Minireview

Intracellular and Molecular Aspects of Ca^{2+}-Mediated Signal Transduction in Neuronal Cells

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Abstract. Postsynaptic potential is only one aspect of extensive communication between neurons and their synapses. Besides generating of potential changes by activation of ionic channels, neurotransmitters may activate receptors linked with the transient concentration changes of one or several intracellular second messengers, including calcium ions (Ca^{2+}). In the neuronal cells calcium triggers and controls specific processes. Transient changes of Ca^{2+} concentration within the cell play an important signal role by coupling electrical and chemical impulses generated on the plasma membrane with the intracellular systems of responses. Several proteins and/or protein complexes, whose functions are directly controlled by calcium, have been identified in the neuronal cells. Their biochemical properties and physiological importance as well as cellular localization are discussed in this paper.

Key words: Brain — Calcium — Signal transduction — Ca^{2+}-binding proteins and Ca^{2+}-dependent proteins.

Abbreviations: cAMP, cyclic adenosine 3′,5′-monophosphate; AN, annexin family proteins; C, cytoplasm; cGMP, cyclic guanosine 3′,5′-monophosphate; EF, EF-hand motif proteins; ER, endoplasmic reticulum; $InsP_3$, myo-D-inositol (1,4,5) trisphosphate; MAP2, microtubule-associated protein-2; MARCKS, myristoylated alanine-rich C kinase substrate; NMDA, N-methyl-D-aspartate; PDI, protein disulphide isomerase; PM, plasma membrane; SV, synaptic vesicle;

Introduction

It is generally accepted that postsynaptic potential is only one aspect of extensive communication between neurons and their synapses. Besides generating of poten-

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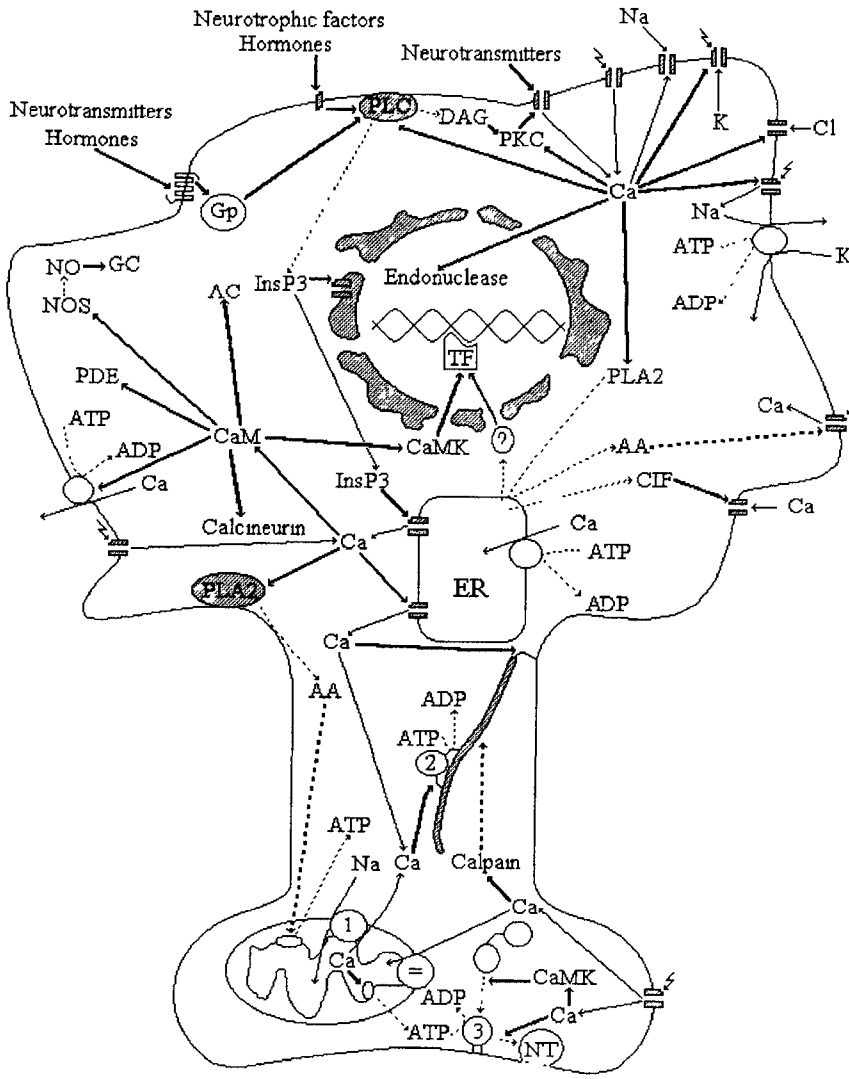

Figure 1. Hypothetical diagram illustrating the Ca^{2+} homeostasis and Ca^{2+}-mediated signal transduction pathways in neuronal cells. In resting non-activated neuronal cells the cytoplasmic concentration of ionized calcium is about 0.1 μmol/l, whereas its extracellular concentration is higher than 1 mmol/l. Activation of cells is associated with Ca^{2+} influx from the extracellular space through voltage- dependent and/or ligand-dependent Ca^{2+} channels localized on the plasma membrane. In addition, activation of some receptors leads to the release of Ca^{2+} from endoplasmic reticulum **(ER)** via inositol (1,4,5) trisphosphosphate-induced **($InsP_3$)** and/or Ca^{2+}-induced Ca^{2+} release. Na^{+}-dependent Ca^{2+} efflux **(1)** from mitochondria is not coupled to receptor activation. Depletion of ER can release small messenger "calcium influx factor" **(CIF)** and

initiate transcription of certain genes via unknown mediator (**?**). CIF triggers entry of extracellular Ca^{2+} via putative, unidentified Ca^{2+} channel. During cell relaxation, Ca^{2+} concentration decreases to resting level via ATP-driven Ca^{2+} transport both to the extracellular space and into ER. Part of Ca^{2+} is extruded through the plasmalemma by Na^+/Ca^{2+} exchanger, and part is sequestered by mitochondria via electrophoretic Ca^{2+} uniporter (=). Ca^{2+} stimulates ATP production in mitochondria.

Transduction of Ca^{2+} signals requires binding of Ca^{2+} to the target protein, and consequently modulation of its biological activity. Calcium regulates several proteins on the plasma membrane level:

- various types of ionic channels: Ca^{2+}-dependent K^+ channel, Ca^{2+}-dependent Cl^- channel and Ca^{2+}-dependent monovalent ion channels. The usual action of Ca^{2+} is to activate these channels, however, Ca^{2+}-induced inhibition of ion channels has also been observed
- phospholipase C (**PLC**) producing $InsP_3$ and diacylglycerols (**DAG**). $InsP_3$ is an important second messenger whose dominant role after activation of its intracellular receptor is to release Ca^{2+} from ER. DAG are signal molecules acting as activators of protein kinase C
- phospholipase A_2 (**PLA_2**) produces predominantly arachidonic acid (**AA**). Arachidonic acid depresses some voltage-dependent neuronal Ca^{2+} channels, and is able to uncouple oxidative phosphorylation.

Calcium regulates also many cytoplasmic proteins:

- protein kinase C (**PKC**) that is synergically activated by Ca^{2+} and DAG. In neurons PKC activates via phosphorylation some ionic channels
- calpain, Ca^{2+}-activated neutral cysteine endopeptidase. Its activation leads to irreversible proteolysis of cytoskeletal proteins
- cytoplasmic phospholipase A_2 with the same biological activity as its membrane isoenzyme
- Ca^{2+}-dependent endonucleases which are able to split chromosomal DNA
- calmodulin (**CaM**), dominant cytoplasmic Ca^{2+} receptor.

Several calcium-calmodulin-dependent proteins have been identified in neuronal cells:

- various calmodulin-dependent protein kinases (**CaMK**), especially protein kinase II. CaMK II activation and subsequent phosphorylation of transcription factors (**TF**) in nuclei triggers transcription of several genes. Presynaptically, synapsin phosphorylation by CaMK II decreases its affinity for synaptic vesicles (**3**) which enables release of neurotransmitters (**NT**) following depolarization
- calcineurin is one of the major CaM-binding proteins in the brain
- adenylyl cyclases (**AC**)
- nitric oxide synthase (**NOS**) produces from L-arginine nitric oxide (**NO**). NO activates synthesis of cyclic 3′,5′-GMP via activation of guanydyl cyclase (**GC**)
- phosphodiesterases (**PDE**) of cyclic nucleotides
- calcium pump from the plasma membrane which extrudes calcium through the plasma membrane into the extracellular space.

Ca^{2+} also plays a role in the axonal transport (**2**) and in the anchoring of cytoskeletal proteins.

The arrows represent:

normal arrow – transport, bold arrow – potentiation or activation, bold dashed arrow – inhibition or proteolysis (in the case of calpain), dashed arrow – generation of products.

tial changes by activation of ionic channels, neurotransmitters may activate receptors linked with the transient concentration changes of one or several intracellular second messengers (Ross et al. 1990), including cyclic nucleotides, diacylglycerol, inositol trisphosphates, nitric oxide and calcium ions (Ca^{2+}). This plasticity which is dependent on biochemical communication between neurons forms the basis for information storage in the brain, and is a base for cerebral adaptation to environmental changes or various injuries.

Generally, in all eukaryotic cells calcium in the cationic form (Ca^{2+}) is an important second messenger which triggers and regulates many different cellular functions (Carafoli 1987), e.g.: fertilization and development of cells, mitotic activity, mobility of intracellular organelles, lipid and carbohydrate metabolism, ATP production, immune response, muscle contraction, endocrine exocytosis, blood clotting, entry and function of toxins and pathogens, cell death etc. Various eukaryotic cells, however, differ in their morphology, metabolism and biological function so that there are some differences in the role of calcium as well. In the neuronal cells calcium triggers and controls specific processes such as:

- synthesis and release of neurotransmitters and hormones
- development and growth of neuronal cells
- neuronal excitability mediated by direct or indirect regulation of ionic channels
- proteosynthesis
- transcription of immediate early genes e. g., c-fos, c-jun
- axonal transport
- induction of long-term potentiation, long-term depression and memory (Kennedy 1989; Henzi and MacDermott 1992; Ghosh and Greenberg 1995).

In resting non-activated eukaryotic cells, including neuronal cells, the cytoplasmic concentration of ionized calcium is about 0.1 μmol/l, whereas its extracellular concentration is higher than 1 mmol/l. Some intracellular Ca^{2+} is present in nonactive form in intracellular stores, such as endoplasmic reticulum and/or mitochondria (Miller 1991), and part is bound to, and buffered with calcium binding proteins (Carafoli 1987). Activation of cells is associated with Ca^{2+} influx from the extracellular space through voltage-dependent and/or receptor-operated Ca^{2+} channels localized on the plasma membrane, and/or by release of Ca^{2+} from intracellular stores to reach Ca^{2+} concentrations up to micromolar levels. During cell relaxation, calcium concentration decreases to resting level via ATP-driven Ca^{2+} transport both to the extracellular space and into intracellular stores (Fig. 1). Thus, transient changes of Ca^{2+} concentration within the cell play an important signal role by coupling electrical and chemical impulses generated on the plasma membrane with the intracellular systems of responses. Transduction of Ca^{2+} signals requires binding of Ca^{2+} to the target proteins, and consequently modulation of their biological activity. Several proteins and/or protein complexes, whose functions

are directly controlled by calcium, have been identified in the neuronal cells. Many of them can be found on the plasma membrane, some in the cytoplasm as well as in intracellular organelle membranes. The localization of Ca^{2+}-regulated proteins within the cell is not uniform, and strong heterogeneity inside the neuronal cell is observed in this respect. Spatial heterogeneity of Ca^{2+}-regulated proteins together with spatial distribution of proteins maintaining Ca^{2+} homeostasis may allow for a large number of physiological responses to be mediated by the Ca^{2+} signal transduction pathway (Miller 1992).

Plasma membrane proteins regulated by calcium

In neuronal cells, several proteins, whose functions are directly regulated by intracellular calcium, have been found on the plasma membrane level, e.g.:

– various types of ionic channels regulated by Ca^{2+}. These may be classified into three broad categories: Ca^{2+}-dependent K^+ channel, Ca^{2+}-dependent Cl^- channel, and Ca^{2+}-dependent monovalent ion channels. The usual action of Ca^{2+} is to activate these channels, however, Ca^{2+}-induced inhibition of ion channels has also been observed. Their role in neurons is likely to modulate propagation of action potentials. Some channels participate in the transport of electrolytes, including regulation of cell osmolality (Marty 1989).

– phospholipase C which hydrolyses phosphatidylinositols and phosphatidylinositol polyphosphates from plasma membranes to generate inositol phosphates and diacylglycerol. $InsP_3$ is an important second messenger (Fisher et al. 1992) whose dominant role after the activation of its intracellular receptor is to release Ca^{2+} from intracellular stores (endoplasmic reticulum) (Berridge 1993). Diacylglycerols are signal molecules acting as activators of protein kinase C (Berridge 1987). Individual isoforms of phospholipase C are coupled with several receptors located on the plasma membrane both by GTP-binding or G-proteins or tyrosine kinases (Berridge 1993; Fisher 1995).

– phospholipase A_2 which preferentially hydrolyses *sn*-2 esteric bond between glycerol and fatty acid in phospholipids present in the plasma membrane. Since the major fatty acid in *sn*-2 position is arachidonic acid, phospholipase A_2 releases predominantly this fatty acid. It also seems that Ca^{2+}-dependent phospholipase A_2 can participate in a Ca^{2+} signalling cascade as well. A cellular role of this cascade has been not yet fully elucidated (Bonventre 1992). Arachidonic acid and products of its oxidation (eicosanoids) are important second messengers in the neuronal cells with broad physiological responses (Piomelli and Greengard 1990; Wolfe and Horrocks 1994; Katsuki and Okuda 1995). Arachidonic acid also affects neuronal Ca^{2+} channels (Keyser and Alger 1990; Schmitt and Meves 1995) and the glutamate NMDA-receptor (Miller et al. 1992), and therefore neuronal excitability. On the other hand, free fatty acids, also liberated by phospholipase A_2 action, are

able to uncouple oxidative phosphorylation (Wojczak and Schönfeld 1993) and thus modulate ATP production.

Cytoplasmic proteins regulated by calcium

As in plasma membrane, several proteins directly dependent on intracellular calcium concentration are also present in the cytoplasm, e.g.:

– protein kinase C, a serine/threonine kinase that is synergically activated by Ca^{2+} and diacylglycerol and by phospholipids (Girard et al. 1986). The majority of nonactivated protein kinase C is located in the cytoplasm; activation of protein kinase C leads to its association with the plasma membrane where protein kinase C phosphorylates and regulates several membrane proteins (Huang 1989). Neurogranin, neuromodulin and MARCKS are among other most prominent substrates of protein kinase C. Phosphorylation of these proteins reduces their affinities for calmodulin, and is involved in axonal regeneration, neurite growth, cytoskeletal rearrangement and synaptic development (Seki et al. 1995). In neurons protein kinase C co-regulates excitability and synaptic transmission via phosphorylation of ionic channels (Kaczmarek 1987), and is thought to play an important role in the initiation and maintenance of long-term potentiation and memory (Malenka et al. 1989; Ben-Ari et al. 1992).

– calpain, Ca^{2+}-activated neutral cysteine endopeptidase, is ubiquitously distributed in all animal cells, including neuronal cells. It forms a family consisting of at least six distinct members, which can be divided into two groups on the basis of the distribution: – ubiquitous and tissue-specific (Saido et al. 1994). It is generally accepted that calpain stands as a unique receptor for Ca^{2+} signals in neuronal cells. Its activation leads to irreversible proteolytic processing of a wide variety of substrate proteins (Johnson 1990), including membrane and cytoskeletal proteins, enzymes and signal peptides (Saido et al. 1994). The potency of calpain inhibitors to inhibit the growth of certain cells (Mellgren 1994) and development of hippocampal long-term potentiation (del Cerro et al. 1990) has also been documented. Its proteolytic modification of plasma membrane Ca^{2+}-ATPase significantly alters the kinetic parameters of this protein (Carafoli 1992); however, the physiological importance of this modification is not yet clear. Besides normal physiological functions, overstimulation of calpain may also exert some cytotoxic effects. Proteolytic conversion of xantin dehydrogenase to xantin oxidase may be an important source of superoxide during reperfusion (McCord 1985).

– cytoplasmic phospholipase A_2 with the same biological activity as its membrane isoenzyme. Various Ca^{2+}-dependent isoforms have been identified in the cytoplasm of eukaryotic cells including neuronal cells (Rodorf et al. 1991). One isoform (molecular weight 14 kDa) is associated with membranes of intracellular organelles, and is directly stimulated by calcium (Farooqui et al. 1992), while

the second Ca^{2+}-dependent isoform (molecular weight 85 kDa) is localized in the cytoplasm. After an increase of the Ca^{2+} concentration to excitation level, this latter isoform associates with the membranes of intracellular organelles, where it hydrolyses preferentially the *sn*-2 bond in glycero-phospholipids (Mayer and Marshall 1993). It also appears that these phospholipases may play some role in a yet unidentified signalling cascade.

– Ca^{2+}-dependent endonucleases which are able to split chromosomal DNA. Their location with respect to neurons is at present not clear. Generally, they are considered to be part of a pathway which is responsible for programmed cell death (apoptosis) (Nicotera and Orrenius, 1992).

– calmodulin is a dominant cytoplasmic Ca^{2+} receptor. It belongs to the group of "EF-hand" Ca^{2+}-binding proteins with regulatory properties ubiquitous to all eukaryotic cells. Concentrations of calmodulin in the cytoplasm of neuronal cells reach 30–50 μmol/l (Kennedy 1989). Each molecule of calmodulin possesses four high-affinity Ca^{2+}-binding sites, however, all are occupied only if calcium concentrations reache excitation level (Klee 1988). Calmodulin is a modulator of biological activity of many proteins localized both in the cytoplasm and on the plasma membrane. Examples of the major proteins regulated by calmodulin are listed in the next section. Calmodulin also binds to a number of other, predominantly cytoskeletal proteins, including MAP2, fodrin, neuromodulin, neurogranin, caldesmon and tubulin (Gnegy 1993).

Neuronal proteins regulated by calcium/calmodulin

Calmodulin with bound Ca^{2+} associates with different affinity with various proteins thus changing their biological functions and activities (Gnegy 1993; Kasai 1993). Several calcium/calmodulin-dependent proteins have been identified in the neuronal cells, e.g.:

– various calmodulin-dependent protein kinases, especially protein kinase II which is the dominant protein kinase of neurons from the cerebral cortex and the hippocampus. It is expressed in all parts of the cytoplasm of neuronal cells, and is the major postsynaptic density protein (Kennedy et al. 1983). Calmodulin-dependent protein kinase II has a broad substrate specificity and is able to trigger a wide variety of physiological responses (Braun and Schulman 1995). As a result of the high concentrations of NMDA receptors in post-synaptic dendrites, this kinase is a target of post-synaptic Ca^{2+} fluxes mediated by this channel as well as by Ca^{2+} currents mediated by L-type Ca^{2+} channels (Bading et al. 1993). It has also been suggested that calmodulin-dependent protein kinase II plays a role in the induction of long-term potentiation and memory (Bliss and Collongridge 1993; Fukunaga et al. 1996). Calmodulin-dependent protein kinase II activation and subsequent phosphorylation of transcription factors CREB and C/EBPβ in

nuclei triggers transcription of several genes including immediate early genes (Vendrell et al. 1993). Presynaptically, calmodulin-dependent protein kinase II phosphorylates synapsin I, a protein which binds to synaptic vesicles and cytoskeleton. Synapsin phosphorylation decreases its affinity for synaptic vesicles (Valtorta et al. 1992; Jahn and Südhof 1994) which enables the release of neurotransmitters following depolarization (Sihra and Nichols 1993; Burgoyne and Morgan 1995). Recently, the phosphorylation of a splice variant of neuronal N-type voltage sensitive calcium channel has been described by this kinase; however, the functional significance of this process remains to be determined (Hell et al. 1994). Another isoform calmodulin-dependent protein kinase III phosphorylates elongation factor-2, the factor which is an essential component of the proteosynthetic apparatus. This phosphorylation inactivates elongation factor-2, and consequently terminates proteosynthesis (Nairn and Palfrey 1987).

– calcineurin (protein phosphatase 2B, calmodulin-stimulated protein phosphatase) is one of the major calmodulin-binding proteins in the brain (Tallant and Cheung 1983), but it has a rather narrow substrate specificity (Liu and Storm 1989). It has been implicated in the regulation of the Ca^{2+}-mediated signalling pathway that affects many aspects of neuronal functions. In neurons, this phosphatase dephosphorylates DARRP-43, an endogenous inhibitor of low specific brain phosphatase I (King et al. 1984). Thus, calcineurin activation can trigger cascade dephosphorylations of proteins previously phoshorylated by kinases with particular functional implications. Because of co-localization of calcineurin with protein kinase C substrates, it is likely that calcineurin is a phosphatase with potential to reverse the action of protein kinase C (Seki et al. 1995). The role of calcineurin in transcription of immediate early genes (Enslen and Soderling 1994) and nuclear import of transcription factor NF-AT (Shibasaki et al. 1996) has also recently been documented.

– various adenylyl cyclases which catalyze the synthesis of the important signal molecule cAMP. Several isoforms have been identified in neurons both activated or inhibited by calmodulin binding (Cooper et al. 1995). Variable distribution in various parts of the brain as well as in different types of neurons has been demonstrated. All isoforms likely exert some role in coordinated control of the physiological function of neuronal cells.

– nitric oxide synthase produces from L-arginine nitric oxide, an important signal molecule, which mediates vascular smooth muscle relaxation (Schmidt et al. 1993). It has been suggested to have a role in retrograde signalling and modulation of synaptic plasticity in neuronal cells (Kerwin and Heller 1994). On the other hand, its free radical nature and chemical reactivity (Stamler et al. 1992) makes him a potent cytotoxic agent involved in several pathophysiological conditions (Kerwin and Heller 1994). Nitric oxide synthase activation also leads to amplification of Ca^{2+} signals which trigger expression of immediate early genes (Peunova and Enikolopov

1993). In neurons as in brain vascular smooth muscle cells, nitric oxide activates synthesis of cGMP (Mayer et al., 1993, Schmidt et al., 1993), also an important second messenger, whose physiological role in neuronal cells, except for some sensory neurons, is not yet clearly elucidated (Nestler and Duman 1994). In addition, nitric oxide modulates Ca^{2+} channel currents in sympathetic neurons (Chen and Schofield 1993). Recent results indicate that nitric oxide-evoked neurotransmitter release is mediated by two distinct release systems, a Ca^{2+}-dependent system and the reverse process of an Na^{+}-dependent carrier-mediated neurotransmitter transport system (Kuriyama and Ohkuma 1995).

– phosphodiesterases of cyclic nucleotides, which catalyse degradation of major second messengers cAMP and cGMP, have been identified as first molecular targets of calmodulin (Kakiuchi and Yamazaki 1970). In neurons, due to coexpression of calmodulin-dependent-nitric oxide synthase and calmodulin-dependent-cGMP-phosphodiesterase, the later terminates the action of nitric oxide in stimulated cell by hydrolysis of produced cGMP. Thus, calmodulin-dependent-cGMP-phosphodiesterase accomplishes the function of nitric oxide as a retrograde signal molecule (Mayer et al. 1993).

– calcium pump from plasma membrane which extrudes calcium through the plasma membrane into the extracellular space. Binding of calmodulin with bound calcium significantly affects the kinetic parameters of this protein (Carafoli 1992) exerting its complex physiological regulation in the brain (Lehotský 1995).

– ryanodine receptor was originally characterized as Ca^{2+} and a caffeine-sensitive intracellular Ca^{2+} channel (Henzi and MacDermott 1992) which contributes to the Ca^{2+} current after depolarisation or NMDA stimulation of neurons (Simpson et al. 1993). In non- muscle cells, including neuronal cells, another activator of the ryanodine receptor, cyclic ADP ribose, has been identified (Mészáros et al. 1993). Recently, cyclic ADP ribose activation of the ryanodine receptor has been shown to be mediated by calmodulin (Lee et al. 1994). Similarly, there is evidence that one isoform of the $InsP_3$ receptor, the second endoplasmic reticulum membrane Ca^{2+} channel, contains a calmodulin-binding domain (Yamada et al. 1995).

Calcium binding proteins

Along with Ca^{2+}-dependent proteins exhibiting enzymatic or ion channel activities, the major role of Ca^{2+}-binding proteins in brain structures is to bind, buffer and transport intracellular Ca^{2+} as well as to regulate various enzyme systems which are dependent on Ca^{2+} (Neher and Augustine 1992). These proteins have been extensively studied during the past decade due to their potential use as selective markers for identification of a variety of neuronal cells, functional brain systems and their circuitries (Andressen et al. 1993), as well as because of their implications

Table 1. Calcium binding proteins of neuronal cells (Heizmann and Braun 1992; Milner et al. 1992; Andressen et al. 1993; Kasai 1993) AN – annexin family proteins; C – cytoplasm; EF – EF-hand motif proteins; ER – endoplasmic reticulum; PDI – protein disulphide isomerase; PM – plasma membrane; SV – synaptic vesicle

	K_d (Ca^{2+}) (μmol/l)	Ca^{2+} binding domain	Cellular localization	Function
Calmodulin	3	EF	C	mediates many Ca^{2+}-dependent processes
α-Actinin	0.2	EF	C	microfilament anchoring
Fodrin	0.03	EF	PM	cytoskeleton interaction
S-100α	1–10	EF	C	
S-100β	1–10	EF	C	involved in growth and differentiation of glial cells
Calbindin		EF	C	intracellular Ca^{2+} acceptor
Calretinin		EF	C	intracellular Ca^{2+} acceptor
Parvalbumin	0.1	EF	C	intracellular Ca^{2+} acceptor
Synaptotagmin	$\approx$ 10		SV	docking of synaptic vesicles with presynaptic membrane
Calreticulin			ER	Ca^{2+} storage
T3BP/PDI			ER	Ca^{2+} storage
Endoplasmin			ER	Ca^{2+} storage
Synexin	200	AN	PM	
Lipocortin I	10	AN	PM	
Calpactin I	1.8	AN	PM	

in the pathophysiology of some neurodegenerative diseases (Heizmann and Braun 1992). In the neuronal cells, Ca^{2+}-binding proteins can be found in the cytoplasm, on the plasma membrane and in the lumen of intracellular organelles, e.g. the endoplasmic reticulum and the nucleus. Among others, the role of some Ca^{2+}-binding proteins in the modulation of neuronal excitability has also been suggested (Baimbridge et al. 1992). Synaptotagmin, Ca^{2+}-binding protein from the membrane of synaptic secretory vesicles, is involved in Ca^{2+}-dependent vesicle fusion with presynaptic membrane during synaptic transmission (Littleton and Bellen 1995). Table 1 summarizes the major Ca^{2+}-binding proteins which have been identified in neurons, their basic properties and functions (except for proteins exhibiting enzymatic or ion channel activity which were discussed above).

Regulation of neuronal cell functions by luminal Ca^{2+} concentration in the endoplasmic reticulum

There is growing evidence that Ca^{2+} entry and many aspects of cell signalling are

regulated by the state of filling of the calcium stores (Berridge 1995). By analogy with a capacitor in an electric circuit, the calcium stores prevent entry when they are charged up. They begin to promote Ca^{2+} entry as soon as calcium is discharged. The mechanism of such capacitative control of the cell functions is still controversial, although the existence of a novel small messenger released after the emptying of intracellular Ca^{2+} stores has recently been postulated (Randriamampita and Tsien 1993; Parekh et al. 1993). It seems that depletion of intracellular stores can affect the cytoplasmic signal transduction pathways such as the tyrosine kinase cascade (Tepel et al. 1994) and cGMP-pathway (Xu et al. 1994). In various eukaryotic cells it has, however, been demonstrated that depletion of intracellular Ca^{2+} stores triggers and regulates several biological responses:

- entry of extracellular Ca^{2+} via putative, unidentified Ca^{2+} channel (Fasolato et al. 1994)
- transcription of certain genes (Li et al. 1993)
- cell growth and progression of the cell through the cell cycle (Waldron et al. 1994)
- activation of nitric oxide synthase (Xu et al. 1994)
- inhibition of protein synthesis (Srivastava et al. 1995)

Although the capacitative mechanism of Ca^{2+} entry into neuronal cells has not yet been described in detail, there is suggestion that such a mechanism is a common feature of mammalian cells (Fasolato et al. 1994).

Regulation of mitochondrial enzymes by calcium

Energy demand of neuronal tissue is enormous since neurons involve ion motive ATPases for neuronal relaxation and signal transduction pathways. Several intramitochondrial dehydrogenases, especially pyruvate dehydrogenase, are activated by elevated intramitochondrial Ca^{2+} concentrations (Huang et al. 1994). Dehydrogenases which participate in Krebs cycle are stimulated by Ca^{2+} in the concentration range expected within the mitochondrial matrix after sequestration of cytosolic Ca^{2+} (Hansford 1994). However, the mechanism which activates dehydrogenases is not uniform, and several differences exist in this respect. Activation of pyruvate dehydrogenase is mediated by Ca^{2+}-sensitive phosphorylase, whereas isocitrate and α-ketoglutarate dehydrogenases have been shown to be allosterically activated by the binding of elevated Ca^{2+} (Gunter et al. 1994). Furthermore, in mitochondria Ca^{2+} stimulates other biochemical processes such as amino acid catabolism, fatty acid oxidation, electron transport, F1-ATPase and adenine nucleotide translocase (Gunter et al. 1994). Although most results have been obtained through the study of heart mitochondria, the mechanism of energy production stimulated by Ca^{2+} has also been documented in neuronal cells (Hansford 1994).

Conclusions

Neuronal activity leads to marked increases in the concentration of cytosolic calcium functioning as a second messenger that activates distinct intracellular signalling pathways. Whereas plasma membrane Ca^{2+} channels are the major routes by which Ca^{2+} enters the cell from the extracellular space, release of Ca^{2+} from the endoplasmic reticulum contributes significantly to the elevated cytosolic Ca^{2+} concentration. Depending on the route by which Ca^{2+} enters the cytosol, highly localized Ca^{2+} increases differentially affect neuronal processes. Calcium stimulates the activity of a variety of preexisting enzymes and proteins and effects short-term responses. Long-lasting responses require changes in gene expression, and are involved in neuronal survival and/or in Ca^{2+} mediated neuronal death. As it has been presented in this review, the spatial intracellular localization of the proteins regulated by Ca^{2+} is strongly heterogeneous. This heterogeneity may allow for a large number of physiological responses to be mediated by the Ca^{2+} signal transduction pathway. From this point of view, the exact cellular localization and biochemical characterization of these proteins is of great interest. Big progress in this field has been achieved using several techniques of molecular and cellular biology (in situ hybridization, immunodetection ...) as well as optical techniques monitoring the intracellular calcium dynamics. Despite this progress, there are several unclear questions concerning Ca^{2+}-dependent protein distribution, their exact physiological role, molecular mechanism of Ca^{2+}-mediated signal transduction and interference of various signal routes. Thus, future strategies and techniques are needed to improve the view on neuronal Ca^{2+} effector systems and their contributions to neuronal function. The techniques of molecular biology, hand in hand with comprehensive biochemical characterization, genetic and optical methods could answer the remaining questions. Finally, we have to consider not only heterogeneity at the intracellular level but also the strong heterogeneity among the various types of the neuronal cells.

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