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Calcium signaling in the brain

Claus W. Heizmann

Department of Pediatrics, Division of Clinical Chemistry,
University of Zurich, 75 Steinwiesstr., CH-8032 Zurich, Switzerland

Abstract. Calcium ions regulate many processes in the central nervous system via interaction with intracellular calcium-binding proteins. One class of these proteins shares a common structural motif, the EF-hand. A consensus amino acid sequence for this motif has aided the identification of many new members of this family. Some of these proteins, like parvalbumin, calbindin, and calretinin, proved to be useful neuronal markers for a variety of functional brain systems and their circuitries. Their major role is assumed to be buffering, transport of Ca^{2+}, and regulation of various enzyme systems. Cellular degeneration is often accompanied by Ca^{2+} overload. It has been assumed that neurons containing certain intracellular Ca^{2+}-binding proteins may have a greater capacity to buffer Ca^{2+} and therefore would be more resistant to degeneration.

Key words: Ca^{2+}-binding proteins, brain, EF-hand structure, annexin, chromosomal localization, neurodegenerative disorders

INTRODUCTION

Several biological processes in the central nervous system are regulated, directly or indirectly, by the ubiquitous second messenger calcium. It is the increase of the free Ca^{2+} concentration within stimulated cells that triggers fundamental processes such as release of neurotransmitters, electric activity, fast axonal flow or memory storage.

In normal cell homeostasis, the resting level of intracellular calcium is approximately 200 nM compared to approximately 5,000 times higher concentrations in the extracellular space. Cells thus have developed sophisticated mechanisms that precisely control influx and extrusion of calcium in the presence of this high transsarcolemmal calcium gradient. Long-term maintenance of Ca^{2+} homeostasis is the concerted action of the importing systems, the Ca^{2+} channels (Neher 1992), the exporting systems (the Ca^{2+} pump, the Na^{+}-Ca^{2+} exchanger) (Carafoli 1991), and the Ca^{2+}-binding/buffering/-transporting/regulating proteins (Kretsinger et al. 1991, Croall and Demartino 1991, Heizmann and Hunziker 1991).

The calcium signal is transmitted into the intracellular response, in part by Ca^{2+}-binding proteins that are involved in the regulation of many cellular activities illustrated in Fig. 1.

According to their structural features these proteins can be subdivided into the following families: (1)EF-hand Ca^{2+}-binding proteins: Proteins belonging to this evolutionary family (Moncrief et al. 1990, Lee et al. 1991, Nakayama et al. 1992) and sharing a type of Ca^{2+}-binding domain known as the EF-hand (Heizmann and Hunziker 1991, Heizmann 1991, Kretsinger et al. 1991). (2)The annexins,

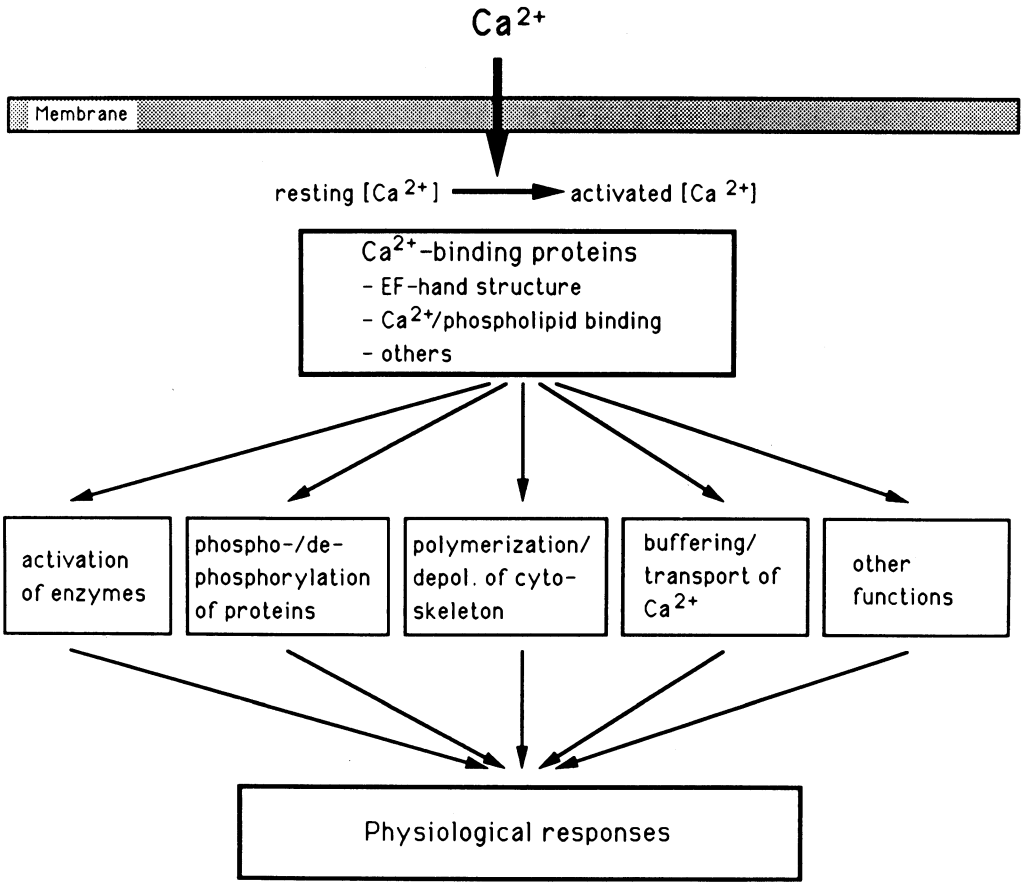

Fig. 1. Involvement of calcium in the intracellular signal transmission. Reprinted by permission of W.B. Saunders Company, Seminars in Cell Biology (1990) 1: 277-282.

Ca^{2+}-dependent phospholipid-binding proteins: The members of this protein family interact with phospholipids and cellular membranes in a calcium-dependent manner (Klee 1988, Cirino and Flower 1991, Moss et al. 1991). They are also present in neuronal and non-neuronal cells of the brain (Woolgar et al. 1990) and have recently also been examined in relation to neurological diseases (Rothwell and Flower 1992). One member of this family, annexin I (a phosphoprotein and a major substrate of the epidermis growth factor receptor, previously known as lipocortin), appears to be selectively distributed in glia cells of the human CNS (Johnson et al. 1989). The appearance of immunoreactivity to annexin I in reactive astrocytes and macrophages surrounding lesions indicated an involvement of this protein in CNS inflammation and repair. Interestingly, some of these annexins, which are integral membrane proteins, were reported to exhibit voltage-dependent Ca^{2+} channel activities (Burns et al. 1989, Brisson et al. 1991).

In this review I would like to focus on the EF-hand Ca^{2+}-binding proteins and summarize their protein and gene structures, localization, tissue-specific expression in the CNS, their physiological functions, as well as their altered expression in several human degenerative disorders.

PROTEIN AND GENE STRUCTURES, AND CHROMOSOMAL LOCALIZATION OF EF-HAND Ca^{2+}-BINDING PROTEINS

Calmodulin, parvalbumin, calbindin, S-100 proteins, calpain, and altogether more than 200 proteins belong to this family (Heizmann and Hunziker 1991, Kretsinger et al. 1991). All of these proteins exhibit a common structural motif, the EF-hand, which is present in multiple copies and binds calcium selectively and with high affinity. Each of these domains consists of a loop of 12 amino acids (a variant loop with 14 amino acids is present in the S-100 protein subfamily) that is flanked by two -helices. This structural principle was first identified

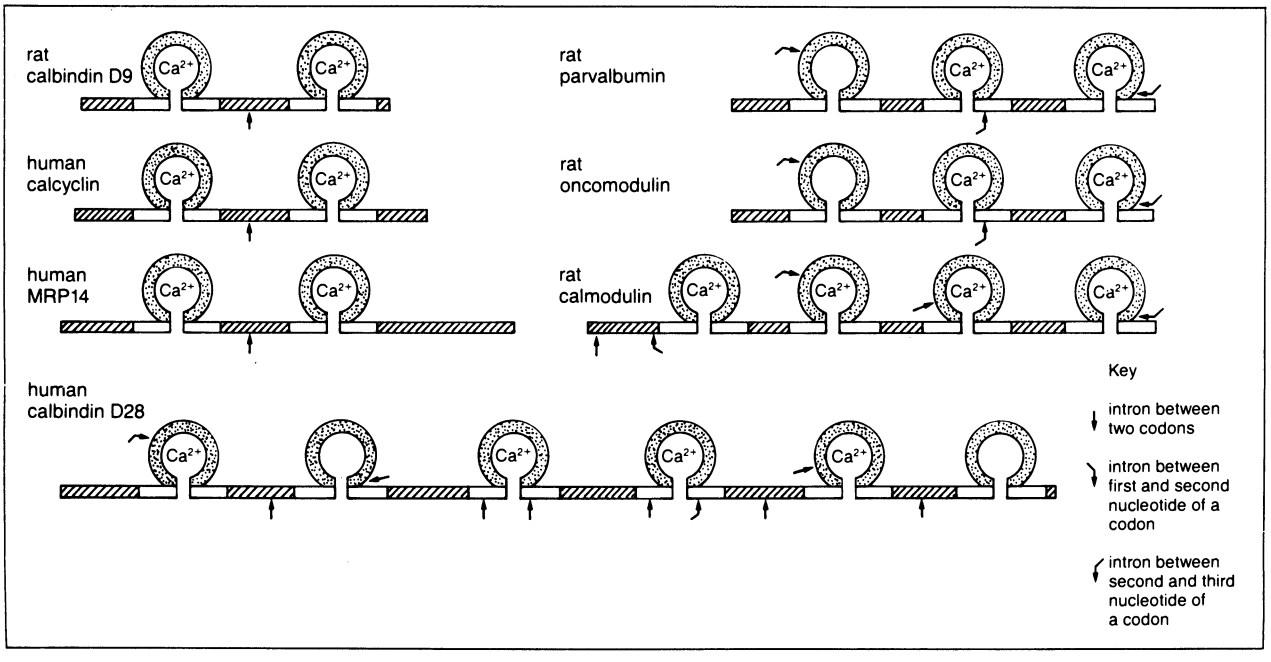

Fig. 2. The known genes encoding the EF-hand calcium-binding proteins can be divided into three subgroups on the basis of intron positions and numbers. These subgroups may reflect an evolutionary family relationship. Loops are stippled, helices are unshaded, and linker regions are hatched (reprinted by permission of Elsevier Trends Journals, TIBS (1991) 16: 98-103).

with the crystal structure of the calcium-binding carp parvalbumin and is designated as EF-hand after the E- and F-helices of parvalbumin (Kretsinger and Nockolds 1973). This structure has been refined recently (Kumar et al. 1990, Roquet et al. 1992).

From this structural information, the putative EF-hand-dependent calcium-binding ability of a protein can be predicted or confirmed on the basis of the amino acid or cDNA sequence, leading to the discovery of many new EF-hand proteins. The model even allows us to predict whether an EF-hand domain is still functional or whether it has lost its calcium-binding ability due to mutations.

So far the genes of only a few members of the EF-hand Ca^{2+}-binding proteins have been analyzed, but already a picture of a family tree is emerging from comparisons of the numbers and positions of the introns (Fig. 2).

One branch of the family is the S100-like proteins. All genes of this group that have been analyzed contain an intron in the linker region separating the two EF-hand domains.

The second family branch contains proteins with four EF-hands (e.g. calmodulin, troponin C) and a related protein with three EF hands (parvalbumin), related on the basis of intron positions and numbers.

The third family branch contains the six EF-hand domain proteins, calbindin D-28K, and calretinin.

Interestingly, in the S-100 branch the introns separate functional domains whereas in the other two branches the introns are preferentially located within the EF-hand domains (Heizmann and Hunziker 1991).

Adjacent localization of two related genes on the same chromosome can be indicative of a recent gene duplication event and therefore suggests a close kinship. Chromosome localization of a gene in conjunction with the chromosome localization of certain inherited diseases could yield some clues as to the physiological function of the protein. Several members of the S-100 protein family (e.g. S-100α, calcyclin, MRPs) are located on chromosome 1. As the only exceptions, the human S-100β and calbindin D-9K, members of the same subfamily, are located on chromosome 21 and X, respectively. It has been speculated that some of the impaired brain functions observed in trisomy 21 might be a consequence of an additional copy of the S-100β gene. Elevated S-100β levels in the blood and lymphocyte fractions of patients with Down's syndrome have been reported (Duneau et al. 1989). The known chromosomal assignments of other EF-hand calcium-binding proteins are listed in Table I.

S-100 proteins are expressed in a cell type-dependent fashion (for reviews see Hilt and Kligman 1991, Longbottom and van Heyningen 1991). The amino acid sequences of a number of S-100 proteins have been conserved in a wide variety of organisms ranging from protozoa to man. This strong conservation argues for an important (and conserved) biological role for S-100 proteins.

The number of S-100 proteins is steadily increasing. We cloned S-100α, CAPL, and other S-100 proteins from human tissue (Engelkamp et al. 1992) and detected two novel S-100 proteins (unpublished).

The biological functions of the S-100 proteins are less clear. They likely exert their biological effects by interacting with secondary effector proteins in a Ca^{2+}-dependent fashion. This mode of protein-protein interaction and modulation of the activity of the secondary effector protein is similar to that seen with calmodulin. S-100 proteins interact, however, with a different set of binding proteins than does calmodulin. One member of the S-100 protein family, the p11, is associated with the tyrosine kinase substrate p36 (or annexin II) (Gerke 1991). It has been suggested that the $p36_2p11_2$ complex might be involved in the membrane-cytoskeletal linkage and/or the control of membrane fusion events during exocytosis (Burgoyne and Geisow 1989). Calcyclin was found to be associated with a new member of the annexin family (Tokumitsu et al. 1992).

S-100β also acts extracellulary, e.g, as a neurite extension factor (Kligman and Marshak 1985, Hilt and Kligman 1991). The most difficult and interesting question to date remains that of the precise bi-

TABLE I

Ef-hand calcium-binding proteins in the brain

Protein	Chromosomal assignment[a]	Localization[b]	Suggested functions[b]	Neurodegenerative disorders associated with abnormal proteinb
Calmodulin		Ubiquitous	Mediates many Ca^{2+}-dependent processes	Alzheimer's disease
α-Parvalbumin	22q12-q13.1	Neurons	Ca^{2+}-buffering and transport protective role in Ca^{2+} overload	Alzheimer's disase epilepsy, ischemia Pick's disease Down's syndrome meningiomas neurofibromatosis
Calbindin D-28K	8q21.3-q22.1	Neurons (expression regulated by corticosterone in hippocampus)	Ca^{2+}-buffering and transport, protective role in Ca^{2+} overload	Alzheimer's disase epilepsy, ischemia Parkinson's disease Down's syndrome
Calretinin	16q22-q23	Neurons	Ca^{2+}-buffering and transport, phosphorylation	?
Calcineurin	4, 8, 10	Neurons	Calmodulin- dependent phosphatase, target of cyclophilin-cyclosporin complexes	?
Calpain	1,11,15,19	Neurons, astroglia and microglia	Ca^{2+}-activated protease	Alzheimer's disase ischemia
S-100α	1	Neurons	?	?
S-100β	21q22	Glia cells	Involved in growth and differentiation, assembly and disassembly of microtubules and actin filaments, posphorylation, neurite extension (extracellular function)	Alzheimer's disase Down's syndrome acquired immunodeficiency disease (AIDS)
Recoverin, or visinin, frequinin, p26	17	Photoreceptor layer in retina	Phototransduction, activates guanylate cyclase to restore dark state	Retinopathy

[a]For references see TIBS (1991) 16: 98-103
[b]For references see TINS (1992) 15: 259-264

ological function of these proteins. Recently, Barger and Van Eldik (1992) reported on the stimulation of calcium fluxes in glial and neuronal cells by S-100β. Toward this goal experiments of over- and underexpression of S-100 proteins in various cell lines and studies of transgenic animals have been started in several laboratories.

DISTRIBUTION AND LOCALIZATION

Calmodulin is present in all cells but most other Ca^{2+}-binding proteins, including those listed in Table II, are expressed only in some tissues and cells. Several of these proteins (parvalbumin, cal-

bindin D-28K, calretinin, calcineurin, S-lOO proteins) have been found in high concentrations in the central and peripheral nervous systems of many species, including man (for reviews see Heizmann and Braun 1990, Heizmann 1991), where many processes depend on calcium. The tissue-specific and developmental pattern expression of different Ca^{2+}-binding proteins in the central nervous system can be quite distinct (Table II).

Parvalbumin, for example, is present in a subpopulation of neurons in mammals containing the inhibitory neurotransmitter γ-aminobutyric acid (GABA) (Heizmann and Braun 1990). Some exceptions to this colocalization have been found in the retina of the cat and in some nuclei of birds. Generally, parvalbumin is associated with neurons that have a high firing rate and a high oxidative metabolism. The presence of parvalbumin in fast-spiking cells (firing at high frequency and showing no adaptation of spike frequency with sustained depolarization) in the rat hippocampus (CA2 region) has been demonstrated by injection of Lucifer yellow *in vitro* in combination with postembedding parvalbumin immunohistochemistry (Kawaguchi et al. 1987).

It is suggested that parvalbumin may be involved in the buffering/transport of calcium in a subset of neurons with specialized electrophysiological properties. Calbindin D-28K is present in a subpopulation of neurons scattered in most but not all areas of the central nervous system. The role of this protein in neurons, as in other tissues, is suggested to be Ca^{2+}-buffering/transport.

Calcineurin is a Ca^{2+}- and calmodulin-dependent protein phosphatase in which the EF-hand subunit (calcineurin B) is regulatory (Cohen 1989, Kuno et al. 1992). Immunoreactivity is observed in many neurons throughout the brain but not in glial cells. Recently it was found (Liu and Storm 1989) that calcineurin is able to dephosporylate neuromodulin, a major neuronspecific calmodulin-binding protein. On the basis of these and other results it was proposed that levels of free calmodulin in neurons may be regulated by the activities of protein kinase C and calcineurin through reversible phosphorylation/dephosphorylation of neuromodulin. Recently calcineurin has also been identified as a key signalling enzyme in T-lymphocyte activation (Clipstone and Crabtree 1992, Fruman et al. 1992, O'Keefe et al. 1992, Swanson et al. 1992).

CALCIUM-BINDING PROTEINS IN HEALTH AND DISEASE

Calcium-binding proteins are involved in a wide variety of activities, such as cytoskeletal organiza-

TABLE II

Immunocytochemical localization of some Ca^{2+}-binding proteins in mammalian cerebellum

	Rat				Cat		
Brain area	PV	Calbindin	CaN	CalR	PV	Calbindin	CaN
Cerebellum-Purkinje cells	+++	+++	+++	-	+++	+++	+
Basket/stellate cells	+++	-	+	-	+++	-	?-
Granule cells	-	-	-	+	-	-	+-
Golgi cells		-		-			-
Cerebellar nuclei	+f	+f		+			
Lugaro cells		-		+			

PV, parvalbumin; calbindin, calbindin D-28K; CaN, calcineurin; CalR, calretinin; f, fibrous neutropil; +,++,+++, density of stained structures; -, no staining (reprinted by permission of Elsevier Trends Journals, TIBS (1991) 16: 98-103)

tion, cell motility and differentiation, cell cycle regulation, calcium buffering and transport. One might therefore suppose that altered levels of some calcium-binding proteins (e.g. due to deletion or mutation of the corresponding genes) should lead to an impaired calcium homeostasis in cells and to pathological conditions.

Several research groups have now started to search for an altered expression of the Ca^{2+}-binding proteins, parvalbumin, calbindin D-28K, and S-100 proteins, in affected brain regions of patients (Heizmann and Braun 1992). Cases examined include those suffering from acute insults such as stroke and epileptic seizures and from chronic neurodegenerative disorders, such as Alzheimer's, Huntington's, Parkinson's, and Pick's diseases. In addition, altered calcium levels have been found in platelets of patients with bipolar affected disorders (Dubovsky et al. 1986, Dubovsky et al. 1989), and calcium antagonists have been suggested for treatment of psychotic depression.

Calcium overload as a result of seizures or ischemia is supposed to activate biochemical processes leading to enzymatic breakdown of proteins and lipids, to malfunctioning of mitochondria, energy failure, and ultimately to cell death (Barry 1991). There is reason to assume that neurons that contain certain intracellular calcium-binding proteins, and therefore have a greater capacity to buffer calcium, would be more resistant to degeneration. Investigations of the vulnerability of such neurons in human brain as well as in experimental animal models have revealed contradictory results concerning the postulated protective role of calcium-binding proteins (see Table I and Heizmann and Braun 1992).

The increase in intracellular calcium that occurs as a result of excitatory amino acid receptor activation has been suggested to be the initiating factor in seizure-associated degeneration and neuronal death. Since only certain subsets of neurons are susceptible to irreversible damage the positive correlation between parvalbumin or calbindin D-28K content and relative resistance to seizure-induced neuronal damage in certain hippocampal neuron populations is in support of the neuroprotective hypothesis of calcium-binding proteins. However, in human epileptic brain tissue some authors report that the parvalbumin- and calbindin D-28K-immunoreactive neurons in the hippocampus are relatively spared from degeneration and only some calbindin D-28K-positive granule cells are lost, while other authors find a clear loss of parvalbumin- and calbindin D-28K-immunoreactive neurons.

The levels of Ca^{2+}-binding proteins have also been investigated in chronic neurodegenerative diseases, e.g., Alzheimer's disease and Parkinson's disease, however, results from studies on human pathological material are also inconsistent.

Ca^{2+}-binding proteins have also been suggested as diagnostic tools. S-100 protein has been measured in cerebrospinal fluid and blood of patients with cerebral infarction, transient ischemic attack, hemorrhage, and head injury (Persson et al. 1987). An enhancement of S-100β was also found in the blood of patients with Down's syndrome (Kato et al. 1990). These and other Ca^{2+}-binding proteins might be selective markers in the future to estimate the extent of brain damage in the various neurological disorders and also to classify various brain tumors in children (Ishiguro et al. 1983) and in adults (Fagnart et al. 1988).

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