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Neuronal Calcium Signaling

Review

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Introduction

Calcium plays an important role in regulating a great variety of neuronal processes. Like other cells, neurons use both extracellular and intracellular sources of calcium. The mechanisms responsible for regulating the influx of external calcium are well established (Figure 1). For example, voltage-operated channels are used to trigger the release of neurotransmitter at synaptic junctions and they contribute to dendritic action potentials. In addition, neurotransmitters can induce an influx of calcium using receptor-operated channels such as the NMDA receptors located primarily at postsynaptic sites. While much is known about these influx pathways, there is less information on the mechanism and role of the intracellular supply of calcium stored within the endoplasmic reticulum of neurons. Inositol 1,4,5-trisphosphate receptors ($InsP_3Rs$) or ryanodine receptors (RYRs) distributed throughout the endoplasmic reticulum are responsible for releasing Ca^{2+} from this internal store (Figure 1). Since the endoplasmic reticulum is a continuous network distributed throughout the cell, it may be considered as a neuron-within-a-neuron, a concept that becomes all the more interesting because the endoplasmic reticulum, like the plasma membrane, has both integrative and regenerative properties that could play important roles in neural signaling. This review explores the idea that the endoplasmic reticulum and the plasma membrane form a binary membrane system that functions to regulate a variety of neuronal processes including excitability, associativity, neurotransmitter release, synaptic plasticity, and gene transcription.

Structural Organization of the Neuronal Endoplasmic Reticulum

Neurons have an elaborate endoplasmic reticulum that extends throughout the cell. Perhaps the most remarkable aspect of this endoplasmic reticulum network is that it appears to be a continuous membrane system (Figure 2), as has been demonstrated by both conventional microscopy (Droz et al., 1975; Broadwell and Cataldo 1984; Martone et al., 1993) and by monitoring the diffusion of a fluorescent, lipophilic dye (Terasaki et al., 1994). This endoplasmic reticulum network has a number of regional specializations, which are of particular significance with regard to Ca^{2+} signaling. Beginning in the soma, the outer nuclear membrane is continuous with an anastomising endoplasmic reticulum network that extends up into the dendrites and down into the axon (Figure 2). Within the soma and the initial dendritic region, portions of the endoplasmic reticulum come into close contact with the plasma membrane to form subsurface cisternae (Rosenbluth, 1962; Herndon, 1963; Siegesmund, 1968; Takahashi and Wood, 1970; Henkart et al., 1976). These subsurface cisternae have been classified into different types depending on how closely they approach the plasma membrane (Takahashi and Wood, 1970). The type I come within 40–80 nm, whereas the type II and III come much closer (20 nm) and often follow the contours of the plasma membrane, suggesting that the two membranes are bound together (Rosenbluth, 1962). Indeed, the two membranes are separated by a fuzzy material with a distinct periodicity reminiscent of the triadic junction of muscle cells (Henkart et al., 1976). In the axonal initial segment of certain neurons (e.g., cortical principal cells, spiny stellate cells, and dentate granule cells) the subsurface cisternae appear as multilayered structures called cisternal organelles (Peters et al., 1968; Kosaka, 1980; Takei et al., 1992; Benedeczky et al., 1994). In the case of the initial segment of CA3 hippocampal neurons, both subsurface cisternae and cisternal organelles coexist (Kosaka, 1980). These subsurface cisternae and cisternal organs may play an important role in regulating neuronal excitability (see below).

Within the axon, the endoplasmic reticulum consists of connecting tubules running parallel with the axon (Droz et al., 1975; Broadwell and Cataldo, 1984; Lindsay and Ellisman, 1985). Occasionally the tubules connect to hypolemmal cisternae, which are flattened sheets that lie close to the plasma membrane and thus resemble the subsurface cisternae. This axonal endoplasmic reticulum network extends into the synapse where it is often closely associated with a mitochondrion (Bird, 1978; McGraw et al., 1980; Westrum and Gray, 1986).

At the opposite end of the cell, the endoplasmic reticulum network extends throughout the dendritic tree and into the spines, where it finally terminates as the spine apparatus (Figure 2) (Harris and Stevens, 1988; Mignery et al., 1989; Satoh et al., 1990; Martone et al., 1993; Spacek and Harris, 1997). As indicated by Spacek and Harris (1997), ~50% of the spines contain a spine apparatus. The latter consists of closely stacked plates of endoplasmic reticulum separated by a densely staining material. The important point to stress is that the dendritic endoplasmic reticulum network is continuous with the spine apparatus (Figure 2). There is considerable variation in the structure of the endoplasmic reticulum within the neck region of the spine; in some cases it is a narrow tubule, whereas in others it can occupy up to 36% of the neck. There is no evidence for the endoplasmic reticulum occluding the neck, which means that ions and messengers are relatively free to move between the spine and the dendrites (Svoboda et al., 1996; Spacek and Harris, 1997). The spine apparatus usually lies within the middle of the spine, but tubular branches sometimes come into close contact with the postsynaptic density (Figure 2) (Spacek and Harris, 1997).

The endoplasmic reticulum neuronal network contains $InsP_3Rs$ and RYRs, both of which are capable of regenerative Ca^{2+} release, which enables the endoplasmic reticulum to play an active role in neuronal calcium signaling. Immunohistochemical studies have revealed that the $InsP_3Rs$ are distributed throughout the endoplasmic reticulum network of Purkinje cells and spiny

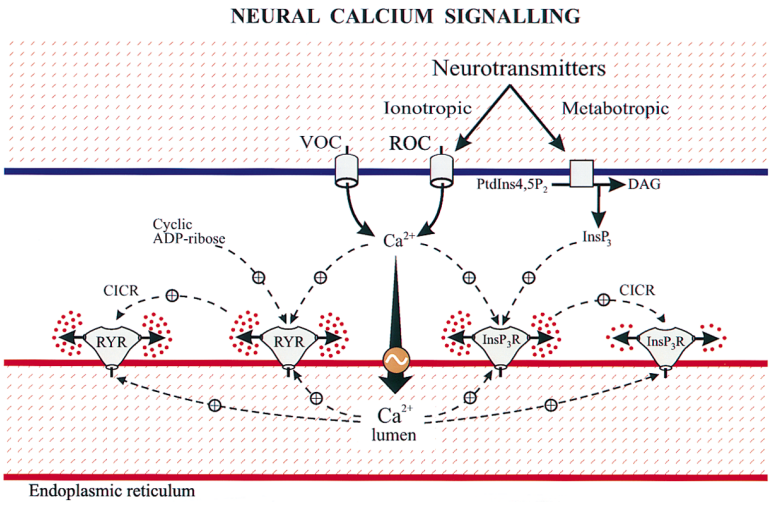


Figure 1. Neural Calcium Signaling

The entry of Ca^{2+} from the outside is regulated by voltage-operated channels or by receptor-operated channels controlled by the ionotropic neurotransmitters. Metabotropic neurotransmitters stimulate the formation of inositol 1,4,5-trisphosphate ($InsP_3$), which acts on $InsP_3$ receptors ($InsP_3R$) to release Ca^{2+} from the endoplasmic reticulum. The latter also has ryanodine receptors (RYR) sensitive to cyclic ADP ribose. Both the $InsP_3Rs$ and the RYRs are also sensitive to Ca^{2+}, and this process of calcium-induced calcium release can set up propagated Ca^{2+} waves. Uptake of Ca^{2+} into the lumen primes these receptors by enhancing their Ca^{2+} sensitivity.

striated neurons, including that within the spines and the synaptic ending (Mignery et al., 1989; Otsu et al., 1990; Satoh et al., 1990; Walton et al., 1991; Takei et al., 1992; Martone et al., 1993, 1997). They were particularly evident on the endoplasmic reticulum forming the subsurface cisternae and hypolemmal cisternae (Ross et al., 1989; Takei et al., 1992). They were also present on the endoplasmic reticulum network located within the synaptic terminal (Takei et al., 1992). The predominant RYR isoform in the brain is the cardiac RYR2. The exception is that the cerebellar Purkinje neurons express mainly the skeletal muscle RYR1 isoform. The RYRs are primarily located in the soma of neurons (Kuwajima et al., 1992; Seymour-Laurent and Barish, 1995), but they can be found within other regions. Like the $InsP_3$ receptors, the RYRs are found on the subsurface cisternae and were also found to coexist in other locations. In most regions, therefore, these two receptor types display similar distributions, but there are some interesting differences. For example, the spines of Purkinje neurons contain $InsP_3Rs$ but no RYRs (Walton et al., 1991). The opposite arrangement is found in the spines of CA1 hippocampal cells where the RYRs are present in greater abundance than the $InsP_3Rs$ (Sharp et al., 1993).

Calcium Signaling by the Endoplasmic Reticulum

The neuronal endoplasmic reticulum network contributes to the dynamics of Ca^{2+} signaling by acting either as a source or as a sink of signal Ca^{2+} (Miller, 1991; Simpson et al., 1995). The existence of this internal reservoir of Ca^{2+} can have a profound effect on shaping neuronal Ca^{2+} signals (Garaschuk et al., 1997). Elevations in Ca^{2+} can be highly localized within compartments such as the spines or the terminals or they can spread through neurons as global Ca^{2+} waves.

The Endoplasmic Reticulum as a Ca^{2+} Source

Calcium stored within the endoplasmic reticulum of neurons represents an important source of signal Ca^{2+} that is released upon activation of either the $InsP_3Rs$ or the RYRs (Henzi and MacDermott, 1992; Kostyuk and Verkhratsky, 1994; Simpson et al., 1995). These two intracellular channels integrate information from both the cytoplasm and from within the lumen (Figure 1). Perhaps their most important property is their sensitivity to Ca^{2+}, in that they both display the phenomenon of Ca^{2+}-induced Ca^{2+} release. It is this regenerative process that is responsible for amplifying Ca^{2+} signals coming from the outside and for setting up Ca^{2+} waves (Figure 1). While Ca^{2+}-induced Ca^{2+} release is usually associated with the activity of RYRs, it is important to stress that the $InsP_3Rs$ are equally capable of displaying this autocatalytic property. The Ca^{2+} sensitivity of these two receptors can be influenced by a variety of factors including other messengers (e.g., $InsP_3$ and cyclic ADP ribose) or by the degree of store loading (Figure 1).

Ca^{2+} Release from Inositol Trisphosphate Receptors

The phosphoinositide system is particularly well developed in the brain (Nahorski, 1988; Chuang, 1989; Fowler and Tiger, 1991; Nahorski et al., 1991; Fisher et al., 1992; Furuichi and Mikoshiba 1995). A large number of different receptors respond by stimulating the hydrolysis of phosphatidylinositol 4,5-bisphosphate (PtdIns[4,5]P_2) to form the second messengers diacylglycerol (DAG) and $InsP_3$. The latter acts by releasing Ca^{2+} from the $InsP_3$ receptors, which are widely distributed throughout the brain (Sharp et al., 1993; Furuichi and Mikoshiba, 1995). Much of the evidence for $InsP_3$-induced Ca^{2+} release has come from studying cultured neurons (Irving et al., 1992; Linden et al., 1994; Seymour-Laurent and Barish, 1995; Geiling and Schild, 1996), but there are some reports of neurotransmitter-induced Ca^{2+} release from neurons in situ, which may result from the activation of metabotropic receptors (Frenguelli et al., 1993; Miller et al., 1996).

Release of intracellular Ca^{2+} by $InsP_3$ is complicated by the fact that its receptor is sensitive to a variety of factors. The most effective activation of the receptor is achieved when Ca^{2+} and $InsP_3$ are presented together (Figure 1). This dual activation has a number of interesting implications for neural signaling. First, the requirement for two separate messengers means that the $InsP_3$ receptor might act as a coincidence detector during synaptic plasticity (Berridge, 1993; Simpson et al., 1995). Already there is evidence that release of Ca^{2+} from the spine apparatus is likely to provide the highly localized

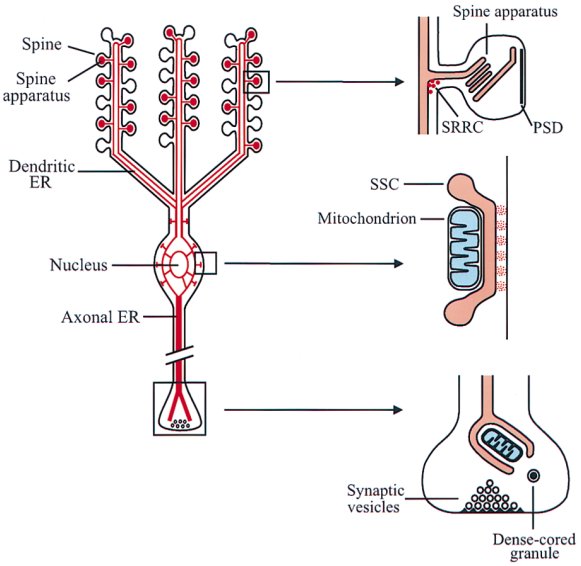


Figure 2. Structural Organization of the Endoplasmic Reticulum in Neurons

The endoplasmic reticulum is a continuous network that extends to all parts of the neuron. The three insets provide details of the arrangement of the endoplasmic reticulum in the spines, cell soma, and synaptic ending. PSD, postsynaptic density; SRRC, synapse-associated polyribosome complex; and SSC, subsurface cistern.

and input-specific Ca^{2+} signals to induce synaptic plasticity (Korkotian and Segal, 1998). Second, small elevations of $InsP_3$, which are not able to stimulate release directly, can enhance the Ca^{2+} sensitivity of the $InsP_3$ receptor, thereby converting the cytoplasm into an excitable medium capable of producing regenerative Ca^{2+} waves. For example, the responsiveness of neurons to synaptic stimulation was enhanced by inhibiting the hydrolysis of $InsP_3$ (Miller et al., 1996). In PC12 cells, raising intracellular Ca^{2+} levels by membrane depolarization failed to induce Ca^{2+} waves, which only occurred following the activation of metabotropic receptors, which increased the level of $InsP_3$ (Lorenzon et al., 1995). Another factor that regulates excitability is the degree of luminal Ca^{2+} loading (Figure 1). Through a mechanism that has yet to be defined, a buildup of Ca^{2+} within the lumen of the endoplasmic reticulum can exert a positive feedback effect by increasing the Ca^{2+} sensitivity of both the $InsP_3$Rs and the RYRs (Figure 1).

Ca^{2+} Release from Ryanodine Receptors

Ryanodine receptors (RYRs) resemble the $InsP_3$Rs in that they are sensitive to Ca^{2+} entering either from outside or from neighboring receptors (Figure 1) (Verkhratsky and Shmigol, 1996). The basic idea is that Ca^{2+} entering through either the voltage-operated channels or receptor-operated channels provides the trigger Ca^{2+} to stimulate Ca^{2+} release from the internal stores (Lipscombe et al., 1988; Barish, 1991; Friel and Tsien, 1992a; Alford et al., 1993; Hua et al., 1993; Llano et al., 1994; Shmigol et al., 1995; Li and Hatton, 1997; Usachev and Thayer, 1997). The amplification of an influx signal by release of internal Ca^{2+} in neurons is very similar to the process of excitation-contraction coupling in cardiac muscle (Shmigol et al., 1995; Verkhratsky and Shmigol, 1996). Calcium release in cardiac cells is mediated by the type 2 RYR, which is the predominant isoform found in the brain. In cardiac cells, these RYR2 channels are closely apposed to the Ca^{2+} channels in the plasma membrane across the 15 nm junctional gap that separates the sarcolemma from the sarcoplasmic reticulum at the triadic junction. An analogous mechanism may occur in neurons at the specialized subsurface cisternae, which resemble triadic junctions as described earlier (Figure 2).

The extent to which this internal amplification process operates varies considerably between neurons. Clear evidence that action potentials can trigger a release of Ca^{2+} from internal stores has been described in dorsal root ganglion cells (DRG) (Shmigol et al., 1995; Usachev and Thayer, 1997), rat cerebellar Purkinje neurons (Kano et al., 1995), nodose neurons (Cohen et al., 1997), and hippocampal neurons (Alford et al., 1993; Jacobs and Meyer, 1997). An internal release can be triggered by either a single action potential (e.g., nodose cell) or by trains of action potentials (e.g., DRGs and hippocampal cells). The degree to which this internal amplification process operates depends upon a number of factors.

As for the $InsP_3$ receptors, the Ca^{2+} sensitivity of the RYRs can be influenced by both cytosolic and luminal factors. Increasing the resting cytosolic level of Ca^{2+} greatly increases the sensitivity of these receptors to caffeine (Kano et al., 1995). Application of low doses of glutamate to telencephalic neurons activates Ca^{2+} influx through NMDA receptors, resulting in a large potentiation of caffeine-induced Ca^{2+} release (Tsai and Barish, 1995). There also is evidence that the putative second messenger cyclic ADP ribose (Galione, 1994; Lee, 1994) can enhance Ca^{2+} release in neurons. A transmembrane glycoprotein resembling CD38 in lymphocytes, which can catalyse both the synthesis and hydrolysis of cyclic ADP ribose, has been localized in neurons (Yamada et al., 1997). Injection of cyclic ADP ribose into sympathetic neurons failed to release Ca^{2+} by itself, but it appeared to enhance the amplification factor by increasing the Ca^{2+} sensitivity of the RYRs such that they released more Ca^{2+} following each depolarization (Hua et al., 1994). The pharmacological agent caffeine mobilizes Ca^{2+} in neurons through a similar mechanism of enhancing the Ca^{2+} sensitivity of the RYRs.

Another important factor determining the degree of amplification is the Ca^{2+} content of the endoplasmic reticulum (Friel and Tsien 1992a; Usachev and Thayer, 1997; Hernández-Cruz et al., 1997). Loading of the endoplasmic reticulum plays a crucial role in determining just how much of the internal store will contribute Ca^{2+} to the amplification process. As Ca^{2+} enters across the plasma membrane, it is likely to activate those RYRs or $InsP_3$Rs located near the cell surface, but the remainder that lie deeper within the cell may or may not respond depending on their sensitivity. The Ca^{2+}-induced Ca^{2+} release process may thus be restricted to the subsurface cisternal sites near the cell surface, or it may spread deeper into the neuron in those cases where the internal receptors are sufficiently sensitive to excite each other by setting up a regenerative calcium wave. A key determinant of this variation in sensitivity is the degree of store loading.

Spontaneous Miniature Outward Currents (SMOCs)

Using confocal microscopy, the opening of either individual or small groups of $InsP_3Rs$ or RYRs have been visualized and have been referred to as elementary events (Bootman and Berridge 1995; Berridge, 1997; Lipp and Niggli, 1997). So far, these elementary events have not been visualized in neurons, but such events may be responsible for the spontaneous miniature outward currents (SMOCs) recorded in dorsal root ganglion cells (Mathers and Barker, 1984) and sympathetic ganglion cells (Brown et al., 1983; Satin and Adams, 1987; Marrion and Adams, 1992). It was proposed that these SMOCs resulted from the release of a small "packet" of Ca^{2+} from stores located near the membrane (Brown et al., 1983; Adams et al., 1985). The time course of these SMOCs, with their rapid rising phase and slower recovery, closely resemble the Ca^{2+} sparks observed in cardiac cells (Lipp and Niggli, 1997). Since the frequency of these neuronal SMOCs is accelerated by caffeine (Marrion and Adams, 1992), it is reasonable to propose that they result from elementary release events involving the RYRs and that this release may come from the subsurface cisternae (Figure 2). Also, the elementary events responsible for the SMOCs may be the building blocks of the slow afterhyperpolarization that follows bouts of neural activity (see below) (Adams et al., 1985).

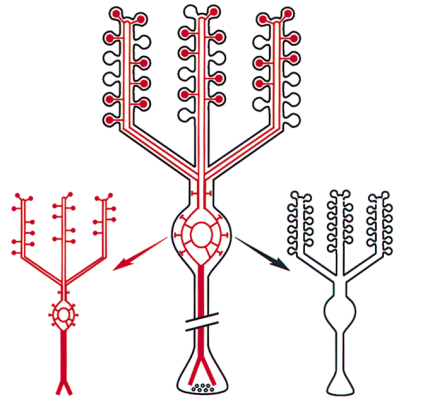
Calcium Spikes and Waves

The neuronal endoplasmic reticulum is an excitable organelle capable of regenerative release to produce repetitive Ca^{2+} spikes and waves (Kuba and Nishi, 1976; Lipscombe et al., 1988; Friel and Tsien, 1992b; Jaffe and Brown, 1994; Lorenzon et al., 1995; Rathouz et al., 1995). As discussed earlier, such regenerativity depends upon the excitability of the $InsP_3Rs$ and the RYRs. Why neurons employ both intracellular receptors is still a mystery. Hernández-Cruz et al. (1997) have proposed that the RYRs may function to amplify the Ca^{2+} signals emanating from the $InsP_3$ receptors. In the case of PC12 cells, the Ca^{2+} waves in the neurites are driven by $InsP_3Rs$ (Lorenzon et al., 1995). However, it is important to remember that both receptors are capable of regenerative Ca^{2+} release.

Loading up the stores with sufficient Ca^{2+} seems to be a critical event in setting the stage for the initiation of this regenerative process. For example, hippocampal neurons respond to the local application of ACPD by generating a Ca^{2+} wave that spreads through the neuron, which then becomes refractory to further stimulation (Jaffe and Brown, 1994). However, the cell can be reprimed by at least three bursts of action potentials induced by 1 s depolarizing current pulses. This depolarization opens the voltage-operated channels to introduce a pulse of Ca^{2+}, which is then taken up by the endoplasmic reticulum (Figure 1). Since one burst of Ca^{2+} entry was not sufficient to prime the stores, Jaffe and Brown (1994) proposed that "there may be a nonlinear relationship between levels of sequestered Ca^{2+} and the ability to trigger Ca^{2+} release." Such Ca^{2+} waves may function to spread Ca^{2+} signals bidirectionally throughout the cytoplasm, that is, both toward and away from the soma. For example, this regenerative signaling mechanism may be important to transfer Ca^{2+} signals from the cell periphery into the nucleus (Hernandez-Cruz et al., 1990; Usachev and Thayer, 1997) or to transmit information throughout the neuron (Jaffe and Brown, 1994), or it could function much more locally, for example within the dendrites, to spread a localized Ca^{2+} signal between synaptic spines. The existence of such Ca^{2+} waves is very dependent on the progressive loading of the endoplasmic reticulum, which thus reflects its dual role as both a source and a sink for Ca^{2+}.

The Endoplasmic Reticulum as a Ca^{2+} Sink

The amount of Ca^{2+} stored within the lumen of the endoplasmic reticulum is highly variable. There are indications that the endoplasmic reticulum stores in central neurons may only be partially full under resting conditions (Garaschuk et al., 1997). Since the endoplasmic reticulum is capable of rapidly sequestering Ca^{2+} through its SERCA pumps, it represents a major buffering system that functions as a sink for Ca^{2+} signals (Lipscombe et al., 1988; Friel and Tsien, 1992a; Markram et al., 1995). In neocortical pyramidal neurons, for example, a large proportion of the Ca^{2+} that enters the dendrites during the invasion of a back-propagating action potential is removed by being pumped into the endoplasmic reticulum (Markram et al., 1995). Recovery from such depolarization-induced Ca^{2+} signals is much slower if the sequestering ability of the endoplasmic reticulum is reduced either by locking the RYRs in an open state using ryanodine (Shmigol et al., 1995) or by inhibiting the SERCA pump with thapsigargin (Markram et al., 1995). A consequence of store loading is an increase in the sensitivity of the $InsP_3Rs$ and RYRs, which are thus primed to release Ca^{2+} as has been described for the Ca^{2+} waves in CA1 hippocampal neurons (Jaffe and Brown, 1994). Likewise, the release of internal Ca^{2+} by synaptic stimulation in hippocampal CA3 neurons is very dependent on store loading. Brief periods of membrane depolarization bring in extracellular Ca^{2+}, which is then taken up by the stores, making them responsive to synaptic stimulation (Miller et al., 1996). Such priming is not permanent because, in the absence of ongoing neural activity, the sequestered Ca^{2+} gradually dissipates. For example, the stores in cerebellar neurons can be loaded up by Ca^{2+} that enters during membrane depolarization, but the sequestered Ca^{2+} then runs down in 2–3 min (Brorson et al., 1991). Since the endoplasmic reticulum has a large capacity, it can function as a Ca^{2+} sink for a large number of spikes, but as its load increases the intracellular channels will become increasingly excitable, and Ca^{2+} may be released back into the cytoplasm through the process of Ca^{2+}-induced Ca^{2+} release described earlier (Blaustein, 1988; Miller, 1991). This conclusion has important implications with regard to the neuron-within-a-neuron concept because it implies that the endoplasmic reticulum can function as an integrator or "memory" of neuronal activity (Figure 3). By soaking up and storing the brief pulses of Ca^{2+} associated with each action potential, the endoplasmic reticulum may keep track of neuronal activity and may be able to signal this information to the nucleus through periodic bursts of Ca^{2+}. For example, brief bursts of neuronal activity generate small localized pulses of Ca^{2+} that are rapidly buffered, but prolonged firing may charge up the endoplasmic reticulum sufficiently for it to transmit regenerative global signals to the nucleus to initiate gene transcription (see below).



	ENDOPLASMIC RETICULUM	PLASMA MEMBRANE
Signal Integration	Second messengers (Ca^{2+}, $InsP_3$, cyclic ADPR)	Excitatory (+) and inhibitory (-) neural inputs
Regenerative process	Ca^{2+} waves	Action potential
Regenerative mechanism	Slow propagation by Ca^{2+} diffusion (~ 40 μm/s)	Fast propagation by local circuits (~100 m/s)
Conduction system	Ca^{2+}-dependent $InsP_3Rs$ and RyRs	Voltage-dependent Na^+ and Ca^{2+} channels
'Memory' mechanisms	Ca^{2+} release and storage?	Phosphorylation of ion channels
Reciprocal interactions	Ca^{2+} release modulates excitability	Ca^{2+} entry sensitizes ER Ca^{2+} release

Figure 3. Binary Membrane System of Neural Calcium Signaling

As summarized in the table, the endoplasmic reticulum may be considered as a neuron-within-a-neuron, in that it has many of the properties normally associated with the plasma membrane.

The Neuron-within-a-Neuron Concept

The basic concept is that neuronal Ca^{2+} signaling depends upon a binary membrane system (Figure 3). The outer plasma membrane integrates external information (e.g., excitatory and inhibitory inputs) and generates fast propagating action potentials using voltage-dependent Na^+ and Ca^{2+} channels. The endoplasmic reticulum system monitors internal signals (e.g., Ca^{2+}, $InsP_3$, cyclic ADP ribose, and cyclic AMP) and can produce slowly propagating regenerative Ca^{2+} signals using a conduction system based on the $InsP_3Rs$ or the RYRs. Both systems are highly nonlinear. The plasma membrane rapidly integrates large amounts of excitatory and inhibitory inputs and triggers an action potential when the membrane potential declines below a critical threshold. Likewise, the excitability of the endoplasmic reticulum system is highly variable and also has to reach a critical threshold before it will elicit a regenerative Ca^{2+} wave. Both systems seem to display a “memory” for previous events. In the case of the plasma membrane, the memory depends upon the phosphorylation of ion channels, whereas the endoplasmic reticulum may use the accumulation of luminal Ca^{2+} as a memory of previous events. Finally, these two membranes are intimately tied together through a variety of reciprocal interactions to regulate specific neuronal processes such as excitability, associativity, transmitter release, synaptic plasticity, and gene transcription.

Neuronal Excitability

Release of Ca^{2+} from the endoplasmic reticulum modulates neuronal excitability by altering membrane potential. For example, many neurons display pronounced after-hyperpolarizations (AHPs) or depolarizing afterpotentials (DAPs) following either a single action potential or bursts of action potentials. Such AHPs and DAPs modify neuronal activity by suppressing or promoting firing patterns, respectively. The Ca^{2+}-dependent ion channels responsible for these variations in membrane potential are controlled either by Ca^{2+} entering through voltage-operated channels or by Ca^{2+} released from internal stores (Figure 4).

After-Hyperpolarizations (AHPs)

AHPs have been described in many neurons, such as those from the bullfrog sympathetic ganglion (Fujimoto et al., 1980; Koketsu et al., 1982; Kuba et al., 1983; Pennefather et al., 1985; Hua et al., 1993), the mesenteric plexus (North and Tokimasa, 1983; Hirst et al., 1985), sympathetic preganglion (Yoshimura et al., 1986), inferior mesenteric ganglion (Dun et al., 1986), olfactory cortical region (Constanti and Sim, 1987), nodose ganglion of the vagus (Fowler et al., 1985), rat superior cervical ganglion (Kawai and Watanabe, 1989; Davies et al., 1996), visceral pelvic ganglion (Nishimura et al., 1988), coeliac ganglion (Cassell and McLachlan, 1987; Jobling et al., 1993), preganglionic neurons of dorsal motor nucleus (Sah and McLachlan, 1991), otic ganglion cells (Yoshizaki et al., 1995), and the hippocampus (Cole and Nicoll, 1984; Lancaster and Nicoll, 1987). Many of the neurons are found in the autonomic nervous system. Since this AHP can often last for seconds, it can regulate the rate of firing by interrupting high frequency discharges.

These AHPs arise from the opening of two separate Ca^{2+}-activated K^+ channels (gK_{Ca1} and gK_{Ca2}) (Lancaster and Adams, 1986; Sah and McLachlan, 1991), which are controlled by two different Ca^{2+} signaling pathways (Figure 4). During the course of the action potential, Ca^{2+} entering through voltage-operated Ca^{2+} channels rapidly activates gK_{Ca1}, which thus helps to repolarize the membrane, and this fast AHP (fAHP) rapidly recovers back to the resting potential in 100–200 ms. In some neurons, this fAHP is followed by a slow AHP (sAHP), which depends upon the opening of a separate conductance gK_{Ca2}, which activates more slowly and remains open for a lot longer. In some neurons, gK_{Ca2} switches on after the fAHP has begun to recover, thus producing a distinct hump preceding the onset of the sAHP (Figure 4). What is so interesting about these two hyperpolarizing processes is that they display clear spatial and temporal differences, even though they are both activated by Ca^{2+} entry.

The activation of gK_{Ca1} appears relatively straightforward. Ca^{2+} entering from the outside during the course of an action potential switches on neighboring gK_{Ca1} channels to produce the fAHP (Figure 4) (Sah, 1992). Sah and McLachlan (1991) have pointed out that the kinetics of such fAHPs is entirely consistent with the time course of the Ca^{2+} microdomains that build up beneath the plasma membrane following an action potential (Hernandez-Cruz et al., 1990). By contrast, there is a large temporal discrepancy between the Ca^{2+} entry

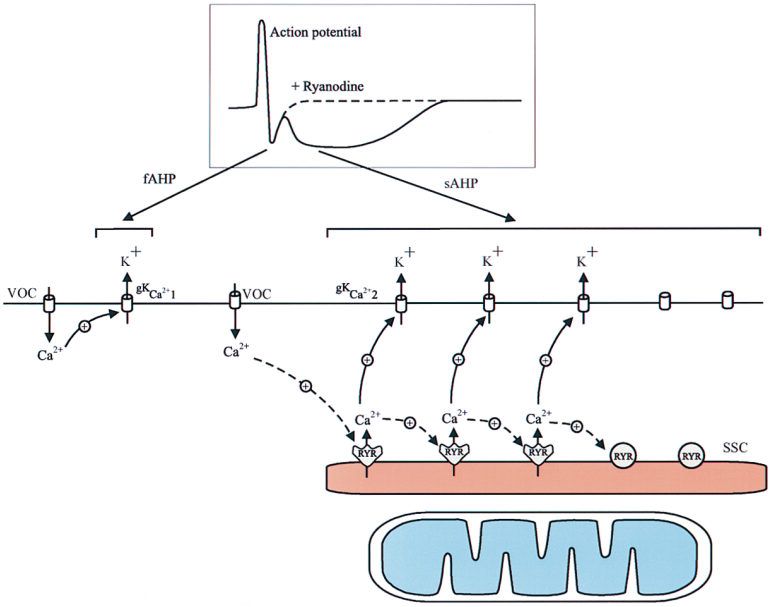


Figure 4. The Role of Calcium Release in the Regulation of Neuronal Excitability

The trace at the top illustrates the appearance of typical fast and slow afterhyperpolarizations (fAHP and sAHP). The fAHPs are mediated by a type 1 Ca^{2+}-activated K^+ channel (gK_{Ca1}), whereas the sAHPs result from the opening of gK_{Ca2} regulated by the release of Ca^{2+} from ryanodine receptors (RYR) most likely to be located on the subsurface cisternae (SSC).

events that occur during the action potential and the delayed onset of the sAHP. All the evidence suggests that gK_{Ca2} is activated by Ca^{2+} released from an internal store through the process of Ca^{2+}-induced Ca^{2+} release described earlier. The mechanism resembles that found in cardiac cells, in that Ca^{2+} entering through voltage-operated channels triggers RYR2s to release internal Ca^{2+} through a process of Ca^{2+}-induced Ca^{2+} release (Berridge, 1997). The analogy with cardiac cells can be extended because the sAHP in neurons can be inhibited by ryanodine (Kawai and Watanabe, 1989; Sah and McLachlan, 1991; Hua et al., 1993; Jobling et al., 1993; Usachev et al., 1993; Yoshizaki et al., 1995; Davies et al., 1996; Moore et al., 1998) or they can be prolonged by caffeine (Fujimoto et al., 1980). There is a clear correlation between those neurons that release Ca^{2+} when stimulated by caffeine and those that display sAHPs in response to action potentials (Kuba et al., 1983). There seems to be general agreement, therefore, that the sAHP is regulated by Ca^{2+} released from an internal store through a process of Ca^{2+}-induced Ca^{2+} release. The slow acetylcholine-induced inhibition of hair cells is also mediated by Ca^{2+} release from the subsurface cisternae (Sridhar et al., 1997).

The problem is to understand how a brief pulse of Ca^{2+} entering across the plasma membrane can bring about the delayed release of Ca^{2+} from the internal stores.

In addition to this temporal delay, the two channels also appear to be separated spatially, because the entry of Ca^{2+} through voltage-operated channels cannot activate gK_{Ca2} when the stores are inhibited by ryanodine. Therefore, any model must take into account this apparent spatial and temporal separation between the Ca^{2+} entry channels and gK_{Ca2} (Figure 4). Since such sAHPs only occur when the action potential invades the soma (North and Tokimasa, 1983; Fowler et al., 1985), it seems that the mechanism is localized in the cell body close to the spike initiation zone. The subsurface cisternae, which are particularly prevalent on the cell body (Figure 2), may be the sites where such sAHPs are generated (Henkart, 1980; Fujimoto et al., 1980). Adams et al. (1985) have proposed that the SMOCs described earlier represent the individual Ca^{2+} release events that sum to generate the sAHPs. As described earlier, the subsurface cisternae have much in common with the triadic junctions in muscle and may thus be responsible for the process of Ca^{2+}-induced Ca^{2+} release as proposed in Figure 4. The narrow cleft between the plasma membrane and the subsurface cisternae may provide a compartment that separates the surface Ca^{2+} channels from gK_{Ca2}. In order to activate the latter, Ca^{2+} must be released into this cleft (Figure 4). There are two ways in which the entry of Ca^{2+} might trigger the delayed release of internal Ca^{2+} responsible for the sAHPs. First, the Ca^{2+} that enters across the plasma membrane may take time to diffuse into the subsurface cisternal space to activate RYRs to initiate a process of Ca^{2+}-induced Ca^{2+} release (Figure 4). Release of Ca^{2+} into the narrow interspace between the subsurface cisternae and the plasma membrane is then responsible for activating gK_{Ca2}. Second, the entry of external Ca^{2+} may act indirectly by first stimulating a Ca^{2+}-sensitive phospholipase C to generate the second messenger $InsP_3$. Neurons are known to possess Ca^{2+}-sensitive phospholipase C isoforms. Diffusion of $InsP_3$ into the cleft of the subsurface cisternae would begin to release Ca^{2+}, and this initial signal may be amplified further by the RYRs. For such a mechanism to operate, the subsurface cisternae must contain both $InsP_3$Rs and RYRs, and such a colocalization has been described in various neurons (Ross et al., 1989; Takei et al., 1992). The interpolation of an enzymatic step to synthesise a diffusible messenger such as $InsP_3$ would account for the slow kinetics and the temperature dependence of the sAHPs (Fowler et al., 1985; Cassell and McLachlan, 1987; Sah and McLachlan, 1991). While the precise mechanism remains to be determined, it is clear that Ca^{2+} release from internal

stores, most likely the subsurface cisternae, is responsible for regulating the onset of sAHPs.

Modulation of sAHPs

A characteristic feature of the sAHPs is their modulation by a variety of neurotransmitters such as norepinephrine (NE) (Madison and Nicoll, 1986; Constanti and Sim, 1987; Lancaster and Nicoll 1987; Sah and McLachlan, 1991), acetylcholine (ACh) (Koketsu et al., 1982; Cole and Nicoll, 1984; Lancaster and Nicoll, 1987), PGE_1 (Fowler et al., 1985), and glutamate (Gereau and Conn, 1995). In all cases, these neuromodulators act by abolishing the sAHP. There is evidence that NE acts through cyclic AMP but the site of action has not been determined. Since glutamate suppresses the sAHP in CA1 hippocampal neurons by acting on mGluR5 receptors, it seems that a phosphoinositide pathway may also be involved (Gereau and Conn, 1995). Similarly, release of intracellular Ca^{2+} by metabotropic glutamate receptors can modulate the excitability of cerebellar Purkinje neurons (Netzeband et al., 1997). By stimulating the production of $InsP_3$, neuromodulators may act by draining Ca^{2+} from the stores. Consistent with such a mechanism is the observation that the cholinergic excitation of CA1 hippocampal neurons is preceded by an initial hyperpolarization (Benardo and Prince, 1982), which would be consistent with the emptying of Ca^{2+} from the internal stores. This hyperpolarization is followed by membrane depolarization associated with an increase in input resistance and a large acceleration of spike frequency. If this interpretation is correct, it implies that under resting conditions, the membrane potential may be regulated by a tonic release of Ca^{2+}. The SMOCs described earlier may be indicative of such a tonic release mechanism.

Depolarizing After-Potentials (DAPs)

Magnocellular neurons within the supraoptic nucleus display DAPs that develop after a single spike or trains of spikes. Release of Ca^{2+} from ryanodine-sensitive Ca^{2+} stores contributes to the buildup of Ca^{2+} responsible for activating the DAP (Li and Hatton, 1997). These DAPs contribute to the phasic firing of these supraoptic nucleus cells, which are responsible for releasing vasopressin.

Potentiation of IPSCs in Cerebellar Purkinje Cells

The size of IPSCs in cerebellar Purkinje cells can be potentiated by applying a train of depolarizing pulses (Hashimoto et al., 1996). This potentiation seemed to depend upon a contribution of Ca^{2+} released from $InsP_3$-sensitive stores, because it was suppressed either by inhibiting the $InsP_3$ receptors with heparin or by emptying the internal stores using thapsigargin.

Associativity

Association of Synaptic Signals

Studies on Purkinje cells have uncovered a mechanism of synaptic integration whereby the metabotropic glutamate receptor pathway of the parallel fiber input can be primed by prior activation of the climbing fibers (Batchelor and Garthwaite, 1997). A brief tetanic stimulation (three to six pulses at 50 Hz) of the parallel fibers caused an immediate depolarization due to the activation of AMPA receptors and a brief burst of firing, which was then followed by a much slower depolarizing potential. The latter results from the activation of the mGluR1s that are coupled to the phosphoinositide system and thus generate $InsP_3$ and DAG. This $mGluR_{EPSP}$ was potentiated by activating the climbing fiber, which triggered a Ca^{2+} spike that invaded the dendrites and hence the spines innervated by the parallel fibers. Subsequent activation of these parallel fibers gave a much larger $mGluR_{EPSP}$, and this potentiated state persisted for up to 2 min after the climbing fiber Ca^{2+} signal had disappeared. Somehow the transient Ca^{2+} rise induced by the climbing fiber left behind a relatively long-lasting "memory" capable of augmenting the parallel fibers and thus represents an associative mechanism for integrating noncoincident synaptic inputs (Batchelor and Garthwaite, 1997). The mechanism responsible for this "memory" is not known. Perhaps it can be explained by Ca^{2+} loading of the endoplasmic reticulum leading to sensitization of the $InsP_3$Rs that respond to parallel fiber stimulation.

Release of Neurotransmitter

Influx of Ca^{2+} through voltage-operated channels is the classical mechanism of transmitter release. The docked vesicles are engulfed in the microdomain of Ca^{2+} that forms when the voltage-operated channel opens during depolarization. In addition to this role of extracellular Ca^{2+}, there is increasing evidence that exocytosis might be regulated by release of calcium from intracellular stores (Blöchl and Thoenen, 1995; Peng, 1996; Smith and Cunnane, 1996; Tse et al., 1997). The basic premise is that the elementary events resulting from the opening of either $InsP_3$Rs or RYRs are capable of either enhancing or generating the localized high concentration burst of Ca^{2+} necessary to trigger exocytosis. For this mechanism to operate, therefore, it is necessary for portions of the endoplasmic reticulum to come into close contact with the plasma membrane and the associated secretory vesicles, as has been described by Westrum and Gray (1986). A role for $InsP_3$ receptors in controlling exocytosis has been well characterized in gonadotrophs (Tse et al., 1997). It was estimated that the level of Ca^{2+} in the vicinity of the subsurface cisternae was 5-fold higher than the global level of Ca^{2+}. In the case of cholinergic synapses in *Aplysia*, release of transmitter was enhanced by cyclic ADP ribose, which acts by potentiating the release of Ca^{2+} by RYRs (Mothet et al., 1998). Release of Ca^{2+} from internal stores can enhance transmitter release from lamprey reticulospinal neurons in response to activation of metabotropic glutamate receptors (Cochilla and Alford, 1998).

Such local release of Ca^{2+} from internal stores may also account for the release of nerve growth factor from hippocampal neurons (Blöchl and Thoenen, 1995). The activity-dependent release of nerve growth factor is unusual in that it takes place over much of the neuron and particularly from the soma and dendrites (Blöchl and Thoenen, 1996). Release from the latter site is of interest because it has been implicated in neuronal plasticity. Another unusual aspect of nerve growth factor release is that it can occur independently of external Ca^{2+} but does seem to require an influx of Na^+. An interesting possibility is that the entry of Na^+ may act

to speed up the efflux of Ca^{2+} from mitochondria, which then loads up the endoplasmic reticulum, thus enhancing the frequency of the elementary events responsible for triggering exocytosis. Such a mechanism would be consistent with the close association between the endoplasmic reticulum and the mitochondria, especially in the soma, dendrites, and synaptic ending (Figure 2).

A similar loading phenomenon might also explain the residual release of neurotransmitters that occurs when sympathetic nerve terminals are stimulated at high frequencies (Smith and Cunnane, 1996). Single impulses fail to induce release, but when given repetitively they begin to load up the endoplasmic reticulum to a point where the latter can release sufficient Ca^{2+} to stimulate exocytosis. This might be another example where the endoplasmic reticulum functions as an integrator; as it becomes progressively loaded, it may be capable of generating meaningful signals such as the triggering of exocytosis (Blaustein, 1988; Miller, 1991). In the case of sympathetic ganglionic neurons, release of Ca^{2+} from RYR-sensitive stores contributes to the Ca^{2+} signal in the synaptic terminals. The Ca^{2+} that enters through the voltage-operated channels triggers the release of small synaptic vesicles, whereas the Ca^{2+} released from the internal stores may raise the level above the threshold for the release of the dense-cored vesicles (Peng, 1996).

In summary, there is increasing evidence that the elementary events associated with Ca^{2+} stores located next to the plasma membrane may be capable of either generating or modulating the high intensity Ca^{2+} signals necessary to release certain neurotransmitters.

Synaptic Plasticity

Connectivity between neurons is mediated through synapses, which can undergo rapid and long-lasting changes in effectiveness. Such synaptic plasticity is thought to be the basis for learning and memory. Long-term potentiation (LTP) and long-term depression (LTD) are typical examples of how synaptic transmission can be reversibly enhanced or reduced, respectively. In the case of LTP, it is necessary to distinguish between early LTP (E-LTP), which fades after about an hour, and late-LTP (L-LTP), which persists for much longer. The induction of L-LTP, which requires gene transcription and protein synthesis, will be described in the following section. Here, we concentrate on the rapid events localized at the synapse responsible for short-term modifications in synaptic plasticity.

Much of our information on the mechanisms that modify synaptic strength in mammalian neurons has come from studies in the hippocampus (Bliss and Collingridge, 1993) or cerebellum (Linden and Connor, 1993; Linden, 1994; Bear and Malenka, 1994). Studies on the CA1 region of the hippocampus have revealed that the same synapses can be switched rapidly between LTP and LTD (Heynen et al., 1996). What is remarkable about this bidirectional conversion is that both LTP and LTD are mediated by Ca^{2+} (Linden, 1994; Bear and Malenka 1994; Linden and Connor, 1995). There is general agreement that an elevation of Ca^{2+} is the single most important factor for inducing changes in synaptic plasticity, but how can this messenger mediate the opposite effects of potentiation and depression within the same synapse? Based on the fact that a stronger stimulus is usually required to induce LTP than LTD, it was proposed that this bidirectional switch may depend upon the amplitude of the Ca^{2+} signal (Lisman, 1989; Artola and Singer 1993; Cummings et al., 1996). In addition to changes in Ca^{2+} concentration, it may also be important to consider other parameters such as the spatial and temporal organization of the Ca^{2+} signal (Linden, 1994; Linden and Connor, 1995).

One way of altering the spatial organization of Ca^{2+} signals is to vary the source of signal Ca^{2+} by using variable combinations of extracellular or intracellular stores. Although influx of Ca^{2+} through NMDA receptors or through voltage-operated channels is of major significance (Bliss and Collingridge, 1993), there is increasing evidence that the internal stores, functioning either as sinks or as additional sources of Ca^{2+}, may contribute to the onset of both LTP and LTD. As described earlier (Figure 1), access to these stores from the plasma membrane can be mediated either through Ca^{2+} itself (i.e., the process of Ca^{2+}-induced Ca^{2+} release) or through the generation of $InsP_3$ by activating metabotropic receptors (Figure 1). The receptors that have received the most attention are the metabotropic glutamate receptors (mGluRs), but there is considerable controversy concerning their role in synaptic plasticity (Nakanishi, 1994). Those metabotropic receptors that are coupled to phosphoinositide signaling have been implicated in synaptic plasticity in both the hippocampus (Bashir et al., 1993; Bolshakov and Siegelbaum, 1994; Reyes and Stanton, 1996; Riedel and Reymann, 1996) and the cerebellum (Kohda et al., 1995) and in the visual cortex (Kato, 1993; Komatsu, 1996). Cholinergic receptors may also play a role in the hippocampus because carbachol alone can induce LTP in the absence of external Ca^{2+}, strongly indicating a role for the internal stores (Auerbach and Segal, 1994). Of greater significance is the observation that subthreshold cholinergic stimulation induced LTP when coupled with subthreshold tetanic stimulation. Such a synergistic effect could be explained by the $InsP_3R$ functioning as a coincidence detector. As described earlier, the opening of the $InsP_3R$ requires both Ca^{2+} and $InsP_3$ (Figure 1). In the case of the hippocampus, therefore, it is conceivable that the cholinergic stimulation provides a small elevation of $InsP_3$, which then enhances the Ca^{2+} sensitivity of the $InsP_3R$ such that it will respond to the subthreshold Ca^{2+} signals entering through either the voltage-operated channels or the receptor-operated channels.

Further evidence for a role of internal stores in mediating synaptic plasticity has come from experiments using a variety of pharmacological agents. Thapsigargin, which specifically inhibits the Ca^{2+} pump on the internal store, was found to inhibit the onset of both LTP (Harvey and Collingridge, 1992; Auerbach and Segal 1994; Behnisch and Reymann, 1995; Wang et al., 1996) and LTD (Kohda et al., 1995; Reyes and Stanton, 1996). Of particular interest is the observation that this inhibitory effect of thapsigargin in the hippocampus was related to stimulus intensity. Thapsigargin was ineffective at high stimulus intensities but began to inhibit the onset of LTP induced by weaker stimuli (Behnisch and Reymann, 1995). Perhaps at these lower intensities the Ca^{2+} signal

is recruited from both extra- and intracellular sources. Injecting neurons with heparin, which inhibits Ca^{2+} release by $InsP_3Rs$, prevented the onset of LTD (Kato, 1993) or the induction of LTP at inhibitory synapses in the visual cortex (Komatsu, 1996).

Another useful drug for manipulating the intracellular Ca^{2+} stores is ryanodine, which has a range of effects on RYRs. Low concentrations of ryanodine were found to switch the response of CA1 hippocampal neurons to low frequency stimulation from LTD to LTP (Wang et al., 1996). It was concluded that the induction of LTD by low frequency stimulation depends upon an influx of external Ca^{2+} through voltage-operated channels, which is then augmented by Ca^{2+} release from the internal stores through the RYRs (Wang et al., 1997). Reyes and Stanton (1996) suggest that the induction of hippocampal LTD depends upon the release of Ca^{2+} from both postsynaptic $InsP_3$-sensitive stores and from presynaptic stores regulated by RYRs. Similarly, Ca^{2+} release from RYRs has been implicated in the induction of LTD in cultured cerebellar Purkinje cells (Kohda et al., 1995). It is difficult to draw general conclusions from all of these studies, but what seems abundantly clear is that internal stores can contribute either as sources or as sinks of Ca^{2+} during activity-dependent changes in synaptic plasticity.

Gene Transcription

Long-term memories are laid down when the short-term modifications in synaptic plasticity, such as those described in the previous section, are consolidated by more permanent changes brought about through gene transcription and protein synthesis. A fundamental unsolved problem in neurobiology concerns the way in which activated synapses communicate with the nucleus.

There are a number of ways by which activated synapses might send signals to the nucleus. One mechanism might entail the local generation of signaling intermediates that then diffuse into the nucleus. There already is evidence that the cyclic AMP effector molecule protein kinase A and the ras pathway intermediate MAP kinase are translocated into the nucleus following neural activity in *Aplysia* (Backsai et al., 1993; Martin et al., 1997). Another suggestion is that activated transcription factors, such as NFkB, may transmit information from activated synapses into the nucleus (Meberg et al., 1996). One problem with such mechanisms is that they are slow and the messengers are likely to be corrupted en route to the nucleus, especially in the case of those synapses located on the more distal dendrites. One way of avoiding signal degradation is to resort to regenerative processes such as an action potential or a Ca^{2+} wave. In both cases, the endoplasmic reticulum may play a role in transferring information into the nucleus. For example, the large Ca^{2+} signal that occurs at the activated synapse could spawn a regenerative dendritic Ca^{2+} wave that could travel down to the nucleus (Figure 5). As noted earlier, such a Ca^{2+} wave has already been described in CA1 neurons (Jaffe and Brown, 1994). A more likely scenario is that the activated synapses communicate with the soma via an electrical signal (passive electrotonic spread or a dendritic action potential). The resulting depolarization of the soma will open voltage-operated channels, and the entry of Ca^{2+} can then communicate with the nucleus through a number of mechanisms, including a somatic Ca^{2+} wave (Figure 5). The microdomain of Ca^{2+} near the plasma membrane can activate adenylyl cyclase to produce cyclic AMP (Cooper et al., 1995), which can either diffuse into the nucleus (Figure 5) or can activate protein kinase A, causing it to translocate into the nucleus (Backsai et al., 1993). Ca^{2+} can also stimulate CAM kinase to phosphorylate transcription factors such as Ca^{2+}/cAMP response element binding protein (CREB) (Deisseroth et al., 1996), or it can activate the ras signaling pathway (Rosen et al., 1994). In the latter case, MAP kinase has been shown to translocate into the nucleus during the induction of long-term synaptic plasticity in *Aplysia* (Martin et al., 1997). Through this extensive network of interactions, Ca^{2+} entering at the plasma membrane can induce a number of signaling pathways known to be essential for gene activation. There is general agreement that second messengers such as cyclic AMP and Ca^{2+} play a critical role in neuronal gene transcription (Bailey et al., 1996; Finkbeiner and Greenberg, 1997; Bito et al., 1997; Hardingham et al., 1997). One of the functions of these signaling pathways is to phosphorylate CREB (Figure 5). In order for CREB to induce transcription, it is necessary for a Ca^{2+} signal to invade the nucleus (Hardingham et al., 1997). It may gain access to the nucleus either by diffusion or by a somatic wave if the trigger Ca^{2+} entering across the membrane can be amplified and relayed by release from the endoplasmic reticulum (Usachev and Thayer, 1997) (Figure 5). In summary, therefore, Ca^{2+} appears to have multiple roles in nuclear gene activation. Not only can it initiate the onset of a number of signaling pathways at the plasma membrane, but it can also invade the nucleus to play a more direct role in gene activation. In the latter case, the endoplasmic reticulum may play an active role in relaying Ca^{2+} signals from the cell surface into the nucleus.

An intriguing aspect of the endoplasmic reticulum, based on its function as a Ca^{2+} sink, is its potential to function as a buffer to prevent information accessing the nucleus during normal neural processing. The endoplasmic reticulum can effectively filter out random events and only reverts to its capacity to amplify and transmit a Ca^{2+} signal once its buffering capacity is saturated. A frequent observation is that synaptic stimulation induces a large and rapid elevation of Ca^{2+} within the dendrites with a much smaller and slower Ca^{2+} signal reaching the soma (Tank et al., 1988; Geiling and Schild, 1996). The need to circumvent this endoplasmic reticulum buffering system may explain why gene transcription and memory consolidation requires either an unusual juxtaposition of events or repetitive stimulation. In the case of hippocampal neurons, for example, a 1 s stimulation at 100 Hz induces E-LTP, but there was no evidence of the L-LTP that requires gene transcription and protein synthesis. The latter events were induced when the above stimulus was repeated three times at 10 min intervals (Frey et al., 1993; Nguyen et al., 1994). Despite being given 10 min apart, the 1 s periods of stimulation must somehow leave behind a “memory”

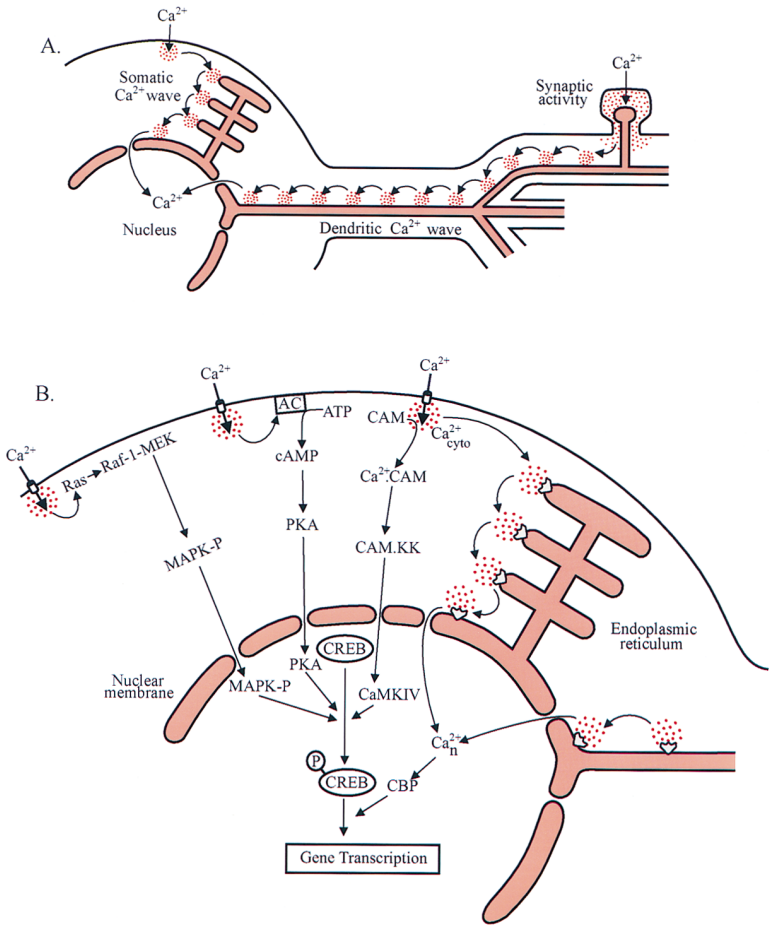


Figure 5. Proposed Role of Ca^{2+} Signaling in Neuronal Gene Transcription

(A) Waves of Ca^{2+} may facilitate the transfer of a Ca^{2+} signal into the nucleus. An elevation of Ca^{2+} at synapses may trigger a dendritic Ca^{2+} wave. Alternatively, a somatic Ca^{2+} wave initiated at the cell surface may help to transfer a Ca^{2+} signal into the nucleus.

(B) Summary of signaling pathways sensitive to Ca^{2+} entry across the plasma membrane. AC, adenylyl cyclase; CAM, calmodulin; CAM.KK, calmodulin kinase kinase; cAMP, cyclic adenosine monophosphate; CREB, Ca^{2+}/cAMP response element binding protein; CBP, CREB-binding protein; MAPK, mitogen-activated protein kinase; and PKA, protein kinase A.

that enables subsequent stimuli to build up a meaningful signal to induce transcription. The nature of this "memory" is still a mystery. One interesting possibility is that the endoplasmic reticulum may function as such a "memory" by integrating the Ca^{2+} signals associated with each stimulation period. Once primed, the endoplasmic reticulum would be capable of releasing this Ca^{2+} in a regenerative manner, thus enabling the signal to spread through the cell to invade the nucleus. Such an intracellular Ca^{2+} wave emanating either from the synapses or from the surface membrane of the soma may provide one of the mechanisms for relaying information into the nucleus (Figure 5).

Conclusion

Neurons employ two sources of signal Ca^{2+}. Entry of external Ca^{2+} through voltage- or receptor-operated channels plays a major role, but there also is an important contribution of Ca^{2+} released from the endoplasmic reticulum. The latter is a continuous membrane network that extends throughout the neuron. Both structurally and functionally, the endoplasmic reticulum may be considered as a neuron-within-a-neuron. Indeed, the endoplasmic reticulum network has many properties resembling those of the plasma membrane. In particular, it maintains a large concentration gradient of Ca^{2+} that can be released in a regenerative manner, thus allowing information to spread long distances through Ca^{2+} waves. This regenerativity is mediated by either $InsP_3$ or ryanodine receptors, which display a process of Ca^{2+}-induced Ca^{2+} release. One of the important factors determining the sensitivity of these two Ca^{2+} channels is the content of Ca^{2+} in the lumen. During bouts of neuronal activity, the Ca^{2+} that enters from the outside is taken up by the endoplasmic reticulum, which then becomes primed; i.e., the $InsP_3$ receptors and the RYRs become sufficiently sensitive to release the accumulated Ca^{2+} back into the cytosol. In effect, the endoplasmic reticulum provides a short-term memory of neural activity by integrating the brief Ca^{2+} pulses associated with each discharge. When the accumulated Ca^{2+} reaches the critical threshold for regenerative release, the large explosive and global release of Ca^{2+} could provide the neuron with information concerning previous levels of neuronal activity.

The important point to stress, therefore, is that neuronal Ca^{2+} signaling is controlled by the endoplasmic reticulum and the plasma membrane operating as a binary membrane system. The two membranes interact with each other to control a wide range of processes including excitability, neurotransmitter release, associativity, plasticity, and gene transcription.

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