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THE ALZHEIMER'S PLAQUES, TANGLES AND MEMORY DEFICITS MAY HAVE A COMMON ORIGIN; PART I; A CALCIUM DEFICIT HYPOTHESIS

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1. ABSTRACT

Review of the literature reveals that several biochemical events implicated in the pathology of Alzheimer's disease (AD) are calcium dependent processes. These processes include normal processing of β-amyloid precursor protein, dephosphorylation and degradation of tau, neurotransmitter release and memory formation. Since all of these processes appear to be inactivated during progression of AD, we propose that a "deficit" of intracellular calcium levels may occur in the early phase of the disease. We also propose several experiments to test this hypothesis. The hypothesis predicts that presenilins most likely act as calcium channels *in vivo* and that their gene mutations may cause the disease by diminishing the Ca^{2+} channeling function.

2. INTRODUCTION

Alzheimer's disease (AD) is a cognitive disorder with unknown pathological origin. However, the invariable presence of amyloid plaques and neurofibrillary tangles in most AD patients (1-4) would suggest that the biological processes underlying the cognitive impairments and the formation of the histological lesions may be related. Amyloid plaques are mainly comprised of ß-amyloid protein (Aß), which is derived from ß-amyloid precursor protein (APP). Processing of APP normally occurs within the Aß region by a putative α-secretase thus precluding the formation of Aß. For unknown reasons, APP in AD is excessively cleaved by two other proteases, ß-/γ-secretases, resulting in an overproduction of Aß (1,2).

3. DISCUSSION

3.1. Calcium-dependent α-secretase

Based on an analysis of a number of the reported data, we proposed that α-secretase is a calcium-dependent protease (5). Although some discrepancies on this issue await further clarification, this view may still permit a consideration of AD features from a new perspective. When the abnormality of APP processing is reconsidered, it appears to us that the mechanism of Aß overproduction probably involves an "inactivation" of α-secretase because: (i) the two pathways of APP processing are mutually exclusive (5), thus the increased product of one reaction (Aß in AD) should come from the decrease of the other (α-secretase cleavage)(figure 1); (ii) soluble APP (APP_s, a product of α-secretase) is indeed reduced in the body of AD patients (6); and (iii) such a reduction of APP_s may not be solely due to an "overactivation" of ß-/γ-secretases, since Ca^{2+} signaling should be more sensitive (as such more vulnerable to pathological insults) in regulating the protease than other cellular elements, if they can "regulate" ß-/γ-secretases at all. The inactivation of α-secretase would overcharge the amyloidogenic pathway leading to the overproduction of Aß.

If this consideration is correct, then it should follow that the inactivation of α-secretase, as calcium-dependent, could occur as a result of a "deficit" in the free intracellular calcium in the brain of AD patients. This outcome is unexpected. It is a widely held notion today that the intracellular calcium levels are elevated in AD brain, which in turn are responsible for the neurodegeneration and cell death (7,8). However, calcium levels, if elevated, would be expected to enhance the α-secretase activity and thus would reduce Aß, a situation opposite to that in AD. This conceptual disparity, together with the central role of calcium in brain functions and in AD pathogenesis (7,8), prompted us to examine additional features of AD for indications of calcium states in the disease.

3.2. Neurofibrillary tangles (NFTs)

NFTs contain paired helical filaments (PHFs),

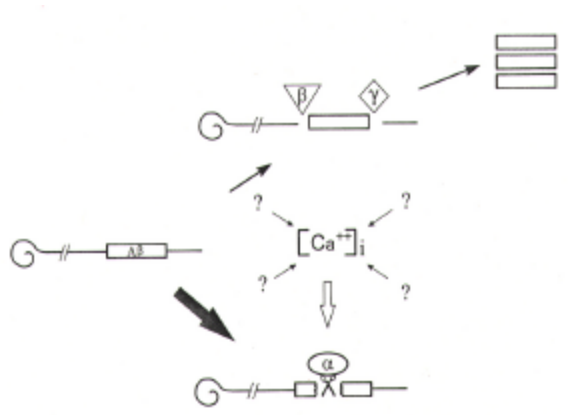

Figure 1. Two pathways of APP processing. APP processing is depicted here emphasizing the central role of calcium homeostasis. Actions of α-secretase (a dominant activity, large arrow), and ß-/γ-secretases (α,ß,γ) are shown. The two pathways appear to operate reciprocally in most, if not all, cells, competing for the same pool of intact APP (5). Whereas APP trafficking and processing are exceedingly complex, the overall process outlined here suggests that an inactivation of α-secretase would lead to an increase of Aß, and α-secretase should be vulnerable to many pathological insults (questions marks) in AD that affect calcium homeostasis or mobilization. The scheme also predicts that elevation of calcium levels by many calcium agonists (estrogen, phorbol esters, etc.) would reduce Aß.

which are mainly composed of phosphorylated tau protein. Trojanowski and Lee (9) and others (3,10) have elegantly demonstrated that abnormal phosphorylation of tau is the primary mechanism underlying the NFT accumulation, and that the apparent hyperphosphorylation of PHF tau is mainly the result of an "inactivation" of protein phosphatases including calcium-dependent calcineurin (PP2B). This favors a calcium deficit in AD as discussed (11).

In addition to the phosphorylation mechanism, tau in vivo undergoes dynamic turnover by proteolytic degradation preferentially by calpain (12), a known calcium-dependent protease (13). Degradation of tau in cells is promoted by calcium mobilization and inhibited by tau phosphorylation (14). These studies altogether indicate that tau is like many other cytoskeletal proteins, whose dynamic turnover through a signal-mediated phosphorylation/ dephosphorylation/ proteolysis scheme is an integral part of the cellular homeostasis, as proposed by several investigators including us (12,13,15-17). If both dephosphorylation and degradation of tau are necessary steps in its turnover, and if both events involve calcium-dependent processes, then the apparent hyperphosphorylation and accumulation of tau would point to a calcium deficit, which would inactivate calcineurin and calpain in the early phase of AD [though in advanced AD patients, calpain can be overactivated (11)].

3.3. Neurotransmission and memory formation

Two more lines of consideration from a broader background seem to further support our view. First, neurotransmitter release and long-term potentiation are both highly calcium-dependent processes (18). The former is quantitatively controlled by the calcium ions entering the presynaptic cells (activation of protein kinase C and Ca^{2+}-kinase II is involved), and the latter depends on the calcium entry into the postsynaptic cells (calpain and calcineurin are involved)(18-20). Although brain synaptic activities are enormously complex, their calcium-dependent nature would nevertheless suggest that a sufficient concentration of calcium is probably essential for maintaining the normal cognitive functions; hence the deficits of such functions in AD would indicate decreased calcium levels in the brain. Second, in addition to synaptic transmission, calcium-dependent processes are essential for such activities as muscle contraction, cell division and growth, and protein synthesis (12,13,18). There is probably a general decline of such activities in aged and AD individuals (18).

3.4. The drug effects

If there is a calcium deficit in the early phase of AD, then it would be anticipated that drugs that can elevate intracellular calcium levels should have protective effects in at-risk individuals. In this regard, several existing drugs have been shown to have such effects. They include estrogen, nicotine, indomethacin and ibuprofen (21-24). Although their mechanisms of action are currently believed to be heterogeneous, it is noteworthy that estrogen and nicotine are known calcium agonists (25,26); indomethacin and ibuprofen have also been shown to induce concentration-dependent calcium rises in cultured cells (27-29). These drugs obviously have many distinct actions in the body, but Ca^{2+} signaling pathway can convert part of those actions into the same downstream calcium-dependent processes. It thus seems likely that these four drugs might exert their protective actions partly through a common effect, but the protective actions would not be expected if the calcium levels in at-risk individuals were already in excess [though controversies exist, i.e., nimodipine (30); see below].

3.5. The basis of the current hypothesis

The current calcium elevation hypothesis of AD is partly based on the experimental results that Aß at supraphysiological doses can induce calcium rises, and that calpain is overactivated in postmortem AD brain (7,31). We believe that these results demonstrate calcium rises at late stage or endpoint of the disease (i.e., as a result of the Aβ and tau accumulation), and they may not represent an "early defect" that triggers the accumulation of Aß and tau, as discussed (11). It may be necessary to consider the early and late phases separately in order to distinguish an early defect from its end results.

Also, it has been observed that cells from AD subjects respond more sensitively to exogenous calcium agonists such as glutamate (7,8). However, this effect is observed only when AD cells are treated with equal amounts of the agonists compared to the control cells. Such presumed "equal amounts" of the agonists may not reflect the conditions in AD, since many calcium agonists are severely reduced in AD brain [e.g., glutamate by as much as 83% (32); as are acetylcholine, estrogen, and others (18,33)]. As such, the observed effect should not be interpreted to indicate higher resting calcium levels in AD cells.

Yet, ß-like secretase activity has been suggested to be calcium-dependent (this implies that elevated calcium could lead to overproduction of Aß); we however considered this possibility to be theoretically unfavorable (5). Moreover, AD is often compared with cerebral ischemia, in which calcium rises are known to be responsible for neuronal death (34). But, it is our opinion that the cell death process in ischemia, an acute disorder, may be different from that in AD, a slow and progressive disorder characterized by the accumulation of plaques and tangles. This feature suggests that the cell death process in AD should be related to the mechanism by which the plaques and tangles are being formed.

3.6. A "biphasic" proposal for calcium alterations in AD

In view of the forgoing, we propose that a chronic and persistent deficit of free intracellular (cytosolic) calcium levels, or a down-regulation of calcium mobilization (see below), might occur early in AD and to a lesser extent in aging (the initial causes of the deficit are unknown but should be heterogeneous). This deficit would compromise the functional integrity of various calcium-dependent processes including α-secretase, calpain, calcineurin and neurotransmission. As a result, this could trigger or contribute to the accumulation of plaques and tangles along with the depression of synaptic transmission. Over time, these lesions, if passed a certain limit, would progress into an advanced stage in which a heterogeneity of cellular impairments would occur such as membrane disruption, occurrence of oxidative stress and calcium rises (to list but a few; perhaps every biological process would be impaired at this stage)(4,7,8). These would intensify the memory deficits and would lead to cell death, the proximal course of dementia. This proposal implies that: (i) plaques, tangles and early memory reduction might have a common origin; (ii) calcium is altered in the early and late phases in opposite directions; and (iii) the calcium rises in the late stage are unable to functionally compensate for the early deficits because cells in the late stage have been irreversibly damaged (plaques and tangles formed, membranes disrupted and synapses lost).

3.7. Predictions and experimental testing

An intrinsic obstacle associated with the study of calcium in AD is that a direct evaluation of the dynamic intracellular calcium fluctuations in living human brain has not been possible thus far (postmortem tissues are inadequate for such use). Nevertheless, the following proposed experiments might provide some indications for the calcium states in AD. If estrogen, nicotine, indomethacin and ibuprofen exert their protective actions partly via calcium elevation as we proposed, then these drugs would be expected to increase APP_s secretion (and decrease Aß) in cultured cells (by activating α-secretase)(figure 1). Notably, such effects of the first three listed drugs have been reported (5,35). According to our proposal, other calcium agonists (e.g., many hormones, growth factors, excitatory neurotransmitters and plant alkaloids) would have similar effects on APP processing. Nitsch *et al*. (36) and Buxbaum *et al*. (37) have proposed a number of compounds for AD treatment (glutamate, thrombin, ACh, IL-1, phorbol esters, etc.) based on their ability to promote APP_s secretion and reduce Aß. Perhaps all of the proposed compounds can be considered as calcium agonists (5). Our proposal predicts that such agents should, in principle, not only reduce Aß, but also slowdown the processes of tangle formation and neurotransmission depression in at-risk individuals. On the other hand, if nimodipine, a calcium antagonist, is to be used in such individuals, then this too should be justified partly by testing its effects on APP processing. We recently observed that, as expected, nimodipine reduces APP_s and increases Aß in cultured cells (unpublished data).

Gene mutations of presenilins (PS-1 and PS-2) account for most of the familial early-onset AD (38,39), but their physiological functions are not yet clear. Because their molecular structures are typical of those of ion channels, and they have some sequence homologies with an actual Ca^{2+} channel (38,39), we predict that presenilins might act as Ca^{2+} channels *in vivo* (or channels for cations including Ca^{2+}; or channel-related receptors/transporters in subcellular trafficking). As Ca^{2+} channels, their gene mutations, which are all located within or near the transmembrane domains (40), would be expected to "diminish" Ca^{2+} supply, thereby down-regulating many calcium-dependent processes including α-secretase, calcineurin, calpain and neurotransmission. This would lead to typical and severe lesions of AD. Functional reconstitution and electrophysiological studies should directly reveal whether or not presenilins in artificial membranes could act as Ca^{2+} channels, and if so, whether the mutations would diminish the channeling function. This is a critical test of our proposal.

The actual determinations of the resting intracellular calcium levels in the isolated cells, an important parameter that may reflect to a certain extent the calcium states in the brain, have been controversial (7,41). However, Peterson *et al*. (42) and Müller *et al*. (41) have repeatedly reported that cells from AD subjects and aged animals display lower concentrations of free cytosolic Ca^{2+}. It is possible that the controversies may be related to the mild calcium changes in sporadic AD (an insidious and decade-long disorder) and dynamic pulses of Ca^{2+} signaling *in vivo* (18). The protective effects of estrogen (21) have suggested that calcium agonists such as some hormones can have a direct role in cognition. Assuming that such a role is in part via calcium mobilization (25), then a decrease of hormone levels, a widespread phenomenon in aging and AD (33), would be expected to cause a decline in frequency or height of the Ca^{2+} pulses. This would give rise to a "functional" down-regulation of calcium mobilization, and may partly underlie the difficulties in the direct calcium evaluation in isolated cells from sporadic AD subjects.

Nevertheless, since some presenilin mutant human hosts display the earliest onset ages of AD (38,39), and the presenilin gene knock-outs in animals cause premature death (43)(Ca^{2+} channels are essential for life), we further predict that cells from such human subjects and particularly from the gene knocked-out animals (surviving embryos) may display more prominent lower levels of cytosolic Ca^{2+} than the cells from sporadic AD subjects. It is also possible that, as a result of calcium deficits, these

cells may exhibit lower levels of the activated forms of calpain.

Measurement of the activated form of calpain by immunoreactivity (31) provides an indicator for calpain activity in the living cells. The state of calpain in AD is further suggested by: (i) activation of protein kinase C, a key event in neurotransmission (18), depends on its cleavage by calpain (13); (ii) inhibition of calpain blocks the long-term potentiation in rats (44); and (iii) catalytic cysteine of calpain is vulnerable to oxidative stress, a known threat to the aging and AD brains (45). These observations, together with its role in tau degradation, all indicate that calpain is "inactivated" in the early phase of sporadic AD. To evaluate the calpain state in this phase, one needs to examine, in our opinion, the postmortem brains of "normally aged" subjects (it is difficult to distinguish such subjects from those in early phase of AD) or slightly memory-impaired individuals [but not of severe AD patients, in whom brain neurons are severely damaged or dead, and calcium raised (11)]. It is possible that, if compared to young subjects, the aged subjects should display a decrease of the activated form of calpain (although the decrease could be only marginal).

Furthermore, our proposal implies that a presenilin mutant-based animal model, in concept, would be fully successful only if an endogenous presenilin gene has been knocked out (otherwise it would continue to supply Ca^{2+}) and the mutant gene expressed at normal levels (overexpression of the mutant gene may compensate for its diminished ability of Ca^{2+} supply). Finally, there is a possibility that an animal model that mimics the sporadic AD pathologies might be created by the use of intracellular calcium antagonists (further discussed elsewhere). Altogether, these experiments will substantially prove, or disprove, our proposal.

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