INTRACELLULAR CALCIUM SIGNALING – BASIC MECHANISMS AND POSSIBLE ALTERATIONS

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INTRACELLULAR CALCIUM SIGNALING – BASIC MECHANISMS AND POSSIBLE ALTERATIONS

PLATON G. KOSTYUK*
Bogomoletz Institute of Physiology, National Academy of Sciences, Kiev, Ukraine

ELENA A. LUKYANETZ
Bogomoletz Institute of Physiology, National Academy of Sciences, Kiev, Ukraine

Abstract. The main property of neuronal and other excitable cells is their capability to transform excitatory waves into intracellular signals, where they trigger or modulate practically all cellular functions. Influx of calcium ions from the extracellular medium ("calcium signals") plays a key role in this process. Correspondingly alterations in intracellular calcium signaling are an important component of the physiological process of aging and of the most frequent and complicated forms of pathology, and their clarification is of basic medical importance. Therefore in the present paper we will discuss the main molecular mechanisms determining such signaling as well as their possible alterations

Keywords: Calcium channels, Calcium stores, Calcium, Endoplasmic reticulum, IP3 receptors, Mitochondria, RyR receptors, Store-operated channels, TRP channels

* To whom correspondence should be addressed: Platon Kostyuk, Bogomoletz Institute of Physiology, National Academy of Sciences, Kiev, Ukraine, E-mail: pkostyuk@biph.kiev.ua

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1. Calcium channels in cell membrane

Calcium-selective voltage-operated ion channels form a main pathway for transmembrane calcium currents (I_{Ca}). A comprehensive analysis of the function of these channels became possible after the elaboration of special techniques allowing the recording of I_{Ca} separately from other types of transmembrane currents (I_{NA}, I_K). The intracellular perfusion (or dialysis) approach (Kostyuk *et al.*, 1975; Kostyuk and Krishtal, 1977) was especially helpful in this respect (Fig.1). Quite soon it became obvious that calcium channels, contrary to previously analysed sodium channels, are not homogeneous in their properties and actually form a whole family of different channels.

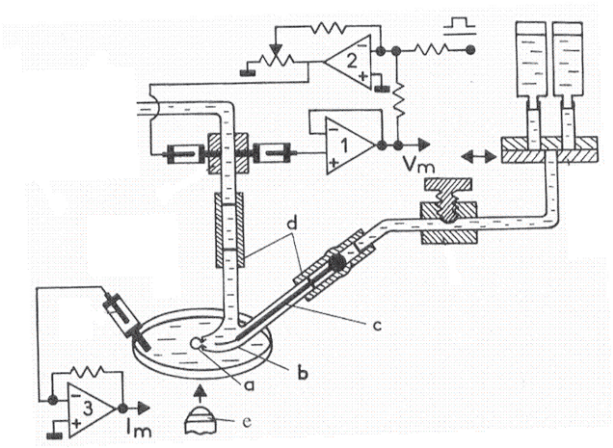

Figure 1. Schematic presentation of intracellular perfusion method. **a** - perfused cell, **b** – plastic micropipette, **c** – nylon thread for destruction of the part of cell membrane, **d** – holders, **e** – microscope objective for visual control.

The presence of two main subtypes of Ca^{2+} channels was first detected in our laboratory when analysing the current-voltage characteristics of I_{Ca} in dorsal root ganglion (DRG) neurones: this current could be clearly separated into low-voltage and high-voltage activated (LVA and HVA) components (Veselovskii and Fedulova, 1983). Contrary to already known HVA currents, the LVA current could be activated at very negative membrane potentials (between -60 and -40 mV) and rapidly inactivated in a potential-dependent way. Separation of Ca^{2+} channels into these two main groups according to their potential dependence has been confirmed in several subsequent papers (Carbone and Lux, 1984; Fedulova *et al.*, 1985; Nowycky *et al.*, 1985). The existence of both groups of channels has been demonstrated in a large variety of excitable cells.

Concerning their functional properties, LVA channels seemed to form a quite homogeneous group. However, in some neurons a component in the corresponding I_{Ca} has been observed with different kinetics: somewhat slower activation and extremely slow inactivation with an almost potential-independent rate constant (thalamic reticular neurons—Huguenard and Prince, 1992; DRG neurons— (Kobrinsky *et al.*, 1994).

The situation with HVA channels is much more complex. Nowycky *et al.* (Nowycky *et al.*, 1985) separated the corresponding component of I_{Ca} into an inactivating and a steady component and denoted them as N and L currents. These symbols together with symbol T for the LVA current are now widely used in the literature. The three types of calcium channels have been distinguished in mouse and rat sensory neurons, rat or guinea-pig granule and pyramidal hippocampal neurons, clonal endocrine cells etc. The situation seemed to be more or less clear and convenient for functional analysis. However, when other methods for differentiation of Ca^{2+} channels became popular, they rapidly prompted the conclusion that the present classification is too simple and that Ca channels are much more diverse. The recognition of this fact coincided with a rapid increase in the list of cellular functions triggered or modulated by the influx of Ca^{2+} through calcium channels. Therefore the question concerning the diversity of Ca^{2+} channels and their functional implications is still under consideration. The existing classifications of calcium selective voltage-operated channels are summarized in Fig. 2.

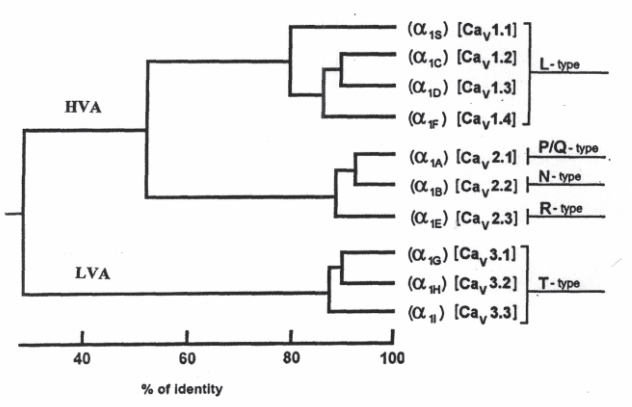

Figure 2. Classifications of calcium selective voltage-operated channels.

A natural way to distinguish different types of voltage-operated Ca^{2+} channels and reveal their role in pathological conditions could be a possible modulation of their activity by physiologically active substances (transmitters,

hormones and metabolites). The presence of such modulation is a unique property of Ca^{2+} channels, differentiating them from many other types of ion channels; the classical example is the potentiation of Ca^{2+}- channel activity in cardiomyocytes by β-adrenoreceptor agonists mediated through cAMP-dependent protein phosphorylation (Reuter, 1974).

Again, the division of Ca^{2+}- channels into LVA and HVA types seems to fit well with their susceptibility to metabolic modulation. HVA channels are very sensitive to interruption of their connections with cytoplasmic processes; if the cell is perfused with saline solutions, these channels rapidly pass into a "silent" state (channel "run-down"). Conversely, LVA channels continue to function even in isolated membrane patches (Fedulova *et al.*, 1985). This difference may indicate that HVA Ca^{2}-channels have to be continuously phosphorylated in order to remain in an active state. This has been clearly demonstrated in cardiac cells; in neuronal cells also dephosphorylation by phosphatases "downregulates" HVA channels, and phosphatase inhibition may "run-up" these channels. A systematic study of possible tonic regulation of HVA Ca^{2+} channel activity was made in our laboratory on identified snail neurones (Kostyuk and Lukyanetz, 1993). Upregulation of I_{Ca} could be induced by lowering $[Ca^{2+}]_i$, injection of calmodulin (CM) antagonists (TFP), inhibition of phosphodiesterase by isobutylmethylxanthine (IBMX) and inhibition of Ca-CM-dependent phosphatase (calcineurin) by okadaic acid. It is important to notice that additional upregulation of I_{Ca} remained possible by application of a natural Ca^{2+} channel modulator in these neurons: 5-hydroxytryptamine (5-HT, serotonin). 5-HT obviously exerted its action through the same channel-phosphorylating system based on Ca^{2+}-CM-dependent enzymes, as its effect could be blocked by elevation of $[Ca^{2+}]_i$ or supported by inhibition of the activities of phosphodiesterase and phosphatase. In fact both enzymes form here a negative feedback system for tonic downregula tion of Ca^{2+} channels activated by elevation of $[Ca^{2+}]_i$; the intervention of both enzymes may occur in subsequent order, as the K_D of their activation by Ca^{2+} differ substantially (0.04 μM and 0.69 μM respectively).

Experiments on identified snail neurons have shown that different secondary messenger systems may be involved in Ca^{2+} channel modulation in different cells. There are neurones in which HVA Ca^{2+} channels are upregulated by a cAMP-dependent mechanism triggered by activation of 5-HT receptors in the neuronal membrane (Kostyuk *et al.*, 1992a). In other snail neurones upregulation is exerted through activation of protein kinase C (PK-C), and the natural agonist can be a parathyroid hormone-like peptide (Kostyuk and Doroshenko, 1990; Kostyuk *et al.*, 1992b). Parathyroid hormone is an endogenous upregulator of calcium channels also in cardiomyocytes and snail neurones, however, in neuroblastoma cells and smooth muscle fiber synthetic

parathyroid hormone inhibited the activity of L-type Ca^{2+} channels (Pang *et al.*, 1990). In some neurons both mechanisms could be found; they were additive and had a different time course. Mediation of the response to 5-HT through activation of cGMP-dependent protein kinase has also been demonstrated in certain neurons. Finally, upmodulation of HVA Ca^{2+} channels triggered by activation of muscarinic receptors and mediated through a still unknown secondary messenger system may also occur (Gerschenfeld *et al.*, 1991).

The molecular mechanisms of the effect of phosphorylation on channel function are still unknown; this process remains after transplantation of the HVA channel in phospholipid bilayer and is manifested mainly by prolongation of the mean open time. In skeletal muscle it is connected structurally to the C terminal of the α_1 subunit, as the cleavage of its main part removes the major site for cAMP-dependent phosphorylation (De Jongh *et al.*, 1994).

Much more complicated are the results obtained in vertebrate neuronal cells. Here the possibility of direct interaction between GTP-binding proteins (G proteins) involved in the adenylate cyclase complex and Ca^{2+} channels has been postulated. Therefore the activity of Ca^{2+} channels can be modulated here both through a short intramembrane and a longer cytosolic pathway, which has been shown for cardiomyocytes (Shuba *et al.*, 1990; Shuba *et al.*, 1991), DRG neurones (Dolphin and Scott, 1990) and other structures. Both effects can be opposite in nature. Rapid β-adrenergic potentiation of cardiac Ca^{2+} channels by a fast G_s protein pathway has been demonstrated on cardiomyocytes and their inhibition by activation of cGMP-dependent PK. However, for cardiac Ca^{2+} channels the possibility of direct regulation by G proteins is still questioned (Hartzell and Fischmeister, 1992).

The possible effects of G proteins on neuronal Ca^{2+} channels were studied mostly by testing the influence of application of pertussis-toxin (PTX) or intracellular introduction of GTP analogues (GTPγS or GDPβS) which either promote or inhibit their action. The results indicated the possible existence of a tonic inhibitory action of G proteins on neuronal Ca^{2+} channels which can be removed by loss of intracellular GTP and subsequent inactivation of the corresponding G protein. After initial inhibition by GTPγS a delayed augmentation of Ca^{2+} channel currents has been observed in chick sensory neurones, which represented not relief from inhibition but a distinct upregulatory process prevented by PK-C inhibitor (Zong and Lux, 1994); its mechanism is unclear and may involve intracellular phosphorylating systems. More complicated mechanisms of the action of G proteins have been also suggested: promotion of the effects of different receptor agonists on Ca^{2+} channels (Scott and Dolphin, 1987) and involvement in the interaction between DHPs and channels (Schettini *et al.*, 1991). The possible mechanisms of

physiological modulation of the functioning of Ca^{2+} channels are presented schematically in Fig. 3.

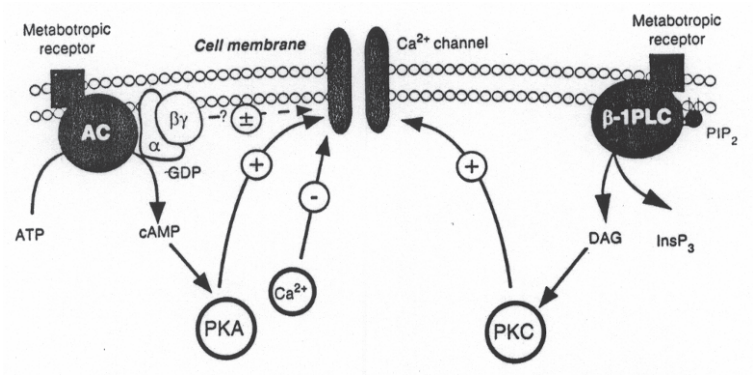

Figure 3. Schematic presentation of intracellular mechanisms modulating the functioning of Ca^{2+} channels. AC, adenylate cyclase; GDP, G protein; PKA/ protein kinase A; PKC, protein kinase C; DAG, diacylglycerol.

Extremely variable up- and downmodulatory effects were shown on different neuronal structures under the action of various receptor agonists. In fact, all known neurotransmitters and hormones modulate the activity of voltage-gated Ca^{2+}channels, mainly inhibiting it.

From all the data presented we may conclude that the members of a family of voltage-operated Ca^{2+} channels have common basic properties, indicating the presence of identical features in the structure of the channel-forming protein molecule (its α_1 subunit). The most essential subdivision of this family is between low- and high voltage-activated (LVA and HVA) channels; their principal differences in the properties of natural and artificial gene expression and in the mechanisms of interaction with cytosolic processes. The group of LVA channels is quite homogeneous in their features; they do not seem to be susceptible to phosphorylation by intracellular protein kinases - a mechanism highly important for the functioning of HVA channels.

Contrary to LVA channels, HVA channels show immense variability in their properties, leaving little hope for strict classification based on kinetic characteristics, pharmacological sensitivity, etc. However, one should not be distressed by this fact and should not spend too much time in classifying, but use this feature for applied purposes to find ways for selective modification of Ca^{2+}-dependent cellular functions in different species, different tissues and

different cells of the same tissue. This may be of major importance not only for medicine but even more so for effective analysis of the unprecedented role of Ca^{2+} ions in the living process.

2. Intracellular calcium signals

The temporal characteristics of the cytoplasmic calcium signal are determined by (1) the amount of calcium entering the cytoplasm via plasmalemmal channels and the amount of calcium released from the stores; (2) cytoplasmic calcium buffering by fast Ca^{2+} chelators; (3) calcium uptake by mtracellular organelles; and (4) calcium extrusion into the extracellular space. Here we will summarize the current data concerning the basic features of the mentioned components of the calcium signal generation chain.

The most abundant stimulus which practically always induces a prominent elevation of intracellular Ca^{2+} in nerve cells is the depolarization of the cellular membrane. This depolarization opens plasmalemmal voltage-operated Ca^{2+} channels, which deliver Ca^{2+} ions in the form of a transmembrane calcium current. These Ca^{2+} ions in turn may trigger Ca^{2+}-induced Ca^{2+} release (CICR), which further amplifies the depolarization-induced $[Ca^{2+}]_i$ signal. It is quite obvious that while the depolarization and, respectively, transmembrane calcium current develop in a millisecond time range, the $[Ca^{2+}]_i$ transients last for several seconds. This time dissociation between I_{Ca} and $[Ca^{2+}]_i$ transient is quite a consistent observation for many neuronal types (Mironov *et al.*, 1993; Llano *et al.*, 1994; Shmigol *et al.*, 1995). It may equally well reflect either Ca^{2+} redistribution within the cellular cytoplasm or the amplifying effect of CICR, triggered by calcium entry (see below). Calcium removal from the bath also abolished depolarization-induced $[Ca^{2+}]_i$ transients, indicating that I_{Ca} and $[Ca^{2+}]_I$ elevation are causally related. Further evidence for such a relation comes from a comparison of the voltage dependence of peak I_{Ca} with the voltage dependence of the peak $[Ca^{2+}]_i$ transient

After entering the cell through Ca^{2+} -permeable channels either from the extracellular space or from intracellular stores, Ca^{2+} ions immediately face the strongest challenge from a wide complex of mechanisms trying to exclude them from their possible physicochemical activity. They include direct binding by cytosolic buffers, expulsion back to the extracellular space by plasmalemmal transporting systems (Ca-ATPase, Na^+/Ca^{2+} exchange) and reabsorption into intracellular stores. These mechanisms have different affinity and different kinetic characteristics, and their separation and analysis depend entirely on the availability of technical possibilities for recording intracellular changes of free

Ca^{2+} with adequate time and space resolution. This is especially important for the evaluation of the first of the mentioned steps—binding by cytosolic buffers—which obviously takes place with much faster time constants (in the millisecond range) compared with mechanisms involving energy-consuming ion-transporting systems.

The major part of Ca^{2+} ions entering the cell is almost instantly buffered by cytoplasmic calcium-binding sites. Only a small amount of calcium which penetrated into the cytosol shows up as free Ca^{2+}. An extensive analysis of such binding has been made recently on chromaffin cells using digital imaging and photometry in conjunction with the fluorescent indicator fura-2 (Neher and Augustine, 1992). It was established that the endogenous buffer capacity in these cells is about 75. It is created mostly by some immobile molecules, since it did not decrease substantially even during long-lasting dialysis of the cell, and has a low affinity for Ca^{2+} ions, because it did not saturate even with 1 mM Ca^{2+} inside the cell. They obviously represented by Ca^{2+} -binding proteins which belong to the so-called EF-hand family, where EF corresponds to a Ca^{2+} -coordinatmg helix-loop-helix sequence. Possible candidates are calmodulin, calreticulin, parvalbumin and calbindin. Intracellular administration of calbindin and parvalbumin into rat sensory neurons did not significantly alter the basal $[Ca^{2+}]_i$ but substantially reduced the peak amplitude of the Ca^{2+} signal obtained by membrane depolarization, decreased its rate of rise and altered the kinetics of decay to a single slow component, calbindin being more effective (Chard *et al.*, 1993). However, Neher and Augustine have considered calmodulin as more compatible with the physiological characteristics of cytosolic Ca^{2+} buffering, while parvalbumins are not (because their Ca^{2+} affinities are in the submicromolar range). In addition, cytosolic buffer capacity can be mediated by ATP, which seems to be able to bind a significant amount of Ca^{2+} ions; in this case they represent mobile intracellular buffers which could be functionally important by supporting cytoplasmic diffusion of Ca^{2+} ions and facilitating the spreading of Ca^{2+} signals (Zhou and Neher, 1993).

Despite the presence of this rapid buffering capacity, Ca^{2+} ions entering the cell during depolarization still produced in these cells substantial spatial gradients, being highest in the vicinity of the plasmalemma and declining towards its centre.

Direct analysis of calcium buffering in nerve cells in our group this has been done using a different technical approach which can be called “Ca-clamp” (Belan *et al.*, 1993). In this technique the intracellular free Ca^{2+} concentration has been fixed at different physiologically significant levels in large snail neurons by a feedback system between the fluorescent signal of the fura-2 probe loaded into the cell and ionophoretic injection of Ca^{2+} ions through a $CaCl_2$-loaded microelectrode. The membrane potential of the neuron has also

been clamped. Clamping of $[Ca^{2+}]_i$ at a new increased level was accompanied by a transient of the Ca^{2+}-injecting current corresponding to injection of 36 ± 20 2μM Ca^{2+} for a change in resting level of 0.1 μM. Obviously, this transient represents the filling of a fast cytosolic buffer which has to be done before reaching a new increased level of $[Ca^{2+}]_i$. Taking into account that some additional capacity is added by fura-2, the endogenous buffer capacity here equals about 300; this is much higher than in chromaffin cells.

These differences in the buffer capacity may indicate diversity of cytosolic properties in different types of cells. On the other hand, cell conditions during measurement also should be taken into account intracellular dialysis through the pipette may affect the properties of endogenous buffers. Obviously, special studies of the cytosolic buffering in different types of nerve cells would be quite important.

Calcium induced Ca^{2+} release plays a defined and very important role as an amplifier of $[Ca^{2+}]_i$ signal in a variety of excitable cells. Most of the current knowledge concerning the CICR mechanism came from experiments on muscle cells where CICR plays a crucial role in initiation of the contraction. In myocytes, plasmalemmal Ca^{2+} influx is responsible for only 10-20% of actual $[Ca^{2+}]_i$ signal; the rest is due to internal Ca^{2+} release. In neural cells the role and importance of the CICR mechanism is less clear. As was mentioned above, the CICR mechanism is associated with the activity of Ca^{2+}-gated Ca^{2+} release channels incorporated into the membrane of the endoplasmic reticulum. Using various modulators of these channels it appeared possible to describe major physiological properties of CICR in nerve cells.

Caffeine is one of the most popular and convenient tools for studying the properties of Ca^{2+}-sensitive Ca^{2+} stores. It belongs to methylxanthines, which have a number of well-defined pharmacological effects/ related to the blockade of adenosine receptors, inhibition of phosphodiesterases etc. From the pharmacological point of view methylxanthines are effective stimulators of the central nervous system.

An additional feature of caffeine and several other methylxanthines (theophylline and IBMX) which is important for investigations of $[Ca^{2+}]_i$ signaling is their ability to activate Ca^{2+}-gated Ca^{2+}- release channels, thus inducing Ca^{2+} liberation from internal stores. The discovery of caffeine as a potent Ca^{2+} mobilizer from the internal stores came from investigations of caffeine-induced contractures in skeletal and cardiac muscle. This elevation persists in Ca^{2+}-free extracellular solution, thus suggesting its origination from intracellular structures, cf. (Ganitkevich and Isenberg, 1992).

Effects of caffeine on $[Ca^{2+}]_i$ in peripheral neurons were studied on freshly isolated and cultured sensory and sympathetic neurons from both non-mammalian and mammalian preparations. The major properties of caffeine-

evoked $[Ca^{2+}]_i$ transients appeared to be quite similar in these preparations. Supervision of peripheral neurons by solutions containing 10-20 mM caffeine induced the elevation of $[Ca^{2+}]_i$ from the resting level to 300-400 nM with the maximal rate of rise in the range of 150-200 nM/s. After reaching a peak, the $[Ca^{2+}]_i$ level started to decline in the presence of caffeine and within 80-100 sec cytoplasmic calcium returned to the initial resting value. If the caffeine was washed out during this recovery phase $[Ca^{2+}]_i$ immediately dropped to the basal level. In the absence of extracellular calcium caffeine produced a similar rise in $[Ca^{2+}]_i$ indicating that caffeine released Ca^{2+} from the internal store. The amplitude of caffeine-triggered $[Ca^{2+}]_i$ transients in sensory neurones was reported to be modulated by basal $[Ca^{2+}]_i$ level (Mironov and Hermann, 1994): the maximal amplitudes of caffeine-induced $[Ca^{2+}]_i$ transients were observed at basal $[Ca^{2+}]_i$ close to 300-400 nM. At lower and higher $[Ca^{2+}]_i$; the amplitudes of calcium release became smaller, indicating presumably the bell-shaped regulation of the CICR channel by cytoplasmic Ca^{2+} concentration.

The action of methylxanthines on $[Ca^{2+}]_i$ is clearly intracellular and at millimolar drug concentrations $[Ca^{2+}]_i$ elevation develops rapidly, indicating that methylxanthines are approaching their intracellular targets quite fast. Fortunately, while measuring $[Ca^{2+}]_i$ with one of the ratiometric dyes, namely indo-1, it appeared possible simultaneously to monitor the intracellular caffeine concentration. Monitoring of the intracellular caffeine (as well as other methylxanthines) concentration was facilitated by the fact that methylxanthines bind to a Ca^{2+} indicator indo-1 (Usachev and Verkhratsky, 1995) and quench its fluorescence in a wavelength-independent way. An important feature of methylxanthine-dependent quenching of indo-1 is its concentration dependence. Using the concentration-dependent quenching of indo-1 fluorescence by methylxanthines, it has been shown that methylxanthines indeed freely penetrate cellular membrane and the intracellular caffeine concentration equilibrates with the external concentration with a time constant of about 8 sec (O'Neill *et al.*, 1990).

Caffeine and other methylxanthines released Ca^{2+} from the internal stores in nerve cells in a concentration-dependent manner. The threshold concentrations for caffeine are in the range of 0.5-1 mM and saturation is reached at about 5 mM (experiments on rat sensory neurons); IBMX and theophylline show similar concentration dependence (Usachev *et al.*, 1993; Usachev and Verkhratsky, 1995). Moreover, the submaximal caffeine concentrations could not fully discharge the calcium store. In experiments performed on cultured DRG neurons we have found that these cells responded to application of 2 mM caffeine by transient $[Ca^{2+}]_i$ elevation with an average amplitude of 76 ± 31 nM. The successive challenge with 2 mM caffeine applied 30 s later failed to produce a $[Ca^{2+}]_i$ response; however, 10 mM caffeine induced $[Ca^{2+}]_i$ transients

with an average amplitude of 232 ± 17 nM. This property matches the 'quantal' or "incremental" calcium release from InsP3- sensitive stores.

The concentration dependence presumably reflects the number of activated CICR channels, while “quantal” release might indicate the existence of various ER compartments bearing different sensitivity to caffeine. In addition, such a property predicts a “gradual”, rather than an “all-or-nothing” responsiveness of the CICR mechanism in nerve cells.

Based on the general scheme of caffeine-induced calcium liberation, it is obvious that the kinetics of the caffeine-induced $[Ca^{2+}]_i$ transient is determined by the balance between $Ca^{2=}$ release from the internal store, Ca^{2+} reuptake into the store, cytoplasmic Ca^{2+} buffering and $Ca^{2=}$ extrusion to the extracellular space. Ca^{2+} release by itself is determined by the driving force for Ca^{2+} ions (assuming that ER Ca^{2+} channels are open throughout the caffeine application). After initiation of the release the driving force for $Ca^{2=}$' ions falls because of (1) deprivation of the intraluminal free Ca^{2+} content and (2) increase of the cytoplasmic Ca^{2+} concentration. During release some Ca^{2+} ions are reloaded back into the store, and some are buffered and/or extruded outside. However, it seems that the most important mechanism responsible for the decay of the caffeine-induced $[Ca^{2+}]_i$; transients is associated with the depletion of ER-releasabIe Ca^{2+}.

The major question is whether the refilling of internal stores could be fulfilled by calcium already existing in the cytoplasm, or whether it is necessary to initiate an additional calcium inflow from the external environment. A characteristic property of the peripheral neurons is the ability of caffeine-sensitive stores to restore their responsiveness to caffeine in steady-state conditions (Friel and Tsien, 1992; Usachev *et al.*, 1993). Such a recovery of the amplitude of the caffeine-induced $[Ca^{2+}]_i$, transient obviously reflects the complete replenishment of the calcium stores.

The deprivation of internal stores of releasable Ca^{2+} stimulates Ca^{2+} uptake from the ER. Quite often following the washout of caffeine a subresting drop of $[Ca^{2+}]_i$ is observed (so-called “post-caffeine undershoof” This undershoot probably reflects the increased activity of SERCA pumps and is believed to be a sign of the activation of SERCA pumps following the depletion of internal stores. The speed of refilling of ER stores is greatly enhanced if additional Ca^{2+} has been injected into the cytoplasm: if the cell was depolarized after depletion of stores by caffeine the succeeding application of caffeine induced a full-size $[Ca^{2+}]_i$ response. Moreover, quite often the amplitude of this $[Ca^{2+}]_i$ response (elicited immediately after the end of depolarization-triggered $[Ca^{2+}]_i$ transient) was significantly larger as compared with the initial caffeine-induced $[Ca^{2+}]_i$ elevation (Thayer *et al.*, 1988; Usachev *et al.*, 1993).

Thus Ca^{2+} entry via voltage-operated calcium channels may serve as an additional source of calcium ions which could be trapped by caffeine-sensitive stores. In addition, Ca^{2+} influx during the depolarization can overload these stores/ indicating an involvement of the caffeine-sensitive calcium pools in sequestration of cytoplasnuc calcium during depolarization-induced $[Ca]_i$ transients. Our investigations of caffeine-sensitive Ca^{2+} release in mammalian sensory neurons demonstrated that caffeine-triggered $[Ca^{2+}]_i$ transients can be elicited only in a certain types of cells. It is widely accepted that the diameters of somata of sensory neurons in dorsal root ganglia correlate with the conduction velocity of their axons and sensory modalities: rapidly conducting Aβ axons which presumably transmit proprioceptive and tactile information belong to neurons with the largest cell bodies, whereas slow conducting Aγδ and C-type axons which are believed to transmit pain and thermal information are attached to neurons with small somata. In addition, DRG neurons of different size generate action potentials with characteristic features and express distinct patterns of voltage-gated Ca^{2+} channels. We found that caffeine is able to elevate $[Ca^{2+}]_i$ only in large DRG neurons; in contrast to large DRG neurones, cells with small soma diameter displayed either no response to caffeine/ or responded by low-amplitude steady-state $[Ca^{2+}]_i$ elevation. Moreover, depolarization-triggered $[Ca^{2+}]_i$ transients did not modulate the subsequent responses to caffeine. This led us to suggest that caffeine-sensitive ER stores are almost absent in small-diameter DRG neurons. Similar to our observations, a subpopulation of chick DRG neurons was also found to be insensitive to caffeine, suggesting the absence of functioning CICR in these cells (Mironov *et al.*, 1993). Furthermore, CICR-reIated ER Ca^{2+} stores may be unevenly distributed within the same neurone: caffeine usually evoked higher $[Ca^{2+}]_i$ transients in the soma of cultured DRG neurones as compared with their processes (Thayer *et al.*, 1987).

Investigation of the properties of caffeine-induced Ca^{2+} release in mammalian central neurones revealed some differences as compared to the peripheral ones. First of all, generally, the amplitudes of caffeine-induced $[Ca^{2+}]_i$ transients recorded at rest were substantially smaller in central neurons. However, in many cell types a depolarization-induced Ca^{2+} entry into these neurons markedly increased the amplitudes of consecutive caffeine-triggered $[Ca^{2+}]$i transients.

These results led us to the suggestion that, although having quite similar pharmacological properties, Ca^{2+} stores in peripheral and central neurons differ in Ca^{2+} ion handling. In peripheral sensory and sympathetic neurons under resting conditions the Ca^{2+} stores are continuously filled by releasable Ca^{2+} and after discharging they spontaneously refill. In contrast, in central neurons caffeine-sensitive stores have a minute amount of releasable $Ca^{2=}$ under resting

conditions; nevertheless they can be rapidly but transiently charged by depolarization-triggered Ca^{2+} entry. The Ca^{2+} stores in central neurons, in contrast to peripheral ones, display a spontaneous depletion of releasable Ca^{2+}.

Various substances known to interact with Ca^{2+}-gated Ca^{2+}- release channels effectively inhibit caffeine-induced $[Ca^{2+}]_i$ transients in peripheral and central neurons. Among different blockers ryanodine demonstrated clear use dependence, while other substances inhibit caffeine-induced $[Ca^{2+}]_i$ transients in a concentration-dependent way. As has been pointed above, Ca^{2+} uptake into the ER stores is achieved via the activity of SERCA pumps, which can be selectively blocked by the tumor promoter thapsigargin. Treatment of peripheral neurons by thapsigargin (20-50 nM) inhibited caffeine-induced $[Ca^{2+}]_i$ transients by preventing the reloading of ER calcium stores (Shmigol *et al.*, 1994; Shmigol *et al.*, 1995). Thapsigargin effectively blocked both steady-state replenishment of calcium stores and the loading of them by depolarization-triggered Ca^{2+} entry.

For quite a long time methylxanthines and ryanodine were the only one known modulators of Ca^{2+} release via Ca^{2+}-gated Ca^{2+}-release channels, and certainly it was important to find an endogenous substance able to modulate the state of these channels. Such an endogenous substance which may function as a physiological modulator (or perhaps a second messenger) of Ca^{2+}-induced Ca^{2+} release has been found. The candidate for such a role is an NAD metabolite, cyclic ADP ribose (cADPR; molecular weight 541), which derives from NAD due to the activity of ADP ribosyl-cyclase.

At the molecular level cADPR was reported to bind to the putative ATP-binding site of the CICR channel-RYR molecule; moreover, it was found that cADPR competes with ATP for this binding site (Sitsapesan *et al.*, 1994). Assuming much higher concentrations of ATP, NAD^- and other adenine nucleotides in the cell, it seems unlikely that cADPR may resume its ability to activate CICR under physiological conditions, although this suggestion has to be confirmed. The resting cADPR concentration in brain tissue was estimated to be close to 100-200 nM, which is much higher than the K_D for Ca^{2+} release activation determined in sea urchin egg. This may indicate either a different sensitivity of ER Ca^{2+} release channels in mammalian tissue or, in fact, cADPR may serve as a regulator, rather than an activator of ER Ca^{2+} release channels in neurons. It is still a question whether cADPR may act as a distinct second messenger, or whether its action is confined to the sensitization of Ca^{2+} release channels to calcium, which would potentiate CICR in turn. It is also not clear what kind of signal-transducing pathway may control the production of cADPR. One of the possible ways suggested is the involvement of cGMP as an intermediate transmitter which activates cGMP-dependent protein kinase and the latter subsequently activates cADP ribosyl cyclase.

Several attempts have been made to reveal the action of cADPR on $[Ca^{2+}]_i$ in neuronal cells. The action of cADPR on $[Ca^{2+}]_i$ was directly studied on voltage-clamped bullfrog sympathetic (Hua *et al.*, 1994) and mouse DRG (Shmigol *et al.*, 1995) neurons. In both species intracellular administration of cADPR did not induce Ca^{2+} release but significantly potentiated CICR triggered by plasmalemmal Ca^{2+} entry. In cADPR-loaded neurons a significant increase of the unit Ca^{2+} transient was observed, suggesting enhancement of CICR evoked by Ca^{2+} entry.

The mechanism of neurotransmitter-evoked $[Ca^{2+}]_i$ signals is less straightforward as compared with depolarization-induced $[Ca^{2+}]_i$ elevation. The majority of neurotran-smitters interact with several receptor subtypes, which may be co-localized in the same cell or in the same postsynaptic regions. Synaptic transmission is often associated with the generation of intracellular Ca^{2+} signals the characteristics of which are determined by a number of distinct mechanisms. The pathways for excitatory neurotransmitter-induced $[Ca^{2+}]_i$ elevation comprise the following: (1) binding to ionotropic receptors resulting in membrane depolarization, which causes activation.of voltage-dependent plasmalemmal calcium channels; (2) a number of subsets of ionotropic receptors possess significant Ca^{2+} permeability (see below) which also participate in Ca^{2+} delivery to the cytoplasm; (3) Ca^{2+} influx via both voltage- and ligand-operated channels may induce CICR, which will further amplify the signal; and (4) neurotransmitters activate several subclasses of metabotropic receptors coupled with PI turnover and subsequent Ca^{2+} release from the InsPs-sensitive calcium stores. All these pathways may act simultaneously, producing complicated cytoplasmic responses.

The ability of excitatory amino acids (EAA: glutamate and its agonists kainate and AMPA) to induce $[Ca^{2+}]_i$ elevation has been demonstrated in many types of mammalian neurons. The action of EAA on $[Ca^{2+}]_i$ is quite complex and is determined by the wide variety of their receptors; the latter are responsible either for the generation of inward cationic currents (sometimes with significant Ca^{2+} component) and the alteration of intracellular levels of second messengers ($InsP_3$ and cAMP) which also might activate intracellular Ca^{2+} release. Whatever the particular mechanisms of cellular excitation by EAA are, usually they depolarize the cell and trigger Ca^{2+} entry via voltage-gated channels. The relative involvement of different Ca^{2+}-gated channels subtypes in EAA-triggered $[Ca^{2+}]_i$ elevation remains to be elucidated.

Acetylcholine ionotropic responses in the nervous system are mediated by various subclasses of NChRs. Activation of NChRs effectively depolarizes neurons with subsequent Ca^{2+} entry via voltage-gated channels. In addition, Ca^{2+} may permeate neuronal NChRs (see below).

The existence of ATP-gated ionic currents in nerve tissue was first discovered in 1983 in rat sensory neurons (Krishtal *et al.*, 1983). Later, ATP-gated cation-selective ion channels (coupled with P2X and P2Y purinoreceptors) were found in various peripheral and central mammalian neurons. ATP-induced cation conductance effectively depolarized nerve cells and the ATP-triggered elevation of $[Ca^{2+}]_i$ mediated via activation of P2Y purinoreceptors was observed in chick ciliary ganglion cells (Abe *et al.*, 1995), whereas in. rat hypothalamic neurons ATP-driven $[Ca^{2+}]_l$ rise was mediated by P2X purinoreceptors (Chen *et al.*, 1994).

GABA (γ-aminobutyric acid) is the most common inhibitory transmitter in the nervous system. GABA acts through the activation of two subtypes of ionotropic (GABAA and GABAc) and one metabotropic (GABAb) receptors. Both GABAA and GABAc receptors generate transmembrane flux of chloride ions (Bormann, 1988), which usually enter the cells down the electrochemical gradient (assuming an E_{Cl} of -70 mV and a resting membrane potential of —70 to -50 mV) thus causing neuronal hyperpolarization. However, the existence of a novel GABAA receptor subtype linked to the cationic (Na^+- and presumably Ca^{2+}-permeable) channel which is responsible for depolarization in certain populations of nerve cells or in cells during early ontogenetic stages was suggested (Cherubini *et al.*, 1991; Lambert *et al.*, 1991). In addition Ca^{2+} even acting through the conventional GABAA anion-permeable channel GABA might depolarize the cell if the latter creates the outward driving force for Cl^- ions. Possibly, both mechanisms are accounted for the generation of $[Ca^{2+}l_i$ transients recorded from several types of vertebrate neurons in response to GABA applications.

The activation of GABAA receptor-coupled cationic channels was suggested to produce depolarization with subsequent voltage-gated Ca^{2+} channel openings and $[Ca^{2+}]_i$ elevation in cultured hippocampal neurons (Segal, 1993). In dorsal horn neurons GABA also triggered $[Ca^{2+}]_i$ elevation; however, the mechanism of depolarization was completely different, being associated with Cl^-permeable channels (Reichling *et al.*, 1994). These neurons seem to maintain unusually high intracellular C1~ concentrations (>22 mM) which allows Cl^- efflux (and, therefore, generation of depolarizing current) at resting membrane potential. This Cl^- current-associated depolarization was high enough in order to activate voltage-gated Ca^{2+} channels. Pharmacological analysis of GABA-triggered $[Ca^{2+}]_i$ transients with Ca^{2+} channel agonists revealed that both L- (nimodipine-sensitive) and N- (ω-conotoxin-sensitive) Ca^{2+} channels were involved in the generation of Ca^{2+} fluxes.

Similarly to GABA, glycine receptors are permeable to Cl^- ions. Glycine was reported to generate $[Ca^{2+}]_i$ elevation in dorsal horn neurons due to Cl^--

dependent depolarization and subsequent opening of voltage-gated Ca^{2+} channels (Reichling *et al.*, 1994).

The second major route for Ca^{2+} entry induced by neurotransmitters is associated with direct Ca^{2+} inflow through ligand-gated channels. For quite a long time it was believed that activation of neuronal ligand-gated channels may induce Ca^{2+} influx only indirectly, due to the generation of Na^{+} depolarizing currents with subsequent opening of voltage-gated Ca^{2+} channels. This paradigm was first broken after the discovery of high calcium permeability of one of the subclasses of neuronal glutamate-gated channels—the NMDA receptors (Mayer and Westbrook, 1987).

The activation of NMDA ionotropic receptors with subsequent Ca^{2+} entry via both NMDA-gated and voltage-gated Ca^{2+}' channels was found to be responsible for Ca^{2+} signal generation in many types of central neurons. Subsequently appreciable calcium permeability was discovered for other subtypes of glutamate receptors. The indication for Ca^{2+}-permeable AMPA/KA receptors was found for many types of cultured central neurons.

Almost simultaneously with the discovery of Ca^{2+} influx through GluR channels, relatively high calcium permeability was found for nicotinic cholinoreceptors, expressed in the central nervous system. As compared to the muscle subtype, neuronal NChRs have higher Ca^{2+} permeability and additionally current through neuronal NChRs is modulated by extracellular Ca^{2+} concentration. The fractional permeability of neuronal NChR determined for bovine and rat chromaffin cells varied between 2.5% and 4.1% (Vernino *et al.*, 1994) in physiological solutions.

Calcium permeability was also suggested for some other ligand-gated channels, including purinoreceptors; GABAA receptors (cation-permeable), etc. For neuronal ATP-gated channels the relative Ca^{2+}/Na^{+} permeability ratio varied between 2:1 for isolated nucleus tractus solitarii neurons (Ueno *et al.*, 1992) and 1:3 in sensory neurons (Bean, 1992). The fractional Ca^{2+} contribution to the ionic currents induced by all these ligands has to be elucidated.

Another pathway for induction of intracellular Ca^{2+} release from the endoplasmic reticulum is the production of inositol-3-phosphate (InsP3). Although InsP3 turnover and InsP3-induced Ca^{2+} release (IICR) have been studied in a wide variety of cells, and IICR has been suggested to play an important role in development of a number of important brain functions (like neuronal integrative function, long-term potentiation, learning and memory) there are still a very limited number of experimental data describing IICR peculiarities in different nerve cells

Under physiological conditions the activation of the phospholipase C family, which underlie the synthesis of InsP3, is controlled by. a number of metabotropic receptors widely expressed in the nervous system. These receptors

share many structural and functional properties; in particular, all of them are composed of seven membrane-spanning domains and are coupled to phospholipase C via various subsets of G proteins. The metabotropic receptor family is responsible for the effects of numerous neurotransmitters and neuromodulators—glutamate, acetylcholine, ATP, noradrenaline, 5-hydroxytryp-tamine (serotonin), etc.

Metabotropic glutamate receptors (mGluRs) belong to a broad gene family. At least six types of mGluRs with distinct phannacological properties have been discovered and molecularly characterized so far. Similarly to other G protein-linked receptors mGluRs comprise seven transmembrane domains; however, their amino acid structure differs significantly from the other representatives of the G protein-coupled receptor family. The six isoforms of mGluRs represent two groups which are distinguished by their intracellular effects. Only two mGluRs namely mGIuRl and mGluR5, are coupled with phospholipase C, thus participating in InsP3 formation; four other isoforms (mGIuR2, 3, 4 and 6) are linked to the inhibitory shoulder of the cAMP messenger system, see (Nakanishi, 1992; Hollmann and Heinemann, 1994) for review. The mGluR-controlled activation of PLC is mediated via an ample family of G proteins. based on pharmacological studies the existence of an additional mGluR coupled with InsP3 formation was postulated. Apart from stimulation of InsP3 production, mGIuRl is coupled with cAMP production and arachidonic add release presumably via different subsets of G proteins (Aramori and Nakanishi, 1992); mGluR5 solely interferes with InsP3 turnover. The successful expression of mGIuRl and mGluR5 in a transfected cell line demonstrated that both of them stimulate cleavage of PIP2, InsP3 production and subsequent Ca^{2+} mobilization from the internal stores.

Both mGIuRl and mGluR5 are expressed throughout the brain, see (Hollmann and Heinemann, 1994; Tanabe *et al.*, 1993), with some regions of preferential expression of one of the subtypes. mGIuRl is preferentially expressed in cerebellum, substantia nigra, olfactory bulb and superior colliculus, whereas mGluR5 dominates in cerebral cortex, CA1 region of hippocampus and nucleus accumbens. Interestingly, in cerebellum mGluR5 appears only for a short period during development; adult cerebellar cells completely lack it. A high concentration of mGluR5 was also found in nociceptive dorsal horn neurons of the rat (Walker *et al.*, 2001).

The metabotropic effect of acetylcholine is mediated via the family of muscarinic receptors (M1 to M5) which are typical G protein-coupled metabotropic receptors. Among them the M5 subtype is coupled with PLC and InsP3 production. In cultured cerebellar granule neurons activation of MChRs releases Ca^{2+} from the same pool as glutamate, although the MChR-sensitive

pathway was insensitive to pertussis toxin, suggesting the probable involvement of Gq/G_{11} proteins (Irving *et al.*, 1992).

Metabotropic purinorepectors have been characterized at the molecular level and cDNAs for both P2Y and P2U receptors have been cloned (Lustig *et al.*, 1993; Webb *et al.*, 1993). Structurally P2Y/U receptors are similar to other G protein-coupled receptors and demonstrate a seven-transmembrane domain structure. P2Y receptors are thought to activate PLC/βS-1 in a pertussis toxin-sensitive manner, which suggests the involvement of G proteins, while P2U receptors interact with PLC in pertussis toxin-sensitive and insensitive pathways. These pathways involve the newly identified Gq/G_{11} class of heterotrimeric G proteins, which do not show pertussis toxin-mediated ADP ribosylation The ATP-induced $Ca2^{2+}$ release from internal stores has been characterized for hippocampal and thalamic neurons kept in short-term culture (Mironov, 1994). In these neurons ATP clearly evoked intracellular Ca^{2+} release mediated through P2Y purinoreceptors. Such ATP-triggered Ca^{2+}' transients were not associated with generation of membrane current and persisted in Ca^{2+}-free extracellular media. The ATP-triggered IICR was antagonized by the SERCA blockers thapsigargin and BHQ; surprisingly it was also effectively blocked by ryanodine in micromolar concentrations.

All results we have discussed previously concern changes in $[Ca^{2+}]_i$ all over the cell. However, in reality the distribution of cytoplasmic free calcium is not homogeneous, and a number of steep intracellular Ca^{2+} gradients may occur accompanying physiological cellular reactions. This intracellular heterogeneity of $[Ca^{2+}]_i$ signals are especially important for neurons endowed with striking specialization of various subcellular compartments. There are at least two manifestations of spatial organization of intracellular calcium signals: one which can be seen at a "macrocellular level" (represented by $[Ca^{2+}]_i$ waves or local $[Ca^{2+}]_i$ spikes due to a peculiar concentration of Ca^{2+} channels or receptors involved in calcium signalling) and the second represented by appearance of "microdomains" of elevated calcium.

The problem of "micro" calcium signals seems to be even more intriguing. Initially the idea about local organization of $[Ca^{2+}]_i$ signals came from theoretical assumptions which simulate the diffusion of Ca^{2+} ions in the cytoplasm after their entry via membrane channels, see review by Augustine and Neher (Augustine and Neher, 1992). These assumptions predict that $[Ca^{2+}]_i$ may rise to hundreds of micromoles in tiny compartments, and the microdomains with high Ca^{2+} concentration may survive for several milliseconds. Unfortunately the currently available techniques for recording spatial development of $[Ca^{2+}]_i$ signals are far from the detection of micromolar Ca^{2+} transients in a time range of several milliseconds which has made the problem of local $[Ca^{2+}]_i$ signalling one of the most challenging questions in the

whole calcium field. However, in recent years evidence (mostly indirect) supporting the idea of local $[Ca^{2+}]_i$ signals has appeared.

First, Llinas *et al.* (Llinas *et al.*, 1992)using synthetic aequorin-based Ca^{2+} recordings demonstrated that synaptically activated Ca^{2+} entry via plasmalemmal channels can indeed elevate $[Ca^{2+}]_i$ to levels >100 μM in a tiny compartment in close proximity with postsynaptic active zones. The duration or these high-Ca^{2+} microdomains did not exceed several milliseconds. Using mitochondria-targeted aequorin Rizzuto *et al.* (Rizzuto *et al.*, 1993) demonstrated that Ca^{2+} fluxes through InsP3-gated channels may also generate micromolar local Ca^{2+} gradients which are sensed by neighbouring mitochondria.

Second, a number of studies have revealed that neuronal dendritic spines form a very specialized compartment, which are relatively isolated from the rest of the neuron, and the spines might be a very good place for generating the local Ca^{2+} signals. Initially the isolation or dendritic spines in respect to $[Ca^{2+}]_i$ signals was shown by Connor *et al.,* (Connor *et al.*, 1994), who found that synaptic activation produced a $[Ca^{2+}]_i$ rise in individual spines, leaving dendrites unaffected. Similarly, purely dendritic $[Ca^{2+}]_i$ transients were not transmitted to the spines (Guthrie *et al.*, 1991), thus indicating their unique segregation. Keeping in mind that dendritic spines play an important role in synaptic transmission in the central nervous system, local $[Ca^{2+}]_i$ signals in spines might be highly important for neuronal integrative functions. Similarly, local $[Ca^{2+}]_i$ signals arising near the ER Ca^{2+} release channels may serve as a signal targeting specifically localized enzymes.

Being always under high pressure from intracellularly aimed Ca^{2+} gradients, eukaryotic cells have developed a sophisticated system for elimination of Ca^{2+} from the cytoplasm. This system includes (1) Ca^{2+} extrusion into the extracellular space by an ATP-driven plasmalemmal Ca^{2+} pump and an Na^+/Ca^{2+} exchanger mechanism and (2) calcium uptake by ER stores and intracellular organelles. The overlapping activity of these transporters and their competition with calcium buffers for Ca^{2+} entering into the cytoplasm actually terminate the $[Ca^{2+}]_i$ signal and determine the recovery kinetics of $[Ca^{2+}]_i$ transients.

The plasmalemmal calcium pump (PMCA) serves as a low-affinity system which is able to operate at low $[Ca^{2+}]_i$ thus effectively participating in maintaining $[Ca^{2+}]_i$ at physiological concentrations. The activity of PMCA is controlled by cytoplasmic Ca^{2+} and by a number of biologically active substances. The most important among them are Ca^{2+} ions themselves and calmodulin. Increase in cytoplasmic calcium activates the pump and calmodulin increases the affinity of the calcium pump and its maximal transport rate. The most commonly used blockers of the Ca^{2+} pump are vanadate and lantanides.

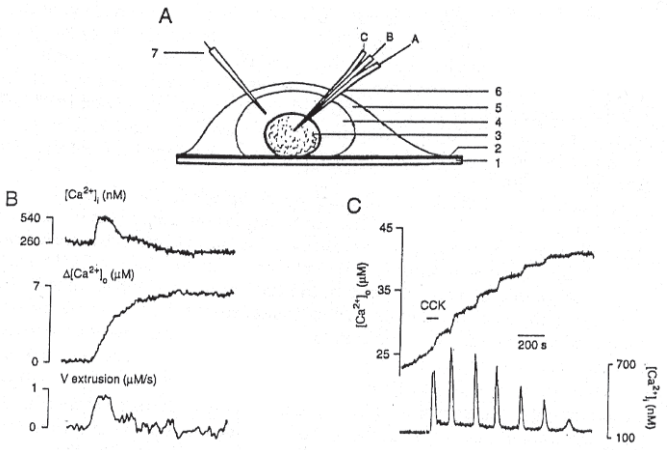

Figure 1. Extrusion of Ca^{2+} from a single cell measured by the microdroplet technique. (A) Scheme of the microdroplet: (1) glass plate; (2) silicon layer; (3) isolated cell; (4) extracellular solution; (5) non-fluorescent oil; (6) three-barrel microelectrode with barrels A and B filled with 2,5 M KCI for voltage-clamp and barrel C with 0.2 M $CaCl_2$ for Ca^{2+} injection; (7) micropipette for drug application. (B) Short injection of Ca^{2+} into a snail neuron inducing a Ca^{2+} transient (top record) and parallel changes in extracellular Ca^{2+} level (middle record); rate of Ca^{2+} extrusion is shown in the bottom record. (C) Rhythmic Ca^{2+} transients triggered in a pancreatic islet cell by short application of cholecystokinin (CCK) (lower record) and changes in extracellular Ca^{2+} level induced by immediate Ca^{2+} extrusion.

Methods for measurement of Ca^{2+} extrusion from cells are much less developed compared to measurement of intracellular Ca^{2+} levels. Usually radioisotope techniques and multicellular preparations are used for this purpose, and they estimate only slow extrusion processes. In our group a technique has been developed which allows measurement of the extrusion of Ca^{2+} ions in parallel with the changes of their level in the cytosol, and it has provided the first direct data about the kinetics and intensity of this process (Tepikin *et al.*, 1991; Tepikin *et al.*, 1994). The technique is based on the formation of a microchamber around the isolated cell with a volume of extracellular solution of 4-7 nl (approximately 10 times greater than that of the cell). The microchamber was in fact a drop of extracellular solution covered with a layer of non-fluorescent oil to avoid evaporation. An isolated cell loaded with fluorescent indicator (fura-2) was placed in the centre of the drop, which contains another Ca^{2+} indicator working at different wavelengths. This allows parallel measurement of Ca^{2+} level changes both inside and outside the cell. Measurements have shown that Ca^{2+} ion extrusion from the cell starts in parallel with the rise of the intracellular Ca^{2+} signal (Fig. 4). During increase of $[Ca^{2+}]_i$ to 0.2-0.5 μM, the velocity of Ca^{2+} extrusion from a snail neurone varied

between 0.3 and 4.6 μM/s per cell volume. During caffeine-induced Ca^{2+} transients a stimulation of calcium extrusion took place, reaching a velocity of 5.0μM/s per cell volume. An approximate comparison indicates that at least 30% of Ca^{2+} injected into the cytosol is immediately extruded into the extracellular solution. This value may be somewhat overestimated because of the low free Ca^{2+} level in the drop, which may facilitate Ca^{2+} extrusion.

The fundamental property of the Na^+/Ca^{2+} exchange mechanism is its ability to translocate Ca^{2+} ions from the cytoplasm to the extracellular space, i.e. against a high electrochemical gradient by utilizing the electrochemical gradient of sodium ions. This mechanism has been found and extensively characterized in various excitable and non-excitable cells, reviewed by (Blaustein *et al.*, 1991). In nervous cells the involvement of the Na^+/Ca^{2+} exchanger in $[Ca^{2+}]_i$ regulation was initially observed in experiments on invertebrate axons and isolated neurons.

In mammalian neurons the question of the relative importance of Na^+/Ca^{2+} - exchanges in maintaining the resting $ICa^{2+}]_i$ and in Ca^{2+} extrusion after neuronal excitation was addressed in a limited number of studies. However, the data available show a clear difference between peripheral and central neurons. In peripheral neurons (rat DRG cells) Na^+ removal from the extracellular solution did not change appreciably either basal $[Ca^{2+}]_i$ or recovery kinetics of $[Ca^{2+}]_i$ transients (Duchen *et al.*, 1990; Shmigol *et al.*, 1995). the Na^+/Ca^{2+} exchanger was shown to affect significantly the recovery phase of $[Ca^{2+}]_i$ transients and (sometimes) alter resting $[Ca^{2+}]_i$. The importance of Na^+/Ca^{2+} homeostasis in $[Ca^{2+}]_i$ handling in central neurons is also supported by the finding that blockade of the exchanger potentiates glutamate neurotoxicity (Andreeva *et al.*, 1991). Furthermore, Ca^{2+} entry via the Na^+/Ca^{2+} exchanger mechanism functioning in reverse mode was reported to enhance and prolong the glutamate-triggered $[Ca^{2+}l_i$ transient in cerebellar granule neurons.

Mitochondria are also intracellular organelles found to be associated with the regulation of $[Ca^{2+}]_i$. The accumulation of Ca^{2+} ions by mitochondria is achieved via an electrogenic uniporter; that is the high proton electrochemical gradient, which provides the driving force for Ca^{2+} flow into the mitochondrial matrix. The H^+ gradient created by mitochondrial proton transport systems makes the inner mitochondrial membrane potential highly negative (up to -200 mV). The mitochondrial membrane appears almost impermeable to Na^+ and K^+- but it displays a highly active Ca^{2+} electrogenic transporter which allows massive Ca^{2+} influx down the electrochemical gradient, so that the intramitochondrial Ca^{2+} concentration may reach levels of hundreds of micromoles. The nature of this transporter remains unclear, although it was hypothesized that it might be a highly mitochondria-specific calcium channel.

Simultaneously, the mitochondrial membrane contains electrically neutral H^+/Ca^{2+} and Na^+/Ca^{2+} exchangers which prevent mitochondria from Ca^{2+} overload.

Collapsing of the mitochondrial membrane potential (by uncouplers of oxidative phosphorylation or blockers of electron transport) causes release of Ca^{2+} ions. This release is mediated by several pathways, see review (Pozzan *et al.*, 1994), including passive Ca^{2+} outflow, persistent activity of electroneutral exchanger and activation of a 'mega' cationic channel with enormously high conductance (~1 nS) (Bernardi, 1992). The common assumption is that mitochondria start to sequester Ca^{2+} when the $[Ca^{2+}]_i$ rises above a certain "set-point" which for neurons may lie in the range of 300-600 nM. Mitochondrial Ca^{2+} uptake may limit the peak of cytosolic $[Ca^{2+}]_i$ elevation, thus serving as a neuroprotector factor. In addition, mitochondria may represent a dynamic system which is not only responsible for fast Ca^{2+} accumulation, but may also release Ca^{2+} when $[Ca^{2+}]_i$ is below the "set-point", thus leading to the prolongation of $[Ca^{2+}]_i$ signal recovery. Such a prolongation was indeed found in sensory (Thayer; Miller, 1990) and sympathetic (Friel and Tsien, 1994) neurons, and it might be important for temporal amplification of the calcium signal. In addition, Ca^{2+} ions accumulated by mitochondria could provide Ca^{2+} signal to metabolic processes. Another important role of mitochondrial Ca^{2+} uptake might be determined by their preferential intracellular localization in the neighbourhood of Ca^{2+} entry sites (e.g. near plasma membrane) where local Ca^{2+} concentration could be exceptionally high (>1-10 μM*)*. In particular, mitochondria were suggested to be preferentially localized near the sites of InsP3 release, where they can face micromolar calcium concentrations produced by IICR in a small microdomain (Rizzuto *et al.*, 1993). In this case mitochondria may be involved in regulation of $[Ca^{2+}]_i$ in such a microcompartment.

It is still very unclear whether the nucleus can act as a functionally distinct $[Ca^{2+}]_i$-handling subcompartment. Initial studies of the permeability of nuclear membrane revealed that many hydrophilic compounds with a molecular weight as high as 20 kDa can freely penetrate the nuclear envelope. However, video-imaging and especially high-resolution confocal microscopy recordings of intracellular Ca^{2+} distribution have demonstrated the existence of Ca^{2+} concentration gradients between nucleus and cytosol. Several groups have reported that calcium concentration in the nerve cell nucleus increased faster and reached higher levels than cytosolic Ca^{2+} after depolarization-induced Ca^{2+} entry. Such heterogeneity of nuclear Ca^{2+} signal presumes the existence of a specialized amplification system, located in the neuronal envelope. The generation of an intranuclear Ca^{2+} signal might be of special importance taking into account that gene expression and other intranuclear reactions are strongly

influenced by calcium ions. Indeed, there are several clues indicating the possible association of neuronal development and nuclear Ca^{2+} signals. Nuclear Ca^{2+} transients were reported to be coupled to neurite outgrowth in DRG neurons (Birch *et al.*, 1992). Furthermore, nuclear Ca^{2+} signals undergo developmental changes: prominent nuclear Ca^{2+} signals were observed in embryonic DRG neurons, whereas they became much smaller in postnatal neurons (Utzschneider *et al.*, 1994).

Simultaneously with experimental evidence favoring the idea of intranuclear Ca^{2+} gradients, a number of authors have failed to observe differences in $[Ca^{2+}]_i$ between nucleus and cytoplasm (Marrion and Adams, 1992; Nohmi *et al.*, 1992; Neher and Augustine, 1992). O'Malley made a very careful examination of Ca^{2+} permeability in the nuclear envelope of sympathetic bullfrog neurons using fluo-3 confocal microscopy and intracellular perfusion with Ca^{2+} buffers (O'Malley, 1994). His experiments suggest that Ca^{2+} freely penetrates the nuclear envelope, and there is no evidence supporting the existence of Ca^{2+} gradients between nucleus and cytoplasm. Generally, based on currently available knowledge, it is impossible to confirm unequivocally or rule out the importance of the nucleus as a separate component of $[Ca^{2+}]$ homeostasis.

In conclusion we can state that the injection of Ca^{2+} ions into the cell through the highly coordinated activity of plasmalemmal and intracellular calcium-permeable channels is the main source of temporary elevation of free Ca^{2+} level in the cytosol (the "calcium signal") during cellular activity. However, this signal is substantially modified by several cellular mechanisms functioning on different time scales. The most rapid one is the buffering of injected ions by cytosolic buffers, mainly by Ca^{2+}-binding proteins, which occurs in a time range of milliseconds. Despite this speed, substantial spatial gradients of free Ca^{2+} still occur inside the cell, reaching millimolar concentrations near the injection sites; this might be important for triggering further cellular reactions having comparatively low sensitivity to Ca^{2+}. Altogether, not more than 1% of the injected ions remain free for exerting their physicochemical activity inside the cell.

Two other mechanisms—extrusion of Ca^{2+} ions back into the extracellular space and their uptake or release by intracellular stores, both depending on the activity of membrane ATPases—function on a time scale of seconds; nevertheless they can substantially modify the amplitude and kinetics of the calcium signal. Obviously, the relative role of these mechanisms might be substantially different depending on the type of cell, the stage of its development, as well as on possible pathological conditions. However, data on the individual features of the Ca^{2+} -handling mechanisms in nerve cells are still quite scarce, and this problem should be extensively studied.

3. Role of store-operated channels.

Capacitative calcium entry (CCE) is the response of a large population of channels that are activated by a feed-back mechanism – the depletion of intracellular stores (store-operated channels –SOCs –cf. (Putney, Jr., 2003)). Between them the group of so called CRAC channels can be identified in which the main mechanism of activation is the release of Ca^{2+} during depletion of intracellular calcium-activated stores. CRAC channels are members of the group of non-selective cationic channels populating the cell membrane. Their density is augmented by removing intracellular Ca^{2+}. Reducing Ca^{2+} uncovers channels whose conductance is inconveniently low in physiological solution (Kostyuk and Krishtal, 1977). CRAC current are typically induced by store depletion protocol in which thapsigargin, InsP3 or dialysis using Ca^{2+} chelators evoke inward currents of 10-50 pA amplitude. Analysis of CRAC current variance estimated CRAC single channel conductance even at 11 mM $[Ca^{2+}]_0$ in the range of fS. The low conductance in monovalent conditions lacking Ca^{2+} raises an interesting point about the nature of high Ca^{2+} selectivity of CRAC channels. Probably there are selective to calcium ions because they have high-affinity sites for them in the mouth of the pore, usually implemented by a ring of negatively charged glutamate or aspartate amino acid.

At the same time Kerrshbaum and Cahalan (Kerschbaum and Cahalan, 1998)recorded a much larger and non-inactivating monovalent current when internal Mg^{2+} was also omitted. The presumed CRAC single channel conductance in monovalent solution was 35-49 pS in size The monovalent channel conduction was 40 times higher than in the presence of $[Ca^{2+}]_o$ solution and blocked in a voltage-dependent manner. Several investigations have also shown that these monovalent currents differ significantly from typical CRAC-currents. They can be separated from classic CRAC currents by their kinetics and internal Mg^{2+} sensitivity. It was proposed to call channels with such characteristics MIC channels (but still they belong to the group of CRAC channels, although they are not very similar to classic CRAC channels (Clapham, 2002).

Ryanodine-sensitive endoplasmic reticulum is tightly connected with L-type voltage-dependent calcium channels, capacitative calcium entry or store depletion by CICR. This connection is supported by different mechanism in skeletal muscle, cardiomyocytes and neurons. In cardiomyocytes and skeletal muscle such connection with L-type of channels is called “foot-by foot”, whereas in neurons there are practically on-line connections. In skeletal muscle their interconnections are formed with the help of calmodulin. The binding site of calmodulin on L-type channels may stabilize the contact between these two proteins and help the proteins to regulate there interaction (Sencer *et al.*, 2001).

Probably such effective interconnection is due to direct influence on the α-subunit of L-type voltage-gated channels and can be limited by the affinity of the binding events. It was shown that in neurons there is also a tight functional coupling between ryanodine receptors and L-type channels (Chavis *et al.*, 1996). However, whether the possibilities of interconnections between L-type voltage channels and ryanodine sensitive endoplasmatic reticulum have all the same features in central and DRG neurons is under question.

It was also shown that such interaction in neurons include activation of mGlutR1 receptors and results in large oscillations which lead to an increase of Ba^{2+} currents through L-type calcium channels (Ba^{2+} was used as a charge carrier because of its permeability through L-type calcium channels and RYR). The amplitude of Ba^{2+} current remained constant during repetitive voltage-steps applied before the agonist application. This activation was blocked by caffeine and by ryanodine. The kinetics of this blocking was dependent on the frequency of Ba^{2+} current. It must be noted that ryanodine did not affect the Ba^{2+} currents in resting cells but progressively inhibited the oscillatory increase in Ba^{2+} current triggered by depletion of ER. Ryanodine suppressed both the mGluR1 and caffeine induced connection between ER and L-type channels. Possible such process is partly connected also with the activity of NMDA channels. At the same time the activity of mGluR1 agonist were completely blocked by nimodipine, providing evidence that t-ACPD acted at mGlutR1 to increase current through L-type channels. Probably mGlutR1 act through PTX-insensitive G-proteins. Their facilitation was not a response to the activity of PLC-coupled receptors, was independent from InsP3 and protein kinase C, arachidonic acid and PCA action (for instance, application of arachidonic inhibitor quinacrine did not influences these processes). The use-dependence of ryanodine blockade suggests that RYR activity depends critically on coupling with L-type calcium channel activity and probably with mGluR1 activation. The activation of mGLutR1 also triggered a cyclic facilitation of L-type channels. Such coupling between RYRs and calcium entry through L-type channels and the participation in this process of mGlutR1 may be an important factor in the regulation of neuronal activity and synaptic plasticity (for instance it leads to the induction of long term depression in cerebellar cells).

It is of interest that the DRG neurons give a great response to depletion of calcium store whereas in neurons of the dorsal horn the answer to caffeine is much lower. A conclusion can be made that in different types of neurons the mechanisms of L-type calcium channel connection with ER may differently influence the activation of capacitative Ca^{2+} entry, and even a little difference in these interconnection (probably including also mitochondrial Ca^{2+} activated mechanisms) can lead to significant differences in such entry.

It must be added that in neurons capacitative calcium entry can be induced by a short-term receptor- and longer cellular-control mechanisms (Emptage *et al.*, 2001). It is possible that in neurons capacitative calcium entry due to InP3 ER activation also exists, although the main role plays the ryanodine-induced capacitative calcium entry. It is possible that in non-excitable cells InP3 ER activation is quite enough to induce capacitative calcium entry, while in neurons the ryanodine-induced capacitative calcium entry plays the main role in neuronal signaling.

In some cells neurotransmitters and hormones can engage mainly the phosphoinositide pathway to evoke a biphasic increase in intracellular free Ca^{2+} concentration – an initial transient release of Ca^{2+} from intracellular stores with followed it sustained phase of Ca^{2+} influx. A scheme is proposed that InsP3 reticulum action in this case is a result of connection between endoplasmic reticulum, mitochondria and metabotropic activated plasma membrane channels (here the channels proposed on the role of store operated channels are from the group of TRP (transient receptor potential) type of channels. It is also proposed that such capacitative calcium entry is the major rout of calcium entry in non-excitable cells in contrast to neurons.

That fact that single store-activated channel conductance is <1pS indicates that they have a unique inward rectifying property (CRAC-like). This gives the possibility to propose that Ca^{2+} selective TPRV6 or TPRV7 channels (in general about 7 groups of these channels are known) having similar monovalent single channel conductance may be the part of the group of CRAC channels. It seems that from all the TRP channels the main role as a channels for capacitative calcium entry (CCE) play just the TRPV6 and TRPV7 groups of these channels that are involved in nociceptive signaling (Voets *et al.*, 2001). They are localized in single complexes by scaffolding proteins. The mechanism of their activation is still not clear but receptor-mediating activation of these channels by different activators including tyrosine kinase is most probable. Hormones or growth factors inhibit the single channel activity of this group of channels. But the main feature of these channels is that they are also inhibited by Mg^{2+}. Almost all TRP channels, including TPRV6 and TPRV7 channels, dribble Ca^{2+} into the cells at potentials near to 0. Probably classical CRAC, TPRV6 and TPRV7 channels can provide localized Ca^{2+} increases through spatially defined single transductions ways. Due to some authors, these channels are very close to above mentioned MIC type of channels (Emptage *et al.*, 2001).

There are two possible mechanisms for induction of CCE – the natural signal from endoplasmic reticulum that activates the channels and the molecular identity of the channels themselves. Some investigations have shown direct interaction between InsP3 receptors and TRP channels as a result of close

location of membrane channels and receptors. It was proposed that in this case exogenously located TRP channels can be activated by an InsP3 conformational coupling and that channel activity can be lost but restored by InP3 or members of TRPM and TRPC groups of channels. Another theory proposed such interaction due to protein-protein connections. At the same time several authors still put the question whether the TRP channels in any case can play a role of store-operated channels for InsP3 endoplasmic reticulum or whether such role can play another group of calcium regulated channels (for instance stretch-activated ones).

Certain role in the regulation of capacitative calcium entry can also play changes in the activity of the sarco-endoplasmatic Ca^{2+} pumps (SERCAs). By investigations of the roles of the PLC, InsP3 and capacitative calcium entry and calcium release-activated calcium currents it was shown that in acinar cells inhibition of PLC or SERCA blocked capacitative calcium entry (Putney, Jr., 1999). Several types of cells do not express capacitative calcium entry, and a great role for Ca^{2+} entry begins to play the sodium/calcium exchanger.

It is possible that in non-excitable cells capacitative calcium entry due to InsP3 ER activation is quite enough for their functioning while in neurons the main role plays RYR-induced capacitative calcium entry, being significant for neuronal signaling. Such difference may be quite important for the differentiation of excitable and non-excitable cells. It is of interest that in the chromaffine cells (the model of sympathetic neurons) capacitative calcium entry has not been found (Cheek, 1989).

Currents due to the activation of CRAC can be subdivided into the microscopic and macroscopic ones. As it was shown, mitochondria as well as ER can participate in the activation of CRAC. This pathway includes microscopic store-operated calcium currents. At the same time it includes and probably controls the period of cytosolic and mitochondria – endoplasmic reticulum oscillations. They can be slowly inactivated following significant rise in intracellular calcium (this phenomenon is called Ca^{2+} dependent slow inactivation). It can contribute to the frequency of intracellular Ca^{2+} oscillations as well to the shaping the profile of calcium signals. It was shown that Ca^{2+} dependent slow inactivation of CRAC can be regulated by mitochondria during buffering cytosolic Ca^{2+}. It seems that for the activation of Ca^{2+} waves and oscillations the activation of CRAC is necessary due to the substantial depletion of stores and that InsP3 itself can not mediate such effect.

At the same time respiring mitochondria can do it under physiological condition (Gilabert and Parekh, 2000). In connection with mitochondria the InsP3 ER can generate even macroscopic CRAC currents. In the case of week intracellular calcium buffering InP3 endoplasmic reticulum fails to activate the whole cell macroscopic I_{CRAC}, despite substantial store empting, probably

exceeding the apparent low threshold that is needed for activation of I_{CRAC} (Parekh *et al.*, 1997). This fact is underlined by the observation that oligomycine does not prevent I_{CRAC}. Due to the investigations of several authors, mitochondria have profound effects on both the activation and inactivation of I_{CRAC} - in the case of facilitated activation of mitochondria I_{CRAC} will be increased. So, under the physiological conditions mitochondria in cooperation with InsP3 reticulum in the non-excitable cells (and probably also in excitable cells) can play a pivotal role in all stages of store-operated Ca^{2+} entry, determining whether macroscopic I_{CRAC} became activated, to what extent and for how long Ca^{2+} entry stays operational.

Macroscopic I_{CRAC} are an important factor that determines the formation of Ca^{2+} signals. In general in excitable cells the capacitative calcium entry provides a rapid replenishment of calcium stores so that the cell is quickly ready for another stimulus. It also provides a mean for prolonged sustained elevation of $[Ca^{2+}]_i$. In the case of sustained $[Ca^{2+}]_i$ signals CCE provides a "looping out" of calcium stores to maintain the constant amplitude of each $[Ca^{2+}]_i$ spike. It must be also mentioned that chronic absence of capacitative calcium entry and store depletion as well as changes in the function of mitochondria and calcium channels can lead to such serious pathology as Alzheimer disease, different channelopathia and even result in cell apoptosis (Bian *et al.*, 1997).

4. Functional meanings of intracellular calcium trafficking

All the above described aspects of intracellular calcium trafficking may be summated in an interesting functional picture. Discrete Ca^{2+} discharge events take place in many cell types following spontaneous InsP3 or RYR activation with possible activation of autoregenerative processes (calcium waves). Starting from discrete sites of Ca^{2+} influx or intracellular pacemakers, the release of Ca^{2+} from intracellular stores can take part in the formation of such processes as action potential after-hyperpolarization. The initial phase of this response is connected with the opening of Ca^{2+}-dependent K^+ channels. In most cases this phase is followed by another one sustained by another family of K^+ channels. In the later the response can be connected with the involvement of InsP3 receptors. This after-hyperpolarization response may be modulated negatively by preactivation of receptors coupled with InsP3 ER. The question is whether such response can be found in all types of cells. $[Ca^{2+}]_i$ rises generated by store discharge can also induce activation of PMCA and surface Ca^{2+} sensing receptors (Hernandez-Cruz *et al.*, 1997).

In excitable cells mitochondrial Ca^{2+} can be involved in the post-tetanic potentiation of synaptic transmission (PTP) that arises from a persistent presynaptic $[Ca^{2+}]_i$ elevation. Several inhibitors of mitochondrial uptake and

release blocked PTP and the persistence of presynaptic residual $[Ca^{2+}]_i$, while ER Ca^{2+} pump inhibitors and release channel activators had no effect. PTP can also result from the slow efflux of Ca^{2+} from tetanically accumulated mitochondria. If the protonophore CCCP was applied after tetanic stimulation, it caused a large transient increase of Ca^{2+} dependent transmitter release. Ruthenium red is specific blocker of the mitochondria Ca^{2+} uniporter with little effect on ATP production. If it was injected presynaptically, it blocked the formation of PTP. The pretetanic levels of transmission were unaffected. Caffeine exerted no effect on tetanic enchantment and in higher concentration even caused the inhibition of synaptic transmission.

So, mitochondria play a definite role in the generation of PTP. ATP production seems not to be responsible for this process. When mitochondrial uptake is blocked, $[Ca^{2+}]_i$ rises to higher levels during tetanic stimulation. This elevated level of Ca^{2+} appears to act by triggering enhanced transmitter release. Then the cytoplasmic $[Ca^{2+}]_i$ decays in seconds determined by membrane Ca^{2+} extrusion pumps, and PTP becomes blocked. Tetanic stimulation can also lead to hyperloading of mitochondria with Ca^{2+} and loading of terminals with Na^+, reducing transmembrane Na^+ gradients and slowing the efflux of cytoplasmic Ca^{2+}. This process also may contribute to the prolongation of residual Ca^{2+} responsible for PTP. As a result $[Ca^{2+}]_i$ reaches higher levels during tetanus, but the posttetanic plateau phase of residual $[Ca^{2+}]_i$ may be greatly reduced (Tang and Zucker, 1997).

Mitochondria can play a great role in these processes because of close connection with cytosolic Ca^{2+} and ER channels due to so called mitochondria-induced calcium release that has some features close to that of ER-induced CCE. The resulting changes in transmitter release or depletion of vesicles available for release can not be excluded, as all calcium regulating stores play an active role in the exocytotic processes. Within presynaptic terminals the ER cysternae are distributed often in the proximity of one or more mitochondria. So the stimulating effects of calcium waves and oscillation may have a significant role for the accumulation of Ca^{2+} and the genesis of spontaneous synaptic potentials.

The important role of cytosolic Ca^{2+}-accumulating structures in the formation of calcium signals becomes quite obvious in pathological conditions, as they are more sensitive to alterations of metabolic processes comparing with plasmalemmal ion channels. A detailed analysis of this role has been made on neurons from aged animals. Aging is characterized by a decrease in the peak amplitudes of calcium signals with parallel substantial prolongation of their decay time (Kostyuk *et al.*, 1993; Verkhratsky *et al.*, 1994; Kirischuk and Verkhratsky, 1996; Kostyuk, 1998). On one hand, they could be due to changes in the regulation of Ca^{2+} channels (slow-down in inactivation);.on the other

hand –to alterations of the mechanisms responsible for removal of excessive Ca^{2+} from the cytosol. The intracellular stores in sensory neurons were found to be overfilled with Ca^{2+} (judging by the amplitudes of transients which could be induced by activation of CICR by caffeine) and probably less capable to accumulate excessive Ca^{2+} from the cytosol. Changes in mitochondrial Ca^{2+} uptake have also already been found. Such alterations in the kinetics of Ca^{2+} transients should definitely impair interneuronal synaptic transmission and especially its components dependent on repetitive activity, for example long-term potentiation and depression, creating the basis of senile changes in perception and learning.

Another example of this importance is the changes in calcium signaling in neurons from animals with experimentally-induced diabetes, which also demonstrate substantial alterations in synaptic transmission of sensory volleys. The recovery of Ca^{2+} transients to the basal $[Ca^{2+}]_I$ level becomes extremely prolonged in both primary and secondary sensory neurons (Kostyuk *et al.*, 1995; Kostyuk *et al.*, 2001). Changes in Ca^{2+}-accumulating function of the endoplasmic reticulum are substantial in this case (Kruglikov *et al.*, 2004), as well as the corresponding function of mitochondria (Svichar *et al.*, 1998). Such changes may lead to the activation of calcium-dependent proteolytic enzymes (caspases) and promotion of necrotic processes which may step by step exclude from the functioning in neuronal networks of more and more integrated neurons, leading to the progressing weakening of the corresponding brain functions.

Hypoxia also may induce dramatic effects by inducing excessive elevation of cytosolic calcium level. Depending on the level of hypoxia and its duration it can develop either fast apoptotic processes or more delayed necrotic changes. The initial source of such elevation is excessive influx of ions through massive activation by released glutamate of calcium-permeable NMDA-channels, resulting in membrane depolarization and additional activation of voltage-operated calcium channels. An important further step is a substantial increase of ion accumulation by mitochondria, which may lead to the opening of permeability transition pores and subsequent processes leading to apoptotic cell death. The dysfunction of other above mentioned structures which participate in accumulation and transport of calcium ions may aggravate such damaging effects, cf. (Kostyuk *et al.*, 2003; Lukyanetz *et al.*, 2003).

5. Conclusion

Thus we have summarized recent data about the role of calcium ions in brain functions in physiological conditions, as well as during most general forms of pathology. They confirm that intracellular calcium signaling is of

primary importance for neuronal activity, and influx of calcium ions plays a dominant role in it. The characteristics of such calcium signals are determined by extremely complicated molecular mechanisms. The characteristics of different components of this complex and their interactions can now be analyzed in detail due to technical progress in direct measurements of changes in free calcium in the cytosol and intracellular structures and their interactions. Such measurements are of primary importance for the understanding of basic mechanisms of possible generalized pathological statuses and for the search of effective ways of their compensation.

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