of electrogenic Na\textsuperscript{+} pumping. The isolation of single cardiac cells (140) rendered possible \(I_p\) measurements (48) by means of patch-clamp techniques (77). Reliable \(I_p\) measurements require patch pipettes with a large tip diameter of \(\sim 4\)–5 \(\mu m\) and a low resistance (<2 M\(\Omega\)). In addition, membrane currents other than \(I_p\) have to be suppressed by adequate experimental conditions. The measurements demonstrated the activation of \(I_p\) by various intra- and extracellular cations and established the voltage dependence of \(I_p\) under a variety of conditions. Furthermore, they revealed the existence of transient pump currents in the Na\textsuperscript{+} and K\textsuperscript{+} limb of the Na\textsuperscript{+}-K\textsuperscript{+} pump cycle (130, 135). Clearly, electrophysiological investigations of the Na\textsuperscript{+}-K\textsuperscript{+} pump in noncardiac preparations have likewise produced exciting new insight into active cation transport. For example, studies of \(I_p\) in Xenopus oocytes (110, 147) and combined electrophysiological and tracer flux measurements in squid axons (53, 144) provided important data for our current understanding of structural and functional properties of the Na\textsuperscript{+}-K\textsuperscript{+} pump in animal cells (111, 145, 150, 180).

B. Physiological Significance of Electrophysiological Studies on the Cardiac Na\textsuperscript{+}-K\textsuperscript{+} Pump

The electrogenicity of ion pumps cannot be considered an epiphenomenon which is inevitably linked to ion pumping. Although the electrical potential difference set up by the pump across the cell membrane is thermodynamically equivalent to the simultaneously generated osmotic gradient, the former and the latter display quite different kinetic characteristics. As a consequence, the pump-generated membrane potential is, under many conditions, more efficient as a driving force for secondary active transports than the osmotic (ionic) gradient produced by the pump (see Ref. 111, p. 13–14). In addition, the pump current \(I_p\) directly affects the automaticity, the resting and action potential, and thereby the conduction of electrical impulses in excitable membranes. \(I_p\) is a direct indicator of Na\textsuperscript{+}-K\textsuperscript{+} pumping, since the coupling ratio 3Na\textsuperscript{+}:2K\textsuperscript{+}:1ATP per pump cycle remains constant under a variety of conditions including changes of the intracellular Na\textsuperscript{+} or extracellular K\textsuperscript{+} concentration and of the membrane potential. This applies for cardiac (46, 65) and noncardiac tissues (reviewed in Ref. 32). Measurements of \(I_p\) by means of electrophysiological methods offer the possibility to study the Na\textsuperscript{+}-K\textsuperscript{+} pump in its physiological environment, i.e., in the cell membrane separating two compartments of different ionic compositions (intra- and extracellular space) and to measure pump-mediated Na\textsuperscript{+} and K\textsuperscript{+} fluxes. In cardiac myocytes as in most animal cells, a membrane potential exists across the cell membrane. Because a translocation of electrical charge across the membrane constitutes the pump current, the \(I_p\) amplitude must depend on the membrane potential. The interaction between the electrogenic Na\textsuperscript{+}-K\textsuperscript{+} pump and the membrane potential is best studied by electrophysiological techniques. The measured voltage dependence of external and internal ionic binding to the cardiac pump has inspired our imagination of the molecular shape of the Na\textsuperscript{+}-K\textsuperscript{+} pump as a channel-like structure. Furthermore, electrophysiological studies have rendered possible the identification of additional partial reactions in the pump cycle displaying voltage sensitivity. They also revealed effects of Na\textsuperscript{+}-K\textsuperscript{+} pumping on currents produced by cardiac ionic channels or transporters in the vicinity of pump molecules. A major advantage of electrophysiological methods for studies on Na\textsuperscript{+}-K\textsuperscript{+} pump-generated Na\textsuperscript{+} and K\textsuperscript{+} fluxes across the sarcolemma over, for instance, tracer measurements is the much better time resolution (up to the microsecond range). Corresponding measurements of \(I_p\) provided new insights into the kinetics of the interaction between the Na\textsuperscript{+}-K\textsuperscript{+} pump and drugs with hitherto unrivalled precision.

This paper reviews some characteristics of the Na\textsuperscript{+}-K\textsuperscript{+} pump as a current-generating molecule in single cardiac cells. The pump current has been investigated in cells isolated from various regions of the heart of different mammalian species. These regions differ in morphology and function. They include the primary pacemaker (sinoatrial node), the cardiac conducting system (Purkinje fibres), and the working myocardium (atrial and ventricular). Especially the ventricular myocytes are easy to isolate and exhibit a high density of pump molecules in the cell membrane. Their cellular geometry is adequate for studies on the Na\textsuperscript{+}-K\textsuperscript{+} pump by means of patch-clamp techniques. For these reasons, several investigations that are pivotal for our understanding of the electrogenicity of the Na\textsuperscript{+}-K\textsuperscript{+} pump have been carried out on these cells.

II. ACTIVATION OF THE CARDIAC PUMP CURRENT BY MONOVALENT CATIONS

A. Activation of \(I_p\) by Intracellular Na\textsuperscript{+}

1. Mean affinity constant values for \(I_p\) activation by intracellular Na\textsuperscript{+} solution vary according to the experimental conditions

Earlier studies in multicellular cardiac preparations showed that intracellular Na\textsuperscript{+} (Na\textsubscript{i}) activates \(I_p\) (45, 65).
However, due to experimental problems, a quantitative relationship between [Na⁺] and Iₚ was difficult to establish. Whole cell recording from single cardiac cells certainly facilitated investigations on this issue, and, during the last decade, various mean affinity constant (K₀.₅) values for half-maximal Iₚ activation by intracellular Na⁺ ([Na⁺]pip) have been reported. In view of the variable numbers published, the following points should be kept in mind. Apart from species differences (including the expression of different Na⁺-K⁺-ATPase isoforms), the experimental procedure chosen for estimation of a K₀.₅ value is likely to affect the result. A low tip resistance (~ 1 MΩ) of the patch pipette is a prerequisite for correct measurements of Iₚ as a function of [Na⁺] (121). There is evidence suggesting the existence of a "fuzzy space" (112), in which the ionic concentration deviates from that in the bulk cytosolic solution (23, 159). As to the cardiac Iₚ-[Na⁺]pip relationship, it was demonstrated that the sub-sarcolemmal [Na⁺] is not always controlled by the [Na⁺] of the pipette solution. This is true not only during strong Iₚ activation but also in the steady state, at least in certain cells (16). Furthermore, a relatively high patch-pipette resistance might cause an additional Na⁺ gradient across the cytosol if active Na⁺/K⁺ exchange is strongly activated. Thus a thoughtful procedure is required to obtain reliable results. In addition, intracellular K⁺ are known to be competitive inhibitors of Na⁺ at intracellular Na⁺ binding sites of the Na⁺-K⁺ pump (73). Consequently, one would expect a lower [Na⁺]pip for half-maximal Iₚ activation (K₀.₅ value) from measurements where the main cation of the pipette solution is a weaker competitor than K⁺ [Cs⁺ or even tetraethylammonium ion (TEA⁺)]. It might be helpful to remember these points when reading the data presented in Table 1. They were mainly obtained at 30–37°C. Since, under physiological conditions, K⁺ is the main cation in cardiac cells, the “physiological” K₀.₅ value for Iₚ activation by Na⁺ may be in the range of ~20 mM Na⁺.

Figure 2 compares the Iₚ activation as a function of [Na⁺]pip using pipette solutions containing different main cations in guinea pig ventricular myocytes at 0 mV holding potential. Obviously, half-maximal Iₚ activation occurs at higher [Na⁺]pip if Cs⁺ instead of TEA⁺ or N-methyl-d-glutamate ion (NMDG⁺) is used as main pipette cation.

In summary, cardiac Iₚ is activated by intracellular Na⁺. The [Na⁺]pip reported for half-maximal Iₚ activation varies widely according to the experimental procedure and the ionic conditions chosen.

2. Is Iₚ activation by intracellular Na⁺ voltage dependent?

Whether or not binding of intracellular Na⁺ to the cardiac Na⁺-K⁺ pump is voltage sensitive is still a point of controversy. The sensitivity of the pump in guinea pig ventricular cells to [Na⁺]pip in the range between 3 and 50 mM increased with depolarization (131). An e-fold drop of the K₀.₅ value (i.e., [Na⁺]pip for half-maximal Iₚ activation) was estimated for a depolarization by 250 mV. However, the effect was present only in Na⁺-rich superfusion media but absent in Na⁺-free solution where the apparent affinity of the pump to Na⁺pip seemed to be voltage independent. Consequently, it was concluded that cytoplasmic Na⁺ binding to the cardiac Na⁺-K⁺ pump is voltage insensitive (131). The weak membrane potential dependence of the K₀.₅ value seen in myocytes superfused with Na⁺-rich media was ascribed to a voltage-dependent redistribution of enzyme intermediates in the pump cycle. In line with the findings described above, an increasing sensitivity of the pump to low [Na⁺]pip (5 mM) was ob-

### TABLE 1. Activation of Iₚ by Na⁺pip

<table>
<thead>
<tr>
<th>[Na⁺]pip, Value, mM</th>
<th>Species</th>
<th>Cells</th>
<th>Internal Cation</th>
<th>Holding Potential, mV</th>
<th>Reference No.</th>
</tr>
</thead>
<tbody>
<tr>
<td>~10</td>
<td>Guinea pig</td>
<td>Ventricular</td>
<td>Cs⁺</td>
<td>0</td>
<td>49</td>
</tr>
<tr>
<td>11</td>
<td>Guinea pig</td>
<td>Ventricular</td>
<td>Cs⁺</td>
<td>0</td>
<td>131</td>
</tr>
<tr>
<td>2.7</td>
<td>Guinea pig</td>
<td>Ventricular</td>
<td>TEA⁺, NMDG</td>
<td>0</td>
<td>9</td>
</tr>
<tr>
<td>~3</td>
<td>Guinea pig</td>
<td>Ventricular</td>
<td>TEA⁺</td>
<td>0</td>
<td></td>
</tr>
<tr>
<td>~43 or 1 (K₀.₅) and 29 (K₀.₀ = K₀.₅)</td>
<td>Guinea pig</td>
<td>Ventricular</td>
<td>K⁺ + TEA⁻</td>
<td>~40</td>
<td>128</td>
</tr>
<tr>
<td>14</td>
<td>Guinea pig</td>
<td>Atrial</td>
<td>Cs⁺</td>
<td>0</td>
<td>109</td>
</tr>
<tr>
<td>~40</td>
<td>Rat</td>
<td>Ventricular</td>
<td>K⁺ + Cs⁺</td>
<td>+20 to ~120</td>
<td>167</td>
</tr>
<tr>
<td>21.4</td>
<td>Rat</td>
<td>Ventricular</td>
<td>K⁺</td>
<td>0</td>
<td>84</td>
</tr>
<tr>
<td>7.8</td>
<td>Rat</td>
<td>Ventricular</td>
<td>TEA⁺</td>
<td>0</td>
<td>84</td>
</tr>
<tr>
<td>21.4</td>
<td>Rabbit</td>
<td>Ventricular</td>
<td>K⁺ + TMA⁺</td>
<td>~40</td>
<td>184</td>
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<tr>
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<td>Cs⁺</td>
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<td>161</td>
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<td>84</td>
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<tr>
<td>14</td>
<td>Rabbit</td>
<td>Sinoatrial node</td>
<td>Cs⁺</td>
<td>0</td>
<td>151</td>
</tr>
<tr>
<td>17.4</td>
<td>Chicken</td>
<td>Embryonic cardiac myocytes</td>
<td>K⁺</td>
<td>~70</td>
<td>168</td>
</tr>
<tr>
<td>9</td>
<td>Sheep</td>
<td>Purkinje cardioballs</td>
<td>Cs⁺</td>
<td>~40</td>
<td>66</td>
</tr>
</tbody>
</table>

TEA⁺, tetraethylammonium ion; NMDG, N-methyl-d-glutamate; TMA⁺, tetramethylammonium ion; K₀.₅, mean affinity constant; Iₚ, pump current.
served with depolarization in sheep cardiac Purkinje cells superfused with Na\(^+\)-rich medium (66). Similarly, an increased apparent Na\(^+\) affinity of the pump to low [Na\(^+\)]\(_{\text{pip}}\) with depolarization was reported for guinea pig atrial myocytes superfused with Na\(^+\)-containing or Na\(^+\)-free solution (109). However, little evidence for voltage-dependent internal Na\(^+\) binding between 20 and 85 mM Na\(^+\)\(_{\text{pip}}\) was found during whole cell recording from rat ventricular cells in Na\(^+\)-containing medium (167). The results described so far were obtained with pipette solutions containing K\(^+\) or Cs\(^+\), which may compete with Na\(^+\) for internal cation binding sites of the Na\(^+\)-K\(^+\) pump and may thereby obscure the mechanism of Na\(^+\) binding. In a more recent study, TEA\(^+\) or NMDG\(^+\) were used as noncompetitive main cations in the pipette solution, and at ≤5 mM Na\(^+\)\(_{\text{pip}}\), voltage-sensitive binding of internal Na\(^+\) to the pump of guinea pig ventricular myocytes was clearly shown in Na\(^+\)-free solution (9). Figure 3 is from this work and demonstrates that the \(K_{0.5}\) value for \(I_p\) activation by [Na\(^+\)]\(_{\text{pip}}\) varies with membrane potential in myocytes containing either NMDG\(^+\) (A) or TEA\(^+\) (B) as the main cation species. Thus experimental evidence is accumulating that the apparent affinity of the cardiac Na\(^+\)-K\(^+\) pump to intracellular Na\(^+\) is voltage dependent and increases with depolarization. This conclusion is supported by observations from experiments on cell-free Na\(^+\)-K\(^+\)-ATPase systems (74, 89, 134, 171).

B. Activation of \(I_p\) by Extracellular K\(^+\) and Its Congeners

1. Measurements in Na\(^+\)-containing solution

Under physiological conditions, the Na\(^+\) pump of animal cells is activated by extracellular K\(^+\). The activation of the pump by external K\(^+\) follows sigmoid saturation kinetics that can be described by a Hill equation. Earlier studies on the pump activation by extracellular K\(^+\) in multicellular cardiac preparations have been reviewed in useful articles (45, 65). The majority of the data suggest half-maximal \(I_p\) activation by [K\(^+\)]\(_{\text{ext}}\) in the low millimolar range. Similar \(K_{0.5}\) values were obtained from experiments on single cardiac myocytes. The data obtained at 30–37°C are presented in Table 2. The \(I_p\) activation by two K\(^+\) congeners, Ti\(^+\) and NH\(_4\)\(^+\), was tested in rabbit Purkinje cells (15). The authors derived \(K_{0.5}\) values of 0.4 mM Ti\(^+\) and 5.7 mM NH\(_4\)\(^+\) and noted no alteration in the shape of the \(I_p\)-Voltage (V) curve if equipotent concentrations of K\(^+\), Ti\(^+\), or NH\(_4\)\(^+\) were applied. The following relative potency of extracellular monovalent cations to activate \(I_p\) was observed in rabbit sinoatrial node cells: K\(^+\) > Rb\(^+\) > Cs\(^+\) > Li\(^+\) (151). The order of potency is the same as that observed for the activation of the isolated Mg\(^{2+}\)-dependent, Na\(^+\)- and K\(^+\)-activated ATPase, which is the
molecular basis of the Na\(^+\)-K\(^+\) pump (155). In addition, the order of potency confirmed the sequence deduced from voltage-clamp measurement on guinea pig papillary muscles and sheep Purkinje fibers (39).

2. \(K_{0.5}\) values for \(I_p\) activation by external cations in Na\(^+\)-free media

Since the early observation on erythrocytes it is known that external Na\(^+\) and K\(^+\) compete for common binding sites (138). As a consequence, the \(K_{0.5}\) value for pump activation by extracellular K\(^+\) is appreciably lower in Na\(^+\)-free than in Na\(^+\)-containing media. Thus one would expect for cardiac cells a lower \(K_{0.5}\) value for the \(I_p\) activation by K\(^+\) and its congeners in Na\(^+\)-free than in Na\(^+\)-containing solution. In fact, a \(K_{0.5}\) value of 0.22 mM was derived for the activation of \(I_p\) by \(K_o\) in guinea pig ventricular myocytes superfused with Na\(^+\)-free medium (holding potential 0 mV) (131). This is shown in Figure 4, where normalized pump currents are plotted versus [K\(^+\)]\(_o\). The holding potential is 0 mV. The \(K_{0.5}\) value for \(I_p\) activation by \(K_o\) in these cells decreases from 1.5 mM K\(^+\)\(_o\) in Na\(^+\)-containing solution to 0.22 mM K\(^+\)\(_o\) in Na\(^+\)-free medium (131). Similarly, a \(K_{0.5}\) value of 0.2 mM was reported for the \(I_p\) activation by \(K_o\) in rat and guinea pig

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

**FIG. 4.** Activation of Na\(^+\)-K\(^+\) pump current in guinea pig ventricular myocytes at 0 mV by [K\(^+\)]\(_o\) at high [Na\(^+\)]\(_o\) \((A)\) or at zero [Na\(^+\)]\(_o\) \((B)\). In both cases, pump current amplitude at each [K\(^+\)]\(_o\) was normalized to control runs at 5.4 mM K\(^+\). The graphs show mean values ± SE of the resulting relative pump currents plotted against [K\(^+\)]\(_o\) and the curves show least-squares fits of the Hill equation to the unweighted data. [Na\(^+\)]\(_o\) was 50 mM in all cells. \(A\): data from 17 cells at high [Na\(^+\)]\(_o\); best-fit parameters: maximal relative current = 1.30 ± 0.10; \(K_{0.5}\) = 1.54 ± 0.31 mM K\(^+\); \(n_H\) = 0.96 ± 0.13. \(B\): data from 4 cells at zero [Na\(^+\)]\(_o\); best-fit Hill equation parameters: maximal relative current = 1.03 ± 0.05, \(K_{0.5}\) = 0.22 ± 0.03 mM K\(^+\), \(n_H\) = 1.12 ± 0.14. [Adapted from Nakao and Gadsby (131).]

**TABLE 2.** Activation of \(I_p\) by \(K_o^+\) in Na\(^+\)-containing solution

<table>
<thead>
<tr>
<th>(K_{0.5}) Value, mM</th>
<th>Species</th>
<th>Cells</th>
<th>Internal [Na(^+)](_o), mM</th>
<th>Holding Potential, mV</th>
<th>Reference No.</th>
</tr>
</thead>
<tbody>
<tr>
<td>1.5</td>
<td>Guinea pig</td>
<td>Ventricular</td>
<td>50(_{pip})</td>
<td>0</td>
<td>131</td>
</tr>
<tr>
<td>1.1</td>
<td>Guinea pig</td>
<td>Ventricular</td>
<td>50(_{pip})</td>
<td>-20</td>
<td>84</td>
</tr>
<tr>
<td>1.2</td>
<td>Guinea pig</td>
<td>Atrial</td>
<td>50(_{pip})</td>
<td>0</td>
<td>109</td>
</tr>
<tr>
<td>1.2</td>
<td>Rat</td>
<td>Ventricular</td>
<td>10(_{pip})</td>
<td>-40</td>
<td>107</td>
</tr>
<tr>
<td>3.5</td>
<td>Rat</td>
<td>Ventricular</td>
<td>85(_{pip})</td>
<td>-40</td>
<td>107</td>
</tr>
<tr>
<td>0.9</td>
<td>Rat</td>
<td>Ventricular</td>
<td>100(_{pip})</td>
<td>-20</td>
<td>84</td>
</tr>
<tr>
<td>1.9</td>
<td>Chicken</td>
<td>Embryonic cardiac cells</td>
<td>Augmented</td>
<td>-70</td>
<td>168,170</td>
</tr>
<tr>
<td>1.4</td>
<td>Rabbit</td>
<td>Sinoatrial node</td>
<td>30(_{pip})</td>
<td>0</td>
<td>151</td>
</tr>
<tr>
<td>1.9</td>
<td>Rabbit</td>
<td>Purkinje cells</td>
<td>10(_{pip})</td>
<td>-20</td>
<td>15</td>
</tr>
<tr>
<td>0.8</td>
<td>Dog</td>
<td>Purkinje cells</td>
<td>Not determined</td>
<td>-30</td>
<td>25</td>
</tr>
</tbody>
</table>

The subscript pip refers to the Na\(^+\) concentration of the patch pipette solution.
ventricular cells (84). Furthermore, the maximum $I_p$ amplitude that can be activated in Na$^+$-free media by K$^+$ and congeners declines with increasing $K_{0.5}$ values of the various monovalent cations. $K_{0.5}$ values for the $I_p$ activation by external K$^+$ or Tl$^+$ of $\sim 0.22$ mM K$^+$ and $\sim 0.08$ mM Tl$^+$ were estimated in rat ventricular myocytes superfused with Na$^+$-free solution (holding potential 0 mV) (13). In Na$^+$-free media, the $K_{0.5}$ values for the activation of the pump current by K$^+_o$ or K$^+$ congeners in rabbit Purkinje cells amount to 0.05 mM Tl$^+_o$, 0.08 mM K$^+_o$, 0.4 mM extracellular NH$_4^+$, and 1.5 mM Cs$^+_o$ (holding potential 0 mV) (18). The authors concluded that in general the $K_{0.5}$ values for the Na$^+$-K$^+$ pump activation in cardiac cells superfused with Na$^+$-free solution are lower by a factor of 10–20 than those obtained in Na$^+$-containing media. The same is true for the $K_{0.5}$ values reported from $I_p$ measurements at the squid axons (144) and Xenopus oocytes (133), although the absolute numbers are somewhat larger than those estimated for cardiac cells.

3. Activation of $I_p$ by K$^+$ in cardiac cells is voltage dependent

The negative slope of the $I_p$-V relationship in Xenopus oocytes is probably due to voltage-sensitive binding of extracellular K$^+$ to the Na$^+$-K$^+$ pump (147). It is considered that K$^+$ binding to the pump occurs at the bottom of a "high field, narrow access channel" (see Ref. 111, p. 74–83). To reach their binding sites, the K$^+$ have to migrate through the channel within the electrical field across the cell membrane. The membrane potential thereby affects the local K$^+$ concentration at the binding sites which, in general, differ from [K$^+$] of the extracellular medium. Depolarization of the cell membrane decreases the local [K$^+$] and increases the estimated (apparent) $K_{0.5}$ value for $I_p$ activation by K$^+$. Vice versa, hyperpolarization increases the local [K$^+$] at the binding sites and decreases the $K_{0.5}$ value. Under certain experimental conditions, the cardiac $I_p$-V relationship also exhibits a region of negative slope. This is true for myocytes superfused with Na$^+$-containing solution (13, 15, 84, 167) or Na$^+$-free (poor) media (13, 15, 18, 52), especially at low (<$K_{0.5}$ value) concentrations of K$^+_o$ or its congeners. A detailed study on the activation of $I_p$ by K$^+_o$, Tl$^+_o$, extracellular NH$_4^+$, and Cs$^+_o$ at various membrane potentials ($V_c$) was carried out with rabbit cardiac Purkinje cells in Na$^+$-free solution (18) (Fig. 5; see also Table 3). Figure 5 shows that the apparent affinity of the cardiac Na$^+$-K$^+$ pump to K$^+_o$, Tl$^+_o$, Cs$^+_o$, or extracellular NH$_4^+$ depends on voltage. The affinity decreases with depolarization, whereas the corresponding $K_{0.5}$ values increase according to the Boltzmann equation

$$K_{0.5} = K_{0.5(V_c=0\ mV)} \cdot \exp(\alpha \cdot FV_c/RT)$$

(1)

where $K_{0.5(V_c=0\ mV)}$ denotes the $K_{0.5}$ value at zero potential. $\alpha$ is a steepness factor, and $F$, $V_c$, $R$, and $T$ have their usual meanings. $K_{0.5(V_c=0\ mV)}$ values for $I_p$ activation by various external cations and the respective $\alpha$-values are collected in Table 3 (from Ref. 18). Because the steps subsequent to K$^+$ binding in the K$^+$ limb of the pump cycle are supposed to be voltage independent (135), it is assumed that the binding of K$^+_o$ or its congeners to the Na$^+$-K$^+$ pump probably is the voltage-sensitive process involved. From the $\alpha$-values and the Hill coefficients derived, it can be estimated that the external activator cations sense $\sim 0.2$ of the membrane potential at their binding sites (see Ref. 150). In summary, the binding of K$^+_o$ and isst congeners to the cardiac Na$^+$-K$^+$ pump is most probably voltage dependent.

C. Li$^+$ Binds to Extra- and Intracellular Binding Sites of the Cardiac Na$^+$-K$^+$ Pump and Activates $I_p$

It is known from earlier studies on multicellular cardiac preparations that Li$^+$ is a weak external pump activator cation (see Ref. 64 for references). As to isolated

**Table 3. $K_{0.5(V_c=0\ mV)}$ and $\alpha$-values for $I_p$ activation by external cations in Na$^+$-free solution**

<table>
<thead>
<tr>
<th>Cation</th>
<th>$K_{0.5(V_c=0\ mV)}$</th>
<th>$\alpha$</th>
</tr>
</thead>
<tbody>
<tr>
<td>Ti$^+$</td>
<td>0.05</td>
<td>0.24</td>
</tr>
<tr>
<td>K$^+$</td>
<td>0.08</td>
<td>0.29</td>
</tr>
<tr>
<td>NH$_4^+$</td>
<td>0.4</td>
<td>1.5</td>
</tr>
<tr>
<td>Cs$^+$</td>
<td>1.5</td>
<td>0.18</td>
</tr>
</tbody>
</table>

[From Bielen et al. (18). Copyright 1993 The Physiological Society.]
cardiac cells, \( I_p \) is activated by \( \text{Li}^+ \) in single rabbit Purkinje cells (15). In isolated sinoatrial cells, \( \text{Li}^+ \) is an ~14-fold weaker external activator than \( \text{K}^+ \) (151). Interestingly, intracellular \( \text{Li}^+ \) activates \( \text{Li}^+/\text{K}^+ \) and \( \text{Li}^+/\text{Li}^+ \) exchange via the \( \text{Na}^+/\text{K}^+ \) pump in rabbit ventricular myocytes (83). However, internal \( \text{Li}^+ \) is a much less effective activator than intracellular \( \text{Na}^+ \). The pump current density elicited by 160 mM \( \text{Li}^+ \) was clearly lower than the current density measured at high \( [\text{Na}^+]_o \). A \( K_{\text{m},1} \) value for \( I_p \) activation by \( [\text{Li}^+]_o \) of 36 mM was found for guinea pig ventricular myocytes superfused with \( \text{Na}^+ \)-free (chocline+) medium containing 1 mM K+ (holding potential ~20 mV) (87). Half-maximal \( I_p \) activation was observed at 23 or 73 mM \( \text{Li}^+ \) in myocytes containing either 50 to 100 mM \( [\text{Na}^+]_o \) or 100 mM \( [\text{Li}^+]_o \), respectively. Binding of \( \text{Li}^+ \) to the pump is voltage sensitive. At the locus of their binding, \( \text{Li}^+ \) sense ~0.2 of the membrane potential across the sarcolemma. Thus \( \text{Li}^+ \), like the other \( K^+ \) congeners and \( K^+ \) itself, probably binds to the \( \text{Na}^+/\text{K}^+ \) pump at the bottom of an access channel. Regions of positive and/or negative slope persist in the cardiac \( I_p \)-V curve under conditions where the intra- and extracellular cation binding sites of the \( \text{Na}^+/\text{K}^+ \) pump should be saturated by \( \text{Li}^+ \). Therefore, mechanisms apart from binding/rebinding of the transported cations are voltage dependent and affect the shape of the cardiac \( I_p \)-V relationship (87).

D. Pump Current Densities, Pump Site Densities, and Maximum Turnover Rate

Usually, in experiments designed to measure \( I_p \) the \( \text{Na}^+/\text{K}^+ \) pump of cells is strongly activated by nearly saturating concentrations of \( \text{Na}^+ \) and \( [\text{K}^+]_o \). The measured \( I_p \) densities depend not only on these ionic concentrations, but also on the membrane potential and the temperature at which the measurements were carried out. In addition, cells in culture often exhibit lower \( I_p \) densities than freshly isolated cells. Furthermore, the species of the main intracellular cation used (\( \text{K}^+ \), \( \text{Cs}^+ \), or \( \text{NMDG}^+ \)) affects the \( I_p \) density. Ionic species that do not compete with \( \text{Na}^+ \) for intracellular binding sites of the pump (for instance \( \text{NMDG}^+ \)) tend to evoke higher \( I_p \) densities (166). It might be helpful for the reader of the data presented below to keep these points in mind. The first measurement of the \( I_p \) density in isolated guinea pig ventricular myocytes yielded 1–1.5 \( \mu \)A/cm\(^2\) (48). The maximum \( I_p \) density of these cells was estimated to be ~1.6 \( \mu \)A/cm\(^2\) (131). In sheep Purkinje cardioballs (1–3 days in culture), an \( I_p \) density of 1.1 \( \mu \)A/cm\(^2\) has been observed (66). A maximum \( I_p \) of ~1.9 \( \mu \)A/cm\(^2\) has been derived from \( I_p \) measurements in rabbit sinoatrial node cells (151). Guinea pig atrial cardioballs (1 day in culture) exhibit an \( I_p \) density of 0.66 \( \mu \)A/cm\(^2\) (109), whereas rabbit ventricular myocytes display \( I_p \) densities between ~1.7 and ~2.2 \( \mu \)A/cm\(^2\) (14, 75, 76). \( I_p \) densities reported for rat ventricular cells vary between 1.6 \( \mu \)A/cm\(^2\) (166) and ~4.3 \( \mu \)A/cm\(^2\) (101). All these numbers were obtained from measurements at membrane potentials greater than ~45 mV and near body temperature. They agree reasonably well with earlier data for the active \( \text{Na}^+ \) efflux via the cardiac \( \text{Na}^+ \) pump obtained by various nonelectrophysiological methods (63). (An \( I_p \) density of 1 \( \mu \)A/cm\(^2\) translates to a pump-mediated flux of ~30 pmol/cm\(^2\)s\(^{-1}\)). They are also in line with previous estimates of \( I_p \) density in multicellular cardiac preparations (28). For comparison, simultaneous measurements of \( I_p \) density and active \( ^{22}\text{Na} \) efflux in squid axons yielded 0.89 \( \mu \)A/cm\(^2\) and ~25 pmol/cm\(^2\)s\(^{-1}\) (144), again in accordance with earlier flux data (94).

From the maximum quantity of movable charge derived from measurements of transient pump currents in single cardiac cells (see sect. nB), the pump site density of the myocytes has been estimated assuming a single charge (1.6 \( \times \) \( 10^{-10} \) C) to be transferred per pump molecule and the specific membrane capacitance to be 1 \( \mu \)F/cm\(^2\). The first number obtained by this procedure for the pump site density of single cardiac cells was published by Nakao and Gadsby (130). According to the authors, the pump site density of guinea pig ventricular myocytes is ~1,200/\( \mu \)m\(^2\). Later estimates include slightly higher numbers of 2,200 to 2,800 pumps/\( \mu \)m\(^2\) for guinea pig ventricular cells (108) and ~2,600 sites/\( \mu \)m\(^2\) for rat ventricular myocytes (35). These values are higher by a factor 2 to 4 than an earlier number derived from \( I_p \) measurements in a guinea pig multicellular ventricular preparation (28).

The maximum turnover rate of the charge transfer by the cardiac \( \text{Na}^+/\text{K}^+ \) pump can be calculated if the maximum \( I_p \) density and the pump site density are known. Accordingly, a maximum turnover rate of ~80/s has been obtained from the data mentioned above for guinea pig ventricular myocytes (51). A higher maximum turnover rate of ~200/s has been derived from measurements of transient pump currents in excised patches of rat ventricular cells (42). Both numbers apply to turnover rates at 0 mV and 36°C.

The pump site densities reported above are within the range of earlier estimates in a variety of cell species by means of different methods, mainly cardiac glycoside binding (see Table 3 in Ref. 32). These estimates vary considerably among the cell types, whereas the calculated turnover rates are much more similar and amount to ~100/s at body temperature (32).

III. THE REVERSAL POTENTIAL OF THE CARDIAC PUMP CURRENT

A. Theoretical Considerations

The free energy of intracellular ATP hydrolysis \( (\Delta G_{\text{ATP}}) \) fuels the active \( \text{Na}^+/\text{K}^+ \) transport by the \( \text{Na}^+/\text{K}^+ \)
pump. It amounts to about $-60 \, \text{kJ/mol}$ in many animal cells (31). The energy is used to transport $3 \, \text{mol} \ Na^+$ and $2 \, \text{mol} \ K^+$ against their respective concentration gradient (osmotic work) and the electric charge of $1 \, \text{mol} \ Na^+$ against the electrical field across the cell membrane (electrical work). Thus the physiological active $Na^+/K^+$ transport proceeds as long as

$$|\Delta G_{\text{ATP}}| > 3 \times RT \ln(a^o_{Na}/a^i_{Na}) + 2 \times RT \ln(a^o_{K}/a^i_{K}) - F \cdot E_m$$

where $a^o$ and $a^i$ represent the activities of the transported ions at both sides of the cell membrane, $E_m$ denotes the membrane potential, and $R$, $T$, and $F$ have their usual meanings. According to Equation 2, there should be a membrane potential where $\Delta G_{\text{ATP}}$ equals the energy required for the active cation transport. This potential is called the reversal potential ($E_{\text{rev}}$) of the $Na^+/K^+$ pump. No active $Na^+/K^+$ fluxes occur at $E_{\text{rev}}$, and $I_p$ vanishes. If the membrane potential becomes more negative than $E_{\text{rev}}$, the $Na^+/K^+$ pump runs backward producing an active $Na^+$ influx into the cell and generating an inwardly directed $I_p$. $E_{\text{rev}}$ can be derived from Equation 2 by

$$E_{\text{rev}} = \Delta G_{\text{ATP}}/F + 3E_{Na} - 2E_K$$

where $E_{Na}$ and $E_K$ stand for the Nernst potential of the respective cation. From Equation 3, $E_{\text{rev}}$ can be calculated to be $E_{\text{rev}} = -60 \times 10^3 (V \cdot A \cdot s/mol)/9.65 \times 10^4 (A \cdot s/mol) + 3 \times 0.07 V + 2 \times 0.09 V = -0.232 \, \text{V}$ (3a)

Thus under physiological conditions $E_{\text{rev}}$ is beyond the membrane potentials that are experimentally accessible. However, according to Equation 3, it is possible to shift $E_{\text{rev}}$ to more positive membrane potentials by lowering $\Delta G_{\text{ATP}}$ and steepening the ionic gradients. $\Delta G_{\text{ATP}}$ is given by

$$\Delta G_{\text{ATP}} = \Delta G_{\text{ATP}}^0 + RT \ln([ADP] \cdot [P_i]/[ATP])$$

where $\Delta G_{\text{ATP}}^0$ denotes the standard free energy of ATP hydrolysis and $[ATP]$, $[ADP]$, and $[P_i]$ represent the intracellular concentration of adenosine triphosphate, adenosine diphosphate, and inorganic phosphate, respectively. Thus a lower (less negative) $\Delta G_{\text{ATP}}$ can be obtained by increasing the $[ADP] \cdot [P_i]/[ATP]$ ratio in the cell studied.

B. Evidence for Backward Running $Na^+/K^+$ Pump and Determination of the $Na^+/K^+$ Pump $E_{\text{rev}}$

The procedure outlined above was first applied to squid giant axons, and an inwardly directed $I_p$ was demonstrated (34). Under similar experimental conditions, an inwardly directed $I_p$ was measured in guinea pig ventricular myocytes over a range of membrane potentials between $+40$ and $-120 \, \text{mV}$ (6). Figure 6 presents some of the results. Figure 6A (bottom trace) demonstrates that the strophanthidine-

![Figure 6](http://physrev.physiology.org/)

**Figure 6.** Voltage dependence of inward current generated by the inwardly directed $Na^+/K^+$ pump. A: chart recording of membrane potential (top) and current (bottom); holding potential, $-40 \, \text{mV}$. The horizontal bar marks exposure to $0.5 \, \text{mM}$ strophanthidin (str). The K$^+$-free external solution contained $5 \, \text{mM}$ Ba$^{2+}$; the pipette solution was Na$^+$ and Cs$^+$ free and contained $145 \, \text{mM}$ K$^+$, $5 \, \text{mM}$ MgATP, $5 \, \text{mM}$ Tris$_2$ADP, and $5 \, \text{mM}$ phosphate. B: superimposed records of strophanthidin-sensitive currents for 80-ms pulses to $+40$, $0$, $-60$, and $-100 \, \text{mV}$, obtained by subtracting each trace recorded in the presence of strophanthinidin from the average of control traces recorded during pulses to the same potential just before and just after the exposure to strophanthidin. C: whole cell current-voltage relationships from the experiment in A, determined before (○), during (△), and after (■) exposure to strophanthidin. Ordinate, steady current levels; abscissa, membrane potential. D: current-voltage relationship of the backward-running $Na^+/K^+$ pump. Ordinate, steady levels of the strophanthidin-sensitive currents represented in B; abscissa, membrane potential. [Adapted from Bahinski et al. (6).]
inhibited pump current \( I_p \) of a guinea pig ventricular myocyte is inwardly directed under the conditions chosen. As shown in Figure 6B, \( I_p \) remains an inward current even at positive potentials. Figure 6C displays I-V curves before, during, and after application of the cardiac steroid, a specific inhibitor of the Na+-K+ pump. The I-V relationships were derived from the experiment illustrated in Figure 6A. Figure 6D exhibits the \( I_p-V \) curve of the backward running Na+-K+ pump obtained from the strophanthidin-sensitive current \( I_p \) at various potentials, as illustrated in Figure 6B. \( I_p \) was small at +40 mV, increased with hyperpolarization, and reached an apparent plateau of about −0.32 μA/cm² near −100 mV. By means of a similar approach, an inward \( I_p \) between +30 mV and −110 mV that did not reach a plateau at the most negative membrane potential tested was observed in cardiac Purkinje cells. The \( I_p \) density amounted to −0.13 μA/cm² at −95 mV (71). Furthermore, \( E_{rev} \) shifted to more positive potentials at less negative \( \Delta G_{ATP} \) values. Shifting \( \Delta G_{ATP} \) to less negative values also diminished \( I_p \) over the entire voltage range studied. However, the concentration of extracellular Cs⁺ required for half-maximal \( I_p \) activation remained unchanged. Equation 3 predicts changes of \( E_{rev} \) by variation of the transmembrane gradients of the pumped cations at constant \( \Delta G_{ATP} \). This prediction was verified (69). Flattening the ionic gradients increased \( I_p \) over the entire voltage range studied and shifted \( E_{rev} \) toward more negative potentials. Conversely, steepening the gradients diminished \( I_p \) and shifted \( E_{rev} \) to more positive potentials.

The \( I_p-V \) curve of the backward running Na⁺-K⁺ pump was also studied in internally dialyzed squid axons under voltage clamp (143). There was a steady decline of \( I_p \) density from an apparent plateau at −80 to −100 mV (−0.24 μA/cm²) to practically zero at +30 mV. In contrast to earlier observations (34), a negative slope of the \( I_p-V \) relationship was not found. In K⁺-free solution, an inwardly directed \( I_p \) was measured in Xenopus oocytes with a reduced intracellular [Na] and an augmented [ADP]·[P]/[ATP] ratio. The \( I_p \) density amounted to about −0.1 μA/cm² at −100 mV without an apparent plateau and declined with depolarization. Under these conditions the \( I_p \) reversal potential was obviously at positive membrane potentials (38).

In summary, the studies demonstrate under suitable conditions in various cell species a backward running Na⁺-K⁺ pump generating an inward \( I_p \) in line with thermodynamic considerations.

IV. VOLTAGE DEPENDENCE OF CARDIAC PUMP CURRENTS

A. Cardiac Steady-State \( I_p-V \) Relationships

1. Basic characteristics of the \( I_p-V \) curve

Whole cell recording is a mode of the patch-clamp technique (77) which permits a much better control of the membrane potential and the ionic composition of the intracellular compartment than earlier voltage-clamp techniques applied to multicellular preparations. Gadzby et al. (48) were the first to study the voltage dependence of \( I_p \) by whole cell recording from isolated, single guinea pig ventricular cells in Na⁺-containing solution after minimizing passive Na⁺, Ca²⁺, and K⁺ currents. According to the authors, the \( I_p-V \) relationship of the myocytes is sigmoid in shape with a steep positive slope between −100 and 0 mV, a less steep slope at more negative potentials, and nearly no voltage dependence of \( I_p \) at positive membrane potentials (see also Fig. 7A, circles). A further careful study by the authors confirmed these characteristics of the cardiac \( I_p-V \) curve (51). However, a region of negative slope in the \( I_p-V \) curve was not observed. A decrease of \( I_p \) with hyperpolarization in guinea pig ventricular cells was also reported in 1987 by others (123) in abstract form. The pump current of adult isolated rat cardiac myocytes displays a similar voltage dependence as in guinea pig myocytes (167). This is also true for the voltage dependence of \( I_p \) in rabbit ventricular cells dialyzed with a pipette solution containing 80 mM Na⁺ (79).

For unknown reasons, some authors were unable to detect any voltage dependence of \( I_p \) in guinea pig ventricular myocytes (127). In the cardiac conducting system, the \( I_p-V \) curve shows little voltage dependence at membrane potentials positive to −20 mV. The pump current declines with hyperpolarization (66).

2. Effects of internal Na⁺ on the \( I_p-V \) relationship of cardiac cells

Already in 1987 it was reported that lowering internal [Na⁺] diminishes the pump current of guinea pig ventricular cells in Na⁺-rich solution and shifts the \( I_p-V \) curve to the right (to more positive potentials), whereas the voltage dependence of \( I_p \) persists (50). However, in the absence of external Na⁺, lowering [Na⁺]pip from 50 to ≤17 mM scaled down the \( I_p-V \) relationship of these cells without a marked shift toward more positive potentials (131). A similar shift of the normalized \( I_p-V \) curve to the right and a reduction of \( I_p \) if [Na⁺]pip was lowered from 50 to 5 mM occurred in sheep cardiac Purkinje cells superfused with Na⁺-rich medium (66). Reducing [Na⁺]pip from 85 to 20 mM scales down the \( I_p-V \) relationship of rat myocytes at 145 mM Na⁺ without any shift of the \( I_p-V \) curve along the voltage axis (167). It seems likely that [Na⁺]pip in these experiments was not low enough to produce this shift. By way of contrast, both a scaling down and a rightward shift of the \( I_p-V \) relationship were observed in guinea pig atrial myocytes superfused with Na⁺-free or Na⁺-containing media (109). Recently, a scaling down and a shift of the normalized \( I_p-V \) curve toward more positive potentials were reported from measurements on guinea pig ventricular cells in Na⁺-free solution after lowering
[\text{Na}^+]_{\text{pip}}$ from 50 to $\leq$5 mM (main cation in pipette: TEA$^+$ or NMDG$^+$) (9). Some of the findings are displayed in Figure 7, B and C. Lowering [\text{Na}^+]_{\text{pip}}$ from 50 to 5 mM or below reveals the effect of [\text{Na}^+]_{\text{pip}}$ on the cardiac $I_p$-$V$ curve in myocytes containing NMDG$^+$ (Fig. 7B) or TEA$^+$ (Fig. 7C) as main cation. The relative $I_p$ activation at low [\text{Na}^+]_{\text{pip}}$ increases with depolarization, i.e., the $K_{0.5}$ value for $I_p$ activation by [\text{Na}^+]_{\text{pip}}$ declines (Fig. 3). In Na$^+$-free pipette solutions, $I_p$ is absent in the entire voltage range tested.

In summary, reducing [\text{Na}^+]_{\text{pip}}$ to values well below the $K_{0.5}$ value reveals not only a scaling down of the $I_p$-$V$ relationship in cardiac cells but also a rightward shift of the $I_p$-$V$ curve to more positive membrane potentials. The effect is observed in Na$^+$-free and Na$^+$-containing solution. The reason why Nakao and Gadsby (131) did not detect the shift of the $I_p$-$V$ curve to the right at low [\text{Na}^+]_{\text{pip}}$ and [\text{Na}^+]_o remains unclear. The shift may be produced by the voltage sensitivity of internal Na$^+$ binding to the Na$^+$-K$^+$ pump. At [\text{Na}^+]_{\text{pip}}$, lower than saturating concentrations, negative potentials will inhibit the binding of internal Na$^+$ to the pump and thereby the activation of $I_p$. As a consequence, the normalized $I_p$-$V$ relationship is shifted toward more positive membrane potentials. However, without additional information it cannot be excluded that the voltage dependence of internal Na$^+$ binding is only apparent and is due to the voltage sensitivity of any slower step subsequent to Na$^+$ binding in the pump cycle.

3. The voltage dependence of $I_p$ varies with the extracellular Na$^+$ concentration

The sigmoid shape of the cardiac $I_p$-$V$ relationship in Na$^+$-containing solution is characterized by a steep positive slope of the curve at negative membrane potentials up to $-100 \text{ mV}$ and a less positive slope at even more negative potentials. Lowering [\text{Na}^+]_o reduces the voltage dependence of $I_p$ in cardiac cells in a concentration-dependent manner, as first reported from measurements on isolated guinea pig ventricular myocytes (50, 131). Figure 7A shows, for example, that $I_p$ of a guinea pig ventricle cell in Na$^+$-poor solution (1.5 mM Na$^+$) is clearly less voltage dependent than in Na$^+$-rich control medium (150 mM Na$^+$). The effect of Na$^+$ on the $I_p$-$V$ curve was also observed in rabbit cardiac Purkinje cells (15), rat ventricular myocytes (167), Xenopus oocytes (158), and squid axons (144). Most probably, a step late in the Na$^+$ translocation by the pump is affected by Na$^+$. The Na$^+$ effect on the potential dependence of $I_p$ is voltage dependent (131). The $K_{0.5}$ value (i.e., [\text{Na}^+]_o exerting the half-maximum effect) increases fourfold from 91 to 357 mM.
Na\textsuperscript{+} between −120 and −40 mV. The authors were able to fit their \(I_p-V\) data obtained at various [Na\textsuperscript{+}]\textsubscript{o} by a kinetic equation derived within the framework of the pseudo two-state model of Na\textsuperscript{+}-K\textsuperscript{+} pumping (81; see sect. ivA6). The mechanism by which Na\textsuperscript{+} affects the voltage dependence of \(I_p\) is probably voltage-sensitive Na\textsuperscript{+} binding to the Na\textsuperscript{+}-K\textsuperscript{+} pump. The voltage dependence is not a characteristic of the binding per se but is rather due to a voltage-dependent variation of [Na\textsuperscript{+}] at the bottom of a “narrow, high-field access channel” (see Ref. 111, p. 75–83) where Na\textsuperscript{+} binding to the pump occurs (147). The increasing inhibition of \(I_p\) by Na\textsuperscript{+} at increasingly more negative potentials is probably not mediated via an increasing unidirectional Na\textsuperscript{+} influx as a consequence of enhanced voltage-dependent Na\textsuperscript{+} binding. The reason is that both unidirectional active \(^{22}\text{Na}\) efflux through the pump and pump current in the squid giant axon exhibit the same voltage dependence in Na\textsuperscript{+}-free and Na\textsuperscript{+}-containing artificial sea water (144). Of course, the voltage dependence of efflux and current is steeper in the latter solution. Thus an almost irreversible partial reaction seems to be present in the Na\textsuperscript{+} transport limb of the Na\textsuperscript{+}-K\textsuperscript{+} pump.

4. Effect of \(K_o^+\) on the \(I_p-V\) relationship

In contrast to the sigmoid \(I_p-V\) relationship of cardiac cells (48), endogenous Na\textsuperscript{+}-K\textsuperscript{+} pumps of Xenopus oocytes (110, 158) and Torpedo pumps expressed in Xenopus oocytes (157) display \(I_p-V\) curves exhibiting a maximum and a region of negative slope positive to +20 mV. The authors concluded that their measurements suggest that both unidirectional active \(^{22}\text{Na}\) efflux through the pump and pump current in the squid giant axon exhibit the same voltage dependence in Na\textsuperscript{+}-free and Na\textsuperscript{+}-containing media at low [K\textsuperscript{+}]\textsubscript{o} (less than \(K_{o,5}\) value) and, therefore, represent an artifact caused by passive currents overlapping \(I_p\) (146). A later study finally established beyond any reasonable doubt that the \(I_p-V\) relationship of Xenopus oocytes shows a region of negative slope (147). According to the authors, the negative slope of the \(I_p-V\) curve is most probably due to voltage-dependent binding of K\textsuperscript{+} to the Na\textsuperscript{+}-K\textsuperscript{+} pump. The extent of the region depends on the experimental conditions chosen.

Since then, \(I_p-V\) curves displaying a region of negative slope were also observed under special conditions in cardiac cells. A region of negative slope in the \(I_p-V\) curve of rabbit cardiac Purkinje cells was measured in Na\textsuperscript{+}-containing media at low [K\textsuperscript{+}]\textsubscript{o} (less than \(K_{o,5}\) value) and positive membrane potentials. The region extends to negative voltages in Na\textsuperscript{+}-free solution containing low concentration of K\textsuperscript{+} or its congeners Tl\textsuperscript{+}, NH\textsubscript{4}\textsuperscript{+}, and Cs\textsuperscript{+} (15, 18). An example is presented in Figure 8. \(I_p-V\) curves of rabbit Purkinje cells in Na\textsuperscript{+}-free (choline) solution containing 10.8 or 0.05 mM K\textsuperscript{+} are shown. Figure 8A displays \(I_p-V\) curves from three cells. \(I_p\) is presented in absolute values (pA). Figure 8B exhibits normalized mean \(I_p-V\) relationships. In Figure 8, A and B, the \(I_p-V\) curves of cells in K\textsuperscript{+}-poor solution have an extended region of negative slope. The solid symbols in Figure 8A indicate that the currents measured are blocked by dihydroouabain (DHO) and, therefore, represent \(I_p\). Similarly, an extended region of negative slope in the \(I_p-V\) relationship of guinea pig ventricular myocytes was observed at low [Na\textsuperscript{+}]\textsubscript{o} and [K\textsuperscript{+}]\textsubscript{o} (52). Furthermore, an increasingly negative slope of
the \( I_p-V \) curve of rat ventricular cells was measured in \( K^+ \)-containing media at positive membrane potentials as \([K^+]_o \) was lowered (167).

5. The 3Na\(^+\):2K\(^+\) stoichiometry of the Na\(^+\)-K\(^+\) pump is voltage independent

For an adequate interpretation of the shape of \( I_p-V \) relationships it is essential to know whether the 3Na\(^+\):2K\(^+\) stoichiometry of the Na\(^+\)-K\(^+\) pump is voltage dependent. Two studies of \( I_p-V \) curves in isolated noncardiac cells have provided relevant informations. Simultaneous measurements of \(^{22}\)Na efflux and pump current revealed that the Na\(^+\)-K\(^+\) stoichiometry of Torpedo californica pumps expressed in Xenopus oocytes is voltage independent between +50 and −100 mV (157, 179). Comparable experiments on squid giant axons also demonstrated that the Na\(^+\)-K\(^+\) stoichiometry is voltage independent (144). Both the pumped Na\(^+\) efflux and the Na\(^+\)-K\(^+\) pump current declined by roughly the same amount upon hyperpolarization. This voltage dependence implies an effect of membrane potential on Na\(^+\) release/rebinding at the extracellular face of the cell membrane rather than on a hypothetical reverse Na\(^+\) transport via Na\(^+\)-K\(^+\) pump.

6. Interpretation of \( I_p-V \) relationships

The sigmoid shape of the \( I_p-V \) curve in guinea pig ventricular myocytes (48) was interpreted in the following way (51). Since the pump rate (and therefore \( I_p \)) is nearly voltage independent at positive membrane potentials, a voltage-independent partial reaction rate limits the pump cycle in this potential range. At more negative potentials a step with voltage-sensitive transition rates limits the cycle rate, either directly, or via the control of the level of an intermediate that enters the rate-limiting partial reaction (see also Ref. 33). In view of the experimental data only a single voltage-dependent partial reaction was assumed. Several studies on cells and cell-free systems (see Refs. 33, 142) suggested that the Na\(^+\)-extruding limb of the pump cycle contains this voltage-sensitive step. More precisely, it seems likely that the deocclusion and release/rebinding of Na\(^+\) to/from the extracellular space are voltage dependent (see Ref. 4). This step in turn controls the concentration of an intermediate participating in the rate-limiting partial reaction, which, under the experimental conditions chosen, is probably the K\(^+\) translocation to the cell interior (see Ref. 33). Thus the shape of the \( I_p-V \) relationship in guinea pig ventricle cells was explained by assuming that depolarization of the sarclemma enhances the voltage-dependent step of the Na\(^+\) translocation such that the voltage-insensitive K\(^+\) import becomes rate limiting for the Na\(^+\)-K\(^+\) pump cycle at positive membrane potentials (6).

The quantitative kinetic analysis of the data (51) was based on a model mentioned above (81). According to this model for the interpretation of \( I_p-V \) curves, any multistate (unbranched) pump cycle containing a single voltage-sensitive step can be treated as a pseudo two-state cycle (scheme 1)

\[
\begin{array}{c}
\alpha \\
\downarrow \\
E_1 \\
\beta \\
\downarrow \\
E_2 \\
\end{array}
\]

(Scheme 1)

where \( E_1 \) and \( E_2 \) represent the two states of the pump cycle, \( \alpha \) and \( \beta \) denote empirical voltage-dependent rate constants, and \( c \) and \( d \) signify lumped empirical rate constants that are voltage insensitive. If an asymmetrical Eyring barrier exists for charge translocation (see Ref. 111, p. 68–69) and a single charge is moved in the pump cycle (130), the voltage-sensitive rate constants are given by

\[
\alpha = \alpha_0 \cdot \exp[\delta \cdot V \cdot F/R \cdot T] \quad \text{and} \quad \beta = \beta_0 \cdot \exp[-(1 - \delta) \cdot V \cdot F/R \cdot T],
\]

where \( \alpha_0 \) and \( \beta_0 \) represent the forward and backward rate constant at zero potential, respectively; \( \delta \) indicates the location of the barrier in the cell membrane; and \( V, F, R, \) and \( T \) have their usual meaning. By assuming numbers for the rate constants suggested by the experimental data, and \( \delta = 0.1 \), it was possible to fit the turnover rate-voltage relationship derived from the \( I_p-V \) curve by means of an equation deduced on the basis of the pseudo two-state model for the pump cycle (51).

As mentioned above, this interpretation assumes a single voltage-sensitive partial reaction in the Na\(^+\)-K\(^+\) pump cycle. However, to understand \( I_p-V \) curves displaying a region of negative slope, an additional potential-dependent step in the pump cycle has to be postulated (147). It was hypothesized that binding of extracellular K\(^+\) to the Na\(^+\)-K\(^+\) pump might occur within the cell membrane. To reach their binding sites, the K\(^+\) have to cross a part of the electrical field over the membrane. As a consequence, the membrane potential affects the local K\(^+\) concentration at the binding sites. Depolarization decreases the K\(^+\) concentration at the sites and thereby diminishes the pump cycle rate and \( I_p \). This mechanism produces the negative slope of the \( I_p-V \) relationship. The reader is referred to a brilliant, introductory review (Ref. 111, p. 75–83) for a detailed discussion of this “high field, narrow access channel hypothesis.” Some early observations (6, 74) suggested that the steps of K\(^+\) translocation by the pump beyond K\(^+\) binding may be voltage independent, and recent experimental evidence (135) supports this view.
B. Transient Pump Currents

1. Identification of transient pump currents during electroneutral Na\(^+\)/Na\(^+\) exchange in cardiac cells

To identify voltage-dependent partial reactions in the pump cycle, it is extremely helpful to constrain the active cation transport to only a few steps of the cycle. The physiological Na\(^+\)/K\(^+\) exchange via the pump can be blocked by application of K\(^+\)-free solution and thereby the transporter is forced to carry out Na\(^+\)/Na\(^+\) exchange (130). Under these conditions a steady-state pump current is absent, i.e., the Na\(^+\)/Na\(^+\) exchange is electroneutral. Nevertheless, cardiac glycoside-sensitive transient currents were observed after abrupt changes in membrane potential. These currents were outwardly directed upon depolarization and inwardly directed upon hyperpolarization. They declined monoexponentially to zero current (Fig. 9A, inset). They were blocked by Na\(^+\)-free internal or external media, cardiac glycosides, or oligomycin B and required metabolic energy. The characteristics of the currents strongly suggest that they are generated by the Na\(^+\) pump and represent transient pump currents. Since the physiological Na\(^+\)/K\(^+\) exchange mode of the pump does not exist under the experimental conditions chosen, the observed charge translocation most probably occurs in the Na\(^+\) limb of the Na\(^+\)/K\(^+\) pump (Fig. 9C). More specifically, it seems likely that the charge translocating step is Na\(^+\) release/rebinding to/from the exterior, whereas binding and occlusion of intracellular Na\(^+\) do not cause charge translocation under comparable conditions (5). The voltage dependence of the charge moved by the transient pump currents could be fitted by a Boltzmann-Fermi relation assuming that a single positive electrical charge crosses the entire membrane dielectric (Fig. 9A). The rate constant of transient current decline depends on membrane potential. It remains nearly constant at positive membrane potentials and increases steeply at negative voltages (Fig. 9B). The same rate constant was found for the electrogenic Na\(^+\) translocation by analyzing transient pump currents at 24°C and zero potential when either voltage or ATP concentration jumps were applied to the same giant patch from a guinea pig ventricular myocyte (43). The possible effect of \(\beta\)-adrenergic stimulation on the transient pump current was studied in adult rat cardiac myocytes (35). According to the authors, 10–50 \(\mu\)M norepinephrine did not alter the total charge transferred during the transient pump current. The density of pump molecules was calculated from the maximal charge moved during the transient current. It remained unchanged in the presence of the drug. Most recently, an altered voltage dependence of the charge transfer by the the Na\(^+\)/K\(^+\) pump was observed upon application of forskolin to guinea pig ventricular cells (108; see sect. viiiA1).

2. Hypothetical mechanism of the transient pump currents

The characteristics of the transient pump currents were explained by the hypothesis that the Na\(^+\) binding
sites of the pump molecule provide two negative charges and thus exhibit one positive charge if three Na\(^+\) are bound (130). As a consequence, the rate of Na\(^+\) translocation by the pump is expected to be voltage sensitive. Since a positive charge is moved, in general the forward rate constant of the translocation should be increased upon depolarization, whereas the backward rate constant should be diminished. Vice versa, a hyperpolarization of the cell membrane should reduce the forward rate constant and augment the backward rate constant. Thus the steady-state concentrations of the enzyme intermediates participating in the translocation step depend on membrane potential. After a voltage jump, the rate constants immediately reach their new values, whereas the concentrations of the intermediates approach their new steady-state more slowly. During this approach the forward and backward fluxes of the intermediates differ and cause a transient pump current that declines monoeXponentially with a rate constant that is the sum of the new forward and backward rate constants at the new membrane potential. The rate constant of decline of the transient pump current increases steeply with hyperpolarization but is nearly constant at positive potentials (Fig. 9B). Since at positive voltages the backward rate constant of the translocation step should be small, the rate constant of current decline will be dominated by the forward rate constant, which seems to be nearly voltage independent. Thus the observed potential dependence of the rate constant of current decline is largely due to the voltage dependence of the backward rate constant. By comparing the forward and backward rate constants near zero potential with data in the literature, the authors suggested that the studied voltage-dependent step of the pump cycle might be the translocation/deocclusion \(E_P \cdot Na_3 \rightleftharpoons E_P \cdot Na_2\). Furthermore, they discussed the possibility that the voltage dependence of this partial reaction might fully account for the electrogenicity and voltage sensitivity of the physiological Na\(^+/K\(^+\) exchange carried out by the Na\(^+/K\(^+\) pump (130). By application of a giant-patch technique (90) to guinea pig ventricular myocytes, two components of transient pump current were distinguished, suggesting Na\(^+\) release/rebinding from/to \(E_P\) in two partial reactions with different rate constants, amounts of charge \(Q\) moved and slope factors of the corresponding Boltzmann-Fermi equations (91). Both \(Q_{fast}\) (within the first 100 \(\mu\)s during a potential step) and \(Q_{slow}\) (in the millisecond range) depend on [Na\(^+\)]\(_o\). These observations were interpreted to mean that the Na\(^+\) binding sites of the pump open to the exterior in two voltage-sensitive partial reactions. Deocclusion of the first Na\(^+\) rate limits the strongly voltage-dependent release of this ion constituting \(Q_{slow}\), whereas a further electroneutral conformational change enables the last two Na\(^+\) to dissociate from their binding sites in a weakly voltage-sensitive partial reaction (\(Q_{fast}\)). The further analysis of transient pump currents during 50-\(\mu\)s transient steps revealed additional current components probably related to Na\(^+\) release and rebounding from or to the \(E_P \cdot Na_3 / E_P \cdot Na_2\) intermediates of the pump cycle (92). Similarly, measurements on squid axons succeeded in differentiating at least three components of transient pump current which might be related to Na\(^+\) release to the exterior (ultrafast component) and two distinct transitions involved in the Na\(^+\) deocclusion/release (142). High-speed voltage jumps revealed that the pre-steady-state charge movements relax in three phases that represent the deocclusion and release of the three Na\(^+\) in a strictly sequential order (95).

3. Identification and properties of transient pump currents during electroneutral \(K^+ / K^+\) exchange

Transient pump currents might likewise occur under conditions that constrain the Na\(^+\)/K\(^+\) pump to \(K^+_o / K^+_i\) exchange. Although transient currents in guinea pig ventricular myocytes were observed when the pump carried out forward (physiological) or backward Na\(^+\)/K\(^+\) transport or Na\(^+\)/Na\(^+\) exchange, they were never recorded under conditions permitting only \(K^+_o / K^+_i\) exchange (6). Therefore, the authors concluded that K\(^+\) translocation is not associated with a net charge transport across the membrane dielectric and is, most probably, voltage independent. Furthermore, they made the interesting point that the transient pump currents observed during forward and backward Na\(^+\)/K\(^+\) transport and Na\(^+\)/Na\(^+\) exchange indicate that the forward and the backward mode of Na\(^+\)/K\(^+\) transport is rate-limited not by a voltage-dependent partial reaction but by a voltage-insensitive step of the pump cycle, probably K\(^+\) translocation. However, the data presented were, on the one hand, consistent with the notion that the actual \(E_K \cdot K_3\) \(\rightleftharpoons E_K \cdot K_2\) K\(^+\) translocation is probably electroneutral, but, on the other hand, they did not exclude voltage-dependent K\(^+\) binding to the pump, since the experiments were conducted at saturating \([K^+]_o\) (Ref. 111, p. 216). In fact, ouabain-sensitive transient (pump) currents were more recently measured under \(K^+_o / K^+_i\) exchange conditions at \([K^+]_o\) or \([Tl^+]_o\) submaximally activating the pump of rat ventricular myocytes (135). The currents were observed at the beginning and immediately after the end of steep changes in membrane potential. The maximum charges moved during "on" and "off" transients amounted to 9.6 and 9.1 fC/pF with an 10.2 \pm 0.3 pF with an effective valency of 0.48 (compare this value to the effective valency of 1.0 derived from the Q-V curve for Na\(^+\)/Na\(^+\) exchange, Ref. 130). The rate constant of current decline remained constant at positive membrane potentials but increased with increasingly more negative potentials, reminiscent of characteristics of the kinetics of transient pump currents during Na\(^+\)/Na\(^+\) exchange (130) (see Fig. 9B). The transient pump currents seen under conditions of K\(^+\)/K\(^+\) exchange were absent in K\(^+\)-free (Tl\(^-\))
free) external solution or at 2 mM Tl\(^+\) (135), a [Tl\(^+\)]\(_o\) saturating the external cation binding sites of the pump (18). These properties of the transient currents strongly suggest voltage-dependent \(K_p\) binding to the pump. Lowering [Tl\(^+\)]\(_o\) from 0.1 to 0.025 mM did not affect the rate constant of current decline at positive potentials but decreased the rate constant at negative membrane potentials. Again, this finding is reminiscent of the effect of lowering [Na\(^+\)]\(_o\) on the rate constant of current decline during Na\(^+\)/Na\(^+\) exchange (52). By comparing the potential- and temperature-dependent characteristics of the transient \(I_p\) under conditions of Na\(^+\)/Na\(^+\) and K\(^+\)/K\(^+\) exchange, it was concluded that binding of extracellular K\(^+\) and Na\(^+\) to the Na\(^+\)-K\(^+\) pump occurs at different sites in the ATPase or to different conformations of the enzyme (135). The maximal charge moved, the effective valency, and the activation energy of the transient charge movements are all larger under Na\(^+\)/Na\(^+\) exchange (135), a [Tl\(^+\)]\(_o\) dependence of the active Na\(^+\)/K\(^+\) transport in cardiac cells containing a high intracellular Na\(^+\) and K\(^+\) concentration.

V. DEPENDENCE OF PUMP CURRENT ON INTRACELLULAR ATP

The molecular basis of the Na\(^+\)-K\(^+\) pump is the Mg\(^{2+}\)-dependent, Na\(^+\)-K\(^+\)-activated ATPase. Thus one would expect that the activity of the pump, including the generation of \(I_p\), depends on intracellular ATP. In 1983 it was verified that in dog cardiac preparations both Na\(^+\)-K\(^+\) pump and sarcolemmal Na\(^+\)-K\(^+\) ATPase display identical dependencies on Na\(^+\) and ATP (136). The ATP concentration required for half-maximum pump activation in the sarcolemmal vesicles studied was estimated to be \(\sim 210 \mu\text{M}\). From studies on the steady-state \(I_p\) in isolated cardiac Purkinje cells it seems likely that the Na\(^+\)-K\(^+\) pump is preferentially fuelled by glycolytic ATP synthesis (70), although other ATP sources contribute to the ATP supply for the cardiac Na\(^+\)-K\(^+\) pump (see Ref. 141). The pump current of giant patches from guinea pig ventricular cells is half-maximally activated at \(\sim 80 \mu\text{M}\) cytosolic MgATP (98). Similarly, a saturable ATP dependence of \(I_p\) with a Michaelis constant (\(K_m\)) value of \(\sim 150 \mu\text{M}\) was observed in giant patches from rat and guinea pig ventricular myocytes (42). Figure 10 illustrates these findings. Figure 10A displays outward (pump) currents (bottom trace) evoked by various MgATP concentrations (top trace) at 0 mV in a giant patch from a rat ventricular myocyte. The pump current increases with increasing [MgATP]. Figure 10B shows normalized mean \(I_p\) values as a function of [MgATP]. The experiments were carried out on giant patches from rat and guinea pig ventricular cells under various ionic conditions at zero potential. The dashed curve represents the fit of a Michaelis-Menten equation to the data. The \(K_{2.5}\) values were estimated to be 146 to 165 \mu\text{M} MgATP and suggest ATP binding to the low-affinity ATP binding site of the Na\(^+\)-K\(^+\) pump. A strong reduction of the transient pump currents evoked by voltage jumps was noted in guinea pig cells upon internal perfusion with ATP-poor or -free solutions (130). ATP concentration jumps induced by photolytic ATP release from caged ATP activate cardiac \(I_p\). The kinetics of the transient pump currents induced by ATP jumps or voltage jumps were
studied in giant patches from guinea pig cardiomyocytes superfused with K\(^{+}\)-free media (43). The rate constants of the fast component from the transient pump currents evoked by an ATP concentration jump were very similar to the rate constants of the transient pump currents induced by voltage jumps to zero or positive potentials (∼200 s\(^{-1}\) at 24°C) and displayed the same activation energy. Furthermore, the two techniques revealed a comparable amount of charge moved during the transient pump currents in the same giant patch. This result implies that the same charge-carrying mechanism was studied by both techniques.

VI. TEMPERATURE DEPENDENCE OF STEADY-STATE AND TRANSIENT PUMP CURRENTS

Because sarcolemmal Na\(^{+}\)-K\(^{+}\) transport by the pump is performed by a vectorial enzyme reaction, a marked temperature dependence of the Na\(^{+}\)-K\(^{+}\) pump activity is anticipated. As to the steady-state \(I_p\) of single rabbit sinoatrial node cells, a \(Q_{10}\) value of 2.1 in a temperature range between 25 and 37°C has been reported (151). This means a change of the \(I_p\) amplitude by a factor of 2.1 following an alteration of the temperature by 10°C. Similar \(Q_{10}\) values were found for the steady-state \(I_p\) of isolated sheep cardiac Purkinje cells (\(Q_{10} = 2.9\)) and single guinea pig ventricular myocytes (\(Q_{10} = 2.2\)) (187).

Concerning transient pump currents, the rate constants of current decline during Na\(^{+}\)/Na\(^{+}\) exchange in guinea pig ventricle cells exhibited a voltage-independent \(Q_{10}\) value of ∼3 (130). In voltage-jump experiments on excised giant patches from guinea pig ventricular myocytes, the activation energy for the forward rate constants of the transient Na\(^{+}\)/Na\(^{+}\) pump currents was estimated to be ∼80 kJ/mol. Nearly the same value (84 kJ/mol) was observed for the rate of the electrogenic step (or an electroneutral partial reaction preceding and rate-limiting the faster electrogenic step) in these patches during ATP concentration jumps (43). Interestingly, an Arrhenius plot yielded an activation energy of 27.5 kJ/mol for the off-rate constants of transient pump currents observed during Tl\(^{+}\)/K\(^{+}\) exchange in rat cardiac ventricular myocytes. A clearly higher activation energy of 87 kJ/mol was found for the off-rate constants of the current transitions during Na\(^{+}\)/Na\(^{+}\) transport via the pump in the same cell species (135). Therefore, it was concluded that the Na\(^{+}\)/Na\(^{+}\) and Tl\(^{+}\)-evoked charge movements do not reflect the characteristics of the same partial reaction in the Na\(^{+}\)-K\(^{+}\) pump cycle. It seems likely that different conformational states of the Na\(^{+}\)-K\(^{+}\)-ATPase control the rate of the electrogenic transitions and/or the binding of Na\(^{+}\) and K\(^{+}\) to the pump (135).

VII. SIGNIFICANCE OF ELECTROGENIC SODIUM-POTASSIUM PUMPING FOR THE MEMBRANE POTENTIAL OF CARDIAC CELLS

A. Contribution of Electrogenic Na\(^{+}\)-K\(^{+}\) Pumping to the Cardiac Resting Potential

1. Theoretical note

The Na\(^{+}\)-K\(^{+}\) pump directly generates a potential difference across the cell membrane. Under physiological conditions this potential difference \(E_p\) hyperpolarizes the cell membrane and, therefore, increases the absolute value of the resting potential. According to Ohm’s law, the magnitude of \(E_p\) depends on both the \(I_p\) amplitude and the membrane resistance. The \(I_p\) amplitude is determined by the pump molecule density in the cell membrane and by the turnover rate of the pump. The membrane resistance depends on the density and characteristics of mainly the ionic channels in the membrane. Accordingly, the contribution of electrogenic Na\(^{+}\)-K\(^{+}\) pumping to the resting potential of various cells varies widely. There are animal cells in which the resting potential is predominantly generated by the Na\(^{+}\)-K\(^{+}\) pump. These cells include T lymphocytes of mice (100), rat mast cells (21), and vomeronasal chemoreceptor neurons of the frog (177). In cells where the passive Na\(^{+}\) and K\(^{+}\) fluxes are certainly determining factors for the setting of the resting potential, the effect of electrogenic Na\(^{+}\)-K\(^{+}\) pumping on the resting potential (\(E_{m}\)) may be described by the Mullins-Noda equation (129)

\[
E_m = \frac{R \cdot T}{F} \ln \frac{P_{Na}[Na^{+}]_o + r \cdot P_K[K^{+}]_o}{P_{Na}[Na^{+}]_i + r \cdot P_K[K^{+}]_i}
\]

where \(r\) denotes the coupling ratio between the pumped Na\(^{+}\) and K\(^{+}\) fluxes (\(r = 1.5\) under physiological conditions) and the other symbols have their usual meaning. According to Ascher (see Ref. 176), the potential difference \(E_p\) set up by the Na\(^{+}\)-K\(^{+}\) pump in these cells cannot exceed

\[
E_p = \frac{R \cdot T}{F} \ln \frac{1}{r}
\]

or 10 to 11 mV in resting cells. Although the passive Na\(^{+}\) and K\(^{+}\) fluxes are certainly determining factors for the cardiac resting potential, it seems questionable whether the Mullins-Noda equation adequately describes the contribution of electrogenic Na\(^{+}\)-K\(^{+}\) pumping to the cardiac resting potential. The assumption implied in the equation of voltage-independent Na\(^{+}\) and K\(^{+}\) permeabilities probably does not hold for cardiac...
cell membranes. More specifically, due to the typical N-shaped steady-state I-V relationship of heart cells, the inhibition of the Na\(^+-\)K\(^+\) pump probably causes a much larger depolarization of the sarcolemma than the predicted 10 mV (45). Similarly, the strong inward rectification of the cardiac steady-state I-V curve might evoke a considerably stronger depolarization upon pump blockade than expected from the slope conductance of the sarcolemma at the resting potential (10).

2. Experimental data

Experimental estimations of the pump contribution to the cardiac resting potential in multicellular preparations yielded 5–10% of the resting potential (reviewed in Refs. 40, 65). As to single cells, an \( E_p \) of 4.2 mV was derived contributing to the resting potential of canine cardiac Purkinje cells (25). In chick cardiac myocytes, \( I_p \) hyperpolarizes the sarcolemma by 6.5 mV (170). An \( E_p \) value of only 0.4 mV was reported from measurements on guinea pig ventricular myocytes (114). A much higher \( E_p \) of \( \sim 20 \) mV can be calculated from data obtained in experiments on isolated rabbit sinoatrial node cells (151). Although, in general, the contribution of electrogenic Na\(^+-\)K\(^+\) pumping to the cardiac resting potential amounts to only a few millivolts, it is of physiological significance. This is because the steady-state inactivation of Na\(^+\) (and Ca\(^{2+}\)) channels and thus their availability for the production of action or pacemaker potentials depends steeply on membrane voltage near the resting (or maximal diastolic) potential.

B. Importance of Electrogenic Na\(^+-\)K\(^+\) Pumping for the Cardiac Action Potential

As mentioned above, the potential difference \( E_p \) contributed by the electrogenic Na\(^+-\)K\(^+\) pump to the cardiac membrane potential depends on the \( I_p \) amplitude and the membrane resistance. Compared with the values at resting potential, both factors are increased at the plateau of the cardiac action potential. The higher membrane resistance is mainly due to a reduced potassium conductance at the plateau level (see Ref. 132, p. 28). The cardiac \( I_p \)-V relationship predicts an increase of \( I_p \) by a factor \( \sim 2 \) at the action potential plateau (15, 131) (see also Fig. 7A). Furthermore, the enhanced Na\(^+\) influx during an action potential additionally augments \( I_p \) via an increased subsarcolemmal Na\(^+\) concentration. For these reasons one would expect that electrogenic Na\(^+-\)K\(^+\) pumping markedly affects the shape of the cardiac action potential during the plateau phase. Indeed, in the first voltage-clamp study of the cardiac \( I_p \) in a multicellular preparation, a prolongation of the action potential was observed in Purkinje fibers as an early effect of the Na\(^+-\)K\(^+\) pump inhibition by the cardiac glycoside DHO (99). Vice versa, the activation of the pump in Purkinje fibers following a short period of increased stimulation frequency or in K\(^+\)-free solution considerably shortens the action potential duration (47).

In isolated cardiac cells too, an early effect of cardiac glycosides is a lengthening of the action potential. Figure 11 shows an example. The action potential of an isolated guinea pig ventricular myocyte is prolonged by ouabain in a concentration-dependent manner. The higher the concentration applied, the stronger is the Na\(^+-\)K\(^+\) pump inhibition and the longer is the action potential duration (at 90% repolarization; APD\(_{90}\)) since the repolarizing \( I_p \) is increasingly diminished. Comparable effects of other cardiac steroids have been reported (e.g., Ref. 113). The effects are in accordance with calculations based on the membrane resistance and \( I_p \) amplitude at the plateau level of the action potential (113, 115, 116). These calculations predict a

![Graph](image)
plateau depolarization of up to 9–16 mV following a blockade of $I_p$. The significance of $I_p$ and its modulation by adrenergic transmitters for the shape of the cardiac action potential under physiological conditions have recently been reemphasized (183).

VIII. MODULATION OF CARDIAC PUMP CURRENT BY AUTONOMIC TRANSMITTERS AND RELATED COMPOUNDS

It seems likely that autonomic transmitters adjust the Na$^+$/K$^+$ pump activity to the functional demands of the cells and the organism. In fact, various effects of the transmitters (and related compounds) on Na$^+$/K$^+$ pumping of single cardiac cells via adrenergic and muscarinic acetylcholine receptors have been described. The mechanisms of the intracellular signal transduction from receptor stimulation to the cardiac Na$^+$/K$^+$ pump are outlined in scheme 2, which is partially hypothetical. β-Adrenergic agonists exert their effects via β-receptors in the sarcolemma. The receptors are coupled to G$\alpha$ proteins which activate adenyl cyclase (AC) to facilitate the synthesis of cAMP.

cAMP stimulates protein kinase A (PKA) which enhances active Na$^+$/K$^+$ exchange by phosphorylation of Na$^+$/K$^+$ pump molecules. Stimulation of adrenergic $\alpha_1$-receptors causes, via a G protein, the activation of phospholipase C (PLC) which cleaves phosphatidylinositol 4,5-diphosphate (PIP$_2$) to diacylglycerol (DAG) and inositol trisphosphate (IP$_3$). DAG and Ca$^{2+}$ (released from the sarcoplasmic reticulum by IP$_3$) activate protein kinase C, which in turn stimulates cardiac Na$^+$/K$^+$ pumping by pump phosphorylation. Binding of acetylcholine to a muscarinic acetylcholine receptor (M$_2$ receptor) causes inhibition of adenyl cyclase, decrease of [cAMP], and thereby inhibition of PKA and Na$^+$/K$^+$ pump activity.

A. Effect of Adrenergic Agonists on $I_p$

1. β-Adrenergic stimulation of cardiac $I_p$

A stimulatory action of these substances on the Na$^+$/K$^+$ pump in multicellular cardiac preparations has been reported by various authors during the last 45 years (for references, see Ref. 67). However, the mechanism of action remained unclear. More recent studies on isolated cardiac cells failed so far to elucidate the mechanism.
Even worse, according to the information available at present, it seems questionable whether a universal mechanism of catecholamine action exists for cardiac cells of different animal species. Apart from species differences, technical problems (impaired receptor function following cell isolation, use of patch pipettes with relatively high resistance (>2 MΩ)) may also contribute to the inconsistent observations.

Isoprenaline (10⁻⁷ to 10⁻⁶ M) induced a decrease of the intracellular Na⁺ activity (dNa) by ~25% in isolated rabbit ventricular myocytes and an enhanced dNa$_{Na}$ decline following an increase of the extracellular K⁺ concentration (30). Unfortunately, the sensitivity of the effects to cardiac glycosides, specific inhibitors of the Na⁺-K⁺ pump, was not tested. The authors concluded from their results that isoprenaline directly stimulates cardiac active Na/K⁺ transport and not indirectly via a drug-induced accumulation of external K⁺. The latter mechanism had previously been considered to explain the stimulation of the cardiac Na⁺-K⁺ pump by catecholamines in multicellular preparations. In contrast to the experiments just mentioned, more indirect measurements failed to demonstrate any direct β-adrenergic stimulation of the Na⁺-K⁺ pump in guinea pig ventricular myocytes (120). However, other observations on the same cell species revealed a direct β-adrenergic effect (56). The β-adrenergic modulation of $I_p$ was not due to drug-induced changes of the intracellular Na⁺ or extracellular K⁺ concentration but directly to the variation of the maximum pump turnover rate. Interestingly, $I_p$ was reduced by isoprenaline at low (<150 nM) intracellular [Ca²⁺], but increased at higher [Ca²⁺]. In a further patch-clamp study (54) it was shown that the β-adrenergic increase of $I_p$ at high [Ca²⁺] was mediated by a phosphorylation step via the cAMP-PKA cascade. A study of the β-adrenergic effects of isoprenaline on the $I_p$-V relationship in guinea pig ventricular cells (59) showed that the increase of $I_p$ at high [Ca²⁺] (1.4 μM) was voltage dependent. At positive voltages isoprenaline had little effect on the $I_p$ amplitude, whereas the drug increased $I_p$ at negative membrane potentials. However, the inhibition of $I_p$ caused by isoprenaline at low [Ca²⁺] (15 nM) was voltage independent. The authors suggested two effects of intracellular Ca²⁺ on the β-adrenergic modulation of cardiac $I_p$. First, it counteracts the inhibition of $I_p$ induced by protein phosphorylation via PKA activation (55), and second, it shifts the $I_p$-V curve in the negative direction during the β-adrenergic, PKA-mediated phosphorylation of the cardiac Na⁺-K⁺ pump. The physiological significance of the increase of $I_p$ during β-adrenergic stimulation at high [Ca²⁺], may consist of a partial compensation for β-adrenergic effects on membrane conductances, which tend to prolong the cardiac action potential. Most recently, an increase of $I_p$ by the adenylyl cyclase activator forskolin in guinea pig ventricular myocytes has been reported. The increase was observed at nanomolar and subnanomolar [Ca²⁺] and was mediated, at least in part, by facilitation of a partial reaction in the Na⁺ limb of the pump cycle, different from Na$_i$ binding (108).

In contrast to the just described observations on guinea pig ventricle myocytes, a study on rat ventricular cells did not detect any β-adrenergic regulation of the Na⁺-K⁺-ATPase (101). Surprisingly, other experiments using the same cell species, similar solutions, and electrophysiological techniques but a different protocol showed an $I_p$ stimulation by norepinephrine and isoprenaline (35). Figure 12 illustrates some of these results. Under the conditions chosen, the membrane current of a rat myocyte clamped at −40 mV is nearly identical to the ouabain-sensitive $I_p$ (Fig. 12, A and B). Norepinephrine (10 μM) and isoprenaline (10 μM) increase $I_p$. This is shown in Figure 12, A and B, respectively. Mean values of

![Figure 12](http://physrev.physiology.org/)

**FIG. 12.** Stimulation of $I_p$ by norepinephrine (NA) and isoprenaline (Iso) in rat cardiac myocyte. A: example recording of the holding current ($I_h$) at −40 mV as affected by 10 μM NA (marked by arrows) and 1 mM ouabain (Ou). The peak effect of NA was measured as shown by arrow labeled $I_{ho}$. Ouabain was applied to the cell for a short time before and after NA application to measure $I_p$ as shown by arrow $I_{po}$.

B: example record of $I_p$ before and during the application of NA (1–10 μM, columns on left) or Iso (1–10 μM, columns on right). n indicates the number of cells studied. [Adapted from Dobretsov et al. (35).]
the $I_p$ activation by the two catecholamines are presented in Figure 12C. Both substances augment $I_p$ by $\sim 40\%$. The stimulation of $I_p$ occurred at low [Ca$^{2+}$]$_i$ (20 nM), was voltage independent, and was not due to Na$^+$ or K$^+$ accumulation, changes in the pump affinity to Na$^+$ or K$^+_o$, or in the pump site density. The effect of isoprenaline increased with increasing the intracellular K$^+$ concentration from 0 to 100 mM K$^+$. From simulation of the experimental data with a simplified Post-Albers cycle, the authors suggested that the $\beta$-adrenergic stimulation of $I_p$ in rat myocytes might be caused by a drug-induced facilitation of K$^+$ deocclusion and K$^+$ release into the cell interior. Furthermore, the adrenergic modulation of $I_p$ was examined in adult rat cardiac myocytes in short-term culture (up to 4 days). The $I_p$ stimulation by norepinephrine and isoprenaline (at 20 nM free Ca$^{2+}$) displayed $K_{0.5}$ values of 36 and 1.5 nM, respectively, and was $\beta$-receptor mediated (166). In contrast to experiments on guinea pig ventricular cells (56, 59), a reduction rather than an increase of the adrenergic $I_p$ stimulation with increasing [Ca$^{2+}$]$_i$ was observed, and no effect of adrenergic stimulation on the shape or position of the $I_p$-V relationship could be detected. Because there was no change in the voltage dependence of $I_p$ during $\beta$-adrenergic stimulation of the Na$^+$-K$^+$ pump, it was concluded that the Na$^+$ release from the pump to the extracellular medium is not modulated by $\beta$-agonists and that the mechanism of $\beta$-adrenergic $I_p$ modulation in rat ventricular cells is qualitatively different from that proposed for guinea pig cardiac myocytes (166).

2. $\alpha$-Adrenergic stimulation of $I_p$ in cardiac cells

In canine Purkinje myocytes, the $\alpha$-adrenergic drug phenylephrine increases the Na$^+$-K$^+$ pump activity and thereby $I_p$ and decreases the background K$^+$ conductance. Both effects are mediated by $\alpha_1$-adrenergic receptors and are blocked by a pretreatment of the Purkinje fibers with pertussis toxin. The $\beta$-antagonist propranolol is ineffective. The effect of phenylephrine is abolished by $10^{-4}$ M DHO, a specific blocker of the Na$^+$-K$^+$ pump, in Ba$^{2+}$-containing external solution. The known negative chronotropic effect of phenylephrine might be due to activation of electrogenic Na$^+$-K$^+$ pumping. The pump seems to be coupled to a pertussis toxin-sensitive G protein (160). Norepinephrine and the $\alpha$-adrenergic agonists phenylephrine, methoxamine, and metaraminol increase $I_p$ of guinea pig ventricular myocytes in propranolol-containing media. The increase is blocked by the $\alpha_1$-antagonist prazosin and is unaffected by the $\alpha_2$-antagonist yohimbine. The stimulation of $I_p$ is not due to accumulation of intracellular Na$^+$ or extracellular K$^+$ and is independent of voltage. The norepinephrine concentration required for half-maximal $I_p$ stimulation ($K_{0.5}$ value) depends on [Ca$^{2+}$]$_i$, and decreases from 219 nM at 15 nM Ca$^{2+}$ to 3 nM at 1.4 $\mu$M Ca$^{2+}$ (183). $I_p$ measurements during application of (non-specific) activators or inhibitors of PKC (60, 61) are in line with the hypothesis that cardiac Na$^+$-K$^+$ pump is coupled to $\alpha_1$-adrenoceptors via PKC. The sensitivity of the coupling depends on [Ca$^{2+}$]$_i$. The maximal increase in $I_p$ is independent of membrane potential and [Ca$^{2+}$]$_i$ (183). An electropharmacological study demonstrated that the $\alpha_1$-adrenoceptor stimulation of $I_p$ in rat ventricular myocytes is mediated by the $\alpha_{1b}$-subtype of the $\alpha_1$-adrenergic receptors (185). It is helpful to note that adrenergic modulation of the Na$^+$-K$^+$ pump (Na$^+$-K$^+$-ATPase) might result in different effects, depending on cell species and/or experimental conditions. For further discussion and additional references, the reader is referred to a relevant review (180). In a most recent paper, evidence is presented that two isoforms of the $\alpha$-subunit of the Na$^+$-K$^+$-ATPase (\(\alpha_1, 82\% \); \(\alpha_2, 18\% \)) are present in guinea pig ventricles. The $\beta$-adrenergic, PKA-mediated effects on the cardiac Na$^+$-K$^+$ pump are targeted to the $\alpha_1$-isoform, whereas the $\alpha$-adrenergic, PKC-dependent effects are targeted to the $\alpha_2$-isoform (62).

B. Modulation of Cardiac $I_p$ by Acetylcholine

To our knowledge, so far only one report has been published on the action of acetylcholine (ACh) on the cardiac $I_p$ (58). According to this study ACh does not modulate the basal $I_p$ at any voltage in guinea pig ventricular myocytes containing a high or low [Ca$^{2+}$]$_i$. However, it reverses the effect of isoprenaline on $I_p$, regardless of [Ca$^{2+}$]$_i$, with a $K_{0.5}$ value of $\sim 16$ nM. Figure 13 presents an original record of membrane current from a guinea pig ventricular cell clamped at $-60$ mV (58). $I_p$ is estimated as current blocked by 1 mM DHO. At low internal [Ca$^{2+}$] (15 nM), isoprenaline (1 $\mu$M) decreases $I_p$. This effect is abolished by acetylcholine (ACh; 1 $\mu$M). The ACh effect is mediated by muscarinic receptors since it is blocked by atropine. The ACh-induced stimulation of the receptor leads most probably via a G$\alpha$ protein to an inhibition of adenyl cyclase and in consequence to a decrease in [cAMP] (see scheme 2). Thus a high vagal tone per se does not modulate the cardiac Na$^+$-K$^+$ pump, but activation of muscarinic receptors reverses the modulation of the pump induced by a high sympathetic tone.

IX. CARDIAC GLYCOSIDES AND CARDIAC PUMP CURRENT

A. Binding of Cardiac Glycosides to Various Isoforms of the Cardiac Na$^+$-K$^+$ Pump Is Species Dependent

As mentioned in section i, different isoforms of the $\alpha$-subunit of the Na$^+$-K$^+$-ATPase are expressed in the

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cardiac cells of various animal species. In general, two of three $\alpha$-subunit isoforms are detected in the heart. There is agreement in the literature that rat myocytes (e.g., Refs. 12, 164, 175) and myocytes from many other species, including human cells (e.g., Refs. 175, 182), express more than one Na$^+$-K$^+$ pump isoform (see Ref. 175). Because the $\alpha$-isoforms represent the receptors for cardiac glycosides and normally differ in their sensitivity for the drugs (see Ref. 19, but also Ref. 27), more than one receptor class for cardiac steroids should be found in heart cells. However, only a single $\alpha$-isofrom is expressed in sheep cardiac cells (175) and, perhaps, in guinea pig ventricular myocytes (175; but see Refs. 11, 62). Thus only one receptor class may be present in the heart cells of these two species. The sensitivity of the $\alpha$-isoforms for cardiac steroids varies widely among the animal species. For instance, the rat $\alpha_1$-subunit is known to be extremely resistant to the drugs.

The chapter below describes the interaction between cardiac glycosides and the Na$^+$-K$^+$ pump as studied by electrophysiological techniques in isolated cardiac cells. It is not in the focus of the present article to review the immense literature on cellular and subcellular actions of cardiac steroids in other preparations and/or investigated by other physiological or by biochemical methods. Clearly, this literature is at least as important for our understanding of the glycoside action as the few observations reported here. For a more extensive overview the reader is referred to relevant reviews (2, 40, 78, 116).

Ever since the pump current was first described in a cardiac preparation, cardiac glycosides, which are specific inhibitors of the Na$^+$-K$^+$ pump (153), have been used to identify cardiac $I_p$ (99). For example, in the first paper on the voltage dependence of $I_p$ in single cardiac cells, the pump current of guinea pig ventricular myocytes was measured as the ouabain-blockable current (48). As just described, it is still controversial whether in these cells more than one $\alpha$-subunit isoform of the Na$^+$-K$^+$-ATPase (more than one class of cardiac steroid-receptors) is expressed and functioning. On the one hand, two components of the total $I_p$ with different sensitivities to cardiac glycosides have been described (57, 127). According to the authors, the highly sensitive component contributes up to 30–40% of the total $I_p$ under quasi-physiological conditions. This is in line with a biochemical-immunological study (11) which described a component with high affinity toward cardiac glycosides (dissociation constant $\sim$10 nM ouabain, DHO, or digoxigenin) that contributes as much as $55\%$ to the total Na$^+$-K$^+$-ATPase activity of guinea pig ventricular cells. Furthermore, a recent paper (62) showed by a molecular biological approach that 18% of the pump mRNA consists of mRNA for the $\alpha_2$-subunit of the pump which exhibits a high affinity for cardiac glycosides. On the other hand, $I_p$ inhibition by strophanthidin occurs as reversible 1:1 binding to a single class of pumps in guinea pig ventricular cells (114). Similarly, the $I_p$ inhibition by DHO in rat and guinea pig cardiac myocytes in a range between $\sim$20 and 95% inhibition can be analyzed by assuming simple saturation kinetics and a single population of pump molecules (86). Of course, this does not exclude the presence of a minor class of pumps (additional $\alpha$-isoform), displaying a different affinity to cardiac glycosides. However, application of immunologic methods did not detect any $\alpha_2$-isoenzyme of the Na$^+$-K$^+$ pump in guinea pig ventricular myocytes (122, 175). Finally, the $I_p$ inhibition-concentration curve (range $10^{-8}$ to $5 \times 10^{-5}$ M) for guinea pig ventricular myocytes could not be fitted by assuming two isoymes showing different sensitivities toward DHO (84). So far, the reason for the conflicting results with guinea pig cardiac cells is com-

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**Fig. 13.** Acetylcholine (ACh) reverses the effects of Iso on $I_p$ at low [Ca$^2+$], and $-60$ mV. The guinea pig ventricular myocyte was held at $-60$ mV, and the pipette solution contained 15 nM Ca$^{2+}$. The continuous line indicates the application of Iso (1 $\mu$M), and the dashed line indicates the application of Iso plus 1 $\mu$M ACh (Iso + ACh). The vertical line labeled $I_p$ indicates the size of $I_p$ and how it is measured. The effect of ACh on $I_p$ was reversible. [Adapted from Gao et al. (58).]

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B. Kinetics of Cardiac Steroid Binding to the Cardiac Na⁺-K⁺ Pump

1. Reversible one-to-one binding to a single class of receptors on the pump

To study the inhibition of the cardiac $I_p$ by cardiac glycosides, DHO is often used since its action on the Na⁺-K⁺ pump is readily reversible. The inhibition of $I_p$ by DHO was investigated in isolated canine Purkinje myocytes at 8 mM K⁺o by means of a discontinuous voltage-clamp technique (25). The data were analyzed by assuming a reversible one-to-one interaction between the cardiac steroid and a single receptor class R on the Na⁺-K⁺ pump (28)

$$DHO + R \rightleftharpoons DHO \cdot R$$  \hspace{1cm} (7)

The equilibrium dissociation constant $K_D$ of this reaction is given by

$$K_D = \frac{k_2}{k_1}$$  \hspace{1cm} (8)

The association rate constant $k_1$ is related to $\tau_{on}$, the time constant of the process of DHO binding, by

$$\frac{1}{\tau_{on}} = k_1 \cdot [DHO] + k_2$$  \hspace{1cm} (9)

whereas the dissociation rate constant $k_2$ is defined as

$$k_2 = \frac{1}{\tau_{off}}$$  \hspace{1cm} (10)

$\tau_{off}$ is the time constant of DHO unbinding. By applying the above equations to their data, an apparent $K_D$ value (i.e., the [DHO] which causes half-maximal inhibition of $I_p$) of $3.7 \times 10^{-6}$ M DHO ($K_{0.5}$ value), an apparent association rate constant $k_1'$ of $2.5 \times 10^{9}$ (M x s)⁻¹ and a dissociation rate constant $k_2'$ of 0.009 s⁻¹ were calculated (25). The agreement between measured steady-state $I_p$ inhibition as a function of [DHO] and calculated $(k_2'/k_1')K_{0.5}$ value suggested that the kinetic model outlined above is reasonable (25). A similar but more detailed study was carried out on single rabbit cardiac Purkinje cells (17). Half-maximal inhibition of $I_p$ by $1 \times 10^{-5}$ M DHO was found in a medium containing 2 mM K⁺o. This $K_{0.5}$ value increased to $3.5 \times 10^{-5}$ M DHO at 10.8 mM K⁺o. The antagonistic effect of K⁺o on the $I_p$ inhibition by DHO was studied in some details as shown in Figure 14 (17). Figure 14A, top panel, displays the membrane current of a Purkinje cell at 2 mM K⁺o before, during, and after application of $10^{-4}$ M DHO. The steroid inhibits $I_p$ and therefore shifts the membrane current in the inward directions. A new stable current level is reached in ~10 s. Short intermittent test pulses of K⁺-free medium block $I_p$ and indicate the current level in the absence of Na⁺-K⁺ pumping. It is clear from the current trace that $10^{-4}$ M DHO inhibits ~90% of $I_p$ at 2 mM K⁺o. Figure 14A, bottom panel, demonstrates that at 10.8 mM K⁺o, the $I_p$ inhibition by $10^{-4}$ M DHO proceeds more slowly to a final level of only ~65% of $I_p$ in drug-free solution. Figure 14B emphasizes that the time constant of $I_p$ decline in DHO-containing solution is smaller at 2 mM K⁺o than at 10.8 mM K⁺o. By way of contrast, washout of the drug is very similar at both [K⁺o] (Fig. 14C). Application of the reaction scheme presented above yielded apparent association rate constants ($k_1'$) of $1 \times 10^{4}$ (M x s)⁻¹ at 2 mM K⁺o and $2.8 \times 10^{3}$ (M x s)⁻¹ at 10.8 mM K⁺o, the apparent dissociation rate constant $k_2'$ turned out to be almost K⁺o independent and amounted to ~0.06 s⁻¹ at both [K⁺o]. Increasing [Na⁺] of the patch-pipette solution (for intracellular perfusion) from 5 to 50 mM increased the $k_1'$ value but left the $k_2'$ value unchanged. As a consequence, the $K_{0.5}$ value decreased by a factor of 3–5. The main changes occurred after an increase of [Na⁺]pip from 5 to 15 mM. The observed dependence of $K_{0.5}$ on [Na⁺]pip is reminiscent of an earlier paper (169) which demonstrated a decrease of the $K_{0.5}$ value of ouabain for the pump inhibition in chick cardiac myocytes upon an increase in the intracellular Na⁺ concentration. In accordance with a model published previously (88), it was concluded that an increase of Na⁺ augments the probability for a distinct pump molecule to take on a conformation that binds ouabain with high affinity. The $K_{0.5}$ value for $I_p$ inhibition in rat and guinea pig ventricular myocytes amounted to $2.4 \times 10^{-3}$ and $1.4 \times 10^{-2}$ M DHO, respectively ($K_{0.5}$, 5.4 mM; holding potential, ~20 mV; Na⁺ pip 50 or 100 mM). The higher $K_{0.5}$ value for binding of DHO to rat cells was due to a smaller apparent association rate constant and a larger dissociation rate constant. There was little evidence for the presence of an isoform with higher DHO affinity in guinea pig myocytes, and only a few percent of the pump molecules in rat cells displayed a higher affinity to DHO (86). Half-maximal $I_p$ activation by K⁺o re-
mained nearly unchanged, but \( I_{p(max)} \) decreased following addition of [DHO] near the respective \( K_{0.5} \) value at 5.4 mM \( K^+_0 \). The \( K_{0.5} \) value of DHO for \( I_p \) inhibition and the activation of \( I_p \) showed a similar dependence on \( K^+_0 \) (86). The authors also reported that the \( K_{0.5} \) value was larger in Na\(^+\)-free than in Na\(^+\)-containing media under conditions where \( I_p \) activation by \( K^+_0 \) was nearly the same, suggesting that DHO binding occurred preferentially to a Na\(^+\)-bound conformational state of the cardiac Na\(^+\)-K\(^+\) pump. In line with earlier biochemical evidence (reviewed in Ref. 78), the data were interpreted to mean that the antagonism between DHO and \( K^+_0 \) as to the activation of \( I_p \) is noncompetitive. \( K^+_0 \) and the cardiac glycoside bind to different conformational states of the pump transiently exposed to the external face of the cell membrane during the pump cycle. DHO-bound states are unable to generate \( I_p \) (86).

2. Binding of cardiac glycosides to two classes of pump molecules

In line with reports on two isoforms of the \( \alpha \)-subunit present in guinea pig ventricular myocytes (11, 62), inhibition of \( I_p \) in these cells has been occasionally described to display a biphasic concentration dependence (127). The data were fitted best by the equation

\[
I_{DHO} = \frac{I_{DHO,max} \times [DHO]}{K_{Dih} + [DHO]} + \frac{I_{DHO,max1} \times [DHO]}{K_{Dil} + [DHO]}
\]

where \( I_{DHO} \) denotes the current blocked by a distinct [DHO], \( I_{DHO,max} \) and \( I_{DHO,max1} \) represent the DHO-blockable current of pump isoforms showing a high or low affinity toward DHO, respectively, and \( K_{Dih} \) and \( K_{Dil} \) are the respective equilibrium dissociation constants of the interaction between DHO and the two classes of pumps displaying either high or low affinity to DHO. \( K_{Dih} \) was calculated to be \( 5 \times 10^{-8} \) M DHO, and \( K_{Dil} \) was derived to be \( 6.5 \times 10^{-5} \) M DHO. Under the conditions chosen (temperature, 34°C; Na\(^+\)pip, 30 mM; \( K^+_0, 5.4 \) mM; holding potential, ~40 mV), the pumps with a high DHO affinity contributed as much as 40% to the total \( I_p \). A detailed analysis of electrogenic Na\(^+\)-K\(^+\) pumping in guinea pig ventricular myocytes also indicated the presence of more than one \( \alpha \)-subunit isoform (57). Obviously, two classes of pump molecules displaying different affinities to DHO contribute to the total pump current at ~60 mV holding potential. The high-affinity pumps are half-maximally inhibited by 7.5 \( \times \) \( 10^{-7} \) M DHO and the low-affinity pump molecules by 7.2 \( \times \) \( 10^{-5} \) M DHO. Although the latter \( K_{0.5} \) value agrees nicely with the corresponding \( K_{Dil} \) value above (127), the former is larger by a factor of 15. The high-affinity pumps produced ~30% of the total pump current. They were half-maximally activated by 0.4 mM \( K^+_0 \), whereas the low-affinity pumps required 3.7 mM \( K^+_0 \) for half-maximal activation.

As reported above, a well-established kinetic competition exists between \( K^+_0 \) and binding of cardiac glycosides to the cardiac Na\(^+\)-K\(^+\) pump (8; reviews in
Refers to 2, 40, 156). Surprisingly, the \( I_p \) inhibition observed at various [DHO] in guinea pig (and canine) ventricular myocytes in media containing 1 or 4.6 mM \( K_o^+ \) could not be fitted by assuming this type of competition between \( K_o^+ \) and DHO. In contrast to observations on cultured chick cardiac myocytes (169) and rabbit cardiac Purkinje cells (17), increasing [Na\(^+\)]\(_{pip} \) from 10 to 50 mM had little effect on the \( K_{0.5} \) value of DHO for half-maximal \( I_p \) inhibition (57).

3. At therapeutic concentrations cardiac glycosides preferentially bind to high-affinity pump molecules in human cardiac tissue

Studies on electrogenic Na\(^+-\)K\(^+\) pumping of human atrial tissue in media containing various acetylstrophanthidin concentrations (149) or of atrial tissue from digoxin-treated patients (148) suggest that at least two classes of pump molecules displaying different glycoside sensitivity are present in the human heart. If administered therapeutically, cardiac glycosides seem to bind mainly to high-affinity pumps that may represent up to \( \sim \)33% of the pumps (149). These findings are in qualitative agreement with observations on \( I_p \) inhibition by cardiac glycosides in isolated ventricular myocytes from the human failing heart (182). According to the latter authors, the \( I_p \) inhibition-concentration curve for DHO is biphasic. The \( K_{0.5} \) value for inhibition of the high-affinity pumps amounted to \( 6 \times 10^{-9} \) M DHO. This class of pump molecules generated \( \sim 14\% \) of the total \( I_p \) under the conditions chosen. The low-affinity pumps displayed a \( K_{0.5} \) value of \( 2 \times 10^{-6} \) M DHO, 10 times lower than for pumps in guinea pig ventricular cells. The difference is obviously due to a larger dissociation rate constant for DHO in guinea pig myocytes. The \( K_{0.5} \) values of digoxin for high-affinity Na\(^+-\)K\(^+\) pumps were estimated to be \( \sim 10^{-9} \) M and for low-affinity pumps \( 3 \times 10^{-7} \) M, respectively (at [Na\(^+\)]\(_{pip} \) = 100 mM). Thus this report suggests that therapeutic doses probably block preferentially high-affinity pump molecules and inhibit 7–10% of the total \( I_p \) or Na\(^+-\)K\(^+\) pump activity (182).

4. Evaluation of new cardiac steroids as Na\(^+-\)K\(^+\) pump modulators by measurements of the cardiac \( I_p \)

A convenient method to study the modulating effect of new cardiac steroids on the cardiac Na\(^+-\)K\(^+\) pump is to measure the change of \( I_p \) caused by the drugs in cardiac cells. This method was used in an attempt to identify C-22-substituted derivatives of digoxigenin, which might be suitable for affinity labeling of the binding site(s) of the lactone ring on the Na\(^+-\)K\(^+\)-ATPase. Whole cell recording from sheep cardiac Purkinje cells revealed that esters derived from 22-hydroxy-digitoxigenin are useful for this purpose (41).

C. Cardiac Glycosides Alter the Cardiac \( I_p-V \) Relationship

Alterations of the cardiac \( I_p-V \) curve by cardiac steroids were shown when the interaction between DHO or ouabain, and the Na\(^+-\)K\(^+\) pump of rat and guinea pig ventricular myocytes was studied at various [K\(^+\)]\(_o \) and membrane potentials by means of whole cell recording (85). The glycoside-induced changes of the cardiac \( I_p-V \) relationship are probably mediated by potential-evoked alterations of the local [K\(^+\)]\(_o \), near the K\(^+\) binding sites of the Na\(^+-\)K\(^+\) pump at the bottom of an extracellular access channel. The alterations were estimated by means of the Boltzmann-Fermi equation and compared with the effect of various [K\(^+\)]\(_o \) on the binding of DHO to the cardiac Na\(^+-\)K\(^+\) pump at 0 mV. Upon a change of [K\(^+\)] in the access channel, a new equilibrium of glycoside binding to the pump is reached. The time required to establish the new binding equilibrium depends on the glycoside, the concentration of the cardioactive steroid, and on the sensitivity of the cells toward the drug. The findings lend further support to the access-channel hypothesis and thus suggest that most probably K\(^+\) binding to the cardiac Na\(^+-\)K\(^+\) pump occurs within the electrical field across the sarcolemma (85).

X. MODULATION OF THE CARDIAC SODIUM-POTASSIUM PUMP BY HORMONES

A. Aldosterone

A short-term exposure of cardiac cells to the mineralocorticoid aldosterone affects the Na\(^+-\)K\(^+\) pump. This was shown in isolated rabbit ventricular myocytes by whole cell recording of \( I_p \) and measurements of \( a_{Na}^i \) and pH in right ventricular papillary muscles using ion-sensitive microelectrodes (125). After exposure to 10 nM aldosterone for 20 min, \( a_{Na}^i \) was significantly higher (9.9 \pm 0.7 mM) than in controls (7.7 \pm 0.7 mM), whereas intracellular pH remained unchanged (even after 60 min in 100 nM aldosterone). Furthermore, after blockade of the Na\(^+-\)K\(^+\) pump by DHO, the rise of \( a_{Na}^i \) was enhanced in aldosterone-treated papillary muscles. The effect of aldosterone on \( I_p \) varied with the transmembranal Na\(^+\) and Cl\(^-\) gradients. There was an aldosterone-induced increase of \( I_p \) if these gradients were steeply inward. Flattening the Na\(^+\) and Cl\(^-\) gradients resulted in an aldosterone-evoked decrease of \( I_p \). Bumetanide (10 \mu M), an inhibitor of the Na\(^+-\)K\(^+\)-2Cl\(^-\) transporter, abolished the increase of \( I_p \) and Na\(^+\) influx evoked by aldosterone. Similarly, the mineralocorticoid receptor blocker potassium canrenoate completely blocked the aldosterone-induced \( I_p \) increase. The data strongly suggest that stimulation of \( I_p \) and Na\(^+\) influx in cardiac myocytes exposed to aldosterone in vitro is
most probably due to activation of the Na\(^{+}\)-K\(^{+}\)-2Cl\(^{-}\) transporter by the hormone (125).

Interestingly, when the hormone (50 µg/kg body wt) was administered to rabbits for 7 days, \(I_p\) of isolated ventricular cells decreased at 10 mM but not at 80 mM Na\(_{\text{pip}}\). The \(a_{Na}^i\) of papillary muscles from treated animals increased, whereas the Na\(^+\) influx remained unchanged, in contrast to the observations during short-term exposure (see above). Aldosterone had no effect on the density of pump molecules in the sarcolemma. The aldosterone antagonist spironolactone blocked both the \(I_p\) decrease at 10 mM Na\(_{\text{pip}}\) and the increase of \(a_{Na}^i\) in papillary muscles. Thus the mineralocorticoid decreased the Na\(^+\) affinity of the cardiac Na\(^{+}\)-K\(^{+}\) pump but did not affect Na\(^+\) influx following infusion for several days (126).

**B. Angiotensin-Converting Enzyme Inhibition**

By means of the same methods and cardiac preparations, a decrease of \(a_{Na}^i\) from 8.2 ± 0.4 mM (controls) to 3.6 ± 0.4 mM, but no change in intracellular pH was found in ventricular papillary muscles of rabbits treated with the angiotensin-converting enzyme (ACE) inhibitor captopril for 8 days (97). This treatment also caused an increased \(I_p\) of isolated ventricular myocytes at near-physiological [Na\(^+\)]\(_{\text{pip}}\). No captopril effect was seen at [Na\(^+\)]\(_{\text{pip}}\) < 2.5 mM and >10 mM. The effect on \(I_p\) was imitated by enalapril, an ACE inhibitor containing no sulfhydryl group, but not by the vasodilator hydralazine or by an exposure to captopril in vitro (20–55 min). According to the authors, captopril induces an intrinsic increase in cardiac Na\(^{+}\)/K\(^{+}\) pumping at near-physiological [Na\(^+\)]. One year later, the same group showed that the modulation of the cardiac Na\(^{+}\)/K\(^{+}\) pump activity by ACE inhibitors is due to an interference with the physiological mechanism of angiotensin metabolism (98). Compared with controls, myocytes from rabbits treated with the angiotensin II type 1 receptor antagonist losartan exhibited an increased \(I_p\) (at approximately physiological [Na\(^+\)]) similar to \(I_p\) of myocytes from captopril-treated animals. In addition, the latter cells displayed an \(I_p\) near the control level following exposure to angiotensin II (10 nM) for 45 min. There was a reduction of \(I_p\) rather than an interference with the pump synthesis. The modulation of \(I_p\) by captopril in ventricular myocytes from rabbits was blocked by pertussis toxin and the PKC inhibitors staurosporine and bis-indolylmaleimide I. It was mimicked by the PKC activator phorbol 12-myristate 13-acetate (PMA). Thus modulation of \(I_p\) (and cardiac Na\(^{+}\)/K\(^{+}\) pump activity) by angiotensin II is mediated via the angiotensin II type 1 receptor, a G protein, and PKC.

Figure 15 is from a recent paper on the subject (22) and shows that the increase of \(I_p\) caused by captopril or losartan can be blocked by PMA-induced PKC stimulation, by inclusion of the PKC fragment PKCF (see legend to Fig. 15) in the patch pipette solution, or by exposure of myocytes from captopril-treated rabbits to angiotensin II. The modulation of cardiac \(I_p\) by angiotensin II is probably based on a decrease of the pump’s selectivity for Na\(^+\) over K\(^+\) at binding sites near the cytosolic surface of the sarcolemma. The change in selectivity includes a phosphorylation by PKC. The \(I_p-V\) curve is not affected by the hormone.

C. Thyroid Status and Cardiac Na\(^{+}\)/K\(^{+}\) Pump

The dependence of the cardiac Na\(^{+}\)/K\(^{+}\) pump activity on the thyroid status was studied in isolated ventricular myocytes and papillary muscles from the rabbit (37). \(I_p\) of single ventricular cells was measured by means of whole cell recording and \(a_{Na}^i\) in papillary muscles by means of Na\(^+\)-sensitive microelectrodes. At −40 mV and 10 mM Na\(_{\text{pip}}\), \(I_p\) amounted to 0.24 ± 0.02 pA/pF in myocytes from hypothyroid rabbits and to 0.48 ± 0.05 pA/pF in cells from animals treated with 3,3’,5-triiodothyronine (T\(_3\)). In cells from hypothyroid rabbits, \(a_{Na}^i\) was 5.2 ± 0.42 mM, whereas \(a_{Na}^i\) amounted to 7.6 ± 0.69 mM in papillary muscles from T\(_3\)-treated rabbits. The increase in \(I_p\) was not due to an increased passive Na\(^+\) influx not compensated for by the intracellular dialysis. Using the Oxsoft Heart computer model (Oxsoft Heart Program Manual; Oxford, UK; Oxsoft, 1993), the effect of the T\(_3\)-induced
changes in $I_p$ and $a_{Na}^i$ on the cardiac action potential was simulated and found to be similar to the action potential shortening observed in papillary muscles. A subsequent paper by the same group demonstrated that the $a_{Na}^i$ increase in papillary muscles of T$_3$-treated rabbits was caused by an enhanced Na$^+$/H$^+$ exchange due to an augmented acid production (36).

D. Insulin Changes the Cardiac $I_p$-$V$ Curve

Insulin (100 mU/ml) flattens the $I_p$-$V$ curve of rabbit ventricular myocytes at negative and positive membrane potentials (80). The flattening, observed at 10 mM Na$_{pip}$, was absent if the pipette solution contained 100 $\mu$M tyrphostin A-25, a tyrosine kinase inhibitor. It seems likely that the tyrosine kinase activity of the insulin receptor modulates the voltage dependence of cardiac $I_p$. The topic was further examined in a most recent paper (79). The flattening effect of insulin on the $I_p$-$V$ relationship of the rabbit cardiac myocytes depends on Na$_{pip}$ and voltage. When Na$_{pip}$ was 80 mM and thus nearly saturating the internal Na$^+$ binding sites of the pump, or when the membrane potential was positive to +20 mV, the insulin effect on the $I_p$-$V$ curve disappeared. However, the flattening persisted in Na$_{pip}$- or K$_1$-poor media at high [K$^+$]$_o$, suggesting that competition of Na$^+$ and K$^+$ at external or internal cation binding sites of the pump was not involved. Apart from the inhibition of the insulin effect by tyrphostin A-25, wortmannin (1 $\mu$M$_{pip}$ a phosphatidylinositol 3-kinase inhibitor) abolished the $I_p$ stimulation by insulin at the holding potential of −40 mV. The hormone most probably facilitates the voltage-dependent binding of Na$^+$ to the highly specific Na$^+$-binding site within the internal access channel of the Na$^+$-$K^+$ pump via activation of the insulin receptor tyrosine kinase, phosphatidylinositol 3-kinase and protein phosphatase 1, PKC and protein phosphatase 2 are unlikely to be involved.

The effects of catecholamines on cardiac electrogenic Na$^+$-$K^+$ pumping have already been reviewed in section VIII A.

XI. MISCELLANEOUS

A. Anisosmolar External Solution Affects the Activity of the Cardiac Na$^+$-$K^+$ Pump

The role of the Na$^+$-$K^+$-ATPase in the short-term volume regulation of cardiac and other cells is controversial. The effect of hypo- and hyperosmolar bathing solutions on $I_p$ and $a_{Na}^i$ was examined in cardiac preparations by whole cell recording from isolated ventricular myocytes and $a_{Na}^i$ recording by means of Na$^+$-sensitive microelectrodes in right ventricular trabeculae of the guinea pig heart, respectively (184). Figure 16 displays $I_p$ density as a function of [Na$^+$]$_{pip}$ in ventricular myocytes at different osmolarities of the external media. As shown in Figure 16, $I_p$ measurements revealed an increase in the apparent Na$^+$ affinity of the Na$^+$-$K^+$ pump in myocytes from a $K_{D,5}$ value of 21.4 mM (isosmolar medium) to 12.8 mM Na$_{pip}$ during cell swelling in hyposmolar solution. Half-maximum $I_p$ activation occurred at 39 mM Na$_{pip}$ during cell shrinkage in hyperosmolar medium (data not shown). Maximum $I_p$ remained unaltered, and Hill coefficients were similar. In ventricular trabeculae, exposure to the hyposmolar external medium for 20 min caused a sustained Na$^+$ decrease. Na$^+$ was augmented in hyperosmolar solution. Thus osmotic myocyte swelling stimulated the Na$^+$-$K^+$ pump near physiological Na$^+$ levels, whereas shrinkage inhibited the pump. The mechanism of the changes in the apparent Na$^+$ affinity was not investigated. However, the changes were not caused by alterations in [Ca$^{2+}$]$_i$, [K$^+$]$_o$, or intracellular pH. The mechanism was further studied in a more recent paper (14). Whole cell recording of $I_p$ from isolated rabbit ventricular myocytes confirmed an increase in $I_p$ at near-physiological [Na$^+$]$_{pip}$ and no effect on $I_p$ at 80 mM Na$_{pip}$ in hyposmolar, Ca$^{2+}$-free solution at −40 mV holding potential. Cell swelling also induced a flattening of the $I_p$-$V$ curve at negative

![Figure 16](http://physrev.physiology.org/)

FIG. 16. Dependence of $I_p$ on [Na$^+$]$_{pip}$ in isosmolar (○) and hyposmolar (△) solutions. Values are means ± SE of $I_p$ normalized for cell capacitance plotted against [Na$^+$]$_{pip}$. Data are from a total of 37 rabbit ventricular myocytes exposed to isosmolar solution and 38 myocytes exposed to hyposmolar solution. Numbers in parentheses are number of cells studied at each [Na$^+$]$_{pip}$. Four experiments using 0 mM [Na$^+$]$_{pip}$ were performed at each osmolarity. [Na$^+$] in both isosmolar and hyposmolar solutions was reduced to 70 mM. Na$^+$ in isosmolar solution was substituted with 140 mM sucrose. Curves represent least-squares fit of the Hill equation to data. Note that [Na$^+$]$_{pip}$ vs. $I_p$ relationship is shifted to left in hyposmolar solution relative to that in isosmolar solution. [Na$^+$]$_{pip}$ for half-maximal current ($K_{0.5}$) values derived from the fit were 12.8 ± 2.0 and 21.4 ± 3.0 mM, respectively. Maximal $I_p$ was similar in both solutions (1.25 ± 0.10 vs. 1.24 ± 0.17 pA/pF). [Adapted from Whalley et al. (184).]
potentials and a region of negative slope at positive voltages. Thus $I_p$ stimulation by hypotonic solution is voltage dependent. The effect on $I_p$ at the holding potential was independent of $[Ca^{2+}]_o$ and $[Na^{+}]_o$. In contrast to the decrease of $I_p$ upon reexposure to isosmolar solution, $I_p$ stimulation in hypotonic media was not mediated by PKC. It was blocked by the tyrosine kinase inhibitor tyrphostin A-25, which also completely inhibited the variation of the $I_p$-$V$ relationship observed in hypotonic solution. Furthermore, LY-294002, a specific inhibitor of phosphatidylinositol 3-kinase, and okadaic acid, an inhibitor of protein phosphatase 1 (PP1) and 2A (PP2A), also blocked the $I_p$ stimulation at $-40 \text{ mV}$. Tyrosine kinase has been shown to activate protein phosphatase 1, whereas phosphatidylinositol 3-kinase is activated in tyrosine kinase-dependent intracellular signaling. The results suggest that $I_p$ stimulation by cell swelling involves the activation of tyrosine kinase, phosphatidylinositol 3-kinase, and protein phosphatase 1 and may be mediated by a dephosphorylation of the Na$^+$-K$^+$-ATPase. In contrast to the findings reported above (184), an $I_p$ stimulation at high $[Na^{+}]_{pip}$ (>55 mM) was observed in about one-half of the isolated guinea pig ventricular myocytes exposed to hypotonic medium and an $I_p$ decrease at comparable $[Na^{+}]_{pip}$ in 50% of the myocytes superfused with hypotonic solution. However, only 1 of 10 experiments with cells dialyzed with 10 mM Na$^+$ showed a modest $I_p$ increase (27%) in hypotonic solution (152).

B. Dietary Cholesterol Alters Cardiac Na$^+$-K$^+$ Pumping

A modest diet-induced increase in serum cholesterol (from $-0.9$ to $-4$ to $-6 \text{ mM}$) augmented $I_p$ at 10 mM Na$^+$ and $-40 \text{ mV}$ from $-0.3$ to $-0.5 \text{ pApF}$ in isolated rabbit ventricular myocytes (75). The $I_p$-[Na$^+$]$_{pip}$ relationship revealed an increased $I_p$ in a range of [Na$^+$]$_{pip}$ between 2.5 and $<30 \text{ mM}$. At higher or lower [Na$^+$]$_{pip}$, the dietary cholesterol was without effect. Thus a modest, but not a strong, increase ($>12 \text{ mM}$) in serum cholesterol raises the apparent affinity of the pump for cytoplasmic Na$^+$. The apparent pump affinity for $K_+^*$ was unaffected, as was the $I_p$-$V$ curve. In right ventricular papillary muscles from rabbits displaying a serum cholesterol of 4.9 mM, $a_{Na}^{i}$ decreased to 5 from 7.7 mM $a_{Na}^{i}$ in untreated controls (serum cholesterol 1.1 mM) (75). These observations clearly suggest that dietary cholesterol can alter the Na$^+$-K$^+$ pump activity in quiescent cardiac preparations.

C. Amiodarone Inhibits the Cardiac Na$^+$-K$^+$ Pump Following Acute and Chronic Treatment by Different Mechanisms

The antiarrhythmic agent amiodarone produces (among other effects) a state of cellular hypothyroidism. Because the cardiac Na$^+$-K$^+$ pump is sensitive to changes of the thyroid status (see above), the effect of the antiarrhythmic drug on the pump was studied by whole cell recording of $I_p$ from isolated rabbit ventricular cells and by $a_{Na}^{i}$ measurements in rabbit ventricular papillary muscles (76). Treatment for 4 wk resulted in an $I_p$ decrease regardless of whether [Na$^+$]$_{pip}$ was 10 or 80 mM. However, acute exposure to $\sim1 \text{ mM}$ amiodarone reduced $I_p$ at 10 mM but not at 80 mM Na$^+$. The drug had no effect on the $I_p$-$V$ curve, and the pump’s apparent affinity to $K_+^*$. The observations suggest two mechanisms of amiodarone action: chronic treatment reduces the Na$^+$-K$^+$ pump capacity, whereas acute exposure to the drug decreases the apparent affinity of the pump to Na$^+$. The inhibition of $I_p$ may contribute to the prolongation of the cardiac action potential evoked by amiodarone. The drug might exert its effects on the Na$^+$-K$^+$ pump via “a modification of the physicochemical properties of the lipid membrane” (76).

XII. EFFECTS OF THE CARDIAC SODIUM-POTASSIUM PUMP ON ION TRANSPORTERS AND CHANNELS MEASURED BY ELECTROPHYSIOLOGICAL TECHNIQUES

A. Modulation of the Cardiac Na$^+$/Ca$^{2+}$ Exchange

During the past decade, the interaction of Na$^+$-K$^+$ pumping with currents generated by other cardiac ion transporters and channels has been studied by patch-clamp methods. In internally dialyzed isolated cardiac cells, alterations of the Na$^+$-K$^+$ pump activity cause changes not only of the pump current $I_p$ but also of the Na$^+$/Ca$^{2+}$ exchange current $I_{Na/Ca}$. Figure 17 presents an example. Figure 17A shows the membrane current of a rabbit cardiac Purkinje cell at $-20 \text{ mV}$ holding potential. After 8 min in K$^+$-free solution, electrogenic Na$^+$-K$^+$ pumping is initiated by application of a medium containing 10.8 mM K$^+$ (uppermost line). The K$^+$-rich solution immediately evokes an outward current (largely $I_p$) that declines from an initial peak toward a steady-state level. The Na$^+$/Ca$^{2+}$ exchanger current $I_{Na/Ca}$ is simultaneously measured as current blocked by 5 mM Ni$^{2+}$. Both the direction and the amplitude of the exchanger current vary during the decline of $I_p$. The $I_{Na/Ca}$ amplitude is linearly related to the pump current (Fig. 17B). The reason probably is that the subsarcolemmal [Na$^+$] varies with the activity of the Na$^+$-K$^+$ pump and directly affects the currents generated by both transporters (16). A similar conclusion was reached from measurements of the Na$^+$/Ca$^{2+}$ exchange in the same cell species (120). A detailed, quantitative study on guinea pig ventricular myocytes pointed out that the activities of the Na$^+$/Ca$^{2+}$ exchanger and Na$^+$-K$^+$ pump are tightly coupled via the intracellular...
Na⁺ concentration in an interactive space representing ~14% of the total cell volume (44). This space seems to be much larger than the subsarcolemnal “fuzzy space” originally proposed (112). Furthermore, electrophysiological measurements on isolated mouse ventricular myocytes showed a similar dependence of \( I_{\text{p}} \) and Na⁺/Ca²⁺ exchanger current \( I_{\text{Na/Ca}} \) on prior Na⁺/K⁺ activity, presumably because both ion transporters generating the currents “sense” a similar subsarcolemnal Na⁺ concentration that differs from the bulk [Na⁺] of the cytosol. The data suggest the “functional presence of so-called Na⁺ fuzzy space” (172), although several questions as to the existence of such a space remain to be answered. Furthermore, the findings are consistent with the view that the exchanger and the pump molecules may be colocalized in the sarcolemma (see Ref. 122). The colocalization of special isoforms of the Na⁺-K⁺ pump and the Na⁺/Ca²⁺ exchanger may have important physiological and pharmacological implications. Generally speaking, any modulation of the cardiac pump activity affects the contractile function of the heart by modifying the Na⁺/Ca²⁺ exchange. It is widely accepted that cardiac steroids cause their positive inotropic effect by increasing the amount of Ca²⁺ released from the sarcoplasmic reticulum and available for contraction during an action potential. The increase of [Ca²⁺] is probably mainly brought about by an inhibition of the Ca²⁺ efflux via the Na⁺/Ca²⁺ exchanger following Na⁺-K⁺ pump inhibition by the drugs. The pump inhibition induces an increase in [Na⁺] and thereby a reduction of the driving force for the Ca²⁺ efflux (7, 68). Additional cellular mechanisms of cardiac steroid action and the relation between Na⁺-K⁺ pump inhibition and cardiac contraction have been discussed elsewhere in detail (116). It has recently been shown in rat smooth muscle cells of mesenteric arteries, neurons, and astrocytes that highly cardiac glycoside-sensitive \( \alpha_{1c} \) and \( \alpha_{2} \)-subunits (astrocytes) of the Na⁺-K⁺ pump and the Na⁺/Ca²⁺ exchanger are expressed in a reticular distribution within the cell membrane overlying functional sarcoplasmic (endoplasmic) reticulum. Cardiac steroids (and endogenous digitalis-like factors) may exert their pharmacological (hormonal) effect by binding to these specialized plasmalemmal domains, modulating [Na⁺] and [Ca²⁺] only in the cleft between cell membrane and sarcoplasmic reticulum (“plasmerosome”) and thereby the Ca²⁺ content of the adjacent sarcoplasmic reticulum. By way of contrast, Na⁺-K⁺ pump molecules containing the cardiac glycoside-insensitive “housekeeping” \( \alpha_{1} \)-isoform are uniformly expressed in the rat cell membranes studied (20, 102).

B. Interaction Between the Cardiac Na⁺-K⁺ Pump and Kₐ₅ Channels

The effect of altered Na⁺-K⁺ pump activity on \( I_{\text{K(ATP)}} \) was investigated by means of patch-clamp measurements on guinea pig ventricular myocytes (141). Inhibition of the forward-running pump (i.e., the physiological mode) by the cardioactive steroid strophantidin, K⁺-free bathing solution, or strong hyperpolarization decreased \( I_{\text{K(ATP)}} \). Correspondingly, blocking the backward-running (ATP-
A K⁺ channel that is gated by intracellular [Na⁺] >20 mM exists in guinea pig ventricular myocytes. The probability of opening for this channel depends on Na⁺ with a $K_{0.5}$ value of 66 mM, but not on Ca²⁺ or voltage. In passing it was noted that the inhibition of the Na⁺-K⁺ pump might be important for the activation of $I_{K(Na)}$ through the channel under pathological conditions (104). This aspect was emphasized by the demonstration that inhibition of cardiac Na⁺-K⁺ pumping either by ouabain or in K⁺-free solution activated $I_{K(Na)}$ even at a cytosolic [Na⁺] too low for the gating of the channel (118). It seems likely that [Na⁺] at the inner mouth of the channel is, in general, different from bulk [Na⁺]. The activity of the Na⁺-K⁺ pump is one important factor governing [Na⁺] at the channel mouth. [Na⁺] increases following pump inhibition and augments the open probability of the $I_{K(Na)}$ channel (24). The mechanism tends to stabilize the resting potential after an impairment of Na⁺-K⁺ pumping.

D. Blockade of the Na⁺-K⁺ Pump Activates $I_{K(ACh)}$ in Atrial Myocytes

A novel gating mechanism of the cardiac muscarinic K⁺ channel, independent of G protein activation, was identified in chick atrial cells (173). The mechanism involves two steps. First, the functional state of the channel is modified by ATP hydrolysis. Second, [Na⁺]i >3 mM gates the modified channel with a $K_{0.5}$ value of ~40 mM. Inhibition of the Na⁺-K⁺ pump either in K⁺-free medium or by ouabain (5 × 10⁻⁴ M) activates $I_{K(ACh)}$ in the same manner as an increase of [Na⁺]i and implies that the blockade of the pump activates $I_{K(ACh)}$ via an augmented subsarcomembral [Na⁺]. The findings demonstrate for the first time a Na⁺-mediated effect of cardiac glycosides on an atrial ionic channel that is involved in the regulation of the cardiac rhythm.

XIII. CONCLUSION

The Na⁺-K⁺ pump of animal cells generates the pump current $I_p$. Under physiological conditions $I_p$ is an outward current. It can be measured by electrophysiological methods. The introduction of the patch-clamp techniques has rendered possible a hitherto unequalled experimental control of membrane potential and composition of the intracellular medium in single cells. The techniques of whole cell recording and recording from giant patches permit the study of Na⁺-K⁺ pump characteristics by measuring $I_p$. For this purpose, cardiac cells are especially suitable, since they exhibit a high density of sarcosomal pump molecules and an adequate cellular geometry and are easily obtainable. In fact, measurements of the cardiac $I_p$ have been pivotal for the understanding of Na⁺-K⁺ pumping in electrically excitable cells. The cardiac Na⁺-K⁺ pump can be studied in its physiological environment, i.e., in the sarcolemma which separates cytosol and extracellular medium with their different ionic compositions and across which a membrane potential exists. The effect of intra- and extracellular cations on the cardiac pump, the interaction between membrane potential and Na⁺-K⁺ pump, and the modulation of the pump activity by transmitters, hormones, and drugs have been studied with unrivaled precision and time resolution by means of patch-clamp techniques. Through the recording of $I_p$ and transient pump currents, several voltage-dependent partial reactions of the pump cycle have been identified, including the binding and unbinding of monovalent cations to or from the pump in an access channel. The fundamental mechanism of ion translocation by the
pump, however, remains to be clarified. Furthermore, the quantitative contribution of electrogenic Na⁺-K⁺ pumping to the cardiac action potential under physiological conditions has still to be assessed. The knowledge of pump modulation by transmitters, hormones, and drugs in cardiac cells is preliminary and requires further detailed studies. Finally, the interaction between Na⁺-K⁺ pump molecules and ion channels or transporters colocalized in the sarcolemma is a promising field of future research.

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