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Review

Parameters of calcium homeostasis in normal neuronal ageing

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ABSTRACT

The last decade has witnessed a significant turn in our understanding of the mechanisms responsible for the decline of cognitive functions in aged brain. As has been demonstrated by detailed morphological reassessments, the senescence-related changes in cognition cannot be attributed to a simple decrease in the number of neurons. It is becoming clearer that a major cause of age-induced deterioration of brain capability involves much subtler changes at the level of synapses. These changes are either morphological, i.e. reduction in the number of effective synapses and/or functional alterations, i.e. changes in the efficacy of remaining synapses. Important questions are now raised regarding the mechanisms which mediate these synaptic changes. Clearly, an important candidate is calcium, the cytotoxic role of which is already firmly established. The wealth of evidence collected so far regarding the changes of Ca^{2+} homeostasis in aged neurons shows that the overall duration of cytoplasmic Ca^{2+} signals becomes longer. This is the most consistent result, demonstrated on different preparations and using different techniques. What is not yet clear is the underlying mechanism, as this result could be explained either through an increased Ca^{2+} influx or because of a deficit in the Ca^{2+} buffering/clearance systems. It is conceivable that these prolonged Ca^{2+} signals may exert a local excitotoxic effect, removing preferentially the most active synapses. Uncovering of the role of Ca^{2+} in the synaptic function of the aged brain presents an exciting challenge for all those involved in the neurobiology of the senescent CNS.

Key words: Cognition; synaptic efficiency; excitotoxicity.

INTRODUCTION

Decline of human mental capabilities which accompany ageing is one of the major concerns of modern gerontology. Indeed, the increase of longevity occurring in the developed world has greatly increased the number of old people and the attendant social pressures of an ageing population. Compared with the advanced treatment of age-associated somatic abnormalities, which has contributed significantly to the overall increase of longevity, our achievements in coping with age-associated cognitive decline look pale. Moreover, we have to admit that the mechanisms of CNS ageing remain far from being understood.

Amazingly a decade ago the situation appeared much better. The model of neuronal ageing that was generally accepted was simple: neuronal ageing was believed to be a result of neurodegeneration; i.e. old brain was believed to contain fewer neurons. Severe neuronal loss (e.g. in Alzheimer's disease, AD) resulted in debilitating dementia, less pronounced neurodegeneration resulted in milder forms of mental decline. The theory was well advanced and fairly logical. Numerous morphological studies almost unequivocally demonstrated age-associated decreases in the number of neurons in various regions of the brain, even in cases when senile plaques (the hallmark of AD) were absent. Everything changed however, when several morphological studies performed recently, using more advanced stereological methods

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(West et al. 1994; Gomez-Isla et al. 1996) demonstrated that decrease in neuronal numbers does not accompany normal ageing of the brain—only when brain is affected by AD or other neurodegenerations does the neuronal loss become detectable.

These anatomical studies thus forced a revision of the old paradigm. Inevitably we have to admit the existence of 2 distinct versions of age-associated mental decline: pathological (neurodegeneration) associated with neuronal loss and physiological (functional abnormalities) with mild mental decline unassociated with neuronal loss but possibly related to synaptic alterations. While the former is well investigated, the latter remains mostly unexplored.

In the present paper we will overview one of the hypothesis of neuronal ageing the so-called 'Ca hypothesis', trying to show that age-dependent changes in calcium homeostasis may indeed be one of the reasons for mental decline in senescence.

CALCIUM AND NEURONAL FUNCTION

The intracellular calcium signalling system is involved in the regulation of a wide range of physiological reactions (Verkhratsky & Toescu, 1998*b*). This ancient system, which developed very early in evolution, utilises the tremendous transmembrane gradient for Ca^{2+} ions which by far exceeds transmembrane gradients for all the other physiologically relevant ions. Therefore, even tiny changes of membrane Ca^{2+} permeability result in huge changes in cytoplasmic Ca^{2+} concentration, providing a signalling system with an intrinsic high signal-to-noise ratio. Various intracellular enzymes bind Ca^{2+} ions with different affinities, determining the amplitude coding of the signalling system. These enzymes act as effectors of the signalling system, as on Ca^{2+} binding they change their catalytic activity. In the nervous system the best example for the role of Ca^{2+} ions is synaptic transmission. Calcium, entering presynaptic terminals following the depolarisation caused by the arrival of the action potential, triggers the fusion of the neurotransmitter vesicles with plasmalemma, thus allowing the chemical transduction of nervous impulse. Nevertheless, this calcium entry could be toxic and, in certain conditions, neurons overloaded with Ca^{2+} die, a process referred to as excitotoxicity (Choi, 1994; Toescu, 1998*a*). Obviously these are not the only roles of Ca^{2+} in nerve cells, and changes in $[Ca^{2+}]_i$ regulate many other neuronal functions (e.g. excitability, metabolism and gene expression (Ghosh & Greenberg, 1995), but these 2 faces of Ca^{2+} action are important for our further discussion.

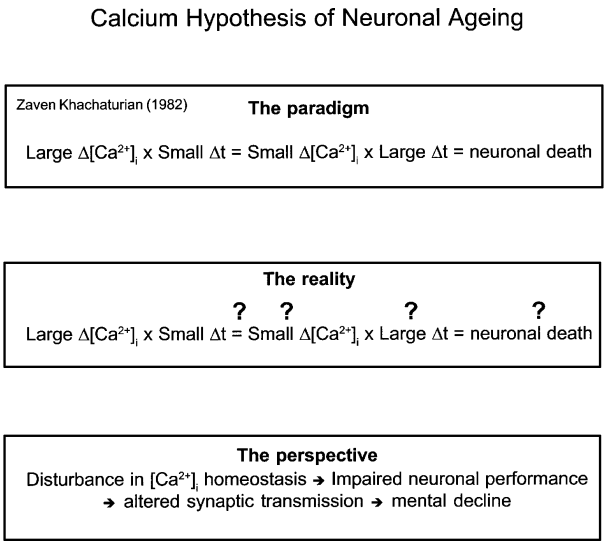

Fig. 1. Calcium hypothesis of ageing: initial sequence, problems and perspectives.

CALCIUM HYPOTHESIS OF AGEING

The calcium hypothesis of ageing was promulgated in 1982 by Zaven Khachaturian (Khachaturian et al. 1989; Khachaturian, 1991, 1994) and was based solely on the toxic effects of excess Ca^{2+} on nerve cells. The core idea of the hypothesis was simple: it postulated that the underperformance of Ca^{2+} homeostatic systems in aged neurons causes chronic $[Ca^{2+}]_i$ elevation which eventually results in neuronal death. In other words the hypothesis assumed that long-lasting small elevations of $[Ca^{2+}]_i$ may cause the same neurotoxic effects as rapid and large $[Ca^{2+}]_i$ increase. In the end, this chronic $[Ca^{2+}]_i$ dysregulation results, according to the calcium hypothesis, in neuronal death.

In fact, neither of the assumptions of the calcium hypothesis has been established by experiments (Fig. 1). First, we do not have evidence that long but small $[Ca^{2+}]_i$ increases have the same effects as large but short $[Ca^{2+}]_i$ elevations. Second, we know now that physiological neuronal ageing is not necessarily accompanied by neuronal demise. Nevertheless, the calcium hypothesis of ageing, which appeared when neuronal calcium studies were in an inchoate state, stimulated experiments aimed at investigating the age-dependent changes in calcium homeostasis.

$[Ca^{2+}]_i$ REGULATION IN AGED NEURONS

As we have already mentioned, cellular $[Ca^{2+}]_i$ homeostasis is regulated by numerous molecular cascades which balance Ca^{2+} fluxes through extracellular and intracellular membranes (Ca^{2+} channels

and transporters) and cytoplasmic Ca^{2+} buffering (Ca^{2+} binding proteins). The precise mechanisms of $[Ca^{2+}]_i$ homeostasis are the subject of many reviews which give a detailed image of Ca^{2+} regulation in various cell types, including nerve cells (e.g. Baimbridge et al. 1992; Pozzan et al. 1994; Verkhratsky & Shmigol, 1996; Ichas & Mazat, 1998; Verkhratsky & Petersen, 1998). It appears that in aged neurons several Ca^{2+} homeostatic systems are affected. Moreover, it seems that their impairment could be very different in different regions of the nervous system.

Before turning to a detailed account of the state of the art of $[Ca^{2+}]_i$ regulation in aged neurons an important part of experimental design, namely the validity of experimental preparation merits discussion. Direct measurements of $[Ca^{2+}]_i$ could now be considered as a routine physiological technique. A wealth of Ca^{2+}-sensitive intracellular probes are supported by advanced microfluorimetric techniques, which allow $[Ca^{2+}]_i$ recordings in a limited ($\sim$ 1 μm^3) intracellular compartments in a millisecond time range (Toescu & Verkhratsky, 1999). Almost any type of cell could be examined by these methods. Experimental preparations used for $[Ca^{2+}]_i$ recordings vary between subcellular particles (microsomes, isolated mitochondria, etc.), acutely isolated or cultured cells and cells in situ in manifold tissue preparations (acutely prepared brain slices being the most popular example). Naturally each has advantages and disadvantages, and each of these preparations was used in studying $[Ca^{2+}]_i$ homeostasis in aged nervous tissue. The results, as expected, were controversial (Verkhratsky & Toescu, 1998*a*), the controversies being determined, to a large extent, by the particular properties of the cellular and subcellular preparations used. Old brain tissue presents a special challenge for cell isolation/culturing, and only recently has a certain success been achieved (Cowen et al. 1997). The preparation of choice for studying the age-dependent changes of $[Ca^{2+}]_i$ homeostasis is the acutely isolated brain slice (see Thibault et al. 1998 and Verkhratsky & Toescu, 1998*a* for extended discussion). Very recently, another preparation has been proposed, somewhat surprisingly, as a possible experimental model, the long-term (3–4 wk) primary culture of embryonic or perinatal neurons (Thibault et al. 1998; Toescu & Verkhratsky, unpublished). A careful and detailed analysis of the Ca^{2+} currents expressed by the hippocampal neurons derived from late embryonic animals and maintained long-term in culture showed that the L-type Ca^{2+} channels are expressed, over the 3–4 wk of maintenance in culture, in a manner which

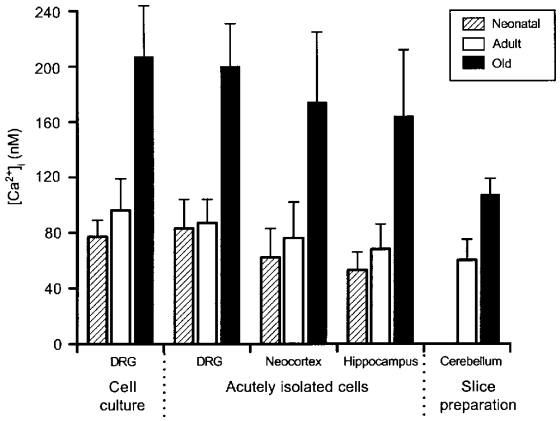

Fig. 2. Age-dependent changes in resting $[Ca^{2+}]_i$ in different types of neurons studied in different preparations. Data taken from (Kirischuk et al. 1992, 1996; Verkhratsky et al. 1994).

was strikingly similar to that observed in the ageing animals (Thibault et al. 1998). In a similar fashion, the cerebellar granule neurons, which in brain slice preparations show a decrease of the amplitude of Ca^{2+} signal and an increase in the time of recovery following a Ca^{2+} signal (Kirischuk & Verkhratsky, 1996 and see below), display the same type of changes in the pattern of Ca^{2+} response when maintained in long-term (3 wk) cultures (Toescu & Verkhratsky, unpublished). While, obviously, the long-term primary cultures cannot be used as a direct substitute for the changes taking place during ageing, these initial observations might indicate that these time-dependent changes in Ca^{2+} homeostasis in cultured neurons could be a useful model for studying at least some functional implications and sequencing of the processes normally associated with ageing.

Ultimately, however, the best experimental model would allow the possibility of measuring $[Ca^{2+}]_i$ in neurons from undamaged brain, in situ, and recent advances in multiphoton confocal microscopy (Svoboda et al. 1997) could offer such a possibility in the near future. Be that it as it may, we will now present a brief account on age-dependent changes of $[Ca^{2+}]_i$ regulation in old nerve cells.

Resting calcium in aged neurons

The data on the resting calcium concentration in old nerve cells are quite limited. The $[Ca^{2+}]_i$ measurements on synaptosomes showed that resting $[Ca^{2+}]_i$ was somewhat increased in old preparations (Martinez et al. 1988; Giovannelli & Pepeu, 1989). The $[Ca^{2+}]_i$ recordings performed both on cultured central neurons and on neurons in acutely prepared cerebellar slices demonstrated a consistent increase in resting $[Ca^{2+}]_i$ in aged nerve cells (Kirischuk et al. 1992, 1996;

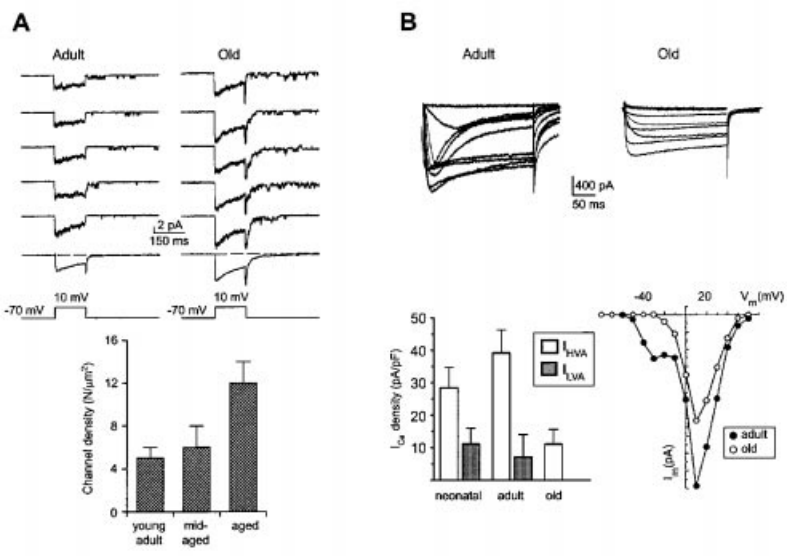

Fig. 3. Heterogeneity of age-dependent changes of voltage-operated calcium currents in different regions of the nervous system. (*A*) Age-dependent increase in calcium current density in hippocmapal neurons (from Thibault & Landfield, 1996). (*B*) Age-dependent decrease in calcium current density in dorsal root ganglion neurons (from Kostyuk et al. 1993).

Verkhratsky et al. 1994; Fig. 2.) Nevertheless, it is still impossible to unequivocally consider that ageing is associated with increase in neuronal resting $[Ca^{2+}]_i$ in all brain regions. Moreover, an experimentally observed age-dependent increase in resting $[Ca^{2+}]_i$ was much more pronounced in single cell preparations as compared with acute slices (Fig. 3). The final conclusion on this matter needs substantially more experimental evidence.

Plasmalemmal calcium channels

Calcium permeable channels present in the neuronal plasma membrane are the main route for Ca^{2+} entry during neuronal excitation. These channels fall into 2 major classes (Hofmann et al. 1994; Hollmann & Heinemann, 1994; Catterall, 1998), voltage-gated Ca^{2+} channels (VOCCs) and agonist-gated Ca^{2+} channels (ionotropic neurotransmitter receptors). Ageing does not affect the molecular physiology of VOCCs: their elementary biophysical properties remain unchanged through the life (Kostyuk et al. 1993; Thibault & Landfield, 1996; Thibault et al. 1998). Their density, however, is affected in old nerve cells and, even more strikingly, the number of VOCCs in the plasmalemma is altered differently in different regions of the nervous system (Fig. 4). In hippocampal neurons, ageing leads to a substantial (2–3 fold) increase in L-type calcium channel density, increasing Ca^{2+} entry accordingly (Landfield & Pitler, 1984; Thibault & Landfield, 1996; Thibault et al. 1998). In basal forebrain neurons, ageing does not affect significantly the density of L-type VOCCs, but increases the number of T-type channels (Murchison & Griffith, 1995, 1996). Conversely, in the peripheral nervous system, in sensory neurons the T-channels disappear completely and the amount of L-type VOCCs in cellular membrane undergoes a dramatic (3-fold) decrease, thus limiting considerably depolarisation-induced Ca^{2+} entry (Kostyuk et al. 1993). We still lack more extended mapping of the nervous system in respect to age-associated changes of VOCCs expression, although we may expect that heterogeneity could be very substantial.

The electrophysiology of ionotropic neurotransmitter receptors in aged neurons is almost completely unexplored, although there are some indirect data indicating that even their molecular composition could be affected by ageing. Hippocampal neurons in old animals may express glutamate receptors lacking a GluR-B subunit (Pagliusi et al. 1994), thus increasing the overall Ca^{2+} permeability of glutamate ionotropic receptors.

Intracellular Ca^{2+} channels

The second important route for Ca^{2+}appearance in the cytoplasm is associated with intracellular Ca^{2+}-permeable channels incorporated in the membrane of endoplasmic reticulum calcium stores (see Furuichi & Mikoshiba, 1995; Verkhratsky & Shmigol, 1996). These intracellular Ca^{2+} channels are represented by 2 subfamilies of ligand-gated channels, that is by Ca^{2+}-gated channels (commonly known as ryanodine receptors) and $InsP_3$-gated channels dubbed $InsP_3$ receptors. Being activated either by cytosolic Ca^{2+}

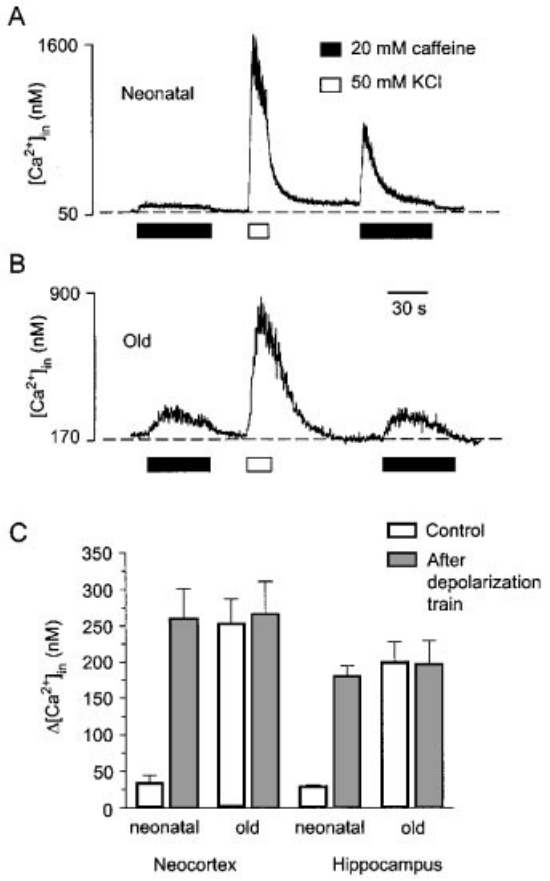

Fig. 4. Age-dependent changes in the status of intracellular calcium stores (from Verkhratsky et al. 1994). To activate Ca^{2+}-induced Ca^{2+} release (CICR) 20 mM caffeine was applied to isolated central neurons obtained from neonatal and old rats. Caffeine-induced $[Ca^{2+}]_i$ transients were recorded before and after charging depolarisation in neocortical neonatal (*A*) and old (*B*) neurons. In C the mean amplitudes of initial caffeine-triggered $[Ca^{2+}]_i$ transients and the same amplitudes after cell depolarisation are summarised for neocortical and hippocampal neurons obtained from neonatal and old rats. Note that in old neurons depolarisation-induced Ca^{2+} entry does not increase the releasable Ca^{2+} content within the calcium store.

(ryanodine receptors) or by the increase in cytoplasmic $InsP_3$ ($InsP_3$ receptors; synthesis of $InsP_3$ is controlled by numerous plasmalemmal metabotropic receptors) they both trigger Ca^{2+} release from the stores, which may result in substantial intracellular $[Ca^{2+}]_i$ gradients. The age-dependent fate of intracellular channels is largely unknown. Certain biochemical experiments (Igwe & Ning, 1993; Martini et al. 1994) suggest that their density could be affected in old nerve cells, although the physiological correlate for these changes has not yet been found.

Calcium clearance

Besides the amount of Ca^{2+} entry, the amplitude and the temporal characteristics of the $[Ca^{2+}]_i$ signal are determined by cytoplasmic Ca^{2+} binding (Baimbridge et al. 1992) and Ca^{2+} removal from the cytoplasm either into intracellular Ca^{2+} stores (endoplasmic reticulum and mitochondria) or into the extracellular space due to the activity of plasmalemmal Ca^{2+} pumps and/or exchangers. The activity of these systems mainly determines the shape and length of the $[Ca^{2+}]_i$ signal recovery. Indeed, in neurons, the period of Ca^{2+} entry is limited in time, occurring mostly during the action potential, and in space, being confined, at least in the early stages of the response, to the restricted postsynaptic densities, with the highest densities of receptors which have Ca^{2+} permeability. Thus the periods of effective Ca^{2+} entry are constrained to several milliseconds. Similarly, cytoplasmic Ca^{2+} buffering occurs very rapidly, limiting mostly the peak amplitude of $[Ca^{2+}]_i$ elevation. Therefore, Ca^{2+} recovery (or in other words, periods of elevated $[Ca^{2+}]_i$) after elementary neuronal excitation is solely determined by the performance of Ca^{2+}extrusion/accumulation systems.

Ageing of the nervous system affects both clearance processes: the Ca^{2+} buffers and the Ca^{2+} removal systems. Overall the Ca^{2+} buffering capacity of nerve cell bodies and their processes decreases during ageing (Villa et al. 1994; Duckles et al. 1996). This means, in essence, that old neurons are exposed to a higher $[Ca^{2+}]_i$ elevation even if Ca^{2+} entry remains unchanged. If the latter increases, as in the case of the hippocampal neurons, the fall in buffer capacity would further multiply effects of increased Ca^{2+} entry, thus exposing the aged hippocampal neurons to a very high magnitude of $[Ca^{2+}]_i$ elevation.

Calcium removal systems are also affected in aged neurons and this effect is best demonstrated by the large increase in the time taken for the neurons to recover following normal stimulation. Using acutely isolated cells and cerebellar granule neurons from brain slices, both obtained from aged animals, it has been shown that, despite the fact that the amplitude of the Ca^{2+} signal evoked by KCl depolarisation (50 mM) was diminished, the time taken to recover the resting $[Ca^{2+}]_i$ levels was significantly increased (Kirischuk et al. 1992; Verkhratsky et al. 1994; Kirischuk & Verkhratsky, 1996). In adult central neurons (neocortex), the half-time for recovery towards resting $[Ca^{2+}]_i$ was 10 ± 1 s (mean $\pm$ S.E.M.), whereas this value increased to 27 ± 3 s in the aged neurons. Similarly, for peripheral neurons (DRG neurons), the value for the half-time recovery increased from 25 ± 3 s to 43 ± 5 s (adult vs aged neurons, respectively). Furthermore, in experiments performed in brain slices, it was found that larger Ca^{2+} loads, as evoked by longer depolarisation of the neurons and from which adult

neurons invariably recover, frequently resulted, in aged slices, in irreversible increases in $[Ca^{2+}]_i$.

A principal candidate for explaining these experimental observations is the activity of the plasma membrane Ca^{2+} ATPase (PMCA), which is ultimately responsible for the final removal of Ca^{2+} from the neurons. Indeed, detailed biochemical characterisation of the PMCA showed that, even under optimal conditions of saturating Ca^{2+} and ATP, neuronal Ca^{2+} ATPase activity decreases with age (Michaelis et al. 1996). From this work it appears that an important reason for this decreased effectiveness of the ATPase activity is the development of age-dependent structural modifications within the primary sequence of calmodulin, a Ca^{2+}-binding protein, which mediates many of the metabolic effects of Ca^{2+}, including the activation of the cellular Ca^{2+}-dependent ATPases. These changes in calmodulin primary structure could be reproduced in younger animals by the judicious use of a range of reactive oxygen species, supporting the view that the continuous production, even at low levels, of free radicals is an important mechanism in the expression of the age-related changes.

The other Ca^{2+} removal system which participates in the clearing of the Ca^{2+} load following neuronal stimulation is the accumulation of Ca^{2+} into the internal stores (endoplasmic reticulum), a process mediated by a different type of ATPase, the sarco-(endo)plasmic reticulum Ca^{2+} ATPase (SERCA). These stores can participate as a 'Ca^{2+} sink' during the initial phases of the Ca^{2+} response (Toescu, 1998*b*), and the amount of Ca^{2+} loaded into these stores can be assessed experimentally by exposure of neurons to caffeine (Verkhratsky et al. 1994). When adult neocortical neurons are challenged with caffeine following stimulation, they respond with a very large cytosolic Ca^{2+} signal, indicating significant loading of the internal stores. In contrast, when the same protocol is applied to the aged neurons, caffeine fails to reveal a store loading and thus points to deficiencies in the activity of the SERCA pump (Fig. 4). To what extend these deficiencies are related to the structural changes in calmodulin conformation mentioned above is not yet clear.

Finally, another important system which contributes to the removal of Ca^{2+} from the cytosol, is the mitochondria. To date, relatively little is known about the activity of mitochondria in aged intact neurons. The only available information is derived from studies on synaptosomes (Satrustegui et al. 1996), and these have shown an impairment in the activity of the mitochondrial Ca^{2+} uniporter, which is the main system of mitochondrial Ca^{2+} uptake and is driven by the significant membrane potential difference between the mitochondrial matrix and cytosol (> 150 mV).

A MODEL OF NEURONAL AGEING: Ca-DEPENDENT DECLINE AND FALL OF SYNAPSES

Can these changes in cytoplasmic calcium homeostasis result in functional abnormalities relevant to neuronal ageing? The obvious point at which disruption of $[Ca^{2+}]_i$ handling may significantly deteriorate the information processing in the brain is the synapse. Indeed, as we have mentioned above, the synaptic transmission is heavily Ca^{2+} dependent. The age-dependent decrease of $[Ca^{2+}]_i$ recovery following neuronal excitation would promote Ca^{2+} accumulation in the presynaptic terminal, which in turn may affect the efficacy of synaptic transmission. Moreover, the chronic increase in $[Ca^{2+}]_i$ in the limited volume of a presynaptic terminal may trigger a local excitotoxic effect, leading to the elimination of the most active synapses. Certain, although faint, hints exist showing that both synaptic morphology and number of active synapses are affected in old brain (Geinisman et al. 1995). Similarly, synaptic plasticity may be also attenuated in aged animals (Barnes, 1994). These synaptic malfunctions could result in a rewiring of neuronal circuits in an old CNS and may play a key role in the age-dependent decline of mental capabilities. Further investigation of the link between intracellular calcium and synaptic efficacy in the old brain could open a new horizons both for understanding brain ageing and for appropriate treatment of senescence-associated brain failure.

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REFERENCES

BAIMBRIDGE KG, CELIO MR, ROGERS JH (1992) Calcium-binding proteins in the nervous system. *Trends in Neuroscience* **15**, 303–308.

BARNES CA (1994) Normal aging: regionally specific changes in hippocampal synaptic transmission. *Trends in Neuroscience* **17**, 13–18.

CATTERALL WA (1998) Structure and function of neuronal Ca^{2+} channels and their role in neurotransmitter release. *Cell Calcium* **24**, 307–323.

CHOI DW (1994) Calcium and excitotoxic neuronal injury. *Annals of the New York Academy of Sciences* **747**, 162–171.

COWEN T, JENNER C, SONG GX, SANTOSO AW, GAVAZZI I (1997) Responses of mature and aged sympathetic neurons to laminin and NGF: an in vitro study. *Neurochemistry Research* **8**, 1003–1111.

DUCKLES SP, TSAI H, BUCHHOLZ JN (1996) Evidence for decline in intracellular calcium buffering in adrenergic nerves of aged rats. *Life Science* **58**, 2029–2035.

FURUICHI T, MIKOSHIBA K (1995) Inositol 1,4,5-trisphosphate receptor-mediated Ca^{2+} signaling in the brain. *Journal of Neurochemistry* **64**, 953–960.

GEINISMAN Y, DETOLEDO-MORRELL L, MORRELL F, HELLER RE (1995) Hippocampal markers of age-related memory dysfunction: behavioral, electrophysiological and morphological perspectives. *Progress in Neurobiology* **45**, 223–252.

GHOSH A, GREENBERG ME (1995) Calcium signaling in neurons: molecular mechanisms and cellular consequences. *Science* **268**, 239–247.

GIOVANNELLI L, PEPEU G (1989) Effect of age on K^+-induced cytosolic Ca^{2+} changes in rat cortical synaptosomes. *Journal of Neurochemistry* **53**, 392–398.

GOMEZ-ISLA T, PRICE JL, McKELL DW, JR , MORRIS JC, GROWDON JH, HYMAN BT (1996) Profound loss of layer II entorhinal cortex neurones occurs in very mild Alzheimer's disease. *Journal of Neuroscience* **16**, 4491–4500.

HOFMANN F, BIEL M, FLOCKERZI V (1994) Molecular basis for Ca^{2+} channel diversity. *Annual Reviews in Neuroscience* **17**, 399–418.

HOLLMANN M, HEINEMANN S (1994) Cloned glutamate receptors. *Annual Reviews in Neuroscience* **17**, 31–108.

ICHAS F, MAZAT JP (1998) From calcium signaling to cell death: two conformations for the mitochondrial permeability transition pore. Switching from low- to high-conductance state. *Biochemica Biophysica Acta* **1366**, 33–50.

IGWE OJ, NING L (1993) Inositol 1,4,5-trisphosphate arm of the phosphatidylinositide signal transduction pathway in the rat cerebellum during aging. *Neuroscience Letters* **164**, 167–170.

KHACHATURIAN ZS (1991) Calcium and the aging brain: upsetting a delicate balance? *Geriatrics* **46**, 78–79.

KHACHATURIAN ZS (1994) Calcium hypothesis of Alzheimer's disease and brain aging. *Annals of the New York Academy of Sciences* **747**, 1–11.

KHACHATURIAN ZS, COTMAN CW, PETTEGREW W (1989) Calcium, membranes, aging, and Alzheimer's disease. *Annals of the New York Academy of Sciences* **568**, 1–292.

KIRISCHUK S, PRONCHUK N, VERKHRATSKY A (1992) Measurements of intracellular calcium in sensory neurons of adult and old rats. *Neuroscience* **50**, 947–951.

KIRISCHUK S, VERKHRATSKY A (1996) Calcium homeostasis in aged neurones. *Life Science* **59**, 451–459.

KIRISCHUK S, VOITENKO N, KOSTYUK P, VERKHRATSKY A (1996) Age associated changes of cytoplasmic calcium homeostasis in cerebellar granule neurons in situ: investigation on thin cerebellar slices. *Experimental Gerontology* **31**, 475–487.

KOSTYUK P, PRONCHUK N, SAVCHENKO A, VERKHRATSKY A (1993) Calcium currents in aged rat dorsal root ganglion neurones. *Journal of Physiology*, *London* **461**, 467–483.

LANDFIELD PW, PITLER TA (1984) Prolonged Ca^{2+}-dependent afterhyperpolarizations in hippocampal neurones of aged rats. *Science* **226**, 1089–1092.

MARTINEZ A, VITORICA J, SATRUSTEGUI J (1988) Cytosolic free calcium levels increase with age in rat brain synaptosomes. *Neuroscience Letters* **88**, 336–342.

MARTINI A, BATTAINI F, GOVONI S, VOLPE P (1994) Inositol 1,4,5-trisphosphate receptor and ryanodine receptor in the aging brain of Wistar rats. *Neurobiology of Aging* **15**, 203–206.

MICHAELIS ML, BIGELOW DJ, SCHONEICH C, WILLIAMS TD, RAMONDA L, YIN D et al. (1996) Decreased plasma membrane calcium transport activity in aging brain. *Life Science* **59**, 405–412.

MURCHISON D, GRIFFITH WH (1995) Low-voltage activated calcium currents increase in basal forebrain neurones from aged rats. *Journal of Neurophysiology* **74**, 876–887.

MURCHISON D, GRIFFITH WH (1996) High-voltage-activated calcium currents in basal forebrain neurons during aging. *Journal of Neurophysiology* **76**, 158–174.

PAGLIUSI SR, GERRARD P, ABDALLAH M, TALABOT D, CATSICAS S (1994) Age-related changes in expression of AMPA-selective glutamate receptor subunits: is calcium-permeability altered in hippocampal neurons? *Neuroscience* **61**, 429–433.

POZZAN T, RIZZUTO R, VOLPE P, MELDOLESI J (1994) Molecular and cellular physiology of intracellular calcium stores. *Physiological Reviews* **74**, 595–636.

SATRUSTEGUI J, VILLALBA M, PEREIRA R, BOGONEZ E, MARTINEZ SERRANO A (1996) Cytosolic and mitochondrial calcium in synaptosomes during aging. *Life Science* **59**, 429–434.

SVOBODA K, DENK W, KLEINFELD D, TANK DW (1997) In vivo dendritic calcium dynamics in neocortical pyramidal neurons. *Nature* **385**, 161–165.

THIBAULT O, LANDFIELD PW (1996) Increase in single L-type calcium channels in hippocampal neurons during aging. *Science* **272**, 1017–1020.

THIBAULT O, PORTER NM, CHEN KC, BLALOCK EM, KAMINKER PG, CLODFELTER GV et al. (1998) Calcium dysregulation in neuronal aging and Alzheimer's disease: history and new directions. *Cell Calcium* **24**, 417–433.

TOESCU EC (1998*a*) Apoptosis and cell death in neuronal cells: where does calcium fit in? *Cell Calcium* **24**, 387–403.

TOESCU EC (1998*b*) Intraneuronal Ca^{2+} stores act mainly as a 'Ca^{2+} sink' in cerebellar granule neurones. *Neuroreport* **9**, 1227–1231.

TOESCU EC, VERKHRATSKY A (1999) Principles of fluorescence measurements–dyes and hardware required. In *Calcium Measurements* (ed. Petersen OH). Stuttgart: Springer.

VERKHRATSKY A, SHMIGOL A, KIRISCHUK S, PRONCHUK N, KOSTYUK P (1994) Age-dependent changes in calcium currents and calcium homeostasis in mammalian neurons. *Annals of the New York Academy of Sciences* **747**, 365–381.

VERKHRATSKY A, SHMIGOL A (1996) Calcium-induced calcium release in neurones. *Cell Calcium* **19**, 1–14.

VERKHRATSKY A, PETERSEN OH (1998) Neuronal calcium stores. *Cell Calcium* **24**, 333–343.

VERKHRATSKY A, TOESCU EC (1998*a*) Calcium and neuronal ageing. *Trends in Neurosciences* **21**, 2–7.

VERKHRATSKY A, TOESCU EC (ed) (1998*b*) *Integrative Aspects of Calcium Signalling*, p. 428. London: Plenum Press.

VILLA A, PODINI P, PANZERI MC, RACCHETTI G, MELDOLESI J (1994) Cytosolic Ca^{2+} binding proteins during rat brain ageing: loss of calbindin and calretinin in the hippocampus, with no change in the cerebellum. *European Journal of Neuroscience* **6**, 1491–1499.

WEST MJ, COLEMAN PD, FLOOD DG, TRONCOSO JC (1994) Differences in the pattern of hippocampal neuronal loss in normal ageing and Alzheimer's disease. *Lancet* **344**, 769–772.