Physiology and Pathophysiology of the Calcium Store in the Endoplasmic Reticulum of Neurons

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| I. Endoplasmic Reticulum and Cellular Physiology: Historical Remarks | 202 |
| A. The neuronal ER as a continuous endomembrane structure | 203 |
| B. The ER as a universal signaling organelle | 205 |
| C. The ER as an excitable organelle | 205 |
| II. Organization of the Endoplasmic Reticulum | 203 |
| A. The neuronal ER as a continuous endomembrane structure | 203 |
| B. The ER as a universal signaling organelle | 205 |
| C. The ER as an excitable organelle | 205 |
| III. Endoplasmic Reticulum Calcium Release in Nerve Cells | 207 |
| A. The discovery of Ca\(^{2+}\) release from the ER | 207 |
| B. Ca\(^{2+}\)-induced Ca\(^{2+}\) release | 209 |
| C. IICR in nerve cells | 226 |
| D. Do InsP\(_3\)Rs and RyRs share a common Ca\(^{2+}\) pool? | 233 |
| E. Luminal Ca\(^{2+}\) as a regulator of Ca\(^{2+}\) release | 234 |
| F. Refilling the Ca\(^{2+}\) store and store-operated Ca\(^{2+}\) entry | 236 |
| G. Pharmacology of Ca\(^{2+}\) release | 242 |
| IV. Endoplasmic Reticulum Calcium Signaling and Regulation of Neuronal Functions | 244 |
| A. Neuronal excitability | 244 |
| B. Neurotransmitter release | 244 |
| C. Synaptic plasticity | 250 |
| D. Gene expression, ER Ca\(^{2+}\) waves, and ER Ca\(^{2+}\) tunneling | 253 |
| E. Neuronal growth and morphological plasticity | 254 |
| F. Neurohormone release | 255 |
| G. Circadian rhythms | 255 |
| V. Endoplasmic Reticulum Calcium Homeostasis and Neuronal Pathophysiology | 255 |
| A. ER stress response and neurodegeneration | 255 |
| B. ER stores and ischemia | 256 |
| C. ER stores and human immunodeficiency virus | 257 |
| D. ER stores and diabetes | 257 |
| E. ER stores and gangliosidoses (Gaucher's and Sandhoff's disease) | 258 |
| F. ER stores and Alzheimer's disease | 258 |
| G. ER stores and ageing | 260 |
| H. Spinocerebellar ataxia type 1 | 261 |
| I. ER and neuronal trauma | 261 |
| J. ER and Parkinson's disease | 261 |
| K. ER and epileptiform activity | 261 |
| L. ER and Huntington's disease | 261 |
| VI. Concluding Remarks | 261 |

Verkhratsky, Alexei. Physiology and Pathophysiology of the Calcium Store in the Endoplasmic Reticulum of Neurons. *Physiol Rev* 85: 201–279, 2005; doi:10.1152/physrev.00004.2004.—The endoplasmic reticulum (ER) is the largest single intracellular organelle, which is present in all types of nerve cells. The ER is an interconnected, internally continuous system of tubules and cisterns, which extends from the nuclear envelope to axons and presynaptic terminals, as well as to dendrites and dendritic spines. Ca\(^{2+}\) release channels and Ca\(^{2+}\) pumps residing in the ER membrane provide for its excitability. Regulated ER Ca\(^{2+}\) release controls many neuronal functions, from plasmalemmal excitability to synaptic plasticity. Enzymatic cascades dependent on the Ca\(^{2+}\) concentration in the ER lumen integrate rapid Ca\(^{2+}\) signaling with long-lasting adaptive responses through modifications in protein synthesis and processing. Disruptions of ER Ca\(^{2+}\) homeostasis are critically involved in various forms of neuropathology.
I. ENDOPLASMIC RETICULUM AND CELLULAR PHYSIOLOGY: HISTORICAL REMARKS

In 1674 Antonius van Leeuwenhoek, after discovering the cross-striations of muscle fibers using one of his ingenious light microscopes, reflected “… who can tell, whether each of these filaments may not be enclosed in its proper membrane and contain within it an incredible number of still smaller filaments.” (236). One century later, Luigi Galvani discovered “animal electricity” (178). To explain his observations, Galvani suggested that nerve and muscle are capable of generating electricity by accumulating positive and negative charges on two opposite surfaces. Moreover, the electrical flow necessary to produce voltage changes was explained by Galvani in terms of specific fluid-filled pores between the internal and external surfaces, which were the harbingers of ion channels.

The 19th century witnessed the emergence of the cellular theory. Furthermore, it became apparent that the cell is a complex structure containing numerous organelles. Great histologists, such as Rudolf Virchow, Gustav Retzius, Santiago Ramon y Cajal, and Jan Purkinje, to name but a few, developed various techniques to look inside the cells, and it was Camillo Golgi who, using his “black (silver chromate) reaction” technique in 1898 discovered the “fine and elegant reticulum hidden in the cell body” of owl cerebellar Purkinje neurons (187; see also Refs. 37, 384), the structure known to us as the “Golgi complex.” The first detailed description of the endoplasmic reticulum was made by Golgi’s pupil, Emilio Veratti, who, by using black reaction technique on muscle fibers, was the first to discover a “true reticular apparatus constituted of filaments” (385, 668, 669; Fig. 1). This finding remained almost unnoticed for 50 years, and only when the endoplasmic reticulum (ER) was rediscovered by Keith Porter and George Palade, who used electron microscopy (481), were Veratti’s achievements generally recognized.

Insights into the physiological function of the ER and the appearance of the concept of intracellular Ca$^{2+}$/H$^{+}$ stores resulted from studies of muscle contraction. Sidney Ringer’s experiments (535), which clearly identified Ca$^{2+}$ as an ion mediating muscle contraction, did not address directly the question of the Ca$^{2+}$ source, although Ringer noticed that contractions of skeletal muscle can be maintained in Ca$^{2+}$/H$^{+}$-free media for hours and even days (536, 537), suggesting the existence of some barriers hampering the exchange of ions between the muscle interior and the external milieu. Further notions of the possible importance of intracellular Ca$^{2+}$-containing systems were stimulated by experiments in which it was shown that move-

![Fig. 1. Early drawings of intracellular reticular structures. A: original drawings of Camillo Golgi. The drawings present dorsal root ganglion neurons of an adult horse (Figs. 1–3), bovine fetus (Fig. 4), a neonatal cat (Fig. 6), and a young dog (Fig. 8). Black reticular structures (“internal reticular apparatus”) are visualized by metallic impregnation (188). B: original drawing of Emilio Veratti. Longitudinal section of a limb muscle of the water beetle *Hydrophilus piceus* (668). The black reticular structure represents sarcoplasmic reticulum. [A from Bentivoglio et al. (37); B from Mazzarello et al. (385).]
ments of ameba are particularly sensitive to chelation of intracellular Ca²⁺. Injection of Ca²⁺-precipitating agents such as phosphates, sulfates, or alizarine sulfonate froze the movements of the amebas almost instantly (513, 532). The first direct evidence for the existence of a sizable intracellular Ca²⁺ storage compartment was obtained by Weise, who found that excessive physical exercise led to the appearance of Ca²⁺ in the ultrafiltrate of rat skeletal muscle (691). Later, an increased release of radioactive Ca²⁺ from stimulated frog sartorius muscle was demonstrated by Woodward (698), thus proving the existence of intracellularly stored calcium.

The idea of an intracellular Ca²⁺ store and the importance of regulated intracellular Ca²⁺ release for muscle contraction was formulated by Heilbrunn, who was also the first to test these concepts experimentally (210, 211). His theory was not readily accepted, and for many years K⁺ were considered to be the regulators of contraction and relaxation, a theory very much supported by Szent-Gyorgyi (619). Only after the free intracellular Ca²⁺ in skeletal muscle was measured directly by murexide (263) or aequorin (15, 533) could internal Ca²⁺ release be visualized and the intracellular Ca²⁺ store became the legitimate member of a Ca²⁺ signaling cascade. In the meantime, the concept of intracellular Ca²⁺ pumps associated with the endomembrane was developed by W. H. Hasselbach, Setsuro Ebashi, and Anne-Marie Weber (see Ref. 159), explaining how the ER could accumulate Ca²⁺. At the same time, the first indications of ER-residing regulated ion channels, which could provide a means of Ca²⁺ release, appeared (158).

At the beginning of the 1970s the crucial role of the ER (in its sarcoplasmic reticulum disguise) in regulation of contraction became generally accepted and the concepts of Ca²⁺-induced Ca²⁺ release and depolarization-induced Ca²⁺ release firmly established (see Refs. 140, 160 for review). At the same time, the receptor-activated, second messenger-induced intracellular Ca²⁺ release was discovered (81, 457), and a possible role for inositol phospholipids in signal transduction was suggested (410). Another decade, however, passed before the seminal experiments of Hanspeter Streb, Robin Irvine, Irene Schulz, and Michael Berridge (607) showed that this alternative pathway for intracellular Ca²⁺ release is mediated by the second messenger inositol 1,4,5-trisphosphate (InsP₃). The intracellular Ca²⁺ release channels, residing within the endomembrane, were soon molecularly characterized, revealing two main families: the Ca²⁺-gated and InsP₃-gated channels (505, 613). Thus the general concept of intracellular Ca²⁺ stores was established, and the stage was set for identification of their functional role in various cell types.

The ability of nerve cells to actively buffer cytosolic Ca²⁺ was initially associated with Ca²⁺ accumulation by mitochondria, the latter utilizing H⁺ transport to develop an electrochemical gradient favoring Ca²⁺ entry (334, 544). The functional importance of the neuronal ER as a dynamic Ca²⁺ pool was first appreciated after the demonstration that neuronal microsomal fractions can accumulate Ca²⁺ via an ATP-dependent mechanism (649). Almost simultaneously it was also discovered that various neuronal preparations, such as the squid axon (64), extruded axoplasm (21), and permeabilized synaptosomes (51, 52), are able to sequester Ca²⁺ even when mitochondrial uptake was obliterated (see also Ref. 416 for review). Finally, this nonmitochondrial Ca²⁺ uptake was unequivocally associated with the ER (212–214, 682), and the latter became firmly established as a dynamic intracellular Ca²⁺ store, deeply involved in neuronal signaling.

II. ORGANIZATION OF THE ENDOPLASMIC RETICULUM

A. The Neuronal ER as a Continuous Endomembrane Structure

The endoplasmic reticulum is arguably the largest single intracellular organelle, which appears as a three-dimensional network formed by an endomembrane and organized in a complex system of microtubules and cisternae. In neurons, the ER extends from the nucleus and the soma to the dendritic arborization and, through the axon, to presynaptic terminals. Classical histology subdivides the ER into smooth ER, rough ER, and the nuclear envelope (32). The continuity of the endomembrane that forms these three structures was initially deduced from microscopic observations (130, 131, 371, 682). Further insights into the continuity of the ER were gained by fluorescent microscopy on cells injected with the lipophilic probe 1,1′-dihexadecyl-3,3′,3′,3′-tetramethylindocarbocyanine perchlorate (DiIC₁₆), which diffuses within strictly continuous lipid membranes. When injected into cells, DiIC₁₆ labeled the intracellular membrane network extending throughout all parts of the cell (Fig. 2, A and B). The kinetics of dye spread was consistent with intramembrane diffusion; moreover, the ER labeling occurred even when slices were fixed 2 min after dye injection, thus excluding the possibility of active transport (634). Very similar ER labeling was observed in cultured hippocampal neurons (634). The concept of a continuous ER was further substantiated by experiments on nonneuronal cells. Initially, the continuity of the intra-ER aqueous space was directly demonstrated in pancreatic acinar cells, where soluble molecules, such as Ca²⁺ and fluorescent dyes, were shown to move throughout the ER lumen with remarkable ease (491, 509). Later on, it was found
that much larger molecules of green fluorescence protein (GFP) targeted against the ER and expressed in Chinese hamster ovary (CHO) cells, were also able to freely diffuse within the ER lumen. Repeated local laser illumination of a spot 2 μm in diameter eventually bleached all GFP present in the cell, thus demonstrating the continuity of the ER lumen (115). Similarly, in RBL cells transfected with even larger ER-targeted elastase-GFP fusion protein (molecular weight 60 kDa), fluorescence recovery after photobleaching revealed rapid (diffusion coefficient ~0.5 μm²/s) movements of this, rather hefty, protein within the entire ER lumen (610).

Although internally continuous, the ER exhibits an extraordinary heterogeneity in both its morphology and the functional properties. The ER can be represented as tubular networks as well as by flattened cisternae, the latter sometimes arranged in complex cisternal ER stacks or even crystalloid structures found in photoreceptors (682) and Purkinje neurons (625). In neuronal somata, the ER network is omnipresent (Fig. 2), and part of it forms the nuclear envelope. Portions of the ER in the soma and in the distal dendrites are molded into specialized structures known as subsurface cisternae, which come into close contact with the plasma membrane (620). These cisternae exist in several shapes and forms: type I is separated from the plasmalemma by a distance of ~60–80 nm, whereas types II and III reside much closer, at a distance of ~20 nm from the plasmalemma. These latter types of ER cisternae often follow the contour of the surface membrane (543). Sometimes the surface cisternae are organized in more complex structures, the cisternal organelles, which have been found in the proximal parts of axons (34, 267, 305, 548).

In axons, the ER mostly has a tubular structure and extends into the presynaptic terminals, where it often closely enwraps the mitochondria (48, 391, 693). The ER also spreads throughout the dendrites, terminating in the spine apparatus and thereby connects the latter with the entire ER lumen (604).

An important feature of the ER is its morphological plasticity. The ER network constantly remodels itself through multiple forms of tubular movements. These
movements occur quite rapidly; new structures emerge within tens of seconds (57). Even large formations such as crystalline cisternal organelles are not static and may swiftly surface as part of a neuronal adaptive response, for example, against hypoxia (625).

The topographical heterogeneity of the ER is matched by its functional diversity. Protein synthesis within the rough ER occurs in specific compartments, with different mRNAs being specifically selected and targeted towards distinct regions of the reticulum (209, 525, 682). Sometimes mRNA is engaged in a long-distance travel to end up in the ER in dendrites, where the actual synthesis of a locally demanded protein (e.g., InsP$_3$ receptors, Ref. 606) takes place (180, 566). Here, the ER closely interacts with another reticular structure, the Golgi apparatus, the outposts of which are present in dendrites and are involved in final trafficking of membrane proteins and proteins destined for secretion (237, 238). Although the precise mechanisms of the intra-ER mRNA sorting remain unclear, they appear to be an important part of proteins targeting cascades. The synthesis of phospholipids and cholesterol similarly appears to be spatially heterogeneous, with different steps of the process occurring in different ER compartments (32). The enormous functional heterogeneity of the ER is nonetheless integrated into a coherent system by the continuous lumen.

B. The ER as a Universal Signaling Organelle

The functions of the ER are many. First and foremost, the ER is the site of synthesis and maturation of proteins. Protein synthesis is accomplished in the rough ER, while posttranslational processing of proteins is governed by an extended family of ER-resident chaperones, which form complexes with newly synthesized proteins, assist their folding into the final tertiary structure and prevent them from aggregation. If the process of folding somehow fails, the chaperones remain assembled with misfolded proteins, thereby preventing them from proceeding through the ER exit sites and towards the Golgi complex. Whenever the concentration of unfolded proteins rises dangerously high, the ER develops a specialized reaction known as ER stress, as a result of which transcription-affecting signals are sent to the nucleus, which, in turn, adjusts gene expression according to environmental requirements. In addition to the synthesis of proteins, the ER is the primary site of formation of phospholipids, glucosylphosphatidylinositolos, and leukotrienes. The ER may also serve as a graveyard for various unwanted molecules and toxins. Owing to its continuous lumen, the ER serves as a highway allowing haulage of transport RNAs, secretory products, numerous structural proteins and, as we shall see later, ions between different parts of polarized cells. The ER is also intimately involved in rapid cellular signaling, because it is a dynamic store of Ca$^{2+}$ that regulates the cytosolic Ca$^{2+}$ concentration and generates Ca$^{2+}$ fluxes between the cytosol and the ER lumen in response to extracellular stimulation. Finally, the ER coordinates all these diverse processes with the physiological state of the cell. Therefore, the ER may be defined as a multifunctional organelle able to detect and integrate incoming signals and generate output signals in response to environmental changes (40, 57). For a comprehensive overview of ER functions, see References 65, 257, 364, 388, 392–402, 479, 487, 508, 509, 511, 677.

The intimate mechanisms of ER integration remain largely undiscovered, yet a central role for Ca$^{2+}$ is emerging. First, Ca$^{2+}$ is a key input and output signal of the ER. Indeed, Ca$^{2+}$ concentration increases in the cytosol affect its concentration in the ER, and in turn, the ER Ca$^{2+}$ release and uptake influence the cytosolic Ca$^{2+}$ concentration. Second, a number of intra-ER chaperones, such as calreticulin, calnexin, grp78/BiP, endoplasmin (or glucose-regulated protein, grp94), are Ca$^{2+}$ binding proteins, and changes in free Ca$^{2+}$ concentration in the lumen of the ER ([Ca$^{2+}]_{l}$) profoundly affect their functional activity (106, 409). Therefore, fluctuations in the ER Ca$^{2+}$ content may provide the link between rapid signaling and long-lasting cellular adaptive responses.

C. The ER as an Excitable Organelle

The basic physiology of the ER calcium store has been extensively characterized in a variety of excitable and noneexcitable cells (for key reviews, see Refs. 38–42, 56, 75, 76, 147, 177, 308, 309, 311, 381, 507, 508, 539, 670–672, 712). The ER acts as a dynamic Ca$^{2+}$ store due to concerted activity of Ca$^{2+}$ channels and transporters residing in the endomembrane, and intraluminal Ca$^{2+}$-binding proteins, which serve as a high-capacity Ca$^{2+}$ buffering system. Ca$^{2+}$ efflux from the ER is executed by two families of Ca$^{2+}$ channels, the Ca$^{2+}$-gated Ca$^{2+}$ channels, generally referred to as the ryanodine receptors (RyRs), and the InsP$_3$-gated channels, commonly known as InsP$_3$ receptors (InsP$_3$Rs). Ca$^{2+}$ accumulation into the ER lumen results from the activity of Ca$^{2+}$ pumps of the sarco(endo)plasmic reticulum Ca$^{2+}$-ATPase (SERCA) family. The molecular physiology of ER channels and SERCA pumps has been comprehensively reviewed recently (RyRs, Refs. 59, 148, 161, 396, 545, 605; InsP$_3$Rs, Refs. 44, 411, 412, 631; SERCA pumps, Refs. 121, 134, 587, 618, 699); hence, I shall limit myself to a brief discussion of their key features in the context of neuronal signaling.

1. Ca$^{2+}$ release channels

The Ca$^{2+}$ release channels, the RyRs and the InsP$_3$Rs, are large tetrameric channel proteins that share a rather peculiar four-leaf clover-like structure when observed by
electron microscopy. The RyRs were the first to receive experimental attention due to their vital role in excitation-contraction coupling in muscle. The RyR family comprises three major receptor subtypes, which were historically classified as “skeletal muscle,” “heart,” and “brain” types, currently referred to as RyR1, RyR2, and RyR3, respectively. The historic classification had a certain physiological sense, as the RyR1 type, dominant in skeletal muscle, possesses a rather unique activation mechanism; it can be activated solely by membrane depolarization by virtue of a direct link with the plasmalemmal Ca\(^{2+}\) channels through a huge cytosolic “foot” extension. All three RyRs can be activated by Ca\(^{2+}\) from the cytosolic side; the rank order of their sensitivity to cytosolic free Ca\(^{2+}\) concentration ([Ca\(^{2+}\)]\(_i\)) being RyR1 > RyR2 > RyR3 (93, 435). On the basis of studies on reconstituted channels in lipid bilayer membranes, the RyRs can be biophysically described as not perfectly selective Ca\(^{2+}\) channels (P\(_{Ca}/P_k \sim 6–7\) with fast activation kinetics (\(\tau\) of activation \(~0.5–1\) ms) and a large conductance (100–500 pS, depending on the nature of ion carrier) (148). Yet, taking into account the imperfect selectivity toward monovalent cations and the virtual absence of selectivity between divalents, the physiological unitary Ca\(^{2+}\) current through RyRs can be as small as 0.5–0.9 pA at 2 mM intraluminal Ca\(^{2+}\) concentration (397).

All three types of RyRs have been detected in neurons; however, expression of the RyR2 isoform dominates. Interestingly, although the RyR3 was initially called the “brain” subtype, its total expression in the central nervous system does not exceed 2% of all RyRs (434). The RyR1 is heavily expressed in cerebellar Purkinje neurons, whereas RyR3 is found in hippocampal structures, the corpus striatum, and the diencephalon (170, 185, 434, 474, 574).

However, these are only general trends; in reality, many neurons coexpress either two or all three types of RyR isoforms. RyRs are found in high densities in neuronal somata; in axons of hippocampal (574), cerebellar (475), and cortical (723) neurons; in cerebellar mossy fibers (475); and in presynaptic terminals (355). RyRs are also widely expressed in the dendritic tree; their expression is particularly high in dendritic spines of hippocampal CA1 neurons, where they represent the dominant Ca\(^{2+}\) release channel (574). Incidentally, RyRs were visualized (with fluorescent ryanodine) in the Golgi complex of sympathetic neurons, although the functional relevance of this discovery remains unclear (98).

The expression of RyRs changes considerably during development; for instance, throughout the embryonic stage, RyR1 mRNA levels are highest in the rostral cortical plate, whereas RyR3 mRNA was most prominent in the caudal cortical plate and hippocampus. Low levels of RyR2 mRNA were detected in the diencephalon and the brain stem. However, from postnatal day 7 onward, RyR2 mRNA became the major isoform in many brain regions, while RyR1 mRNA became prominent in the dentate gyrus and in the Purkinje cell layer. Postnatal downregulation in the caudal cerebral cortex restricted RyR3 mRNA expression to the hippocampus, particularly the CA1 region (427).

In vivo imaging of RyRs can be achieved using fluorescent BODIPY-ryanodine (e.g., Refs. 389, 603; Fig. 2C). Because ryanodine binding to the RyR is use dependent, the intensity of BODIPY-ryanodine staining may reflect the overall density of open channels. With the use of this technique, a redistribution of active RyRs from soma to dendrites was observed in developing hippocampal neurons (611); moreover, L-type Ca\(^{2+}\) channel-RyR coupling was identified at early developmental stages (611), reflecting a developmental switch between RyR1 and RyR2 expression in nerve cells (427).

Similar to RyRs, the InsP\(_3\) receptor family comprises three homotetrameric isoforms, InsP\(_3\)R1, InsP\(_3\)R2, and InsP\(_3\)R3, although further diversity may arise from alternative splicing of InsP\(_3\)R1 (up to 6 variants; Ref. 463) and heteromeric expression (399). The most important difference between isoforms lies in their different sensitivity to [Ca\(^{2+}\)]; the InsP\(_3\)R1 has the “classical” bell-shaped dependence on [Ca\(^{2+}\)] with a maximal activation at \(~300–400\) nM (45); the InsP\(_3\)R2 and InsP\(_3\)R3 are also stimulated by [Ca\(^{2+}\)], but are not readily inhibited by larger [Ca\(^{2+}\)] increases (180).

In the central nervous system, the InsP\(_3\)R1 and InsP\(_3\)R2 were initially discovered in the cerebellum (172, 613), but thereafter all three isoforms were identified throughout the brain, with particularly strong expression in Purkinje neurons and in somas of CA1 cells (170, 171, 574). The InsP\(_3\)R1 is the most abundant isoform in the brain, whereas InsP\(_3\)R2 dominates in the spinal cord and is also highly expressed in glial cells. The InsP\(_3\)R3 are expressed to a much lesser extent but are nonetheless clearly detectable in several brain regions, such as the cerebellar granule layer and the medulla (575). There is a prominent heterogeneity in the intracellular distribution of InsP\(_3\)Rs. For example, InsP\(_3\)R1 is found in dendrites, dendritic spines, cell bodies, axons, and axonal terminals of cerebellar Purkinje cells, but is mostly confined to somatic regions and proximal dendrites in other neurons (122, 551, 575). The InsP\(_3\)R3 is very much concentrated in the neuropil and neuronal terminals (575).

2. SERCA pumps

The SERCA pump belongs to the family of P-type Ca\(^{2+}\)-ATPases and includes three gene products, SERCA1, SERCA2, and SERCA3; alternative splicing produces two distinct isoforms of SERCA2, namely, 2a and 2b. In central neurons, the SERCA2b is ubiquitously expressed, whereas SERCA2a and SERCA3 are found al-
most exclusively in cerebellar Purkinje neurons (18). The physiological role of SERCA3 remains quite mysterious, and genetic deletion of this Ca\(^{2+}\) pump does not result in any obvious phenotypic change, save minor deficits in relaxation of vascular and tracheal smooth muscles (351).

An important property of the SERCA2b pump that is relevant for ER Ca\(^{2+}\) homeostasis is the tight regulation of its activity by the free Ca\(^{2+}\) concentration in the ER lumen, mediated via the Ca\(^{2+}\)-binding proteins calreticulin (264) and ERp57 (341). Calreticulin senses the actual level of \([\text{Ca}^{2+}]_L\) and, by directly interacting with SERCA2b, activates Ca\(^{2+}\) pumping whenever \([\text{Ca}^{2+}]_L\) decreases. The ERp57 regulates Ca\(^{2+}\) uptake by modulating the redox state of SERCA2b thioles in a Ca\(^{2+}\)-dependent manner.

The SERCA pumps have a distinct pharmacology; they are blocked irreversibly by thapsigargin (TG) in submicromolar concentrations (\(K_D \sim 20\) nM) and reversibly by cyclopiazonic acid (CPA) at concentrations of \(\sim 20–50\) \(\mu\)M. Inhibition of the SERCA pumps results in a relatively slow emptying of releasable Ca\(^{2+}\) from the ER, with Ca\(^{2+}\) leaving the ER through poorly understood pathways (72). It must also be noted that TG is not perfectly specific for SERCAs; it also inhibits plasmalemmal voltage-gated Ca\(^{2+}\) channels of L/N-types in DRG neurons at concentrations of 0.2–2 \(\mu\)M (582). Likewise, micromolar concentrations of TG substantially decreased T and L Ca\(^{2+}\) currents in adrenal glomerulosa cells (546). The concentrations of TG that inhibit Ca\(^{2+}\) channels are comparable to those used in the majority of experiments in which it is intended to block the Ca\(^{2+}\) accumulation by the ER. A certain care, therefore, should be taken when interpreting data arising from such experiments.

3. \(\text{ER Ca}^{2+}\) buffers

\(\text{ER Ca}^{2+}\) buffering is accomplished by several Ca\(^{2+}\)-binding proteins; in neurons, the most abundant is calreticulin, which accounts for nearly one-half of total ER Ca\(^{2+}\) binding. Calreticulin in the neuronal ER is endowed with 20–50 low-affinity Ca\(^{2+}\) binding sites (\(K_D \sim 1\) mM) and therefore can bind a huge amount of Ca\(^{2+}\). As mentioned above, calreticulin acts not only as a Ca\(^{2+}\) buffer, but also as an important chaperone and regulator of SERCA pumps. Calsequestrin, the major ER Ca\(^{2+}\) buffer in skeletal myocytes, is also present in nerve cells, and like calreticulin may bind up to 50 Ca\(^{2+}\) with low affinity. Several other low-affinity Ca\(^{2+}\)-binding proteins, such as endoplasmic (grp94), BiP/grp78, and proteins of the CREC family (reticulocalbin, calumenin, Cab55, etc.) also participate in intra-ER Ca\(^{2+}\) buffering (235, 399, 401, 409).

The most important property of the ER Ca\(^{2+}\) buffers is their low affinity to Ca\(^{2+}\), which allows the maintenance of high intra-ER free Ca\(^{2+}\) levels. There is a fundamental difference in Ca\(^{2+}\) handling in the cytosol and within the ER lumen. The cytoplasmic Ca\(^{2+}\) binding proteins are characterized by a very high (10–100 nM) affinity to Ca\(^{2+}\); therefore, they limit Ca\(^{2+}\) diffusion and favor localization of cytosolic Ca\(^{2+}\) signals. In contrast, the low Ca\(^{2+}\) affinity of the ER buffers allows Ca\(^{2+}\) diffusion throughout the lumen (423).

4. \textit{The ER as an excitable medium}

The extensive complement of endomembrane Ca\(^{2+}\) channels and transporters, together with intra-ER Ca\(^{2+}\) storage proteins, gives rise to the excitable properties of the ER. Ca\(^{2+}\) accumulation in the ER lumen creates a large electrochemical driving force favoring Ca\(^{2+}\) movement from the ER lumen into the cytosol; hence, opening of Ca\(^{2+}\) channels in the ER membrane causes fast efflux of Ca\(^{2+}\) from the ER. In turn, the ER channels are sensitive to signals originating at the plasmalemma and so can be activated by both electrical and chemical stimulation of the cell. Furthermore, the ER channels are subject to positive autoregulation due to their sensitivity to cytosolic Ca\(^{2+}\), leading to a regenerative opening of RyRs and/or InsP\(_4\)Rs beyond a certain threshold of \([\text{Ca}^{2+}]_L\). This regenerative process underlies propagating waves of channel activation along the ER membrane, very similar to the propagating wave of opening and closing of Na\(^+\) and K\(^+\) channels that generate plasmalemmal action potentials. Neuronal function is thus an interplay of two excitable membranes, the outer and the inner, which are coupled through a multitude of reciprocal signaling cascades (39).

In contrast to plasmalemma, which is mostly specialized for integrating extracellular information, the ER uses Ca\(^{2+}\) to couple plasmalemmal and cytosolic events with intraluminal formation and transport of proteins, as well as with gene expression within the nucleus. The periodic fluctuations of the ER Ca\(^{2+}\) concentration are instrumental for this intracellular integration, because \([\text{Ca}^{2+}]_L\) regulates the excitability of the ER membrane by controlling ER Ca\(^{2+}\) release channels and SERCA pumps, and governs intra-ER enzymatic cascades.

III. ENDOPLASMIC RETICULUM CALCIUM RELEASE IN NERVE CELLS

A. The Discovery of Ca\(^{2+}\) Release From the ER

Historically, the first observations of the release of Ca\(^{2+}\) from an intracellular pool were very much indebted to the previous discovery that caffeine can activate RyRs in muscle cells (688). The pioneering observation indicating that caffeine affects neuronal \([\text{Ca}^{2+}]_L\) was made by K. Kuba and S. Nishi in 1976 (319). When monitoring membrane potential of neurons in isolated sympathetic ganglia of the bullfrog, Kuba and Nishi found that superfusion of the ganglia with an extracellular solution supplemented...
TABLE 1.  Caffeine-induced Ca\(^{2+}\) release

<table>
<thead>
<tr>
<th>Species/Stage/NS Region</th>
<th>Preparation</th>
<th>Technique</th>
<th>Experimental Evidence</th>
<th>Reference Nos.</th>
</tr>
</thead>
<tbody>
<tr>
<td>Rat/newborn/DRG</td>
<td>Primary culture</td>
<td>Aequorin microphotometry</td>
<td>Cells microinjected with aequorin (1–3 mg/ml) responded to caffeine (10 mM) with large [Ca(^{2+})](_i) elevation. The latter was insensitive to 5 mM Cr(^{3+}) applied extracellularly. First application of caffeine (duration ~7 s) depleted the store so that the second application, which arrived ~50 s later, was unable to elevate [Ca(^{2+})](_i). Store can be refilled by 3 APs applied in a sequence, after which caffeine triggered full-blown [Ca(^{2+})](_i) elevation.</td>
<td>452</td>
</tr>
<tr>
<td>Rat/neonatal/superior cervical ganglia</td>
<td>Primary culture</td>
<td>Fura 2-AM or fura 2 microfluorimetry; whole cell voltage clamp</td>
<td>Caffeine (10 mM) induced [Ca(^{2+})](_i) elevation in the absence of extracellular Ca(^{2+}); this [Ca(^{2+})](_i) rise was completely blocked by 1 (\mu)M ryanodine, 10 (\mu)M dantrolene, but not by TMB-8 (10 (\mu)M).</td>
<td>637</td>
</tr>
<tr>
<td>Frog/paravertebral ganglia</td>
<td>Primary culture (up to 5 DIV)</td>
<td>Fura 2-AM imaging</td>
<td>Caffeine (10 mM) triggered [Ca(^{2+})](_i) elevation, which was large in the soma, and smaller in the growth cone. Caffeine responses did not require extracellular Ca(^{2+}).</td>
<td>311, 348, 349</td>
</tr>
<tr>
<td>Helix pomatia/ganglia</td>
<td>Freshly isolated neurons</td>
<td>Fura 2-AM microfluorimetry antipirilaso III microphotometry</td>
<td>Caffeine (6 mM) and extravesicular Ca(^{2+}) (20 (\mu)M) triggered Ca(^{2+}) release inhibited by La(^{3+}) (4 (\mu)M), ryanodine (50 (\mu)M), Mg(^{2+}) (IC(<em>{50} 6 \mu)M for cerebrum and 12 (\mu)M for cerebellum); ruthenium red (IC(</em>{50} 40 \mu)M for cerebrum and 60 (\mu)M for cerebellum) and DAPI (IC(_{50} 90 \mu)M for cerebrum and 150 (\mu)M for cerebellum).</td>
<td>407</td>
</tr>
<tr>
<td>Dog/adult/cerebrum, cerebellum</td>
<td>Microsomes</td>
<td>Stopped-flow photometry of arsenaso III</td>
<td>Caffeine (10 mM) triggered [Ca(^{2+})](_i) elevation, increased frequency of SMOCs and activated an outward current. Caffeine-induced [Ca(^{2+})](_i) elevation was inhibited by ryanodine (0.1–10 (\mu)M) and procaine (5 (\mu)M). Ryanodine by itself caused slow rise in [Ca(^{2+})](_i). Increase in [Ca(^{2+})](_i) levels preceding caffeine application potentiated Ca(^{2+}) release.</td>
<td>368</td>
</tr>
<tr>
<td>Bull frog/adult/lumbar sympathetic ganglia</td>
<td>Primary culture (1–7 DIV)</td>
<td>Whole cell voltage clamp; fura 2 imaging</td>
<td>Caffeine (2–20 mM) triggered [Ca(^{2+})](_i) elevation which did not require extracellular Ca(^{2+}). Caffeine-induced [Ca(^{2+})](_i) transients were blocked by ryanodine (10 (\mu)M), procaine (5 (\mu)M), and Ba(^{2+}) (0.5 (\mu)M).</td>
<td>661</td>
</tr>
<tr>
<td>Rat/P2–P7/DRG</td>
<td>Primary culture (2 DIV)</td>
<td>Indo 1-AM; fura 2-AM microfluorimetry</td>
<td>Caffeine (10 mM) triggered activation of Ca(^{2+})-dependent K(^+) current; inhibition of Ca(^{2+}) release by 10 (\mu)M ryanodine and 0.5–1 mM procaine abolished the current. Caffeine (10 mM) triggered activation of Ca(^{2+})-dependent Cl(^-) current [IC(_{50} 65 \mu)M] and induced [Ca(^{2+})](_i) elevation. Both effects did not require external Ca(^{2+}) and were blocked by 10 (\mu)M ryanodine.</td>
<td>658, 255</td>
</tr>
<tr>
<td>Rat/P14/hippocampus</td>
<td>Freshly isolated cells</td>
<td>Whole cell voltage clamp</td>
<td>Caffeine (10 mM) triggered activation of Ca(^{2+})-dependent K(^+) current; inhibition of Ca(^{2+}) release by 10 (\mu)M ryanodine and 0.5–1 mM procaine abolished the current.</td>
<td>658</td>
</tr>
<tr>
<td>Chicken/P10/DRG</td>
<td>Freshly isolated neurons</td>
<td>Whole cell voltage clamp/indo 1-AM microfluorimetry</td>
<td>Caffeine (10 mM) triggered [Ca(^{2+})](_i) responses in central neurons only after conditioning depolarization by 50 mM KCl; in DRG neurons caffeine was able to induce Ca(^{2+}) release in resting conditions.</td>
<td>581</td>
</tr>
<tr>
<td>Rat/P5–P8/DRG, neocortex, hippocampus CA1, CA3, nucleus cuneatus</td>
<td>Primary culture (2–14 DIV)</td>
<td>Indo 1-AM microfluorimetry</td>
<td>Caffeine (10 mM) and trans-ACPD triggered [Ca(^{2+})](_i) elevations which were considered to be the result of Ca(^{2+}) release from the ER.</td>
<td>553</td>
</tr>
<tr>
<td>Chicken/E16–E19/cerebellum</td>
<td>Freshly dissociated PNs</td>
<td>Fura 2-AM microfluorimetry</td>
<td>Caffeine (10 mM) initiated [Ca(^{2+})](_i) rise, which did not require external Ca(^{2+}), and was blocked by ryanodine in use-dependent manner. Small increase (by ~30 nM) of resting [Ca(^{2+})](_i), by tonic activation of NMDA receptors (0.3 (\mu)M glutamate + 10 (\mu)M glycine in Mg(^{2+})-free solution) significantly (up to 3 times) potentiated caffeine-induced [Ca(^{2+})](_i) response.</td>
<td>650</td>
</tr>
<tr>
<td>Mouse/E15–E16/teIencephalon</td>
<td>Primary cultures (3–17 DIV)</td>
<td>Fura 2-AM imaging</td>
<td>Caffeine (20 mM) caused [Ca(^{2+})](_i) elevation in small proportion of resting neurons; when resting [Ca(^{2+})](_i) was elevated by moderate depolarization caffeine caused [Ca(^{2+})](_i) responses in most of the cells. The amplitude of caffeine-induced [Ca(^{2+})](_i) elevation linearly depended on precaffeine [Ca(^{2+})](_i), level. Caffeine-induced [Ca(^{2+})](_i) responses were blocked by ruthenium red (10 (\mu)M) and by ryanodine (10 (\mu)M, in a use-dependent manner). Caffeine significantly prolonged [Ca(^{2+})](_i) transients in response to depolarization or climbing fiber stimulation; this prolongation was blocked by 10 (\mu)M ruthenium red.</td>
<td>269</td>
</tr>
</tbody>
</table>
with 1–6 mM caffeine resulted in rhythmic hyperpolarizations. Further on, they found that the caffeine-induced effects on membrane potential ($V_m$) were mediated through a $Ca^{2+}$-dependent $K^+$ conductance, because intracellular injection of EDTA inhibited the action of caffeine. Several years later, Kenji Kuba made the logical suggestion that “rhythmic increases in the $G_K$ under the effect of caffeine are due to oscillations of the intracellular $Ca^{2+}$ concentration and that there may be $Ca$ storage sites in the bullfrog sympathetic ganglion cell which are comparable to the sarcoplasmic reticulum in the skeletal muscle fiber” (317). Using $K^+$ permeability as a readout of $[Ca^{2+}]_i$, several groups confirmed these observations (219, 318, 431, 557). The advent of luminescent and fluorescent $Ca^{2+}$ probes allowed direct monitoring of $[Ca^{2+}]_p$ which became instrumental for detailed investigations of ER $Ca^{2+}$ release in nerve cells.

### B. $Ca^{2+}$-Induced $Ca^{2+}$ Release

#### 1. Caffeine-induced $Ca^{2+}$ release

As already mentioned, the methylxanthine caffeine was the first specific agent permitting identification of ER $Ca^{2+}$ release, and it has remained the probe of choice (Table 1). Probably the first direct indication that another methylxanthine, theophylline, triggers $[Ca^{2+}]_i$, rises in vertebrate nerve cells was obtained in bullfrog sympathetic

### Table 1—Continued

<table>
<thead>
<tr>
<th>Species/Age/NS Region</th>
<th>Preparation</th>
<th>Technique</th>
<th>Experimental Evidence</th>
<th>Reference Nos.</th>
</tr>
</thead>
<tbody>
<tr>
<td>Myenteric plexus/P1/guinea pig</td>
<td>Primary culture (2–8 DIV)</td>
<td>Fura 2-AM microfluorimetry</td>
<td>Caffeine (1–100 mM) produced $Ca^{2+}$ release, which was inhibited by dantrolene (10 $\mu$M), CPA (30 $\mu$M), procaine (5 $\mu$M), and ryanodine (1 $\mu$M); the latter block was use dependent (i.e., at least 1 application of caffeine has to be performed in the presence of ryanodine in order to inhibit developments). Multiple caffeine applications depleted the store; store replenishment required plasmalemmal $Ca^{2+}$ influx. Readmission of $Ca^{2+}$ after depletion of the store by caffeine in $Ca^{2+}$-free solution triggered significant $[Ca^{2+}]_i$, increase, indicative of the activation of SOC.</td>
<td>286</td>
</tr>
<tr>
<td>Chicken/E18/brain stem</td>
<td>Acute slices</td>
<td>Fura 2-AM imaging</td>
<td>Caffeine (100 mM) induced $[Ca^{2+}]_i$, increase when applied in $Ca^{2+}$-free media; caffeine-induced $[Ca^{2+}]_i$, responses were inhibited by glutamate and trans-ACPD, suggesting GluR control over CICR.</td>
<td>274</td>
</tr>
<tr>
<td>Rat/P9–P18/hippocampus</td>
<td>Acute slices, CA1 pyramidal neurons</td>
<td>Whole cell voltage clamp; fura 2; fura 2-AM video imaging</td>
<td>Caffeine (~10 mM) triggered $[Ca^{2+}]_i$, elevation in previously unstimulated cells; the caffeine $[Ca^{2+}]_i$, response did not require extracellular $Ca^{2+}$, was blocked by ryanodine (20 $\mu$M) in a use-dependent manner, and was inhibited by CPA (30 $\mu$M) and TG (5 $\mu$M).</td>
<td>179</td>
</tr>
<tr>
<td>Rat/E10/hippocampus</td>
<td>Primary culture (21 DIV)</td>
<td>Calcium green 1 confocal imaging; fura 2-AM imaging</td>
<td>Application of caffeine induced $[Ca^{2+}]_i$, rise in 34 of 48 spines; $[Ca^{2+}]_i$, response did not require extracellular $Ca^{2+}$ and was blocked by TG (1 $\mu$M) and ryanodine (10 $\mu$M). At the same time TG and ryanodine reduced $[Ca^{2+}]_i$, responses to glutamate; the degree of suppression was higher in dendrites vs. spines.</td>
<td>303</td>
</tr>
<tr>
<td>Rat/adult/tongue</td>
<td>Freshly isolated taste receptor cells</td>
<td>Fura 2-AM microfluorimetry</td>
<td>Caffeine (10 mM) induced $Ca^{2+}$ release, blocked by preincubation with 1 $\mu$M TG (25–40 min); the release was independent on extracellular $Ca^{2+}$ and insensitive to ryanodine (50 mM–100 $\mu$M).</td>
<td>715</td>
</tr>
<tr>
<td>Bullfrog/adult/sympathetic ganglia</td>
<td>Primary culture (14 DIV)</td>
<td>Fura 2-AM, fura 6-AM microfluorimetry</td>
<td>Repetitive applications of low (2 mM) concentrations of caffeine resulted in sensitization of CICR and gradual increase in the amplitude of caffeine-induced $Ca^{2+}$ release most probably due to an increase in ER $Ca^{2+}$ content.</td>
<td>226</td>
</tr>
<tr>
<td>Tiger salamander/larval stage/retina</td>
<td>Freshly isolated rods and cones</td>
<td>Fura 2-AM imaging; fluo 4-AM confocal imaging</td>
<td>Caffeine (10 mM) evoked $[Ca^{2+}]_i$, transients, which were blocked by ryanodine (20 $\mu$M), CPA (5 $\mu$M), and TG (1 $\mu$M); these drugs also attenuated $[Ca^{2+}]_i$, transients in response to KCl depolarization, indicating CICR.</td>
<td>315</td>
</tr>
<tr>
<td>Mouse/P2–P5/brain stem</td>
<td>Acute slices, hypoglossal motoneurons</td>
<td>Fura 2 imaging; fura 2, calcium green 1 multiphoton imaging</td>
<td>Application of caffeine (5 mM) triggered large $[Ca^{2+}]_i$, rises; $Ca^{2+}$ release was initiated in &quot;hot spots,&quot; which, in somatic regions, coincided with sites of depolarization-induced $Ca^{2+}$ entry. $Ca^{2+}$ release also activated $Ca^{2+}$-dependent $K^+$ currents.</td>
<td>322</td>
</tr>
</tbody>
</table>

DIV, days in vitro; PN, Purkinje neuron; APs, action potentials; CICR, $Ca^{2+}$-induced $Ca^{2+}$ release; DAPI, 4′,6-diamidino-2-phenylindole; ER, endoplasmic reticulum; CPA, cyclopiazonic acid; SOC, store-operated $Ca^{2+}$ entry; TG, thapsigargin.
neurons injected with the bioluminescent Ca$^{2+}$/H11001 probe arsenazo III (597). Yet, the first proper description of caffeine-induced Ca$^{2+}$ release was published in 1984, when Ian Neering and Robert McBurney presented the results of their experiments on DRG neurons, which were isolated from newborn rats and injected with another luminescent Ca$^{2+}$ probe, aequorin (452). When 10 mM caffeine was applied to DRG neurons, they responded by a large light transient, reflecting an elevation of [Ca$^{2+}$]$_i$ (Fig. 3A). In fact, the caffeine-induced [Ca$^{2+}$]$_i$ elevation was three to five times larger than the [Ca$^{2+}$]$_i$ increase evoked by action potentials (the latter artificially prolonged by addition of 20 mM TEA). The caffeine-induced [Ca$^{2+}$]$_i$ response was preserved in an extracellular solution containing 5 mM Co$^{2+}$, suggesting that it was independent of plasmalemmal Ca$^{2+}$ entry. Furthermore, Neering and McBurney (452) showed that caffeine depletes the Ca$^{2+}$ store, which, however, can be rapidly replenished by stimulating the neuron with several action potentials (APs) (Fig. 3B). Further progress in studying caffeine-induced Ca$^{2+}$ release was very much assisted by the introduction of fluorescent Ca$^{2+}$ probes (653, 654) as well as by the widespread use of the patch-clamp technique (200). When combined, these techniques allowed simultaneous monitoring of membrane currents and [Ca$^{2+}$]$_i$ dynamics. In 1988, caffeine-induced Ca$^{2+}$ release was identified in fura 2-loaded sympathetic neurons of the frog (348, 349) and in sympathetic (636), sensory (637), and central (638) neurons of the rat. Within the next several years caffeine-induced Ca$^{2+}$ release was discovered in all types of neurons studied regardless of the preparation; the phenomenon was found in freshly dissociated neu-

![FIG. 3. Identification of caffeine-stimulated ER Ca$^{2+}$ release in nerve cell. A: effect of caffeine on membrane potential ($V_m$) and [Ca$^{2+}$]$_i$ (proportional to changes in $I_{light}$) in a single DRG neuron. The resting $V_m$ was $-55$ mV. Extracellular solution contained 20 mM TEA. Peak of caffeine-induced [Ca$^{2+}$]$_i$ rise was $-10$ μM. B: caffeine depletes the ER calcium store. Similarly to A, the [Ca$^{2+}$]$_i$ and $V_m$ were recorded from a single DRG neuron. First application of caffeine triggered [Ca$^{2+}$]$_i$ rise; however, when caffeine was applied 50 s later, no changes in [Ca$^{2+}$]$_i$ were observed. After the cell was stimulated by three action potentials (AP), caffeine was once more able to produce [Ca$^{2+}$]$_i$ elevation. The time elapsed after the beginning of the experiment is indicated on the graph. [From Neering and McBurney (452), with permission from Nature Publishing Group.]
rons, neurons in primary cultures, and neurons in situ in brain slices (see Table 1).

As a drug aimed at an intracellular target, caffeine is rather convenient as it freely diffuses through the plasma membrane, and its wash-in and wash-out kinetics are quite similar. The process of caffeine entering and exiting the cell can be directly monitored by exploring the ability of caffeine to quench the fluorescence of various Ca\(^{2+}\) probes in a wavelength-independent manner (471, 661, 662). This quenching is most prominent for indo 1, which allows real time recordings of intracellular caffeine concentration simultaneously with [Ca\(^{2+}\)]_i measurements.

The properties of caffeine-induced Ca\(^{2+}\) release are generally similar between different neurons (Table 1). Caffeine induces a [Ca\(^{2+}\)]_i rise without a requirement for extracellular calcium, and the [Ca\(^{2+}\)]_i elevation is not associated with plasmalemmal Ca\(^{2+}\) movements. Caffeine-evoked [Ca\(^{2+}\)]_i transients are sensitive to pharmacological modulators interacting with RyRs or with SERCA pumps; specifically, these [Ca\(^{2+}\)]_i responses are blocked by ryanodine, dantrolene, ruthenium red, and procaine and disappear after inhibition of ER Ca\(^{2+}\) uptake with TG or CPA (Table 1). Caffeine responses are found in all parts of neurons, from soma to dendrites and presynaptic terminals. Caffeine-induced Ca\(^{2+}\) release that is sensitive to TG and ryanodine has even been observed in individual spines of cultured hippocampal neurons, which are rich in ryanodine receptors (303).

Incubation of neurons isolated from bullfrog sympathetic ganglia with 10 mM caffeine frequently induces [Ca\(^{2+}\)]_i oscillations (e.g., Refs. 164, 166, 462), which are often quite diverse in different neurons, ranging from high-amplitude regular oscillatory activity to low-amplitude decaying [Ca\(^{2+}\)]_i fluctuations (111). This diversity of oscillatory patterns reflects complex mechanisms responsible for their generation, which involve ER (Ca\(^{2+}\) -induced Ca\(^{2+}\) release and SERCA-dependent Ca\(^{2+}\) uptake, etc.) as well as non-ER (mitochondrial Ca\(^{2+}\) accumulation, plasmalemmal Ca\(^{2+}\) extrusion) cascades.

When using caffeine in nerve cell preparations, a certain caution is needed, because caffeine (and other methylxanthines) directly affects potassium channels (660) and effectively inhibits delayed rectifier and A-type potassium currents in vertebrate neurons (529). In certain types of neurons, caffeine may also activate plasmalemmal Ca\(^{2+}\) influx, for example, as demonstrated in acutely dissociated rabbit nodose ganglion neurons (229) and leech mechanosensitive neurons (565). The \(K_D\) values for the activation of ER Ca\(^{2+}\) release and plasmalemmal Ca\(^{2+}\) influx by caffeine were close (1.5 and 0.6 mM, respectively); this needs to be taken into account when interpreting caffeine-induced [Ca\(^{2+}\)]_i responses as a sole consequence of ER Ca\(^{2+}\) release.

2. The “quantal” nature of caffeine-induced Ca\(^{2+}\) release

Caffeine releases Ca\(^{2+}\) from neuronal ER in a rather peculiar manner, which has been termed “quantal” release. When the cell is challenged with a low concentration of caffeine (≈ 2–5 mM), a transient Ca\(^{2+}\) response is triggered, and even if caffeine remains in the system, the [Ca\(^{2+}\)]_i recovers back toward the prestimulated level. Applications of caffeine in submaximal concentrations fail to empty the store completely; subsequent application of a higher caffeine concentration induces a further [Ca\(^{2+}\)]_i elevation. Interestingly, it appears that a submaximal caffeine concentration can empty the store “compartment” sensitive to this concentration: when 2 mM of caffeine is applied repetitively, the second application no longer produces a [Ca\(^{2+}\)]_i response (Fig. 4). This peculiar quantal release was found when studying [Ca\(^{2+}\)]_i dynamics in cultured chromaffin cells (92), in rat (661) and mouse (584) DRG neurons, and in myenteric neurons (286). A similar quantal release was also found when monitoring [Ca\(^{2+}\)]_i in chromaffin cells expressing ER-targeted aequorin (9; see Fig. 4A). These observations can be explained by assuming either that different neuronal Ca\(^{2+}\) release channels have different sensitivities to caffeine, or that the ER is composed of several compartments, which are discharged by different concentrations of caffeine (92). Further experiments showed, however, that the quantal caffeine-induced release is controlled by intraluminal Ca\(^{2+}\) concentration, and overcharging the stores with Ca\(^{2+}\) following massive depolarization-induced Ca\(^{2+}\) entry significantly enhances the sensitivity of ER Ca\(^{2+}\) release to caffeine (Fig. 4D, Refs. 300, 584). In the context of ER Ca\(^{2+}\) release, [Ca\(^{2+}\)]_L-dependent regulation of RyRs appears to be a general phenomenon, as the same sort of regulation is well established for another Ca\(^{2+}\) release channel, the InsP\(_3\)R. Similar to RyRs, elevation of luminal Ca\(^{2+}\) increases the sensitivity of InsP\(_3\)R to InsP\(_3\) (419, 465). Changes in “luminal” [Ca\(^{2+}\)]_L in a planar lipid system directly influenced the purified InsP\(_3\)R by affecting their inactivation (643).

3. Cytosolic Ca\(^{2+}\) regulates caffeine-induced Ca\(^{2+}\) release

The nature of interaction of caffeine with RyRs depends on the concentration of the former: direct electrophysiological investigations of isolated ryanodine receptor channels have demonstrated that low (< 2 mM) concentrations of caffeine sensitize the channel to extraluminal (i.e., cytoplasmic) calcium, shifting RyR activation towards submicromolar Ca\(^{2+}\) concentrations. In the presence of low millimolar caffeine concentrations, the RyR channel open probability (\(P_o\)) was significantly increased mostly due to a shortening of the lifetime of the closed state. At higher concentrations (> 5 mM), caffeine...
opens RyRs even at picomolar \([\text{Ca}^{2+}]_i\), and dramatically increases the open time of the channel (e.g., Refs. 221, 547, 593).

These biophysical properties of the caffeine-RyR interactions are also reflected by the \([\text{Ca}^{2+}]_i\) dependence of caffeine action on nerve cells. The amplitude of the \([\text{Ca}^{2+}]_i\) elevation induced by 10 mM caffeine increased sharply when the \([\text{Ca}^{2+}]_i\), preceding the application of the drug was elevated by a conditioning KCl depolarization of rat sympathetic neurons (221). By changing the duration of the KCl application between 0.3 and 7 s, the conditioning \([\text{Ca}^{2+}]_i\), rise could be graded, therefore making it possible to characterize the relation between \([\text{Ca}^{2+}]_i\) and the amplitude of caffeine-induced \([\text{Ca}^{2+}]_i\) release (Fig. 5A). This relation appeared bell-shaped: an increase of \([\text{Ca}^{2+}]_i\), from the resting level of \(\sim 50–70\) to \(100–300\) nM markedly potentiated caffeine-induced \([\text{Ca}^{2+}]_i\), response, but a further increase of \([\text{Ca}^{2+}]_i\), to \(>400\) nM led to an inhibition of the caffeine-evoked \([\text{Ca}^{2+}]_i\), elevation. A similar effect of \([\text{Ca}^{2+}]_i\), on the response to a submaximal (2 mM) caffeine challenge was observed in rat sensory neurons (Fig. 5B), where an increase in \([\text{Ca}^{2+}]_i\), preceding caffeine application greatly potentiated \([\text{Ca}^{2+}]_i\), release (584). Moreover, when precaffeine \([\text{Ca}^{2+}]_i\), reached a level of \(\sim 350\) nM, 2 mM caffeine induced a full-blown \([\text{Ca}^{2+}]_i\), release that almost completely emptied the ER.

An even more striking dependence of caffeine responses on precaffeine \([\text{Ca}^{2+}]_i\), was observed in central neurons. In Purkinje cells studied in rat cerebellar slices, application of 20 mM caffeine to the resting cell (resting \([\text{Ca}^{2+}]_i\), \(\sim 20–40\) nM) rarely resulted in \([\text{Ca}^{2+}]_i\), release. Yet, when \([\text{Ca}^{2+}]_i\), was slightly elevated by moderately depolarizing the cell (experiments were done under voltage-clamp) caffeine produced robust \([\text{Ca}^{2+}]_i\), elevations (Fig. 6). The amplitude of the caffeine-induced \([\text{Ca}^{2+}]_i\), response was linearly dependent on the preceding \([\text{Ca}^{2+}]_i\), level (269). Similarly, in hippocampal neurons, an elevation of precaffeine \([\text{Ca}^{2+}]_i\), from \(\sim 40\) to 284 nM led to a 3.5-fold increase of the caffeine-induced \([\text{Ca}^{2+}]_i\), response (179).

4. Physiological CICR

A) THE ER AS A SOURCE OF AND SINK FOR CYTOSOLIC \([\text{Ca}^{2+}]_i\). Although the ability of the ER to buffer \([\text{Ca}^{2+}]_i\), and at the same time to release \([\text{Ca}^{2+}]_i\), into the cytosol was recognized already by the mid 1980s (213, 214, 386), the concept of the ER \([\text{Ca}^{2+}]_i\), store acting as a “\([\text{Ca}^{2+}]_i\), sink and \([\text{Ca}^{2+}]_i\), source” was for the first time clearly formulated by David Friel and Richard Tsien in 1992 (165). They backed up this concept with a rather thorough analysis of changes in depolarization-induced \([\text{Ca}^{2+}]_i\), transients occurring upon various manipulations of the ER \([\text{Ca}^{2+}]_i\), store. First, they convincingly demonstrated that ER \([\text{Ca}^{2+}]_i\), release participates in the delivery of \([\text{Ca}^{2+}]_i\), to the cytosol during cell depolarization, i.e., that CICR indeed acts as an amplifier of \([\text{Ca}^{2+}]_i\), signals resulting from plasmalemmal \([\text{Ca}^{2+}]_i\), entry. They employed a protocol, which later became a gener-
FIG. 5. Effects of \([\text{Ca}^{2+}]_i\) on caffeine-induced \(\text{Ca}^{2+}\) release in peripheral neurons. A: the \([\text{Ca}^{2+}]_i\), recordings were performed on cultured rat sympathetic neurons. Left: examples of \([\text{Ca}^{2+}]_i\), transients illustrating the effects of applying a conditioning pulse of high KCl of increasing durations (as indicated under the traces) just before a caffeine application of 5 s. The interval between the end of KCl pulse and the caffeine application remained constant at 1.5 s. Right: amplitudes of caffeine \((\Delta[\text{Ca}^{2+}]_{\text{caffe}})\) measured from the records shown on the left and plotted against the “pedestal” \([\text{Ca}^{2+}]_i\) (P), reached immediately before caffeine administration. B: two \([\text{Ca}^{2+}]_i\), recordings taken from the same freshly isolated mouse DRG neuron. Note that increase in \([\text{Ca}^{2+}]_i\), significantly potentiated the amplitude of 2 mM caffeine-induced response (left trace); further increase in \([\text{Ca}^{2+}]_i\), (right trace) triggered even larger response to 2 mM caffeine, which emptied the ER, as judged by very small response to subsequent application of 20 mM caffeine. Timing of caffeine and KCl applications is indicated on the graph. [A from Hernandez-Cruz et al. (221), with permission from Blackwell Publishers Ltd. B from Shmigol et al. (584), copyright 1996 with permission from Elsevier.]

FIG. 6. Effects of \([\text{Ca}^{2+}]_i\) on caffeine-induced \(\text{Ca}^{2+}\) release in Purkinje neurons. A: pseudocolor fluorescence images of \([\text{Ca}^{2+}]_i\), illustrating the \([\text{Ca}^{2+}]_i\), dependence of caffeine-induced \(\text{Ca}^{2+}\) release in various cellular compartments. The gain of intensified CCD camera was set at values that optimized detection from dendrites, but partially saturated the fluorescence signal from the soma (darkened area in the central part of the soma). B: \([\text{Ca}^{2+}]_i\), recordings from the soma and proximal and distal dendrites of Purkinje neuron shown in A. The regions from which the mean \([\text{Ca}^{2+}]_i\), values were calculated are indicated by rectangles on images shown in A. Arrows marked with a, b, and c indicate the time at which the digital fluorescence images in A, labeled a, b, and c, were taken. The baseline \([\text{Ca}^{2+}]_i\), was changed by applying 6 depolarizing pulses (from -60 to 0 mV for 500 ms) immediately before the application of caffeine. [From Kano et al. (269), with permission from Blackwell Publishers Ltd.]
ally accepted test for CICR. This protocol compared depolarization-induced \([\text{Ca}^{2+}]_i\) transients in control conditions and in conditions when 1) ER is recovering following treatment with a high (10–20 mM) concentration of caffeine, 2) RyRs are sensitized by a low (1 mM) concentration of caffeine, and 3) RyRs are activated by 1 \(\mu\)M ryanodine and the stores are permanently depleted. In perfect concordance with the idea of the ER having the dual role of \([\text{Ca}^{2+}]_i\) buffer and \([\text{Ca}^{2+}]_i\) source, these manipulations affected both the rising phase and the recovery of \([\text{Ca}^{2+}]_i\) elevations in response to membrane depolarization with 30–50 mM KCl (Figs. 7 and 8). A complete depletion of the stores decreased the amplitude and rate of rise of KCl-induced \([\text{Ca}^{2+}]_i\) transients, and accelerated their recovery by 1) removing the CICR contribution and 2) upregulating \([\text{Ca}^{2+}]_i\) uptake into the depleted ER. Sensitization of RyRs by 1 mM caffeine increased the rate of rise and the amplitude of the depolarization-induced

\[ \text{FIG. 7.} \] ER \([\text{Ca}^{2+}]_i\) store as a source of and sink for \([\text{Ca}^{2+}]_i\). Top panel represents \([\text{Ca}^{2+}]_i\) traces evoked by 30 mM KCl in control conditions and upon pharmacologically modulated ER \([\text{Ca}^{2+}]_i\) store (b–e) as explained by drawings below. Arrows indicate direction and relative magnitude of the net \([\text{Ca}^{2+}]_i\) flux across the surface membrane and between the cytosol and the store, during the onset and recovery phases of the response. Under control conditions (a), depolarization stimulates \([\text{Ca}^{2+}]_i\) entry and a rise in \([\text{Ca}^{2+}]_i\), which promotes \([\text{Ca}^{2+}]_i\) release from the store (onset). Repolarization permits the \([\text{Ca}^{2+}]_i\) contents of both the cytosol and the store to relax towards their prestimulation levels (recovery); since the store released \([\text{Ca}^{2+}]_i\) during the onset, it must accumulate \([\text{Ca}^{2+}]_i\) during the recovery. b: When continuously present, caffeine (1 mM) enhances \([\text{Ca}^{2+}]_i\)-induced \([\text{Ca}^{2+}]_i\) release (CICR) from the store (onset) so that \([\text{Ca}^{2+}]_i\) increases more rapidly than it does under control conditions. To restore its initial \([\text{Ca}^{2+}]_i\) content, the store also accumulates \([\text{Ca}^{2+}]_i\) more avidly during the recovery; as a result, \([\text{Ca}^{2+}]_i\) declines more rapidly following repolarization. If \([\text{Ca}^{2+}]_i\) entry is induced while the store is filling as in c, net \([\text{Ca}^{2+}]_i\) uptake by the store slows the rate at which \([\text{Ca}^{2+}]_i\) rises during the onset. However, if \([\text{Ca}^{2+}]_i\) is elevated long enough for the \([\text{Ca}^{2+}]_i\) content of both the cytosol and the store to approximate the steady-state values achieved under control conditions (a), then \([\text{Ca}^{2+}]_i\) will recover just as it does in the control (compare a and c, recovery). By increasing passive \([\text{Ca}^{2+}]_i\) exchange between the store and cytosol, ryanodine prevents net \([\text{Ca}^{2+}]_i\) accumulation by the store (d and e). Without the added effect of CICR, \([\text{Ca}^{2+}]_i\) rises more slowly in response to stimulated \([\text{Ca}^{2+}]_i\) entry (compare a and d, onset) and falls more slowly during recovery (compare a and d) than it does under control conditions. Since the store remains discharged in the presence of ryanodine, caffeine has no further effect. Therefore, the postcaffeine response in the presence of ryanodine (e) is essentially the same as the response elicited in the presence of ryanodine without caffeine pretreatment (d). [From Friel and Tsien (165), with permission from Blackwell Publishers Ltd.]
[Ca\(^{2+}\)]_i transient, and moderately accelerated the recovery of the [Ca\(^{2+}\)]_i elevation by 1) enhancing the CICR contribution and 2) augmenting Ca\(^{2+}\) uptake because increased CICR depleted the store to a higher extent compared with control. Finally, permanent emptying of the ER by 1 μM ryanodine decelerated the KCl-induced [Ca\(^{2+}\)]_i transient and slowed down the recovery by 1) removing the CICR contribution and 2) occluding the ER Ca\(^{2+}\) uptake, because the store remained permanently leaky due to constantly open RyRs.

The concept of “sink and source” quite adequately explains CICR behavior in nerve cells, and, most importantly, it emphasizes the role of the intra-ER Ca\(^{2+}\) content, which appears to be an essential regulator of ER function, controlling the mode (source or sink) of Ca\(^{2+}\) store operation. The relatively low ER Ca\(^{2+}\) content would therefore promote [Ca\(^{2+}\)]_i buffering as for example happens in hypoglossal motoneurons, where depletion of the store by caffeine prolonged the recovery, but did not alter the amplitude of AP-evoked [Ca\(^{2+}\)]_i transients (127).

B) EVIDENCE FOR PHYSIOLOGICAL CICR IN NEURONS. With the use of the paradigm described above, the contribution of CICR to the [Ca\(^{2+}\)]_i elevation triggered by plasmalemmal Ca\(^{2+}\) entry has been demonstrated for many types of neurons, in both the peripheral and the central nervous system (Table 2). In addition to the protocols used by Friel and Tsien, several other experimental approaches were developed, e.g., CICR can be completely blocked by high concentrations of ryanodine, or the ER store can be depleted using the SERCA pump blockers TG or CPA. The changes observed in the depolarization-induced [Ca\(^{2+}\)]_i transients caused by these interventions are very much as expected from the sink and source theory: inhibition of CICR by ryanodine slows down the rate of rise and decreases the amplitude of KCl-induced [Ca\(^{2+}\)]_i transients, whereas inhibition of ER Ca\(^{2+}\) uptake has the same effect on the onset of [Ca\(^{2+}\)]_i signals in response to depolarization and significantly decelerates their recovery.

With the use of these methods, the contribution of CICR to depolarization-evoked [Ca\(^{2+}\)]_i signaling was discovered in many, but not in all, neurons (e.g., it was absent in cerebellar granule neurons, Refs. 250, 292). Furthermore, in experiments on brain slices with preserved synaptic inputs, a very significant CICR was recorded in response to synaptic stimulation in dendrites (Fig. 8B) and even spines of hippocampal neurons (6, 138). In these cases the trigger Ca\(^{2+}\) entered the cytosol via Ca\(^{2+}\)-permeable NMDA receptors.

It is worth pointing out that the functionality of the CICR pathway may critically depend on the experimental conditions. For instance, in Purkinje neurons, the parameters of ER Ca\(^{2+}\) release are greatly affected by cell culture. Photorelease of InsP\(_{3}\) or administration of caffeine induced only a tiny [Ca\(^{2+}\)]_i response in a small fraction of cultured Purkinje neurons, whereas the same manipulations resulted in large and frequent [Ca\(^{2+}\)]_i responses in freshly dissociated neurons (697) (however a caveat: the cultures were prepared from the embryonic cerebellum, E16-E18, whereas neurons were acutely isolated from mice at postnatal days 10–16). Conversely, no CICR in response to a single AP or trains of APs (tetanic stimulation) was observed in bullfrog sympathetic neurons, when studied in whole ganglia (462), but a prominent CICR in response to Ca\(^{2+}\) entry was invariably observed when the same cells were kept in culture conditions (240).

In certain cell types, physiological CICR exhibits a remarkable spatial heterogeneity. In leech Retzius neurons, for instance, depletion of the ER by 10 μM CPA reduced AP-induced [Ca\(^{2+}\)]_i transients in dendrites, but not in axons (33). In frog sympathetic ganglion neurons, either within the whole ganglia or in culture, a single AP was able to trigger several local CICR events concentrated in the ER-rich perinuclear region or around axon hillocks, as was revealed by fast fluo 4 confocal imaging (112).

At the subcellular level, activation of CICR is represented by local release events, resulting from spontaneous activation of RyRs and subsequent recruitment of RyR clusters. These elementary [Ca\(^{2+}\)]_i signals (observed in nerve growth factor-differentiated PC12 cells and in cultured hippocampal neurons (298, 398)) are analogs to “sparks” in muscle cell preparations. Neuronal [Ca\(^{2+}\)]_i sparks occur spontaneously, and their frequency increases upon addition of low concentrations of caffeine or following loading of the ER store by conditioning depolarization-induced Ca\(^{2+}\) entry. In contrast to other preparations, neuronal release sites appear to contain both InsP\(_{3}\)Rs and RyRs, since metabotropic stimulation affected elementary Ca\(^{2+}\) release frequency at the same sites as caffeine (298). Spontaneous [Ca\(^{2+}\)]_i sparks were also found in presynaptic boutons of the lizard neuromuscular junction (398) and in cerebellar basket neurons (105, 355).

More accurate characterization of CICR in nerve cells requires a direct comparison between plasmalemmal Ca\(^{2+}\) entry and [Ca\(^{2+}\)]_i elevation. This necessitates simultaneous monitoring of Ca\(^{2+}\) currents (under voltage-clamp) and [Ca\(^{2+}\)]_i; the amount of Ca\(^{2+}\) entering the cell can be precisely calculated by integrating Ca\(^{2+}\) current ($I_{\text{Ca}}$) over time, and hence the $\Delta$[Ca\(^{2+}\)] can be directly related to $I_{\text{Ca}}$/$\Delta t$. Such an experiment was performed for the first time in rat sympathetic neurons (636), revealing a fairly linear relation between Ca\(^{2+}\) entry and $\Delta$[Ca\(^{2+}\)] at short test pulses and its saturation at long test pulses (range of $I_{\text{Ca}}$ durations varied between 10 and 640 ms). When the same experiment was repeated in the presence of 10 mM caffeine and 10 μM ryanodine, suppression of $\Delta$[Ca\(^{2+}\)] was detected; however, this phenomenon was not very robust, leading the authors to conclude that
**TABLE 2. Physiological CICR in nerve cells**

<table>
<thead>
<tr>
<th>Species/Age/NS Region</th>
<th>Preparation</th>
<th>Technique</th>
<th>Evidence for CICR</th>
<th>Reference Nos.</th>
</tr>
</thead>
<tbody>
<tr>
<td>Bullfrog/sympathetic ganglia</td>
<td>Primary culture (up to 7 DIV)</td>
<td>Microelectrodes $V_m$ recordings; fura 2-AM microfluorimetry</td>
<td>Depletion of ER store with 20 mM caffeine reduced the amplitude and speed of subsequent $[Ca^{2+}]_i$ response to 50 mM KCl depolarization. Cell treatment with 1 mM caffeine increased the rate of rise and the amplitude of KCl-induced $[Ca^{2+}]_i$, elevation, whereas ryanodine (1 μM) inhibited caffeine-induced $Ca^{2+}$ release and slowed down and attenuated the amplitude of KCl-triggered $[Ca^{2+}]_i$, transient.</td>
<td>165</td>
</tr>
<tr>
<td>Rat/P2–P7/lumbar DRG</td>
<td>Primary culture (&gt;2 DIV)</td>
<td>Fura 2-AM, indo 1-AM microfluorimetry</td>
<td>Depletion of $Ca^{2+}$ stores with 20 mM caffeine or RyRs inhibition by ryanodine (10 μM) significantly decreased the rate of rise and the amplitude of the subsequent $[Ca^{2+}]_i$, responses to KCl (40–50 mM). Sensitization of RyRs by 1 mM caffeine accelerated the rise and increased the amplitude of $[Ca^{2+}]_i$, transients evoked by KCl.</td>
<td>661</td>
</tr>
<tr>
<td>Snail <em>Helix aspersa</em>/circumoesophageal ganglia</td>
<td>Whole ganglia</td>
<td>Microelectrodes $V_m$ recordings; fura 2 microfluorimetry</td>
<td>Low doses of caffeine (0.2–1 mm) increased the amplitude of depolarization-induced $[Ca^{2+}]_i$, transients. Ryanodine (10 μM) and CPA (50 μM) reduced the size of $[Ca^{2+}]_i$, responses to depolarization and block from effects of caffeine.</td>
<td>472</td>
</tr>
<tr>
<td>Rabbit/P1–P3/otic ganglia</td>
<td>Primary culture (1–3 DIV)</td>
<td>Fura 2-AM microfluorimetry</td>
<td>Ryanodine (10 μM) decreased the amplitude (to ~70% of the control) and the rate of rise (to 100% of the control) of $[Ca^{2+}]_i$, elevation induced by 30 mM KCl and abolished $[Ca^{2+}]_i$, oscillations in the presence of KCl.</td>
<td>711</td>
</tr>
<tr>
<td>Bullfrog/12–18 cm long/sympathetic ganglia</td>
<td>Whole ganglia preparation</td>
<td>Fura 2 microfluorimetry</td>
<td>Sympathetic terminals were loaded by indicator by placing a grain of fura 2K, at the end of sympathetic chain; terminals were filled with the dye within 1–2 h. Ryanodine (10 μM) decreased the amplitude of $[Ca^{2+}]_i$, transients triggered by 100–400 electrical stimuli (20 Hz) by 40% on average (range 12–83%).</td>
<td>502</td>
</tr>
<tr>
<td>Rat/E20/cerebellum</td>
<td>Primary cultured Purkinje neurons</td>
<td>Fura 2-AM imaging</td>
<td>Dantrolene (10 μM), ryanodine (10 μM), preincubation with caffeine (10 μM), and BHQ (10 μM) significantly reduced AMPA-mediated $[Ca^{2+}]_i$, elevation being thus suggestive of CICR.</td>
<td>193</td>
</tr>
<tr>
<td>Rat/P3/hippocampus</td>
<td>Primary culture</td>
<td>Fluo 3-AM confocal imaging</td>
<td>Field stimulation of cultures triggered somatic $[Ca^{2+}]_i$, responses which rose linearly with an increase in number of APs.</td>
<td>256</td>
</tr>
<tr>
<td>Rat/P1–P3/DRG</td>
<td>Primary culture (2–3 DIV)</td>
<td>Whole cell current-voltage clamp; indo 1 microfluorimetry calcium green-1 confocal imaging</td>
<td>In the presence of 5 mM caffeine (acting as a “sensitizer” of CICR) bursts of APs or $Ca^{2+}$ currents triggered all-or-none $[Ca^{2+}]_i$ elevation from the ER when $[Ca^{2+}]_i$, reached the threshold level of ~120 nM). cADPR applied through the pipette was not effective as a substitute to caffeine. In confocal experiments, CICR was shown to facilitate the spread of $Ca^{2+}$ signal from plasmalemma to cell interior.</td>
<td>603</td>
</tr>
<tr>
<td>Rabbit/nodose ganglia</td>
<td>Primary culture (3–4 DIV)</td>
<td>Sharp microelectrode voltage clamp; fura 2-AM microfluorimetry</td>
<td>Amplitude of $Ca^{2+}$-dependent afterhyperpolarization and $[Ca^{2+}]_i$, transient induced by a train of APs (1–8–65) were markedly inhibited by ryanodine (10 μM), TG (100 nM), BHQ (10 μM), or CPA (10 μM). These agents also inhibited caffeine-induced $[Ca^{2+}]_i$, response. In the presence of ER blockers at least 8 APs were necessary to evoke measurable $[Ca^{2+}]_i$, elevation, whereas in control conditions 1 AP was already sufficient to evoke $[Ca^{2+}]_i$, response.</td>
<td>103, 426</td>
</tr>
<tr>
<td>Rat/P10–P17/hippocampus</td>
<td>Acutely isolated slices, CA1 neurons</td>
<td>Whole cell current clamp; fura 2-AM/ bis-fura 2 imaging</td>
<td>CA1 neurons were excited by antidromic field stimulation or directly under current clamp. Low concentrations of caffeine (5 mM) potentiated AP-driven $[Ca^{2+}]_i$, signals, whereas incubation with high (20 mM) concentration of caffeine depressed $[Ca^{2+}]_i$, elevations. AP-driven $[Ca^{2+}]_i$, signals were also suppressed by TG (3μM) and ryanodine (20 μM).</td>
<td>560</td>
</tr>
<tr>
<td>Rat/P10–P18/cortex</td>
<td>Acutely isolated slices</td>
<td>Fura 2/fluo 3 imaging/ microfluorimetry</td>
<td>Stimulation of cortical neurons with depolarization for 2 s evoked burst of APs and biphasic $[Ca^{2+}]_i$, elevation, with sharply rising delayed 2nd phase. This 2nd phase was completely abolished by intracellular perfusion with mixture of ryanodine (30 μM) + heparin (2 mg/ml) or by TG (10 μM).</td>
<td>277</td>
</tr>
</tbody>
</table>
### TABLE 2—Continued

<table>
<thead>
<tr>
<th>Species/Age/NS Region</th>
<th>Preparation</th>
<th>Technique</th>
<th>Evidence for CICR</th>
<th>Reference Nos.</th>
</tr>
</thead>
<tbody>
<tr>
<td>Guinea pig/myenteric plexus</td>
<td>Whole plexus</td>
<td>Microelectrode current clamp; bis-fura 2 imaging</td>
<td>CICR in myenteric neurons was graded by Ca(^{2+}) entry; each AP triggered a release resulting in (-30, \text{nM} , [\text{Ca}^{2+}]_i) increase (which accounted to (-50%) of total ([\text{Ca}^{2+}]_i), rise). CICR controlled the amplitude of AHP; ryanodine (10 (\mu)M) caused (-60%) reduction in both amplitude of AP-induced ([\text{Ca}^{2+}]_i), transient and AHP amplitude.</td>
<td>227</td>
</tr>
<tr>
<td>Mouse WT and (\beta2) NChR KO/9–15 days/substantia nigra pars compacta</td>
<td>Acutely isolated slices</td>
<td>Fura 2-AM imaging</td>
<td>Ca(^{2+}) influx through neuronal (\alpha7)-NChR triggers CICR; inhibition of the ER by dantrolene (10 (\mu)M) or CPA (30 (\mu)M) significantly reduced NChR-mediated ([\text{Ca}^{2+}]_i), elevation.</td>
<td>655</td>
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<tr>
<td>Mouse/vas deference/postganglionic sympathetic terminals</td>
<td>Freshly isolated tissue</td>
<td>Oregon green-BAPTA 1-AM confocal imaging</td>
<td>Perfusion with nicotine (2–30 (\mu)M) triggered small ([\text{Ca}^{2+}]_i), spikes, which were blocked by ryanodine (100 (\mu)M) and caffeine (3 (\mu)M). Authors suggested that activation of NChRs triggers Ca(^{2+}) influx via VGCC, which in turn induces CICR.</td>
<td>63</td>
</tr>
<tr>
<td>Chicken (E15 and P21)/ciliary ganglia</td>
<td>Freshly isolated whole ganglia</td>
<td>Oregon green-1 dextran (10,000 Da) confocal imaging; dye delivery by back-loading</td>
<td>Treatment with ryanodine (10 (\mu)M) and TG (1 (\mu)M) decreased NChR-dependent ([\text{Ca}^{2+}]_i), plateau induced by high-frequency synaptic stimulation. It is assumed that CICR is triggered by Ca(^{2+}) entry through Ca(^{2+})-permeable (\alpha7)-NChRs.</td>
<td>586</td>
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</table>

#### CICR in response to synaptic stimulation

<table>
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<tr>
<th>Species/Age/NS Region</th>
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<th>Evidence for CICR</th>
</tr>
</thead>
<tbody>
<tr>
<td>Rat/P11–P17/hippocampus</td>
<td>Acutely isolated slices, CA1 neurons Organotypic (slice) culture</td>
<td>Whole cell voltage clamp; furo 3 confocal imaging</td>
<td>Tetanic stimulation of SC triggered postsynaptic dendritic ([\text{Ca}^{2+}]_i], response accompanied with NMDAR-mediated EPSC. Ryanodine (10 (\mu)M) and TG (10 (\mu)M) reduced ([\text{Ca}^{2+}]_i), elevation by (-78%) without affecting EPSC.</td>
</tr>
<tr>
<td>Rat/P9/hippocampus</td>
<td>Organotypic culture</td>
<td>Microelectrode current clamp; Oregon green 488 BAPTA-1 confocal imaging</td>
<td>Synaptically evoked ([\text{Ca}^{2+}]_i) transients were measured in individual spines using line-scan mode. These ([\text{Ca}^{2+}]_i), transients resulted from activation of NMDA receptors and subsequent CICR. Inhibition of ER Ca(^{2+}) release by CPA (15 (\mu)M) or ryanodine (10–20 (\mu)M) greatly reduced or even abolished synthetically evoked ([\text{Ca}^{2+}]_i), signals.</td>
</tr>
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</table>

#### Intracellular \([\text{Ca}^{2+}]_i\) transients recordings

<table>
<thead>
<tr>
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<th>Preparation</th>
<th>Technique</th>
<th>Evidence for CICR</th>
</tr>
</thead>
<tbody>
<tr>
<td>Bullfrog/adult/sympathetic ganglia</td>
<td>Primary culture (up to 50 DIV)</td>
<td>Whole cell voltage clamp; fura 2 microfluorimetry; indo 1 confocal imaging</td>
<td>When Ca(^{2+}) entry was graded by changing the duration of (I_{\text{Ca}}) (20–450 ms), the unitary Ca(^{2+}) transient showed a supralinear dependence on Ca(^{2+}) entry when (I_{\text{Ca}} &gt; 300) ms. Ryanodine (10 (\mu)M) depressed ([\text{Ca}^{2+}]<em>i), elevation without affecting (I</em>{\text{Ca}}). Sequence of two or three depolarizations potentiated ([\text{Ca}^{2+}]_i), increase; this effect was blocked by dantrolene. The UT also increased with an increase in resting ([\text{Ca}^{2+}]_i).</td>
</tr>
<tr>
<td>Rat/P8–P14/cerebellum</td>
<td>Acute slice, PN</td>
<td>Whole cell voltage clamp; fura 2 microfluorimetry</td>
<td>The amplitude of ([\text{Ca}^{2+}]<em>i), transients in response to (I</em>{\text{Ca}}) of different durations (20–100 ms) increased supralinearly at depolarizations (-60, \text{ms}). This supralinearity was blocked by ruthenium red (20 (\mu)M) and was potentiated by ryanodine (20 (\mu)M). The supralinearity was more pronounced in dendrites.</td>
</tr>
<tr>
<td>Rat/P2–P7/DRG</td>
<td>Freshly isolated cells</td>
<td>Whole cell voltage clamp; indo 1 microfluorimetry</td>
<td>Unitary Ca(^{2+}) transient was increased by elevation of ([\text{Ca}^{2+}]<em>i), from 2 to 8 (\text{mM}), and by 1 (\text{mM}) caffeine. The UT increased supra-linearly with an increase of (I</em>{\text{Ca}}), duration (-200, \text{ms}) (range 20–500 ms). The supralinearity was blocked after stores depletion with caffeine (20 (\mu)M) and UT in response to 500 ms was greatly suppressed by ryanodine (10 (\mu)M) or after stores depletion with caffeine (20 (\mu)M).</td>
</tr>
<tr>
<td>Rat/P10–P25/dorsolateral geniculate nucleus</td>
<td>Acute slice; freshly isolated neurons</td>
<td>Whole cell voltage clamp; fura 2/bis-fura 2 imaging</td>
<td>Grading of Ca(^{2+}) entry by an increase in the duration of (I_{\text{Ca}}) (60–1,400 ms) resulted in a supralinear increase of the unitary ([\text{Ca}^{2+}]<em>i), transient at (I</em>{\text{Ca}}) longer than 460 ms. This supralinear relation was completely blocked by 20 (\mu)M ryanodine.</td>
</tr>
<tr>
<td>Cockroach Periplaneta americana/dorsal unpaired median (DUM) neurons</td>
<td>Freshly isolated cells</td>
<td>Whole cell voltage clamp; fura 2 microfluorimetry</td>
<td>Unitary Ca(^{2+}) transients showed supralinearly with an increase in (I_{\text{Ca}}), duration from 20 to 1,000 ms; this supralinearity was blocked by ryanodine (100 (\mu)M) or ruthenium red (1 (\mu)M). CICR also amplified ([\text{Ca}^{2+}]_i), responses induced by neurohormone D, which triggered inward Ca(^{2+}) current.</td>
</tr>
<tr>
<td>Bovine/adrenal chromaffin cells</td>
<td>Primary culture</td>
<td>Luminometry of the ER-targeted aequorin</td>
<td>The resting ([\text{Ca}^{2+}]_i), was estimated at (-500–800, \text{mM}). Caffeine and KCl (50 (\mu)M) depolarization triggered ([\text{Ca}^{2+}]_i), decrease being thus indicative of CICR.</td>
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</table>
CICR involvement in amplification of voltage-gated [Ca$^{2+}$]i transients is rather modest, if it exists at all. Several years later, Shao-Yng Hua, Mitsuo Nohmi, and Kenji Kuba (240) added a degree of sophistication to this protocol by inventing a “unitary Ca$^{2+}$ transient” (UT), which legitimized the $\Delta [\text{Ca}^{2+}]_i/\Delta I_{\text{Ca}}$ equation being in essence a measure of how many nanomolar Ca$^{2+}$ will appear in the cytoplasm per nanoCoulombs of Ca$^{2+}$-carried current entering the cell. If the transmembrane calcium current would be the only source for the [Ca$^{2+}$]i increase, the UT should be constant irrespective of the duration or amplitude of $I_{\text{Ca}}$. An increase in UT thus reflects either the occurrence of CICR that delivers additional Ca$^{2+}$, or saturation of cytoplasmic Ca$^{2+}$ buffers and/or fast Ca$^{2+}$ extrusion systems. Several groups, starting with Hua et al. have measured UT or else quantified the relation between Ca$^{2+}$ entry and $\Delta [\text{Ca}^{2+}]_i$ in peripheral (240, 406, 585, 673) and central (68, 353) neurons, and all groups obtained virtually identical results (Table 2).

When Ca$^{2+}$ entry was graded by lengthening the depolarization step, the UT would eventually begin to increase, indicating a supralinear relation between Ca$^{2+}$ entry and $\Delta [\text{Ca}^{2+}]_i$ (Fig. 9); the UT was similarly increased when Ca$^{2+}$ entry was graded by increases in extracellular free Ca$^{2+}$ concentration ([Ca$^{2+}$]o) or by varying the depolarization amplitude (Figs. 9 and 10). Pharmacological inhibition of RyRs, or emptying the ER by preincubation with 20 mM caffeine, would depress UT and remove the supralinear relation between $\Delta [\text{Ca}^{2+}]_i$ and Ca$^{2+}$ entry. Conversely, sensitization of RyRs by 1 mM caffeine results in an increase of UT (i.e., in increase of $\Delta [\text{Ca}^{2+}]_i$ in response to the same $I_{\text{Ca}}$; Fig. 10). These data strongly imply the existence of physiological CICR in nerve cells.

The final proof that CICR can be activated by physiological Ca$^{2+}$ entry came from direct monitoring of [Ca$^{2+}$]i in neural cells (the technique will be discussed in more details below). When performing these [Ca$^{2+}$]i recordings in chromaffin cells (9) and in sensory neurons (603), it was demonstrated that cell depolarization with KCl in the former case, or activation of $I_{\text{Ca}}$ under voltage-clamp conditions in the latter, resulted in a clear decrease in the [Ca$^{2+}$]L, which reflected Ca$^{2+}$ efflux from the ER lumen (Fig. 11). Furthermore, these [Ca$^{2+}$]i transients can be enhanced by low caffeine concentrations and fully inhibited by ryanodine, thus unequivocally linking the [Ca$^{2+}$]i decrease to the activation of RyRs.

C) NEURONAL CICR IS GRADED BY CA$^{2+}$ ENTRY. The activation of neuronal CICR proportionally follows the plasmalemmal Ca$^{2+}$ entry, as was shown in experiments correlating $I_{\text{Ca}}/\Delta t$ and [Ca$^{2+}$]i as described above. The “grading” of CICR by plasmalemmal Ca$^{2+}$ influx was demonstrated even more directly in experiments where $I_{\text{Ca}}$, [Ca$^{2+}$]i, and [Ca$^{2+}$]L were simultaneously monitored (Fig. 12, Ref. 603). The relationship between $I_{\text{Ca}}/\Delta t$ and the magnitude of the transient [Ca$^{2+}$]L decrease activated by Ca$^{2+}$ entry was perfectly linear for $I_{\text{Ca}}$ of increasing (from 50 ms to 3 s) durations. When the “unitary release potency” of $I_{\text{Ca}}$ (defined as the amount of charge translocated necessary to decrease [Ca$^{2+}$]L by 1 $\mu$M) was calculated, it remained the same for Ca$^{2+}$ currents of different durations. This means that a small Ca$^{2+}$ entry may generate only a moderate CICR, which may not significantly affect [Ca$^{2+}$]i. This most likely explains the apparent threshold for CICR observed in unitary [Ca$^{2+}$] transient measurements (68, 240, 353, 406, 585).

### Table 2—Continued

<table>
<thead>
<tr>
<th>Species/Age/NS Region</th>
<th>Preparation</th>
<th>Technique</th>
<th>Evidence for CICR</th>
<th>Reference Nos.</th>
</tr>
</thead>
<tbody>
<tr>
<td>Rat/P1–P3/DRG</td>
<td>Primary culture (1–2 DIV)</td>
<td>Mag-fura 2-AM/fluo 3 imaging</td>
<td>Resting [Ca$^{2+}$]i was estimated at $\sim$300 $\mu$M. Short applications of caffeine (20 nM) triggered [Ca$^{2+}$]i decrease; [Ca$^{2+}$]i recovery was achieved chiefly by TG-sensitive uptake. The $I_{\text{Ca}}$ triggered by cell depolarization resulted in transient decrease in [Ca$^{2+}$]L, which was potentiated by 1 mM caffeine and blocked by 50 $\mu$M ryanodine. The relation between Ca$^{2+}$ entry and [Ca$^{2+}$]i decrease was linear, indicating graded CICR.</td>
<td>603</td>
</tr>
<tr>
<td>Mouse/adult/nerve terminals</td>
<td>Autely isolated</td>
<td>Whole terminal voltage-clamp/fluo 3 imaging</td>
<td>Spontaneous localized [Ca$^{2+}$]i transients (“Ca$^{2+}$ syntillas”) were observed in the terminals held at $\sim$80 mV and bathed in Ca$^{2+}$-free solution. These events were also observed in Ca$^{2+}$-containing solution and in the presence of 200 $\mu$M Cd$^{2+}$. Frequency of Ca$^{2+}$ syntillas was increased by caffeine and by depolarization of the terminal in conditions of blocked plasmalemmal Ca$^{2+}$ influx; ryanodine (100 $\mu$M) inhibited spontaneous Ca$^{2+}$ syntillas. Stronger depolarization (from $-80$ to 0 mV) can trigger global [Ca$^{2+}$]i rise (inhibited by ryanodine) even in the absence of extracellular Ca$^{2+}$.</td>
<td>116</td>
</tr>
</tbody>
</table>

RyR, ryanodine receptor; CICR, Ca$^{2+}$-induced Ca$^{2+}$ release; TG, thapsigargin; VGCC, voltage-gated Ca$^{2+}$ channels; EPSC, excitatory postsynaptic current; CPA, cyclopiazonic acid; $I_{\text{Ca}}$, Ca$^{2+}$ current.

**Depolarization-induced Ca$^{2+}$ release through RyRs**

- The final proof that CICR can be activated by physiological Ca$^{2+}$ entry came from direct monitoring of [Ca$^{2+}$]i in neural cells (the technique will be discussed in more details below). When performing these [Ca$^{2+}$]i recordings in chromaffin cells (9) and in sensory neurons (603), it was demonstrated that cell depolarization with KCl in the former case, or activation of $I_{\text{Ca}}$ under voltage-clamp conditions in the latter, resulted in a clear decrease in the [Ca$^{2+}$]L, which reflected Ca$^{2+}$ efflux from the ER lumen (Fig. 11). Furthermore, these [Ca$^{2+}$]i transients can be enhanced by low caffeine concentrations and fully inhibited by ryanodine, thus unequivocally linking the [Ca$^{2+}$]i decrease to the activation of RyRs.

- The “grading” of CICR by plasmalemmal Ca$^{2+}$ influx was demonstrated even more directly in experiments where $I_{\text{Ca}}$, [Ca$^{2+}$]i, and [Ca$^{2+}$]L were simultaneously monitored (Fig. 12, Ref. 603). The relationship between $I_{\text{Ca}}/\Delta t$ and the magnitude of the transient [Ca$^{2+}$]L decrease activated by Ca$^{2+}$ entry was perfectly linear for $I_{\text{Ca}}$ of increasing (from 50 ms to 3 s) durations. When the “unitary release potency” of $I_{\text{Ca}}$ (defined as the amount of charge translocated necessary to decrease [Ca$^{2+}$]L by 1 $\mu$M) was calculated, it remained the same for Ca$^{2+}$ currents of different durations. This means that a small Ca$^{2+}$ entry may generate only a moderate CICR, which may not significantly affect [Ca$^{2+}$]i. This most likely explains the apparent threshold for CICR observed in unitary [Ca$^{2+}$] transient measurements (68, 240, 353, 406, 585).
The sensitivity of CICR to Ca\(^{2+}\) entry differs very significantly between different neuronal types. For example, in sensory neurons, the contribution of CICR (determined as the ryanodine-sensitive component of the \([\text{Ca}^{2+}]_i\) transient) during physiological electrical activity became apparent only when >50 APs were generated in close succession (20-Hz frequency) (585). Likewise, in thalamocortical neurons, the contribution of CICR began to materialize only when bursts of APs exceeded 25 spikes with inter-AP interval of 7.5 ms (68). Conversely, in myenteric neurons, every single AP triggered a significant CICR, which, however, remained graded because each AP induced the same amount of Ca\(^{2+}\) release (227).

Nonetheless, in certain conditions, and in certain cells, neuronal CICR may acquire a regenerative character, when after reaching a threshold the release develops in an all-or-none fashion. A particularly strong CICR in response to a single AP was observed in nodose neurons, where Ca\(^{2+}\) entry produced by a single AP was amplified 5- to 10-fold by CICR, suggesting a regenerative process. In fact, the inhibition of CICR in these cells resulted in complete disappearance of \([\text{Ca}^{2+}]_i\) elevations in response to less than eight APs (103). In sensory neurons regenerative CICR was also reported, although it required pharmacological sensitization of RyRs. In the presence of 5 mM caffeine, suprathreshold \([\text{Ca}^{2+}]_i\) elevations produced by Ca\(^{2+}\) entry through plasmalemmal channels invariably triggered all-or-none CICR (663). Conversely, when the same protocol was applied to hippocampal neurons in slices, it did not reveal any regenerative CICR (446).

In fact, CICR is tightly regulated by the amount of trigger Ca\(^{2+}\) entering the cell and the rate of rise of \([\text{Ca}^{2+}]_i\) in the close neighborhood of RyRs (222). In the presence of caffeine, the rise of \([\text{Ca}^{2+}]_i\) in the vicinity of RyRs is fast.
enough to trigger a regenerative response, which, by recruiting adjacent RyRs (already sensitized by caffeine), fully depletes the store. In the absence of caffeine, [Ca$^{2+}$]$_i$ rises are slower and inactivation of RyRs prevents the occurrence of all-or-none CICR. One also has to remember the [Ca$^{2+}$]$_{i}$-sequestering ability of the ER, which balances the release, and sometimes may even overcome the latter. Indeed, as was elegantly shown by David Friel and colleagues (4, 234), the ER may switch between net Ca$^{2+}$ release, and sometimes may even overcome the amount of Ca$^{2+}$ entering the cell.

The mechanism that allows smoothly graded CICR in nerve cells is not entirely clear, although the very same phenomenon has been known for a long time from experiments on cardiomyocytes (149). Several theories (e.g., RyR adaptation, Ca$^{2+}$-dependent inactivation of RyRs, [Ca$^{2+}$]$_{i}$ depletion, [Ca$^{2+}$]$_{i}$ regulation of RyRs, etc.) have been proposed to explain the inhibition of autocatalytic CICR (149, 326, 595); most likely a combination of many if not all of these factors is responsible for Ca$^{2+}$ entry-dependent grading of Ca$^{2+}$ release.

5. **Depolarization-induced CICR in nerve cells**

Although many neurons express the “skeletal” RyR1, identification of direct depolarization-induced Ca$^{2+}$ release, which does not involve plasmalemmal Ca$^{2+}$ entry, has long been missing. Some recently published data indicate that direct coupling between the plasmalemmal and ER Ca$^{2+}$ channels in neurons may indeed exist. At the molecular level, for instance, direct coupling between the Ca$^{2+}$ channels Ca$_{v}$1.2 or Ca$_{v}$1.3 and RyR1 was identified in solubilized rat brain membrane preparations (433). Some indirect evidence favoring the expression of depolarization-induced CICR in axons was presented by Ouardouz et al. (473), who demonstrated that inhibition of the voltage sensor of Ca$^{2+}$ channel provides the same degree of protection against ischemic axonal damage as inhibition of RyRs. These authors also observed communoprecipitation (suggestive of a direct physical link) of RyR1 and Ca$_{v}$1.2 and of RyR2 and Ca$_{v}$1.3, both in the whole brain and in the dorsal spinal cord column (such combinations are quite different from skeletal muscle where RyR1 is linked to Ca$_{v}$1.1). Finally, elementary Ca$^{2+}$ release events, which occurred in the absence of Ca$^{2+}$ entry but were potentiated by depolarization, were identified in isolated nerve terminals of magnocellular hypothalamic neurons (116). The localized [Ca$^{2+}$]$_{i}$ transients that resulted from these elementary Ca$^{2+}$ releases were named Ca$^{2+}$ scintillas (a spark or a glimmer in Latin). They were not affected by the removal of extracellular Ca$^{2+}$, they were sensitive to ryanodine, and their frequency increased upon depolarization.

6. **Endogenous CICR modulators**

A) **Cyclic ADP ribose**. The effects of cyclic ADP ribose (cADPR) on Ca$^{2+}$ homeostasis were initially discovered in sea urchin eggs microsomes where this pyridine nucleotide...

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**FIG. 9. Evidence for CICR in neurons:** modulation of unitary Ca$^{2+}$ transients by graded Ca$^{2+}$ entry. **A and B:** nonlinear relations between Ca$^{2+}$ entry and $\Delta$[Ca$^{2+}$]$_{i}$ in Purkinje neurons in cerebellar slice. The amount of Ca$^{2+}$ influx was graded by changing either the duration of Ca$^{2+}$ current ($I_{ca}$; **A**) or the amplitude of test potentials evoking $I_{ca}$ (from $-70$ to $-40$ mV to $0$ mV; **B**). Plots show peak $\Delta$[Ca$^{2+}$]$_{i}$ values as a function of $I_{ca}$ integral. The insets show selected $I_{ca}$ (in response to depolarizations of different durations or different amplitudes) and corresponding [Ca$^{2+}$]$_{i}$ traces. C, similarly to A, the UT was measured from single DRG neurons. Ca$^{2+}$ entry was graded by varying the duration of $I_{ca}$ (evoked by test depolarization from $-60$ to $0$ mV, duration 20–500 ms). Note the clear supralinear increase in $\Delta$[Ca$^{2+}$]$_{i}$, at $I_{ca}$ longer than 200 ms. This supralinearity was completely obliterated when the experiments were repeated in the presence of 20 mM caffeine, which depleted the ER. [A and B modified from Llano et al. (353); C from Verkhratsky and Shmigol (673), copyright 1996 with permission from Elsevier.]
otide applied in low micromolar concentrations was found to trigger Ca\(^{2+}\)/H\(_{11001}\) release (99, 333). The primary target of cADPR is the ryanodine receptor type 2, and cADPR triggers opening of RyR2 in various nonexcitable cells (176, 177). In neuron-related cells or in neuron-derived cell lines, the injection of cADPR was reported to induce Ca\(^{2+}\)/H\(_{11001}\) release. Such direct cADPR-triggered CICR was observed in permeabilized bovine chromaffin cells (430), and PC12 pheochromocytoma cells (100), as well as in neuroblastoma × fibroblast hybrid cells injected with cADPR (251). An increase in [Ca\(^{2+}\)]\(_{i}\) due to Ca\(^{2+}\) release was also observed in buccal Aplysia neurons injected with cADPR (432); moreover, cADPR also potentiated the release of ACh from cholinergic synapses belonging to these neurons. Apart from affecting the ER, cADPR may exert several other effects not connected with RyRs at all, e.g., cADPR was reported to interact with M-type potassium channels by affecting the ACh-induced inhibition of I\(_{K(M)}\) (61). It has to be noted also that cADPR competes with ATP for the same binding site on the RyR2 (592).

FIG. 10. Evidence for CICR in neurons: effects of increase in Ca\(^{2+}\) influx and pharmacological modulators. A: elevation of extracellular Ca\(^{2+}\) (from 2 to 8 mM) and hence increase in I\(_{Ca}\) (evoked by cell depolarization from −60 to 0 mV) leads to an increase in urinary Ca\(^{2+}\) transient (UT) in a single DRG neuron. B: incubation of DRG neuron with 1 mM caffeine results in dramatic increase of Δ[Ca\(^{2+}\)]\(_{i}\), and UT in response to I\(_{Ca}\) after store depletion in the presence of 20 mM caffeine, both UT and Δ[Ca\(^{2+}\)]\(_{i}\), are markedly depressed. Throughout the experiment I\(_{Ca}\) did not change significantly. C: effects of pharmacological inhibition of ryanodine receptors (RyRs) by ryanodine on depolarization-induced [Ca\(^{2+}\)]\(_{i}\) transients. Ryanodine (10 μM) was added into the intrapipette solution, and [Ca\(^{2+}\)]\(_{i}\), and I\(_{Ca}\) were recorded simultaneously from DRG neuron. First depolarization (from −60 to 0 mV, 500 ms) delivered 5 min after the beginning of intracellular perfusion triggered large [Ca\(^{2+}\)]\(_{i}\) transient (left trace). Subsequent application of 20 mM caffeine (middle trace) failed to induce [Ca\(^{2+}\)]\(_{i}\) elevation. The second depolarization (right trace) resulted in I\(_{Ca}\) very similar to the first, yet Δ[Ca\(^{2+}\)]\(_{i}\) was reduced by ~80%, indicating CICR eradication by ryanodine. [A and C modified from Shmigol et al. (585); B modified from Verkhratsky and Shmigol (673)].
[Ca$^{2+}$]$_i$ elevation, the effect being, at least in part, blocked by ryanodine. Apart from facilitating CICR, in these experiments cADPR also potentiated Ca$^{2+}$ entry via L-type channels (204). In another set of experiments on the same neuroblastoma NG108–15, the inclusion of 10 μM cADPR into the pipette solution potentiated [Ca$^{2+}$]$_i$ transients evoked by Ca$^{2+}$ currents and resulted in a strong nonlinearity between Ca$^{2+}$ entry and [Ca$^{2+}$]$_i$ transient amplitude (thus indicating potentiated CICR, Fig. 13A, Ref. 137).

Similarly, intracellular administration of cADPR facilitated depolarization-induced [Ca$^{2+}$]$_i$ transients in CA1 hippocampal pyramidal cells (612) and in thalamocortical neurons (68).

In bullfrog sympathetic neurons, the inclusion of cADPR into the intracellular solution enhances the amplitude of depolarization- and caffeine-induced [Ca$^{2+}$]$_i$ elevations (241). Similar facilitation of CICR by cADPR was found in DRG neurons studied under whole cell voltage-clamp with simultaneous monitoring of [Ca$^{2+}$]$_i$ using fura 2. The inclusion of cADPR into the dialyzing solution significantly enhanced the unitary Ca$^{2+}$ transient, thus reflecting increased nonlinearity between Ca$^{2+}$ entry through voltage-gated Ca$^{2+}$ channels (VGCCs) and the amplitude of the resulting [Ca$^{2+}$]$_i$ elevation (Fig. 13B, Ref. 580). The opposite results, however, were obtained by Usachev and Thayer (663).
who did not observe any potentiation of CICR following intracellular dialysis of rat DRG neurons with 1–10 μM cADPR.

To act as a second messenger, production of cADPR must be controlled by relevant neurotransmitter-activated signaling cascades. Indeed, the activity of ADP-ribosyl cyclase may be regulated by metabotropic receptors, such as muscarinic cholinoreceptors (MChRs) or even glutamate receptors. Coupling between MChR and ADP-ribosyl cyclase was identified in the NG108–15 neuroblastoma, indicating that ACh may regulate cADPR production (225). The neurotransmitter-regulated cADPR-dependent Ca2+ release pathway was also implicated in glutamate-induced [Ca2+]i signaling in midbrain dopamine neurons: local activation of mGluRs triggered propagating Ca2+ waves that were mediated by both InsP3Rs and RyRs, and were blocked by concerted action of heparin and the cADPR antagonist 8-NH2-cADPR. Quite naturally, the idea of mGluRs controlling both phospholipase C (PLC) and ADP-ribosyl cyclase was born (429), although more direct data supporting such a suggestion would be very welcome.

In addition, the activity of ADP ribosyl cyclase may be controlled by the nitric oxide (NO)/cGMP/cGMP-dependent protein kinase cascade (224); NO and cGMP do enhance the formation of cADPR in homogenates of sea urchin eggs (176). Photorelease of cGMP from a caged precursor delivered into cultured DRG neurons via intracellular dialysis triggered a delayed Ca2+–dependent inward current in 50% of all neurons tested (514). This was interpreted as an indication of cADPR-induced Ca2+ release resulting from the activation of ADP ribosyl cyclase caused by cGMP-dependent phosphorylation. In support of this suggestion, the inclusion of the cADPR antagonist 8-NH2-cADPR into the pipette solution inhibited the effects of cGMP. Finally, there is some evidence that cADPR may be involved in assisting CICR necessary for LTP generation in Schaffer collaterals-CA1 neuron synapses in rat hippocampus (531).

All in all, cADPR remains a strong candidate as an endogenous modulator of CICR. In particular, neurotransmitter-regulated synthesis of cADPR in synaptic regions may be very important for local modulation of
[Ca$^{2+}$]$_i$ signals and hence relevant for synaptic plasticity.

B) NICOTINIC ACID ADENINE DINUCLEOTIDE PHOSPHATE. Another nucleotide involved in the regulation of ER Ca$^{2+}$ release in many types of nonexcitable cells is nicotinic acid adenine dinucleotide phosphate (NAADP). This molecule is believed to trigger Ca$^{2+}$ release through specific, but so far unidentified, channels, the NAADPRs (75, 77, 96, 177, 374), although NAADP may also directly activate RyRs (182). In the neural tissue, the NAADP-induced Ca$^{2+}$ release was described in brain microsomes (20) and in neurons from buccal ganglion of Aplysia californica (86). The existence and importance of NAADP-dependent signaling cascade in vertebrate neurons remains unknown.

C) CALEXCITIN. Calexcitin (CE), a Ca$^{2+}$ and GTP-binding protein, is a recently discovered endogenous modulator of neuronal RyRs and CICR. This protein was initially isolated from neurons of conditionally trained Hermisenda (451). CE binds Ca$^{2+}$ by virtue of an EF Ca$^{2+}$-binding domain and undergoes considerable and reversible conformational changes when [Ca$^{2+}$] fluctuates be-
tween 0.1 and 10 μM (14). The physiological effects of CE are exerted through modulation of K⁺ channels and RyRs. CE binds to RyRs in a Ca²⁺-dependent manner (7, 453) and facilitates Ca²⁺ release; injection of CE into hippocampal neurons resulted in a slow-developing [Ca²⁺]ᵢ elevation, which originated from the ER (454). CE also stimulated Ca²⁺ release from squid optic lobe microsomes; this release was blocked by dantrolene and an anti-RyR antibody (454). CE is known to be phosphorylated upon memory consolidation, which raises the interesting possibility that RyRs are involved in this process.

D) VANILLOID RECEPTORS. The effects of capsaicin on nerve cells are executed through the TRPV1/VR1 vanilloid receptor, which belongs to a broad family of TRP channels (35, 49). In cultured DRG neurons, capsaicin increased [Ca²⁺]ᵢ in a dose-dependent manner with ED₅₀ ~13.5 μM. This [Ca²⁺]ᵢ elevation was mimicked by the capsaicin analog resiniferatoxin (100 nM) and blocked by the VR1 receptor antagonist capsazepine (10 mM). Capsaicin-induced [Ca²⁺]ᵢ signaling did not require extracellular Ca²⁺ and disappeared after the ER was depleted by incubating cells with a mixture of 10 mM caffeine and 12 μM ryanodine (144). The [Ca²⁺]ᵢ transients evoked by capsaicin were also inhibited by 10 μM dantrolene or by 10 μM ruthenium red (144), as well as by 2 μM TG (271). Taken together, these data indicate that capsaicin stimulates CICR in sensory neurons.

A rather unusual suggestion of direct involvement of TRPV1/VR1 in Ca²⁺ release from the ER was made recently by Liu et al. (352), who confirmed that capsaicin, when applied in Ca²⁺-free media, produces [Ca²⁺]ᵢ elevation in small DRG neurons. In contrast to previous findings, however, this [Ca²⁺]ᵢ signaling pathway was not affected by TG but was sensitive to the TRPV1/VR1 receptor antagonist capsazepine. Morphological examination revealed a substantial overlap between polyclonal antibody (TRITC labeled) TRPV1/VR1 staining of small DRG neurons and staining of the same cells with ER antibody (TRITC labeled) TRPV1/VR1 staining of small DRG neurons (352). This overlap in staining was considered to indicate specific localization of TRPV1/VR1 in the ER membrane. These data prompted the intriguing speculation that TRPV1/VR1s may act as ER-resident Ca²⁺ release channel. These authors also proposed that the vanilloid receptors act as store-operated channels (but this is disputed by Karai and co-workers, Ref. 271).

E) PROTONS. "It is a truth universally acknowledged that all intracellular reactions are sensitive to pH" wrote Roger Thomas (641), and RyR function as well as CICR in various cell types are sensitive to the concentration of protons (e.g., Refs. 128, 395). In neurons, acidosis inhibits CICR and increases ER Ca²⁺ content (641), whereas alkaline shifts stimulate the release of Ca²⁺ and/or inhibit uptake of Ca²⁺ into the ER (696).

F) GLUTAMATE. It is generally accepted that the action of extracellular glutamate on ER Ca²⁺ release is accomplished via activation of group I metabotropic glutamate receptors (mGluRs) coupled, through G protein pathways, to PLC and InsP₃ metabolism. Data accumulated during the past decade, however, indicate the existence of an alternative route that involves direct interactions of mGluRs and RyRs (145). Initial evidence in support of this pathway was obtained in cell-attached patch-clamp experiments on cultured cerebellar granule cells (90). This study demonstrated mGluR-dependent activation of L-type VGCCs, which could not be mimicked by intracellular dialysis with InsP₃, was not blocked by heparin, but was completely obliterated by 1 μM ryanodine and simulated by caffeine. Subsequently, it was found that ryanodine effectively (~90%) inhibited 1-aminocyclopentane-trans-1,3-dicarboxylic acid (trans-ACPD)-induced [Ca²⁺]ᵢ elevation, while it was much less potent against [Ca²⁺]ᵢ, signals triggered by the other metabotropic agonists carbachol and histamine (119). These data led to an interesting idea about direct functional coupling between mGluRs and RyRs, thought to be mediated by Homer proteins (145). This assembly may be even more complex and may involve voltage-gated Ca²⁺ channels and Ca²⁺-dependent potassium channels. Although extremely stimulating, this hypothesis still requires additional experimentation.

Further evidence for glutamate-mediated regulation of CICR was obtained on neurons from the avian nucleus magnocellularis, in which activation of mGluRs inhibited both CICR and InsP₃-induced Ca²⁺ release (ICR). It is hypothesized that this inhibition provides a mechanism by which glutamate protects highly active neurons from calcium overload (275).

G) NEUROTROPHINS. Neurotrophins, which include nerve growth factor (NGF), brain-derived neurotrophic factor (BDNF), and neurotrophins 3/4/5 (NT-3, -4, and -5), operate through an extended family of TrkA, -B, and -C receptors, tumor necrosis factor (TNF) receptors, and p75 and Fas receptors (89). Among these receptors TrkA and p75 are abundantly expressed in the brain, and their activation is important for neuronal physiological activity; for example, BDNF-dependent neuronal excitation, synaptic plasticity, and release of neurotransmitters have been reported (265, 340, 359, 685, 701). Several of these effects involve intracellular Ca²⁺ release mediated by RyRs. For instance, NGF induces release of glutamate from cultured cerebellar neurons through activation of p75 receptors and ryanodine-sensitive Ca²⁺ mobilization (464). Similar activation of glutamate release, sensitive to dantrolene and to store depletion by caffeine, was observed in cor-

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¹ Assistance from Jane Austin also should be acknowledged: “It is a truth universally acknowledged that a single man in possession of a good fortune must be in want of a wife” (Pride and Prejudice, p. 1).
tical synaptosomes; in this case, NGF acted through TrkA receptors, and glutamate release was sensitive to heparin (527).

Another neurotrophin, NT-3, significantly potentiated the spontaneous neurotransmitter release in the neuromuscular junction; this action required both Ca\(^{2+}\) release via RyR/InsP\(_3\)Rs and the activation of Ca\(^{2+}\)/calmodulin-dependent kinase II (208). In pyramidal neurons from the visual cortex, NT-4 potentiated depolarization-induced [Ca\(^{2+}\)]\(_i\) elevations partly though enhancement of CICR (278).

Alternatively, neurotrophins may regulate CICR by affecting the expression of RyRs. For instance, NGF treatment of cultured adrenal chromaffin cells significantly elevated the expression of RyR2 and dramatically (>1,000% over 10 days) increased the amplitude of caffeine-induced [Ca\(^{2+}\)]\(_i\) responses (260). In cerebellar granule cells, the addition of 10 ng/ml BDNF from the first day in culture augmented the contribution of CICR to depolarization-induced [Ca\(^{2+}\)]\(_i\), transients by a yet unknown mechanism (408).

H) TNF-α/SPHINGOSINE-1-PHOSPHATE. Treatment of cultured rat DRG neurons with TNF-α induced a Ca\(^{2+}\)-dependent current in ~70% of cells and triggered [Ca\(^{2+}\)]\(_i\) increase in ~35% of cells tested; both the current and [Ca\(^{2+}\)]\(_i\) elevation were inhibited by TG (10–50 μM) and ryanodine (10 μM). The action of TNF-α was mimicked by the uncaging of sphingosine or by addition of 0.4 U/ml sphingomyelinase (515). In parallel, two subtypes of TNF-α receptors, TNFR1 and TNFR2, were identified in DRG neurons by immunohistochemical and RT-PCR techniques. Whether the activation of TNFRs represents a novel pathway for Ca\(^{2+}\) release mediated by sphingosine-1-phosphate through separate ER channels or through modulation of RyRs remains rather unclear (712).

I) NO. NO, which acts as a rapidly diffusible gaseous messenger in many tissues including the brain, seems to be another endogenous regulator of RyRs (143). NO-induced activation of RyR-mediated Ca\(^{2+}\) release was observed in hippocampal CA1 neurons; this Ca\(^{2+}\) release was important for both CREB phosphorylation and LTP induction (360). NO-induced activation of Ca\(^{2+}\) release from a ryanodine-sensitive store was also implicated in hypoxic [Ca\(^{2+}\)]\(_i\) rises in brain slices (394). Similarly, NO-mediated ER Ca\(^{2+}\) release via RyRs is considered to be an important pathological step in interleukin (IL)-1β-induced pyrogenic effects (485).

C. IICR in Nerve Cells

1. Direct evidence for neuronal IICR

The initial suggestion of InsP\(_3\)-induced release of Ca\(^{2+}\) in neurons arose from electrophysiological studies, which demonstrated that injection of InsP\(_3\) into various neurons or neuronlike cells [e.g., neuroblastoma × glioma cells (223), rat hippocampal neurons (387), rat dorsal raphe neurons (162), etc.] resulted in activation of Ca\(^{2+}\)-dependent K\(^+\) currents. This suggestion was immediately confirmed by measuring [Ca\(^{2+}\)]\(_i\) (Fig. 14A, Ref. 151), which demonstrated directly that intracellular administration of InsP\(_3\) induces a [Ca\(^{2+}\)]\(_i\) elevation due to Ca\(^{2+}\) release from the intracellular compartments (Table 3).

Similar experiments, in which InsP\(_3\) was administered intracellularly by photorelease from a caged precursor or by InsP\(_3\) application to permeabilized cells, revealed relatively poor sensitivity of neuronal IICR to the agonist. In Purkinje neurons in situ, for example, even 80 μM InsP\(_3\) did not result in an obvious saturation of the [Ca\(^{2+}\)]\(_i\) response (Fig. 14B; Ref. 284). Likewise, the EC\(_{50}\) for InsP\(_3\)-mediated decrease in [Ca\(^{2+}\)]\(_i\) determined in permeabilized embryonic Purkinje neurons loaded with Magfura 2 was 25.5 μM (169). In single permeabilized DRG neurons, maximal depletion of the ER was observed at InsP\(_3\) concentrations of ~20 μM (602). These values are many times greater that effective InsP\(_3\) concentrations determined for purified cerebellar InsP\(_3\)Rs (EC\(_{50}\) ~1 μM; Refs. 45, 362) and InsP\(_3\)R1 heterologously expressed in artificial systems (EC\(_{50}\) ~0.2–2 μM; Refs. 133, 420). Similarly, in many nonexcitable cells, such as astrocytes, hepatocytes, exocrine cells, and vascular epithelium, the maximal IICR was observed at InsP\(_3\) concentrations not exceeding 5–10 μM (285); however, very high doses of InsP\(_3\) (up to 100 μM) were required to trigger maximal response in pancreatic acinar cells (681).

The reasons for low sensitivity of IICR in nerve cells are not quite clear. The effective InsP\(_3\) concentrations in the cytosol can be substantially reduced by an extensive InsP\(_3\) buffering, or else InsP\(_3\) can be quickly degraded by enzymatic systems. These two possibilities, however, are questioned by the experiments on permeabilized cells, where cytoplasm is essentially washed out together with the InsP\(_3\) buffers and InsP\(_3\)-degrading enzymes. Alternatively, the InsP\(_3\) binding to the receptor may be inhibited by endogenous inositol trisphosphate receptor inhibitor (IRI), recently discovered in the cerebellum (686). The low sensitivity of neuronal IICR could be important for the maintenance of synaptic specificity by localization of InsP\(_3\)-induced signaling.

2. Metabotropic receptors and neuronal IICR

Physiological activation of IICR requires stimulation of plasmalemmal receptors coupled to PLC, which in turn hydrolyzes phosphorytidinositol 4,5-bisphosphate and gives birth to InsP\(_3\). These plasmalemmal receptors, which usually share a common seven-transmembrane domains structure, are coupled to G proteins and are generally known as metabotropic receptors. Neurons express an impressive complement of metabotropic receptors, many of which are coupled to the InsP\(_3\) signaling cascade.
Numerous observations have shown that activation of many different types of neuronal metabotropic receptors trigger IICR (Table 4).

The metabotropic receptors are activated upon synaptic transmission (Table 5, Fig. 15) and initiate IICR responsible for several distinct forms of \([\text{Ca}^{2+}/\text{H}^{100}]\) responses, manifested either as propagating \([\text{Ca}^{2+}/\text{H}^{100}]\) waves, discussed below, or in a form of highly localized \([\text{Ca}^{2+}/\text{H}^{100}]\) elevations. The local \([\text{Ca}^{2+}/\text{H}^{100}]\) microdomains are particularly important in cerebellum, where synaptic stimulation of mGluRs results in \([\text{Ca}^{2+}/\text{H}^{100}]\) release strictly confined to postsynaptic spines or defined dendritic areas of the Purkinje neurons (150, 624). This localization of \([\text{Ca}^{2+}/\text{H}^{100}]\) signaling seems to be an intrinsic property of the IICR mechanism, because focal photorelease of InsP3 triggered \([\text{Ca}^{2+}/\text{H}^{100}]\) elevation which did not spread far away from the site of InsP3 appearance (150). This localization may reflect specific properties of the Purkinje neurons such as low sensitivity of IICR to InsP3; high level of cytosolic \([\text{Ca}^{2+}/\text{H}^{100}]\) buffering; large densities of InsP3 receptors/buffers, which bind InsP3; speed of InsP3 degeneration, or the effects of IRI discussed in the previous section. This local IICR modulates the neighboring synapses by inducing long-term depression (LTD); the localized \([\text{Ca}^{2+}/\text{H}^{100}]\) signaling can be important for spatial segregation of synaptic plasticity events.

A further degree of complexity is added by the fact that neuronal InsP3Rs can be directly linked to plasmalemmal mGluRs via Homer proteins (656). This physical link may have important functional consequences, because the expression of a modified form of Homer protein, lacking the ability to cross-link with InsP3R, reduces amplitude and speed of \([\text{Ca}^{2+}/\text{H}^{100}]\) transients mediated by activation of mGluRs (656).

3. CICR-assisted IICR

An important property of InsP3 receptors and IICR is their regulation by \([\text{Ca}^{2+}/\text{H}^{100}]\). An increase in the latter facilitates IICR and may trigger \([\text{Ca}^{2+}/\text{H}^{100}]\) release through InsP3Rs in the presence of a constant level of the second messenger. The synergism between \([\text{Ca}^{2+}/\text{H}^{100}]\) and InsP3 may act as a coincidence detector allowing the system to identify the simultaneous activation of both signaling cascades; furthermore, pairing of InsP3 and \([\text{Ca}^{2+}/\text{H}^{100}]\) elevations may be important in converting local signaling events into propagating waves of ER membrane excitation.

Indeed, IICR was found responsible for generation of propagating \([\text{Ca}^{2+}/\text{H}^{100}]\) waves triggered by synaptic excitation of hippocampal pyramidal neurons. These \([\text{Ca}^{2+}/\text{H}^{100}]\) waves were found almost exclusively in the thick apical shafts of CA1 neurons (445). The generation of the \([\text{Ca}^{2+}/\text{H}^{100}]\) wave has
a threshold nature, which most likely reflects InsP<sub>3</sub> accumulation (717) and amplification of the release by CICR through InsP<sub>3</sub>Rs. This amplification can be seen directly. Pairing of mGluR stimulation (either by synaptic stimulation or by application of a metabotropic receptor agonist) with plasmalemmal Ca<sup>2+</sup> entry, activated by back-propagating action potentials, greatly enhanced [Ca<sup>2+</sup>]<sub>i</sub> signals (Fig. 16, Refs. 444, 446). In fact, the degree of mutual potentiation was quite remarkable: a backpropagated AP by itself resulted in a fast low-amplitude [Ca<sup>2+</sup>]<sub>i</sub> increase, and the application of mGluR antagonists alone did almost nothing to [Ca<sup>2+</sup>]<sub>i</sub>, yet when an AP was generated in the presence of continuous mGluR stimulation, a large (up to 40 μM) [Ca<sup>2+</sup>]<sub>i</sub> increase was produced (446). Similarly, [Ca<sup>2+</sup>]<sub>i</sub> waves could be induced in response to electrical stimulation of mossy fibers, which form synaptic input to CA3 hippocampal neurons. These waves were inhibited by 1 μM heparin and were potentiated by pairing mossy fiber stimulation with AP-driven Ca<sup>2+</sup> influx through VGCC (270). Comparable synaptically activated propagating [Ca<sup>2+</sup>]<sub>i</sub> waves resulting from IICR were also observed in neocortical pyramidal neurons from layers II/III and V. These [Ca<sup>2+</sup>]<sub>i</sub> waves were dependent on stimulation of metabotropic glutamate receptors and were blocked not only by intracellularly applied heparin, but also by 5–20 μM ryanodine, but not by ruthenium red (331). These results may indicate either an amplifying role of RyR-mediated CICR or depletion of a single store shared by RyRs and InsP<sub>3</sub>Rs.

Activation of muscarinic cholinoreceptors in hippocampal CA1 neurons by electrical stimulation of cholinergic synaptic inputs induced [Ca<sup>2+</sup>]<sub>i</sub> waves propagating from dendrites towards soma. These [Ca<sup>2+</sup>]<sub>i</sub> waves were potentiated by AP-induced Ca<sup>2+</sup> entry, disappeared after inhibition of ER Ca<sup>2+</sup> accumulation with TG (100 nM) or CPA (30 μM), and were blocked by intracellular administration of heparin (500 μg/ml), demonstrating the involvement of InsP<sub>3</sub>Rs (518). The CICR from InsP<sub>3</sub>Rs was also described in layer II/III neocortical neurons in which AP-induced [Ca<sup>2+</sup>]<sub>i</sub> transients were remarkably augmented upon stimulation of MChRs; the latter apparently provided an increase in InsP<sub>3</sub>, which sensitized InsP<sub>3</sub>Rs to Ca<sup>2+</sup> entering through the plasmalemma (704). Most interestingly, this CICR via InsP<sub>3</sub>Rs was absent in very young (<P7) rats, although in these young neurons direct application of InsP<sub>3</sub> resulted in IICR, and cell depolarization produced [Ca<sup>2+</sup>]<sub>i</sub> elevation of an appreciable amplitude (705). This developmental difference prompted the suggestion that during development, the

### Table 3. InsP<sub>3</sub>-induced Ca<sup>2+</sup> release in neurons

<table>
<thead>
<tr>
<th>Species/Age/NS Region</th>
<th>Preparation</th>
<th>Technique</th>
<th>Parameters of Release</th>
<th>Reference Nos.</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>Aplysia californica</em> abdominal ganglia</td>
<td>Primary cultures bag cells</td>
<td>Microelectrode current clamp; fura 2 imaging</td>
<td>Microinjection (iontophoresis or pressure) of InsP&lt;sub&gt;3&lt;/sub&gt; (intrapipette concentration 0.8 mM, injection time, 1 s) triggered [Ca&lt;sup&gt;2+&lt;/sup&gt;]&lt;sub&gt;i&lt;/sub&gt; elevation and neuronal hyperpolarization. The [Ca&lt;sup&gt;2+&lt;/sup&gt;]&lt;sub&gt;i&lt;/sub&gt; elevation remained in Ca&lt;sup&gt;2+&lt;/sup&gt;-free media.</td>
<td>151</td>
</tr>
<tr>
<td>Rat/P12–P22/cerebellum</td>
<td>Acute slice, PN</td>
<td>Whole-cell voltage clamp; fluo 3, Mag-fura 2 microfluorimetry</td>
<td>Photorelease of InsP&lt;sub&gt;3&lt;/sub&gt; triggered [Ca&lt;sup&gt;2+&lt;/sup&gt;]&lt;sub&gt;i&lt;/sub&gt;, increase when InsP&lt;sub&gt;3&lt;/sub&gt; concentration exceeded ~9 μM; the amplitude of [Ca&lt;sup&gt;2+&lt;/sup&gt;]&lt;sub&gt;i&lt;/sub&gt; response increased with InsP&lt;sub&gt;3&lt;/sub&gt; concentrations of up to 80 μM. Maximal amplitude of [Ca&lt;sup&gt;2+&lt;/sup&gt;]&lt;sub&gt;i&lt;/sub&gt; elevation was 10–80 μM and maximal rate of rise of 1,400 μM/s at 40 μM InsP&lt;sub&gt;3&lt;/sub&gt;.</td>
<td>284, 285, 467</td>
</tr>
<tr>
<td>Rat/E17/hippocampus</td>
<td>Primary culture (8–14 DIV)</td>
<td>Fura 2-AM imaging</td>
<td>Microinjection of 1 μM InsP&lt;sub&gt;3&lt;/sub&gt; triggered [Ca&lt;sup&gt;2+&lt;/sup&gt;]&lt;sub&gt;i&lt;/sub&gt; elevation.</td>
<td>417</td>
</tr>
<tr>
<td>Rat/P14–P21/cerebellum</td>
<td>Acute slice, PN</td>
<td>Whole-cell voltage clamp; calcium green 1; Oregon green BAPTA-1 confocal imaging</td>
<td>Focal (3–5 μm in diameter) photorelease of InsP&lt;sub&gt;3&lt;/sub&gt; triggered localized [Ca&lt;sup&gt;2+&lt;/sup&gt;]&lt;sub&gt;i&lt;/sub&gt;, elevation in PN dendrites; [Ca&lt;sup&gt;2+&lt;/sup&gt;]&lt;sub&gt;i&lt;/sub&gt; increase dependent on the InsP&lt;sub&gt;3&lt;/sub&gt; concentration (~1 to 160 μM) and did not propagate through the dendrite.</td>
<td>150</td>
</tr>
<tr>
<td>Rat/P1–P21/midbrain</td>
<td>Acutely isolated slices</td>
<td>Whole-cell voltage clamp; fura 6F microfluorimetry</td>
<td>Photorelease of InsP&lt;sub&gt;3&lt;/sub&gt; (1-ms ultraviolet flash; produced concentrations of 0.75–30 μM) triggered [Ca&lt;sup&gt;2+&lt;/sup&gt;]&lt;sub&gt;i&lt;/sub&gt; rise and evoked apamin-sensitive Ca&lt;sup&gt;2+&lt;/sup&gt;-dependent K&lt;sup&gt;+&lt;/sup&gt; currents (SK channels).</td>
<td>428</td>
</tr>
<tr>
<td>Mouse/E18/cerebellum</td>
<td>Primary culture (DIV 21–28), PN</td>
<td>Mag-fura 2 imaging of [Ca&lt;sup&gt;2+&lt;/sup&gt;]&lt;sub&gt;i&lt;/sub&gt;</td>
<td>Application of InsP&lt;sub&gt;3&lt;/sub&gt; to permeabilized cells induced decrease in [Ca&lt;sup&gt;2+&lt;/sup&gt;]&lt;sub&gt;i&lt;/sub&gt; i.e., EC&lt;sub&gt;50&lt;/sub&gt; for InsP&lt;sub&gt;3&lt;/sub&gt;-induced store depletion was 25.8 μM.</td>
<td>169</td>
</tr>
<tr>
<td>Mouse/P17–P25/cortex</td>
<td>Acute slice</td>
<td>Whole-cell voltage clamp; fura 2-photon confocal imaging</td>
<td>Photorelease of InsP&lt;sub&gt;3&lt;/sub&gt; triggered [Ca&lt;sup&gt;2+&lt;/sup&gt;]&lt;sub&gt;i&lt;/sub&gt;, transients in layer V pyramidal neurons. These [Ca&lt;sup&gt;2+&lt;/sup&gt;]&lt;sub&gt;i&lt;/sub&gt;, elevations were potentiated by Ca&lt;sup&gt;2+&lt;/sup&gt;-entry triggered by APs. [Ca&lt;sup&gt;2+&lt;/sup&gt;]&lt;sub&gt;i&lt;/sub&gt;, elevations resulted from IICR regulated neuronal excitability by activation of strong outward K&lt;sup&gt;+&lt;/sup&gt; currents.</td>
<td>609</td>
</tr>
<tr>
<td>Rat/P1–P3/DRG</td>
<td>Primary culture (1–2 DIV)</td>
<td>Mag-fura 2-AM imaging of [Ca&lt;sup&gt;2+&lt;/sup&gt;]&lt;sub&gt;i&lt;/sub&gt;</td>
<td>Application of InsP&lt;sub&gt;3&lt;/sub&gt; (5–20 μM) to permeabilized DRG neurons induced decrease in [Ca&lt;sup&gt;2+&lt;/sup&gt;]&lt;sub&gt;i&lt;/sub&gt;. InsP&lt;sub&gt;3&lt;/sub&gt;-induced [Ca&lt;sup&gt;2+&lt;/sup&gt;]&lt;sub&gt;i&lt;/sub&gt; responses saturated at ~20 μM of agonist.</td>
<td>602</td>
</tr>
</tbody>
</table>

InsP<sub>3</sub>, inositol 1,4,5-trisphosphate; IICR, InsP<sub>3</sub>-induced Ca<sup>2+</sup> release.
<table>
<thead>
<tr>
<th>Species/Age/NS Region</th>
<th>Preparation</th>
<th>Technique</th>
<th>Experimental Evidence</th>
<th>Reference Nos.</th>
</tr>
</thead>
<tbody>
<tr>
<td>Mouse/E17/hippocampus</td>
<td>Primary culture (3–17 DIV)</td>
<td>Fura 2-AM microfluorimetry</td>
<td>Glutamate, NMDA, KA, and quisqualate triggered [Ca(^{2+})], elevations; removal of extracellular Ca(^{2+}) obliterated responses to NMDA and KA, but not to glutamate and quisqualate. Similarly, phenylephrine produced [Ca(^{2+})] transients in Ca(^{2+})-free media.</td>
<td>437, 438</td>
</tr>
<tr>
<td>Rat/P12–P20/cerebellum</td>
<td>Acute slice, PN</td>
<td>Whole cell patch clamp; fura 2 imaging</td>
<td>Applications of glutamate and quisqualate triggered inward currents and [Ca(^{2+})] rise in PN dendrites; [Ca(^{2+})], elevation persisted in Ca(^{2+})-free media. When slices were incubated in Ca(^{2+})-free solution, only 1st agonist application triggered [Ca(^{2+})], signal indicating depletion of the store.</td>
<td>354</td>
</tr>
<tr>
<td>Rat/E17/hippocampus</td>
<td>Primary culture (&gt;20 DIV), PN</td>
<td>Fura 2-AM imaging</td>
<td>Stimulation of PN by trans-ACPD (100–300 μM), DHPG (200-500 μM), and a mixture of quisqualate (1–5 μM) and DNQX (50 μM) induced [Ca(^{2+})], elevation in Ca(^{2+})-free media; these [Ca(^{2+})], signals were inhibited by TG (1 μM), BHQ (10 μM), and dantrolene (10 μM).</td>
<td>193</td>
</tr>
<tr>
<td>Rat/P3/olfactory bulb</td>
<td>Primary neuronal-glial culture</td>
<td>Fluo 3-AM imaging</td>
<td>Stimulation of neurons with glutamate (10–20 μM) or quisqualate (10 μM) triggered Ca(^{2+}) responses in Ca(^{2+})-free media; these responses were blocked by TG (10 μM).</td>
<td>79</td>
</tr>
<tr>
<td>Rat/P13–P27/supraoptic nucleus</td>
<td>Freshly isolated presumed oxytocin/vasopressin neurons</td>
<td>Fura 2-AM imaging</td>
<td>Glutamate, quisqualate, and trans-ACPD evoked [Ca(^{2+})], increase in Ca(^{2+})-free media.</td>
<td>205</td>
</tr>
</tbody>
</table>

**Metabotropic glutamate receptors**

<table>
<thead>
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<th>Species/Age/NS Region</th>
<th>Preparation</th>
<th>Technique</th>
<th>Experimental Evidence</th>
<th>Reference Nos.</th>
</tr>
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<tbody>
<tr>
<td>Mouse/E17/hippocampus</td>
<td>Primary culture (7–20 DIV)</td>
<td>Fura 2-AM imaging</td>
<td>Acetylcholine (&gt;10 μM) triggered [Ca(^{2+})], elevation that was partly independent from extracellular Ca(^{2+}).</td>
<td>320</td>
</tr>
<tr>
<td>Rat/P7–P8/cerebellum</td>
<td>Primary culture, granule cells</td>
<td>Fura 2-AM microfluorimetry</td>
<td>Application of CCh (1 mM) triggered [Ca(^{2+})], transient, which was inhibited by preincubation with TG (10 μM), dantrolene (25 μM), and ryanodine (10 μM). Cell preincubation with caffeine (50 mM) greatly reduced CCh [Ca(^{2+})], response, and vice versa. Pretreatment with CCh inhibited following caffeine [Ca(^{2+})], response.</td>
<td>590</td>
</tr>
<tr>
<td>Rat/P1–P3/hippocampus</td>
<td>Primary culture (6–36 DIV)</td>
<td>Indo 1-AM confocal imaging</td>
<td>Carbachol (1–10 μM) triggered [Ca(^{2+})], elevation in ~20% of resting neurons; conditioning KCl (16.2–25 mM) depolarization made almost all cells respond. Carbachol responses were blocked by TG (2 μM). Replenishment of stores required plasmalemmal Ca(^{2+}) entry mediated through nifedipine (10 μM)-sensitive L-type VGCC.</td>
<td>249</td>
</tr>
<tr>
<td>Rat/P3–P20/spiral ganglion</td>
<td>Freshly isolated cells</td>
<td>Indo 1-AM microfluorimetry</td>
<td>ACh (K(<em>{1/2}) ~8 μM) and muscarine (K(</em>{P}) ~10 μM) triggered [Ca(^{2+})], elevation mostly due to ER Ca(^{2+}) release; responses were slightly attenuated by extracellular Ca(^{2+}) removal and were blocked by 10 μM TG.</td>
<td>540</td>
</tr>
<tr>
<td>Chicken/E18/DRG</td>
<td>Primary culture</td>
<td>Fura 2-AM microfluorimetry</td>
<td>Muscarinic agonists (1 mM muscarine or 1 mM oxotremorine) triggered [Ca(^{2+})], increase mediated through InsP(_3)-induced Ca(^{2+}) release, as [Ca(^{2+})], transients were blocked by 10 μM TG and did not require extracellular Ca(^{2+}).</td>
<td>629</td>
</tr>
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</table>

**Muscarinic cholinoreceptors**

<table>
<thead>
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<th>Species/Age/NS Region</th>
<th>Preparation</th>
<th>Technique</th>
<th>Experimental Evidence</th>
<th>Reference Nos.</th>
</tr>
</thead>
<tbody>
<tr>
<td>Rat/E17/hippocampus</td>
<td>Primary culture (8–14 DIV)</td>
<td>Fura 2-AM imaging</td>
<td>ATP (3–30 μM) produced [Ca(^{2+})], elevation; removal of external Ca(^{2+}) did not affect peak but reduced plateau of the response. ATP did not produce measurable membrane current; ATP responses were not affected by Cd(^{2+}) (1 mM) but were blocked by TG (1 μM). ATP (0.01–1,000 μM) induced [Ca(^{2+})], elevation that was independent on extracellular Ca(^{2+}) and nifedipine (10 μM), hence reflecting Ca(^{2+}) release. The ATP-induced [Ca(^{2+})], responses were blocked by preapplication of CPA (30 μM), by the PLC inhibitor U-73122 (10 μM), and by phorbol ester preincubation (100 nM). Treatment with pertussis toxin (100 ng/ml, 24 h) did not affect Ca(^{2+}) mobilization.</td>
<td>827</td>
</tr>
</tbody>
</table>

**P2Y purinoceptors**

<table>
<thead>
<tr>
<th>Species/Age/NS Region</th>
<th>Preparation</th>
<th>Technique</th>
<th>Experimental Evidence</th>
<th>Reference Nos.</th>
</tr>
</thead>
<tbody>
<tr>
<td>Guinea pig/P1/myenteric plexus</td>
<td>Primary culture (2–8 DIV)</td>
<td>Fura 2-AM microfluorimetry</td>
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<td></td>
</tr>
<tr>
<td>Species/Age/NS Region</td>
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<td>Technique</td>
<td>Experimental Evidence</td>
<td>Reference Nos.</td>
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<tr>
<td>Mouse/P60–P90/DRG</td>
<td>Freshly isolated large (proprioceptive) and small (nociceptive) neurons</td>
<td>Whole cell voltage clamp; indo 1, indo 1-AM microfluorimetry</td>
<td>ATP (100 μM) triggered inward current and [Ca^{2+}]<em>{i} elevation only in proprioceptive, but not in nociceptive, neurons (in the latter, ATP induced inward current only). Both ATP-induced currents and [Ca^{2+}]</em>{i} transients persisted in Ca^{2+}-free media. The [Ca^{2+}]_{i} transient, but not the current, was blocked by intracellular dialysis with 20 μM heparin, TG (200 nM), and ryanodine (50 μM).</td>
<td>615, 616</td>
</tr>
<tr>
<td>Rat/P1/cortex</td>
<td>Primary culture (7–14 DIV)</td>
<td>Whole cell voltage clamp; fura 2-AM imaging</td>
<td>Application of UTP (10 μM) triggered potassium current and [Ca^{2+}]_{i} elevation, which were blocked by intracellularly dialyzed heparin (1 mg/ml) or neomycin (PLC inhibitor, 500 μM).</td>
<td>460</td>
</tr>
<tr>
<td>Rat (14 days old)/neocortex</td>
<td>Acute slices</td>
<td>Fura 2-AM microfluorimetry</td>
<td>ATP-induced [Ca^{2+}]<em>{i} responses were in part mediated through P2Y metabotropic pathway; removal of extracellular Ca^{2+} reduced the amplitude of ATP-induced [Ca^{2+}]</em>{i} elevation by ~40% incubation with 1 μM TG decreased ATP-induced [Ca^{2+}]<em>{i} elevation by ~50% in 2.5 mM [Ca^{2+}]</em>{o}. The P2Y-mediated ATP responses decreased during development: at P14, 90% of neurons were in possession of this mechanism, whereas at P30, only 30% of cells displayed P2Y component of [Ca^{2+}]_{i} response.</td>
<td>324, 325</td>
</tr>
<tr>
<td>Guinea pig/adult/Corti organ</td>
<td>Isolated cochlea preparation; outer hair cells</td>
<td>Calcium green-1 imaging</td>
<td>ATP (local puff application of 1 nM for 100 ms) triggered inward current and two-component (fast and slow) [Ca^{2+}]<em>{i} transient. The slow component of [Ca^{2+}]</em>{i} response persisted in Ca^{2+}-free solution and was inhibited by heparin (7 mg/ml).</td>
<td>366</td>
</tr>
<tr>
<td>Rat/P7–P14/intracardiac ganglia</td>
<td>Primary culture</td>
<td>Fura 2-AM microfluorimetry</td>
<td>ATP and UTP triggered transient [Ca^{2+}]<em>{i} elevation in concentration-dependent manner (EC</em>{50} ~20 and ~40 μM, respectively). These [Ca^{2+}]_{i} responses did not require extracellular Ca^{2+} and were blocked by 10 μM CPA.</td>
<td>350</td>
</tr>
<tr>
<td>Rabbit/nodose ganglia</td>
<td>Primary culture (3–4 DIV)</td>
<td>Whole cell voltage clamp; fura 2-AM, fluo 3-AM microfluorimetry</td>
<td>ATP (100 μM) and InsP_{3}, photorelease triggered [Ca^{2+}]<em>{i} responses in Ca^{2+}-free media; ATP-induced [Ca^{2+}]</em>{i} transients were inhibited by U73122 (1 μM), neomycin (2 mM), heparin (1 mg/ml), and PPADS (10 μM). Ryanodine (10 μM) eliminated ~30% of the ATP-induced response, suggesting a secondary CICR amplifying the ICR.</td>
<td>230</td>
</tr>
<tr>
<td>Rat/P56–P98/DRG</td>
<td>Primary culture (2–3 DIV)</td>
<td>Fura 2-AM microfluorimetry</td>
<td>ATP and UTP (100 μM) triggered [Ca^{2+}]<em>{i} elevation in small (&lt;30 μm diameter) neurons. [Ca^{2+}]</em>{i} responses were blocked by TG (100 mM), suramine (100 μM), and PPADS (10 μM), suggesting P2Y-mediated ICR.</td>
<td>550</td>
</tr>
<tr>
<td>Rat/adult/basal forebrain/cholinergic neurons</td>
<td>Acute slices</td>
<td>Calcium orange/AM multiphoton confocal imaging</td>
<td>Adenosine triggered [Ca^{2+}]<em>{i} elevation selectively in cholinergic neurons; the responses were mimicked by A</em>{1} agonist CHA (100 nM) and inhibited by A_{1} antagonist CPT (1 μM). Adenosine-evoked [Ca^{2+}]_{i} responses were preserved in Ca^{2+}-free media, were blocked by 50 μM TG, and were inhibited by 2-APB (50 μM) and XeC (20 μM).</td>
<td>31</td>
</tr>
<tr>
<td>Rat/P1/cortex, hippocampus, striatum</td>
<td>Primary culture (4–10 DIV)</td>
<td>Fura 2-AM imaging</td>
<td>D1/D5 receptors agonists SKFS1297 (10 μM) or SKF38393 (500 μM) triggered [Ca^{2+}]_{i} responses were associated with Ca^{2+} release after cultured cortical or hippocampal neurons were primed by conditioning stimulations with mGluR agonist DHPG, carbachol, or 100 mM KCl. In striatal neurons, D1/D5 responses were completely absent.</td>
<td>339</td>
</tr>
<tr>
<td>Xenopus/E1/developing neural tube</td>
<td>Primary culture (1 DIV)</td>
<td>Calcium-green dextran confocal imaging</td>
<td>NT-3 induced [Ca^{2+}]_{i} elevation, which was blocked by heparin, XeC (1 μM), or PLC-γ inhibitor U73122 (5 μM).</td>
<td>706</td>
</tr>
<tr>
<td>Rat/adult/nodose ganglia</td>
<td>Primary culture (16–24 HIV)</td>
<td>Fura 2-AM imaging</td>
<td>NGF, BDNF, and GDNF (all at 100 ng/ml) triggered slow [Ca^{2+}]_{i} increase in ~30% of neurons, which remained in Ca^{2+}-free media and was blocked by TG (500 nM) and tyrosine kinase inhibitor K252 (1 μM).</td>
<td>327</td>
</tr>
</tbody>
</table>
plasmalemmal Ca\(^{2+}\) channels and InsP\(_3\)Rs become closer spatially, allowing modulation of InsP\(_3\)Rs by Ca\(^{2+}\) influx.

4. **Endogenous IICR modulators**

4.1 **Protein kinases and calcineurin.** Endogenous regulation of InsP\(_3\)Rs is mainly achieved through phosphorylation/dephosphorylation of the receptor molecule. Phosphorylation of the InsP\(_3\)R, which results in enhanced channel activity, is catalyzed by protein kinases A, C, and G (412, 413) and by tyrosine kinase (258). The latter is also coupled to InsP\(_3\) metabolism and therefore may affect the IICR via several routes. D Dephosphorylation of InsP\(_3\)R and hence reduction of the channel activity is catalyzed by the Ca\(^{2+}\)/calmodulin-dependent phosphatase calcineurin (73), which also regulates the expression of InsP\(_3\)R at the transcriptional level, because pharmacological blockade of calcineurin abolished the expression of InsP\(_3\)Rs in cerebellar granule neurons (181).

### Table 4—Continued

<table>
<thead>
<tr>
<th>Species/Age/NS Region</th>
<th>Preparation</th>
<th>Technique</th>
<th>Experimental Evidence</th>
</tr>
</thead>
<tbody>
<tr>
<td>Guinea pig/P1/myenteric plexus</td>
<td>Primary culture (2–8 DIV)</td>
<td>Indo 1-AM microfluorimetry</td>
<td>Substance P induced Ca(^{2+}) release (blocked by preincubation in TG, 5 (\mu)M, 1 h and independent of extracellular Ca(^{2+})). No SOC was observed in TG pretreated neurons, although it was present in nonneuronal cells from the same culture. <strong>666</strong></td>
</tr>
<tr>
<td>Rat/P3–P30/spinal ganglion</td>
<td>Freshly isolated cells and clusters of cells</td>
<td>Indo 1-AM microfluorimetry</td>
<td>Substance P (EC(_{50}) = 8.8 (\mu)M) initiated [Ca(^{2+})](_i) elevation, which did not require extracellular Ca(^{2+}) and was blocked by U-73122 indicating tachykinin receptor (NKR3)-induced ER Ca(^{2+}) release. <strong>253</strong></td>
</tr>
<tr>
<td>Rat/neonatal/DRG</td>
<td>Primary culture</td>
<td>Fura 2-AM imaging</td>
<td>Bradykinin stimulates InsP(_3) synthesis and releases Ca(^{2+}) from the intracellular store. <strong>637</strong></td>
</tr>
<tr>
<td>Murine neuroblastoma 2a stably expressing (\delta), (\mu), or (\kappa)-opioid receptors</td>
<td>Cultured cell line (neuroblastoma)</td>
<td>Fura 2-AM imaging</td>
<td>Stimulation of opioid receptors triggered Ca(^{2+}) release in neuroblastoma cells stably expressing opioid receptors. <strong>605</strong></td>
</tr>
<tr>
<td>Rat/P9–P14/brain stem/ nucleus raphe magnus</td>
<td>Acute slice</td>
<td>Whole cell voltage clamp</td>
<td>Hyperpolarization-activated current (I_h) was used as a readout of [Ca(^{2+})](_i). (\kappa)-Opioid receptor agonist U69593 (300 nM) enhanced (I_h), this action being inhibited by receptor antagonist BNI (100 nM). Effects of (\kappa)-receptor activation were antagonized by caffeine (10 mM), TG (3 (\mu)M), and heparin (500 (\mu)M). <strong>486</strong></td>
</tr>
<tr>
<td>Mouse (E9.5–E10)/neural crest</td>
<td>Primary culture (&gt;4 DIV)</td>
<td>Oregon green-BAPTA-1-AM imaging</td>
<td>InsP(_3)-induced Ca(^{2+}) release mediated spontaneous [Ca(^{2+})](_i) oscillations; Ca(^{2+}) oscillations were blocked by increased intracellular Mg(^{2+}) (the latter noncompetitively inhibits InsP(_3)-mediated Ca(^{2+}) release) or by ER store depletion with 10 (\mu)M BHQ. Slight increase in [Ca(^{2+})](_i) by reversing Na/Ca(^{2+}) exchanger (removal of Na(^+)) facilitated oscillations, suggesting therefore CICR through InsP(_3)Rs. <strong>78</strong></td>
</tr>
</tbody>
</table>
B) IMMUNOPHILIN FK506 BINDING PROTEIN 12. Immunophilin FK506 binding protein 12 (FKBP12), which is abundantly expressed in brain tissue, was shown to be associated with InsP3R1. This complex is disrupted upon the addition of the immunomodulators FK506 or rapamycin. Dissociation of InsP3R1-FKBP12 causes an increase of Ca\(^{2+}\) flux through the channel, and this can be reversed by adding FKBP12 (74). It seems that the latter serves as a physiological regulator of the conductive properties of InsP3R1s. In biophysical experiments on purified InsP3R1s in planar lipid bilayers, the addition of exogenous recombinant FKBP12 substantially increased the open time of the channels (113).

C) CALMODULIN. The InsP3R type 1, but not type 3, possesses a high-affinity binding site for calmodulin, occupa-

### TABLE 5. Synaptically induced IICR in neurons

<table>
<thead>
<tr>
<th>Species/Age/NS region</th>
<th>Synaptic Contact</th>
<th>Preparation</th>
<th>Technique</th>
<th>Experimental Evidence</th>
<th>Reference Nos.</th>
</tr>
</thead>
<tbody>
<tr>
<td>Rat/P11–P17/hippocampus</td>
<td>SC-CA1</td>
<td>Acute slice, CA1 neurons</td>
<td>Whole cell voltage clamp; fluo 3 confocal imaging</td>
<td>Tetanic stimulation of SC triggered EPSC and [Ca(^{2+})], elevation in dendrites of CA1 neurons (clamped at −35 mV to exclude VGCC activation). The [Ca(^{2+})], elevation was blocked by mGluR antagonist M CPG (250–500 μM); EPSCs were not significantly affected by the drug.</td>
<td>163</td>
</tr>
<tr>
<td>Rat/adult/hippocampus</td>
<td>MF-CA3</td>
<td>Organotypic (4–10 DIV) and acute slices, CA3 neurons</td>
<td>Whole cell voltage clamp; fura 2/fura 2 dextran imaging</td>
<td>Synaptic stimulation triggered [Ca(^{2+})] transient in conditions of pharmacological block of ionotropic GluRs (CNQX 10–50 μM + MK-801, 2–5 μM); these [Ca(^{2+})], responses were blocked by TG (5 μM, 15 min of incubation). Successful induction of Ca(^{2+}) release required conditioning training with several depolarizations to load the stores with Ca(^{2+}).</td>
<td>415</td>
</tr>
<tr>
<td>Rat/P14–21/cerebellum</td>
<td>PF-PN</td>
<td>Acute slice, PN</td>
<td>Calcium green 1; Oregon green BAPTA-1 confocal imaging</td>
<td>Stimulation of PF induced local [Ca(^{2+})], signaling in PN dendrites; the [Ca(^{2+})], response comprised of two components associated with AMPAR mediated Ca(^{2+}) entry and IICR; the latter component was blocked by mGluR antagonist MCPG (1 mM) and intracellularly applied heparin (50 μg/ml).</td>
<td>150</td>
</tr>
<tr>
<td>Mouse/ P20–P32/cerebellum</td>
<td>PF-PN</td>
<td>Acute slice, PN</td>
<td>Whole cell voltage clamp; Oregon green BAPTA-1 confocal imaging</td>
<td>Short repetitive stimulation (5 stimuli, 50 Hz) of PF triggered EPSP and local biphasic [Ca(^{2+})], elevation in dendrites. Rapid phase of Ca(^{2+}) signal was blocked by CNQX (10 μM), whereas delayed phase was inhibited by MCPG (1 mM), intracellular heparin (4 mg/ml) or CPA (20 μM), indicating thus IICR.</td>
<td>624</td>
</tr>
<tr>
<td>Rat/P21–P28/hippocampus</td>
<td>SC-CA1</td>
<td>Acute slice, CA1 neurons</td>
<td>Whole cell voltage clamp; bis-fura 2, Mag-fura 2 imaging</td>
<td>Synaptic stimulation in conditions of blockage of AMPA and NMDA receptors (CNQX 10 μM, AP-5, 100 μM) triggered small dendritic [Ca(^{2+})] transients. When synaptic stimulation was paired with 5–10 APs, the [Ca(^{2+})], transient increased dramatically. Pairing of APs with trans-ACPD (30 μM) resulted in big [Ca(^{2+})], elevations, while APs alone triggered small [Ca(^{2+})], rise. The [Ca(^{2+})], responses triggered by paired synaptic stimulation and APs were inhibited by heparin (1 mg/ml), CPA (20 μM) and ryanodine (10 μM), but not by ruthenium red (120 μM).</td>
<td>444</td>
</tr>
<tr>
<td>Rat/P19–P25/hippocampus CA3</td>
<td>MF-CA3</td>
<td>Acute slice, CA3 neurons</td>
<td>Whole cell voltage clamp; fura 2 imaging</td>
<td>Stimulation of mossy fiber by low-intensity trains of 10- to 100-Hz frequency resulted in [Ca(^{2+})], elevations not associated with AP firing and plasmalemmal Ca(^{2+}) entry. [Ca(^{2+})], responses were blocked by intracellularly applied heparin (1 mM) and potentiated by pairing of mossy fiber stimulation with postsynaptic AP.</td>
<td>270</td>
</tr>
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</table>

MF, mossy fibers; PF, parallel fibers; SC, Schaffer collateral; VGCC, voltage-gated Ca\(^{2+}\) channels; EPSC, excitatory postsynaptic currents.
tion of which inhibits the channel. Inhibition of calmodulin by calmodulin inhibitory peptide sensitizes IICR in Xenopus oocytes (376).

D) CALDENDRIN. The InsP3Rs are also regulated by the cytoplasmic Ca\(^{2+}\)/H\(^{1+}\)-binding proteins caldendrins, which, depending on their structure, may either activate or inhibit IICR. A splice variant of caldendrin was found to bind specifically to all three InsP3R subtypes. This binding activated InsP3Rs, suggesting the possibility of IICR initiation even in the absence of the natural agonist (707). At the same time a long-splice variant of caldendrin, when expressed in PC12 or HeLa cells, inhibited IICR triggered by activation of histamine and purinoreceptors (206).

D. Do InsP3Rs and RyRs Share a Common Ca\(^{2+}\) Pool?

Although morphological and functional evidence (see sect. ii) clearly defined the ER as a single continuous space, the analysis of Ca\(^{2+}\) release divided the field into supporters of a single functional Ca\(^{2+}\) pool available for both CICR and IICR, and supporters of at least two separate pools, selectively sensitive to either InsP3 or Ca\(^{2+}\)/caffeine (see Table 6). The experimental strategy employed by both camps is similar: the cells are treated with maximal concentrations of CICR agonists and thereafter the ability of IICR-stimulated agents to produce Ca\(^{2+}\) release is tested, and vice versa. Provided the cell has a single Ca\(^{2+}\) pool, each agonist should be able to deplete it completely, thus rendering subsequent stimulations with other agents ineffective. Alternatively, if the cell expresses more than one functional pool, each agonist should be able to deplete it independently, thus rendering subsequent stimulations with other agents ineffective.

FIG. 15. Synaptically evoked localized IICR in Purkinje neuron. A: local dendritic [Ca\(^{2+}\)]i signals mediated by repetitive parallel fiber stimulation. a: Confocal image of Purkinje neuron voltage-clamped and loaded with Oregon-green BAPTA-1. The dendritic regions 1 and 2 correspond to sites of stimulation (position of stimulation pipettes are depicted by dotted lines). b: These regions at higher magnification and also shows the kinetics of [Ca\(^{2+}\)]i responses to repetitive parallel fiber stimulation (5 stimuli, 50 Hz). B: dependence of synaptic [Ca\(^{2+}\)]i responses on ER Ca\(^{2+}\) release. a: Almost complete elimination of the delayed phase of [Ca\(^{2+}\)]i transient by intracellularly applied heparin (4 mg/ml). b: Same effect of ER depletion by incubation of slices with cyclopiazonic acid (CPA; 20 \(\mu\)M). [Modified from Takechi et al. (624).]
unidirectional relations between RyR-bearing and InsP₃Rs bearing stores: either ryanodine occluded IICR but not vice versa (541), or else stimulation of IICR depletes caffeine-sensitive pool but stimulation of RyRs does not affect the InsP₃-sensitive one (528). Moreover, in most cases, the separate pools were observed in cell lines or in embryonic cultures, whereas the existence of a single Ca²⁺/H₁₁₀₀₁ store is supported by experiments on primary cultures and brain slices, the latter preparations being rather more physiological (Fig. 17A). When directly investigating intra-ER Ca²⁺/H₁₁₀₀₁ dynamics in sensory neurons, a very clear and full overlap between InsP₃/ryanodine/caffeine/Ca²⁺/H₁₁₀₀₁ and TG/CPA pools was observed (Fig. 17B, Ref. 602).

E. Luminal Ca²⁺ as a Regulator of Ca²⁺ Release

The level of free Ca²⁺ concentration within the ER lumen is critically important for regulation of Ca²⁺ release as it creates the driving force for Ca²⁺ exit and regulates the functional availability of Ca²⁺ release channels. That [Ca²⁺]ᵢ in nerve cells is a labile parameter became obvious from the very beginning of experimental investigations of the ER Ca²⁺ store. The first attempts to induce Ca²⁺ release in single central neurons, either by caffeine or by metabotropic agonists, demonstrated that application of stimulating agents to resting cells triggered very tiny [Ca²⁺]ᵢ increases in a small proportion of neurons. However, when caffeine or a metabotropic agonist was applied shortly after the cell was depolarized, and thus had experienced a large Ca²⁺ influx into the cytosol, a considerable [Ca²⁺]ᵢ elevation due to Ca²⁺ release was apparent in a large proportion of neurons (249, 250, 375, 437, 526, 581). A small depolarization (i.e., with ~10 mM KCl or with low concentrations of NMDA) which led to a steady-state [Ca²⁺]ᵢ increase had the same effect (249).
The stimulating effect of the conditioning depolarization was transient because the amplitude of Ca\(^{2+}\) release decreased progressively when increasing the time gap between the depolarization and the application of a Ca\(^{2+}\) release stimulator (Fig. 18). Quite logically, this peculiar behavior was explained in terms of a low releasable Ca\(^{2+}\) content of the ER in resting central neurons. The empty Ca\(^{2+}\) store favors Ca\(^{2+}\) accumulation and therefore Ca\(^{2+}\) entering the cell following depolarization is taken up by the ER, thus increasing the Ca\(^{2+}\) content within the store and permitting Ca\(^{2+}\) release. An extreme situation, in which the store is empty or almost empty at rest, is usually observed in cultured cells. Application of caffeine to resting neurons in acute hippocampal slices (179) or in acutely isolated thalamocortical neurons (68) routinely evokes [Ca\(^{2+}\)]\(_i\), responses; similarly stimulation of IICR triggered [Ca\(^{2+}\)]\(_i\) elevation in Purkinje neurons (624) and in pyramidal cortical neurons (609) in situ. Nonetheless, the conditioning depolarization dramatically (by 500%) increased the amplitude of a subsequent caffeine-induced [Ca\(^{2+}\)]\(_i\), transient in central neurons studied in acute slices (179); and similarly to what was found in cultured neurons this potentiation was short-lived (Fig. 18B). In peripheral neurons, the effect of conditioning depolarization also existed, although it was much less dramatic; conditioning depolarization increased the amplitude of subsequent Ca\(^{2+}\) release by 10–30% (583, 661). The nature of this rather prominent difference between central and peripheral neurons is unclear; similarly unclear are the mechanisms responsible for the loss of surplus Ca\(^{2+}\) in the ER after the store has been charged.

The ability of the ER of central neurons to rapidly accumulate huge amounts of Ca\(^{2+}\) was confirmed in direct recordings of total ER Ca\(^{2+}\) content using energy dispersive X-ray microanalysis. In these experiments, hippocampal slices were rapidly frozen at different time intervals after tetanic afferent stimulation. It was observed that 30 s after stimulation, the total ER Ca\(^{2+}\) content had increased up to 20 times and stayed elevated for 15–30 min (519, 520). The authors concluded that the ER acts as a major Ca\(^{2+}\) buffering system in dendrites upon synaptic stimulation. However, this may vary between different regions and may depend on heterogeneous distribution of SERCA pumps (365). Importantly, the X-ray microanalysis also showed a remarkable heterogeneity of dendritic ER with respect to Ca\(^{2+}\) accumulation; only ~40% of the reticulum demonstrated an increase in total Ca\(^{2+}\) content. These data once more raise questions about the functional continuity of the ER, although a heterogeneous increase in total Ca\(^{2+}\) may reflect specific concentration of Ca\(^{2+}\) binding proteins in particular ER regions.

The transient Ca\(^{2+}\) loading of the Ca\(^{2+}\) store in central neurons certainly has very important implications. Indeed, a transient increase in [Ca\(^{2+}\)]\(_L\) and the consequent enhancement of Ca\(^{2+}\) release would provide the ER with a coincidence detection mechanism and with (at least short-term) memory. Further understanding of the mechanisms controlling changes in the ER Ca\(^{2+}\) concentration ultimately requires direct monitoring of the free Ca\(^{2+}\) dynamics in the ER lumen.

Several experimental approaches permitting real-time measurements of [Ca\(^{2+}\)]\(_L\) have been developed during the last two decades (Table 7). These techniques employ either ER-targeted Ca\(^{2+}\)-sensing luminescent or fluorescent proteins (10, 120) or conventional fluorescent Ca\(^{2+}\) probes with a sufficiently low Ca\(^{2+}\) affinity (232, 492, 601). The [Ca\(^{2+}\)]\(_L\) measurements in neurons began only recently (Table 7; Fig. 19), and our knowledge about ER Ca\(^{2+}\) dynamics in nerve cells remains very incomplete. The quantitative [Ca\(^{2+}\)]\(_L\) measurements performed in PC12 cells and isolated sensory neurons showed a high resting [Ca\(^{2+}\)]\(_L\) ranging between 100
and 800 μM and demonstrated that activation of Ca\(^{2+}\) release channels by Ca\(^{2+}\), caffeine, ryanodine, and InsP\(_3\) results in a rapid decrease of [Ca\(^{2+}\)]\(_{L}\) reflecting Ca\(^{2+}\) release. Direct [Ca\(^{2+}\)]\(_{L}\) measurements in physiologically intact central neurons have not yet been performed.

**F. Refilling the Ca\(^{2+}\) Store and Store-Operated Ca\(^{2+}\) Entry**

Control of [Ca\(^{2+}\)]\(_{L}\) and refilling of the Ca\(^{2+}\) store after depletion is accomplished by SERCA pumps. Numerous experiments (see Tables 1–5) have demonstrated that inhibition of the latter prevents Ca\(^{2+}\) accumulation and results in progressive depletion of the Ca\(^{2+}\) store due to unopposed leakage of Ca\(^{2+}\) from the ER lumen. Occasional reports mention the possible existence of a TG-resistant Ca\(^{2+}\) store in nerve cells, and a TG-resistant Ca\(^{2+}\) transport was found in brain microsomes and in certain cell lines (687). The relevance of this type of Ca\(^{2+}\) pool for neuronal physiology remains unclear.

Intraluminal Ca\(^{2+}\) recordings revealed an important role of the [Ca\(^{2+}\)]\(_{L}\) level in the regulation of the velocity of ER Ca\(^{2+}\) uptake. Decreases in [Ca\(^{2+}\)]\(_{L}\) significantly (5–7 times) increased the speed of Ca\(^{2+}\) uptake; replenishment of the ER slowed the rate of Ca\(^{2+}\) uptake (603). Most importantly, at least in sensory neurons, the influence of
an increased $[\text{Ca}^{2+}]_{i}$ on the $\text{Ca}^{2+}$ uptake velocity seems to be negligible (Fig. 20). To quantify $\text{Ca}^{2+}$ uptake velocity, $\text{Ca}^{2+}$ release was stimulated by brief application of caffeine, and the recovery kinetics of $[\text{Ca}^{2+}]_{i}$ were monitored during the washout period. After $[\text{Ca}^{2+}]_{i}$ reached the prestimulation level, the SERCA pumps were inhibited by TG, which resulted in a relatively slow decrease in $[\text{Ca}^{2+}]_{i}$ due to $\text{Ca}^{2+}$ leakage. The rate of leakage declined in proportion to the decrease in $[\text{Ca}^{2+}]_{i}$, presumably reflecting a falling electrochemical driving force for $\text{Ca}^{2+}$. It turned out that neither the rate of $\text{Ca}^{2+}$ uptake nor the velocity of $\text{Ca}^{2+}$ leakage was affected when a BAPTA/ $\text{Ca}^{2+}$ (10 mM/2 mM) buffer was washed into the cytosol (Fig. 20, A and B). In the latter condition $[\text{Ca}^{2+}]_{i}$ is clamped, yet plenty of $\text{Ca}^{2+}$ is available for ER accumulation. Therefore, in peripheral neurons store refilling does not require an increase in $[\text{Ca}^{2+}]_{i}$, albeit it requires $\text{Ca}^{2+}$ availability in the cytosol. This seems to be a general mechanism for many cell types, and the very similar dependence of ER $\text{Ca}^{2+}$ uptake on $[\text{Ca}^{2+}]_{i}$ and independence on $[\text{Ca}^{2+}]_{i}$ rises, was shown for pancreatic acinar cells (425; see Fig. 20C) and BHK-21 fibroblasts (231).

In frog sympathetic ganglion neurons, a special mechanism which greatly assists the refilling of the stores after their depletion, the release-activated calcium transport (RACT) was also suggested (110). The nature of this

FIG. 18. Transient overcharging of ER $\text{Ca}^{2+}$ stores in central neurons. A: left panel shows representative $[\text{Ca}^{2+}]_{i}$ recordings from cultured hippocampal neurons challenged with 20 mM caffeine and 50 mM KCl as indicated on the graph. Note that initial application of caffeine to the resting cell fails to trigger $[\text{Ca}^{2+}]_{i}$ elevation, yet caffeine applied immediately after the end of KCl depolarization produces substantial $[\text{Ca}^{2+}]_{i}$ response. Increase in the time gap between conditioning KCl depolarization and application of caffeine leads to progressive decrease in the amplitude of caffeine-induced $[\text{Ca}^{2+}]_{i}$ elevation (right panel shows a summary of similar experiments performed on different types of cultured central neurons), indicating that ER loses surplus $\text{Ca}^{2+}$. The columns on the graph are means ± SD for the amplitudes of caffeine-induced $[\text{Ca}^{2+}]_{i}$ elevation; caffeine was applied 30, 210, and 600 s after the end of KCl depolarization. B: experiment very similar to that described in A except it was performed on CA1 pyramidal neurons in acutely isolated hippocampal slices. Left panel shows examples of $[\text{Ca}^{2+}]_{i}$ recordings demonstrating the changes in caffeine-induced $[\text{Ca}^{2+}]_{i}$ response depending on conditioning KCl (40 mM) challenge. On the right, the bar histogram depicting the relation between the size of caffeine-induced $[\text{Ca}^{2+}]_{i}$ transient versus the time elapsed after a KCl-induced $[\text{Ca}^{2+}]_{i}$ elevation ($\Delta t$). The $\Delta t$ was measured between the peaks of KCl-mediated and caffeine-mediated $[\text{Ca}^{2+}]_{i}$ transients. Each bar represents a mean of 6–11 data points. The amplitudes of caffeine-induced $[\text{Ca}^{2+}]_{i}$ transients evoked after KCl depolarization were normalized with respect to the control (i.e., before KCl) $[\text{Ca}^{2+}]_{i}$ transient. [A from Shmigol et al. (581), with permission from Blackwell Publishers Ltd.; B from Garaschuk et al. (179), with permission from Springer-Verlag.]
pathway remains enigmatic, and it is possible that it simply reflects greater activation of SERCA pumping following severe depletion of the ER (although according to the initial description RACT appeared to be insensitive to TG).

All in all, the regulation of \([Ca^{2+}]_i\) in peripheral neurons seems to be quite straightforward; these stores are full in the resting conditions, and an efficient \([Ca^{2+}]_i\) dependent regulation of SERCA pumping keeps \([Ca^{2+}]_i\) under tight control. This control is assisted by a \([Ca^{2+}]_i\) dependent leakage such that every increase in \([Ca^{2+}]_i\) immediately increases the Ca\(^{2+}\) leak and decreases SERCA activity. Store depletion leads to the opposite effect, i.e., a decrease in Ca\(^{2+}\) leak and rapid upregulation of SERCA pumping velocity. Intuitively, the mechanism controlling \([Ca^{2+}]_i\) in central neurons must be very different as it should permit a high flexibility of the \([Ca^{2+}]_i\) regulation to tolerate the relatively low resting ER Ca\(^{2+}\) content. Nevertheless, this low resting \([Ca^{2+}]_i\) seems to prime SERCAs for rapid accumulation of the bulk of the Ca\(^{2+}\) entering the cytosol upon depolarization or synaptic stimulation. This also assumes the high sensitivity of the Ca\(^{2+}\) uptake mechanism to increases in \([Ca^{2+}]_i\), which represents another clear difference between central and peripheral nerve cells.

The problem of store refilling is also inseparable from the question of neuronal expression of the store-operated Ca\(^{2+}\) entry pathway. This pathway, discovered by Jim Putney (522), is a general feature of nonexcitable cells, which lack other means of Ca\(^{2+}\) entry. A direct relation between ER depletion and SOC activation is firmly established (458, 488, 490, 523). In nerve cells, the replenish-

### TABLE 7. Monitoring of \([Ca^{2+}]_i\) in neurons

<table>
<thead>
<tr>
<th>Species/Age/NS Region</th>
<th>Preparation</th>
<th>([Ca^{2+}]_i) Indicator</th>
<th>Experimental Approach/Data</th>
<th>Reference Nos.</th>
</tr>
</thead>
<tbody>
<tr>
<td>Rat/P35/pituitary gonadotropes</td>
<td>Freshly isolated identified cells</td>
<td>Mag-Indo 1-AM ((K_i = 35 \mu M))</td>
<td>Cells were loaded with Mag-Indo 1-AM by 45-min exposure to 9–18 (\mu M) of the dye at 37°C followed by washout for 30–60 min at 28°C. Cytosolic portion of the dye was removed by intracellular dialysis via patch pipette; 1 mM MnCl(_2) was added to intracellular solution to quench remnants of cytosolic dye fluorescence. Stimulation of gonadotropes with gonadotropin-releasing hormone triggered rhythmic transient ([Ca^{2+}]_i) decreases on top of slow decrease in ([Ca^{2+}]_i).</td>
<td>651</td>
</tr>
<tr>
<td>Frog/adult/sympathetic ganglia</td>
<td>Primary culture (2–6 DIV)</td>
<td>Fluo 3FF-AM ((K_i = 42 \mu M))</td>
<td>Cells were loaded with fluo 3FF-AM (4 (\mu M) for 90 min). The dye was considered to report exclusively ([Ca^{2+}]_i); no attempts to remove cytosolic dye were performed. Recordings reveal rather poor dynamic range (maximal depletion by caffeine was (\sim 0.1 \Delta F/F) unit). More importantly, “on” and “off” kinetics of fluorescence drop in response to caffeine application/washout were almost identical and very rapid. Only responses to caffeine and one small response to chloro-m-cresol were shown without any further pharmacological identification of the mechanisms involved.</td>
<td>110</td>
</tr>
<tr>
<td>Bovine/adrenal medulla rat/cerebellum</td>
<td>Primary cultures of chromaffin cells and cerebellar granule neurons</td>
<td>ER-targeted aequorin</td>
<td>Cells were transfected (HSV-1 virus) with ER-targeted low-affinity aequorin (which allow ([Ca^{2+}]_i) measurements in a range of 20 (\mu M–1 \text{ nM})). Expression required (\sim 16) h. Before the experiment, cells were incubated for 1 h at room temperature in standard medium containing 0.5 mM EGTA, 10 (\mu M) BHQ, and 1 (\mu M) coelenterazine. In some experiments, the plasmalemma was permeabilized with 20 (\mu M) digitonin. To fill up the stores, cells were incubated with 100 mM EGTA-Ca(^{2+}) buffer. Both IICR (histamine stimulated) and CICR (Ca(^{2+}) and caffeine stimulated) were identified.</td>
<td>8–10</td>
</tr>
<tr>
<td>Mouse/E18/cerebellum</td>
<td>Primary culture of PN (21–28 DIV)</td>
<td>Mag-fura 2-AM ((K_i = 50 \mu M))</td>
<td>Cells were loaded with Mag-fura 2-AM by 60-min incubation with 30 (\mu M) of the dye at 24°C. Experiments were performed on cells permeabilized with 60 (\mu M) β-escin (6–10 min); cells were kept in Ca(^{2+})-free/EGTA solutions; to load ER, MgATP and Ca(^{2+}) were added. Application of InsP(_3) induced depletion of the store; no recovery was shown. No attempt to calibrate the system was made.</td>
<td>169</td>
</tr>
<tr>
<td>Rat/P1–P3/DRG</td>
<td>Primary culture (1–2 DIV)</td>
<td>Mag-fura 2-AM ((K_i = 50 \mu M))</td>
<td>Cells were loaded with Mag-fura 2-AM by 30-min incubation with 5 (\mu M) of the dye at 37°C followed by washout for 60 min at 37°C. Cytosolic portion of the dye was removed by either intracellular dialysis or by plasmalemmal permeabilization with 0.001% saponin applied for 7–10 s. Introduction of fluo 3K into intrapipette solution (patch-clamp experiments) allowed simultaneous measurements of ([Ca^{2+}]_i) and ([Ca^{2+}]_i). Both CICR and IICR were identified.</td>
<td>600–603</td>
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</table>
ment of the Ca\textsuperscript{2+} store after depletion by either several consecutive applications of caffeine/metabotropic agonists or prolonged incubation of cells with Ca\textsuperscript{2+} release stimulating agents, requires plasmalemmal Ca\textsuperscript{2+} influx (179, 661). Does this Ca\textsuperscript{2+} influx employ a classical store-operated mechanism? Or, does the Ca\textsuperscript{2+} enter the cell via numerous Ca\textsuperscript{2+}-permeable channels present in the neuronal plasmalemma? This remains a debatable matter. From the general logic of neuronal physiology, the SOC pathway can hardly be justified. Indeed, neurons possess plenty of Ca\textsuperscript{2+} channels, and moreover, these channels are repeatedly activated, as neurons rarely enjoy complete idleness. Furthermore, activation of SOC, according to several reports, has a threshold nature, i.e., it requires a very substantial depletion of the store (202, 489, 490). This is very difficult to reconcile with the observations of empty stores in central neurons; if SOC exists it surely would increase [Ca\textsuperscript{2+}]i up to the point when the stores become replenished.

Nonetheless, several groups have suggested that neurons possess a store-operated Ca\textsuperscript{2+} influx pathway very similar to that of nonexcitable cells (Table 8, Ref. 524), yet the data gathered are not entirely convincing. Quite often SOC expression is judged upon pharmacological sensitivity of [Ca\textsuperscript{2+}]i transients produced by Ca\textsuperscript{2+} readmission after store depletion in Ca\textsuperscript{2+}-free media. Because the pharmacology of store-operated channels is not very well defined, this interpretation is open to criticism.

FIG. 19. Examples of [Ca\textsuperscript{2+}]i recordings. A: an example of [Ca\textsuperscript{2+}]i recording in bovine chromaffin cells transfected by ER-targeted aequorin showing complete overlap of InsP\textsubscript{3}/ryanodine and TG-sensitive store. Left trace shows effects of caffeine (50 mM) and histamine (10 \(\mu\)M) on [Ca\textsuperscript{2+}]i after the ER was refilled by incubation with medium containing 1 mM Ca\textsuperscript{2+}. On the right, the effects of caffeine, histamine, and bradykinin (1 \(\mu\)M) on [Ca\textsuperscript{2+}]i in control conditions and after pretreatment with either ryanodine (10 \(\mu\)M) or thapsigargin (1 \(\mu\)M) are demonstrated. B: example of [Ca\textsuperscript{2+}]i recording using fluo 3FF. The cells were loaded with fluo 3FF-AM, and no attempts to washout the cytosolic portion of the dye were made, which may account for a poor dynamic range of measurements (maximal response \(\sim\)0.1 \(\Delta\text{F/F}\)). Cells were treated with 10 mM caffeine, 50 mM KCl, or 1 mM chloro-m-cresol as indicated on the graph. Caffeine triggered [Ca\textsuperscript{2+}]i response where recovery was as fast (left trace) or even faster (right trace) that the initial [Ca\textsuperscript{2+}]i decrease. C: simultaneous visualization of [Ca\textsuperscript{2+}]i and [Ca\textsuperscript{2+}]L dynamics in DRG neurons. On the left, a series of selected images of the Mag-fura 2 ratio (Ex. 340/380 nm, top panel) taken simultaneously with images of fluo 3 fluorescent intensity (Ex. 488 nm) from the DRG neuron exposed to 20 mM caffeine is shown. Right panel shows calibrated recordings of [Ca\textsuperscript{2+}]i and normalized fluo 3 fluorescent intensity (reflecting changes in [Ca\textsuperscript{2+}]i) taken from the cell shown on the left. Caffeine was applied as indicated on the graph; the positions of the images presented on the left panel are shown near the [Ca\textsuperscript{2+}]L trace. [A] from Alonso et al. (9) by copyright permission from The Rockefeller University Press; [B] from Caerensnes et al. (110), copyright 1997 with permission from Elsevier; [C] from Solovyova et al. (603), with permission from Nature Publishing Group.]
tence of SOC in central neurons, for example, was postulated by Baba et al. (17) based on $[\text{Ca}^{2+}]_i$ imaging of primary cultured pyramidal and granule neurons isolated from Ammon’s horn and dentate gyrus, respectively. After these neurons were incubated with $10^{-6}$ M TG for 5 min in $\text{Ca}^{2+}$-free solution, readmission of external $\text{Ca}^{2+}$ triggered $[\text{Ca}^{2+}]_i$ elevation, which was inhibited by $10^{-5}$ M La$^{3+}$, $3 \times 10^{-5}$ M SKF96365, or $3 \times 10^{-5}$ M 2-aminoethoxydiphenyl borate (2-APB). No attempts, however, were made to activate SOC by more physiological depletion of the ER store such as administration of caffeine or stimulation of metabotropic receptors, yet a conclusion about SOC activation by synaptic transmission (based on partial and rather slight inhibition of $[\text{Ca}^{2+}]_i$ transients evoked by tetanic field stimulation of cultured neurons by 2-APB or SKF96365) was announced. As 2-APB and SKF96365 also attenuated LTP recorded in hippocampal slices, another fundamental conclusion of SOC involvement in synaptic plasticity was made by the same authors (17). Interestingly, La$^{3+}$, 2-APB, and SKF96365 also inhibited the plateau phase of NMDA-induced $[\text{Ca}^{2+}]_i$ elevation, which may reflect their poor selectivity, rather than activation of SOC upon stimulation of ionotropic glutamate receptors.

The protocols of inducing SOC in neurons vary, e.g., cultured cortical neurons were incubated in a $\text{Ca}^{2+}$-free, $50 \mu$M EGTA and $2 \mu$M CPA containing solution for 30 min, and $[\text{Ca}^{2+}]_i$ elevation developing...
after external Ca\(^{2+}\) restoration was regarded as a SOC-related phenomenon (709), yet the possibility that prolonged treatment with a Ca\(^{2+}\)-free solution may affect neuronal status and viability was not explored. Sometimes the existence of SOC, as well as its physiological significance, is deduced from indirect data such as an increase in miniature excitatory postsynaptic potential frequencies in Ca\(^{2+}\)-containing solutions after treatment with CPA (139). These observations favoring neuronal SOC should be balanced by very many experiments, that employed TG/CPA for inhibition of Ca\(^{2+}\) release in nerve cells. In most of these cases, no significant changes in resting [Ca\(^{2+}\)], indicative of SOC were observed (e.g., Refs. 179, 303, 418, 582, 583, 629, 655 to name a few). All in all, the evidence for neuronal SOC derived from pharmacological inhibition of post-TG/CPA-induced [Ca\(^{2+}\)], elevation remains, in my view, unconvincing, and conjectures about the role of SOC in synaptic plasticity, though very stimulating, currently lack real experimental basis.

Perhaps the most convincing case for SOC expression in neurons was presented by Usachev and Thayer, who investigated the process of store replenishment in rat cultured DRG neurons. They demonstrated that Ca\(^{2+}\)-

<table>
<thead>
<tr>
<th>Species/Age/NS Region</th>
<th>Preparation</th>
<th>Technique</th>
<th>Evidence for SOC</th>
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</thead>
<tbody>
<tr>
<td>Rat/E17/hippocampus, thalamus</td>
<td>Primary culture (8-14 DIV)</td>
<td>Fura 2-AM imaging; Mn(^{2+})-dependent fura 2 quenching</td>
<td>Mn(^{2+}) influx into neurons was detected after initiation of Ca(^{2+}) release by intracellular injection of InsP(_3) or stimulation of metabotropic purinoceptors. Reintroduction of extracellular Ca(^{2+}) after the stores were depleted by application of caffeine (10 mM) in Ca(^{2+})-free solution triggered substantial [Ca(^{2+})], elevation. Readmission of Ca(^{2+}) after ER depletion by TG (500 nM) in Ca(^{2+})-free media triggered [Ca(^{2+})], rise sensitive to 10 (\mu)M SKF96365, 1 mM Zn(^{2+}), or 5 mM Ni(^{2+}). This [Ca(^{2+})], rise was prominent at E3 and disappeared at E13. It was also inhibited by genistein, suggesting the involvement of tyrosine phosphorylation in activation of the presumed SOC.</td>
</tr>
<tr>
<td>Myenteric plexus/P1–P3/ guinea pig</td>
<td>Primary culture (2–8 DIV)</td>
<td>Fura 2-AM microfluorimetry</td>
<td></td>
</tr>
<tr>
<td>Chicken/E3–E13/neural retina</td>
<td>Acutely isolated preparation</td>
<td>Fura 2-AM microfluorimetry</td>
<td></td>
</tr>
<tr>
<td>Immortalized hypothalamic GT1 neural cells</td>
<td>Culture</td>
<td>Whole cell voltage clamp; indo 1 microfluorimetry</td>
<td>ER store depletion with TG (5 (\mu)M) induced low-amplitude (–20 pA) Ca(^{2+})-conducting inward current.</td>
</tr>
<tr>
<td>Rat/P1–P3/DRG</td>
<td>Primary culture</td>
<td>Whole cell voltage-current clamp; indo 1/indo 1-AM microfluorimetry; Mn(^{2+})-dependent fura 2 quenching</td>
<td>Refilling of Ca(^{2+}) store after caffeine-induced depletion required Ca(^{2+}) influx, which was inhibited by 2 mM Ni(^{2+}); other VGCC blockers (10 (\mu)M nifedipine, 10 (\mu)M nicardipine, or 1 (\mu)M omega-conotoxin SIA) were ineffective. Refilling of the store was potentiated by hyperpolarizing the cells from –55 to –80 mV. The [Ca(^{2+})], transient elevation upon readmission of Ca(^{2+}) following discharging the store by caffeine/Ca(^{2+})-free solution was found in 68% of cells. Finally, caffeine and CPA potentiated Mn(^{2+}) influx in DRG neurons. Application of 200 (\mu)M carbobol and 0.4 (\mu)M TG triggered [Ca(^{2+})], elevation in Ca(^{2+})-free solution; readmission of external Ca(^{2+}) resulted in secondary [Ca(^{2+})], elevation, which was blocked by 50 (\mu)M La(^{3+}). Readmission of extracellular Ca(^{2+}) after 30 min of cell incubation in Ca(^{2+})-free 50 (\mu)M EGTA and 2 mM CPA triggered [Ca(^{2+})], elevation, which was considered to reflect SOC activation.</td>
</tr>
<tr>
<td>Rat/P3–P5/ hippocampus</td>
<td>Primary culture</td>
<td>Fura 3-AM confocal imaging</td>
<td></td>
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<tr>
<td>Mouse/E15.5/cortex</td>
<td>Primary culture (&gt;1 DIV)</td>
<td>Fura 2-AM imaging</td>
<td></td>
</tr>
<tr>
<td>Rat/P3/hippocampus, Ammon’s horn, and dentate gyrus</td>
<td>Primary culture (7–9 DIV)</td>
<td>Fura 2-AM imaging</td>
<td>[Ca(^{2+})], elevation in response to Ca(^{2+}) readmission after 5 min of incubation with TG/0 Ca(^{2+}); this [Ca(^{2+})], elevation was blocked by 100 (\mu)M La(^{3+}) or 30 (\mu)M 2-APB.</td>
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<tr>
<td>Rat/P22–P28/midbrain</td>
<td>Acute slice</td>
<td>Whole cell voltage clamp; fura 2 imaging</td>
<td>Activation of mGluR I (DHPG, 100 (\mu)M) resulted in cationic current (~300–400 pA at –70 mV. The currents were inhibited by SKF96365 (100 (\mu)M), Gd(^{3+}) (100 (\mu)M), ruthenium red (20 (\mu)M), and 2-APB (30–100 (\mu)M). When the cells were treated with 10 (\mu)M TG in Ca(^{2+})-free media, Ca(^{2+}) readmission resulted in steady-state [Ca(^{2+})], elevation sensitive to 2-APB. RT-PCR revealed expression of multiple TRP channels, with TRPC1 and TRPC5 being the most abundant.</td>
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2-APB, 2-aminoethoxydiphenyl borate; DHPG, S(3,5)-dihydroxyphenylglycine.

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241

Physiol Rev • Vol 85 • January 2005 • www.prv.org

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try, essential for the refilling of ER stores after they have been discharged with caffeine, can be blocked by 2 mM Ni2+ and the refilling of the store is potentiated when the cells are hyperpolarized from \(-55\) mV to \(-80\) mV. Furthermore, Usachev and Thayer (664) substantiated their claim for SOC in nerve cells by demonstrating that ER store depletion potentiates Mn2+ entry into neurons. Still, these data may be explained by a very different mechanism, i.e., Ca2+ entry through T-type Ca2+ channels, which have a “window” current region around \(-75\) to \(-80\) mV, where they may provide for unstimulated Ca2+ influx. Calcium entry through conventional Ca2+ channels following ER depletion was, for instance, observed in cultured guinea pig myenteric neurons, in which the treatment with TG (1 \(\mu\)M) induced long-lasting irregular [Ca2+]i oscillations arising solely from Ca2+ influx, yet these oscillations were completely blocked by \(\omega\)-conotoxin MVIIIA, suggesting that these neurons may use N-type Ca2+ channels for store refilling (665). In the same neurons, application of ATP triggered a biphasic (peak – plateau) [Ca2+]i response; the plateau required extracellular Ca2+ but was blocked by 10 \(\mu\)M nifedipine (287). Likewise, the plasmalemmal voltage-gated Ca2+ channels were responsible for ER store refilling in cultured cerebellar granule cells (249) as well as in cultured motoneurons from larval lampreys (280).

G. Pharmacology of Ca2+ Release

1. Ryanodine receptors/CICR

Mechanisms of pharmacological modulation of RyRs have received much attention, and the properties of drugs that selectively modulate RyRs have been summarized in numerous reviews (e.g., Refs. 135, 216, 217, 259, 308, 310, 483, 614, 703, 720). Therefore, I shall limit myself to a brief discussion of some of these tools, which are instrumental for studying CICR in nerve cells.

A) Ryanodine. Ryanodine was probably the first and the foremost pharmacological agent used for probing for Ca2+ stores and Ca2+ release. Its action on skeletal and cardiac muscle had been known for the last 35 years (259), and its ability to specifically bind to Ca2+-gated Ca2+ release channels led to naming the latter “ryanodine receptor” (505) a little confusing (as no ryanodine was ever in attendance in living tissues, save in roots and stem of the plant \textit{Ryania speciosa}, which is the natural source of ryanodine), but generally accepted. Ryanodine exerts multiple actions on the RyRs, depending on its concentration. At very low concentrations (5–40 nM), ryanodine was reported to increase the frequency of RyR channel opening, without affecting its conductance (67). At low micromolar (1 to \(-5\) \(\mu\)M) concentrations, ryanodine promotes RyR channel opening in a subconducting (~40–60% compared with a normal) state, which is also characterized by a dramatic (~20 times) increase in the channel open time. At higher concentrations (50 to >100 \(\mu\)M) ryanodine completely blocks the channel (67, 440). These multiple actions most likely reflect the existence of high- and low-affinity binding sites for ryanodine within the RyR. Notably, ryanodine binding to the low-affinity site is almost irreversible. An important feature of ryanodine is its “use dependence” (440), i.e., preferential binding of the drug to the open channel. In practical terms, this became apparent as a facilitation of ryanodine action by repetitive activation of RyRs (either by caffeine or by [Ca2+]i increase), a phenomenon that is often observed in nerve cells (29, 92, 269, 308, 636, 661). Ryanodine is still the most specific pharmacological tool used for probing CICR in neurons (see Tables 1 and 2).

B) Ruthenium Red. A polycationic dye, ruthenium red, is widely used as CICR blocker in nerve cells (see Tables 1 and 2). Its effective concentrations for CICR inhibition in muscle preparations range between 1 and 20 \(\mu\)M (720). At the single-channel level, ruthenium red effectively reduces \(P_o\) by prolonging the closed state of the RyR. Unfortunately, as an inhibitor ryanodine red is rather nonspecific as it also inhibits VGCC (97) epithelial Ca2+ channels ECaC1 (459), TRPV channels (194), GABA release from hippocampal synaptic terminals (568), and mitochondrial calcium uptake (534). Moreover, when applied to permeabilized chicken atria, ruthenium red prevented contractile responses induced by InsP3 and potentiated responses to caffeine (676), which makes its employment as a specific CICR inhibitor even more dubious.

C) Dantrolene. Dantrolene was originally synthesized in 1967 (598) as a potent muscle relaxant, and it is currently the only available drug for the specific treatment of malignant hyperthermia, a life-threatening complication of general anesthesia (313). Incidentally, dantrolene may also help against ecstasy intoxication (28). The action of dantrolene on the ER consists of reducing the \(P_o\) of RyRs, thereby inhibiting CICR; the effective concentrations of the drug lie between 10 and 90 \(\mu\)M (469). Dantrolene is widely used as a CICR inhibitor in neurons (Tables 1 and 2), although there are some indications that dantrolene is effective only against RyR1 and does not inhibit RyR2 (167).

D) General Anesthetics. Halothane and enfurane enhance Ca2+ release in cardiac preparation at low micromolar (i.e., in subtherapeutic) concentrations and increase \(P_o\) of RyR channels (720). In the neural cholinergic cell line SN56, halothane triggered Ca2+ release from the ryanodine-, dantrolene- and CPA-sensitive calcium store (189). Considering that volatile anesthetics affect synaptic transmission (94, 95, 293) and neuronal excitability (414), the possibility that they act by modulating CICR should not be forgotten. In addition, however, halothane also inhibits plasmalemmal Ca2+-ATPase (157, 683), so the resulting effects on intracellular Ca2+ dynamics and Ca2+-
dependent processes may be rather complex and difficult to interpret.

E) LOCAL ANESTHETICS. Most of the local anesthetics, such as procaine, tetracaine, lidocaine, prilocaine, the quaternary amines QX 572 and QX 314, and benzocaine, inhibit caffeine-induced Ca\(^{2+}\) release and CICR in millimolar concentrations (720), and this action was also observed in nerve cells (308, 661).

F) DHIDROPROPIDINES. Nifedipine, a well-known voltage-gated channel antagonist, was reported to increase the frequency of miniature end-plate potentials in synaptic terminals studied in the muscular junction of neonatal (up to 3 wk) rats. Effects of nifedipine persisted in Ca\(^{2+}\)-free media and were inhibited by TG (2 \(\mu\)M) and ryanodine (10 \(\mu\)M), which allowed the authors to suggest possible stimulatory action of the drug on RyRs (512).

G) HEPARIN. Heparin, which is customarily used as a selective inhibitor of IICR, does affect RyRs at the very same concentrations that are effective against InsP\(_3\)R. The effects of heparin on RyRs were initially suggested when it was found that heparin stimulates Ca\(^{2+}\) release from skeletal muscle SR vesicles (404, 538). Further experiments on purified RyR channels demonstrated that heparin increases channel openings with EC\(_{50}\) values of \(-0.23 \mu\)g/ml (compared with 1–4 mg/ml concentrations usually employed for IICR inhibition) in a Ca\(^{2+}\)-dependent manner (46, 135).

H) OTHER PHARMACOLOGICAL CICR REGULATORS. The interaction with RyRs and modulation of CICR have been reported for many other pharmacological agents, which generally are not considered to possess such ability. For example, cardiac glycosides and, in particular, digoxin in therapeutic concentrations increase the rate of CICR in cardiac preparations through sensitizing RyRs to [Ca\(^{2+}\)]\(_i\) and increasing channel \(P_o\) (390). Suramin, widely used as a purinoreceptor blocker, induces CICR and increases \(P_o\) of RyRs (594). Another agent, believed to activate CICR and the RyR3 channel, in particular, is chloro-m-cresol, which triggers Ca\(^{2+}\) release in skeletal muscle (719), and in glial cells (383). So far, however, no systematic attempts to use chloro-m-cresol in nerve cells were made. Another class of drugs often employed in experiments with cultured neurons, the aminoglycoside antibiotics neomycin and gentamycin, are potent inhibitors of CICR with effective concentrations in a range between 50 nM and 10 \(\mu\)M (720).

2. Pharmacology of IICR

IICR pharmacology is less well defined than the pharmacology of CICR. From the very beginning of InsP\(_3\)R studies it became apparent that a number of quite different pharmacological agents, including heparin, \(\rho\)-chloromercuribenzoic acid, cinnarasmine, flunarizine, local anesthetics, such as tetracaine and lidocaine, and potassium channel blockers such as TEA, can effectively inhibit InsP\(_3\)-induced Ca\(^{2+}\) release from microsomal ER preparations (see, e.g., Refs. 482, 484). Further complexity was added when it became clear that caffeine, which was believed to selectively interact with RyRs, inhibits InsP\(_3\)R (135, 680), and heparin, the most widely used InsP\(_3\)R blocker, activates RyRs (46, 135). As if this was not enough, caffeine also effectively inhibits InsP\(_3\) production (645). All in all, the ideal inhibitor of IICR, which would penetrate biological membranes and demonstrate high selectivity against InsP\(_3\)R, remains in the realm of dreams.

A) HEPARIN. Heparin was shown to bind to InsP\(_3\)R (613) and block the activity of InsP\(_3\)-gated channels incorporated into lipid bilayers (43). Heparin was also shown to inhibit IICR in many types of nonexcitable and excitable cells, and it is constantly used as a pharmacological probe for neuronal IICR (see Tables 3–5). A big disadvantage of heparin is its membrane impermeability; hence, it must be delivered into the cell interior either by injection or via intracellular dialysis. Although heparin activates RyRs in artificial membranes, I failed to find a single report that would describe heparin-induced Ca\(^{2+}\) release in nerve cells. If that would be the case, however, heparin may still inhibit IICR by depleting ER via activated RyRs, although the interpretation of such data can be difficult.

B) CAFFEINE. The inhibitory action of caffeine on IICR was first discovered by Ole Petersen and co-authors (680) who studied agonist-mediated Ca\(^{2+}\) signaling in pancreatic acinar cells. Later on, the inhibition of IICR by millimolar concentrations of caffeine was found in Xenopus oocytes (493), cerebellar microsomes (66), and permeabilized smooth muscle cells (228). Direct electrophysiological investigations of purified InsP\(_3\)R supported these findings by showing inhibition of unitary InsP\(_3\)R current. The \(K_D\) of caffeine action on InsP\(_3\)R currents was \(\sim1.6\) mM, and at 10 mM caffeine, a complete block of InsP\(_3\)R currents was attained (228).

C) 2-APB. The ability of 2-APB to inhibit IICR was initially found in cerebellar microsomes stimulated with 100 \(\mu\)M InsP\(_3\); 2-APB blocked Ca\(^{2+}\) release with an IC\(_{50}\) value of \(\sim40\) \(\mu\)M. Incubation with 2-APB also inhibited agonist-stimulated IICR in human platelets and neutrophils (373) and in HeLa cells (503). However, further investigations demonstrated that 2-APB lacks selectivity as it affects not only IICR but also Ca\(^{2+}\) pumps and store-operated Ca\(^{2+}\) entry in nonexcitable cells (55, 503).

D) XESTOSPONGINES. Some hope for a selective, membrane-permeable InsP\(_3\)R antagonist appeared in 1997 when Isaac Pessah’s group purified and characterized a group of macrocyclic bis-l-oxaquinolizidines isolated from the Australian sponge Xestospongia species (174). Several of these agents, dubbed Xestospongines (Xes), namely, XeA, C, and D, were reported to be powerful membrane-permeable blockers of IICR when tested on...
ER vesicles isolated from the cerebellum; of these, XeC was the most potent inhibitor with a $K_D \approx 360$ nM. Further experiments have demonstrated that XeC blocks $[\text{Ca}^{2+}]_i$ transients mediated by metabotropic pathways in the PC12 cell line and in primary rat cortical astrocytes. Incubation of cells with $5-20$ μM XeC effectively abolished $[\text{Ca}^{2+}]_i$ responses to bradykinin and carbachol (174). Yet more detailed investigations performed later somewhat dampened the optimism for the action of xestosponges as selective membrane-permeable blockers of InsP$_3$Rs because it emerged that XeC acts as an effective blocker of SERCA pumps and thus empties the ER of Ca$^{2+}$ (84, 123, 600). It is noteworthy that an indication that XeC interacts with SERCA pumps was already present in the initial paper by Gafni et al. (174); Figure 4 of this study clearly shows that incubation of PC12 cells with XeC decreases the amplitude of consequent TG-induced $[\text{Ca}^{2+}]_i$ rise (which was interpreted by authors as an inhibition of leak pathway, yet may reflect depletion of the ER as a result of SERCA blockade).

E) ADENOPHOSTINES. The adenophostines A and B were isolated from metabolites of fungi Penicillium brevicompactum (622) and found to be extremely potent (10–100 times more potent than InsP$_3$) agonists of InsP$_3$Rs (694). They were tested in turtle olfactory sensory neurons (272) and in single InsP$_3$Rs isolated from fish olfactory cilia (70). In both cases adenophostines acted as powerful agonists of InsP$_3$Rs inducing channel opening (70) and promoted IICR as judged by the appearance of InsP$_3$/[Ca$^{2+}]_i$-dependent currents.

IV. ENDOPLASMIC RETICULUM CALCIUM SIGNALING AND REGULATION OF NEURONAL FUNCTIONS

A. Neuronal Excitability

Calcium release from the ER regulates neuronal excitability through several types of plasmalemmal Ca$^{2+}$-activated channels, responsible for K$^+$, Cl$^-$, and nonselective currents (see Tables 9 and 10). In addition, Ca$^{2+}$ release may (at least theoretically) suppress VGCC by increased Ca$^{2+}$-dependent inactivation. The best-characterized effect of CICR is activation of K(Ca) channels, which control postspike afterhyperpolarization (AHP). The AHP comprises three distinct components (554), the fast AHP (mediated by BK channels), the AHP (mediated by SK channels), and the (ultra)slow AHP (mechanism not yet identified). Activation of the fast AHP is mediated solely by plasmalemmal Ca$^{2+}$ entry, while both AHP and slow AHP are sensitive to CICR inhibitors. The contribution of CICR to the AHP is quite substantial: up to 50% of the AHP current is activated by Ca$^{2+}$ released from the ER. The mechanism of CICR-mediated regulation of the (ultra)slow AHP remains entirely unknown. In vagal afferent neurons, Ca$^{2+}$ release activates $I_{\text{AHP}}$ only when CICR is triggered by Ca$^{2+}$ entry through N-type VGCC, which may reflect a very special colocalization of N-type Ca$^{2+}$ channels, RyRs, and Ca$^{2+}$-dependent K$^+$ channels (107). A similar functional colocalization of RyRs and voltage-gated N-type Ca$^{2+}$ channels was also demonstrated in bullfrog sympathetic neurons, where Ca$^{2+}$ entry through N-type VGCC triggers regenerative CICR near the plasma membrane (2). In these cells, RyRs also seem to be functionally coupled to BK channels, thus providing a feedback between ER Ca$^{2+}$ release, membrane excitability, and AP frequency. Finally, the same functional interactions between N-type Ca$^{2+}$ channels and RyRs were confirmed in PC12 cells, where CICR is triggered by Ca$^{2+}$ influx through N-type but not through L-type Ca$^{2+}$ channels (657).

CICR also regulates membrane excitability through Ca$^{2+}$-dependent Cl$^-$ channels underlying depolarizing afterpotentials (DAPs) found in the magnocellular neurons of the supraoptic nucleus, in sympathetic neurons, and in vagal nodose neurons subjected to vagotomy (328, 342, 370).

B. Neurotransmitter Release

The crucial importance of Ca$^{2+}$ for neurotransmission was initially demonstrated by F. S. Locke for the neuromuscular junction (356), and the concept of Ca$^{2+}$-dependent neurotransmitter release was coined by Bernard Katz and colleagues (118, 146). According to this theory, regulated exocytosis, of which neurotransmitter release represents a particular case, requires activation of numerous Ca$^{2+}$-regulated transduction cascades that eventually result in the fusion of vesicles with the plasmalemma and release of vesicle contents into the extracellular space (in the case of neurotransmission, the synaptic cleft). Activation of exocytotic cascades requires a large (up to 100 μM) local $[\text{Ca}^{2+}]_i$ concentration, and the role of plasmalemmal Ca$^{2+}$ entry in neurotransmitter release is well established (36). The possible role of Ca$^{2+}$ originating from the ER store is much less understood. Certainly, exocytosis in several types of nonexcitable and excitable cells almost exclusively depends on ER Ca$^{2+}$ release [good examples are pancreatic acinar cells (506) and pituitary gonadotrophs (652)]. In a nerve-related cell preparation, neuroblastaoma cocultured with rat skeletal muscle cells, bradykinin-induced IICR elevated $[\text{Ca}^{2+}]_i$ transients mediated by metabotropic pathways in the neuromuscular junction (356), and the concept of Ca$^{2+}$-dependent neurotransmitter release was coined by Bernard Katz and colleagues (118, 146). According to this theory, regulated exocytosis, of which neurotransmitter release represents a particular case, requires activation of numerous Ca$^{2+}$-regulated transduction cascades that eventually result in the fusion of vesicles with the plasmalemma and release of vesicle contents into the extracellular space (in the case of neurotransmission, the synaptic cleft). Activation of exocytotic cascades requires a large (up to 100 μM) local $[\text{Ca}^{2+}]_i$ concentration, and the role of plasmalemmal Ca$^{2+}$ entry in neurotransmitter release is well established (36). The possible role of Ca$^{2+}$ originating from the ER store is much less understood. Certainly, exocytosis in several types of nonexcitable and excitable cells almost exclusively depends on ER Ca$^{2+}$ release [good examples are pancreatic acinar cells (506) and pituitary gonadotrophs (652)]. In a nerve-related cell preparation, neuroblastaoma cocultured with rat skeletal muscle cells, bradykinin-induced IICR elevated $[\text{Ca}^{2+}]_i$ and triggered acetylcholine release (468). However, whether ER Ca$^{2+}$ release by itself can trigger exocytosis in presynaptic terminals in vivo remains an unanswered, albeit challenging, question.

Numerous morphological studies confirmed that functional ER is present in presynaptic terminals [e.g.,
## TABLE 9. Neuronal functions regulated by Ca\(^{2+}\)-induced Ca\(^{2+}\) release

<table>
<thead>
<tr>
<th>Function</th>
<th>Brain Region</th>
<th>Experimental Evidence</th>
<th>Reference Nos.</th>
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<tr>
<td>Control of neuronal excitability</td>
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</tr>
<tr>
<td>Activation of (I_{\text{K(Ca)}}) control of AHP</td>
<td>Dorsal motor nucleus of vagus; prevertebral sympathetic neurons</td>
<td>Inhibition of CICR by ryanodine, dantrolene, or intracellular dialysis with ruthenium red selectively inhibited K(Ca) channels responsible for AHP.</td>
<td>262, 555</td>
</tr>
<tr>
<td>Activation of (I_{\text{K(Ca)}})</td>
<td>DRG neurons</td>
<td>Initiation of Ca(^{2+}) release by caffeine (20 mM) or IBMX (5 mM) triggered outward K(^{+}) current; 0.5 mM IBMX triggered SMOCs. In addition, IBMX inhibited M current in a Ca(^{2+})-release independent manner.</td>
<td>660</td>
</tr>
<tr>
<td>Activation of (I_{\text{K(Ca)}})</td>
<td>Otic ganglion</td>
<td>Ryanodine (10 μM) almost completely blocked the activation of late AHP component without affecting fast (I_{\text{AHP}}).</td>
<td>711</td>
</tr>
<tr>
<td>Activation of (I_{\text{K(Ca)}}) control of AHP</td>
<td>Superior cervical ganglion; sympathetic neurons</td>
<td>Ryanodine (20 μM) inhibited slow component of AHP.</td>
<td>114</td>
</tr>
<tr>
<td>Activation of SMOCs</td>
<td>Parasympathetic cardiac neurons</td>
<td>SMOC activation was potentiated by 2 mM caffeine and blocked by CPA (10 μM) and ryanodine (10–100 μM).</td>
<td>405</td>
</tr>
<tr>
<td>Control of slow AHP</td>
<td>Myenteric afterhyperpolarizing neurons</td>
<td>Ryanodine (10 μM) inhibited slow component of AHP.</td>
<td>227</td>
</tr>
<tr>
<td>Control of slow AHP</td>
<td>Hippocampal pyramidal neurons</td>
<td>Ryanodine (10 μM) reduced amplitude of slow AHP by ~30%.</td>
<td>58, 572</td>
</tr>
<tr>
<td>Control of slow AHP</td>
<td>Myenteric plexus neurons</td>
<td>Incubation of ganglia with ryanodine (10–20 μM) or caffeine (2–5 mM) inhibited both slow AHP and (I_{\text{AHP}}).</td>
<td>678</td>
</tr>
<tr>
<td>Activation of STOCs</td>
<td>Dentate gyrus; granule neurons</td>
<td>In voltage-clamped granule neurons, Ca(^{2+}) release events potentiated by intracellularly applied cADPR and InsP(_3) controlled STOCs and regulated neuronal excitability.</td>
<td>579</td>
</tr>
<tr>
<td>Activation of SMOCs</td>
<td>Meunert nucleus basalis neurons</td>
<td>Frequency of SMOCs was reduced by ryanodine (10–100 μM) and potentiated by caffeine (1 mM). ER inhibition by TG (100 nM) or CPA (10 μM) blocked SMOCs.</td>
<td>13</td>
</tr>
<tr>
<td>Activation of (I_{\text{K(Ca)}}) channels; modulation of AP</td>
<td>Stellate ganglion; sympathetic neurons</td>
<td>Inhibition of CICR by ryanodine (20 μM) or TG (1 μM) decreased the latency to AP generation in response to depolarizing current ramps. It is suggested that CICR normally activates BK K(Ca) channels, therefore limiting neuronal excitability. The effects of CICR were potentiated following mitochondrial blockade, which suggested some role of the mitochondria in rapid buffering of Ca(^{2+}) released from the ER.</td>
<td>30, 357</td>
</tr>
<tr>
<td>Control of DAP</td>
<td>Supraoptic nucleus; magnocellular neurons</td>
<td>Inhibition of CICR by ryanodine (10 μM), dantrolene (10 μM), ruthenium red (20 μM), or ER depletion with TG (3 μM) or CPA (15 μM) suppressed DAP amplitudes by ~50%, shortened their duration, and eliminated phasic patterns of firing. Application of caffeine (5–10 mM; 5–10 min) increased DAP amplitudes and increased firing rates. Heparin (2–4 mg/ml) either did not affect or potentiated DAP (possible stimulation of RyRs?).</td>
<td>342</td>
</tr>
<tr>
<td>Control of DAP; activation of (I_{\text{K(Ca)}})</td>
<td>Superior cervical ganglion; sympathetic neurons</td>
<td>Ryanodine (20 μM) markedly (~75%) inhibited Ca(^{2+})-activated Cl(^{-}) current and associated afterdepolarization.</td>
<td>370</td>
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<tr>
<td>Control of DAP</td>
<td>Nodose ganglion</td>
<td>DAPs were recorded in neurons, isolated 4–6 days after vagotomy; they were absent in control cells. Ryanodine (10 μM) reduced DAP amplitudes by 37%.</td>
<td>328</td>
</tr>
<tr>
<td>Activation of (I_{\text{K(Ca)}}) and nonselective cation currents</td>
<td>DRG</td>
<td>Application of ryanodine (10 μM) activated Cl(^{-}) and nonselective cation currents when holding the cell at resting potential. Ryanodine also suppressed (I_{\text{Cl}}) evoked by depolarization.</td>
<td>16</td>
</tr>
<tr>
<td>Spontaneous hyperpolarization</td>
<td>Midbrain dopaminergic neurons</td>
<td>Caffeine in a low (1 mM) concentration potentiated spontaneous hyperpolarization; at high (10 mM) concentration, caffeine transiently increased the frequency of hyperpolarization, followed by its inhibition. Spontaneous hyperpolarization was reversibly blocked by CPA (10 μM) and irreversibly by TG (10 μM).</td>
<td>570</td>
</tr>
<tr>
<td>Spontaneous GABA release from presynaptic terminals</td>
<td>CA3 hippocampal neurons</td>
<td>Slice incubation with Ca(^{2+})-free media did not significantly affect mIPSPs; short (5 s) pulses of caffeine (10 mM) increased mIPSP frequency, whereas long incubation with caffeine decreased mIPSP amplitude and frequency. TG (10 μM) increased mIPSP frequency, while ryanodine (30 μM) was without effect.</td>
<td>563</td>
</tr>
</tbody>
</table>

*Physiol Rev • VOL 85 • JANUARY 2005 • www.prv.org*
<table>
<thead>
<tr>
<th>Function</th>
<th>Brain Region</th>
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<th>Reference Nos.</th>
</tr>
</thead>
<tbody>
<tr>
<td>Synaptic ACh release</td>
<td>Cholinergic synapses in <em>Aplysia californica</em> buccal ganglion</td>
<td>CICR induced by intracellular injection of cADPR (10–1,000 μM) potentiated ACh release. The injection of cADPR per se triggered [Ca$^{2+}$] elevation. Effects of cADPR were prevented by 8-amino-cADPR and ryanodine.</td>
<td>432</td>
</tr>
<tr>
<td>Glutamate release</td>
<td>Rod photoreceptors</td>
<td>Application of caffeine (10 mM) triggered transient [Ca$^{2+}$], elevation followed by prominent undershoot. In the presence of caffeine the glutamate release was depressed due to substantial (~50%) inhibition of $I_{\text{Ca}}$. Ryanodine (20 μM) inhibited the effect of caffeine on $I_{\text{Ca}}$ but not on glutamate release.</td>
<td>314</td>
</tr>
<tr>
<td>GABA release</td>
<td>Cerebellum; basket cell–PN synapse</td>
<td>High level of RyR expression was found in basket cell terminals on PN. Treatment with high concentration of ryanodine (100 μM) decreased the amplitude of depolarization-evoked [Ca$^{2+}$], transients and significantly reduced amplitude and frequency of spontaneous miniature IPSCs. Low ryanodine concentration (5–10 μM) enhanced mIPSC's frequency, likely due to facilitating a multivesicular GABA release.</td>
<td>355</td>
</tr>
<tr>
<td>Synaptic ACh release</td>
<td>Frog neuromuscular junction</td>
<td>TG (2 μM) prolonged presynaptic depolarization-induced [Ca$^{2+}$], transients and induced a rapid rise in the amplitude of the EPP, without affecting mEPPs. It appears as ER limits neurotransmitter release by providing a Ca$^{2+}$ clearance mechanism.</td>
<td>83</td>
</tr>
<tr>
<td>Synchronization of EPSPs oscillations</td>
<td>Neocortex</td>
<td>In acute slices of visual cortex, caffeine (4–6 mM) potentiated and thapsigargin (20–80 μM) inhibited the appearance of oscillatory synaptic potentials.</td>
<td>710</td>
</tr>
<tr>
<td>Synaptic glutamate release</td>
<td>Pyramidal neurons in layer II of barrel cortex</td>
<td>Frequency and amplitude of mEPSPs was inhibited by loading the slice with BAPTA-AM, by blocking SERCA pumps with CPA (20 μM), and by ryanodine (10 μM). Caffeine (10 mM) enhanced mEPSPs; this enhancement was observed for up to 20-min incubation with caffeine. Administration of 2-APB (14 μM) reduced and activation of metabotropic receptors by DHPG (20 μM) enhanced frequency and amplitude of mEPSPs.</td>
<td>589</td>
</tr>
<tr>
<td>Spontaneous GABA release from presynaptic terminals</td>
<td>Cerebellum; PN</td>
<td>Incubation with ryanodine (10 μM) and CPA (25 μM) affected frequency of spontaneous mIPSCs in conditions of limited Ca$^{2+}$ entry through VGCCs.</td>
<td>26</td>
</tr>
<tr>
<td>Synaptic glutamate release</td>
<td>Hair cells</td>
<td>Caffeine (1–10 mM) triggered [Ca$^{2+}$], elevation accompanied by an increase of $C_m$ (indicative of exocytosis) and potentiated depolarization-evoked [Ca$^{2+}$], responses. Inhibition of CICR by ryanodine (40 μM) depressed afferent transmission as judged from a reduced frequency of mEPSPs.</td>
<td>337</td>
</tr>
<tr>
<td>Synaptic GABA release</td>
<td>Cerebellum; basket cell–PN synapse</td>
<td>Ryanodine (100 μM) induced ~70% inhibition of evoked GABA-mediated mIPSCs in basket cell–PN synapses studied in acute rat slices. Low concentrations of ryanodine (5–10 μM) had a biphasic effect: IPSPs were transiently enhanced, which was followed by their depression.</td>
<td>175</td>
</tr>
<tr>
<td>Regulation of frequency and amplitude of spontaneous mEPSPs</td>
<td>Hippocampus; CA3 neurons</td>
<td>In hippocampal slices CICR activated by Ca$^{2+}$ entry following opening of presynaptic NChRs potentiated frequency and increased amplitude of mEPSPs. Inhibition of ER Ca$^{2+}$ accumulation by TG (5 μM) or blockade of CICR by ryanodine (100 μM) abolished effects of nicotine; transient application of 20 mM caffeine mimicked effects of nicotine.</td>
<td>573</td>
</tr>
</tbody>
</table>

**Presynaptic [Ca$^{2+}$]$^{2+}$, signaling and presynaptic plasticity**

[$\text{Ca}^{2+}$]$^{2+}$], signaling in presynaptic terminal
- MF terminals
  - [$\text{Ca}^{2+}$]$^{2+}$], elevation in synaptic terminals (“bulk” loaded with fura 2-AM; microfluorimetric recordings from many terminals simultaneously), triggered by electrical stimulation of MFs, was, in part, due to CICR, as TG (10 μM), CPA (30 μM), ryanodine (20 μM), and ruthenium red (100 μM) decreased [Ca$^{2+}$], signal in the terminal. CICR contributed to ~50% of intraterminal [Ca$^{2+}$], elevation.
  - 344

[$\text{Ca}^{2+}$]$^{2+}$], signaling in presynaptic terminal; short-term plasticity
- Frog motor nerve terminals
  - CICR contributes to intraterminal [Ca$^{2+}$], signals; CICR can be primed by depolarization-induced Ca$^{2+}$ influx and can participate in regulated exocytosis of neurotransmitter and in short-term presynaptic plasticity. Priming of CICR by conditioning stimulation train can markedly increase neurotransmitter release.
  - 449, 450
### TABLE 9—Continued

<table>
<thead>
<tr>
<th>Function</th>
<th>Brain Region</th>
<th>Experimental Evidence</th>
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</tr>
</thead>
<tbody>
<tr>
<td>Short-term synaptic plasticity/synaptic glutamate release</td>
<td>Hippocampus; CA3 neurons/associational-commissural (AC) synapse</td>
<td>Confocal recordings of ([Ca^{2+}]_i), in a single synaptic boutons of CA3 neurons in organotypic slices showed that inhibition of CICR by 30 (\mu)M ryanodine or emptying the ER with CPA (30 (\mu)M) or TG (3.8 (\mu)M) reduced intraterminal ([Ca^{2+}]_i), transient and abolished paired pulse facilitation. Ryanodine also obliterated spontaneous mEPSPs, thus linking CICR to the release of neurotransmitter.</td>
<td>139</td>
</tr>
<tr>
<td>Presynaptic LTP</td>
<td>Hippocampus; CA1 neurons</td>
<td>Application of caffeine triggered LTP of SC–CA1 pyramidal neuron synapses, which was blocked by incubation of slice with ryanodine (20 (\mu)M), but remained unaffected when ryanodine was included into the patch pipette, suggesting presynaptic mechanism.</td>
<td>369</td>
</tr>
<tr>
<td><strong>Postsynaptic effects, including LTP and LTD</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>LTD</td>
<td>Hippocampus/dentate gyrus</td>
<td>Dantrolene (50 (\mu)M) completely blocked LTD in rat dental gyrus.</td>
<td>470</td>
</tr>
<tr>
<td>Inhibition of postsynaptic GABA A currents LTD</td>
<td>Retina; ganglion cells</td>
<td>Caffeine (10 mM) and intracellular dialysis of InsP 3 (50 (\mu)M) significantly inhibited GABA A-mediated currents. Effect of caffeine was blocked by TG (10 (\mu)M) and ryanodine (20 (\mu)M).</td>
<td>3</td>
</tr>
<tr>
<td>LTD</td>
<td>Hippocampus; CA1 neurons</td>
<td>Induction (but not maintenance) of LTD in SC–CA1 synapses was blocked by slice superfusion with TG (1 (\mu)M), CPA (1 (\mu)M) or ryanodine (10 (\mu)M).</td>
<td>530</td>
</tr>
<tr>
<td>LTD</td>
<td>Hippocampus; CA3 neurons</td>
<td>Induction of LTD of GABA A-receptor mediated synaptic responses was blocked by intracellularly applied ruthenium red (20 (\mu)M) but not by heparin (2 mg/ml). The LTD was also blocked by bath application of ryanodine (10 (\mu)M); intracellular administration of ryanodine prevented LTD only when LTD induction protocol was preceded by conditioning depolarizations (use dependence of ryanodine?).</td>
<td>71</td>
</tr>
<tr>
<td>LTD</td>
<td>Hippocampus; CA3 neurons</td>
<td>LTD in MF-CA3 synapses is expressed postsynaptically and is controlled by (Ca^{2+}) influx through L-type VGCC and IICR/CICR through InsP 3Rs; inhibition of the InsP 3Rs by heparin (0.2 mg/ml) or emptying the ER by TG (10 (\mu)M) prevented LTD.</td>
<td>335</td>
</tr>
<tr>
<td>Homosynaptic potentiation in Aplysia</td>
<td>Sensory–motor neuron synapses</td>
<td>Homosynaptic potentiation produced by mild titanic stimulation required IICR and CICR in both pre- and postsynaptic compartments. This form of potentiation was blocked by TG (10 (\mu)M), by 2-APB (10 (\mu)M), and by 8NH 2-cADPR (20 (\mu)M) and ryanodine (100 (\mu)M); low concentrations of ryanodine (1 (\mu)M) facilitated this type of synaptic plastic response.</td>
<td>261</td>
</tr>
<tr>
<td>Mossy fibers LTP</td>
<td>Hippocampus</td>
<td>LTP of mossy fibers recorded in hippocampal slices were critically dependent on CICR in presynaptic terminal; CICR was initiated by (Ca^{2+}) entry through presynaptic (Ca^{2+})-permeable KA receptors/L-type (Ca^{2+}) channels and CICR inhibition by ryanodine (10 (\mu)M) inhibited LTP.</td>
<td>332</td>
</tr>
<tr>
<td>Circadian rhythm</td>
<td>Suprachiasmatic nucleus</td>
<td>In organotypic cultures of SCN, pharmacological manipulation with ryanodine receptors/ER stores (caffeine, 1 mM; TG, 0.5 (\mu)M; dantrolene, 20 (\mu)M; or ruthenium red, 10 (\mu)M) modulated clock resetting mediated through iGluRs in the early night phase.</td>
<td>126</td>
</tr>
<tr>
<td>Circadian rhythm</td>
<td>Suprachiasmatic nucleus</td>
<td>In SCN slice cultures, the circadian rhythms of ([Ca^{2+}]_i), which closely matched electrical circadian rhythms, were recorded using chameleon probes transfected into SCN neurons. Inhibition of CICR by ryanodine (up to 100 (\mu)M) dumped both electrical and ([Ca^{2+}]_i) rhythms.</td>
<td>244</td>
</tr>
<tr>
<td>Tonic activity of thalamocortical neurons</td>
<td>Dorsolateral geniculate nucleus</td>
<td>CICR triggered by plasmalemnal (Ca^{2+}) entry produced tonic activity in thalamocortical neurons associated with state of wakefulness.</td>
<td>68</td>
</tr>
<tr>
<td>Olfactory sensation/propagating (Ca^{2+}) wave</td>
<td>Olfactory receptor neurons</td>
<td>Odor-stimulating agents triggered (Ca^{2+}) waves propagating from cilia towards dendrites and soma through activation of CICR.</td>
<td>722</td>
</tr>
<tr>
<td>BDNF release</td>
<td>Hippocampus; cultured neurons</td>
<td>BDNF release from electrically stimulated rat neonatal cultured hippocampal neurons was inhibited by TG (10 (\mu)M) and dantrolene (50 (\mu)M). Caffeine (30 (\mu)M) induced BDNF release even in the absence of electrical stimulation.</td>
<td>22</td>
</tr>
</tbody>
</table>
Table 10. Neuronal functions regulated by InsP₃-induced Ca²⁺ release

<table>
<thead>
<tr>
<th>Function</th>
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</tr>
</thead>
<tbody>
<tr>
<td>Modulation of GABA&lt;sub&gt;ᵦ&lt;/sub&gt; receptors affinity</td>
<td>Retinal neurons</td>
<td>Glutamate-induced ER Ca²⁺ release reduced GABA&lt;sub&gt;ᵦ&lt;/sub&gt; receptor affinity via Ca²⁺-calmodulin system.</td>
<td>576</td>
</tr>
<tr>
<td>Regulation of locomotor network activity</td>
<td>Motoneurons; spinal cord preparation</td>
<td>Activation of mGluR1/5 by DHPG (100 μM) produced [Ca²⁺]i oscillations in primary cultured motoneurons from larval lampreys. These oscillations were blocked by TG (1 μM, 45-min incubation), but not by ryanodine (100 μM, 45 min), implying sole activation of InsP₃Rs. mGluRs are activated during locomotion as was ascertained in experiments on spinal cord preparation, and [Ca²⁺]i oscillations are believed to regulate locomotor activity. One of the pathways is associated with stimulation of mGluR1/5 autoreceptors, which trigger Ca²⁺ release, and the latter, in turn, enhances release of glutamate.</td>
<td>280, 621</td>
</tr>
<tr>
<td>Activation of Ca²⁺-activated cation-selective channels</td>
<td>Hippocampal neurons</td>
<td>Activation of IICR following stimulation of SC in a conditioned system inhibited ionotropic receptors, or following application of mGluR agonist trans-ACPD, resulted in potentiation of Ca²⁺-activated cation selective channels (CAN).</td>
<td>494</td>
</tr>
<tr>
<td>Potentiation of neurotransmitter release</td>
<td>Neuronal muscular junction</td>
<td>Neurotrophin (NT-3) potentiated neurotransmitter release by simultaneous activation of Trk-receptor/PLC-γ mediated IICR and stimulation of PI 3-kinase; both cascades were necessary for potentiation of neurotransmitter release.</td>
<td>706</td>
</tr>
<tr>
<td>Potentiation of neurotransmitter release</td>
<td>Cholinergic synapses in buccal ganglion (Aplysia californica)</td>
<td>Intracellular injections of InsP₃ and NAADP increased the amplitude of IPSCs. Effects of InsP₃, but not of NAADP, were blocked by heparin (1 mM) and ryanodine (100 μM).</td>
<td>86</td>
</tr>
<tr>
<td>LTD</td>
<td>PF-PN synapses</td>
<td>Local photorelease of InsP₃ and subsequent IICR triggered LTD in PF to PN synapse without requirement for climbing fiber input.</td>
<td>150</td>
</tr>
<tr>
<td>LTD</td>
<td>Visual cortex, layers II/III</td>
<td>Cortical LTD required a simultaneous IICR in postsynaptic cell (achieved by photorelease of caged InsP₃, delivered to the neuron via patch pipette) and tectal stimulation of nearby cortex.</td>
<td>276</td>
</tr>
<tr>
<td>Synaptic potentiation</td>
<td>Hippocampus; SC-CA3</td>
<td>Synaptic potentiation induced by BDNF and NT-3 was blocked by pretreatment with TG (5 μM, 30 min), suggesting the role for ER Ca²⁺ release (IICR?).</td>
<td>288</td>
</tr>
</tbody>
</table>

LTD, long-term depression; PLC, phospholipase C; PI, phosphatidylinositol; NAADP, nicotinic acid adenine dinucleotide phosphate; BDNF, brain-derived neurotrophic factor.

Refs. 50, 82, 203, 391; see also an excellent review by Ron Bouchard et al. (59)], and moreover, ER structures are located in close proximity (as near as 40–400 nm) to the presynaptic active zone. As demonstrated already in 1986 by Westrum and Grey (693), the ER within the presynaptic terminal appears to be in close association with microtubules and synaptic vesicles and, moreover, ER portions impinge directly on the presynaptic membrane forming “tubular-fibrillar” extensions. Such close apposition of the ER and the presynaptic membrane may perfectly well allow Ca²⁺ released from the store to participate in creation of [Ca²⁺]i microdomains relevant for exocytosis. Yet the role of ER Ca²⁺ release in regulation of neurotransmitter release remains controversial, with arguments supporting (59) or denying (721) its importance.

Indeed, there are not many indications that ER Ca²⁺ release in the absence of plasmalemmal Ca²⁺ entry can induce massive exocytosis in synaptic terminals, although numerous data (see Tables 9 and 10) demonstrate that both CICR and IICR may modulate/amplify neurotransmitter release from presynaptic compartments.

Probably the first to contemplate the importance of presynaptic Ca²⁺ release in the regulation of neurotransmission were Erulkar and Rahaminoff (141), who arrived at this idea based on analysis of tetanic stimulation-induced changes in the frequency of the miniature end-plate potentials at varying Ca²⁺ electrochemical gradients. Further experimentation, aimed at investigating the effects of pharmacological manipulations with ER Ca²⁺ handling on either spontaneous or evoked postsynaptic currents, demonstrated that both Ca²⁺ release from and Ca²⁺ uptake into the ER may significantly modulate exocytosis of neurotransmitter in synaptic terminals. It appeared that spontaneous RyR-mediated presynaptic [Ca²⁺]i transients may trigger neurotransmitter release in resting conditions. This conclusion was based on experiments demonstrating that the frequency or amplitude of miniature postsynaptic currents (mIPSCs as well as mEPSCs) is sensitive to inhibition of RyRs (by ryanodine) or SERCA pumps (by TG and CPA). This phenomenon was observed in cerebellar neurons (26, 355) as well as in hippocampal pyramidal (139, 563) and cortical (589) neurons. In parallel, Ca²⁺ imaging of presynaptic terminals indeed revealed the spontaneous [Ca²⁺]i release events (139, 355). Moreover, in the presynaptic terminals of basket cells, the amplitude of [Ca²⁺]i signals resulting from spontaneous...
Ca\(^{2+}\) release was comparable with \([Ca^{2+}]_i\) rises produced by single AP (105).

At the same time, CICR triggered by depolarization-induced Ca\(^{2+}\) entry was detected in single presynaptic terminals in intact bullfrog sympathetic ganglia, where it accounted for \(-40\%\) of the total \([Ca^{2+}]_i\) elevation induced by electrical stimulation of the nerve (502) and in the presynaptic mossy fiber terminals, where it contributed as much as \(-50\%\) of the \([Ca^{2+}]_i\), elevation induced by a train of action potentials (344). In hair cells (which are presynaptic to primary afferent neurons), direct activation of RyR-mediated Ca\(^{2+}\) release by caffeine induced a \([Ca^{2+}]_i\), elevation and an increase in membrane capacitance that may indicate activation of exocytosis (337). Finally, pharmacological inhibition of ER reduced not only spontaneous but also evoked GABA-mediated IPSCs in basket cell-Purkinje neuron synapses in the cerebellum (175), whereas potentiation of CICR by intracellular addition of cADPR augmented ACh release from terminals of Aplysia buccal neurons (432). Likewise, CICR activated by Ca\(^{2+}\) entry via VGCC enhanced neurotransmitter release in the frog neuromuscular junction (450). In this preparation, a close apposition of RyRs and plasmalemmal Ca\(^{2+}\) channels was postulated, and it was also suggested that Ca\(^{2+}\) entry primes and activates CICR, which in turn contributes to neurotransmitter release (449).

The intimate mechanism of CICR-dependent potentiation of evoked neurotransmitter release may, however, be more complicated than simple amplification of Ca\(^{2+}\) entry and therefore an increase in \([Ca^{2+}]_i\) domains near the active zone. The simplest hypothesis argues that CICR events trigger neurotransmitter release and moreover favor the multiquantal release from the same active zone (105, 355). However, analysis of ryanodine effects on the amplitude and frequency of evoked IPSCs in basket cell-Purkinje neuron synapse, led Galante and Marty (175) to suggest that Ca\(^{2+}\) released by CICR arrives at the active zone too late to affect exocytosis, but it does sensitize the exocytotic machinery to a subsequent depolarization.

Sometimes the effective modulation of neurotransmitter release requires synergism between IICR and CICR. When recording simultaneously from presynaptic axons and postsynaptic motoneurons in a Lamprey spinal cord preparation, Cochilla and Alford (102) observed potentiation of synaptic transmission by Ca\(^{2+}\) release from the ER; both IICR and CICR were necessary as this potentiation could be blocked by pharmacological inhibition of mGluRs as well as by 20 \(\mu\)M ryanodine.

Incidentally, the CICR responsible for spontaneous neurotransmitter release may also be activated by Ca\(^{2+}\) entry through presynaptic Ca\(^{2+}\)-permeable ionotropic receptors. The frequency and amplitude of spontaneous mEPSCs in CA3 hippocampal neurons were greatly enhanced following activation of presynaptic nicotinic cholinoreceptors (NChRs). Subsequent analysis revealed that CICR triggered in the presynaptic terminal by Ca\(^{2+}\) entry through Ca\(^{2+}\)-permeable NChRs was instrumental in the action of nicotine on mEPSCs. Pharmacological inhibition of presynaptic CICR completely abolished NChR-dependent modulation of mEPSCs (573). Most importantly, CICR in the presynaptic terminal remodeled neurotransmitter release quite substantially, by a shift towards a multiquantal mode, or else in synchronizing release across several active zones, as judged by a dramatic (\(-3\) times) increase in mean mEPSC amplitude and appearance of a relatively high number of extralarge mEPSCs reaching \(-200\) pA (as compared with a mean mEPSC amplitude of 20 pA in control conditions) upon activation of NChRs. This remodeling has important functional consequences as nicotine-induced facilitation of neurotransmitter release was able to trigger a burst of action potentials in the postsynaptic cell, thus indicating the existence of synaptic transmission without AP-induced depolarization of the presynaptic membrane (573). Local CICR in response to Ca\(^{2+}\) entry through NChRs was also found in nerve terminals in vas deferens; this CICR facilitated neurotransmitter release, which resulted in a 70% increase in the amplitude of excitatory junction potentials (63).

Very much in line with the concept of Ca\(^{2+}\) store/sink duality, the presynaptic ER may not only amplify, but also limit neurotransmitter release, by buffering Ca\(^{2+}\) in the terminal, thus reducing the lifetime of elevated \([Ca^{2+}]_i\) microdomains. This, for example, happens in the frog neuromuscular junction, where inhibition of SERCAs facilitates synaptic transmission (83). In the rat neuromuscular junction, CICR also limits the transmission, albeit in a completely different way, by limiting Ca\(^{2+}\) entry through VGCC as a result of increased Ca\(^{2+}\)-induced inactivation of the latter (567).

However, comprehensively the data discussed above favor the role of presynaptic Ca\(^{2+}\) release in the regulation of neurotransmission, it may not be regarded as an ubiquitous mechanism always present in all synapses. For example, Carter et al. (80) while investigating transmission in the hippocampus (associational-commissural synapses and mossy fiber synapses in CA1 and CA3 neurons) and cerebellum (parallel fiber-Purkinje neuron synapse) did not observe any involvement of CICR in the regulation of neurotransmitter release. Moreover, they failed to detect either caffeine-induced Ca\(^{2+}\) release or CICR in cerebellar parallel fibers. Likewise, a study of giant EPSCs [which were quite similar to extralarge ACh-mediated mEPSCs observed by Sharma and Vijayaraghavan (573)] in the mossy fiber-CA3 synapse did not show dramatic effects of either TG/CPA or ryanodine/caffeine on occurrence of giant EPSPs (215). Furthermore, the inhibition of Ca\(^{2+}\) release affected the parameters of normal EPSPs rather slightly, if at all (e.g., 10 \(\mu\)M ryanodine changed EPSP frequency to 126 \(\pm\) 42% of the control; Ref. 215). No significant CICR (as probed by 3 mM caffeine, 10

Physiol Rev • VOL 85 • JANUARY 2005 • www.prv.org
μM ryanodine, or 1 μM TG) was found during [Ca^{2+}]_i recordings from the single synaptic boutons of rat sympathetic preganglionic terminals (347) and from sympathetic varicosities (as probed by 10 μM ryanodine; Ref. 62). CICR was also not found in presynaptic terminals of goldfish retinal bipolar cells (296). Or was it? The same group published results supporting the importance of intracellular Ca^{2+} release for amplification of intraterminal [Ca^{2+}]_i transients and for facilitation of transmitter release from the same goldfish retinal bipolar cells (295); these two contradictory papers are separated by exactly 6 wk and were published in the same journal. These discrepancies most likely reflect the complexity of the system responsible for synaptic transmission. In particular, they may arise from differential expression of ER in various terminals, from differences in ER functional conditions (e.g., the degree of Ca^{2+} loading), and, naturally, from the experimental difficulties, as all these measurements are technically quite demanding and are performed at the very edge of the sensitivity of existing equipment. One also has to consider that incubation of whole brain slices with rather powerful pharmacological tools such as TG or ryanodine may trigger obscure indirect effects, thus complicating data interpretation.

C. Synaptic Plasticity

The living brain constantly remodels and modifies its cellular networks. Throughout life, synapses weaken and strengthen or else form and die. These processes underlie the adaptation of the brain to the constantly changing environment and represent what we know as learning and memory. Modification of synaptic transmission efficacy is an important part of this adaptation, which is manifested in short- and long-term synaptic plasticity. The electrophysiological correlates of long-term synaptic plasticity are long-term potentiation (LTP) or depression (LTD) of synaptic potentials in response to either high-frequency stimulation of single synaptic inputs or to simultaneous activation of several distinct synaptic inputs.

LTP, discovered by Tim Bliss and Terje Lomo in hippocampal neurons (53), was also the first form of synaptic plasticity, which highlighted the importance of cytoplasmic Ca^{2+} signals, as intracellular injection of the Ca^{2+} chelator EGTA effectively obliterated the potentiation of synaptic strength (363). Very soon thereafter, the importance of the cytoplasmic Ca^{2+} rise was also appreciated for cerebellar LTD (136, 301). More recent experiments also clearly demonstrate that the ER Ca^{2+} store plays an important role in the generation of [Ca^{2+}]_i elevations relevant to the induction of synaptic plasticity (154, 542, 552).

1. Short-term synaptic plasticity

Short-term synaptic plasticity is a form of presynaptic strengthening of neurotransmitter release, and as such, it ultimately depends on presynaptic Ca^{2+} signaling as discussed above. The involvement of CICR in a form of short-term plasticity, known as paired-pulse facilitation (PPF), was recently proposed for CA3 hippocampal neurons studied in organotypic slices (139). In contrast, a detailed investigation of PPF in excitatory synapses in the hippocampus and cerebellum failed to reveal any contribution of ER Ca^{2+} release to this phenomenon (80).

2. Long-term synaptic plasticity

Historically, LTP and LTD were mostly investigated in the hippocampus and cerebellum, respectively. Incidentally, as far as ER Ca^{2+} release is concerned, the mechanisms of hippocampal and cerebellar plasticity clearly differ in that cerebellar LTD involves IICR, whereas CICR is important for hippocampal LTP.

A) SYNAPTIC PLASTICITY IN THE HIPPOCAMPUS: A ROLE FOR CICR.

Much of our understanding of hippocampal synaptic plasticity comes from investigations of synapses formed by CA3 neurons through Schaffer collaterals on CA1 pyramidal cells. A peculiarity of this synapse is that it possesses both LTP and LTD depending on the stimulation protocol. LTP is usually induced by high frequency (~100 Hz; 100 stimuli or so) stimulation, whereas LTD requires prolonged low-frequency stimulation of Schaffer collaterals (many 100s of stimuli at 1–2 Hz) (154). Both LTP and LTD critically require a [Ca^{2+}]_i increase. The precise mechanisms of differentiating between the different types of synaptic input in favor of potentiation versus depression are unclear, yet the signals arising from the ER may hold the key to further understanding.

The first and foremost task in the elucidation of the role of the ER in synaptic plasticity is to show that synaptic stimulation can result in Ca^{2+} release in dendritic spines and proximal dendrites, i.e., where exactly do the signaling events responsible for LTP/LTD occur. Hippocampal neurons have a rather peculiar distribution of the ER and Ca^{2+} release channels. First, in vivo the ER is not present in every spine. Only ~25–50% of the small spines contain ER. In contrast, the latter is present in ~80–90% of the big mushroom-like spines (604). Second, hippocampal pyramidal neurons have a peculiar distribution of Ca^{2+} release channels (617). Only RyRs (particularly RyR3) are present in the spines, while both RyRs and InsP3Rs reside in the dendritic ER [although prominent species-dependent differences exist, i.e., RyR expression in the rabbit hippocampus appears to be almost negligible compared with the rat (588)].

Such a distribution of Ca^{2+} release channels suggests strong CICR in those CA1 neuron spines, which possess a
functional ER. Because the distribution/localization of the latter may change depending on the experimental conditions (i.e., the animal’s age), conflicting results could be anticipated safely, and, sure enough, the controversy did not wait long to emerge. To begin with, a robust caffeine-induced $\text{Ca}^{2+}$ release, sensitive to TG and ryanodine, and CICR in response to glutamate application, were observed in spines from cultured hippocampal neurons (303). This observation was confirmed by $[\text{Ca}^{2+}]_i$ imaging on hippocampal neurons in organotypic slices (138), which revealed that CICR is responsible for by far the largest ($\sim 80-90\%$) part of the $[\text{Ca}^{2+}]_i$ elevation triggered by stimulation of NMDA receptors (Fig. 21A). Yet, when confocal $\text{Ca}^{2+}$ imaging was applied to hippocampal neurons in acute slices, no signs of significant CICR following NMDA-mediated $\text{Ca}^{2+}$ influx were detected (312), and the NMDA-induced $\text{Ca}^{2+}$ flux was found to be almost solely responsible for the $[\text{Ca}^{2+}]_i$ elevation (Fig. 21B). All in all, the question about functional CICR in spines remains unresolved, which most likely reflects the complexity and heterogeneity of the preparations. Yet, physiological data support the notion of at least some involvement of CICR

FIG. 21. CICR in spines of CA1 hippocampal neurons: evidence pro and contra. A: recordings of $[\text{Ca}^{2+}]_i$ in a single spine of hippocampal CA1 neuron filled with Oregon green BAPTA-1 through the tip of sharp microelectrode. Top row shows $[\text{Ca}^{2+}]_i$ traces; the row on the bottom demonstrates voltage recordings. In control conditions, synaptic stimulation triggers $[\text{Ca}^{2+}]_i$ elevation and excitatory postsynaptic potentials (EPSPs); incubation of slice with 15 $\mu$M CPA completely abolished $[\text{Ca}^{2+}]_i$ increase without any apparent effects on EPSPs. One hour of washout with normal saline restores the parameters of $[\text{Ca}^{2+}]_i$ responses. B: similar recordings demonstrating a minor role played by CICR in synaptically induced $[\text{Ca}^{2+}]_i$ signals in hippocampal slices. a: EPSP-associated spine $[\text{Ca}^{2+}]_i$ signal (color-coded image and top trace) was reduced by $\sim 30\%$ in the presence of ryanodine ($25 \mu$M, bottom trace). b: Time course of the effect of ryanodine (indicated by the bar) on the peak amplitudes of $[\text{Ca}^{2+}]_i$ transients. Same experiment as in A. Single-shock stimuli were repeatedly delivered every 4 min; each data point represents a single trial. The dashed lines represent the values during control conditions (1.0) and in the presence of ryanodine (0.72). The numbers point to the individual recordings displayed in A. c: Application of CPA ($30 \mu$M, indicated by the bar) reduced the amplitude of synaptic $\text{Ca}^{2+}$ signals in spines by $\sim 36\%$. d: Bar graph summarizing the effects of ryanodine (20 or 25 $\mu$M) and CPA (30 $\mu$M). Data points were normalized to control conditions. The first two bars represent the effect on synaptically evoked $[\text{Ca}^{2+}]_i$ signal in spines ($n = 7$ and 9 cells for ryanodine and CPA, respectively). The last two bars show that both drugs effectively abolish dendritic $[\text{Ca}^{2+}]_i$ responses evoked by local application of caffeine (20–40 mM, $n = 5$ for ryanodine and for CPA). Data points were normalized to the mean control value. Experiments were done at 30°C. [A from Emptage et al. (138) copyright 1999 with permission from Elsevier; B from Kovalchuk et al. (312) copyright 2000 by the Society for Neuroscience.]
in LTP in SC-CA1 synapses, since emptying the ER with TG (1 μM) or CPA (1 μM), or blocking CICR with 10 μM ryanodine, inhibited the induction but did not affect the maintenance of LTD in acute hippocampal slices (530).

The next step in investigating the role of ER Ca\(^{2+}\) release in LTP quite naturally involved genetic manipulation of Ca\(^{2+}\) release channels. The obvious candidate to check was RyR3, because of its expression in CA1 neurons. In any case deletions of RyR1 and RyR2 are not compatible with life. Genetic deletion of RyR3 led to facilitated LTP and a complete disappearance of LTD in CA1. Furthermore, RyR3-deficient mice demonstrated improved spatial learning on a Morris water maze task (173). LTP in the RyR3 knockout mouse was, however, principally different from the wild type; it could not be blocked by NMDA receptor inhibitors and was partially dependent on Ca\(^{2+}\) influx through VGCC, and on metabotropic glutamate receptors. Another RyR3 knockout mouse, also generated in Japan, displayed a smaller magnitude of LTP and diminished synaptic AMPA responses, although this was not due to downregulation of AMPAR expression and habituation, but had an increased speed of locomotion and a mild tendency to circular running” (23). To add to the degree of complexity, the genetic deletion of InsP3R1 led to an increased magnitude of CA1 hippocampal LTD. Homosynaptic LTD remained unchanged, whereas heterosynaptic LTD of nonassocciated pathway was blocked (168, 441). In summary, the role of ER Ca\(^{2+}\) release in synaptic plasticity in CA1 hippocampal neurons remains obscure.

Some indications of the importance of CICR in generation of LTP/LTD are coming from other neurons (CA3 hippocampal cells, cortical neurons), although they often involve both pre- and postsynaptic mechanisms (see Table 9). IICR seems to play a more important role in CA3 neurons, as deletion of InsP\(_3\)Rs facilitated LTD and completely inhibited LTD in mossy fiber-CA3 neuron synapses (254).

B) SYNAPTIC PLASTICITY IN THE CEREBELLUM: A ROLE FOR IICR. The role for IICR in LTD generation in the cerebellum is rather well established. The LTD of parallel fibers, which form their synapses on dendrites of Purkinje neurons, occurs as a result of simultaneous activation of parallel and climbing fibers. Stimulation of climbing fibers can be fully substituted by depolarization of the neuronal soma and [Ca\(^{2+}\)]\(_i\) elevation (301). Whatever protocol of LTD induction is used, [Ca\(^{2+}\)]\(_i\) elevation in the PN is necessary for LTD development. In fact, stimulation of climbing fibers or somatic depolarization may be replaced by an artificial increase in [Ca\(^{2+}\)]\(_i\) due to photorelease of caged Ca\(^{2+}\) (338) (incidentally, stimulation of parallel fibers can be fully substituted by NO generation, Ref. 338).

Genetic manipulation of components of the mGluR-IICR signaling cascade evidently demonstrated the crucial importance of this pathway for cerebellar LTD. Deletion of mGluR1 resulted in the appearance of a clear cerebellar phenotype, with ataxia, motor discoordination, and impaired LTD (1, 104). Both LTD and locomotive deficits can be restored by expression of mGluR1 under the control of a Purkinje neuron-specific promoter (243).

Almost simultaneously, the involvement of IICR in cerebellar LTD was suggested by physiological experiment. Direct activation of IICR by photorelease of caged InsP\(_3\) in Purkinje neurons produced LTD when coincident with somatic depolarization, thus fully substituting for the stimulation of parallel fibers (282). Furthermore, as shown by Finch and Augustine (150), local InsP\(_3\) uncaging and subsequent IICR were sufficient to induce local LTD in synapses close to the uncaging foci.

It has to be noted, though, that IICR appeared not to contribute to LTD in cultured or freshly isolated Purkinje neurons, as no difference was found in [Ca\(^{2+}\)]\(_i\) transients induced by depolarization and combination of depolarization and glutamate application. Moreover, LTD was insensitive to XeC, and diacylglycerol was able to substitute for mGluR activation in the induction of LTD (448). Yet, these results most likely reflect certain changes in signaling pathways introduced by tissue culture conditions or the isolation procedure.

Further evidence favoring the key role of IICR in the generation of cerebellar LTD was obtained from mice with genetically deleted InsP\(_3\)R1s, in which LTD completely vanished (Fig. 22A) (247). Interestingly, InsP\(_3\)R1\(^{-/-}\) animals never survived beyond the 23rd day. The same authors also reported that a similar disappearance of LTD can also be achieved by perfusing neurons with specific antibodies raised against InsP\(_3\)R1. Not only the expression but also the localization of InsP\(_3\)R1s matters, as was demonstrated on animals mutated for myosin Va. This mutation causes the ER not to enter PN spines, which therefore effectively eliminates IICR specifically from this compartment. The LTD in mutant mice and rats was completely lost (Fig. 22B); nonetheless, it could be rescued by Ca\(^{2+}\) uncaging within the spine (421).

C) SYNAPTIC MODIFICATION. The most amazing results were obtained when studying GABAergic synapses formed by basket interneurons on CA1 hippocampal pyramidal cells. When the CICR mechanism was primed by intracellular cADPR, postsynaptic depolarization triggered a large [Ca\(^{2+}\)]\(_i\) elevation that changed GABA-mediated IPSPs into...
excitatory postsynaptic potentials (612). The actual mechanism underlying such remodeling is not precisely understood, although it is sensitive to carbonic anhydrase inhibition. This may indicate certain changes in the GABA-operated Cl\[^{-}\]/HCO\[^{-}\] channel, resulting in the appearance of a depolarizing HCO\[^{-}\]/HCO\[^{2-}\] efflux.

D) MEMORY PROCESSES. Not much is known about the role of Ca\[^{2+}\]/HCO\[^{3-}\] release in consolidative memory processes. There are some indications that synchronous oscillations of membrane potential in the neocortex, which were suggested to be important for cognitive brain function (218), are governed by CICR triggered by Ca\[^{2+}\] entering through NMDA receptors (710). A certain importance of ER Ca\[^{2+}\] release may be also deduced from the observation that a significant increase in RyR2 expression, at both the mRNA and protein level, was found in the hippocampus of rats subjected to intensive water maze training (716).

D. Gene Expression, ER Ca\[^{2+}\] Waves, and ER Ca\[^{2+}\] Tunneling

The importance of cytoplasmic and nuclear Ca\[^{2+}\] signals in the regulation of gene expression is well established, and a variety of Ca\[^{2+}\]-dependent cascades controlling transcription have been characterized (19, 91, 152, 183, 184, 692), which shall not be discussed here. However, the problem of how Ca\[^{2+}\] reaches the nucleus during physiological activity of the geometrically complex and highly polarized nerve cell remains unsolved. It is well established that cytosolic Ca\[^{2+}\] buffering hampers Ca\[^{2+}\] diffusion from often distant entry sites towards the cell interior. The ER can be instrumental in conveying Ca\[^{2+}\] over long distances and therefore providing nuclear systems with appropriate Ca\[^{2+}\] signals. Intracellular propagation of Ca\[^{2+}\] signals can be achieved by virtue of endo-

FIG. 22. InsP\[^{3}\]-induced Ca\[^{2+}\] release is critical for cerebellar long-term depression (LTD). A: loss of LTD in InsP\[^{3}\]R1\(^{−/−}\) Purkinje cells. a: Pairing depolarization and PF stimulation (1 Hz, 240 times) induced long-lasting depression of the PF-EPSP amplitude in control experiments using a wild-type (WT) InsP\[^{3}\]R1\(^{+/-}\) cerebellar slice. b: LTD was lost in an InsP\[^{3}\]R1\(^{−/−}\) cerebellar slice. Insets show an average of 10 consecutive sweeps at time points indicated. c: Averaged time course of normalized EPSP amplitude. Results are presented as means ± SE. B: LTD is absent in dilute-opithotonus (dop) mutant rats (117), which lack ER Ca\[^{2+}\] stores in Purkinje cell dendritic spines. Top panel shows traces of PF-EPSPs measured in control (left) and mutant (right) Purkinje cell before (pre) and 25 min after conjunctive PF and CF stimulation. Bottom panel shows the time course of changes in EPSP amplitudes after PF-CF stimulation (incidence of stimulation is indicated on the graph) for control (left) and mutant (right) rat. LTD is completely absent in mutant animal. [A from Inoue et al. (247) copyright 1998 by the Society for Neuroscience; B from Miyata et al. (421) copyright 2000 with permission from Elsevier.]
membrane excitability. Either RyR-mediated CICR or CICR-assisted Ca\(^{2+}\) release from InsP\(_3\)Rs or their combination may create propagating Ca\(^{2+}\) waves transforming near-plasmalemmal [Ca\(^{2+}\)]\(_{p}\), elevations into nuclear Ca\(^{2+}\) rises. Indeed, such propagating [Ca\(^{2+}\)]\(_{p}\) waves have been observed experimentally. For instance, CICR maintains spreading Ca\(^{2+}\) waves between the plasmalemma and the nucleus of the frog sympathetic ganglion neurons. Moreover, the speed of this wave remains constant all the way to the nucleus, suggestive of an active propagation, whereas at the moment the Ca\(^{2+}\) signal reaches the nucleus, its spread becomes diffusion governed (112, 389). Similarly, InsP\(_3\)Rs-dependent propagating Ca\(^{2+}\) waves have been described in a variety of central neurons (see Ref. 25 for review). Most interestingly, the Ca\(^{2+}\) wave can become amplified when approaching the center of the cell. For example, pairing of muscarinic stimulation of CA1 hippocampal neurons with trains of action potentials resulted in a focal Ca\(^{2+}\) elevation, which triggered a propagating Ca\(^{2+}\) wave, and the [Ca\(^{2+}\)]\(_{i}\) increase resulting from this wave was largest in the soma and the nuclear region (518). This amplification critically depended on ER entry (by 10\(^{-8}\) M ryanodine, however, the administration of netrin-1 to the neuronal culture created a local concentration gradient, which, in turn, forced the neurites to turn towards the source of netrin-1. This turning movement was modified by pharmacological inhibition of either plasmalemmal Ca\(^{2+}\) entry (by 50 \(\mu\)M Cd\(^{2+}\)) or Ca\(^{2+}\) release from the ER (by 10 \(\mu\)M BHQ or 1 \(\mu\)M TG). When the cultures were treated with 100 \(\mu\)M ryanodine, however, the administration of netrin-1 produced repulsion of the cone. Most interestingly, applications of low concentrations of ryanodine (~100 nM around the growth cone) caused a [Ca\(^{2+}\)]\(_{i}\) elevation within the cone and turning of the latter towards the source of the drug (233). Thus, by regulating the pattern of local [Ca\(^{2+}\)]\(_{i}\) signaling in the growth cone, CICR determines the direction and rate of axon extension/repulsion. A somewhat similar role of CICR was observed in the embryonic chick retina, where local CICR stabilized dendrite formation during development. Inhibition of this local Ca\(^{2+}\) release induced rapid retraction of dendrites (358).

In contrast, in embryonic chick DRG neurons, the regulation of growth cone motility seems to be regulated with substantial peripheral Ca\(^{2+}\) influx and [Ca\(^{2+}\)]\(_{i}\), elevation in dendrites, which then had to be conveyed to the nucleus to activate CREB. Bading and co-workers (201) found that inhibition of ER Ca\(^{2+}\) accumulation by TG completely prevented the signal from reaching the nucleus. In the described experimental conditions, Ca\(^{2+}\) entry into the ER must occur in dendrites, precisely where [Ca\(^{2+}\)]\(_{i}\) is elevated, and therefore, the possibility of Ca\(^{2+}\) diffusing through the ER lumen to the nucleus appears probable. Clearly many more experiments are needed to test for ER Ca\(^{2+}\) tunneling in nerve cells.

E. Neuronal Growth and Morphological Plasticity

Neurite outgrowth is governed by [Ca\(^{2+}\)]\(_{i}\) fluctuations within the growth cone, where both the level of [Ca\(^{2+}\)]\(_{i}\), and patterns of [Ca\(^{2+}\)]\(_{i}\) fluctuations regulate the velocity of cone extension (273). For example, complex [Ca\(^{2+}\)]\(_{i}\) transients are produced in growth cones during their migration in the embryonic spinal cord. The larger the frequency and magnitude of these [Ca\(^{2+}\)]\(_{i}\) spikes, the slower the outgrowth. Inhibition of [Ca\(^{2+}\)]\(_{i}\), elevation accelerates the rate of neurite extension, whereas artificial increase of [Ca\(^{2+}\)]\(_{i}\), by Ca\(^{2+}\) photorelease inhibits axonal extension (190).

The [Ca\(^{2+}\)]\(_{i}\) signals relevant for neurite outgrowth are produced by both Ca\(^{2+}\) entry and Ca\(^{2+}\) release, and the particular importance of CICR was appreciated quite early (297, 330). Recent analysis of growth cone movements of Xenopus neurons treated with netrin-1, an established regulator of neurite outgrowth, has further substantiated the importance of CICR for growth cone extension (233). Focal administration of netrin-1 to the neuronal culture created a local concentration gradient, which, in turn, forced the neurites to turn towards the source of netrin-1. This turning movement was modified by pharmacological inhibition of either plasmalemmal Ca\(^{2+}\) entry (by 50 \(\mu\)M Cd\(^{2+}\)) or Ca\(^{2+}\) release from the ER (by 10 \(\mu\)M BHQ or 1 \(\mu\)M TG). When the cultures were treated with 100 \(\mu\)M ryanodine, however, the administration of netrin-1 produced repulsion of the cone. Most interestingly, applications of low concentrations of ryanodine (~100 nM around the growth cone) caused a [Ca\(^{2+}\)]\(_{i}\) elevation within the cone and turning of the latter towards the source of the drug (233). Thus, by regulating the pattern of local [Ca\(^{2+}\)]\(_{i}\) signaling in the growth cone, CICR determines the direction and rate of axon extension/repulsion. A somewhat similar role of CICR was observed in the embryonic chick retina, where local CICR stabilized dendrite formation during development. Inhibition of this local Ca\(^{2+}\) release induced rapid retraction of dendrites (358).
by InsP$_3$Rs. Growth cones of these neurons are particularly rich in InsP$_3$Rs. Depletion of the ER store with TG (10 μm) arrested the extension of the cones, and inhibition of IICR with intracellulary injected heparin induced neurite retraction (626).

ER Ca$^{2+}$ release controls not only the extension of growth cones, but also the extension of dendritic spines. Caffeine-activated release of Ca$^{2+}$ from the ER triggers rapid elongation of spines in cultured embryonic hippocampal neurons. Spine elongation was already obvious 30 min after a short application of 5–10 mM caffeine, and after 90 min, the spine length had increased by 0.35 μm (~33%; Ref. 304). Short puffs of caffeine even triggered a de novo formation of spines, although this was not very frequent (new spines appeared in 4 of 22 preparations). The effects of caffeine were a specific consequence of ER Ca$^{2+}$ release, as 10 μM ryanodine obliterated them completely (304).

In summary, ER Ca$^{2+}$ release acts as a powerful regulator of the motility of growth cones and dendrites, most likely through interaction with enzymes controlling polymerization/dem polymerization of the cytoskeleton.

F. Neurohormone Release

An interesting hypothesis postulating that the ER Ca$^{2+}$ store may regulate dendritic (i.e., extraterminal) release of oxytocin was developed as a result of in vivo experiments, which measured oxytocin release in the supraoptic nucleus of anesthetized rats in response to local dialysis of ER-specific agents. TG, but not caffeine or ryanodine, induced release of the neurohormone and greatly potentiated depolarization-induced oxytocin secretion. Interestingly, this potentiation was not observed shortly after (5 min) application of TG, but a large potentiation was detected 30–90 min later (361). The authors explained their results in terms of TG-induced sustained Ca$^{2+}$ release, which, in fact, does not seem a plausible explanation. First, [Ca$^{2+}$]$_i$, rises caused by SERCA inhibition are short-lived, and second, depletion of the ER store renders additional Ca$^{2+}$ release from this compartment impossible. If anything, post-TG potentiation of depolarization-induced oxytocin release may be explained by larger [Ca$^{2+}$]$_i$ rises in the presence of TG due to exclusion of ER Ca$^{2+}$ buffering, or else due to activation of an alternative Ca$^{2+}$ entry pathway.

IICR triggered by stimulation of P2Y purinoreceptors stimulated the release of a pain-related neuromodulator, calcitonin gene-related peptide (CGRP), from small (presumed nociceptive) cultured DRG neurons (559).

Finally, the release of BDNF from electrically active neurons required activation of Ca$^{2+}$ influx through N-type Ca$^{2+}$ channels and CICR, as BDNF secretion was inhibited by ω-conotoxin GVIA, TG, and dantrolene (22).

G. Circadian Rhythms

Ca$^{2+}$ release via ryanodine receptors is intimately involved in the control of circadian rhythms in the suprachiasmatic nucleus (SCN). Transfection of organotypic SCN slices with cytosol- and nucleus-targeted chameleon YC2.1 (422), by employing gene gun technology, allowed long-term monitoring of intracellular Ca$^{2+}$ in neurons that maintain circadian rhythms (244). These long-lasting recordings revealed a circadian rhythm for cytosolic but not nuclear [Ca$^{2+}$]$_i$, and the former was fully synchronized with the electrical circadian rhythm measured by a multiple electrode array. Treatment with ryanodine (100 μM) depressed both the [Ca$^{2+}$]$_i$ and electrical rhythms, although TG (1 μM) was ineffective (244). Hence, the precise role played by the ER store remains to be investigated in much greater detail.

Most interestingly, the expression of RyR2 in SCN neurons is also governed by the circadian rhythm, with the maximal level in the second half of the day phase. This rhythm of RyR2 expression was preserved even when the animals were constantly kept in dim light, suggesting that it has a fundamental importance in setting the circadian clock (125). A role for InsP$_3$Rs in regulation of circadian rhythms has also been suggested, although the only evidence for this is the inhibitory action of 2-APB (199), which may not be associated with suppression of IICR at all due to the lack of specificity.

V. ENDOPLASMIC RETICULUM CALCIUM HOMEOSTASIS AND NEURONAL PATHOPHYSIOLOGY

A. ER Stress Response and Neurodegeneration

Since ER is responsible for transcription, posttranslational protein modification, and selective transport of proteins to different destinies, it has developed a highly sophisticated system for controlling the quality of the final protein products. Any imbalance of protein handling triggers a specific reaction generally defined as the ER stress response. This response is triggered either by accumulation of unfolded proteins (the “unfolded protein response,” UPR) or by overexpression of particular proteins (“the ER overload response,” EOR). The ER stress results in activation of several transcription factors (most notably IRE1 and its receptor PERK) that upregulate the expression of chaperones and reduce ER protein load by inhibiting overall protein synthesis. In addition, the ER triggers the activation of NF-κB and the transcription factor C/ERP-homologous protein, which in turn control synthesis of proinflammatory and hemopoetic proteins and act as inhibitors of growth as well as promoters of apoptosis (see Refs. 12, 155, 364, 477, 478, 500). If the ER
stress persists, it may result in the release of necrotic factors, which trigger cell death. As already discussed above, the activity of many intra-ER enzymatic cascades ultimately depends on the intraluminal Ca\textsuperscript{2+} concentration, and a strong link between ER Ca\textsuperscript{2+} homeostasis, ER stress, and neurodegeneration therefore has been postulated (495–498). This hypothesis is supported by several lines of evidence, which show that 1) depletion of ER stores induces upregulation of ER stress markers, 2) depletion of ER stores causes neuronal cell death, 3) exposure of neuronal cells to various insults upregulates ER stress markers, and 4) inhibition of ER Ca\textsuperscript{2+} release protects against neurotoxicity. Several examples of such data are discussed below.

The depletion of ER Ca\textsuperscript{2+} stores in primary cultured rat embryonic cortical neurons by incubation with TG (1 μM), CPA (30 μM), or caffeine (10 mM) reduced protein synthesis to 10–70% of the control and caused complete disaggregation of polyribosomes (129, 499). A significant decrease in protein synthesis was also observed in cultured embryonic rat cortical neurons treated with 100 nM ryanodine, a concentration which depletes the ER store by opening RyRs (5). At the same time, ER depletion following 30-min incubation with 1 μM TG led to an almost 200-fold increase in the expression of ER stress markers grp78, grp94, gadd34, and gadd153 and in a 6-fold increase of SERCA2b and BCl-2 (403). Therefore, severe disruption of ER Ca\textsuperscript{2+} homeostasis triggers ER stress and has a clear neurotoxic effect.

ER stress is also induced by exposure of nerve cells to various neurotoxic conditions. For example, application of excitotoxic concentrations of glutamate as well as oxidative insults elevated the level of grp78 in rat cultured hippocampal neurons (713). This increase in grp78 expression was neuroprotective, as treatment of cultures with grp78 antisense substantially increased neuronal cell death. Hypoxia/reperfusion increased the expression of another chaperon, grp94, in neuroblastoma cells. The increased level of grp94 was also cytoprotective, as treatment of neuroblastoma cells with grp94 antisense exacerbated cell death (24). Similarly, CA1 hippocampal neurons overexpressing grp94 were resistant to ischemic damage in gerbils subjected to transient carotid artery occlusion (24).

Finally, disturbances of ER Ca\textsuperscript{2+} handling cause neuronal death. Treatment of cultured cerebellar granule neurons with the neurotoxin mastoparan triggered massive ICR, which in turn initiated apoptosis and cell death. Inhibition of PLC (and hence InsP\textsubscript{3} production) had a neuroprotective effect (346). Depletion of Ca\textsuperscript{2+} stores with TG (300 nM), CPA (300 μM), and BHQ (300 μM) resulted in 50–60% neuroblastoma cell death within 48 h and in an almost complete demise of the culture within 72 h (455). Interestingly, loading the cells with BAPTA-AM did not protect against TG toxicity, although it prevented TG-induced [Ca\textsuperscript{2+}]\textsubscript{i} increases, thus demonstrating that the ER depletion, rather than [Ca\textsuperscript{2+}]\textsubscript{i} disturbances, were responsible for neurotoxicity (455). Experiments on the same cell line demonstrated that toxicity induced by oxygen-glucose deprivation is also mediated through Ca\textsuperscript{2+} depletion of the ER (684). In Caenorhabditis elegans, necrotic neuronal death can be prevented by loss of function mutations in Ca\textsuperscript{2+} release channels; at the same time, treatment with TG invariably exacerbated necrosis (e.g., Ref. 702).

The ER stress response may regulate neuronal death through another pathway, which involves caspase-12. This enzyme resides within the ER lumen and is activated upon various insults initiating ER stress (577). Most notably, ER stress triggered by disruption of ER Ca\textsuperscript{2+} homeostasis also activated caspase-12 cascade (442). This pathway is endowed with a degree of specificity as cortical neurons isolated from caspase-12-deficient mice were resistant to apoptosis induced by amyloid-β proteins, but not to apoptosis induced by staurosporine or trophic factor deprivation (442). Caspase-induced cell death in embryonic hippocampal neurons similarly depended on ER Ca\textsuperscript{2+} signaling and required the activation of ryanodine receptors (239).

Overall, it is quite clear that nerve cells do develop ER stress upon severe disruption of ER Ca\textsuperscript{2+} homeostasis. Yet, the maneuvers employed for damaging ER Ca\textsuperscript{2+} handling, such as the irreversible block of SERCA pumps by TG, are rather brutal and are very far from the conditions that neurons may encounter even in a diseased state. Clearly more experimentation is needed to precisely characterize the link between [Ca\textsuperscript{2+}]\textsubscript{L} and ER stress. Another very important point lies in the determination of harmful levels of [Ca\textsuperscript{2+}]\textsubscript{L}. Indeed, it is evident that dramatic falls in intra-ER free Ca\textsuperscript{2+} are detrimental for the cell; however, increased [Ca\textsuperscript{2+}]\textsubscript{L} can also be toxic (147, 511). It seems that a certain normal “window” of [Ca\textsuperscript{2+}]\textsubscript{L} exists, and when ER Ca\textsuperscript{2+} rises or falls beyond a particular limit, grave consequences follow. Lastly, changes in ER Ca\textsuperscript{2+} concentration may cause severe fragmentation of the ER (610, 634), which may also have pathological consequences.

B. ER Stores and Ischemia

As a rule, glucose/oxygen deprivation triggers a substantial elevation of [Ca\textsuperscript{2+}]\textsubscript{L} in nerve cells (521, 630, 708), part of which is due to ER Ca\textsuperscript{2+} release (569, 644). In striatal slices exposed to hypoxic conditions, Ca\textsuperscript{2+} release from the ER (partially mediated by NO) was responsible for a large part of the \textsuperscript{45}Ca\textsuperscript{2+} liberation (394). When cultured hippocampal neurons were subjected to an anoxic environment, mimicked by incubation with NaCN, a large [Ca\textsuperscript{2+}]\textsubscript{L} increase was induced. This [Ca\textsuperscript{2+}]\textsubscript{L} elevation
was blocked by dantrolene and ruthenium red, therefore implying an important role of CICR (132) The NaCN-induced [Ca\(^{2+}\)]\(_i\) elevation in hippocampal neurons in slices (\([\text{Ca}^{2+}]\), increased up to 28 μM), and this increase was invariably followed by cell edema and death. Similar massive (up to 30 μM) increases in [Ca\(^{2+}\)]\(_i\) following an interruption in blood flow were observed in hippocampal neurons impaled with ion-sensitive electrodes (588). Depletion of the ER stores by preincubation of hippocampal slices from young Wistar rats with TG (1 μM, 30 min) dramatically (~5 times) reduced the [Ca\(^{2+}\)]\(_i\) elevation triggered by energy deprivation (192). Promotion of ER Ca\(^{2+}\) release (by intracellular injection of InsP\(_3\) into gerbil hippocampal neurons) greatly facilitated the irreversible depolarization induced by ischemia (288). Dantrolene significantly reduced neuronal death in the CA1 area of the hippocampus in adult rats, subjected to global cerebral ischemia, but this effect could be partly attributed to reduced release of glutamate (447). Interestingly, however, the glutamate release was diminished to an equal extent by dantrolene as well as by DMSO (used as a solvent for dantrolene); DMSO alone also protected neurons against ischemia, yet dantrolene was twice as potent. Inhibition of ER Ca\(^{2+}\) release also prevented the liberation of neurotoxic free fatty acids from the ischemic cortex (510).

Ca\(^{2+}\) release from the ER also appears instrumental in ischemic damage of white matter. Destruction of axons upon ischemic stress is mediated through an increase in cytosolic Ca\(^{2+}\). Experiments on rat dorsal columns showed that this [Ca\(^{2+}\)]\(_i\) elevation persisted after removal of Ca\(^{2+}\) from the perfusate; similarly, ischemic injury of axons persisted in Ca\(^{2+}\)-free conditions. At the same time, the axonal [Ca\(^{2+}\)]\(_i\) increases were sensitive to ER depletions by TG or by inhibition of CICR by ryano dine (473). Most intriguingly, the CICR in axons may be of the “skeletal muscle” variety, i.e., involving direct coupling between plasmalemmal Ca\(^{2+}\) channels and RyRs. This suggestion is based on the fact that blockage of the Ca\(^{2+}\) channel voltage sensor (by dihydropyridines), but not of the Ca\(^{2+}\) channel pore (by Cd\(^{2+}\)), was hugely protective against ischemia. At the same time, simultaneous inhibition of RyRs (by ryano dine) and of Ca\(^{2+}\) channels by nimodipine was not additive, thus favoring interdependence of two molecules. In concordance with this suggestion, replacement of extracellular Na\(^+\) with impermeable cations, limiting depolarization, protected axons against ischemia, whereas substitution of Na\(^+\) with Li\(^+\) (which is carried through Na\(^+\) channels and hence allows depolarization) was not effective (473). Together, these findings provide very important information on mechanisms of ischemic damage of white matter, which seem to be different from those in gray matter. White matter damage is accomplished by intracellular Ca\(^{2+}\) release, whereas injury of gray matter very much depends on plasmalemmal Ca\(^{2+}\) entry.

The link between ischemia-induced changes in ER Ca\(^{2+}\) homeostasis and intra-ER chaperone activity has also been described recently, when the ischemia-induced ER chaperone orp150 (oxygen-regulated protein of 150 kDa) was isolated and characterized. Orp150 is strongly upregulated in astrocytes following ischemic stress, which may explain their exceptional resistance to ischemic conditions. Overexpression of orp150 in cultured rat cortical neurons protected them against ischemia, and vice versa, inhibition of orp150 synthesis in cultured astrocytes increased their vulnerability to hypoxic shock (627). Transgenic mice expressing orp150 under control of a platelet-derived growth factor B-chin promoter displayed high levels of orp150 in the cortex, hippocampus, and cerebellum and were much more resistant to global cerebral ischemia compared with wild-type controls (627). Interestingly, in cultured neurons with an increased level of orp150, glutamate-induced [Ca\(^{2+}\)]\(_i\) loads were substantially reduced, implying that this chaperone may also stabilize cellular Ca\(^{2+}\) homeostasis, most likely by limiting Ca\(^{2+}\) release from the ER, which accompanies excessive glutamate stimulation (294).

C. ER Stores and Human Immunodeficiency Virus

The human immunodeficiency virus (HIV) envelope glycoprotein GP120 was shown to induce large [Ca\(^{2+}\)]\(_i\) increases in cultured hippocampal neurons and promote their degeneration. Both the [Ca\(^{2+}\)]\(_i\) elevation and the neurodegeneration can be prevented by the CICR blocker dantrolene, or by ER depletion produced by preincubation of cells with caffeine, carbachol, or TG (393).

D. ER Stores and Diabetes

The involvement of the ER Ca\(^{2+}\) store in diabetic peripheral neuropathies can be suggested on the basis of a rather limited number of observations demonstrating a reduction in the size of caffeine-induced Ca\(^{2+}\) release in peripheral neurons isolated from animals with experimental (usually streptozotocin induced) diabetes type I. Such a decrease was found in diabetic DRG neurons (242, 306, 316), and the reduced [Ca\(^{2+}\)]\(_i\) responses to caffeine were especially prominent in cells isolated from lumbar ganglia (242), which are the first to be affected by diabetic neuropathies. A very substantial decrease in caffeine-induced [Ca\(^{2+}\)]\(_i\) transients (from 270 nM in control animals
to 30 nM in diabetic ones) was also observed in dorsal horn neurons from streptozotocin-induced diabetic rats (316, 679). In vivo experiments, ryanodine and TG significantly modified the formalin-induced nociceptive response in streptozotocin-diabetic mice (266) providing further evidence that the ER Ca\(^{2+}\) store is involved in the pathogenesis of diabetic neuropathies.

E. ER Stores and Gangliosidoses (Gaucher’s and Sandhoff’s Disease)

Mutations of the human gene encoding glucocerebrosidase result in a reduction of activity of this enzyme and accumulation of glycosylceramide, which, in turn, promotes neurodegeneration in the central nervous system. Clinically this type of neurodegenerative disorder is known as Gaucher’s disease (27). When the activity of glucocerebrosidase in cultured hippocampal neurons was blocked by incubation with conduritol-B-epoxide, accumulation of glycosylceramide occurred, thus mimicking the pathological conditions associated with Gaucher’s disease. This condition was accompanied by a significant increase in ER tubular elements and by a great increase in the release of Ca\(^{2+}\) from the ER in response to both metabotropic stimulation and caffeine (302). Furthermore, hippocampal neurons with inhibited glucocerebrosidase were significantly more vulnerable to excitotoxic shock (exposure to high glutamate concentrations), and this increased vulnerability was blocked by ryanodine (302). Therefore, remodeling of the ER Ca\(^{2+}\) homeostasis which results in an increase of ER Ca\(^{2+}\) content may be regarded as a key element of neurodegeneration associated with Gaucher’s disease.

Another type of gangliosidoses, when gangliosides accumulate in cells due to a defect in lysosomal proteins catalyzing their degradation, the GM2 gangliosidoses, or Sandhoff’s disease, also affects the ER, as the rate of SERCA-driven Ca\(^{2+}\) uptake was found to be considerably lower in the Sandhoff mouse model (501).

F. ER Stores and Alzheimer’s Disease

Alzheimer’s disease (AD) is a form of senile dementia characterized by the advent of plaques in the brain matter (54, 153) and appearance of fibrillar structures within the neuronal cytoplasm (11). It receives much attention because of its social impact affecting the progressively ageing mankind. The importance of cellular Ca\(^{2+}\) homeostasis in the pathogenesis of AD was perceived at the beginning of 1980s, when Zaven Khachaturian promulgated his “Ca\(^{2+}\) hypothesis of AD and neuronal ageing” (281). This hypothesis postulated that long-lasting subtle changes in [Ca\(^{2+}\)]\(_{i}\) homeostasis would eventually result in the neurodegeneration so characteristic of AD.

Investigations in the last decade confirmed this early reflection by clearly demonstrating that disruption of cellular Ca\(^{2+}\) homeostasis is a prominent feature of AD pathophysiology. Furthermore, these investigations identified ER Ca\(^{2+}\) dysregulation as a key step in the development of AD.

As already mentioned, the histopathology of AD is represented by plaques formed by aggregates of amyloid-\(\beta\) peptide (A\(\beta\)) and by the appearance of intracellular filamentous structures of a macrotubule-associated protein tau (378). This reflects several complex alterations of enzymatic systems controlling the synthesis and processing of A\(\beta\). The familial form of AD (FAD) is caused by mutations of genes encoding the A\(\beta\) precursor protein (APP, chromosome 21) and presenilins 1 (PS1, chromosome 14) and 2 (PS2, chromosome 1). Mutations in PS1 are responsible for the majority of FAD cases. The central question for the pathophysiology of AD is related to events proximal to the malfunction of A\(\beta\), as they could represent potential therapeutic targets.

Disrupted Ca\(^{2+}\) homeostasis has been identified as a feature of AD. Increased levels of free and bound Ca\(^{2+}\) have been detected in neurons containing neurofibrillary tangles (439). Likewise, neurons possessing tangles showed increased activity of various Ca\(^{2+}\)-dependent proteases (461). Early studies on fibroblasts isolated from AD patients also demonstrated impairment of Ca\(^{2+}\) mobilization in response to metabotropic stimulation (186, 252). Direct administration of A\(\beta\) to nerve cells caused elevation in resting [Ca\(^{2+}\)]\(_{i}\), and greatly diminished the ability of cells to cope with Ca\(^{2+}\) loads (207, 367, 379; see also Ref. 377 for review). Most importantly however, cellular Ca\(^{2+}\) dishomeostasis occurs at the initial stages of AD (142), and studies on transgenic mice with APP or PS mutations found that impaired Ca\(^{2+}\) handling occurs long before any histopathological changes (195, 323). The mechanisms of AD-related disruption of [Ca\(^{2+}\)]\(_{i}\), homeostasis can be multifaceted and involve plasmalemmal systems as well as intracellular organelles. The effects of A\(\beta\) and its fragments, for instance, are reported to influence plasmalemmal Ca\(^{2+}\) transport by forming Ca\(^{2+}\)-permeable pores (47, 279), although acute application of A\(\beta\) additionally augments intracellular Ca\(^{2+}\) release (207, 299). Several recently conducted studies suggest that disrupted ER Ca\(^{2+}\) homeostasis may be regarded as a specific feature of the AD-related neuronal Ca\(^{2+}\) dysregulation (see Table 11 and Refs. 323, 378, 380, 608, 709). Among molecules involved in the pathogenesis of AD, presenilins are particularly relevant for disruption of ER function. Presenilins are integral membrane proteins that contain eight transmembrane domains and serve as part of a multiprotein protease complex, the \(\gamma\)-secretase, which is responsible for the intramembranous cleavage of the APP and the Notch receptors (124). Most importantly, presenilins are involved in integrative neuronal functions,

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TABLE 11. Involvement of ER Ca\(^{2+}\) homeostasis in neurodegenerative changes associated with Alzheimer’s disease

<table>
<thead>
<tr>
<th>Effects</th>
<th>Preparation</th>
<th>Experimental Evidence</th>
<th>Reference Nos.</th>
</tr>
</thead>
<tbody>
<tr>
<td>Increase of InsP(_3) production and Ca(^{2+}) release</td>
<td>Primary cultured hippocampal embryonic neurons</td>
<td>Applications of a secretory form of (\beta)-amyloid precursor protein APP(\beta) induced [Ca(^{2+})](_i) rise in Ca(^{2+}),-free media.</td>
<td>299</td>
</tr>
<tr>
<td>Increase of ER stress response marker grp78</td>
<td>Primary cultured hippocampal embryonic neurons</td>
<td>Exposure of cultures to (\alpha\beta) fragment (\alpha\beta)(_{25-35}) increased expression of grp78.</td>
<td>713</td>
</tr>
<tr>
<td>Increased kainate and glutamate excitotoxicity</td>
<td>Mutant PS1 mice, hippocampus primary hippocampal cultures</td>
<td>Increased kainite (in vivo) and glutamate (in vitro) excitotoxicity correlated with increased amplitude of glutamate-induced [Ca(^{2+})](_i) elevation in PS1 mutant neurons. Dantrolene effectively prevented excitotoxicity in culture.</td>
<td>195</td>
</tr>
<tr>
<td>Increase in VGCC and stimulation of Ca(^{2+}) release from the ER</td>
<td>Primary cultured DRG</td>
<td>In voltage-clamped neurons, application of (\alpha\beta) fragment (\alpha\beta)(<em>{25-35}) increased L- and T-type Ca(^{2+}) currents; in fura 2-AM-loaded cells, (\alpha\beta)(</em>{25-35}) induced [Ca(^{2+})](_i) increase in Ca(^{2+}),-free media.</td>
<td>207</td>
</tr>
<tr>
<td>Increased Ca(^{2+}) release</td>
<td>PC12 cells</td>
<td>In PC12 cells expressing mutant PS1 (L286V), carbachol, bradykinin, and TG induce larger [Ca(^{2+})](_i) responses compared with control. These cells also show larger [Ca(^{2+})](_i) elevation and increased cell death upon application of (\alpha\beta); effects of Ca(^{2+}) can be inhibited by nifedipine and dantrolene or expression of Bcl-2.</td>
<td>196, 197</td>
</tr>
<tr>
<td>Upregulation of RyRs expression and increased Ca(^{2+}) release</td>
<td>PC12, primary cultured hippocampal neurons</td>
<td>In PC12 cells expressing mutant PS1 (L286V or M146V) and in hippocampal neurons isolated from mutant PS1 (M146V) mice, the greater levels of RyRs coincided with increased amplitude of caffeine-induced Ca(^{2+}) release.</td>
<td>88</td>
</tr>
<tr>
<td>PS1 effects on SOC</td>
<td>SY5Y neuroblastoma, CHO cells, primary cultured cortical neurons</td>
<td>In cultured neurons from PS1 knockout and SY5Y cells expressing inactive PS1 the SOC (determined as [Ca(^{2+})](<em>i), increase after depletion of ER by CPA and Ca(^{2+}) readmission) was potentiated. SY5Y cells expressing PS1 mutant (M146L) or PS2 mutant (N141I) as well as cortical neurons isolated from PS2 (N141I) mutant mice displayed reduced SOC. In CHO cells with stable expression of PS1 mutant gene, the I(</em>{CRAC}) was severely inhibited.</td>
<td>709</td>
</tr>
<tr>
<td>Increased Ca(^{2+}) release and inhibition of SOC</td>
<td>SH-SY5Y neuroblastoma cell line</td>
<td>Expression of wild-type PS1 or mutant PS1 (\Delta E) increased the amplitude of muscarine-induced [Ca(^{2+})](_i) transients recorded in Ca(^{2+})-free media; the SOC measured as [Ca(^{2+})](_i) increase after readmission of Ca(^{2+}) into external solution was inhibited in cells expressing PS1 (\Delta E) only.</td>
<td>596</td>
</tr>
<tr>
<td>Increased sensitivity to oxidative stress</td>
<td>PS1 knock-out mice; neocortical and hippocampal embryonic primary cultures</td>
<td>Neuronal cultures prepared from PS-deficient mice exhibited much higher cell death in response to 24-h incubation with 100 (\mu)M (\text{H}_2\text{O}_2) and to 100–1,000 (\mu)M glutamate.</td>
<td>443</td>
</tr>
<tr>
<td>Increased LTP and vulnerability to kainite excitotoxicity</td>
<td>Transgenic mutant PS1 mice; hippocampal brain slices; primary hippocampal cultures</td>
<td>Depolarization (KCl 50 mM), glutamate (50 (\mu)M), TG (1 (\mu)M), and bradykinin produced higher [Ca(^{2+})](_i) elevation in PS1-derived cells studied in both slices and culture. Mutant PS1 neurons were more vulnerable to kainite- (in vivo) and glutamate-induced excitotoxicity. LTP in response to weak stimulation was facilitated in PS1 mutant neurons.</td>
<td>564</td>
</tr>
<tr>
<td>Increased ER Ca(^{2+}) release and increased SOC</td>
<td>Various transgenics containing mutant PS1, APP(^{-/-}) and their combinations, hippocampal brain slices; primary hippocampal cultures</td>
<td>The direction was decreased in PS1 mutants, but not in APP-deficient animals (the SOC was measured as SKF-96365 sensitive component of [Ca(^{2+})](_i) rise in response to readmission Ca(^{2+}) after the period of Ca(^{2+})-free media with CPA). In mice deficient for PS1 and APP TG as well as in PS1 mutant mice TG triggered much larger [Ca(^{2+})](_i) elevation compared with wild-type animals.</td>
<td>220</td>
</tr>
<tr>
<td>Increased InsP(_3)-mediated Ca(^{2+}) release</td>
<td>Cortical brain slices</td>
<td>The amplitude of InsP(_3)-evoked (flash photolysis of caged InsP(_3)) [Ca(^{2+})](_i) transients was &gt;3 times larger in cortical neurons from mice expressing mutant PS1 (M146V) as compared with wild-type age-matched controls. Expression of mutant PS1 greatly increased the number of neurons responding to caged InsP(_3) photolysis by [Ca(^{2+})](_i) transient. The level of InsP(_3)Rs was not affected by PS1 knock-in, suggesting increased [Ca(^{2+})](_i), as a main reason for the differences observed.</td>
<td>608</td>
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LTP, long-term potentiation; \(\alpha\beta\), \(\beta\)-amyloid protein.
as conditioning knockout of both PS1 and PS2 in the forebrain of adult mice causes a remarkable impairment of memory, LTP, NMDA responses, and CREB/CBP-mediated gene expression and promotes neurodegeneration (562).

Presenilins are highly expressed in neurons and reside mostly in the ER membrane. Not much is known about the involvement of presenilins in ER Ca\(^{2+}\) handling, although it has been shown that PS1 can bind to RyRs (88) and interact with sorcin (476), calsenilin (69), and calpain (372), i.e., proteins known to regulate Ca\(^{2+}\) release channels. Nonetheless, every alteration of PS, be it in the form of its genetic deletion or of expression of mutant (FAD associated) PS, results in impairment of ER function. As a rule, the latter is manifested as an increased amplitude of the Ca\(^{2+}\) response to metabotropic agonists, caffeine, or the SERCA inhibitors TG and CPA (see Table 11). This increase in [Ca\(^{2+}\)]\(_i\) transients is generally regarded as a consequence of an elevated [Ca\(^{2+}\)]\(_E\). The changes in ER Ca\(^{2+}\) content are not confined to neurons, but are also observed in various peripheral cells such as, e.g., fibroblasts (336).

Manipulations of presenilin expression affect several Ca\(^{2+}\)-dependent functions as well as neuronal vulnerability to various types of insults. In transgenic mice expressing mutant PS1, neurons in hippocampal slices show an increased sensitivity to kainate excitotoxicity and demonstrate facilitated LTP induction (564). Both effects are attributable to an increased Ca\(^{2+}\) release from the ER. Similarly, expression of mutant PS1 increased the size of infarction following middle cerebral artery occlusion and reperfusion in vivo and increased cell death in cortical neurons in vitro, when exposed to hypoxia and glucose deprivation (382). Likewise, cultured hippocampal neurons from the same animals displayed increased vulnerability to glutamate excitotoxic shock (195). Inhibition of ER stores by dantrolene protected PS1 mutant-bearing neurons (382). Higher susceptibility of PS1 mutant neurons to various insults correlated with failures of the Ca\(^{2+}\) homeostatic machinery. Recordings of [Ca\(^{2+}\)]\(_i\) in PS1 mutant neurons revealed larger [Ca\(^{2+}\)]\(_i\) elevations induced by hypoxia, glutamate, and intracellular delivery of InsP\(_3\) (195, 382, 608). Modulation of PS functions by overexpressing calsenilin, a cytoplasmic protein interacting with PS1 and PS2, enhanced apoptosis and increased the amount of TG-released Ca\(^{2+}\), once more pointing to a possible increase in [Ca\(^{2+}\)]\(_L\) (345).

The PS-mediated alterations of ER function were accompanied by biochemical changes characteristic of the ER stress response. Specifically, expression of mutant PS1 in hippocampal neurons was associated with an abnormal activation of ER resident caspase-12 (87), which, as has been mentioned previously, mediates ER stress-related apoptosis. Expression of an aberrant spliced form of PS2 (PS2V) altered UPR and increased production of Aβ40 and Aβ42 (561). Furthermore, neurons overexpressing the mutant (but not the wild-type) form of PS1 were more susceptible to spontaneous apoptosis and apoptosis induced by ER stress, through decreased UPR (635).

An extremely important achievement in studying the cellular mechanisms of AD occurred recently after several genetic AD models were created. Conceptually these models aimed at the insertion of various forms of APP, which replicated Aβ deposition and plaque formation, or inclusion of mutant PS genes, which produced numerous AD-specific neuropathological changes. The recently discovered tau gene mutations (191), associated with frontotemporal dementia, have provided an additional tool for genetic modeling. A big step forward was made by Frank LaFerla, Mark Mattson, and co-workers who succeeded in creating a triple transgenic mouse bearing presenilin 1 (PS1m146V), amyloid precursor protein (APPSwe), and tau (tauP301L) transgenes (466). These animals possessed several hallmarks of AD such as progressively developing plaques and tangles as well as deficiencies in LTP, which preceded the appearance of the histological markers of AD. The functional changes were clearly age dependent, as both the amplitude of field-stimulation evoked EPSPs and LTP were significantly impaired in 6-mo-old triple transgenic animals, whereas at 1 mo there was no difference between the transgenic mice and wild-type controls (466). However, a detailed analysis of the ER signaling changes associated with this model has yet to be performed.

Although there is no doubt about the AD-related impairment of ER Ca\(^{2+}\) homeostasis, the important question of whether ER changes precede Aβ formation or conversely Aβ malsynthesis causes ER dysfunction remains open.

G. ER Stores and Ageing

The process of physiological ageing (646), which is not associated with any prominent neuronal loss, yet is accompanied by cognitive decline, involves changes in neuronal Ca\(^{2+}\) homeostasis, which comprise alterations of Ca\(^{2+}\) influx, mitochondrial and cytoplasmic Ca\(^{2+}\) buffering, and Ca\(^{2+}\) extrusion (108, 156, 289, 307, 329, 516, 639, 640, 647, 675, 700). The amplitudes of caffeine-induced [Ca\(^{2+}\)]\(_i\) responses are moderately decreased in aged DRG, neocortical, and cerebellar granule cells (290, 291, 674) and in acutely dissociated rat basal forebrain neurons (436). This may reflect the decreased [Ca\(^{2+}\)]\(_L\), and indeed, the studies mentioned above found a decreased Ca\(^{2+}\) sequestering capacity of the ER in aged nerve cells. In contrast, old cultured hippocampal neurons (101) demonstrated prolonged CICR in response to glutamate exposure, although the size of the caffeine-
releasable pool was similar in young and old cultures. Experiments in hippocampal slices isolated from old (22–24 mo) rats found that increased ER Ca$^{2+}$ release affects LTP induction in the aged hippocampus (321). All in all, however, the age-dependent changes in ER Ca$^{2+}$ homeostasis are rather mild when compared with the ER alterations in AD. Moreover, no obvious increase in the ER Ca$^{2+}$ content has been discovered in old neurons, which may reflect a fundamental difference between the physiological processes of brain ageing and age-dependent neurodegeneration.

H. Spinocerebellar Ataxia Type 1

Spinocerebellar ataxia type 1 (SCA1) is an autosomal dominant neurological disorder characterized by loss of Purkinje neurons and neurons in pontine nuclei, which is clinically manifested as a malfunction of the brain stem and ataxia. SCA1 is caused by the expansion of a CAG trinucleotide repeat, which results in an expanded polyglutamine tract in its gene product, ataxin-1 (248). In SCA1 transgenic mice, which carry the expanded allele, a significant reduction in the expression of the InsP$_3$R1 and the SERCA2 pump was observed, although quantitatively parameters of [Ca$^{2+}$]$_i$ signaling and mGluR-mediated Ca$^{2+}$ release did not differ much from wild-type controls (248).

I. ER and Neuronal Trauma

A direct link between neurotrauma and ER Ca$^{2+}$ homeostasis has been demonstrated for cultured cortical neurons subjected to mechanical deformation. It appeared that such a crude traumatization of neurons resulted in a remarkable decrease in the proportion of cells able to produce ER-associated Ca$^{2+}$ release. The percentage of cells responding to caffeine fell from 70% in control to 30%, and the percentage of neurons producing IICR from ~90% in normal cells to ~20% already 15 min after the trauma (690). Three hours later the ER Ca$^{2+}$ release was normalized, although an increase in SOC was observed (689). SOC was determined as SKF96365-sensitive component of the TG-induced [Ca$^{2+}$]$_i$ elevation.

J. ER and Parkinson’s Disease

A possible role for the ER in the pathogenesis of Parkinson’s disease (PD) has been suggested based on investigations of a cellular model of PD, namely, exposure of cells to 6-hydroxydopamine, an agent which causes a type of neurodegeneration similar to that observed in PD (659). When PC12 cells were exposed to 100 μM 6-hydroxydopamine for 8 h, they responded by an upregulation of the expression of numerous genes indicative of UPR (550). Similarly, the ER stress response was evoked by rotenone and 1-methyl-4-phenyl-pyridinium, other agents used for cellular modeling of PD. Finally, the recently identified PD-related G protein-coupled receptor protein, termed the Pael receptor, was found to accumulate in the ER and cause ER-related cell death (623). Whether these biochemical changes become translated into impairments of ER Ca$^{2+}$ homeostasis remains totally unexplored.

K. ER and Epileptiform Activity

There is some evidence indicating that incubation of hippocampal slices with 1–5 μM TG or with 30–100 μM dantrolene suppresses epileptiform activity in CA3 hippocampal area (549). Similarly, TG is shown to inhibit bicuculline-induced epileptiform activity in hippocampal neuronal networks in culture (599). In a pilocarpine model of epilepsy in hippocampal neuronal cultures, a reduction of TG-sensitive Ca$^{2+}$ uptake in “epileptic” neurons together with augmented CICR and altered IICR were described (480). Whether these findings reflect any involvement of the ER Ca$^{2+}$ store in the pathogenesis of epilepsy remains unknown.

L. ER and Huntington’s Disease

Huntington’s disease is a fatal autosomal dominant neurodegenerative disorder, which is characterized by a triad of motor (chorea), cognitive (progressive loss of mental abilities), and psychiatric/behavioral disturbances. This neuropathology is caused by mutations (polyglutamine expansion) of the protein huntingtin (85). The mutant huntingtin sensitizes the InsP$_3$R1 to InsP$_3$, and moreover, transfection of medium spiny striatal neurons with this mutant protein facilitates [Ca$^{2+}$]$_i$ responses to mGluR1/5 activation (628).

VI. CONCLUDING REMARKS

The ER is deeply engaged in neuronal integration. This integration involves coordinated activities of numerous molecular cascades that provide the ER with incoming signals, intra-ER information processing, and generation of output signals which control rapid neuronal signaling and long-lasting adaptive responses (Fig. 23). The movements of Ca$^{2+}$ across the ER membrane and within the ER lumen are responsible for many of these duties. Regulated Ca$^{2+}$ release from the ER is implicated in rapid neuronal responses to synaptic inputs and action potentials and is important for synaptic plasticity. Fluctuations of the intra-ER Ca$^{2+}$ concentration couple these rapid
signaling events to protein synthesis and modification. Finally, disruptions of ER Ca\(^{2+}\) homeostasis may be intimately involved in numerous neuropathological processes.

Our understanding of the ER function in nerve cells is clearly incomplete, and future investigations will undoubtedly result in many exciting discoveries in this field.

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