[Frontiers in Bioscience 7, d1255-1279, May 1, 2002]

MODULATING CA^{2+} CLEARANCE FROM NEURONS

Stanley A. Thayer, Yuriy M. Usachev and William J. Pottorf, II

Department of Pharmacology, University of Minnesota, 6-120 Jackson Hall, 321 Church Street, SE, Minneapolis, MN 55455-0217

TABLE OF CONTENTS

1. Abstract
2. Introduction
3. Mitochondria
- *3.1. Mitochondria damp the amplitude and prolong the duration of $[Ca^{2+}]_i$ increases*
- *3.2. Modulation of mitochondrial uptake, storage and release of Ca^{2+}*
- *3.3. Modulating mitochondrial Ca^{2+} buffering alters neuronal function*

4. Endoplasmic Reticulum (ER)
- *4.1. ER Ca^{2+} buffering and release – a capacity-dependent switch*
- *4.2. Modulation of sarcoplasmic or endoplasmic reticulum Ca^{2+} ATPase (SERCA)*
- *4.3. Modulation of ER Ca^{2+} storage capacity*
- *4.4. Functional consequences of switching between Ca^{2+} release and uptake*

5. Ca^{2+} Binding Proteins
6. Plasma Membrane Ca^{2+} ATPase (PMCA)
- *6.1. Alternative splicing generates Ca^{2+} pump isoforms with unique properties*
- *6.2. PMCAs provide high affinity Ca^{2+} extrusion*
- *6.3. Pharmacologic modulation of PMCAs*
- *6.4. Selective modulation of PMCA isoforms by endogenous signaling pathways*
- *6.5. PMCAs are sites where signaling pathways converge*

7. Plasmalemmal Na^+/Ca^{2+} Exchange
- *7.1. Na^+/Ca^{2+} exchange provides low affinity, high turnover Ca^{2+} extrusion*
- *7.2. Thermodynamic modulation of Na^+/Ca^{2+} exchange*
- *7.3. Modulation of Na^+/Ca^{2+} exchange by second messengers*
- *7.4. Pharmacologic modulation of Na^+/Ca^{2+} exchange*
- *7.5. Functional consequences of modulating Na^+/Ca^{2+} exchange in neurons*

8. Perspectives
- *8.1. Competing for Ca^{2+} - the integrated response*
- *8.2. Future directions*

9. Acknowledgements
10. References

1. ABSTRACT

Neurons are exquisitely sensitive to the duration, amplitude and localization of transient increases in intracellular Ca^{2+} concentration ($[Ca^{2+}]_i$). Modulation of Ca^{2+} uptake into the mitochondrion and endoplasmic reticulum, and efflux via the plasma membrane Ca^{2+} pump and Na^+/Ca^{2+} exchange profoundly affect the shape of $[Ca^{2+}]_i$ signals. Ca^{2+} clearance mechanisms are modulated by other signaling pathways, are sensitive to metabolic state and have a memory of the recent history of cell activation. We present here examples of pharmacologic and endogenous regulation of Ca^{2+} sequestration and efflux in neurons. Ca^{2+} clearance mechanisms differentially shape $[Ca^{2+}]_i$ signals based on their affinity, capacity and location; their modulation alters specific neuronal functions. The increasingly apparent diversity of the molecular entities that make up the $[Ca^{2+}]_i$ regulatory system reveals new sites for modulation. Specialized Ca^{2+} clearance mechanisms participate in unique cellular functions and thus, are important targets for pharmacological and physiological regulation of the neuron.

2. INTRODUCTION

Transient increases in the intracellular Ca^{2+} concentration ($[Ca^{2+}]_i$) trigger many neuronal functions including excitability, neurotransmitter release, gene expression and neurotoxicity (1). The rate at which Ca^{2+} is cleared from the cytoplasm following excitation affects the duration, amplitude and spread of $[Ca^{2+}]_i$ signals. This article focuses on the modulation of the processes responsible for removing Ca^{2+} from the cytoplasm of neurons primarily using results from studies with sensory

neurons as examples. Modulation of Ca^{2+} influx and release is well established and recent reviews discuss pharmacologic and second-messenger-dependent modulation of Ca^{2+} channels gated by voltage (2, 3), ligands (4), temperature (5) and second messengers (6, 7). We examine here the processes that shape transient increases in $[Ca^{2+}]_i$ such as uptake into the mitochondrion and endoplasmic reticulum (ER) and efflux via the plasma membrane Ca^{2+} ATPase (PMCA) and Na^+/Ca^{2+} exchanger. It is increasingly apparent that Ca^{2+} clearance mechanisms are not simple housekeepers of Ca^{2+} homeostasis but are modulated by cross talk with other signaling pathways, are sensitive to metabolic state and have a memory of the recent history of cell activation. Specialization of function and state-dependence of activation are central to understanding the modulation of Ca^{2+} clearance mechanisms in neurons. This review will provide examples demonstrating that regulation of sequestration and efflux processes is far more dynamic than previously thought.

The versatility of Ca^{2+} as a second messenger is made possible by the varied and complex Ca^{2+} regulatory system. Each cell expresses a set of Ca^{2+} transporters tailored to suit its specialized function (8). The mitochondrion acts as a low affinity $[Ca^{2+}]_i$ buffer. The ER has a high affinity for $[Ca^{2+}]_i$, limited capacity and depending on refilling state can either take up or release Ca^{2+}. The PMCA has a high affinity for $[Ca^{2+}]_i$, has an infinite capacity and predominantly influences $[Ca^{2+}]_i$ recovery near basal levels. The Na^+/Ca^{2+} exchanger has a low affinity for $[Ca^{2+}]_i$, is sensitive to electrical and Na^+ gradients, and is responsible for rapid reduction in $[Ca^{2+}]_i$ following intense stimulation. Sequestration and efflux processes play different roles in shaping $[Ca^{2+}]_i$ signals based on their affinity, capacity and location.

Ca^{2+}-activated processes display a complimentary sensitivity to the amplitude and duration of changes in $[Ca^{2+}]_i$. A number of enzymes are exquisitely sensitive to the duration of increases in $[Ca^{2+}]_i$. For example, prolonged elevation of $[Ca^{2+}]_i$ enables the autocatalytic activation of Ca^{2+}/calmodulin-dependent protein kinase II (9) enabling subsequent long lasting effects on synaptic plasticity (10). The neurosecretory machinery has both high and low affinity components. The Ca^{2+}-dependent initiation of membrane fusion has a low affinity for Ca^{2+} and is very sensitive to the amplitude of $[Ca^{2+}]_i$ increases (11, 12). In contrast, the size of the readily releasable pool of vesicles is regulated by calmodulin, is sensitive to modest increases in $[Ca^{2+}]_i$ and is thus, very sensitive to $[Ca^{2+}]_i$ recovery kinetics and residual $[Ca^{2+}]_i$ (13). Thus, modulation of Ca^{2+} clearance alters important functional responses in neurons.

3. MITOCHONDRIA

3.1. Mitochondria damp the amplitude and prolong the duration of $[Ca^{2+}]_i$ increases

Mitochondria have a tremendous capacity to take up Ca^{2+}. The low affinity of isolated mitochondria for Ca^{2+} was thought to limit their participation in Ca^{2+} signaling to toxic processes. It is now clear that mitochondrial Ca^{2+} buffering shapes physiological $[Ca^{2+}]_i$ transients in neurons (14, 15). This more physiological role may be due to a higher affinity for Ca^{2+} *in vivo* relative to isolated mitochondria or exposure to higher $[Ca^{2+}]_i$ near the mouths of Ca^{2+} channels than previously realized (16-19). Mitochondria do not appear to retain Ca^{2+}, at least not as free cation, but instead, slowly release Ca^{2+}. The net result is a powerful buffer that attenuates the amplitude and increases the duration of the $[Ca^{2+}]_i$ response. As shown in Figure 1A, this places a ceiling on $[Ca^{2+}]_i$ such that increasing stimulus strength lengthens the duration of a shoulder in the recovery phase of the $[Ca^{2+}]_i$ transient (14). In sensory neurons, this shoulder forms a distinct plateau following application of large Ca^{2+} loads. Rapid mitochondrial uptake followed by slow release has also been observed in adrenal chromaffin cells (20, 21), sympathetic neurons (15), central neurons (22-24) and motor nerve endings (25). Thus, the mitochondrion acts as a powerful buffer to shape physiological $[Ca^{2+}]_i$ signals.

Changes in mitochondrial Ca^{2+} uptake and release affect Ca^{2+}-sensitive processes both within and outside the mitochondrion. Changes in matrix Ca^{2+} regulate Ca^{2+}-sensitive dehydrogenases, coupling the increased metabolic demand signaled by elevated $[Ca^{2+}]_i$ to increased aerobic metabolism (26, 27). Ca^{2+} buffering by the mitochondrion alters $[Ca^{2+}]_i$ gradients, which affects secretory processes (18, 28) and the refilling and release of Ca^{2+} from the ER (29-31). The mitochondrial contribution to Ca^{2+} regulation is location specific. Slow, prolonged Ca^{2+}-release from mitochondria provides the residual $[Ca^{2+}]_i$ necessary for post-tetanic potentiation at the crayfish neuromuscular junction (32). In contrast, mitochondria at a ribbon synapse only affect synaptic transmission indirectly, by supplying ATP (33). Mitochondrial Ca^{2+} uptake seems to play a dual role during bursts of activity. Increases in $[Ca^{2+}]_{mt}$ activate metabolism, while rapid Ca^{2+} uptake preserves the phasic nature of individual action-potential-induced increases in $[Ca^{2+}]_i$ (34, 35). When elevated $[Ca^{2+}]_i$ reaches toxic levels, neurodegenerative processes are triggered by the resulting Ca^{2+} overload (36, 37). It was previously thought that mitochondrial Ca^{2+} buffering protected the cell from Ca^{2+} triggered cell death, however, it is now clear that mitochondria are targets for Ca^{2+} overload and are capable of initiating both necrotic and apoptotic processes (38). In summary, mitochondria play an important physiological role in shaping the amplitude and duration of transient increases in $[Ca^{2+}]_i$ and in so doing, link excitation to metabolism. Ca^{2+} overload triggers cell death and the excessive accumulation of Ca^{2+} within the mitochondrial matrix is an early event in Ca^{2+}-induced toxicity.

3.2. Modulation of mitochondrial uptake, storage and release of Ca^{2+}

Mitochondrial Ca^{2+} uptake and release occur by different pathways (Figure 1B). The mitochondrial membrane potential ($\Delta\psi$) provides the driving force for Ca^{2+} uptake via the uniporter (39, 40). Within the matrix, free Ca^{2+} is in equilibrium with Ca bound to an anion, mostly phosphate (41). Free Ca^{2+} is removed from neuronal mitochondria primarily by a Na^+/Ca^{2+} exchange process (42-45). In addition, under certain conditions

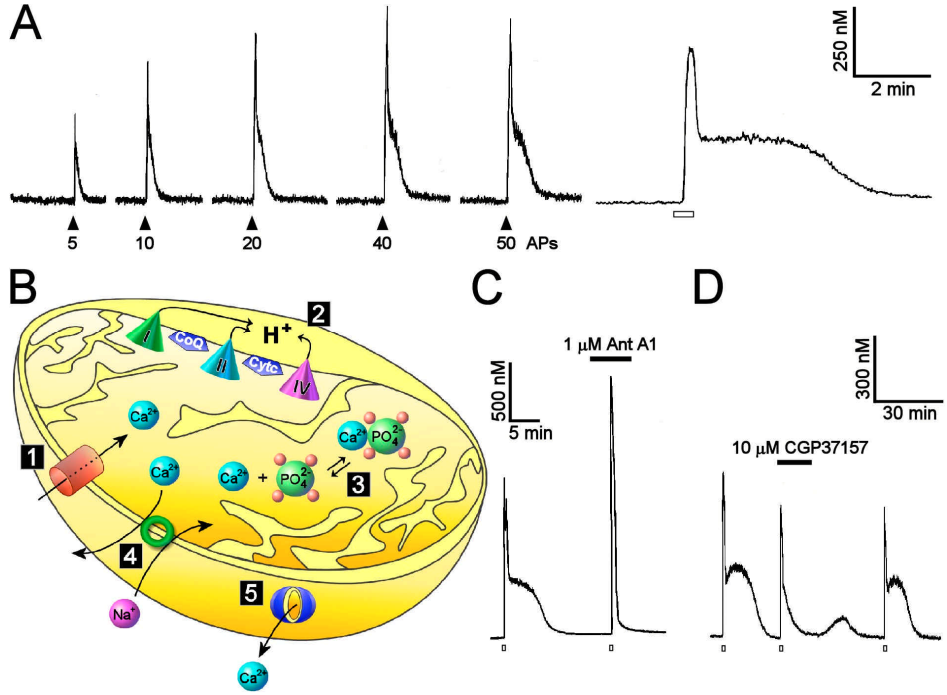

Figure 1. Mitochondria decrease the amplitude and increase the duration of depolarization-induced increases in $[Ca^{2+}]_i$. A. Trains of action potentials with the indicated number of spikes (*APs*) were elicited by electric field stimulation (▲) of a single sensory neuron. The same neuron was depolarized for 30 s with 50 mM K^+ (ϒ). $[Ca^{2+}]_i$ was recorded with indo-1-based photometry as previously described (14). B. Scheme shows important sites for modulation of mitochondrial Ca^{2+} regulation: *1*, uniporter; *2*, Δψ; *3*, Ca^{2+} phosphate complex formation; *4*, Na^+/Ca^{2+} exchange; and *5*, PTP. C. Depolarization induced (50 mM K^+, 30 s, ϒ) increases in $[Ca^{2+}]_i$ displayed a marked plateau in the recovery to baseline. Antimycin A1 (1 μM) applied at the time indicated by the horizontal bar increased the amplitude and decreased the duration of the mitochondrial-mediated plateau. D. CGP37157 (10 μM) applied after depolarization (ϒ) accelerated recovery to basal $[Ca^{2+}]_i$. Removal of the drug evoked a rebound increase in $[Ca^{2+}]_i$ as Ca^{2+} trapped in the matrix was allowed to enter the cytoplasm. Figures A and C, and D were reproduced with permission from references (14) and (87), respectively.

matrix Ca^{2+} is released via the mitochondrial permeability transition pore (PTP) (46-48) or via a Na^+-independent Ca^{2+} efflux pathway (49). Thus, sites for modulation include the uptake mechanism, including the uniporter and Δψ, capacity, and release via Na^+/Ca^{2+} exchange and the PTP (Figure 1B). An abbreviated list of agents that act at these sites is presented in Table 1.

Changes in Δψ alter Ca^{2+} uptake into the mitochondrion. Agents that poison electron transport (50), such as antimycin A1, provide a clear demonstration of how mitochondria shape transient increases in $[Ca^{2+}]_i$. As shown in Figure 1C, when Δψ was dissipated by treatment with antimycin A1, the amplitude of the evoked $[Ca^{2+}]_i$ response increased and the plateau phase was absent, dramatically shortening the duration of the response. Proton ionophores such as *p*-trifluoromethoxyphenylhydrazone (FCCP) uncouple electron transport to dissipate Δψ and produce similar changes in $[Ca^{2+}]_i$ buffering (14). FCCP is a particularly useful agent for inhibiting mitochondrial Ca^{2+} uptake in intact cells because it is potent, membrane permeant and reversible. It is not, however, selective for mitochondrial membranes (51). Agents that activate ATP-sensitive K^+ channels such as diazoxide also decrease the potential across the inner membrane and reduce Ca^{2+} uptake into the matrix; diazoxide affords protection from ischemia/reperfusion injury in cardiac myocytes (52, 53). The primary complication from dissipating Δψ is reduced ATP production and in some circumstances the actual consumption of ATP via reversal of the ATP synthase (54). Δψ is also changed by endogenous factors. Weak lipophilic acids such as palmitic acid produce a proton leak that decreases Δψ (55). Neurons express uncoupling proteins homologous to those responsible for thermogenesis in brown fat cells (56). Uncoupling proteins reduce mitochondrial production of reactive oxygen species, which may be their primary function in neurons. The availability of metabolic substrates alters Ca^{2+} uptake into the mitochondrion in a manner predicted by effects on Δψ (57, 58). Thus, the energy status of the cell modulates mitochondrial Ca^{2+} uptake. The actual transport of Ca^{2+} across the inner membrane requires energy and large Ca^{2+} loads depolarize mitochondria and reduce further Ca^{2+} uptake (59, 60). In contrast, modest increases in $[Ca^{2+}]_{mt}$ increase the proton motive force by stimulating Ca^{2+}-sensitive dehydrogenases (61). Generally, metabolic stress impairs and aerobic metabolism enhances, Ca^{2+} uptake into mitochondria.

Table 1. Selected modulators of mitochondrial Ca^{2+} uptake and release

Target[1]/modulator	**Concentration[2] (mM)**	**Effect**	**References**
1. **uniporter**			
Ruthenium Red	0.007	Inhibit	64
Ru360	0.0002	Inhibit	64
Spermine	100-400	Increase	67, 68
2. Dy			
Uncouplers – e.g. FCCP	0.1	Decrease	296
e^- transport inhibitors – e.g. antimycin A1	0.024-1.0	Decrease	14, 50
$MitoK_{ATP}$ channel opener – e.g. diazoxide	100	Decrease	52, 53
Substrates - e.g. pyruvate/malate	10000	Increase	57, 58
3. matrix capacity			
pH_{mt}	↓	Decrease	41
phosphate	250	Increase	41
4. Na^+/Ca^{2+} exchange			
CGP37157	4	Inhibit	87
$[Na^+]_i$	↓	Inhibit	42, 44
5. PTP			
Bongkrekic acid	1	Inhibit	45, 297
Atractylate	500	Induce	45, 298
Cyclosporins – e.g. cyclosporin A	0.005-0.1	Inhibit	78, 299
Prooxidants – e.g. t-butylhydroperoxide	600	Induce	48, 300
SH reagents – e.g. N-ethylmaleimide	5	Inhibit	301
$\uparrow[Ca^{2+}]_{mt}$	1000	Induce	83, 302
ADP	4-40	Inhibit	300
pH_{mt}	↓	Inhibit	45
P_i	5000	Induce	300
$\Delta\psi$	↓	Induce	45

[1]Numbers preceding targets refer to figure 1B. , [2] Concentrations are approximate values that were shown effective for the conditions cited.

Ca^{2+} enters the mitochondrion via the uniporter, a Ca^{2+} permeable channel of unknown molecular identity (39). The channel is activated by elevated $[Ca^{2+}]_i$ and in some cells displays a rapidly desensitizing high-conductance mode (62). ATP and Mg^{2+} inhibit the uniporter by acting on its cytoplasmic face (63). Ruthenium red and a purified component, RU360, will block this channel preventing Ca^{2+} entry (64, 65). The use of these agents is limited due to their lack of specificity, the poor membrane permeability of ruthenium red and the poor stability of RU360. Cobalt complexes inhibit Ca^{2+} uptake by isolated mitochondria similar to ruthenium red and may prove more stable and cell permeant (66). Polyamines, particularly spermine, increase the rate and affinity of Ca^{2+} uptake into mitochondria (67, 68). Polyamine levels in brain fluctuate during stress, intense electrical activity and development (69, 70). Taurine also appears to enhance mitochondrial Ca^{2+} uptake by acting on the uniporter at millimolar concentrations (71). As noted above, there are a number of physiologic and pharmacologic agents that modulate the uniporter, providing a direct mechanism to alter $[Ca^{2+}]_{mt}$. A clear demonstration of the endogenous modulation in intact neurons is lacking.

Once inside the mitochondrion, Ca^{2+} is reversibly bound as a phosphate complex (72). Recent speculation concerning the dynamic nature of this interaction focuses on the effects of pH and phosphate (28). A decrease in matrix pH or a decrease in phosphate concentration reduced complex formation in isolated mitochondria (41), although experiments testing this hypothesis *in situ* have not been performed. An adjustable capacity for Ca^{2+} uptake could have significant effects on the role of the mitochondrion in shaping physiological signals and coping with potentially toxic Ca^{2+} loads.

The PTP is an inner membrane channel of unknown structure (45, 48). Opening of the large conductance PTP is enhanced by decreases in $\Delta\psi$, elevated $[Ca^{2+}]_{mt}$, increased matrix pH and oxidants (73). Because these changes accompany Ca^{2+} overload combined with metabolic stress, this channel is thought to contribute to the collapse of $\Delta\psi$ and release of mitochondrial factors that trigger apoptosis (74, 75). Arachidonic acid (76) and cytotoxic agents, such as doxirubicin (77), also activate the PTP. Agents that interact with cyclophillins inhibit the PTP (78). Cyclosporin A inhibits the PTP and calcineurin; N-Me-Val-4-cyclosporine is more selective for the PTP and FK506 selectively inhibits calcineurin aiding in the differentiation between the two cellular targets (79, 80). Carboxyatractylate promotes and bongkrekic acid and ADP inhibit opening of the PTP (45). Because these agents modulate the adenine nucleotide translocase, it has been suggested that the translocase forms the pore (81). The PTP appears to have a small conductance state that participates in physiological signaling in the form of Ca^{2+}-induced Ca^{2+}-release (46) and may underlie channel activity recorded from mitochondria *in situ* during synaptic

transmission (82). Mitochondrial Ca^{2+}-induced Ca^{2+}-release is activated by increases in matrix pH making it very sensitive to Ca^{2+}-induced increases in respiration (83). The Ca^{2+}-induced Ca^{2+}-release mode of the PTP is inhibited by the cyclosporin analog, SDZ PSC833. This drug decreased the amplitude of IP_3-mediated $[Ca^{2+}]_i$ responses, suggesting that under certain conditions mitochondrial Ca^{2+} release will amplify cytosolic $[Ca^{2+}]_i$ increases (84). Changing from this physiological small-conductance state to the large conductance state is triggered by an increase in $[Ca^{2+}]_{mt}$ and is associated with mitochondrial toxicity (83). Inhibition of the high-conductance PTP affords neuroprotection in some excitotoxicity models (85).

Ca^{2+} release from mitochondria in sensory neurons is primarily via a Na^+/Ca^{2+} exchange process (44). Derivatives of the calcium channel blocker diltiazem, the most specific being CGP37157, inhibit this process (86, 87). As shown in Figure 1D, application of CGP37157 at the start of the mitochondrial Ca^{2+} release phase of the $[Ca^{2+}]_i$ transient traps Ca^{2+} within the matrix, allowing $[Ca^{2+}]_i$ to fall to basal levels. Removal of the drug produced a rebound increase in $[Ca^{2+}]_i$ as release resumed. Modulation of Na^+/Ca^{2+} exchange produces concentration-dependent modulation of the duration and amplitude of the plateau phase of the $[Ca^{2+}]_i$ response. Of more physiological importance are the similar effects produced by reduced intracellular Na^+ concentration ($[Na^+]_i$). Reduced $[Na^+]_i$ decreased the amplitude of the plateau phase and increased its duration, consistent with slowed Ca^{2+} release from the matrix (44). Thus, the large Na^+ load that accompanies intense bursts of action potentials or activation of ligand-gated Na^+ channels reduces the ability of the mitochondrion to retain Ca^{2+} (88, 89).

In summary, the separation of Ca^{2+} uptake, storage and release mechanisms provides a high degree of flexibility to mitochondrial control of $[Ca^{2+}]_i$, enabling this organelle to differentially affect the amplitude and duration of $[Ca^{2+}]_i$ increases and to adjust $[Ca^{2+}]_{mt}$ levels. Identification of the proteins that actually transport Ca^{2+} across the inner membrane will be an important step in furthering our understanding of the modulation of Ca^{2+} handling by mitochondria and will aid in developing agents to selectively control mitochondrial Ca^{2+} uptake and release.

3.3. Modulating mitochondrial Ca^{2+} buffering alters neuronal function

Mitochondrial Ca^{2+} buffering inhibits Ca^{2+}-dependent processes triggered by intense stimuli that produce large increases in $[Ca^{2+}]_i$ and enhances processes activated by prolonged exposure to modest increases in $[Ca^{2+}]_i$. For example, modulation of mitochondrial Ca^{2+} uptake and release alters Ca^{2+}-mediated toxicity and neurosecretory responses. Mitochondria within a cell form a surprisingly heterogeneous group in terms of $\Delta\psi$, shape and distribution (90). Assuming this heterogeneity affects Ca^{2+} buffering then it seems likely that mitochondria are modulated individually by their local environment, for example by polyamines, $[Na^+]_i$ or the availability of metabolic substrates, enabling them to create local Ca^{2+} signaling domains.

Inhibition of Ca^{2+} uptake into mitochondria with metabolic poisons will actually delay cell death triggered by glutamate-induced Ca^{2+} loads (91, 92). Protection likely results from a decrease in $\Delta\psi$ leading to decreased $[Ca^{2+}]_{mt}$ and reduced formation of reactive oxygen species (93, 94). Bcl-2 and Bax are members of a family of proteins that inhibit and activate apoptosis, respectively (95). Bcl-2 is localized to ER, mitochondrial and nuclear membranes (96). Bcl-2 increases mitochondrial Ca^{2+} uptake (97), prevents the release of proapoptotic factors such as cytochrome C (98, 99) and affords protection from Ca^{2+}-triggered toxicity (100). Bax binds to Bcl-2 on the mitochondrial membrane (101) and may activate apoptosis by oligomerization to form ion channels (102).

Microdomains of $[Ca^{2+}]_i$ on the order of 200-300 μM occur near the mouths of Ca^{2+} channels (103) and the vesicular release machinery in nerve terminals is linked to these channels (104). Inhibition of mitochondrial Ca^{2+} uptake increases secretion of catecholamines from adrenal chromaffin cells (18) and increased hormone release from pituitary gonadotropes (28), consistent with the idea that lost buffering capacity allows $[Ca^{2+}]_i$ to reach higher levels and thus, more effectively trigger exocytosis. In nerve terminals the role of the mitochondrion is less clear. At some synapses mitochondria seem to affect $[Ca^{2+}]_i$ indirectly by supplying ATP (33), while in other preparations inhibition of Ca^{2+} uptake with ruthenium red or by dissipating $\Delta\psi$, reduces residual $[Ca^{2+}]_i$ and impairs post-tetanic potentiation of neurotransmitter release (32, 105). Peptide release from sympathetic neurons is proportional to the time-integral of $[Ca^{2+}]_i$ above a threshold, providing an example of exocytosis that is especially sensitive to the duration of elevated $[Ca^{2+}]_i$ (106). The precise spatial relationship between the Ca^{2+} source, the mitochondrion and the affinity of the neurosecretory machinery for Ca^{2+} determines the role of mitochondria in a given exocytotic process. Mitochondria damp exocytosis of fast neurotransmitters triggered by large localized increases in $[Ca^{2+}]_i$. In contrast, release activated by prolonged elevation of $[Ca^{2+}]_i$ to more modest levels is actually enhanced by the prolongation of the $[Ca^{2+}]_i$ increase produced by mitochondrial Ca^{2+} buffering.

In summary, the specialized role of mitochondria in buffering large $[Ca^{2+}]_i$ increases makes this organelle an important target for modulating processes activated by intense stimulation. The special role of mitochondria in damping amplitude and prolonging the duration of $[Ca^{2+}]_i$ increases enables mitochondria to modulate rapid Ca^{2+}-induced exocytosis and the availability of vesicles for release. Thus, mitochondria are poised to influence the synaptic enhancement that follows repetitive presynaptic activity (105). While clearly an important regulator of physiological $[Ca^{2+}]_i$ responses, some of the most significant roles for mitochondrial Ca^{2+} uptake are seen in response to toxic stimuli. Excessive accumulation of matrix Ca^{2+} triggers processes that lead to cell death. The exciting prospects of neuroprotective drugs or agents that modulate synaptic plasticity by acting on mitochondria must be balanced with the hazards of adversely affecting cellular energy supplies.

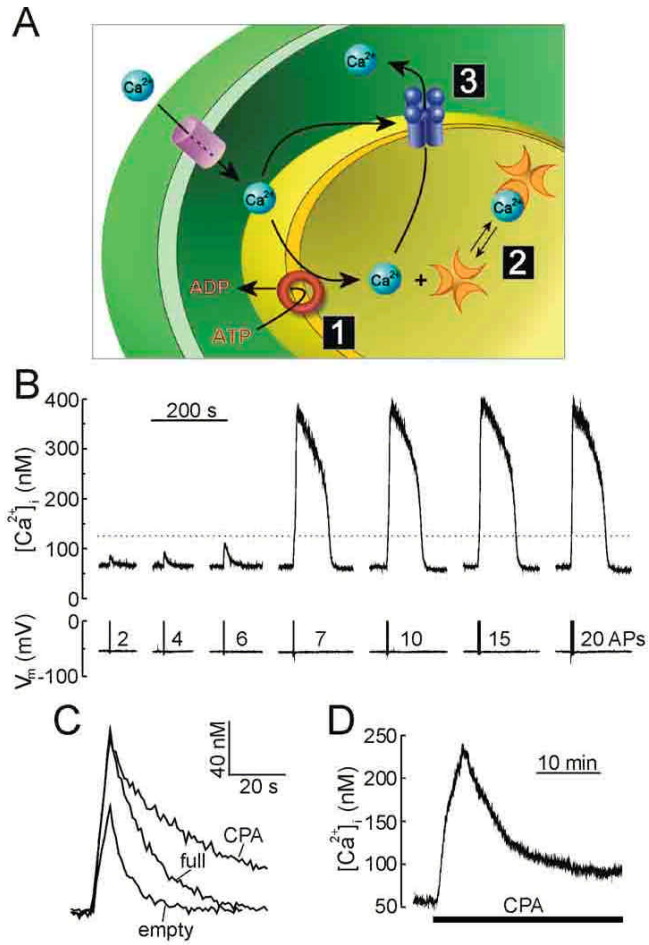

Figure 2. ER Ca^{2+} stores amplify or attenuate $[Ca^{2+}]_i$ increases depending on the refilling state of the store. A. Schematic shows ER Ca^{2+} regulation by *1*, SERCA; *2*, luminal binding proteins; and *3*, Ca^{2+} release channels. B. Regenerative $[Ca^{2+}]_i$ transients were evoked in a rat sensory neuron with full Ca^{2+} stores. $[Ca^{2+}]_i$ transients were measured with indo-1 and elicited by 2 Hz trains of action potentials (*APs*) in the presence of 5 mM caffeine. Action potentials were evoked in current-clamp, and the number of action potentials in each stimulus train is indicated above the voltage trace. The *horizontal dashed line* indicates the threshold for $[Ca^{2+}]_i$ for triggering regenerative Ca^{2+}-induced Ca^{2+}-release. Trains of action potentials were evoked every three minutes. C. Action potential-induced $[Ca^{2+}]_i$ transients were elicited in indo-1 AM-loaded sensory neurons under three states of Ca^{2+} store refilling. $[Ca^{2+}]_i$ transients are compared before (*full*), after application of 5 mM caffeine (*empty*) and after application of 5 μM cyclopiazonic acid (*CPA*). The recovery phase of the $[Ca^{2+}]_i$ transients were well fit by a monoexponential equation with time constants of 6, 13 and 29 s for empty, full and CPA, respectively. D. Application of 5 μM cyclopiazonic acid to a sensory neuron evoked an increase in $[Ca^{2+}]_i$. Figures B, C and D were reproduced with permission from (107), (109) and (223), respectively.

4. ER

4.1. ER Ca^{2+} buffering and release – a capacity-dependent switch

ER Ca^{2+} stores, in contrast to mitochondria, are poised for rapid release of Ca^{2+} via ligand gated ion channels and take up Ca^{2+} via a relatively slow ATP dependent Ca^{2+} pump. The sarcoplasmic or endoplasmic reticulum Ca^{2+} ATPase (SERCA) has a high affinity for Ca^{2+} enabling the ER to retain Ca^{2+} at high concentration (100 μM), even while $[Ca^{2+}]_i$ is low (100 nM). Stored Ca^{2+} can be rapidly released upon activation of ligand gated ion channels. The capacity of the ER to store Ca^{2+} is limited; this confers a marked state-dependence on the Ca^{2+} uptake and release process such that immediately following release the store is a powerful Ca^{2+} clearance mechanism, in contrast to full stores that are incapable of taking up Ca^{2+} and instead, are poised to amplify increases in $[Ca^{2+}]_i$ (107-111).

The SERCA, luminal buffering and Ca^{2+} release channels are all sites of modulation, and because of their interdependence, exert complex effects on the Ca^{2+} uptake properties of the ER as a whole (Figure 2A *1-3* respectively). Agents acting on these targets are listed in Table 2. The 1,4,5-inositol trisphosphate receptor (IP_3R) and ryanodine receptor (RyR) are Ca^{2+} release channels on the ER membrane. A full description of the pharmacology and modulation of these proteins is beyond the scope of this article and has been reviewed elsewhere (6, 112, 113). The feature of these channels that is of particular relevance to

Table 2. Modulation of ER Ca^{2+} uptake and release

Target[1]	Modulator [2]	EC_{50}, mM	Effect	References
1. SERCA				
(all isoforms)	thapsigargin	0.01-0.02	Inhibit	129
	cyclopiazonic acid	0.4	Inhibit	134
	tBHQ	0.4	Inhibit	131-133
	CaM kinase		Stimulate	148
SERCA2b	calnexin (PKC-dependent)		Inhibit	155
SERCA2b	calreticulin		Inhibit	153
2. Capacity				
	oxalate	4 mM	Increase	170
	pyrophosphate	5.8 mM	Increase	171
3. Ca^{2+} release channels[2]				
IP_3R	IP_3	0.24	Sensitizes to Ca^{2+}	303
	$[Ca^{2+}]_i$	<0.2	Increase	304
	(in 2 μM IP_3)	>0.2	Decrease	
	xestospongin	0.36	Inhibit	305
RyR	ryanodine	0.01-10	Sensitizes to Ca^{2+}	306, 307
		>10	Inhibits	
	caffeine	20 mM	Sensitizes to Ca^{2+}	308
	dantrolene	25	Inhibit	309, 310
	$[Ca^{2+}]_i$	0.01-100	Increase	304
	(in 500 μM ATP)	>100	Decrease	

[1]Numbers preceding targets refer to figure 2A., [2] Modulators of release channels were limited to a few key examples.

$[Ca^{2+}]_i$ clearance mechanisms is sensitivity of the release process to Ca^{2+} within the lumen of the ER. The release channels are modulated by luminal Ca^{2+}, possibly by Ca^{2+} binding proteins within the lumen (114-116). The degree to which the store refills alters the coupling of release channels by Ca^{2+}-induced Ca^{2+}-release. As shown in Figure 2B, action-potential-induced Ca^{2+} influx triggers regenerative Ca^{2+} release from ryanodine-sensitive Ca^{2+} stores in sensory neurons (107). This all-or-none response displays a discrete threshold for activation. The refilling state of the store is one factor that determines threshold and presumably results from the ability of Ca^{2+} released from one channel to activate neighboring sites.

When the ER is depleted of Ca^{2+} it can act as a powerful and highly localized Ca^{2+} clearance mechanism changing both the amplitude and duration of $[Ca^{2+}]_i$ signals. In Figure 2C, action potential-evoked increases in $[Ca^{2+}]_i$ from the same sensory neuron are superimposed. Each trace was recorded with the ER under a different state of refilling. When empty, the ER acted as a powerful Ca^{2+} clearance mechanism, consistent with increased Ca^{2+} uptake when Ca^{2+} levels in the lumen were low (109, 117-119). When the ER was allowed to refill, uptake was greatly reduced and $[Ca^{2+}]_i$ recovery kinetics were slowed. Thus, a loss of ER Ca^{2+} storage capacity inhibited SERCA-mediated Ca^{2+} uptake resulting in slowed $[Ca^{2+}]_i$ clearance kinetics.

In summary, the ER can act to buffer or amplify $[Ca^{2+}]_i$ increases depending on refilling state. Modulation of Ca^{2+} uptake into the ER will favor a particular state and the resulting effect on $[Ca^{2+}]_i$ will depend on whether the predominant influence of the ER was as a source or sink for Ca^{2+}.

4.2. Modulation of SERCA

The SERCA-type Ca^{2+} pumps are responsible for Ca^{2+} uptake into the ER. The three SERCA genes display tissue-specific expression with type 2 and 3 expressed in brain (120-123). Alternative splicing of SERCA2 primary transcripts results in two isoforms (124, 125) of which only the "b" isoform is expressed in neurons (126). Both SERCA isoforms found in brain (2b and 3) are equally sensitive to currently available pharmacologic inhibitors.

Several membrane-permeant inhibitors of SERCA type Ca^{2+} pumps are available. They are highly selective for SERCA relative to PMCA type Ca^{2+} pumps. Thapsigargin is a sesquiterpene lactone isolated from the plant, *Thapsia garganica*. This compound is an irritant, probably resulting from its ability to activate mast cells (127). Thapsigargin is also a weak tumor promoter, although it does not activate protein kinase C (128). Thapsigargin inhibits SERCAs with a half-maximal potency of approximately 10-20 nM by binding irreversibly to stabilize the Ca^{2+}-bound state of the enzyme (129). Because this drug titrates available SERCA in one to one stoichiometry, potency is affected by pump density, exposure time and whether it is bath applied or perfused through a recording chamber (130). It is a highly lipophilic compound and has a tendency to adsorb to glass and plastic recording chambers. 2,5-Di(*tert*-butyl)hydroquinone (tBHQ) also inhibits SERCA-type Ca^{2+} pumps, although

this compound appears to be less selective than other SERCA inhibitors (131-133). The mycotoxin cyclopiazonic acid is a selective inhibitor of SERCA type Ca^{2+} pumps having no detectable effects on Na^+/K^+ ATPase, H^+/K^+ ATPase or PMCA type pumps (134). Cyclopiazonic acid is less potent than thapsigargin, but is readily reversible, has fewer adsorption problems and has become a widely used tool for SERCA inhibition. Other non-specific inhibitors of SERCAs include vanadate and fluoride (124, 135-138).

When SERCA inhibitors are applied to resting cells with a full ER Ca^{2+} load, the compounds evoke a transient elevation of $[Ca^{2+}]_i$ (Figure 2D). The $[Ca^{2+}]_i$ increase results from slow release of stored Ca^{2+} followed by influx of Ca^{2+} triggered by depletion of the store. This capacitative Ca^{2+} influx (139, 140) is pronounced in non-excitable cells and is mediated by a family of store-operated channels; some of these channels are homologues of the *Drosophila* transient receptor potential protein (141). In neurons, this secondary Ca^{2+} influx is small. In Figure 2D, capacitative Ca^{2+} influx contributed to the elevated basal $[Ca^{2+}]_i$ observed in the presence of cyclopiazonic acid (109, 142, 143). Interestingly, neurons expressing certain mutant presenilin proteins exhibit greatly enhanced capacitative Ca^{2+} entry (144, 145). Presenilins are localized to the ER and are known to modulate γ secretase activity to yield mis-processed β amyloid proteins in Alzheimer's disease (146, 147). How presenilins modulate capacitative Ca^{2+} influx is an open question.

SERCA inhibitors are useful tools for studying the Ca^{2+} clearance properties of the ER in neurons. Inhibition of SERCAs in sensory neurons with cyclopiazonic acid greatly slowed the recovery of $[Ca^{2+}]_i$ following a train of action potentials (Figure 2C). Thus, depending on the refilling state of the ER Ca^{2+} store and the pattern of stimulation, cyclopiazonic acid can reduce $[Ca^{2+}]_i$ due to impaired Ca^{2+}-induced Ca^{2+}-release or increase $[Ca^{2+}]_i$ due to lost Ca^{2+} uptake.

Physiological modulation of SERCA type pumps results from phosphorylation, inhibition by accessory proteins, inhibition by Ca^{2+} within the lumen of the ER and possibly by cyclic ADP ribose (cADPr). SERCA isoforms exhibit differential modulation by endogenous signaling pathways. SERCAs are modulated by phosphorylation directly and by phosphorylation of accessory proteins. SERCA type 2 is phosphorylated directly by calmodulin-dependent protein kinase, which causes an increase in pump activity without affecting affinity (148). Phospholamban, a homopentamer of 6 kDa proteins binds to and inhibits SERCA isoforms 1 and 2 (149). Phosphorylation of phospholamban by protein kinase A, C or G or Ca^{2+}/calmodulin-dependent protein kinase inhibits phospholamban binding to SERCA resulting in stimulation of the pump (150). It appears that this type of modulation does not occur in nervous tissue because phospholamban is expressed exclusively in muscle. However, a peptide of unknown function with homology to phospholamban is expressed in developing brain (151). Calnexin and calreticulin are Ca^{2+}-sensitive lectin chaperones that assist protein folding in the ER (152). SERCA2b differs from other Ca^{2+} pump isoforms in that it has a carboxyl-terminal glycosylation site that faces the lumen of the ER enabling this isoform to interact with lectins. Calreticulin and calnexin modulate the Ca^{2+} pumping activity of mature SERCA2b. Calreticulin inhibits Ca^{2+} pumping activity by interacting with SERCA2b from the lumen of the ER (153). Calnexin is localized to the ER membrane and has a luminal Ca^{2+} binding domain and a cytosolic site available for phosphorylation (154). When phosphorylated by protein kinase C, calnexin binds to SERCA2b and inhibits Ca^{2+} pump activity (155). Ca^{2+} release from the store leads to dephosphorylation of calnexin and relief of inhibition of SERCA2b. Calnexin and calreticulin bind N-glycosylated proteins in a manner sensitive to luminal $[Ca^{2+}]$ and are expressed in brain (156-158). The work described above was performed with muscle preparations or heterologous expression systems; direct observation of endogenous modulation of SERCAs in neuronal preparations has not been reported.

cADPr lowers the threshold for Ca^{2+}-induced Ca^{2+}-release from ryanodine sensitive Ca^{2+} stores (159-162). The molecular site of action of cADPr is not known, but a recent study of cardiac myocytes found evidence that cADPr increased Ca^{2+} accumulation by cardiac sarcoplasmic reticulum microsomes suggesting an enhancement of SERCA activity (163). Because increased luminal $[Ca^{2+}]$ enhances regenerative Ca^{2+} release, increased SERCA-mediated Ca^{2+} uptake into the ER could lower the threshold for Ca^{2+}-induced Ca^{2+}-release from ryanodine-sensitive stores.

In summary, selective inhibitors of SERCA such as thapsigargin and cyclopiazonic acid are useful tools for studying ER Ca^{2+} uptake. SERCAs are regulated endogenously by protein-protein interactions and signaling cascades. Modulation of SERCAs by endogenous signals likely occurs in neurons, although it has not yet been reported.

4.3. Modulation of ER Ca^{2+} storage capacity

Ca^{2+} within the lumen of the ER is in the millimolar range (164), a concentration well above the dissociation constant for Ca^{2+} release from the SERCA Ca^{2+} pump (165). Thus, as luminal $[Ca^{2+}]$ increases Ca^{2+} pump activity decreases, reducing the rate of Ca^{2+} clearance from the cytoplasm. In neurons, luminal Ca^{2+} binds to reticular proteins such as calreticulin (166). Expression levels of these proteins are affected by many factors, including stress and disease (167, 168). However, dynamic post-translational regulation of their Ca^{2+} buffering properties does not appear to occur. The anionic composition of the ER lumen affects Ca^{2+} uptake capacity with weak organic acids increasing capacity by binding Ca^{2+} (169). Agents such as oxalic acid have been used as tools to increase the stored Ca^{2+} available for release (170). Similarly, pyrophosphate will reversibly bind Ca^{2+} within the ER to regulate Ca^{2+} uptake and release (171). We have noted that the anionic composition of solutions used in whole-cell patch-clamp recording has a significant effect on the Ca^{2+} store (Usachev and Thayer, unpublished

observations). Luminal Ca^{2+} levels determine whether the store will act in release or uptake mode.

4.4. Functional consequences of switching between Ca^{2+} release and uptake

As described above, there are multiple mechanisms that regulate ER Ca^{2+} signaling by acting on SERCAs to alter Ca^{2+} accumulation. Changes in the ER luminal Ca^{2+} concentration directly affect the folding and trafficking of proteins within the ER (152, 172). Indeed, prolonged blockade of SERCAs results in neuronal death (173) and massive release of Ca^{2+} stores mediates necrotic cell death (174). Ca^{2+} dysregulation associated with altered Ca^{2+} stores may contribute to the neurotoxicity associated with Alzheimer's disease (175). Ca^{2+} release in peripheral neurons regulates cell excitability (176, 177) and the Ca^{2+} content of the ER also affects nuclear transport (178). Ca^{2+}-release from IP_3R and RyR contribute to neurotransmitter release in peripheral neurons (179-181). In contrast, glutamate release from presynaptic terminals in several brain regions is not sensitive to thapsigargin (182). Depletion of Ca^{2+} stores enhances neurotransmitter release from chromaffin cells and hippocampal synaptic boutons by activating Ca^{2+} influx via store-operated Ca^{2+} channels (143, 183). Oscillations in $[Ca^{2+}]_i$, produced by release from Ca^{2+} stores, play an important role in neurite outgrowth (184, 185).

Long-term potentiation and depression (LTP and LTD) of synaptic strength are both initiated by transient increases in $[Ca^{2+}]_i$. Ca^{2+} stores have been implicated in both processes although their precise role is controversial (6, 186, 187). The parallel fiber input to Purkinje neuron dendrites in the cerebellum illustrates the type of role Ca^{2+} stores might play in synaptic plasticity. Repetitive stimulation of parallel fibers produces IP_3-dependent elevation of $[Ca^{2+}]_i$ in Purkinje neuron dendritic spines (188, 189). Blockade of metabotropic glutamate receptors or treatment with thapsigargin prevents Ca^{2+} release and long-term depression (190). The release of Ca^{2+} from the store provides a spatially restricted increase in $[Ca^{2+}]_i$ required for long-term changes in synaptic plasticity. LTD evoked in acutely dissociated cells or cell culture preparations did not require IP_3-mediated Ca^{2+} release (191), indicating that a complete understanding of the role of Ca^{2+} stores in synaptic plasticity has not been achieved.

Linking ER Ca^{2+} uptake to specific physiological processes has not been straightforward. Inhibition of SERCA-mediated Ca^{2+} uptake reduces neurotransmitter release at some synapses and impairs certain forms of synaptic plasticity, but these effects are thought to result from loss of Ca^{2+} release rather than impaired Ca^{2+} clearance. Indeed, Ca^{2+} stores preferentially refill with Ca^{2+} from the extracellular pool, a process aided by activation of capacitative Ca^{2+} entry (109, 192). However, there are several examples in which Ca^{2+} uptake by the ER does appear to be important in the control of Ca^{2+} in spatially restricted spaces within neurons. SERCA-mediated Ca^{2+} uptake reduces mitochondrial Ca^{2+} uptake of small $[Ca^{2+}]_i$ increases, possibly as a result of competition with the uniporter at ER-mitochondrial junctions (193). In dendritic spines, ER Ca^{2+} pumps play a major role in clearing Ca^{2+} following stimulation (194). The hair cell efferent synapse provides an illustration of SERCA modulation of neuronal function (195). Ca^{2+} influx via postsynaptic nicotinic receptors leads to a rapid activation of Ca^{2+}-activated K^+ channels. A second, slower hyperpolarization follows. This slow phase is potentiated by inhibition of SERCA with either cyclopiazonic acid or thapsigargin. SERCA inhibition also prevents the inactivation of the slow, Ca^{2+}-activated K^+-conductance, suggesting that Ca^{2+} uptake by SERCAs is necessary to terminate the response. Sridhar *et al* (195) hypothesize that prolonged stimulation of the cholinergic terminal leads to sufficient postsynaptic Ca^{2+} influx to trigger Ca^{2+}-induced Ca^{2+}-release that spreads to other sub-plasmalemmal cisternae, activating additional K^+ channels. SERCA-mediated Ca^{2+} uptake appears to terminate the response. The slow response may protect the hair cell from over stimulation. Thus, when stimulation exceeds threshold, the Ca^{2+} influx that mediates the rapid response, also initiates Ca^{2+} release from the ER that is orders of magnitude slower and spreads to activate extra-synaptic K^+ channels. The ER changes both the temporal and spatial properties of the Ca^{2+} signal.

Inhibition of SERCAs can inhibit $[Ca^{2+}]_i$ responses resulting from blocked Ca^{2+}-induced Ca^{2+}-release or enhance $[Ca^{2+}]_i$ responses due to lost uptake and increased capacitative Ca^{2+} influx. Thus, the physiological response to modulation of ER Ca^{2+} uptake depends on the Ca^{2+} content of the ER.

5. Ca^{2+} BINDING PROTEINS

Rapid $[Ca^{2+}]_i$ buffering is primarily accomplished by Ca^{2+} binding proteins (196, 197). A large super family of proteins with the EF hand Ca^{2+} binding motif is of particular importance and includes calmodulin, parvalbumin, calbindin, S100 as well as many others (198-204). Mobile buffers account for over 80% of the Ca^{2+} binding sites in the nerve terminal (205). Because rapid modulation (sec to min) of Ca^{2+} binding has not been described, further discussion of this aspect of Ca^{2+} buffering will not be presented here. We note however, that altered expression of these proteins will have marked effects on the $[Ca^{2+}]_i$ transients with consequences ranging from altered synaptic transmission (205) to neurotoxicity (206). Thus, pharmacologic or second-messenger modulation of Ca^{2+} binding affinity could theoretically have significant effects on neuronal function.

6. PMCA

6.1. Alternative splicing generates Ca^{2+} pump isoforms with unique properties

The large number of PMCA isoforms suggests unique and specialized roles for the Ca^{2+} pumps. All four PMCA gene products are expressed in brain (207-209) and PMCAs have been localized to dendritic spines of cerebellar Purkinje neurons (210). PMCA gene products are alternatively spliced to yield at least 30 Ca^{2+} pump isoforms. Alternative splicing affects the localization,

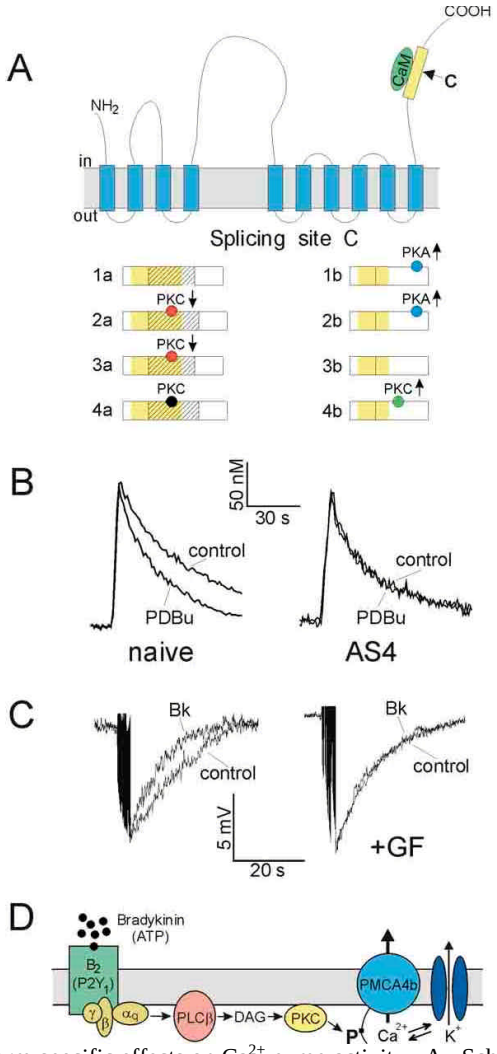

Figure 3. Phosphorylation elicits isoform-specific effects on Ca^{2+} pump activity. A. Scheme shows the general structure of the 4 PMCA gene products. Site C is within the calmodulin-binding domain (*yellow*) and has been expanded to show alternative splicing. The a variants include an exon (*hatched box*) that produces a frameshift. Alternative splicing alters the presence and position of phosphorylation sites for PKA and PKC resulting in isoform-specific effects on pump activity (↑ - stimulation, ↓ - inhibition). A consensus site for phosphorylation by PKA is present in isoforms 1b and 2b (218, 234). PKA stimulation of Ca^{2+} efflux from red blood cells and cardiac myocytes has been observed, but has not been demonstrated with well-defined samples of these isoforms. The exon included in isoforms 2a, 3a and 4a encodes a site that when phosphorylated by PKC, inhibits calmodulin binding in isoforms 2a and 3a but does not affect activity in isoform 4a (218, 232). Phosphorylation of PMCA4b at a site outside of the calmodulin-binding domain stimulates Ca^{2+} pump activity (295). B. Ca^{2+} efflux rate was studied in indo-1 AM loaded sensory neurons. Small Ca^{2+} loads were elicited in cyclopiazonic acid-treated (5 μM) cells before (*control*) and after treatment with 0.5 μM phorbol dibutyrate (*PDBu*). Recordings are from non-transfected cells (*naive*) and cells expressing antisense to PMCA4 (*AS4*). C. Membrane potential was recorded from rat sensory neurons using the perforated-patch technique. Depolarizing current injections (3-5 s, 5-10 Hz) evoked a burst of action potentials (truncated) followed by a Ca^{2+}-dependent slow afterhyperpolarization. Bradykinin (300 nM) accelerated the recovery of the afterhyperpolarization. The PKC antagonist, GF109203x (GF, 5 μM), blocked this effect. D. A model for acceleration of Ca^{2+} efflux by bradykinin and ATP. Binding of bradykinin to B_2 or ATP to $P2Y_1$ receptors activates G_q and phospholipase C. This leads to production of diacylglycerol and activation of PKC. PKC phosphorylates PMCA4b near the carboxyl terminus, resulting in acceleration of Ca^{2+} transport by the pump. Changes in Ca^{2+} efflux alter activation of $[Ca^{2+}]_i$-dependent K^+ channels. Reproduced with permission from (223).

modulation and basal activity of the pump (211, 212). PMCA isoforms are heterogeneously expressed in the nervous system, suggesting specialized functions unique to particular cell types (213). The discussion here will focus on the results of splicing that alters the sequence of the calmodulin binding domain, referred to as site C (214, 215)(Figure 3A). In the absence of Ca^{2+}/calmodulin, the carboxyl tail of the PMCA protein acts as an autoinhibitory

Table 3. The a and b isoforms of PMCA4 have different sensitivities to Ca^{2+}/calmodulin

Isoform	Basal activity (V_{max}, %)	Ca^{2+} affinity ($K_{1/2}$, mM)	Ca^{2+}/Calmodulin activation rate ($t_{1/2}$, s)	Inactivation rate ($t_{1/2}$, min)	CaM affinity ($K_{1/2}$, nM)
4a	39	0.84	20	<1	126
4b	8.1	0.29	60	20	18

Basal activity data are from (239), Ca^{2+} and calmodulin affinity data from (214) and activation/inactivation rates from (217).

domain that blocks Ca^{2+} translocation (216). The binding of Ca^{2+}/calmodulin to the carboxyl tail prevents this intramolecular interaction, stimulating Ca^{2+} pumping activity. Alternative splicing of site C affects the affinity of the resulting PMCA isoform for Ca^{2+}/calmodulin (Table 3)(217). Phosphorylation by protein kinases A and C affects Ca^{2+} pump activity in an isoform specific manner (Figure 3A) (218, 219).

6.2. PMCAs provide high affinity Ca^{2+} extrusion

PMCAs are the predominant mechanism for returning $[Ca^{2+}]_i$ back to basal levels following modest Ca^{2+} loads, such as those produced by short trains of action potentials (220, 221). In sensory neurons, the PMCA appears to be the primary mechanism for extruding Ca^{2+} from the cell and its role in $[Ca^{2+}]_i$ recovery kinetics is particularly apparent during recovery from small Ca^{2+} loads. The kinetics of $[Ca^{2+}]_i$ recovery varies considerably between preparations, due to factors that include Ca^{2+} pump density, surface to volume ratio and method of Ca^{2+} measurement. PMCAs are located near neurotransmitter release zones (222) and are the principal means for $[Ca^{2+}]_i$ recovery at ribbon presynaptic terminals (33), sensory neuron varicosities (223) and in motor nerve terminals (224). This high affinity Ca^{2+} transport sets the resting $[Ca^{2+}]_i$ and determines the duration of small amplitude $[Ca^{2+}]_i$ transients.

6.3. Pharmacologic modulation of PMCAs

Agents acting directly and selectively on the PMCA to modulate Ca^{2+} pumping are limited. Lanthanum acts at an intracellular site to inhibit PMCA function, but this cation is not specific and inhibits many Ca^{2+}-dependent processes including Ca^{2+} channels (225, 226). Carboxyeosin is more selective and inhibits PMCA function at micromolar concentrations (IC_{50}=0.2-1 μM). It acts at an intracellular site; thus, for studies on intact cells, the esterified form is more effective, but also more difficult to reverse (227-230). Screening of a peptide library identified caloxin, an apparently selective PMCA inhibitor (231). Caloxin acts on an extracellular site at millimolar concentrations. Clearly, potent and selective inhibitors of PMCA function will be useful tools for research and, if isoform-selective agents were developed, might have therapeutic potential.

6.4. Selective modulation of PMCA isoforms by endogenous signaling pathways

Recent work from our laboratory has examined the influence of signaling cascades on PMCA function in sensory neurons. An example of modulation of PMCA mediated $[Ca^{2+}]_i$ recovery is shown in Figure 3B. With SERCA type Ca^{2+} pumps blocked, small increases in $[Ca^{2+}]_i$ recovered to basal levels via PMCAs 2 and 4, the predominant isoforms expressed in these cells (223). Activation of PKC accelerated $[Ca^{2+}]_i$ recovery kinetics via a process that was blocked by PKC inhibitors and absent in cells expressing antisense to PMCA4. Thus, activation of metabotropic receptors that couple to phospholipase C, with subsequent activation of PKC, would be predicted to stimulate Ca^{2+} efflux. We found this to be true for sensory neurons in which bradykinin and ATP accelerated PMCA4b-mediated Ca^{2+} efflux kinetics via activation of PKC.

Other potential interactions for the a and b PMCA isoforms with signaling cascades are summarized in Figure 3A. PKC-dependent phosphorylation inhibits the activity of the 2a and 3a isoforms by decreasing the affinity of the pump for calmodulin (232, 233). Phosphorylation of PMCA4a does not alter pump activity, presumably because this site is within a hairpin structure that does not participate in calmodulin binding to this isoform (218). The 1b isoform is phosphorylated by PKA and the 2b isoform also contains a consensus sequence for phosphorylation by PKA (218, 234). Phosphorylation of these isoforms may contribute to enhanced Ca^{2+} efflux from cAMP-stimulated red blood cells and cardiac myocytes (235-237). Protein kinases A and C phosphorylate PMCAs in the CNS in a region specific manner (238). Ca^{2+}/calmodulin stimulates all PMCAs expressed in neurons (211). However, alternative splicing of the particular gene products influences the association and dissociation kinetics of calmodulin binding to the PMCA (Table 3). For example, PMCA isoform 2a rapidly binds Ca^{2+}/calmodulin, enabling its activity to closely track changes in $[Ca^{2+}]_i$ (239). Alternatively, calmodulin dissociates very slowly ($t_{1/2}$= >20 min) from isoform 4b enabling the pump to "remember" an increase in $[Ca^{2+}]_i$ (240). In sensory neurons, PMCA activity remains enhanced for as long as an hour following a large increase in $[Ca^{2+}]_i$ (241).

6.5. PMCAs are sites where signaling pathways converge

The heterogeneous expression of PMCA isoforms that differ in sensitivity to modulation by Ca^{2+}, diacylglycerol, and cAMP signaling cascades identify plasma membrane Ca^{2+} pumps as dynamic regulators of $[Ca^{2+}]_i$ recovery kinetics in neurons. PMCA isoforms specialize in particular neuronal functions, especially those triggered by sub-plasmalemmal increases in $[Ca^{2+}]_i$ such as neurotransmitter release and membrane excitability. For example, sensory neurons exhibit a pronounced slow afterhyperpolarization following bursts of action potentials that is mediated by Ca^{2+}-activated K^+ channels (242, 243). Bradykinin, which acts on metabotropic receptors to stimulate PKC, accelerated PMCA activity. The reduced duration of the $[Ca^{2+}]_i$ increase produced a corresponding decrease in the duration of the afterhyperpolarization

(Figure 3B-D). This excitatory effect may underlie the inhibition of spike frequency accommodation produced by bradykinin (243). Thus, neuronal PMCAs are susceptible to cross talk with other signaling pathways and modulation of a particular splice variant controls a specific Ca^{2+}-sensitive neuronal function. PMCAs are poised to integrate diverse input signals to alter the duration of $[Ca^{2+}]_i$-sensitive membrane events.

7. PLASMALEMMAL Na^+/Ca^{2+} EXCHANGE

All three Na^+/Ca^{2+} exchange gene products (NCX) are expressed in brain with NCX1 most abundant (244). At least three isoforms of the K^+-dependent Na^+/Ca^{2+} exchanger (NCKX) are also expressed in brain (245, 246), although little is currently known about their functional role in neuronal Ca^{2+} regulation. Alternative splicing of NCX1 transcripts can yield 12 isoforms (247). The alternatively spliced NCX transcripts display tissue specific expression suggesting functional specialization of the NCX proteins. However, unique roles for most isoforms have not yet been identified.

7.1. Na^+/Ca^{2+} exchange provides low affinity high turnover Ca^{2+} extrusion

The Na^+/Ca^{2+} exchanger has an approximately 10-fold lower affinity for Ca^{2+} and an approximately 10-50-fold higher turnover rate than the PMCA (244). Thus, the Na^+/Ca^{2+} exchanger is well suited to the rapid removal of large Ca^{2+} loads. Pioneering work on the squid giant axon showed that Ca^{2+} and Na^+ transport across the membrane were coupled and reversible (248). Subsequently, Na^+-dependent modulation of $[Ca^{2+}]_i$ recovery has been reported in neuronal somata (249), including sensory neurons (250), although separating the role of Na^+/Ca^{2+} exchange across the plasma membrane from that resulting from exchange across the mitochondrial inner membrane complicates interpretation of many studies. Immunohistochemistry has shown particularly high levels of NCX-like protein in nerve terminals, consistent with the most robust demonstration of neuronal Na^+/Ca^{2+} exchange in preparations of nerve endings (224, 251, 252). Catecholamine release from adrenal chromaffin cells is an established model for studying neurosecretory processes with properties similar to adrenergic nerve terminals. Figure 4A shows an example from Tang *et al* (253) in which Na^+/Ca^{2+} exchange operates to lower $[Ca^{2+}]_i$ in a chromaffin cell. The recording shows recovery from depolarization-induced increases in $[Ca^{2+}]_i$ in the presence and absence of extracellular Na^+. Na^+-dependent Ca^{2+} efflux plays a significant role in removing Ca^{2+} from the cytoplasm of these cells (253). Factors that modulate Na^+/Ca^{2+} exchange in neurons are presented in Table 4.

7.2. Thermodynamic modulation of Na^+/Ca^{2+} exchange

The Na^+/Ca^{2+} exchanger is electrogenic; 3 Na^+ are transported in exchange for each Ca^{2+} moved across the membrane. Thus, changes in $[Na^+]_i$ and membrane potential (V_m) provide an important means to modulate Ca^{2+} flux via Na^+/Ca^{2+} exchange. Indeed, because Na^+ influx accompanies intense electrical activity, Na^+/Ca^{2+} exchange is reduced and even reversed following high frequency stimulation in crayfish neuromuscular junction (224). Na^+ loads introduced by activation of ligand-gated channels contribute to Ca^{2+} influx and impair $[Ca^{2+}]_i$ recovery in central neurons (23, 254, 255). Thus, modulation of $[Na^+]_i$ can profoundly affect Ca^{2+} clearance in neurons.

7.3. Modulation of Na^+/Ca^{2+} exchange by second messengers

Intracellular ATP levels modulate Na^+/Ca^{2+} exchange even though ATP hydrolysis is not required for catalytic activity of the exchanger (256). Phosphorylation of the Na^+/Ca^{2+} exchanger by protein kinase C accelerates both Ca^{2+} efflux and influx in rat brain synaptosomes (257). Activation of PKC with phorbol esters stimulates Na^+/Ca^{2+} exchange in a number of tissues, including heart (258). However, phorbol esters failed to stimulate Na^+/Ca^{2+} exchange in some neuronal preparations (259), possibly because of differential sensitivity of splice variants to phosphorylation (260). Nitric oxide donors and cGMP analogs stimulate Na^+/Ca^{2+} exchange in rat brain slices and synaptosomes (261). A neuronal isoform of NCX1 is stimulated by PKA when expressed in Xenopus oocytes (262). In some tissues, modulation of Na^+/Ca^{2+} exchange by kinases appears to be mediated by phosphorylation of an accessory protein (263, 264); it is not clear whether this indirect mechanism occurs in mammalian neurons. Genistein inhibited Na^+/Ca^{2+} exchange in cortical neurons in culture, suggesting stimulation by tyrosine phosphorylation as well (259). Clearly, phosphorylation modulates Na^+/Ca^{2+} exchange in neurons. The sensitivity of each isoform varies and likely accounts for some of the discrepant reports. Phosphatidylinositol-4,5-bisphosphate (PIP_2) also modulates Na^+/Ca^{2+} exchange. In heart, elevated ATP increases the formation of PIP_2 with subsequent stimulation of Na^+/Ca^{2+} exchange (265). PIP_2 may bind directly to the autoinhibitory domain on the exchanger (266). In summary, ATP stimulates Na^+/Ca^{2+} exchange by direct phosphorylation of the exchanger and indirectly via phosphorylation of accessory proteins and phospholipids.

Ca^{2+} and Na^+ binding to high affinity regulatory sites also modulate Na^+/Ca^{2+} exchange. As mentioned in section 7.2, elevated $[Na^+]_i$ stimulates Ca^{2+} entry via the Na^+/Ca^{2+} exchanger due to thermodynamic effects. The outward current produced during Ca^{2+} entry rapidly inactivates to a new steady state as a result of a Na^+- and time-dependent process (267). $[Ca^{2+}]_i$ also exerts both thermodynamic and regulatory effects on Na^+/Ca^{2+} exchange. $[Ca^{2+}]_i$ is required for Ca^{2+} entry via Na^+/Ca^{2+} exchange demonstrating a regulatory role (268). A large intracellular loop of the exchanger is required for regulation by both Na^+ and Ca^{2+} (258). Ca^{2+} binds to a regulatory site on the loop; a discrete binding site for Na^+ has not been identified. In summary, increases in $[Ca^{2+}]_i$ and $[Na^+]_i$ produce opposite regulatory effects on Na^+/Ca^{2+} exchange, $[Ca^{2+}]_i$ stimulates and $[Na^+]_i$ inhibits.

7.4. Pharmacologic modulation of Na^+/Ca^{2+} exchange

Chemical analogs of amiloride, such as 3',4'-dichlorobenzamil will inhibit the exchanger at micromolar

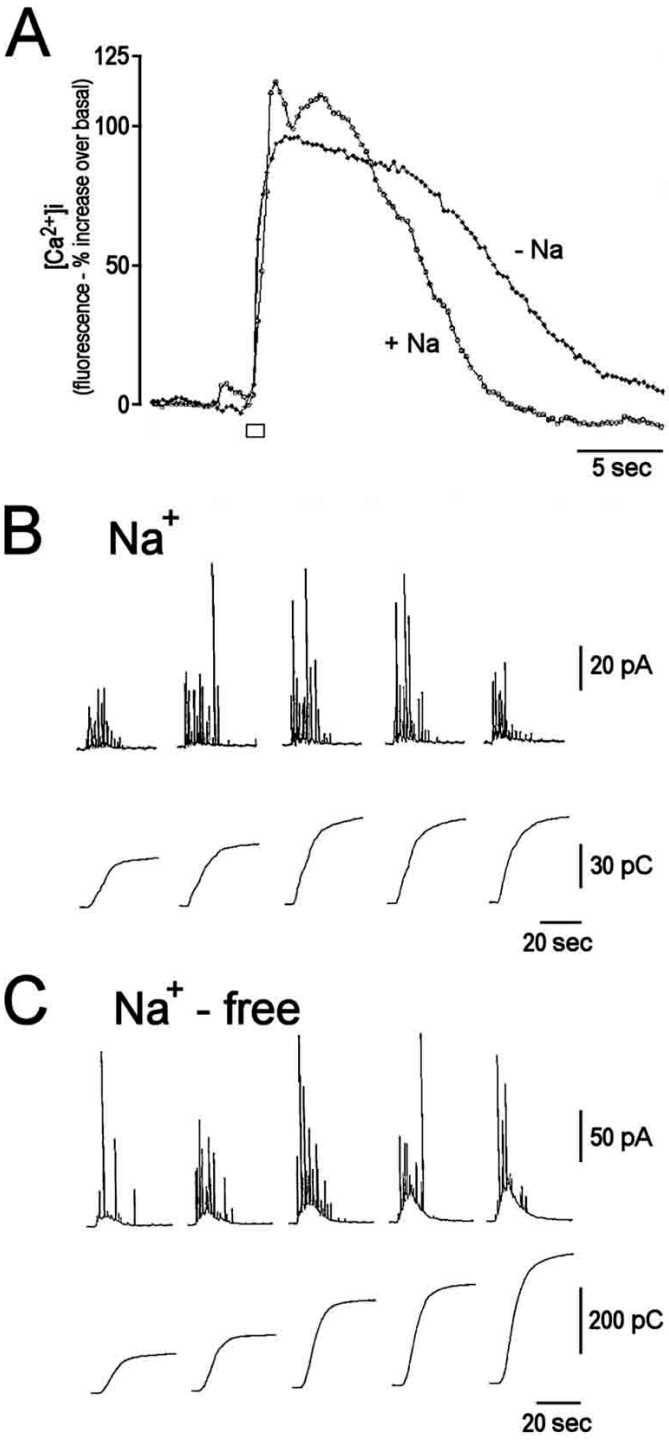

Figure 4. Na^+ dependence of $[Ca^{2+}]_i$ decay and catecholamine release from chromaffin cells. A. Depolarization (75 mM K^+, 1 s, ϒ) induced $[Ca^{2+}]_i$ increases in Oregon Green loaded chromaffin cells. Recordings in the presence (+*Na*) and absence of extracellular Na^+ (-*Na*; Na^+ replaced with N-methylglucamine) recovered with half times of 5.6 and 12.7 s, respectively. B. Amperometric detection of rate of catecholamine release in the presence of Na^+. Exocytotic current spikes are shown from a single chromaffin cell stimulated five times at 90-s intervals with 75 mM K^+ in the presence of Na^+ (*top*). Current traces in upper panel were integrated to show charge as a function of time (*bottom*). C. Same as B above but in the absence of extracellular Na^+. Note the different scales in B versus C and the increase in catecholamine release with repetitive stimulation in the absence of Na^+. Reproduced with permission from author and publisher of (253).

Table 4. Modulation of Na^+/Ca^{2+} exchange

Modulator	EC_{50}, mM	Effect	Mode	References
Pharmacologic				
KB R7943	0.3-2	Inhibit	Ca^{2+} entry	275, 276
	17-30	Inhibit	Ca^{2+} efflux	
3',4'-dichlorobenzamil	17	Inhibit	Ca^{2+} entry & efflux	269, 270
bepridil	30	Inhibit	Ca^{2+} entry (partial) & efflux	274
Zn^{2+}	15	Inhibit	Ca^{2+} influx	278
	>30	Inhibit	Ca^{2+} efflux	
Thermodynamic				
depolarize V_m		Inhibit	Ca^{2+} efflux	244
		Stimulate	Ca^{2+} entry	
↑ $[Na^+]_i$		Inhibit	Ca^{2+} efflux	244
		Stimulate	Ca^{2+} entry	
Signaling				
PKC	-	Stimulate	Ca^{2+} entry & efflux	257
PKA	-	Stimulate	Ca^{2+} efflux	262
PIP_2	<50	Stimulate	Ca^{2+} influx	265
$[Ca^{2+}]_i$	0.01-1	Stimulate	Ca^{2+} influx & efflux	268
$[Na^+]_i$	>40 mM	Inhibit	Ca^{2+} influx	267

concentrations (269, 270). These compounds inhibit both Ca^{2+} entry and Ca^{2+} efflux modes of the exchanger (271) and have additional non-selective inhibitory effects on ion channels at high concentrations (272, 273). The antiarrhythmic drug bepridil also inhibits Na^+/Ca^{2+} exchange (274). KB R7943 preferentially inhibits Na^+/Ca^{2+} exchange in the Ca^{2+} entry mode (275, 276). The selectivity of this compound for inhibition of Ca^{2+} entry versus Ca^{2+} efflux mode depends on the experimental conditions (277). Inorganic cations such as Zn^{2+} also inhibit Ca^{2+} efflux via Na^+/Ca^{2+} exchange (278). La^{3+} is not effective at concentrations that spare other influx and efflux mechanisms (226). Currently available drugs that inhibit Na^+/Ca^{2+} exchange are effective but neither potent nor selective.

7.5. Functional consequences of modulating Na^+/Ca^{2+} exchange in neurons

Na^+/Ca^{2+} exchange appears to play a major role in excitation-secretion coupling in neuronal tissue, analogous to its role in excitation-contraction coupling in heart. The Na^+/Ca^{2+} exchanger acts in either of two modes depending on the activation state of the cell. It serves as a low affinity, high capacity Ca^{2+} extrusion mechanism when $[Na^+]_i$ is low. When $[Na^+]_i$ is high and/or the membrane depolarized, the Na^+/Ca^{2+} exchanger provides a route for Ca^{2+} entry.

Blockade of Ca^{2+} efflux via Na^+/Ca^{2+} exchange enhances neurotransmitter release from rat brain synaptosomes (252), adrenal chromaffin cells (253, 279) and cultured hippocampal neurons (280, 281). For an extensive list of studies that demonstrate Na^+-dependent modulation of synaptic transmission see Blaustein and Lederer (244). Figure 4 shows an example from Tang *et al* (253) in which Na^+/Ca^{2+} exchange operates to lower $[Ca^{2+}]_i$ in a chromaffin cell. Thus, its inhibition by removal of extracellular Na^+ resulted in a slowed return to basal $[Ca^{2+}]_i$ following a depolarizing stimulus. Depolarization-induced release of catecholamines was greatly enhanced in the absence of extracellular Na^+ (Figure 4B and C). Repetitive application of depolarizing stimuli in the absence of Na^+, evoked progressively more release of catecholamines measured by cyclic voltametry. This is consistent with impaired Ca^{2+} efflux allowing $[Ca^{2+}]_i$ to accumulate and more effectively trigger secretion. This result is consistent with the prominent role of Na^+/Ca^{2+} exchange in clearing Ca^{2+} from active secretory zones.

In crayfish motor terminals, the Na^+/Ca^{2+} exchanger actually mediates Ca^{2+} influx. Thus, inhibition of Ca^{2+} entry with KB R7943 reduced the accumulation of Ca^{2+} during tetanus, resulting in decreased post-tetanic potentiation of the neuromuscular junction (224). In amacrine cells, prolonged depolarization induces Ca^{2+} influx via the Na^+/Ca^{2+} exchanger and evokes GABA release; repolarization induces Ca^{2+} efflux via the exchanger and terminates neurotransmitter release (282, 283). There are clearly situations in which Ca^{2+} enters the nerve terminal via Na^+/Ca^{2+} exchange; however, it remains unclear whether these findings can be generalized broadly.

Because Na^+/Ca^{2+} exchange mediates Ca^{2+} entry during intense stimuli that depolarize and/or elevate $[Na^+]_i$, drugs such as KB R7943 that selectively block Ca^{2+} entry may prove effective in preventing excessive excitation in neuronal systems. KB R7943 reduced phospholipase activity following cerebral ischemia (284) and protected hippocampal slices from hypoxic/hypoglycemic injury (285). The neuroprotective effects of some Na^+ channel blockers may also result in part from reduced Ca^{2+} influx via Na^+/Ca^{2+} exchange (286). However, KB R7943 did not protect cortical neurons from glutamate-induced neurotoxicity (255), suggesting that during prolonged glutamate exposure Ca^{2+} influx via the Na^+/Ca^{2+} exchanger is not a major factor. Other studies have found that inhibition of Na^+/Ca^{2+} exchange potentiates neurotoxicity, suggesting that the exchanger operating in Ca^{2+} efflux mode helps to protect neurons from Ca^{2+} overload (287-289). The role of the Na^+/Ca^{2+} exchanger in neuronal injury varies with the preparation and type of insult. Thus, the utility of drugs that modulate Na^+/Ca^{2+} exchange as neuroprotective agents is not clear.

In summary, the low affinity, high turnover rate, and reversibility of the Na^+/Ca^{2+} exchanger make it well suited to participate in the control of neurosecretion. The relative contribution of the exchanger to both Ca^{2+} entry and Ca^{2+} efflux appears to vary between release sites. Although the role of Na^+/Ca^{2+} exchange in neurotoxicity is presently unclear, further investigation may determine conditions in which Na^+/Ca^{2+} exchange inhibitors improve neuronal survival.

8. PERSPECTIVES

8.1. Competing for Ca^{2+} - the integrated response

The various Ca^{2+} uptake and efflux processes compete for cytoplasmic Ca^{2+}. The predominant process is determined by affinity, rate, capacity and location relative to the Ca^{2+} source. Drugs and second messengers principally modulate the affinity and rate with resulting effects on the amplitude, duration, and spatial distribution of the $[Ca^{2+}]_i$ signal. The inherent redundancy in Ca^{2+} clearance mechanisms can make these effects subtle. The cell's ability to compensate for the reduced function of one element of the Ca^{2+} clearance machinery complicates study of the modulation of these processes (290). Sorting out the overlapping and dynamic contributions of Ca^{2+} regulatory processes will be important for determining the specific roles of the individual processes and how their modulation by drugs and second messengers affect the cellular response.

8.2. Future directions

The increasingly apparent diversity of the molecular entities that make up the $[Ca^{2+}]_i$ regulatory system reveals new sites for modulation and links particular $[Ca^{2+}]_i$ clearance processes to specific cellular functions. The diversity and specialization of the PMCA isoforms created by alternative splicing was the specific example discussed here, but heterogeneity in Na^+/Ca^{2+} exchange (247), SERCAs (124, 125), RyR and IP_3R (291, 292) and mitochondria (60, 90, 293) have been described. These Ca^{2+} regulatory mechanisms are expressed in combinations tailored to the needs of specific cell-types and even particular regions within a cell. Future elucidation of the types of signaling in which these molecular targets participate will identify means to modulate specific functions. For example, drugs that reduce mitochondrial Ca^{2+} uptake might protect neurons from Ca^{2+}-induced apoptosis (38, 91). In failing heart, decreased SERCA activity can be restored by ectopic expression of SERCA1a, enhancing contractility and providing a potential therapeutic approach to heart failure (294). As shown in Figure 3, phosphorylation of a particular PMCA isoform alters the excitability of sensory neurons (223). Inhibition of Na^+/Ca^{2+} exchange impaired short-term plasticity of the crayfish neuromuscular junction (224). These recent findings support our contention that specialized Ca^{2+} clearance mechanisms participate in unique cellular functions and thus, represent important targets for pharmacological and physiological regulation of the neuron.

Modulation of $[Ca^{2+}]_i$ clearance mechanisms can influence cell functions ranging from excitability to death. The complex array of proteins that make up the Ca^{2+} clearance system would seem to present attractive pharmacologic targets for modulation of neuronal function. However, compounds discovered to date tend to be toxic and their use limited to research applications. Development of highly selective agents could yield drugs with the potential to alter synaptic transmission, to adjust electrical excitability and afford neuroprotection.

9. ACKNOWLEDGEMENTS

The National Science Foundation (IBN0110409) and the National Institutes of Health (DA07304, DA11806) supported the preparation of this article.

10. REFERENCES

1. Ghosh, A. & M. E. Greenberg: Calcium signaling in neurons: molecular mechanisms and cellular consequences. *Science* 268, 239-247 (1995)
2. Triggle, D. J.: The pharmacology of ion channels with particular reference to voltage- gated Ca^{2+} channels. *Eur J Pharmacol* 375, 311-325. (1999)
3. Herlitze, S., H. J. Zhong, T. Scheuer & W. A. Catterall: Allosteric modulation of Ca^{2+} channels by G proteins, voltage-dependent facilitation, protein kinase C, and Ca(v)beta subunits. *Proc. Natl. Acad. Sci. USA* 98, 4699-4704 (2001)
4. McCleskey, E. W. & M. S. Gold: Ion channels of nociception. *Annu Rev Physiol* 61, 835-856 (1999)
5. Tominaga, M., M. J. Caterina, A. B. Malmberg, T. A. Rosen, H. Gilbert, K. Skinner, B. E. Raumann, A. I. Basbaum & D. Julius: The cloned capsaicin receptor integrates multiple pain-producing stimuli. *Neuron* 21, 531-543 (1998)
6. Berridge, M. J.: Neuronal calcium signaling. *Neuron* 21, 13-26 (1998)
7. Petersen, O. H. & J. M. Cancela: New Ca^{2+}-releasing messengers: are they important in the nervous system? *Trends Neurosci.* 22, 488-494 (1999)
8. Berridge, M. J., P. Lipp & M. D. Bootman: The versatility and universality of calcium signalling. *Nat. Rev. Molec. Cell Bio.* 1, 11-21 (2000)
9. Meyer, T., P. I. Hanson, L. Stryer & H. Schulman: Calmodulin trapping by calcium-calmodulin-dependent protein kinase. *Science* 256, 1199-1202 (1992)
10. Soderling, T. R.: CaM-kinases: modulators of synaptic plasticity. *Curr. Opinion Neurobiol.* 10, 375-380 (2000)
11. Fernandez-Chacon, R., A. Konigstorfer, S. H. Gerber, J. Garcia, M. F. Matos, C. F. Stevens, N. Brose, J. Rizo, C. Rosenmund & T. C. Sudhof: Synaptotagmin I functions as a calcium regulator of release probability. *Nature* 410, 41-49. (2001)
12. Augustine, G. J.: How does calcium trigger neurotransmitter release? *Curr. Opinion Neurobiol.* 11, 320-326 (2001)
13. Sakaba, T. & E. Neher: Calmodulin mediates rapid recruitment of fast-releasing synaptic vesicles at a calyx-type synapse. *Neuron* 32, 1119-1131. (2001)
14. Werth, J. L. & S. A. Thayer: Mitochondria buffer physiological calcium loads in cultured rat dorsal root ganglion neurons. *J. Neurosci.* 14, 348-356 (1994)

15. Friel, D. D. & R. W. Tsien: An FCCP-sensitive Ca^{2+} store in bullfrog sympathetic neurons and its participation in stimulus-evoked changes in $[Ca^{2+}]_i$. *J. Neurosci.* 14, 4007-4024 (1994)
16. Rizzuto, R., P. Pinton, W. Carrington, F. S. Fay, K. E. Fogarty, L. M. Lifshitz, R. A. Tuft & T. Pozzan: Close contacts with the endoplasmic reticulum as determinants of mitochondrial Ca^{2+} responses. *Science* 280, 1763-1766 (1998)
17. Rizzuto, R., M. Brini, M. Murgia & T. Pozzan: Microdomains with high Ca^{2+} close to IP_3-sensitive channels that are sensed by neighboring mitochondria. *Science* 262, 744-747 (1993)
18. Montero, M., M. T. Alonso, E. Carnicero, I. Cuchillo-Ibanez, A. Albillos, A. G. Garcia, J. Garcia-Sancho & J. Alvarez: Chromaffin-cell stimulation triggers fast millimolar mitochondrial Ca^{2+} transients that modulate secretion. *Nature Cell Biology* 2, 57-61 (2000)
19. Pozzan, T. & R. Rizzuto: The renaissance of mitochondrial calcium transport. *European Journal of Biochemistry* 267, 5269-5273 (2000)
20. Herrington, J., Y. B. Park, D. F. Babcock & B. Hille: Dominant role of mitochondria in clearance of large Ca^{2+} loads from rat adrenal chromaffin cells. *Neuron* 16, 219-228 (1996)
21. Babcock, D. F., J. Herrington, P. C. Goodwin, Y. B. Park & B. Hille: Mitochondrial participation in the intracellular Ca^{2+} network. *J Cell Biol* 136, 833-844. (1997)
22. Wang, G. J. & S. A. Thayer: Sequestration of glutamate-induced Ca^{2+} loads by mitochondria in cultured rat hippocampal neurons. *J. Neurophysiol.* 76, 1611-1621 (1996)
23. White, R. & I. Reynolds: Mitochondria and Na^+/Ca^{2+} exchange buffer glutamate-induced calcium loads in cultured cortical neurons. *J. Neurosci.* 15, 1318-1328 (1995)
24. Brocard, J. B., M. Tassetto & I. J. Reynolds: Quantitative evaluation of mitochondrial calcium content in rat cortical neurones following a glutamate stimulus. *J. Physiol. (Lond.)* 531, 793-805 (2001)
25. David, G. & E. F. Barrett: Stimulation-evoked increases in cytosolic $[Ca^{2+}]$ in mouse motor nerve terminals are limited by mitochondrial uptake and are temperature- dependent. *J Neurosci* 20, 7290-7296. (2000)
26. McCormack, J. G. & R. M. Denton: The role of intramitochondrial Ca^{2+} in the regulation of oxidative phosphorylation in mammalian tissues. *Biochem. Soc. Trans.* 21, 793-799 (1993)
27. Duchen, M. R.: Ca^{2+}-dependent changes in the mitochondrial energetics in single dissociated mouse sensory neurons. *Biochem J* 283, 41-50. (1992)
28. Kaftan, E. J., T. Xu, R. F. Abercrombie & B. Hille: Mitochondria shape hormonally induced cytoplasmic calcium oscillations and modulate exocytosis. *J. Biol. Chem.* 275, 25465-25470 (2000)
29. Biden, T. J., C. B. Wollheim & W. Schlegel: Inositol 1,4,5-trisphosphate and intracellular Ca^{2+} homeostasis in clonal pituitary cells (GH3). Translocation of Ca^{2+} into mitochondria from a functionally discrete portion of the nonmitochondrial store. *J Biol Chem* 261, 7223-7229. (1986)
30. Gilabert, J. A. & A. B. Parekh: Respiring mitochondria determine the pattern of activation and inactivation of the store-operated Ca^{2+} current I-CRAC. *EMBO J* 19, 6401-6407 (2000)
31. Arnaudeau, S., W. L. Kelley, J. V. Walsh & N. Demaurex: Mitochondria recycle Ca^{2+} to the endoplasmic reticulum and prevent the depletion of neighboring endoplasmic reticulum regions. *J. Biol. Chem.* 276, 29430-29439 (2001)
32. Tang, Y. G. & R. S. Zucker: Mitochondrial involvement in post-tetanic potentiation of synaptic transmission. *Neuron* 18, 483-491 (1997)
33. Zenisek, D. & G. Matthews: The role of mitochondria in presynaptic calcium handling at a ribbon synapse. *Neuron* 25, 229-237 (2000)
34. Robb-Gaspers, L. D., G. A. Rutter, P. Burnett, G. Hajnoczky, R. M. Denton & A. P. Thomas: Coupling between cytosolic and mitochondrial calcium oscillations: role in the regulation of hepatic metabolism. *Biochim Biophys Acta* 1366, 17-32 (1998)
35. Sparagna, G. C., K. K. Gunter, S. S. Shen & T. E. Gunter: Mitochondrial calcium uptake from physiological-type pulses of calcium - a description of the rapid uptake mode. *J. Biol. Chem.* 270, 27510-27515 (1995)
36. Nicotera, P. & S. Orrenius: The role of calcium in apoptosis. *Cell Calcium* 23, 173-180 (1998)
37. Zipfel, G. J., D. J. Babcock, J. M. Lee & D. W. Choi: Neuronal apoptosis after CNS injury: The roles of glutamate and calcium. *Journal of Neurotrauma* 17, 857-869 (2000)
38. Nicholls, D. G. & S. L. Budd: Mitochondria and neuronal survival. *Physiol. Rev.* 80, 315-360 (2000)
39. Gunter, T. E., L. Buntinas, G. Sparagna, R. Eliseev & K. Gunter: Mitochondrial calcium transport: mechanisms and functions. *Cell Calcium* 28, 285-296 (2000)
40. Nicholls, D. & K. Akerman: Mitochondrial calcium transport. *Biochim Biophys Acta* 683, 57-88. (1982)
41. Lehninger, A. L.: Role of phosphate and other proton-donating anions in respiration- coupled transport of Ca^{2+} by mitochondria. *Proc Natl Acad Sci U S A* 71, 1520-1524. (1974)
42. Crompton, M., M. Kunzi & E. Carafoli: The calcium-induced and sodium-induced effluxes of calcium from heart mitochondria. Evidence for a sodium-calcium carrier. *Eur J Biochem* 79, 549-558. (1977)
43. Crompton, M., E. Sigel, M. Salzmann & E. Carafoli: The sodium-induced efflux of calcium from heart mitochondria. *Eur. J. Biochem.* 69, 429-434 (1976)
44. Thayer, S. A. & R. J. Miller: Regulation of the free intracellular calcium concentration in rat dorsal root ganglion neurones *in vitro*. *J. Physiol. (Lond.)* 425, 85-115 (1990)
45. Bernardi, P.: Mitochondrial transport of cations: channels, exchangers, and permeability transition. *Physiol Rev* 79, 1127-1155. (1999)
46. Ichas, F., L. S. Jouaville & J. P. Mazat: Mitochondria Are Excitable Organelles Capable of Generating and Conveying Electrical and Calcium Signals. *Cell* 89, 1145-1153 (1997)
47. Kristal, B. S. & J. M. Dubinsky: Mitochondrial permeability transition in the central nervous system -

induction by calcium cycling-dependent and -independent pathways. *J. Neurochem.* 69, 524-538 (1997)
48. Zoratti, M. & I. Szabo: The mitochondrial permeability transition. *Biochim Biophys Acta* 1241, 139-176. (1995)
49. Gunter, T. E., L. Buntinas, G. Sparagna, R. Eliseev & K. Gunter: Mitochondrial calcium transport: mechanisms and functions. *Cell Calcium* 28, 285-296. (2000)
50. Slater, E. C.: The mechanism of action of the respiratory inhibitor, antimycin. *Biochim. Biophys. Acta* 301, 129-154 (1973)
51. Wang, G.-J., S. R. Richardson & S. A. Thayer: Intracellular acidification is not a prerequisite for glutamate-triggered death of cultured hippocampal neurons. *Neurosci Lett* 186, 139-144 (1995)
52. Ishida, H., Y. Hirota, C. Genka, H. Nakazawa, H. Nakaya & T. Sato: Opening of mitochondrial K(ATP) channels attenuates the ouabain-induced calcium overload in mitochondria. *Circ Res* 89, 856-858. (2001)
53. Murata, M., M. Akao, B. O'Rourke & E. Marban: Mitochondrial ATP-sensitive potassium channels attenuate matrix Ca^{2+} overload during simulated ischemia and reperfusion: possible mechanism of cardioprotection. *Circ Res* 89, 891-898. (2001)
54. Budd, S. L. & D. G. Nicholls: A reevaluation of the role of mitochondria in neuronal Ca^{2+} homeostasis. *J. Neurochem.* 66, 403-411 (1996)
55. Brustovetsky, N. & J. M. Dubinsky: Limitations of cyclosporin A inhibition of the permeability transition in CNS mitochondria. *J Neurosci* 20, 8229-8237. (2000)
56. Kim-Han, J. S., S. A. Reichert, K. L. Quick & L. L. Dugan: BMCP1: a mitochondrial uncoupling protein in neurons which regulates mitochondrial function and oxidant production. *J Neurochem* 79, 658-668. (2001)
57. Jouaville, L. S., F. Ichas, E. L. Holmuhamedov, P. Camacho & J. D. Lechleiter: Synchronization of calcium waves by mitochondrial substrates in xenopus laevis oocytes. *Nature* 377, 438-441 (1995)
58. Nicholls, D. G. & S. J. Ferguson, in *Bioenergetics* D. G. Nicholls, S. J. Ferguson, Eds. (Academic press, London, 1992) pp. 207-234.
59. Nicholls, D. G.: The regulation of extramitochondrial free calcium ion concentration by rat liver mitochondria. *Biochem. J.* 176, 463-474 (1978)
60. White, R. J. & I. J. Reynolds: Mitochondrial depolarization in glutamate-stimulated neurons - an early signal specific to excitotoxin exposure. *J. Neurosci.* 16, 5688-5697 (1996)
61. Robb-Gaspers, L. D., P. Burnett, G. A. Rutter, R. M. Denton, R. Rizzuto & A. P. Thomas: Integrating cytosolic calcium signals into mitochondrial metabolic responses. *EMBO J* 17, 4987-5000. (1998)
62. Buntinas, L., K. K. Gunter, G. C. Sparagna & T. E. Gunter: The rapid mode of calcium uptake into heart mitochondria (RaM): comparison to RaM in liver mitochondria. *Biochimica et Biophysica Acta - Bioenergetics* 1504, 248-261 (2001)
63. Litsky, M. L. & D. R. Pfeiffer: Regulation of the mitochondrial Ca^{2+} uniporter by external adenine nucleotides - the uniporter behaves like a gated channel which is regulated by nucleotides and divalent cations. *Biochem.* 36, 7071-7080 (1997)
64. Matlib, M. A., Z. Zhou, S. Knight, S. Ahmed, K. M. Choi, J. Krausebauer, R. Phillips, R. Altschuld, Y. Katsube, N. Sperelakis & D. M. Bers: Oxygen-bridged dinuclear ruthenium amine complex specifically inhibits Ca^{2+} uptake into mitochondria *in vitro* and in situ in single cardiac myocytes. *J. Biol. Chem.* 273, 10223-10231 (1998)
65. Wang, G. J. & S. A. Thayer: NMDA-induced Ca^{2+} loads recycle across the mitochondrial inner membrane in hippocampal neurons in culture. *J. Neurophysiol.* 87, 740-749 (2002)
66. Unitt, J. F., K. L. Boden, A. V. Wallace, A. H. Ingall, M. E. Coombs & F. Ince: Novel cobalt complex inhibitors of mitochondrial calcium uptake. *Bioorganic & Medicinal Chemistry* 7, 1891-1896 (1999)
67. Jensen, J. R., G. Lynch & M. Baudry: Polyamines stimulate mitochondrial calcium transport in rat brain. *J. Neurochem.* 48, 765-772 (1987)
68. Rottenberg, H. & M. Marbach: Regulation of Ca^{2+} transport in brain mitochondria. I. The mechanism of spermine enhancement of Ca^{2+} uptake and retention. *Biochim Biophys Acta* 1016, 77-86. (1990)
69. Baudry, M. & I. Najm: Kainate-induced seizure activity stimulates the polyamine interconversion pathway in rat brain. *Neurosci Lett* 171, 151-154. (1994)
70. Gilad, G. M., V. H. Gilad, Y. Eliyayev & J. M. Rabey: Developmental regulation of the brain polyamine-stress-response. *Int J Dev Neurosci* 16, 271-278. (1998)
71. Palmi, M., G. T. Youmbi, F. Fusi, G. P. Sgaragli, H. B. Dixon, M. Frosini & K. F. Tipton: Potentiation of mitochondrial Ca^{2+} sequestration by taurine. *Biochem Pharmacol* 58, 1123-1131 (1999)
72. Posner, A. S.: Intramitochondrial storage of stable amorphous calcium phosphate. *Ann N Y Acad Sci* 307, 248-249. (1978)
73. Petronilli, V., C. Cola & P. Bernardi: Modulation of the mitochondrial cyclosporin A-sensitive permeability transition pore .2. the minimal requirements for pore induction underscore a key role for transmembrane electrical potential, matrix pH, and matrix Ca^{2+}. *J Biol Chem* 268, 1011-1016 (1993)
74. Petronilli, V., D. Penzo, L. Scorrano, P. Bernardi & F. Di Lisa: The mitochondrial permeability transition, release of cytochrome c and cell death. Correlation with the duration of pore openings in situ. *J Biol Chem* 276, 12030-12034. (2001)
75. Reichert, S. A., J. S. Kim-Han & L. L. Dugan: The mitochondrial permeability transition pore and nitric oxide synthase mediate early mitochondrial depolarization in astrocytes during oxygen-glucose deprivation. *J Neurosci* 21, 6608-6616. (2001)
76. Scorrano, L., D. Penzo, V. Petronilli, F. Pagano & P. Bernardi: Arachidonic acid causes cell death through the mitochondrial permeability transition. Implications for tumor necrosis factor-alpha aopototic signaling. *J Biol Chem* 276, 12035-12040. (2001)
77. Solem, L. E. & K. B. Wallace: Selective Activation of the Sodium-Independent, Cyclosporin A-Sensitive Calcium Pore of Cardiac Mitochondria by Doxorubicin. *Toxicol Appl Pharmacol* 121, 50-57 (1993)
78. Griffiths, E. J. & A. P. Jalestrap: Further evidence that cyclosporin A protects mitochondria from calcium overload

by inhibiting a matrix peptidyl-prolyl cis trans isomerase. *Biochem. J.* 274, 611-614 (1991)
79. Khaspekov, L., H. Friberg, A. Halestrap, I. Viktorov & T. Wieloch: Cyclosporin A and its nonimmunosuppressive analogue N-Me-Val-4- cyclosporin A mitigate glucose/oxygen deprivation-induced damage to rat cultured hippocampal neurons. *Eur J Neurosci* 11, 3194-3198. (1999)
80. Ruiz, F., G. Alvarez, M. Ramos, M. Hernandez, E. Bogonez & J. Satrustegui: Cyclosporin A targets involved in protection against glutamate excitotoxicity. *Eur. J. Pharmacol.* 404, 29-39 (2000)
81. Halestrap, A. P., K. Y. Woodfield & C. P. Connern: Oxidative stress, thiol reagents, and membrane potential modulate the mitochondrial permeability transition by affecting nucleotide binding to the adenine nucleotide translocase. *J Biol Chem* 272, 3346-3354. (1997)
82. Jonas, E. A., J. Buchanan & L. K. Kaczmarek: Prolonged activation of mitochondrial conductances during synaptic transmission. *Science* 286, 1347-1350 (1999)
83. Ichas, F. & J. P. Mazat: From calcium signaling to cell death: two conformations for the mitochondrial permeability transition pore. Switching from low- to high-conductance state. *Biochim Biophys Acta* 1366, 33-50 (1998)
84. Ichas, F., L. S. Jouaville, S. S. Sidash, J. P. Mazat & E. L. Holmuhamedov: Mitochondrial calcium spiking: a transduction mechanism based on calcium-induced permeability transition involved in cell calcium signalling. *FEBS Lett* 348, 211-215. (1994)
85. Ankarcrona, M., J. M. Dypbukt, S. Orrenius & P. Nicotera: Calcineurin and Mitochondrial Function in Glutamate-Induced Neuronal Cell Death. *FEBS Lett.* 394, 321-324 (1996)
86. Scanlon, J. M., J. B. Brocard, A. K. Stout & I. J. Reynolds: Pharmacological investigation of mitochondrial Ca^{2+} transport in central neurons: studies with CGP-37157, an inhibitor of the mitochondrial Na^{+}-Ca^{2+} exchanger. *Cell Calcium* 28, 317-327 (2000)
87. Baron, K. T. & S. A. Thayer: CGP37157 modulates mitochondrial Ca^{2+} homeostasis in cultured rat dorsal root ganglion neurons. *Eur J Pharmacol* 340, 295-300 (1997)
88. Zhang, Y. & P. Lipton: Cytosolic Ca^{2+} changes during *in vitro* ischemia in rat hippocampal slices: major roles for glutamate and Na^{+}-dependent Ca^{2+} release from mitochondria. *J Neurosci* 19, 3307-3315 (1999)
89. Rego, A. C., M. W. Ward & D. G. Nicholls: Mitochondria control ampa/kainate receptor-induced cytoplasmic calcium deregulation in rat cerebellar granule cells. *J Neurosci* 21, 1893-1901. (2001)
90. Moudy, A. M., S. D. Handran, M. P. Goldberg, N. Ruffin, I. Karl, P. K. Kranz-Eble, D. C. DeVivo & S. M. Rothman: Abnormal calcium homeostasis and mitochondrial polarization in a human encephalomyopathy. *Pro. Natl. Acad. Sci. USA* 92, 729-733 (1995)
91. Stout, A. K., H. M. Raphael, B. I. Kanterewicz, E. Klann & I. J. Reynolds: Glutamate-induced neuron death requires mitochondrial calcium uptake. *Nature Neuroscience* 1, 366-373 (1998)
92. Nicholls, D. G., S. L. Budd, R. F. Castilho & M. W. Ward: Glutamate excitotoxicity and neuronal energy metabolism. *Ann. NY Acad. Sci.* 893, 1-12 (1999)
93. Castilho, R. F., M. W. Ward & D. G. Nicholls: Oxidative stress, mitochondrial function, and acute glutamate excitotoxicity in cultured cerebellar granule cells. *J. Neurochem.* 72, 1394-1401 (1999)
94. Votyakova, T. V. & I. J. Reynolds: Delta psi(m)-dependent and -independent production of reactive oxygen species by rat brain mitochondria. *J. Neurochem.* 79, 266-277 (2001)
95. Putcha, G. V., M. Deshmukh & E. M. Johnson, Jr.: BAX translocation is a critical event in neuronal apoptosis: regulation by neuroprotectants, BCL-2, and caspases. *J Neurosci* 19, 7476-7485 (1999)
96. Krajewski, S., S. Tanaka, S. Takayama, M. J. Schibler, W. Fenton & J. C. Reed: Investigation of the subcellular distribution of the bcl-2 oncoprotein: residence in the nuclear envelope, endoplasmic reticulum, and outer mitochondrial membranes. *Cancer Res* 53, 4701-4714. (1993)
97. Murphy, A. N., D. E. Bredesen, G. Cortopassi, E. Wang & G. Fiskum: Bcl-2 Potentiates the Maximal Calcium Uptake Capacity of Neural Cell Mitochondria. *Proc. Natl. Acad. Sci. USA* 93, 9893-9898 (1996)
98. Yang, J., X. S. Liu, K. Bhalla, C. N. Kim, A. M. Ibrado, J. Y. Cai, T. I. Peng, D. P. Jones & X. D. Wang: Prevention of apoptosis by Bcl-2 - release of cytochrome C from mitochondria blocked. *Science* 275, 1129-1132 (1997)
99. Kluck, R. M., E. Bossywetzel, D. R. Green & D. D. Newmeyer: The release of cytochrome C from mitochondria - a primary site for Bcl-2 regulation of apoptosis. *Science* 275, 1132-1136 (1997)
100. Prehn, J. H., V. P. Bindokas, C. J. Marcuccilli, S. Krajewski, J. C. Reed & R. J. Miller: Regulation of neuronal Bcl2 protein expression and calcium homeostasis by transforming growth factor type beta confers wide-ranging protection on rat hippocampal neurons. *Proc. Natl. Acad. Sci. USA* 91, 12599-12603 (1994)
101. Mahajan, N. P., K. Linder, G. Berry, G. W. Gordon, R. Heim & B. Herman: Bcl-2 and Bax interactions in mitochondria probed with green fluorescent protein and fluorescence resonance energy transfer. *Nat Biotechnol* 16, 547-552. (1998)
102. Antonsson, B., F. Conti, A. Ciavatta, S. Montessuit, S. Lewis, I. Martinou, L. Bernasconi, A. Bernard, J. J. Mermod, G. Mazzei, K. Maundrell, F. Gambale, R. Sadoul & J. C. Martinou: Inhibition of Bax Channel-Forming Activity By Bcl-2. *Science* 277, 370-372 (1997)
103. Llinas, R., M. Sugimori & R. B. Silver: Microdomains of high calcium concentration in a presynaptic terminal. *Science* 256, 677-679 (1992)
104. Rettig, J., C. Heinemann, U. Ashery, Z. H. Sheng, C. T. Yokoyama, W. A. Catterall & E. Neher: Alteration of Ca^{2+} Dependence of Neurotransmitter Release By Disruption of Ca^{2+} Channel/Syntaxin Interaction. *J. Neurosci.* 17, 6647-6656 (1997)
105. Zucker, R. S.: Calcium- and activity-dependent synaptic plasticity. *Curr Opin Neurobiol* 9, 305-313 (1999)
106. Peng, Y. Y. & R. S. Zucker: Release of LHRH is linearly related to the time integral of presynaptic Ca^{2+} elevation above a threshold level in bullfrog sympathetic ganglia. *Neuron* 10, 465-473 (1993)
107. Usachev, Y. M. & S. A. Thayer: All-or-none Ca^{2+} release from intracellular stores triggered by Ca^{2+} influx

through voltage-gated Ca^{2+} channels in rat sensory neurons. *J. Neurosci.* 17, 7404-7414 (1997)
108. Usachev, Y. M. & S. A. Thayer: Controlling the urge for a Ca^{2+} surge: all-or-none Ca^{2+} release in neurons. *Bioessays* 21, 743-750 (1999)
109. Usachev, Y. M. & S. A. Thayer: Ca^{2+} influx in resting rat sensory neurones that regulates and is regulated by ryanodine-sensitive Ca^{2+} stores. *J. Physiol.* 519, 115-130 (1999)
110. Hongpaisan, J., N. B. Pivovarova, S. L. Colegrove, R. D. Leapman, D. D. Friel & S. B. Andrews: Multiple modes of calcium-induced calcium release in sympathetic neurons II: A $[Ca^{2+}]_i$- and location-dependent transition from endoplasmic reticulum Ca accumulation to net Ca release. *J. Gen. Physiol.* 118, 101-112 (2001)
111. Friel, D. D. & R. W. Tsien: Phase-dependent contributions from Ca^{2+} entry and Ca^{2+} release to caffeine-induced $[Ca^{2+}]_i$ oscillations in bullfrog sympathetic neurons. *Neuron* 8, 1109-1125 (1992)
112. Xu, L., A. Tripathy, D. A. Pasek & G. Meissner: Potential for pharmacology of ryanodine receptor/calcium release channels. *Ann N Y Acad Sci* 853, 130-148. (1998)
113. Wilcox, R. A., W. U. Primrose, S. R. Nahorski & R. A. Challiss: New developments in the molecular pharmacology of the myo-inositol 1,4,5-trisphosphate receptor. *Trends Pharmacol Sci* 19, 467-475. (1998)
114. Beard, N. A., M. M. Sakowska, A. F. Dulhunty & D. R. Laver: Calsequestrin is an inhibitor of skeletal muscle ryanodine receptor calcium release channels. *Biophys J* 82, 310-320. (2002)
115. Thrower, E. C., H. Mobasheri, S. Dargan, P. Marius, E. J. Lea & A. P. Dawson: Interaction of luminal calcium and cytosolic ATP in the control of type 1 inositol (1,4,5)-trisphosphate receptor channels. *J Biol Chem* 275, 36049-36055. (2000)
116. Gyorke, I. & S. Gyorke: Regulation of the cardiac ryanodine receptor channel by luminal Ca^{2+} involves luminal Ca^{2+} sensing sites. *Biophys J* 75, 2801-2810. (1998)
117. Garaschuk, O., Y. Yaari & A. Konnerth: Release and sequestration of calcium by ryanodine-sensitive stores in rat hippocampal neurones. *J Physiol* 502, 13-30. (1997)
118. Friel, D. D. & R. W. Tsien: A caffeine- and ryanodine-sensitive Ca^{2+} store in bullfrog sympathetic neurones modulates effects of Ca^{2+} entry on $[Ca^{2+}]$. *J. Physiol.* 450, 217-246 (1992)
119. Collins, R. O. & R. C. Thomas: The effect of calcium pump inhibitors on the response of intracellular calcium to caffeine in snail neurones. *Cell Calcium* 30, 41-48. (2001)
120. Burk, S. E., J. Lytton, D. H. MacLennan & G. E. Shull: cDNA cloning, functional expression, and mRNA tissue distribution of a third organellar Ca^{2+} pump. *J Biol Chem* 264, 18561-18568. (1989)
121. Gunteski-Hamblin, A. M., J. Greeb & G. E. Shull: A novel Ca^{2+} pump expressed in brain, kidney, and stomach is encoded by an alternative transcript of the slow-twitch muscle sarcoplasmic reticulum Ca-ATPase gene. Identification of cDNAs encoding Ca^{2+} and other cation-transporting ATPases using an oligonucleotide probe derived from the ATP-binding site. *J Biol Chem* 263, 15032-15040. (1988)
122. Baba-Aissa, F., L. Raeymaekers, F. Wuytack, L. Dode & R. Casteels: Distribution and isoform diversity of the organellar Ca^{2+} pumps in the brain. *Mol Chem Neuropathol* 33, 199-208. (1998)
123. East, J. M.: Sarco(endo)plasmic reticulum calcium pumps: recent advances in our understanding of structure/function and biology. *Mol Membr Biol* 17, 189-200. (2000)
124. Lytton, J., M. Westlin, S. E. Burk, G. E. Shull & D. H. MacLennan: Functional comparisons between isoforms of the sarcoplasmic or endoplasmic reticulum family of calcium pumps. *J Biol Chem* 267, 14483-14489. (1992)
125. Verboomen, H., F. Wuytack, H. De Smedt, B. Himpens & R. Casteels: Functional difference between SERCA2a and SERCA2b Ca^{2+} pumps and their modulation by phospholamban. *Biochem J* 286, 591-595. (1992)
126. Wu, K. D., W. S. Lee, J. Wey, D. Bungard & J. Lytton: Localization and quantification of endoplasmic reticulum Ca^{2+}-ATPase isoform transcripts. *Am J Physiol* 269, C775-784. (1995)
127. Thomas, D. & M. R. Hanley: Pharmacological tools for perturbing intracellular calcium storage. *Meth. Cell Biol.* 40, 65-89 (1994)
128. Thastrup, O., P. J. Cullen, B. K. Drobak, M. R. Hanley & A. P. Dawson: Thapsigargin, a tumor promoter, discharges intracellular Ca^{2+} stores by a specific inhibition of the endoplasmic reticulum Ca^{2+}-ATPase. *Proc. Natl. Acad. Sci. USA* 87, 2466-2470 (1990)
129. Fortea, M. I., F. Soler & F. Fernandez-Belda: Unravelling the interaction of thapsigargin with the conformational states of Ca^{2+}-ATPase from skeletal sarcoplasmic reticulum. *J Biol Chem* 276, 37266-37272. (2001)
130. Sagara, Y., F. Fernandez-Belda, L. de Meis & G. Inesi: Characterization of the inhibition of intracellular Ca^{2+} transport ATPases by thapsigargin. *J Biol Chem* 267, 12606-12613. (1992)
131. Missiaen, L., H. Desmedt, G. Droogmans & R. Casteels: 2,5-Di-(tert-butyl)-1,4-benzohydroquinone and cyclopiazonic acid decrease the Ca^{2+} permeability of endoplasmic reticulum. *Eur J Pharmacol* 227, 391-394 (1992)
132. Foskett, J. K. & D. Wong: Calcium oscillations in parotid acinar cells induced by microsomal Ca^{2+} -ATPase inhibition. *Am. J. Physiol.* 262, c656-c663 (1992)
133. Luo, D. L., M. Nakazawa, Y. Yoshida, J. Q. Cai & S. Imai: Effects of three different Ca^{2+} pump ATPase inhibitors on evoked contractions in rabbit aorta and activities of Ca^{2+} pump ATPases in porcine aorta. *General Pharmacology-the Vascular System* 34, 211-220 (2000)
134. Seidler, N. W., I. Jona, M. Vegh & A. Martonosi: Cyclopiazonic acid is a specific inhibitor of the Ca^{2+}-ATPase of sarcoplasmic reticulum. *J. Biol. Chem.* 264, 17816-17823 (1989)
135. O'Neal, S. G., D. B. Rhoads & E. Racker: Vanadate inhibition of sarcoplasmic reticulum Ca^{2+}-ATPase and other ATPases. *Biochem Biophys Res Commun* 89, 845-850. (1979)
136. Pottorf, W. J., S. P. Duckles & J. N. Buchholz: SERCA function declines with age in adrenergic nerves from the superior cervical ganglion. *J Auton Pharmacol* 20, 281-290. (2000)
137. Narayanan, N., N. Su & P. Bedard: Inhibitory and stimulatory effects of fluoride on the calcium pump of cardiac sarcoplasmic reticulum. *Biochimica et Biophysica Acta* 1070, 83-91 (1991)

138. Coll, R. J. & A. J. Murphy: Fluoride-inhibited calcium ATPase of sarcoplasmic reticulum. Magnesium and fluoride stoichiometry. *J Biol Chem* 267, 21584-21587. (1992)
139. Putney, J. W., L. M. Broad, F. J. Braun, J. P. Lievremont & G. S. J. Bird: Mechanisms of capacitative calcium entry. *Journal of Cell Science* 114, 2223-2229 (2001)
140. Parekh, A. B. & R. Penner: Store depletion and calcium influx. *Physiol. Rev.* 77, 901-930 (1997)
141. Philipp, S., J. Hambrecht, L. Braslavski, G. Schroth, M. Freichel, M. Murakami, A. Cavalie & V. Flockerzi: A novel capacitative calcium entry channel expressed in excitable cells. *EMBO J* 17, 4274-4282 (1998)
142. Bouron, A.: Activation of a capacitative Ca^{2+} entry pathway by store depletion in cultured hippocampal neurones. *FEBS Lett.* 470, 269-272 (2000)
143. Emptage, N. J., C. A. Reid & A. Fine: Calcium stores in hippocampal synaptic boutons mediate short-term plasticity, store-operated Ca^{2+} entry, and spontaneous transmitter release. *Neuron* 29, 197-208 (2001)
144. Yoo, A. S., I. Cheng, S. Chung, T. Z. Grenfell, H. Lee, E. Pack-Chung, M. Handler, J. Shen, W. Xia, G. Tesco, A. J. Saunders, K. Ding, M. P. Frosch, R. E. Tanzi & T. W. Kim: Presenilin-mediated modulation of capacitative calcium entry. *Neuron* 27, 561-572 (2000)
145. Leissring, M. A., Y. Akbari, C. M. Fanger, M. D. Cahalan, M. P. Mattson & F. M. LaFerla: Capacitative calcium entry deficits and elevated luminal calcium content in mutant presenilin-1 knockin mice. *J. Cell Biol.* 149, 793-797 (2000)
146. Wolfe, M. S., W. Xia, B. L. Ostaszewski, T. S. Diehl, W. T. Kimberly & D. J. Selkoe: Two transmembrane aspartates in presenilin-1 required for presenilin endoproteolysis and gamma-secretase activity. *Nature* 398, 513-517. (1999)
147. Putney, J. W., Jr.: Presenilins, Alzheimer's disease, and capacitative calcium entry. *Neuron* 27, 411-412. (2000)
148. Narayanan, N. & A. Xu: Phosphorylation and regulation of the Ca^{2+}-pumping ATPase in cardiac sarcoplasmic reticulum by calcium/calmodulin-dependent protein kinase. *Basic Res Cardiol* 92, 25-35. (1997)
149. Frank, K. & E. G. Kranias: Phospholamban and cardiac contractility. *Ann Med* 32, 572-578. (2000)
150. Colyer, J.: Phosphorylation states of phospholamban. *Ann N Y Acad Sci* 853, 79-91. (1998)
151. Dou, D. & R. Joseph: Cloning of human neuronatin gene and its localization to chromosome-20q 11.2-12: the deduced protein is a novel "proteolipid'. *Brain Res* 723, 8-22. (1996)
152. Ellgaard, L. & A. Helenius: ER quality control: towards an understanding at the molecular level. *Curr Opin Cell Biol* 13, 431-437. (2001)
153. John, L. M., J. D. Lechleiter & P. Camacho: Differential modulation of SERCA2 isoforms by calreticulin. *J Cell Biol* 142, 963-973. (1998)
154. Wong, H. N., M. A. Ward, A. W. Bell, E. Chevet, S. Bains, W. P. Blackstock, R. Solari, D. Y. Thomas & J. J. Bergeron: Conserved in vivo phosphorylation of calnexin at casein kinase II sites as well as a protein kinase C/proline-directed kinase site. *J Biol Chem* 273, 17227-17235. (1998)
155. Roderick, H. L., J. D. Lechleiter & P. Camacho: Cytosolic phosphorylation of calnexin controls intracellular Ca^{2+} oscillations via an interaction with SERCA2b. *J. Cell Biol.* 149, 1235-1247 (2000)
156. Michalak, M., P. Mariani & M. Opas: Calreticulin, a multifunctional Ca^{2+} binding chaperone of the endoplasmic reticulum. *Biochem Cell Biol* 76, 779-785 (1998)
157. Ou, W. J., J. J. Bergeron, Y. Li, C. Y. Kang & D. Y. Thomas: Conformational changes induced in the endoplasmic reticulum luminal domain of calnexin by Mg-ATP and Ca^{2+}. *J Biol Chem* 270, 18051-18059. (1995)
158. Treves, S., F. Zorzato & T. Pozzan: Identification of calreticulin isoforms in the central nervous system. *Biochem J* 287, 579-581. (1992)
159. Lee, H. C.: Mechanisms of calcium signaling by cyclic ADP-ribose and NAADP. *Physiol. Rev.* 77, 1133-1164 (1997)
160. Currie, K. P. M., K. Swann, A. Galione & R. H. Scott: Activation of Ca^{2+}-dependent currents in cultured rat dorsal root ganglion neurones by a sperm factor and cyclic ADP-ribose. *Mol Biol Cell* 3, 1415-1425 (1992)
161. Empson, R. M. & A. Galione: Cyclic ADP-ribose enhances coupling between voltage-gated Ca^{2+} entry and intracellular Ca^{2+} release. *J. Biol. Chem.* 272, 20967-20970 (1997)
162. Reyes-Harde, M., R. Empson, B. V. Potter, A. Galione & P. K. Stanton: Evidence of a role for cyclic ADP-ribose in long-term synaptic depression in hippocampus. *Proc Natl Acad Sci U S A* 96, 4061-4066 (1999)
163. Lukyanenko, V., I. Gyorke, T. F. Wiesner & S. Gyorke: Potentiation of Ca^{2+} release by cADP-ribose in the heart is mediated by enhanced SR Ca^{2+} uptake into the sarcoplasmic reticulum. *Circ. Res.* 89, 614-622 (2001)
164. Montero, M., J. Alvarez, W. J. J. Scheenen, R. Rizzuto, J. Meldolesi & T. Pozzan: Ca^{2+} homeostasis in the endoplasmic reticulum - coexistence of high and low $[Ca^{2+}]$ subcompartments in intact Hela cells. *J. Cell Biol.* 139, 601-611 (1997)
165. Pozzan, T., R. Rizzuto, P. Volpe & J. Meldolesi: Molecular and cellular physiology of intracellular calcium stores. *Physiol. Rev.* 74, 595-636 (1994)
166. Treves, S., M. De Mattei, M. Landfredi, A. Villa, N. M. Green, D. H. MacLennan, J. Meldolesi & T. Pozzan: Calreticulin is a candidate for a calsequestrin-like function in Ca^{2+}- storage compartments (calciosomes) of liver and brain. *Biochem J* 271, 473-480. (1990)
167. Park, B. J., D. G. Lee, J. R. Yu, S. K. Jung, K. Choi, J. Lee, Y. S. Kim, J. I. Lee, J. Y. Kwon, A. Singson, W. K. Song, S. H. Eom, C. S. Park, D. H. Kim, J. Bandyopadhyay & J. Ahnn: Calreticulin, a calcium-binding molecular chaperone, is required for stress response and fertility in Caenorhabditis elegans. *Mol Biol Cell* 12, 2835-2845. (2001)
168. Taguchi, J., A. Fujii, Y. Fujino, Y. Tsujioka, M. Takahashi, Y. Tsuboi, I. Wada & T. Yamada: Different expression of calreticulin and immunoglobulin binding protein in Alzheimer's disease brain. *Acta Neuropathol (Berl)* 100, 153-160. (2000)
169. Rys-Sikora, K. E. & D. L. Gill: Fatty acid-mediated calcium sequestration within intracellular calcium pools. *J. Biol. Chem.* 273, 32627-32635 (1998)

170. Favre, C. J., J. Schrenzel, J. Jacquet, D. P. Lew & K. H. Krause: Highly supralinear feedback inhibition of Ca^{2+} uptake by the Ca^{2+} load of intracellular stores. *J. Biol. Chem.* 271, 14925-14930 (1996)
171. Cheek, T. R., V. A. Barry, M. J. Berridge & L. Missiaen: Bovine adrenal chromaffin cells contain an inositol 1,4,5-trisphosphate-insensitive but caffeine-sensitive Ca^{2+} store that can be regulated by intraluminal free Ca^{2+}. *Biochem J* 275, 697-701. (1991)
172. Doutheil, J., M. Treiman, U. Oschlies & W. Paschen: Recovery of neuronal protein synthesis after irreversible inhibition of the endoplasmic reticulum calcium pump. *Cell Calcium* 25, 419-428. (1999)
173. Nguyen, H. N., C. Wang & D. C. Perry: Depletion of intracellular calcium stores is toxic to SH-SY5Y neuronal cells. *Brain Res* 924, 159-166. (2002)
174. Xu, K. L., N. Tavernarakis & M. Driscoll: Necrotic cell death in C-elegans requires the function of calreticulin and regulators of Ca^{2+} release from the endoplasmic reticulum. *Neuron* 31, 957-971 (2001)
175. Mattson, M. P., F. M. LaFerla, S. L. Chan, M. A. Leissring, P. N. Shepel & J. D. Geiger: Calcium signaling in the ER: its role in neuronal plasticity and neurodegenerative disorders. *Trends Neurosci.* 23, 222-229 (2000)
176. Moore, K. A., A. S. Cohen, J. P. Kao & D. Weinreich: Ca^{2+}-induced Ca^{2+} release mediates a slow post-spike hyperpolarization in rabbit vagal afferent neurons. *J Neurophysiol* 79, 688-694. (1998)
177. Kuba, K., K. Morita & M. Nohmi: Origin of calcium ions involved in the generation of a slow afterhyperpolarization in bullfrog sympathetic neurones. *Pflugers Arch* 399, 194-202 (1983)
178. Perezterzic, C., M. Jaconi & D. E. Clapham: Nuclear calcium and the regulation of the nuclear pore complex. *BioEssays* 19, 787-792 (1997)
179. Peng, Y. Y.: Ryanodine-sensitive component of calcium transients evoked by nerve firing at presynaptic nerve terminals. *J. Neurosci.* 16, 6703-6712 (1996)
180. Smith, A. B. & T. C. Cunnane: Ryanodine-sensitive calcium stores involved in neurotransmitter release from sympathetic nerve terminals of the guinea-pig. *J. Physiol. (Lond.)* 497, 657-664 (1996)
181. Tse, F. W., A. Tse, B. Hille, H. Horstmann & W. Almers: Local Ca^{2+} release from internal stores controls exocytosis in pituitary gonadotrophs. *Neuron* 18, 121-132 (1997)
182. Carter, A. G., K. E. Vogt, K. A. Foster & W. G. Regehr: Assessing the role of calcium-induced calcium release in short-term presynaptic plasticity at excitatory central synapses. *J Neurosci* 22, 21-28. (2002)
183. Fomina, A. F. & M. C. Nowycky: A current activated on depletion of intracellular Ca^{2+} stores can regulate exocytosis in adrenal chromaffin cells. *J Neurosci* 19, 3711-3722 (1999)
184. Gomez, T. M., D. M. Snow & P. C. Letourneau: Characterization of spontaneous calcium transients in nerve growth cones and their effect on growth cone migration. *Neuron* 14, 1233-1246 (1995)
185. Spitzer, N. C., N. J. Lautermilch, R. D. Smith & T. M. Gomez: Coding of neuronal differentiation by calcium transients. *Bioessays* 22, 811-817 (2000)
186. Connor, J. A., J. Petrozzino, L. D. Pozzo-Miller & S. Otani: Calcium signals in long-term potentiation and long-term depression. *Canadian Journal of Physiology & Pharmacology* 77, 722-734 (1999)
187. Sabatini, B. L., M. Maravall & K. Svoboda: Ca^{2+} signaling in dendritic spines. *Curr. Opinion Neurobiol.* 11, 349-356 (2001)
188. Finch, E. A. & G. J. Augustine: Local calcium signalling by inositol-1,4,5-trisphosphate in Purkinje cell dendrites. *Nature* 396, 753-756 (1998)
189. Miyata, M., E. A. Finch, L. Khiroug, K. Hashimoto, S. Hayasaka, S. I. Oda, M. Inouye, Y. Takagishi, G. J. Augustine & M. Kano: Local calcium release in dendritic spines required for long-term synaptic depression. *Neuron* 28, 233-244 (2000)
190. Wang, S. S. H., W. Denk & M. Hausser: Coincidence detection in single dendritic spines mediated by calcium release. *Nature Neuroscience* 3, 1266-1273 (2000)
191. Narasimhan, K., I. N. Pessah & D. J. Linden: Inositol-1,4,5-trisphosphate receptor-mediated Ca mobilization is not required for cerebellar long-term depression in reduced preparations. *J Neurophysiol* 80, 2963-2974. (1998)
192. Lo, T.-M. & S. A. Thayer: Refilling the inositol 1, 4, 5-trisphosphate-sensitive Ca^{2+} store in neuroblastoma x glioma hybrid NG108-15 cells. *Am. J. Physiol.: Cell. Physiol.* 33, C641-C653 (1993)
193. Csordas, G. & G. Hajnoczky: Sorting of calcium signals at the junctions of endoplasmic reticulum and mitochondria. *Cell Calcium* 29, 249-262. (2001)
194. Majewska, A., E. Brown, J. Ross & R. Yuste: Mechanisms of calcium decay kinetics in hippocampal spines: role of spine calcium pumps and calcium diffusion through the spine neck in biochemical compartmentalization. *J Neurosci* 20, 1722-1734. (2000)
195. Sridhar, T. S., M. C. Brown & W. F. Sewell: Unique postsynaptic signaling at the hair cell efferent synapse permits calcium to evoke changes on two time scales. *J Neurosci* 17, 428-437. (1997)
196. Chard, P. S., D. Bleakman, S. Christakos, C. S. Fullmer & R. J. Miller: Calcium buffering properties of calbindin D28k and parvalbumin in rat sensory neurones. *J Physiol* 472, 341-357. (1993)
197. Zhou, Z. & E. Neher: Mobile and immobile calcium buffers in bovine adrenal chromaffin cells. *J Physiol* 469, 245-273. (1993)
198. Donato, R.: S100: a multigenic family of calcium-modulated proteins of the EF-hand type with intracellular and extracellular functional roles. *Int J Biochem Cell Biol* 33, 637-668. (2001)
199. DeFelipe, J.: Types of neurons, synaptic connections and chemical characteristics of cells immunoreactive for calbindin-D28K, parvalbumin and calretinin in the neocortex. *J Chem Neuroanat* 14, 1-19. (1997)
200. Chin, D. & A. R. Means: Calmodulin: a prototypical calcium sensor. *Trends Cell Biol* 10, 322-328. (2000)
201. Heizmann, C. W. & J. A. Cox: New perspectives on S100 proteins: a multi-functional Ca^{2+}-, Zn^{2+}- and Cu^{2+}-binding protein family. *Biometals* 11, 383-397. (1998)
202. Heizmann, C. W.: Calcium signaling in the brain. *Acta Neurobiol Exp* 53, 15-23 (1993)
203. Haeseleer, F., Y. Imanishi, I. Sokal, S. Filipek & K. Palczewski: Calcium-binding proteins: intracellular sensors

from the calmodulin superfamily. *Biochem Biophys Res Commun* 290, 615-623. (2002)
204. Kawasaki, H. & R. H. Kretsinger: Calcium-binding proteins 1: EF-hands. *Protein Profile* 2, 297-490 (1995)
205. Burrone, J., G. Neves, A. Gomis, A. Cooke & L. Lagnado: Endogenous calcium buffers regulate fast exocytosis in the synaptic terminal of retinal bipolar cells. *Neuron* 33, 101-112. (2002)
206. Scharfman, H. E. & P. A. Schwartzkroin: Protection of dentate hilar cells from prolonged stimulation by intracellular calcium chelation. *Science* 246, 257-260 (1989)
207. Stahl, W. L., T. J. Eakin, J. J. Owens, J. F. Breininger, P. E. Filuk & W. R. Anderson: Plasma membrane Ca^{2+}-ATPase isoforms: distribution of mRNAs in rat brain by in situ hybridization. *Molec. Brain Res.* 16, 223-231 (1992)
208. Zacharias, D. A., S. J. DeMarco & E. E. Strehler: mRNA expression of the four isoforms of the Human plasma membrane Ca^{2+}-ATPase in the human hippocampus. *Molec. Brain Res.* 45, 173-176 (1997)
209. Stauffer, T. P., D. Guerini, M. R. Celio & E. Carafoli: Immunolocalization of the Plasma Membrane Ca^{2+} Pump Isoforms in the Rat Brain. *Brain Res.* 748, 21-29 (1997)
210. Hillman, D. E., S. Chen, R. Bing, J. T. Penniston & R. Llinas: Ultrastructural localization of the plasmalemmal calcium pump in cerebellar neurons. *Neuroscience* 72, 315-324 (1996)
211. Strehler, E. E. & D. A. Zacharias: Role of alternative splicing in generating isoform diversity among plasma membrane calcium pumps. *Physiol Rev* 81, 21-50. (2001)
212. Keeton, T. P., S. E. Burk & G. E. Shull: Alternative splicing of exons encoding the calmodulin-binding domains and C termini of plasma membrane Ca^{2+}-ATPase isoforms 1, 2, 3, and 4. *J Biol Chem* 268, 2740-2748. (1993)
213. Zacharias, D. A., S. J. Dalrymple & E. E. Strehler: Transcript distribution of plasma membrane Ca^{2+} pump isoforms and splice variants in the human brain. *Molec. Brain Res.* 28, 263-272 (1995)
214. Enyedi, A., A. K. Verma, R. Heim, H. P. Adamo, A. G. Filoteo, E. E. Strehler & J. T. Penniston: The Ca^{2+} affinity of the plasma membrane Ca^{2+} pump is controlled by alternative splicing. *J Biol Chem* 269, 41-43 (1994)
215. Verma, A. K., A. Enyedi, A. G. Filoteo, E. E. Strehler & J. T. Penniston: Plasma membrane calcium pump isoform 4a has a longer calmodulin-binding domain than 4b. *J. Biol. Chem.* 271, 3714-3718 (1996)
216. Carafoli, E.: Biogenesis: Plasma membrane calcium ATPase: 15 years of work on the purified enzyme. *FASEB J.* 8, 993-1002 (1994)
217. Caride, A. J., N. L. Elwess, A. K. Verma, A. G. Filoteo, A. Enyedi, Z. Bajzer & J. T. Penniston: The rate of activation by calmodulin of isoform 4 of the plasma membrane Ca^{2+} pump is slow and is changed by alternative splicing. *J. Biol. Chem.* 274, 35227-35232 (1999)
218. Penniston, J. T. & A. Enyedi: Modulation of the plasma membrane Ca^{2+} pump. *J. Membr. Biol.* 165, 101-109 (1998)
219. Monteith, G. R., Y. Wanigasekara & B. D. Roufogalis: The plasma membrane calcium pump, its role and regulation: New complexities and possibilities. *J Pharmacol Toxicol Meth* 40, 183-190 (1998)
220. Benham, C. D., M. L. Evans & C. J. McBain: Ca^{2+} efflux mechanisms following depolarization evoked calcium transients in cultured rat sensory neurones. *J. Physiol.* 455, 567-583 (1992)
221. Werth, J. L., Y. M. Usachev & S. A. Thayer: Modulation of calcium efflux from cultured rat dorsal root ganglion neurons. *J. Neurosci.* 16, 1008-1015 (1996)
222. Juhaszova, M., P. Church, M. P. Blaustein & E. F. Stanley: Location of calcium transporters at presynaptic terminals. *Eur. J. Neurosci.* 12, 839-846 (2000)
223. Usachev, Y. M., S. J. DeMarco, C. Campbell, E. E. Strehler & S. A. Thayer: Bradykinin and ATP Accelerate Ca^{2+} Efflux from Rat Sensory Neurons via Protein Kinase C and the Plasma Membrane Ca^{2+} Pump Isoform 4. *Neuron* 33, 113-122. (2002)
224. Zhong, N., V. Beaumont & R. S. Zucker: Roles for mitochondrial and reverse mode Na^{+}/Ca^{2+} exchange and the plasmalemma Ca^{2+} ATPase in post-tetanic potentiation at crayfish neuromuscular junctions. *J Neurosci* 21, 9598-9607. (2001)
225. Herscher, C. J. & A. F. Rega: Pre-steady-state kinetic study of the mechanism of inhibition of the plasma membrane Ca^{2+}-ATPase by lanthanum. *Biochem.* 35, 14917-14922 (1996)
226. Shimizu, H., M. L. Borin & M. P. Blaustein: Use of La^{3+} to Distinguish Activity of the Plasmalemmal Ca^{2+} Pump From Na^{+}/Ca^{2+} Exchange in Arterial Myocytes. *Cell Calcium* 21, 31-41 (1997)
227. Shmigol, A., D. A. Eisner & S. Wray: Carboxyeosin decreases the rate of decay of the $[Ca^{2+}]_i$ transient in uterine smooth muscle cells isolated from pregnant rats. *Pflugers Arch* 437, 158-160. (1998)
228. Bassani, R. A., J. W. Bassani & D. M. Bers: Relaxation in ferret ventricular myocytes: role of the sarcolemmal Ca ATPase. *Pflugers Arch* 430, 573-578. (1995)
229. Gatto, C., C. C. Hale, W. Xu & M. A. Milanick: Eosin, a potent inhibitor of the plasma membrane Ca pump, does not inhibit the cardiac Na-Ca exchanger. *Biochem.*, 905-912 (1995)
230. Green, A. K., P. H. Cobbold & C. J. Dixon: Effects On the Hepatocyte $[Ca^{2+}]_i$ Oscillator of Inhibition of the Plasma Membrane Ca^{2+} Pump By Carboxyeosin or Glucagon-(19-29). *Cell Calcium* 22, 99-109 (1997)
231. Chaudhary, J., M. Walia, J. Matharu, E. Escher & A. K. Grover: Caloxin: a novel plasma membrane Ca^{2+} pump inhibitor. *Am J Physiol* 280, C1027-C1030 (2001)
232. Hofmann, F., J. Anagli, E. Carafoli & T. Vorherr: Phosphorylation of the calmodulin binding domain of the plasma membrane Ca^{2+} pump by protein kinase C reduces its interaction with calmodulin and with its pump receptor site. *J Biol Chem* 269, 24298-24303 (1994)
233. Enyedi, A., N. L. Elwess, A. G. Filoteo, A. K. Verma, K. Paszty & J. T. Penniston: Protein kinase C phosphorylates the a forms of plasma membrane Ca^{2+} pump isoforms 2 and 3 and prevents binding of calmodulin. *J. Biol. Chem.* 272, 27525-27528 (1997)
234. James, P. H., M. Pruschy, T. E. Vorherr, J. T. Penniston & E. Carafoli: Primary structure of the cAMP-dependent phosphorylation site of the plasma membrane calcium pump. *Biochem.* 28, 4253-4258 (1989)

235. Caroni, P. & E. Carafoli: Regulation of Ca^{2+}-pumping ATPase of heart sarcolemma by a phosphorylation-dephosphorylation process. *J Biol Chem* 256, 9371-9373. (1981)
236. Dixon, D. A. & D. H. Haynes: Kinetic characterization of the Ca^{2+}-pumping ATPase of cardiac sarcolemma in four states of activation. *J Biol Chem* 264, 13612-13622. (1989)
237. Neyses, L., L. Reinlib & E. Carafoli: Phosphorylation of the Ca^{2+} pumping ATPase of heart sarcolemma and erythrocyte plasma membrane by the cAMP-dependent protein kinase. *J. Biol. Chem.* 260, 10283-10287 (1985)
238. Zylinska, L., D. Guerini, E. Gromadzinska & L. Lachowicz: Protein kinases A and C phosphorylate purified Ca^{2+}-ATPase from rat cortex, cerebellum and hippocampus. *Biochim Biophys Acta* 1448, 99-108 (1998)
239. Caride, A. J., A. G. Filoteo, A. R. Penheiter, K. Paszty, A. Enyedi & J. T. Penniston: Delayed activation of the plasma membrane calcium pump by a sudden increase in Ca^{2+}: fast pumps reside in fast cells. *Cell Calcium* 30, 49-57 (2001)
240. Caride, A. J., A. R. Penheiter, A. G. Filoteo, Z. Bajzer, A. Enyedi & J. T. Penniston: The plasma membrane calcium pump displays memory of past calcium spikes. Differences between isoforms 2b and 4b. *J Biol Chem* 276, 39797-39804. (2001)
241. Pottorf, W. J. & S. A. Thayer: Enhanced Ca^{2+} pump activity following exposure to elevated $[Ca^{2+}]_i$ accelerates Ca^{2+} efflux from rat sensory neurons. *Soc. Neurosci. Abst.* 27 (2001)
242. Sah, P.: Ca^{2+}-activated K^+ currents in neurones: types, physiological roles and modulation. *Trends Neurosci.* 19, 150-154 (1996)
243. Cordoba-Rodriguez, R., K. A. Moore, J. P. Y. Kao & D. Weinreich: Calcium regulation of a slow post-spike hyperpolarization in vagal afferent neurons. *Proc. Natl. Acad. Sci. USA* 96, 7650-7657 (1999)
244. Blaustein, M. P. & W. J. Lederer: Sodium/calcium exchange: its physiological implications. *Physiol. Rev.* 79, 763-854. (1999)
245. Tsoi, M., K. H. Rhee, D. Bungard, X. F. Li, S. L. Lee, R. N. Auer & J. Lytton: Molecular cloning of a novel potassium-dependent sodium-calcium exchanger from rat brain. *J Biol Chem* 273, 4155-4162. (1998)
246. Kraev, A., B. D. Quednau, S. Leach, X. F. Li, H. Dong, R. Winkfein, M. Perizzolo, X. Cai, R. Yang, K. D. Philipson & J. Lytton: Molecular cloning of a third member of the potassium-dependent sodium-calcium exchanger gene family, NCKX3. *J Biol Chem* 276, 23161-23172. (2001)
247. Quednau, B. D., D. A. Nicoll & K. D. Philipson: Tissue specificity and alternative splicing of the Na^+/Ca^{2+} exchanger isoforms NCX1, NCX2, and NCX3 in Rat. *Am J Physiol* 41, C1250-C1261 (1997)
248. Baker, P. F. & R. Dipolo: Axonal calcium and magnesium homeostasis. *Curr. Top. Mem. Trans.* 22, 195-247 (1984)
249. Fierro, L., R. DiPolo & I. I. Llano: Intracellular calcium clearance in Purkinje cell somata from rat cerebellar slices. *J Physiol (Lond)* 510, 499-512. (1998)
250. Verdru, P., C. Degreef, L. Mertens, E. Carmeliet & G. Callewaert: Na^+-Ca^{2+} Exchange in Rat Dorsal Root Ganglion Neurons. *J. Neurophysiol.* 77, 484-490 (1997)
251. Luther, P. W., R. K. Yip, R. J. Bloch, A. Ambesi, G. E. Lindenmayer & M. P. Blaustein: Presynaptic localization of sodium/calcium exchangers in neuromuscular preparations. *J. Neurosci.* 12, 4898-4904 (1992)
252. Sanchez-Armass, S. & M. P. Blaustein: Role of sodium-calcium exchange in regulation of intracellular calcium in nerve terminals. *Am. J. Physiol.* 252, C595-C603 (1987)
253. Tang, Y. M., E. R. Travis, R. M. Wightman & A. S. Schneider: Sodium-calcium exchange affects local calcium signal decay and the rate of exocytotic secretion in single chromaffin cells. *J Neurochem* 74, 702-710. (2000)
254. Kiedrowski, L., G. Brooker, E. Costa & J. Wroblewski: Glutamate impairs neuronal calcium extrusion while reducing sodium gradient. *Neuron* 12, 295-300 (1994)
255. Hoyt, K. R., S. R. Arden, E. Aizenman & I. J. Reynolds: Reverse Na^+/Ca^{2+} Exchange Contributes to Glutamate-Induced Intracellular Ca^{2+} Concentration Increases in Cultured Rat Forebrain Neurons. *Mol. Pharmacol.* 53, 742-749 (1998)
256. DiPolo, R. & L. Beauge: Metabolic pathways in the regulation of invertebrate and vertebrate Na^+/Ca^{2+} exchange. *Biochim Biophys Acta* 1422, 57-71. (1999)
257. Blaustein, M. P., G. Fontana & R. S. Rogowski: The Na^+-Ca^{2+} exchanger in rat brain synaptosomes. Kinetics and regulation. *Ann N Y Acad Sci* 779, 300-317. (1996)
258. Philipson, K. D. & D. A. Nicoll: Sodium-calcium exchange: a molecular perspective. *Annu Rev Physiol* 62, 111-133 (2000)
259. Wang, C., N. Davis & R. A. Colvin: Genistein inhibits Na^+/Ca^{2+} exchange activity in primary rat cortical neuron culture. *Biochem Biophys Res Commun* 233, 86-90. (1997)
260. Pan, C. Y., Y. S. Chu & L. S. Kao: Molecular study of the Na^+/Ca^{2+} exchanger in bovine adrenal chromaffin cells. *Biochem J* 336, 305-310. (1998)
261. Asano, S., T. Matsuda, K. Takuma, H. S. Kim, T. Sato, T. Nishikawa & A. Baba: Nitroprusside and cyclic GMP stimulate Na^+-Ca^{2+} exchange activity in neuronal preparations and cultured rat astrocytes. *J Neurochem* 64, 2437-2441. (1995)
262. He, S., A. Ruknudin, L. L. Bambrick, W. J. Lederer & D. H. Schulze: Isoform-specific regulation of the Na^+/Ca^{2+} exchanger in rat astrocytes and neurons by PKA. *J Neurosci* 18, 4833-4841 (1998)
263. DiPolo, R., G. Berberian, D. Delgado, H. Rojas & L. Beauge: A novel 13 kDa cytoplasmic soluble protein is required for the nucleotide (MgATP) modulation of the Na/Ca exchange in squid nerve fibers. *FEBS Lett* 401, 6-10. (1997)
264. Iwamoto, T., Y. Pan, T. Y. Nakamura, S. Wakabayashi & M. Shigekawa: Protein kinase C-dependent regulation of Na^+/Ca^{2+} exchanger isoforms NCX1 and NCX3 does not require their direct phosphorylation. *Biochem.* 37, 17230-17238 (1998)

265. Hilgemann, D. W. & R. Ball: Regulation of cardiac Na^+,Ca^{2+} exchange and K-$_{ATP}$ potassium channels by PIP_2. *Science* 273, 956-959 (1996)
266. He, Z., S. Feng, Q. Tong, D. W. Hilgemann & K. D. Philipson: Interaction of PIP_2 with the XIP region of the cardiac Na/Ca exchanger. *Am J Physiol* 278, C661-666. (2000)
267. Matsuoka, S. & D. W. Hilgemann: Inactivation of outward Na^+-Ca^{2+} exchange current in guinea-pig ventricular myocytes. *J Physiol* 476, 443-458. (1994)
268. DiPolo, R.: Calcium influx in internally dialyzed squid giant axons. *J Gen Physiol* 73, 91-113. (1979)
269. Siegl, P. K., E. J. Cragoe, Jr., M. J. Trumble & G. J. Kaczorowski: Inhibition of Na^+/Ca^{2+} exchange in membrane vesicle and papillary muscle preparations from guinea pig heart by analogs of amiloride. *Proc Natl Acad Sci U S A* 81, 3238-3242. (1984)
270. Rogister, F., D. Laeckmann, P. Plasman, F. Van Eylen, M. Ghyoot, C. Maggetto, J. Liegeois, J. Geczy, A. Herchuelz, J. Delarge & B. Masereel: Novel inhibitors of the sodium-calcium exchanger: benzene ring analogues of N-guanidino substituted amiloride derivatives. *Eur J Med Chem* 36, 597-614. (2001)
271. Slaughter, R. S., M. L. Garcia, E. J. Cragoe, Jr., J. P. Reeves & G. J. Kaczorowski: Inhibition of sodium-calcium exchange in cardiac sarcolemmal membrane vesicles. 1. Mechanism of inhibition by amiloride analogues. *Biochem.* 27, 2403-2409. (1988)
272. Wettwer, E., H. Himmel & U. Ravens: Amiloride derivatives as blockers of Na^+/Ca^{2+} exchange: effects on mechanical and electrical function of guinea-pig myocardium. *Pharmacol Toxicol* 71, 95-102. (1992)
273. Garcia, M. L., V. F. King, J. L. Shevell, R. S. Slaughter, G. Suarez-Kurtz, R. J. Winquist & G. J. Kaczorowski: Amiloride analogs inhibit L-type calcium channels and display calcium entry blocker activity. *J. Biol. Chem.* 265, 3763-3771 (1990)
274. Garcia, M. L., R. S. Slaughter, V. F. King & G. J. Kaczorowski: Inhibition of sodium-calcium exchange in cardiac sarcolemmal membrane vesicles. 2. Mechanism of inhibition by bepridil. *Biochem.* 27, 2410-2415. (1988)
275. Iwamoto, T., T. Watano & M. Shigekawa: A novel isothiourea derivative selectively inhibits the reverse mode of Na^+/Ca^{2+} exchange in cells expressing NCX1. *J. Biol. Chem.* 271, 22391-22397 (1996)
276. Watano, T., J. Kimura, T. Morita & H. Nakanishi: A novel antagonist, No. 7943, of the Na^+/Ca^{2+} exchange current in guinea-pig cardiac ventricular cells. *Br J Pharmacol* 119, 555-563. (1996)
277. Kimura, J., T. Watano, M. Kawahara, E. Sakai & J. Yatabe: Direction-independent block of bi-directional Na^+/Ca^{2+} exchange current by KB-R7943 in guinea-pig cardiac myocytes. *Br J Pharmacol* 128, 969-974. (1999)
278. Colvin, R. A.: Zinc inhibits Ca^{2+} transport by rat brain Na^+/Ca^{2+} exchanger. *Neuroreport* 9, 3091-3096. (1998)
279. Pan, C. Y. & L. S. Kao: Catecholamine secretion from bovine adrenal chromaffin cells: the role of the Na^+/Ca^{2+} exchanger and the intracellular Ca^{2+} pool. *J Neurochem* 69, 1085-1092. (1997)
280. Bouron, A. & H. Reuter: A role of intracellular Na^+ in the regulation of synaptic transmission and turnover of the vesicular pool in cultured hippocampal cells. *Neuron* 17, 969-978 (1996)
281. Scotti, A. L., J. Y. Chatton & H. Reuter: Roles of Na^+-Ca^{2+} exchange and of mitochondria in the regulation of presynaptic Ca^{2+} and spontaneous glutamate release. *Philos Trans R Soc Lond B Biol Sci* 354, 357-364 (1999)
282. Gleason, E., S. Borges & M. Wilson: Electrogenic Na-Ca exchange clears Ca^{2+} loads from retinal amacrine cells in culture. *J Neurosci* 15, 3612-3621. (1995)
283. Gleason, E., S. Borges & M. Wilson: Control of transmitter release from retinal amacrine cells by Ca^{2+} influx and efflux. *Neuron* 13, 1109-1117. (1994)
284. Pilitsis, J. G., F. G. Diaz, M. H. O'Regan & J. W. Phillis: Inhibition of Na^+/Ca^{2+} exchange by KB-R7943, a novel selective antagonist, attenuates phosphoethanolamine and free fatty acid efflux in rat cerebral cortex during ischemia-reperfusion injury. *Brain Res* 916, 192-198. (2001)
285. Schroder, U. H., J. Breder, C. F. Sabelhaus & K. G. Reymann: The novel Na^+/Ca^{2+} exchange inhibitor KB-R7943 protects CA1 neurons in rat hippocampal slices against hypoxic/hypoglycemic injury. *Neuropharmacol.* 38, 319-321. (1999)
286. Hewitt, K. E., P. K. Stys & H. J. Lesiuk: The use-dependent sodium channel blocker mexiletine is neuroprotective against global ischemic injury. *Brain Res* 898, 281-287. (2001)
287. Andreeva, N., B. Khodorov, E. Stelmashook, E. Cragoe Jr. & I. Victorov: Inhibition of Na^+/Ca^{2+} exchange enhances delayed neuronal death elicited by glutamate in cerebellar granule cell cultures. *Brain Res.* 548, 322-325 (1991)
288. Mattson, M. P., P. B. Guthrie & S. B. Kater: A role for Na^+ - dependent Ca^{2+} extrusion in protection against neuronal excitotoxicity. *FASEB J.* 3, 2519-2526 (1989)
289. Calabresi, P., G. A. Marfia, S. Amoroso, A. Pisani & G. Bernardi: Pharmacological inhibition of the Na^+/Ca^{2+} exchanger enhances depolarizations induced by oxygen/glucose deprivation but not responses to excitatory amino acids in rat striatal neurons. *Stroke* 30, 1687-1693 (1999)
290. Brini, M., D. Bano, S. Manni, R. Rizzuto & E. Carafoli: Effects of PMCA and SERCA pump overexpression on the kinetics of cell Ca^{2+} signalling. *EMBO J* 19, 4926-4935 (2000)
291. Sorrentino, V., V. Barone & D. Rossi: Intracellular Ca^{2+} release channels in evolution. *Curr Opin Genet Dev* 10, 662-667. (2000)
292. Patel, S., S. K. Joseph & A. P. Thomas: Molecular properties of inositol 1,4,5-trisphosphate receptors. *Cell Calcium* 25, 247-264. (1999)
293. Padua, R. A., K. T. Baron, B. Thyagarajan, C. Campbell & S. A. Thayer: Reduced Ca^{2+} uptake by mitochondria in pyruvate dehydrogenase-deficient human diploid fibroblasts. *Am. J. Physiol.* 43, C 615-C 622 (1998)
294. Jane Lalli, M., J. Yong, V. Prasad, K. Hashimoto, D. Plank, G. J. Babu, D. Kirkpatrick, R. A. Walsh, M. Sussman, A. Yatani, E. Marban & M. Periasamy: Sarcoplasmic reticulum Ca^{2+} ATPase (SERCA) 1a structurally substitutes for SERCA2a in the cardiac sarcoplasmic reticulum and increases cardiac Ca^{2+} handling capacity. *Circ Res* 89, 160-167. (2001)
295. Enyedi, A., A. K. Verma, A. G. Filoteo & J. T. Penniston: Protein kinase C activates the plasma membrane

Ca^{2+} pump isoform 4b by phosphorylation of an inhibitory region downstream of the calmodulin-binding domain. *J. Biol. Chem.* 271, 32461-32467 (1996)
296. Terada, H.: Uncouplers of oxidative phosphorylation. *Environ Health Perspect* 87, 213-218 (1990)
297. Budd, S. L., L. Tenneti, T. Lishnak & S. A. Lipton: Mitochondrial and extramitochondrial apoptotic signaling pathways in cerebrocortical neurons. *Proc. Natl. Acad. Sci. USA* 97, 6161-6166 (2000)
298. Fontaine, E., F. Ichas & P. Bernardi: A ubiquinone-binding site regulates the mitochondrial permeability transition pore. *J Biol Chem* 273, 25734-25740. (1998)
299. Narita, M., S. Shimizu, T. Ito, T. Chittenden, R. J. Lutz, H. Matsuda & Y. Tsujimoto: Bax interacts with the permeability transition pore to induce permeability transition and cytochrome C release in isolated mitochondria. *Proc Natl Acad Sci U S A* 95, 14681-14686. (1998)
300. Lapidus, R. G. & P. M. Sokolove: The mitochondrial permeability transition. Interactions of spermine, ADP, and inorganic phosphate. *J Biol Chem* 269, 18931-18936. (1994)
301. Petronilli, V., P. Costantini, L. Scorrano, R. Colonna, S. Passamonti & P. Bernardi: The voltage sensor of the mitochondrial permeability transition pore is tuned by the oxidation-reduction state of vicinal thiols. Increase of the gating potential by oxidants and its reversal by reducing agents. *J Biol Chem* 269, 16638-16642. (1994)
302. Szabo, I., P. Bernardi & M. Zoratti: Modulation of the mitochondrial megachannel by divalent cations and protons. *J Biol Chem* 267, 2940-2946. (1992)
303. Finch, A. E., J. T. Turner & M. S. Goldin: Calcium as a coagonist of inositol 1,4,5-trisphosphate-induced calcium release. *Science* 252, 443-446 (1991)
304. Bezprozvanny, L., J. Watras & E. Ehrlich: Bell-shaped calcium-response curve of Ins(1,4,5,)P_3-and calcium-gated channels from endoplasmic reticulum of cerebellum. *Nature* 351, 751-754 (1991)
305. Gafni, J., J. A. Munsch, T. H. Lam, M. C. Catlin, L. G. Costa, T. F. Molinski & I. N. Pessah: Xestospongins - Potent Membrane Permeable Blockers of the Inositol 1,4,5-Trisphosphate Receptor. *Neuron* 19, 723-733 (1997)
306. Du, G. G., X. Guo, V. K. Khanna & D. H. MacLennan: Ryanodine sensitizes the cardiac Ca^{2+} release channel (ryanodine receptor isoform 2) to Ca^{2+} activation and dissociates as the channel is closed by Ca^{2+} depletion. *Proc Natl Acad Sci U S A* 98, 13625-13630. (2001)
307. Lattanzio, F. A., R. G. Schlatterer, M. Nicar, K. P. Campbell & J. L. Sutko: The effects of ryanodine on passive calcium fluxes across sarcoplasmic reticulum membranes. *J. Biol. Chem.* 262, 2711-2718 (1987)
308. Muller, C. E. & J. W. Daly: Stimulation of Calcium Release by Caffeine Analogs in Pheochromocytoma Cells. *Biochem Pharmacol* 46, 1825-1829 (1993)
309. Zhao, F., P. Li, S. R. Chen, C. F. Louis & B. R. Fruen: Dantrolene inhibition of ryanodine receptor Ca^{2+} release channels. Molecular mechanism and isoform selectivity. *J Biol Chem* 276, 13810-13816. (2001)
310. Szentesi, P., C. Collet, S. Sarkozi, C. Szegedi, I. Jona, V. Jacquemond, L. Kovacs & L. Csernoch: Effects of dantrolene on steps of excitation-contraction coupling in mammalian skeletal muscle fibers. *J Gen Physiol* 118, 355-375. (2001)

Key Words: Intracellular Ca^{2+} concentration, Neuron, plasma membrane Ca^{2+} ATPase, PMCA, Sarcoplasmic and endoplasmic reticulum Ca^{2+} ATPase, SERCA, Mitochondrion, Na^+/Ca^{2+} exchange, NCX, Pharmacology, Phosphorylation, Brain, Alternative splicing, Ca^{2+} signaling, Review

Send all correspondence to: S. A. Thayer, Department of Pharmacology, University of Minnesota Medical School, 6-120 Jackson Hall, 321 Church Street S. E., Minneapolis, MN 55455, Tel: 612-626-7049, Fax: 612-625-8408, E-mail: thayer@med.umn.edu