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Critical Review

Calcium Ions in Neuronal Degeneration

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Summary

Neuronal Ca^{2+} homeostasis and Ca^{2+} signaling regulate multiple neuronal functions, including synaptic transmission, plasticity, and cell survival. Therefore disturbances in Ca^{2+} homeostasis can affect the well-being of the neuron in different ways and to various degrees. Ca^{2+} homeostasis undergoes subtle dysregulation in the physiological ageing. Products of energy metabolism accumulating with age together with oxidative stress gradually impair Ca^{2+} homeostasis, making neurons more vulnerable to additional stress which, in turn, can lead to neuronal degeneration. Neurodegenerative diseases related to aging, such as Alzheimer's disease, Parkinson's disease, or Huntington's disease, develop slowly and are characterized by the positive feedback between Ca^{2+} dyshomeostasis and the aggregation of disease-related proteins such as amyloid beta, alfa-synuclein, or huntingtin. Ca^{2+} dyshomeostasis escalates with time eventually leading to neuronal loss. Ca^{2+} dyshomeostasis in these chronic pathologies comprises mitochondrial and endoplasmic reticulum dysfunction, Ca^{2+} buffering impairment, glutamate excitotoxicity and alterations in Ca^{2+} entry routes into neurons. Similar changes have been described in a group of multifactorial diseases not related to ageing, such as epilepsy, schizophrenia, amyotrophic lateral sclerosis, or glaucoma. Dysregulation of Ca^{2+} homeostasis caused by HIV infection or by sudden accidents, such as brain stroke or traumatic brain injury, leads to rapid neuronal death. The differences between the distinct types of Ca^{2+} dyshomeostasis underlying neuronal degeneration in various types of pathologies are not clear. Questions that should be addressed concern the sequence of pathogenic events in an affected neuron and the pattern of progressive degeneration in the brain itself. Moreover, elucidation of the selective vulnerability of various types of neurons affected in the diseases described here will require identification of differences in the types of Ca^{2+} homeostasis and signaling among these neurons. This information will be required for improved targeting of Ca^{2+} homeostasis and signaling components in future therapeutic strategies, since no effective treatment is currently available to prevent neuronal degeneration in any of the pathologies described here.
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COMMONALITY AND COMPLEXITY OF Ca^{2+} SIGNALING

In all eukaryotic cells, the intracellular Ca^{2+} concentration ($[Ca^{2+}]_i$) determines the physiological status of the cell. Upon cell stimulation, the Ca^{2+} concentration increases from the low level characteristic of the resting state to a higher level sufficient to activate Ca^{2+}-dependent processes. Ca^{2+} transients are characterized by different amplitudes, kinetics and intracellular locations, and are often organized in oscillations. Because of these properties, various Ca^{2+} signals activate a diverse range of crucial generic (proliferation, differentiation, apoptosis, and gene transcription) and cell-type specific processes [reviewed in (*1*)]. Thus, in the case of muscle cells, an increase in $[Ca^{2+}]_i$ switches on the molecular processes leading to contraction. In cells of the immune system, on the other hand, Ca^{2+} signals participate in the regulation of synapse formation between T cells and antigen presenting cells as well as in vesicle exocytosis in cytotoxic T cells (*2*). In neurons, Ca^{2+} regulates such fundamental neuronal processes as plasticity and synaptic transmission. Under physiological conditions the activation of presynaptic neurons leads to the release of neurotransmitters to the synaptic cleft, via a Ca^{2+}-dependent process. Released neurotransmitters, in turn, activate receptors in the membrane of subsequent neurons, thus initiating signal transmission. In post-

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synaptic neurons, activation of certain types of neurotransmitter receptors (namely excitatory ionotropic receptors and some metabotropic receptors) results in the generation of Ca^{2+} signals triggering cellular responses specific to the type of receptor in question.

In the light of the above considerations, it is clear that dysregulation of Ca^{2+} homeostasis (Ca^{2+} dyshomeostasis) compromises the well-being of the neuron. In this article we review Ca^{2+} dyshomeostasis in physiological ageing and in Ca^{2+}-related neurodegenerative diseases. These diseases were selected according to the broad definition of neuronal degeneration, which includes not only neuronal death, but also dysregulation of normal neuronal physiology.

Ca^{2+} HOMEOSTASIS IN HEALTHY NEURONS

Ca^{2+} homeostasis provides a precise way to control $[Ca^{2+}]_i$ and allows the generation of a variety of Ca^{2+} signals, which can be distinguished by distinct spatial dimensions (from nanodomains up to gradients in the whole cell body), temporal dimension, amplitude, frequency in case of oscillations, and localization in the neuron (reviewed in *3*, *4*). The subsequent readout of Ca^{2+} signals employs local and global Ca^{2+}-binding protein sensors and downstream signaling proteins, which transmit the Ca^{2+} message to cellular effectors. Under physiological neuronal conditions, electrical, or receptor-mediated stimuli generate in the neuron different spatiotemporal Ca^{2+} signals in the form of transient (microseconds to minutes) increases in the cytoplasmic concentration of Ca^{2+} which can range from 50–300 nM at rest to 1–500 μM upon activation. The repertoire of these Ca^{2+} signals in neuronal cells is particularly rich; this is reflected in complex homeostatic mechanisms such as the number and diversity of Ca^{2+} channels in the plasma membrane, and a multitude of Ca^{2+}-binding signaling proteins (reviewed in *5, 6*). Ca^{2+} homeostasis comprises mechanisms that turn on Ca^{2+} signals, that activate Ca^{2+} sensitive signaling cascades and that turn signals off, as indicated schematically in Fig. 1 and discussed later.

Mechanisms that Turn Ca^{2+} Signals "On"

An increase in $[Ca^{2+}]_i$, both local and general, can occur as a result of Ca^{2+} influx from the extracellular environment or of its release from the intracellular Ca^{2+} stores, mainly from the lumen of the endoplasmic reticulum (ER), where the Ca^{2+} concentration is ~1,000 times higher than in the cytoplasm.

The principal ways Ca^{2+} enters the cell from the outside are through two types of Ca^{2+} channels in the plasma membrane: ionotropic receptor-operated (ligand-gated) channels (ROCs), and voltage-operated Ca^{2+} channels (VOCCs). Ca^{2+} influx through the ionotropic ROCs is activated by direct binding of specific agonists.

ROCs permeable for Ca^{2+} include the N-methylo-D-aspartate receptors (NMDARs) and some α-amino-3-hydroxy-5-methylisoxazole-4-propionate acid receptors (AMPARs). These are activated by their physiological agonist glutamate, the major excitatory neurotransmitter in the central nervous system (CNS); (reviewed in *7*). NMDARs play a particularly important role in the CNS as efficient routes for Ca^{2+} entry into neurons. The Ca^{2+} signaling mentioned earlier is accompanied by the activation of other NMDARs-associated signaling molecules, which trigger the specific cellular response linked to this particular route of Ca^{2+} entry. NMDARs–associated signaling complexes vary according to the localization of NMDARs thus providing additional levels of response specificity (reviewed in *8*). For example, the influx of Ca^{2+} via extrasynaptic NMDARs can initiate signaling cascades resulting in apoptosis, whereas activation of NMDARs located in the postsynaptic membrane can lead to changes in neuronal plasticity. Depending on the amount of Ca^{2+} entering, synaptic transmission can undergo either long-term potentiation (LTP) or long-term depression (LTD), (reviewed in *6, 9*).

VOCCs, found only in excitable cells, are activated by depolarization of the cell membrane or by the opening of ROCs. There are several molecular types of VOCCs believed to initiate different neuronal functions. For example, N and P/Q-type VOCCs are present in the axonal bouttons of many types of neurons and control Ca^{2+}-dependent release of neurotransmitters, while L-type VOCCs, found on dendrites, can mediate Ca^{2+}-dependent gene activation (reviewed in *10, 11*).

Ca^{2+} release from the ER occurs in neurons via two types of Ca^{2+} channels/receptors: ryanodine receptors (RyRs) and inositol-1,4,5-triphosphate receptors (IP3Rs). IP3Rs are ubiquitously expressed in many cell types whereas RyRs are more characteristic of neurons and muscle cells. Ca^{2+} release through IP3Rs requires binding of the second messenger IP3 generated by phospholipase C (PLC) in response to the activation of various G-protein-coupled receptors (GPCRs) or of tyrosine kinase-linked receptors on the cell membrane (reviewed in *1*). Increased cytoplasmic Ca^{2+} concentration is a major trigger for Ca^{2+} release via RyRs, the phenomenon known as Ca^{2+}-induced Ca^{2+} release (CICR). RyRs are also regulated by other intraneuronal factors, such as cyclic adenosine diphosphate ribose (cADP-ribose). IP3Rs, on the other hand, are regulated by multiple factors both on the cytoplasmic and on the luminal surface of the ER, including apoptosis-linked cytochrome C (*12*), apoptosis-related proteins from the B-cell lymphoma/leukaemia-2 gene (Bcl-2) family, some Ca^{2+}-binding proteins and Ca^{2+} itself. Higher cytoplasmic Ca^{2+} concentrations sensitize IP3R to IP3, initiating CICR process, while low Ca^{2+} concentrations are inhibitory. CICR of IP3Rs and RyRs have critical importance in shaping Ca^{2+} signals because it links and coordinates elementary events representing the opening of a single channel or of an associated group of channels (Ca^{2+} "sparks" or "puffs") into Ca^{2+} waves and oscillations. These waves propagate Ca^{2+} signal in the cytoplasm. Emptying of the internal Ca^{2+} ER store activates the ER refilling mechanism known as Capacitative Ca^{2+} Entry (CCE). CCE is the influx of Ca^{2+}

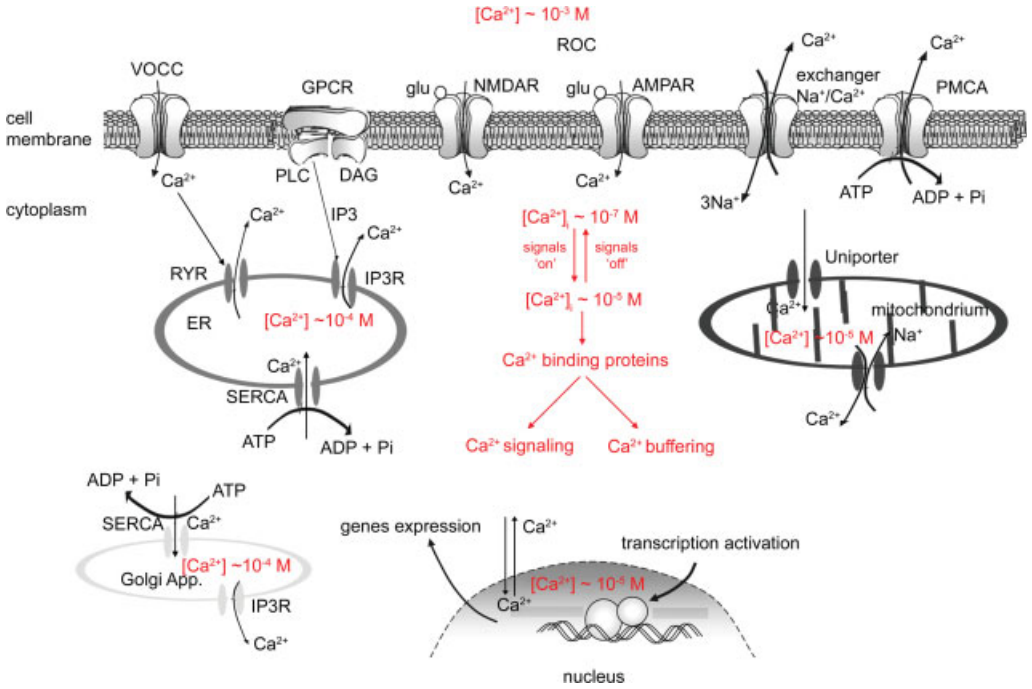

Figure 1. Ca^{2+} homeostasis in healthy neurons. Ca^{2+} signals in the cytoplasm can be turn 'on' by the Ca^{2+} influx from the outside or by the Ca^{2+} mobilization from the intracellular Ca^{2+} stores such as the endoplasmic reticulum (ER) or the Golgi apparatus. Ca^{2+} can enter neurons through voltage-operated Ca^{2+} channels (VOCCs), and through some glutamate (glu)-activated receptor-operated channels (ROCs): the N-methylo-D-aspartate receptors (NMDARs) and some α-amino-3-hydroxy-5-methylisoxazole-4-propionate acid receptors (AMPARs). Activation of some G-protein-coupled receptors (GPCRs) can mobilize Ca^{2+} from the ER via inositol-1,4,5-triphosphate (IP3) channels/receptors (IP3Rs). The release of Ca^{2+} from the ER occurs also via Ca^{2+}-activated ryanodine receptors (RyRs). Ca^{2+} signals are transmitted to cellular effectors by Ca^{2+}-binding protein sensors. Some of Ca^{2+} signals can reach the nucleus and affect gene transcription. Ca^{2+} clearance mechanisms restoring its basal level during 'off' phase comprise Ca^{2+}-binding protein buffers, a plasma membrane Ca^{2+} ATPase (PMCA), a Na^{+}/Ca^{2+} exchanger, and a sarco-endoplasmatic reticulum Ca^{2+} ATPase (SERCA). During the recovery, mitochondria can sequester Ca^{2+} through a uniporter. Ca^{2+} concentration in the nucleus is also controlled. $[Ca^{2+}]i$, Ca^{2+} concentration in the cytoplasm.

through the store-operated channels (SOCs) in the plasma membrane (reviewed in *13*). The identity of SOCs and of the coupling between their opening and ER stores remained unidentified until the recent discovery of the Orai1 and Stim1 proteins. Orai1 functions as a Ca^{2+} channel in the plasma membrane and is gated by a direct interaction with Stim1 and Stim2, both of which are Ca^{2+} binding sensors residing in the ER membranes and sensing Ca^{2+} level in the ER lumen (*14*); (for a recent review on Stim1 see *15*).

Transmission of Ca^{2+} Signals

A Ca^{2+} signal in the form of local or global increase of $[Ca^{2+}]_i$ is sensed and transmitted by a multitude of Ca^{2+}-binding proteins, most of which contain helix-loop-helix motives (EF-hands), where Ca^{2+} is coordinated by several acidic residues in the loop (reviewed in *16*, *17*). Ca^{2+} sensors decode, and differentiate between various Ca^{2+} signals according to differences in the localization of Ca^{2+} sensors, in Ca^{2+} affinity, and in kinetics of ion binding (reviewed in *18*, *19*). The EF-hand proteins involved in sensing and transmitting Ca^{2+} signals in neurons are the ubiquitous calmodulin, S100 proteins, neuronal Ca^{2+} sensors (NCS), and calmyrins which are similar to NCS (reviewed in *19*, *20*). Transduction of Ca^{2+} signals to cellular effectors by the EF-hand proteins is mediated by several mechanisms. Most commonly, Ca^{2+} binding induces a conformational change in an EF-hand protein, enabling its binding to its target proteins (reviewed in *21*, *22*). Transmission of the Ca^{2+} signal can also be mediated by Ca^{2+}-mediated changes in the intracellular localization of EF-hand proteins (*23, 24*). The downstream molecular events transmitting Ca^{2+} signals are complex and are related mainly to phosphorylation cascades. Ca^{2+} signaling employ the following proteins: Ca^{2+} and calmodulin (CaM) dependent kinases (CaMKI-IV), protein kinase C, protein kinase A, IP3 kinase, Ca^{2+}-dependent phosphatase calcineurin B, cyclic AMP phosphodiesterase, adenylyl cyclase, Ca^{2+}-dependent neuronal nitric oxide synthase (NOS) and calpains, which are Ca^{2+}-activated proteases (reviewed in *1*). Some of the Ca^{2+}

signaling cascades propagating in the cytoplasm can reach the nucleus and affect gene transcription (reviewed in *25*). Known transcription factors that are dependent on Ca^{2+} signaling include the calcineurin B-controlled nuclear factor of activated T-cells (NFAT), the cyclic AMP response element-binding protein (CREB), and the Ca^{2+}-binding downstream regulatory element modulator (DREAM), known also as calsenilin. Ca^{2+} concentration in the nucleus is independently controlled, but compartmentalization of Ca^{2+} signaling in the nucleus, as in connected organelles, is simultaneously synchronized with cytoplasmic changes (reviewed in *26*).

Mechanisms that Turn Ca^{2+} Signals "Off"

Ca^{2+} clearance mechanisms in neurons control the duration as well as the spread of Ca^{2+} signals and result in the reduction of free cytoplasmic Ca^{2+} and in the restoration of its basal level during recovery from stimulation (reviewed in *1, 27, 28*). Rapid Ca^{2+} sequestration is attributed to Ca^{2+}-binding EF-hand protein buffers in the cytoplasm, mainly calbindin D-28k, calretinin, and parvalbumin. Slower Ca^{2+} clearance is mediated by Ca^{2+} pumps and exchangers. Ca^{2+} ions are pumped out against a concentration gradient of four order of magnitude by a plasma membrane Ca^{2+} ATPase (PMCA). Ca^{2+} is also removed from the cytoplasm by Na^+/Ca^{2+} exchanger located in the cell membrane. The internal Ca^{2+} stores such as the ER, the Golgi apparatus and mitochondria possess their own release and filling mechanisms which contribute to shaping Ca^{2+} signals and to the clearance of cytoplasmic Ca^{2+} during the "off" phase. Sarco-endoplasmatic reticulum Ca^{2+} ATPase (SERCA) removes cytoplasmic Ca^{2+} and "pump" it into the lumen of the ER, where it can be further sequestered by the EF-hand proteins (reviewed in *29*). Mitochondria, especially those located close to the ER Ca^{2+} release channels, sequester and internalize Ca^{2+} mainly via uniporter, while the release of Ca^{2+} is based on its exchange into Na^+ or H^+ ions. When cytoplasmic Ca^{2+} increases are large, mitochondria become rapidly-sequestering Ca^{2+} buffers, ensuring protection against excess of Ca^{2+} (30; reviewed in *31*).

CAUSES AND EFFECTS OF NEURONAL Ca^{2+} DYSHOMEOSTASIS

The complexity of the Ca^{2+} homeostasis and of the signaling protein systems (Ca^{2+} homeostasome and signalosome) in neurons provides significant compensatory potential, protecting neurons against Ca^{2+} toxicity. However, persistent cellular stress conditions can overcome the compensatory mechanisms and lead to an abnormally increased cytoplasmic Ca^{2+} level resulting in neuronal dysfunction. Especially sensitive to stress conditions are the membranous Ca^{2+} homeostatic components, mainly pumps and Ca^{2+} channels. Oxidative stress, lipids peroxidation or membranous deposition of aggregated proteins impair pumps and Ca^{2+} channels in cell membranes, as well as in membranes of the ER, and contribute to neurodegeneration, as seen in AD or PD (reviewed in *32–34*). Glutamate excitotoxicity is another common mechanism contributing to neurodegeneration in ischemia, glaucoma and many other diseases. It is mediated by excessive release of the excitatory neurotransmitter glutamate (reviewed in *35*). Excessive activation of NMDARs, mediated by glutamate, causes an enhanced influx of Ca^{2+} through this receptor.

Consequences of excessive Ca^{2+} signals vary according to their magnitude, their duration and the type of neuron affected. The extreme effect of Ca^{2+} dysregulation is cell death, and indeed, several Ca^{2+}-activated cascades causing programmed cell death have been described. Excessive accumulation of Ca^{2+} in mitochondria can lead to the formation of a permeability transition pore and to the collapse of transmembrane potential. This triggers the release of cytochrome c and apoptosis (reviewed in *36*). Excessive Ca^{2+} signals can also directly activate Ca^{2+}-binding calpain proteases leading to the degradation of structural and enzymatic proteins (reviewed in *37*). Cell death, however, can be initiated not only rapidly, but also after a prolonged period of subtle changes in Ca^{2+} homeostasis. In recent years, a new hypothesis has been formulated, (*38*), according to which Ca^{2+} dyshomeostasis is more than just a global excess of Ca^{2+} ions in the neuron, activating generalized responses and cell death. Indeed, during physiological ageing or in chronic neurodegenerative diseases, Ca^{2+} dyshomeostasis would be a complex spatiotemporal dynamic process, gradually impairing neuronal mitochondria, the ER, the plasma membrane and signal transduction processes. This hypothesis also points to the interplay between Ca^{2+} homeostasis, signaling and other signal transduction networks. The best example is the excitotoxicity triggered by NMDARs, involving massive influx of Ca^{2+} through this receptor as well as a very complex regulation of NMDARs localization, and of subunit composition and functions (reviewed in *35*). Individual types of neurons are characterized by a specific Ca^{2+} homeostatic and signaling molecular machinery. Thus, diversity of Ca^{2+} homeostatosome and signalosome seems to be underlying distinct responses in various neurons to the same stimuli resulting in the impairment of only one specific type of neurons or region of the brain in physiological ageing or in various types of neurodegeneration.

Ca^{2+} DYSHOMEOSTASIS IN PHYSIOLOGICAL AGEING

Aging of brain neurons is a normal physiological process, distinct from the pathological changes found in neurodegeneration (reviewed in *39*). In contrast to initial findings, significant loss of neurons or other gross morphological changes are not observed in aging. Instead, aging-related changes are subtle and involve a reduced area of synapses accompanied by mild functional deficits in cognition, learning and memory (*40, 41*). Since local Ca^{2+} signaling is indispensable for presynaptic release of neurotransmitters and for triggering of synaptic plasticity, the role of Ca^{2+} in neuronal aging has

been attracting the attention of researchers for some time. In fact, the so-called Ca^{2+} hypothesis of brain aging was proposed 20 years ago (*42–44*). This hypothesis proposed that aging is related to the dysregulation of Ca^{2+} homeostasis and to the sustained increases of $[Ca^{2+}]_i$. Recent studies indicate that Ca^{2+}-dependent functional changes associated with aging are local, subtle, gradual, and take place over a long period of time. Various types of neurons exhibit different profiles of age-related changes in their Ca^{2+} homeostasis and different sensitivities to Ca^{2+} dysregulation, the hippocampal and cortical neurons being the most vulnerable (reviewed in *38*, *45*). Electrophysiological and behavioral studies, as well as neuronal imaging showed that in hippocampal or cortical neurons the resting Ca^{2+} levels are not affected by aging; stimulation, however, triggers higher Ca^{2+} transients (reviewed in *46*). Increased level of Ca^{2+} transients in aged neurons was recently linked to a switch in the main routes of Ca^{2+} entry into neurons: while NMDARs coupled Ca^{2+}-channels play a main role in synaptic stimulation in younger neurons, the role of internal Ca^{2+} stores and the activity of L-type VOCCs increase with age (reviewed in *39*). Since L-type VOCCs are able to interact directly with RyRs-triggering CICR, these two types of Ca^{2+} channels in the disregard membrane and in the ER seem to interact with each other, jointly triggering amplification of Ca^{2+} transients. An abnormally increased Ca^{2+} concentration demands intense Ca^{2+} buffering, a demand that is not met by aging neurons. Decreased age-related Ca^{2+} buffering capacity has been shown in a majority of neurons in the peripheral and CNSs (*47*). The principal dysfunction in Ca^{2+} buffering is observed in mitochondria. In some types of neurons mitochondrial dysfunction is accompanied by decreased expression of Ca^{2+} buffering proteins (*48*). The high demand of neurons for energy production by mitochondrial oxidative metabolism is linked with the generation of free reactive oxygen radicals (ROS) which accumulate with age, and cause oxidative damage and depolarization of the mitochondrial membrane (reviewed in *49*). Depolarization of mitochondria impairs ability of these organelles to sequester excess Ca^{2+}, which, in turn, may expose cytosol to higher Ca^{2+} concentrations, particularly after excitatory stimulation. Mitochondrial dysfunction seems to play a central role in aging and links the two major hallmarks of this process: age-related oxidative stress and impaired Ca^{2+} homeostasis. This view has been supported by the demonstrated prevention against age-related Ca^{2+} buffering changes in the forebrain neurons by caloric restriction (reviewed in *39*). In summary, oxidative stress in aged neurons can slightly impair functions of cell membrane and membranes of intracellular organelles involved in Ca^{2+} homeostasis, causing subtle changes in Ca^{2+}-dependent cell excitability, synaptic plasticity, and connectivity. The degree of disturbance in Ca^{2+} homeostasis is not sufficient to disrupt normal neuronal functions, but makes neurons more vulnerable to additional stress. Aged neurons under neuronal stress may become unable to prevent Ca^{2+} dysregulation and age-related neurodegenerative diseases.

Ca^{2+} DYSHOMEOSTASIS IN NEURONAL PATHOLOGIES

The dysregulation of Ca^{2+} homeostasis is involved in pathogenic mechanisms of various neurodegenerative diseases. Some of these diseases take a long period of time to develop. In others, dysregulation of Ca^{2+} homeostasis initiated by infection or sudden accidents such as ischemia or brain injury, leads to rapid neuronal death. Here we describe neuronal pathologies, in which the crucial role of disrupted Ca^{2+} homeostasis has been established (Table 1).

Ca^{2+} Dyshomeostasis in Slowly Progressing Pathologies

Alzheimer's Disease. AD is the most common age-related neurodegenerative chronic dementia; it develops over a 10 year period until the patients' death. The disease is characterized by slow, gradual degeneration, and death of neurons in the forebrain and particularly in the hippocampus (reviewed in *50, 51*). Over 95% of cases are sporadic (sporadic Alzheimer's disease, SAD) with the age of onset at over 65. Rare familiar cases (familiar Alzheimer's disease, FAD) start earlier and are linked to inherited dominant mutations in the amyloid precursor protein (APP) or in one of the presenilin proteins (PS1 or PS2), responsible for generation of the neurotoxic amyloid beta peptide (A_β) from APP (*52, 53*). Affected brain areas of AD patients showed increased levels of Ca^{2+} (*54*) and increased activation of Ca^{2+}-dependent enzymes (*55*). Moreover, a growing body of evidence indicates that FAD and SAD etiology is based on the interplay between Ca^{2+} dyshomeostasis and either mutated presenilins or neuropathological hallmarks of AD such as A_β and hyperphosphorylated tau protein. In this interplay dysregulation of Ca^{2+} precedes other neuronal malfunctions (reviewed in *34, 46, 56–60*).

SAD can develop from aging-related mild Ca^{2+} dyshomeostasis in forebrain neurons under conditions which also potentiate Ca^{2+} dysregulation. These conditions include a low level of intellectual and physical activity, and a diet rich in calories but containing low amount of folate and antioxidants. Viral infections or brain injuries also increase the risk of AD. Genetic AD risk factors are linked to inherited membrane properties such as those encoded by APOE4 allele. Ca^{2+} contributes to the development of AD by Ca^{2+}-triggered ER and mitochondrial dysfunction, and Ca^{2+}-dependent changes in gene expression (reviewed in *57, 60*). Elevated $[Ca^{2+}]_i$ affects both phosphorylation of tau (*61, 62*) and APP processing resulting in A_β generation (*63–65*). In turn, A_β potentiate Ca^{2+} dyshomeostasis in several ways and can cause further elevation of cytoplasmic Ca^{2+} levels. A_β oligomers can form Ca^{2+}-conducting pores in the cell membrane and generate ROS and oxidative stress, which induce lipid peroxidation and impairs functions of Ca^{2+}

Table 1
Components of Ca^{2+} homeostasis affected by physiological and molecular changes in some neurodegenerative diseases

Component of Ca^{2+} homeostasis	Mechanisms of changes	Diseases	References
Ca^{2+} influx	GluRs overexcitation	PD, HD, epilepsy, TBI, ALS, brain stroke, glaucoma	(*89, 103–108, 115, 122 126, 132, 147, 152*)
	Changes in VOCCs activity	PD, SCAs, glaucoma, epilepsy, TBI	(*90, 113, 118, 127, 129, 131, 150*)
	Reverse activity of Na^{+}/Ca^{2+} exchanger	Glaucoma, TBI	(*119, 150*)
	Effects of neurotoxic proteins (Aβ, AICD, Tat, gp120)	AD, HIV	(*66, 67, 154, 157*)
Intracellular Ca^{2+} buffering	↓ Expression of Ca^{2+}-binding proteins (calbindin D28k, parvalbumin, calretinin)	PD, SCAs, schizophrenia,	(*82–85, 111, 139*)
	Mitochondrial dysfunction	HD, ALS	(*99, 100, 122*)
	↑ Expression of proteins interfering with Ca^{2+} buffering (Aβ, calcyon, NCS-1, GAP 43)	AD, schizophrenia	(*64, 135, 137, 138, 141*)
	Impaired Ca^{2+} extrusion	Brain stroke	(*151*)
ER Ca^{2+} storage	↑ER Ca^{2+} release	AD, HD, schizophrenia, HIV	(*68–72, 101, 136, 156*)
	Impaired ER refilling	AD, SCAs	(*73, 74, 112*)

↑, reported increase; ↓, reported decrease; ER, endoplasmic reticulum; AD, Alzheimer's disease; PD, Parkinson's disease; HD, Huntington's disease; ALS, Amyotrophic lateral sclerosis; SCAs, spinocerebellar ataxias; TBI, Traumatic brain injury.

ATPases and glutamate receptors (reviewed in *66*). In particular, A_β causes impairment of NMDARs signaling and a decrease in the number of NMDARs (*67*). Inside the neurons, A_β-evoked oxidative stress can increase Ca^{2+}-induced malfunctions of mitochondria. Moreover, the APP intracellular domain (AICD), released during APP processing, also modulates Ca^{2+} homeostasis and resting $[Ca^{2+}]_i$, mainly as the regulator of IP3-mediated Ca^{2+} efflux from the ER (*68, 69*).

In FAD pathogenesis, the generation of toxic A_β from mutant APP creates an intense stress leading to an accelerated Ca^{2+} dyshomeostasis, as described above. Mutant PS1 or PS2 themselves contribute to Ca^{2+} dyshomeostasis-affecting functions of the ER Ca^{2+} stores. Presenilins located in the ER membranes affect directly or indirectly RyRs and IP3Rs. Consistently, FAD mutations in presenilins enhance Ca^{2+} release from the ER Ca^{2+} stores, which alters synaptic transmission in hippocampal neurons (*70–74*). Mutations in presenilins are also linked to the marked impairment in the ER Ca^{2+} refilling mechanism CCE (*75, 76*). It is further postulated that presenilins form low conductance cation channels in the ER membrane, and FAD mutations impair this ability (*77*). Another postulated pathway of mutant PS1 and PS2 contribution to dysregulation of Ca^{2+} homeostasis and signaling is the impaired interaction of presenilins with the Ca^{2+}-binding proteins calsenilin and calmyrin. Different mechanisms of calsenilin and calmyrin involvement in AD have been proposed (*78–82*).

Taken together, the above data suggest the following overall picture of AD pathogenesis. Aging-related changes or FAD mutations result in Ca^{2+} dyshomeostasis exacerbated by additional environmental or genetic stress. The positive feedback loop between Ca^{2+} dyshomeostasis and increased production of toxic A_β escalates with time resulting in enhanced cell death signalling and final neuronal loss.

Parkinson's Disease. Parkinson's Disease is the second most common progressive neurodegenerative disease and the most common motor system disorder strongly associated with ageing and affecting mainly the population over age 65 (reviewed in *83*). Bradykinesia, rigidity, tremor and other motor symptoms are attributed to the selective degeneration and loss of dopaminergic neurons in the substantia nigra pars compacta (SNc). Ca^{2+} dyshomeostasis has been recently proposed as the primary age-related condition driving neurodegeneration in the sporadic form of PD (95% of cases). This view is supported by the demonstration that dopaminergic neurons expressing higher levels of protein buffers calbindin D28k, calretinin and parvalbumin seem to be resistant to degeneration in PD (*84–87*). Alfa-synuclein aggregates, neuropathological hallmarks of PD, potentiate neuronal Ca^{2+} dyshomeostasis and overload (*88–90*). Similarly to the situation in AD, intracellular Ca^{2+} overload in PD can be linked to glutamate excitotoxicity (reviewed in *91*). Selective vulnerability of SNc dopamine neurons to aging-related Ca^{2+}

dyshomeostasis has been supported recently by the notion of their specific dependence on the L-type VOCCs which increases with age (reviewed in *92*). L-type VOCCs in aged neurons are opened much of the time causing Ca^{2+} overload. Accordingly, administration of a dihydropiridine, an L-type channel blocker, confers protection against toxins that induce parkinsonism in experimental animal models (*93*). This finding opens a new perspective on potential neuroprotective strategies in PD. However, Ca^{2+} dyshomeostasis alone does not result in PD until it is exacerbated by environmental insults, such as heavy metals, pesticides, neurotoxins or inflammation (reviewed in *94, 95*). Genetic predispositions which can accelerate the pathogenic process in familiar PD are linked to mutations in mitochondrial proteins: PINK1, a mitochondrial kinase, DJ-1 (PARK7), a redox-stress sensor and antioxidative protein, and Parkin, an ubiquitin ligase. These factors converge on the oxidative, mitochondrial, and ER stress, escalating Ca^{2+} dyshomeostasis and neuronal dysfunction (reviewed in *91, 96, 97*).

Huntington's Disease. Huntington's Disease is an inherited autosomal dominant neurodegenerative disease with an age of onset between 20 and 50 years and progression to death within 10–20 years after onset (reviewed in *98, 99*). This disease is characterized by motor and psychiatric symptoms such as chorea and gradual dementia connected to a selective and progressive loss of medium spiny neurons in the striatum (MSN). HD is caused by the abnormal protein huntingtin (Ht), which contains polyglutamine expansion in the N-terminal region. Some toxic functions assigned to mutant Ht convert into Ca^{2+} dyshomeostasis (reviewed in *100*). First, Ht is associated with mitochondria and the mutant form of Ht was demonstrated to disrupt mitochondrial Ca^{2+} homeostasis (*101, 102*). Second, mutant Ht associates directly with IP3R in the ER and is able to sensitize this receptor to its activation by IP3 (*103*). Such malfunction of the ER Ca^{2+} store seems especially significant in MSN neurons, which express a high level of metabotropic glutamate receptors (mGluR5) acting via IP3R-mediated Ca^{2+} release (*104–106*). Finally, many data indicate that MSN loss in HD is mediated by excitotoxicity involving NMDARs, especially the NR2B subtype expressed predominantly on MSN (reviewed in *107*). Mutant Ht was shown to interfere with the binding of NMDARs to the postsynaptic density protein PSD-95, leading to NMDAR hypersensitivity and an increase in Ca^{2+} influx (*108–110*). Ca^{2+} overload, accumulated over time, can eventually trigger the opening of the permeability transition pore in mitochondria and neuronal death.

Autosomal Dominant Spinocerebellar Ataxias. Spinocerebellar Ataxias are a complex group of neurodegenerative disorders. Disease symptoms usually appear between 30 and 50 years of age and are characterized by progressive cerebellar ataxia of gait and limbs variably associated with ophtalmoplegia, pyramidal and extrapyramidal signs, dementia, pigmentary retinopathy and peripheral neuropathy (*111*). At least 28 distinct genetic loci are connected with different forms of SCAs (SCA-1 to SCA-28) (reviewed in *112*). Strong evidence points to an involvement of neuronal Ca^{2+} signaling disturbances in neurodegeneration of SCA. Several neuronal genes abundantly expressed in Purkinje cells and involved in Ca^{2+} signaling or homeostasis are downregulated in the cerebellum of SCA-1 mutant mice characterized by abnormally long polyglutamine tract within the mutated protein. The list includes a decreased expression of Ca^{2+}-binding proteins calbindin-D28k and parvalbumin (*113*). Other examples are IP3R1 and SERCA2, (*114*). In SCA-6 disease, Purkinje cell degeneration is also associated with polyglutamine expansion within the *CACNA1A* gene, which encodes pore-forming subunit of P/Q-type VOCCs (reviewed in *115*).

Glaucoma. Glaucoma represents a group of neurodegenerative diseases and is the second common cause of blindness worldwide (reviewed in *116*). It is characterized by structural damage to the optic nerve and slow progressive death of retinal ganglion cells (RGCs). Subtypes of glaucoma were classified according to changes in intraocular pressure (IOP), state of aqueous outflow channels and the possibility of detecting the cause of elevated IOP (reviewed in *117*). Although the current understanding of the pathogenesis of glaucoma is not complete, there is a considerable evidence pointing to a blood flow deficit at the retina/optic nerve head and development of hypoglycemic, hypoxic or ischemic injury as a result (reviewed in *118–120*). The increased extracellular glutamate level, excessive NMDA and AMPA/kainite receptors stimulation and VOCCs activation are thought to be the principal reasons for RGCs excitotoxic loss (reviewed in *117, 120*). The mechanism of anoxia or ischemia evoked injures in optic nerve are mostly connected with the reverse activity of the Na^{+}/Ca^{2+} exchanger and Ca^{2+} entry into the axoplasm through this route, since Ca^{2+} channel antagonists, effective in retina were relatively ineffective in optic nerve (reviewed in *121*).

Amyotrophic Lateral Sclerosis. Amyotrophic Lateral Sclerosis is a multifactoral neurodegenerative disease, characterized by progressive and highly selective loss of cortical, spinal and brainstem motor neurons and is accompanied by the progressive loss of muscle force and breathing capacity, swallowing difficulties, and limb spasticity (reviewed in *122*). Almost 90% of cases are sporadic and the remaining 10% are of genetic origin. In the latter category, the most common mutation is in superoxide dismutase (reviewed in *123*). The mechanisms leading to the selective degradation of motor neurons are still not clear but several pathogenic factors have been proposed. Among them, disruption of intracellular Ca^{2+} homeostasis, including glutamate excitotoxicity, Ca^{2+} dependent formation of protein aggregates and Ca^{2+}-evoked mitochondrial dysfunction, are thought to play a key role (reviewed in *124*). It has been shown that spinal motor neurons do not express the Ca^{2+} binding proteins parvalbumin and calbindin D28k (*125*) and that they have a high proportion of AMPARs lacking the GluR2 subunit, which favours Ca^{2+} permeability of these channels (*126*). Specific

physiological features, particularly continuous activity-dependent Ca^{2+} cycling and high metabolic demands make motor neurons highly sensitive to any potential excitotoxicty caused by both endogenous and exogenous factors (reviewed in *124, 127*).

Epilepsy. Epilepsy is a common chronic neurological condition, characterized by an uncontrolled, excessive electric discharge by the neurons resulting in unprovoked seizures. There are number of acquired and genetic causes of this disorder; consequently various models of epileptogenesis have been established and studied. It is estimated that up to 50% of all epilepsy cases are initiated by neurological insults and are called acquired epilepsy (AE). It develops in three phases: the injury to the CNS, epileptogenesis, and the chronic epileptic (spontaneous recurrent seizure) phases. Stroke and traumatic brain injury are two examples of common brain injuries that can lead to the development of AE. Recent studies indicate that injury-induced alterations in Ca^{2+} homeostasis play a role in the development and maintenance of AE. Brain damage is caused by an increase in extracellular glutamate concentration that causes increased intraneuronal $[Ca^{2+}]_i$, leading to injury, and/or death of the neurons. The neurons that survive injury sustain long-term changes in intracellular Ca^{2+} and mechanisms of Ca^{2+} homeostasis. These changes are prominent features of the epileptic phenotype (*128*).

Defects in certain types of VOCCs and of their ancillary subunits are important in idiopathic generalized epilepsy. Both T-type and P/Q-type VOCCs appear to mediate important contributions to seizure genesis, to modulation of network activity, and to genetic seizure susceptibility (*129*).

Burst firing of the thalamic neurons is driven by the low threshold Ca^{2+} spike generated by Ca^{2+} influx through T-type Ca^{2+} channels when these channels are activated by membrane hyperpolarization due to inhibitory inputs. The major inhibitory inputs to the thalamocortical neurons come from the GABAergic neurons in the thalamic reticular nucleus. Thalamic burst firings have long been implicated in the pathogenesis of childhood absence epilepsy. This type of epilepsy affects children between 4 and 12 years of age. The patients have recurrent absence seizures that can occur hundreds of times a day. Analysis of mice deficient for the α1G locus, which is the predominant gene underlying the low threshold Ca^{2+} currents in the thalamocortical neurons, has demonstrated the essential role of the thalamocortical bursts in certain forms of absence seizures (*130*).

In patients with the absence epilepsy/ataxia phenotype, genetic marker analysis was consistent with linkage to the *CACNA1A* gene on chromosome 19, which encodes the main pore-forming α_{1A} subunit of $Ca_V2.1$ channels; these channels conduct P/Q-type Ca^{2+} currents. DNA sequence analysis identified a point mutation resulting in an amino acid substitution (E147K) in $Ca_V2.1\alpha1$, which segregated with the epilepsy/ataxia phenotype. Functional expression studies using human *CACNA1A* cDNA demonstrated that the E147K mutation causes an impairment of Ca^{2+} channel function, which may have a central role in the pathogenesis of certain cases of primary generalized epilepsy (*131*). It has been shown that single nucleotide polymorphisms in T-channel genes contribute to neurological disorders characterized by thalamocortical dysrythmia, such as generalized epilepsy (reviewed in *132*). In addition, modulation of the intrinsic firing pattern mediated by α1D T-type Ca^{2+} channels plays a critical role in the genesis of absence seizures in the thalamocortical pathway (*133*).

It has been shown that activation of ionotropic and metabotropic glutamate receptors as well as the TrkB neurotrophin receptors can promote epileptogenesis. These receptors are present in the membranes of the dendritic spine of principal neurons (glutamatergic) and their activation generates an increased Ca^{2+} concentration within the spine. This may activate Ca^{2+}-regulated enzymes implicated in epileptogenesis such as CaMKII, calcineurin, and protein tyrosine kinases Src and Fyn. It has been proposed that limbic epilepsy is a maladaptive consequence of homeostatic responses to increases of Ca^{2+} concentration within dendritic spines induced by abnormal neuronal activity (*134*).

Schizophrenia. Schizophrenia is a psychiatric disorder that has its clinical onset usually in early adulthood. The etiology of schizophrenia is still not clear but this disorder appears to have a complex genetic background, which together with environmental risk factors may contribute to the development of the disease. Neuropathological studies provided evidence that during development dysfunctions in neurotransmitter systems can occur, and altered dopamine-, glutamate- and GABA-mediated neurotransmission appeared to be involved in the pathophysiology of this disorder (reviewed in *135*). The idea that altered intracellular Ca^{2+} signaling may be crucial for the molecular mechanisms leading to schizophrenia was first suggested by Jimerson et al. (*136*) and recently has begun a main course in investigations of the etiology of schizophrenia.

There are nearly 1,700 genes/proteins which changes in the levels of expression are connected with schizophrenia (reviewed in *137*). Many of these proteins play an important role in Ca^{2+} signaling and homeostasis. Regulator of G protein signaling-4 (RGS4), the protein that inhibits Gq protein-induced release of Ca^{2+} from intracellular stores, is down-regulated in the temporal cortex of schizophrenic patients (*138*). Growth-associated protein 43 (GAP 43) controls Ca^{2+}-CaM signaling absorbing free CaM and preventing it from binding to Ca^{2+}. The increase in the level of this protein in the cerebral cortex and hippocampus of schizophrenic patients has been reported (*139, 140*). It was demonstrated that expression of Ca^{2+}-buffering proteins parvalbumin and calbindin D28k, as well as expression of Bcl-2, was decreased in the cerebral cortex of schizophrenic patients. Bcl-2 reduces the amount of Ca^{2+} in intracellular stores, decreasing the release of Ca^{2+} in response to physiological or pathological stimuli (reviewed in *141*). Indeed, decreased level of Bcl-2 protein may signal neuronal vulnerability to proapoptotic stimuli and to neuronal atrophy (*142*).

An increase in the levels of calcyon, the protein which allows D1 receptors to affect Ca^{2+} signaling, and NCS-1, the Ca^{2+}-binding protein inhibiting desensitization of D2 receptors, suggests relationship between Ca^{2+} signaling and dopamine receptors involvement in pathophysiology of schizophrenia (*137, 143*).

Clinical and experimental evidence have shown that NMDARs and some of the intracellular proteins interacting with NMDARs appeared to be dysregulated in schizophrenia (reviewed in *144*). It was postulated that hypofunction and deficit in NMDA receptor mediated neurotransmission is involved in the development of schizophrenia symptoms (reviewed in *145*). Reduction in the number of neuronal cells observed in cortical and subcortical regions of some schizophrenic patients may also be a result of disturbed Ca^{2+} homeostasis (*146, 147*).

Ca^{2+} Dyshomeostasis in Rapid Neurodegeneration

Traumatic Brain Injury. Traumatic Brain Injury is a leading cause of death and disability worldwide (reviewed in *148*). TBI is prevalent in car accidents victims, although falls and assaults also contribute to a large number of traumatically injured patients. The main mechanisms of brain damage after head injury are either due to contact or acceleration/deceleration types of injury (reviewed in *149*). Contact injury usually results in focal brain damage, whereas acceleration/deceleration injuries lead to diffuse brain damage characterized by widespread axons damage, ischemic brain injury and diffuse brain swelling (*150*).

The pathophysiology of TBI consists of two main phases, a primary mechanical phase, which manifests itself shortly after injury and secondary delayed damage that, although initiated at the time of insult, is not detectable for hours or even days after injury (*149*). It has been demonstrated, that ~90% of patients who died showed ischemic damage on histopathological examination of brain tissue and ischemia was suggested as one of the most important mechanisms of secondary brain damage in TBI (*151*). Ischemia causes disturbances in neurotransmission and energy-dependent processes. The extracellular level of glutamate is dramatically increased after TBI resulting in over-stimulation of excitatory amino acids receptors and excessive Ca^{2+} influx into neurons (*149*). This leads to the severe disturbances in Ca^{2+} homeostasis in neurons described earlier in this paper, and eventually results in cell injury and death. It was demonstrated that traumatic deformation of axons induces abnormal sodium influx through mechanically sensitive Na^+ channels, which subsequently triggers an increase in intra-axonal Ca^{2+} via the opening of VOCCs and reversal of the Na^+/Ca^{2+} exchanger (*152*).

Brain Stroke. Brain stroke is also considered as one of the main causes of death and disabilities in the contemporary world. Brain stroke can be either ischemic or hemorrhagic in nature and in both cases the cerebral blood flow is disrupted. Immediately after blocking of blood flow to brain tissue, a complex series of events is initiated, which ultimately leads to the death of brain cells within the ischemic area. Ischemia-evoked neuronal release of glutamate activates pre and postsynaptic glutamate receptors resulting in triggering of excitotoxic mechanisms cascade (reviewed in *35*). Excessive activation of Ca^{2+} permeable NMDA receptors and an increased number of AMPARs raise intracellular Ca^{2+} concentration (*153*). The energy failure caused by blood flow induces an accumulation of intracellular free Ca^{2+} not only by enhancing its entry and release from intracellular stores but also by interfering with ATP-dependent extrusion and sequestration of Ca^{2+}. A prolonged elevation of intracellular Ca^{2+} leads to the activation of Ca^{2+}-dependent enzymes, dysfunction of mitochondria and activation of the prooxidants pathways (reviewed in *154*). Neuronal death by either necrosis or apoptosis is the common result of brain ischemia.

HIV Dementia. HIV dementia is the effect of human immunodeficiency virus type-1 (HIV-1) infection that is the commonest cause of dementia observed in patients under the age of 40. The disease often progresses rapidly over a period of 6 months and may result in even 50% loss of cortical neurons and RGC which is often accompanied by a loss in the complexity of dendritic arborization (reviewed in *155, 156*). Before the combination antiretroviral therapy was available, HIV dementia was noted in nearly 20% of patients with AIDS. In the brain HIV-1 infects mainly perivascular macrophages, resident microglia and some astrocytes (*156*). Neurons themselves are rarely infected, so the observed prominent dendritic pruning, loss of synapses and cell death are probably caused by indirect actions of the viral gp120 and Tat proteins, both identified as neurotoxins. NMDARs play an important role in the neurotoxicity of both gp120 and Tat, but it is believed that their neurotoxicity is a secondary to glutamate release from glial cells rather than a direct effect. However, one of the properties of gp120 is the ability to modify NMDARs kinetics, which results in neuronal Ca^{2+} overload and cellular destruction or death by Ca^{2+} triggered mechanisms (*155*). The Tat protein, via pertussis toxin-sensitive phospholipase C activity, induces Ca^{2+} release from IP3-sensitive intracellular stores, which is followed by glutamate receptor-mediated Ca^{2+} influx (*157*). It was recently shown, that Tat may potentiate glutamate toxicity by phosphorylation of the NMDA receptor subunits NR2A and NR2B (*158*).

COMPONENTS OF Ca^{2+} HOMEOSTASIS AS THERAPEUTIC TARGETS

Concluding from the findings gathered in this paper, we can state that stabilization of Ca^{2+} homeostasis may be a potential therapeutic target capable of attenuating or preventing neuronal degeneration in acute or chronic neuronal pathologies. The potential drug should meet several criteria such as specificity

for the disease target in the broad range of Ca^{2+} homeostasis mechanisms, reach the target at a neuroprotective concentration determined in laboratory models and display neuroprotective activity in clinical trials (reviewed in *117*). Although many different drugs that affect various aspects of Ca^{2+} homeostasis in neuronal cells demonstrated efficacy in animal models, very few have been successful in clinical trials (reviewed in *34*). Until now, the only compounds qualified for clinical trials are those acting on either glutamate receptors or VOCCs. We present below several of the drugs tested in recent years.

Glutamate Receptors

Memantine is the NMDAR antagonist, binding to the ion channel site and is the most promising of presently known NMDAR antagonists accepted for clinical trials. Clinical studies showed that treatment with memantine offers beneficial effects in patients with moderately severe to severe AD in terms of functional and global measurements (reviewed in *159*). Memantine also shows positive effects in open-angle glaucoma and is currently undergoing a 5-year, prospective, phase III clinical trial (reviewed in *117*). It appeared that memantine inhibits Tat and gp120-evoked Ca^{2+} changes in neurons and protects neural cells from death. A clinical trial using this drug is currently under way in patients with HIV dementia (reviewed in *156*, 160). Inhibitors of PI3R and tyrosine kinase Src, the enzyme mediating phosphorylation of NMDAR subunits that attenuate neurotoxicity evoked by viral Tat protein, are also considered as potential therapeutic targets for the treatment of HIV-1 associated dementia (*157*). It was suggested that Ca^{2+} signaling blockers, such as specific inhibitors of NMDARs or metabotropic glutamate receptors 5 and IP3R1, as well as agents promoting the clearance of mutant proteins in the CNS, may also be beneficial for the treatment of some spinocerebellar ataxias subtypes.

EGB 761, an extract of the leaves of the Chinese tree Ginkgo biloba among its many beneficial effects on the whole body also interferes with NMDA receptors and stabilizes Ca^{2+} homeostasis (reviewed in *161*). It was noted that EGB 761 administration improved pre-existing visual field damage in some individuals with normal tension glaucoma .

Excessive activation of ionotropic glutamate receptors is strongly implicated in pathomechanisms of ischemic stroke. However, several recent clinical trials with glutamate antagonists have not been successful in treating ischemic stroke. Both, competitive and non-competitive NMDAR antagonists gave undesirable side effects but also produced worst functional outcome or mortality (reviewed in *162, 163*). On the other hand, gavestine, a selective antagonist at the glycine binding site of the NMDAR seemed at first to be promising in ischemia therapy but recent clinical trials failed to show any beneficial effect (*164*). However, promising clinical results were observed after application of citicoline, the drug that improves glutamatergic transmission by decreasing glutamate release and increasing its uptake by astrocytes (*165*).

The discovery of NMDARs dysfunction in schizophrenia resulted in new propositions of glutamate receptor-related strategies in treatment of this disease. It appeared that combination of antipsychotic drugs and positive modulators of the glycine binding site of the NMDAR such as glycine, D-serine or D-alanine, significantly reduced symptoms in patients with schizophrenia (*166, 167*). Inhibition of glycine reuptake by sarcosine, an endogenous antagonist of glycine transporter 1, which potentiates glycine action on N-methyl-D-aspartate glycine site had beneficial effects on schizophrenia even more effective than direct activation of glycine site by D-serine (*168*).

Riluzole is not exactly a glutamate receptors antagonist, but it is able to act as a glutamate release inhibitor and indirectly blocks glutamate receptors activation. It is the only drug registered for treatment of ALS disorders although it offers only modest benefit (reviewed in *169*). This drug is also currently undergoing industrial trials for Alzheimer's, Parkinson's and Huntington's diseases, stroke and head injury. Lamotrigine, an antiepileptic drug that inhibits glutamate release, is also known to reduce HD chorea symptoms. Three other compounds that undergo phase III for ALS, mecasermin, xaliproden and gabapentin, displayed limited glutamate antagonism activity as well as GABA receptors agonism and interaction with VOCCs. Other glutamate antagonists such as the NMDA channel blocker dextramethrophan, and talampanel - an allosteric inhibitor of AMPARs, are presently qualified for phase I trials in ALS treatment (reviewed in *170*). Drugs commonly used in epilpesy treatment are blockers of voltage dependent sodium channels, blockers of T-type VOCCs, drugs that potentiate the inhibitory effect of GABA, and those that decrease the glutamatergic excitatory transmission.

Voltage Operated Ca^{2+} Channels

Although Ca^{2+} influx through VOCCs plays an important role in some types of neurodegeneration, there are only a few potential therapeutic compounds directed against their activation.

Nimodipine, an antagonist of L-type Ca^{2+} channels has been shown to protect neuronal cells against ischemic damage in the experimental ischemia but in clinical use its vasodilatatory effect was more efficient (reviewed in *171*). However, it was demonstrated that nimodipine protects neurons from gp120 toxicity *in vitro* (*172*) and gave encouraging effects in clinical trial phase I/II for HIV-1 dementia (*173*).

Nimodipine and other L-type Ca^{2+} channel blockers have been shown to improve performance in visual field, color vision and optic disc blood flow in glaucoma patients (reviewed in *174*). Of all the different classes of substances used in glaucoma treatment, two β-blockers, the selective levobetaxol and the non-selective timolol can influence the Na^{+} and Ca^{2+} influx. Osborne et al. (*175*) showed that neuroprotective efficacies of

these two β-blockers are related to their sodium and L-type VOCCs inhibitory effects. Gazulla and Tintore (*176*) suggest that gabapentin and pregabalin, drugs interacting with the P/Q-type VOCCs, might prove beneficial in SCA6, as the ataxia would be expected to improve. Additionally acetazolamide, a carbonic anhydraze inhibitor, temporarily reduced the severity of symptoms in SCA6 (*177*) and episodic ataxia type 2, probably by changing the intracellular pH and transmembraneous potential, thus preventing VOCCs activation (reviewed in *178*).

Electroconvulsive shock used as therapeutic treatment of epilepsy was shown to induce among other genes the T-type Ca^{2+} channel subunit CACNA1G (*179*). Succinimide antileptic drugs are capable of blocking human T-type channels at therapeutically relevant concentrations (*180*).

Intracellular Ca^{2+} Stores

Cellular Ca^{2+}-dysregulation involved in schizophrenia development may be partially inhibited by antipsychotic drugs such as clozapine, fluspirilene, and haloperidol. It was demonstrated that these drugs block IP3-induced Ca^{2+} release from ER (*181*). Moreover, antipsychotic drugs bind to calmodulin and increase its level in the brain (*182*).

The neuroprotective effects of estrogens have been demonstrated in numerous models of acute cerebral ischemia. The therapeutic effect of estrogens was observed in both female and male patients and it appears that the therapeutic window lasts for up to 6 h after ischemic insult (reviewed in *183*). Among many different properties of estrogens, their beneficial effect on the stabilization of mitochondrial mechanisms may play an important role in neuroprotection. Estrogens can stabilize mitochondrial ATP production and prevent cytosolic and mitochondrial Ca^{2+} influx at high levels of excitotoxic stimulation (reviewed in *184*).

FINAL REMARKS

Ca^{2+} homeostasis and Ca^{2+} signaling in neurons affect a large number of neuronal signaling pathways. Therefore it is not surprising that any dysregulations in Ca^{2+} homeostasis lead to significant changes in neuronal functioning. In some cases these dysregulations are temporary and reversible, but they can also become permanent or lead to neuronal degeneration. Neuronal degenerative changes and cell death can occur rapidly as in case of traumatic injury, or slowly as in case of AD or PD. Despite many collected data concerning Ca^{2+} dyshomeostasis in neuronal degeneration, the sequence of pathological events, and the kinetic of degeneration remain incompletely understood. It is not clear, for instance, if in all slowly progressing pathologies, neuronal death is slow, or if there is only a small subset of neurons dying in a particular period of time. The main question that remains to be solved concerns the identification of the particular subset of neurons affected (vulnerability of only a given type of neurons) in a particular type of neuronal degeneration. This information would be pertinent for the development of better focused therapies. Components of neuronal Ca^{2+} homeostasis and signaling have already been identified as therapeutic targets and it is likely that more drugs will be designed to target these processes.

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