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THE ALZHEIMER'S PLAQUES, TANGLES AND MEMORY DEFICITS MAY HAVE A COMMON ORIGIN - PART II: THERAPEUTIC RATIONALE

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1. ABSTRACT

In the previous papers, we hypothesized that there could be an intracellular calcium deficit in the early phase of Alzheimer's disease (AD). In the present study, we consider, from this point of view, several long-lasting questions about AD, which include: why plaques and tangles are only found in the brain; why aging is the primary risk factor; why presenilins are so vulnerable in the disease; and why AD only affects the most delicate function of the brain. Although discrepancies and alternative views exist in many aspects of AD, it came to our attention that our hypothesis perhaps could offer a reasonable and coherent explanation, at least in part, to several such questions by the known biochemical principles. Based on the analyses, we discuss some controversies in therapeutic strategies.

2. INTRODUCTION

Alzheimer's disease (AD) is a major cause of dementia affecting a large aged population. Amyloid plaques and neurofibrillary tangles in brain tissues are routinely used as prominent markers for diagnosing AD, even though other histological changes such as dystrophic neurites, synaptic and neuronal loss may correlate better with dementia (1-4). AD is clinically a multi-factorial disorder and its pathogenesis has been linked to several types of etiological factors including metabolic abnormalities, environmental toxins, genetic elements and social factors (1-5). A wealth of experimental data about AD has been accumulated over the past decades, which has provided a rich resource for developing working models pertaining to the disease origin. Any such models, however, will be subject to scrutinies against a number of the established or repeatedly observed AD features.

3. DISCUSSION

3.1. Calcium deficit in AD

Based on a critical review of the literature, we have recently proposed that there is a deficit of intracellular calcium levels in the early phase of AD. This deficit may underlie or contribute to the formation of amyloid plaques and neurofibrillary tangles, which, in turn, would lead to cell impairments and death (6-8). The hypothesis is summarized in figure 1. While this scenario is subject to debate and experimental confirmation, here we consider additional AD features from the core of the hypothesis in order to see whether or not it would fit with these characteristics of the disease.

AD is a disorder in cognition, the most delicate and most sensitively regulated activity of the human body. Hence, although many cellular defects can be involved in the disease process, it is reasonable to speculate that the primary and initial defect(s) in AD would most likely occur in the sensitive signal transduction systems, or in the factors relevant to signal transduction. Among the possible signaling systems involved in cognition (Ca^{2+}, cAMP, cGMP, NO, etc.)(9), Ca^{2+}-related pathway is perhaps a primary suspect, in part because it is a central factor in brain function (9), and it is relevant to several AD-related events (neurotransmission, long-term potentiation, protease and phosphatase activities)(7,8).

AD is clinically manifested as a multi-factorial disorder (1-5). However, the invariable presence of amyloid plaques and neurofibrillary tangles in most if not all AD patients would imply that the effects of those multiple pathogenic factors during the disease progression may have converged into a common pathway underlying the same hallmark lesions.

In familial and sporadic AD, of which one does and the other does not carry gene mutations on ß-amyloid precursor protein (APP), both cases however exhibit the same pathological consequence (amyloid plaques). From the perspective of a calcium deficit, this phenomenon is probably due to an altered substrate (APP) in the former, and an altered α-secretase activity [a leading enzyme in APP processing (6)]

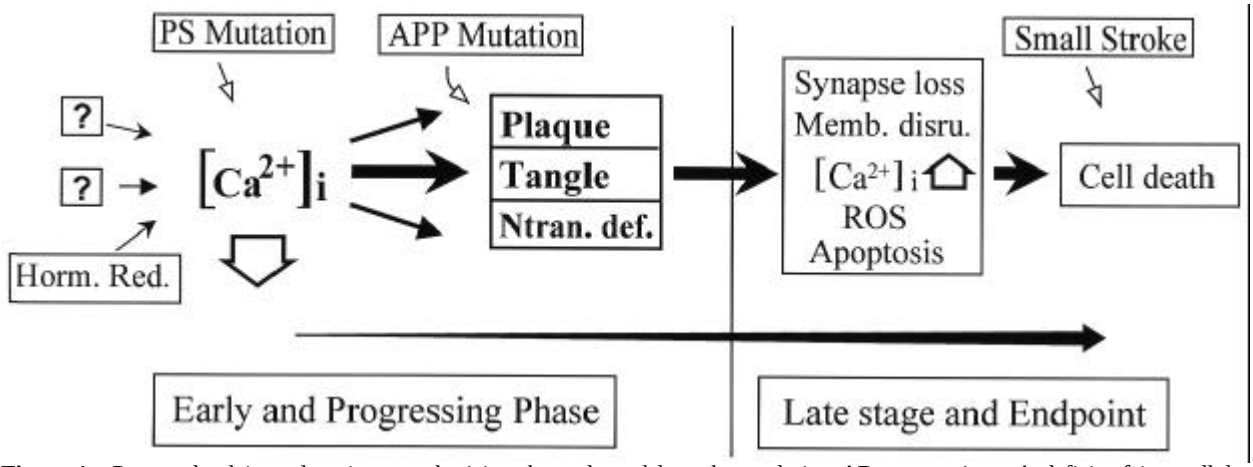

Figure 1. Proposed calcium alterations emphasizing the early and late phases during AD progression. A deficit of intracellular calcium ($[Ca^{2+}]_i$) may occur in the early phase of AD. This may be initiated by a diversity of unidentified factors (question marks), of which we consider hormone reduction (horm. red.) as an important one in sporadic AD (8). The deficit may be the common basis for the accumulation of plaques, tangles and neurotransmission deficits (Ntran. def.). These lesions would slowly lead to synapse losses, membrane disruptions (memb. disru.), $[Ca^{2+}]_i$ rises, formation of reactive oxygen species (ROS) and apoptosis (to list but a few), and eventually to cell death. The memory deficits would progressively intensify throughout this course (long arrow). Presenilins (PS) and APP mutations represent inherited cases. They, together with small strokes, are from different origins, but can merge with the mainstream course of sporadic AD at various points.

in the latter (figure 1). Both alterations would be expected to give rise to the same result: disruption (inhibition) of APP normal processing, leading to the overproduction of Aß.

Overexpression of wild-type APP gene in the transgenic animals has mostly failed to massively produce Aß in the initial efforts to generate animal models for AD (10,11). This, by analogy to humans, is perhaps because at least one alteration is required in the α-secretase/APP pair to be able to efficiently disturb the outcome of APP processing. No alteration has been made in either component of the pair in these animals. Moreover, in the APP mutant-based models (12,13), amyloid plaques are seen, but why are not tangles? Because one substrate of the enzymes has been altered, the other (tau) has not.

The excessive amyloid deposition in the brain leptomeningeal vasculature (1) in AD might also be understood. The Ca^{2+}/enzyme down-regulation, if it occurs, would be a systemic disorder (in both CNS and circulation) as indicated by reduced soluble APP (APP_s) and increased Aß in the peripheral tissues of AD patients (1,14). If so, then why are plaques and tangles only found in the brain? This, in our opinion, is because brain neurons do not proliferate. Although down-regulation of the enzyme activities would occur in both peripheral cells and the brain, the accumulated substrates could be minimized by cellular proliferation and replacement in the former, but not in the latter.

Plaques and tangles manifest various adverse effects to cells (1-4), but it should be clear that plaques and tangles are secondary to an earlier factor(s) that has triggered the accumulation of Aß and tau (i.e., the lesions would not occur without a reason) (figure 1). In an age-related disorder as AD, this early pathogenic factor(s) should remain normal for most part of life and gradually deteriorate only in the later years. The proposed age-related deterioration of calcium-maintaining or mobilization systems parallels with this disease feature.

The calcium deficit, as part of aging process, would intensify as aging advances (partly as a result of the diminution of hormones and growth factors deepens). The deposition of plaques and tangles would increase as a function of time, and would impair cell functions in an "incremental and accumulative" manner. Therefore, time is a prerequisite for such lesions to escalate to a threshold at which their effects would become clinically prominent. This may be relevant to why aging is the primary risk factor for the onset of AD.

As an age-related disorder, sporadic AD patients typically have normal adult life. This can explain why familial AD loci have not been, and will unlikely be, segregated with α-secretase or calpain gene mutations, because such permanent alterations would not allow their hosts to have successful adult life (i.e., the mutations would not be tolerated; instead, most AD-causing mutations occur on the "regulators" of the enzymes, i.e., presenilins, whose mutations would be milder than those in the enzymes *per se*, thus are tolerated; also see below). Calpain is known to be essential for life and large deviation from its normal levels is lethal (15), and α-secretase is likewise (6) [in a recent manuscript, we consider a possibility that these two enzymes may be the same, or closely related, molecular entity(ies)] (manuscript in preparation)].

3.2. Synapse losses vs. plaques and tangles

Cortical synapse loss predicts the severity of dementia more accurately than any other neurological markers (16). Whereas our proposal does not offer a complete answer to this observation, a sketched mechanism

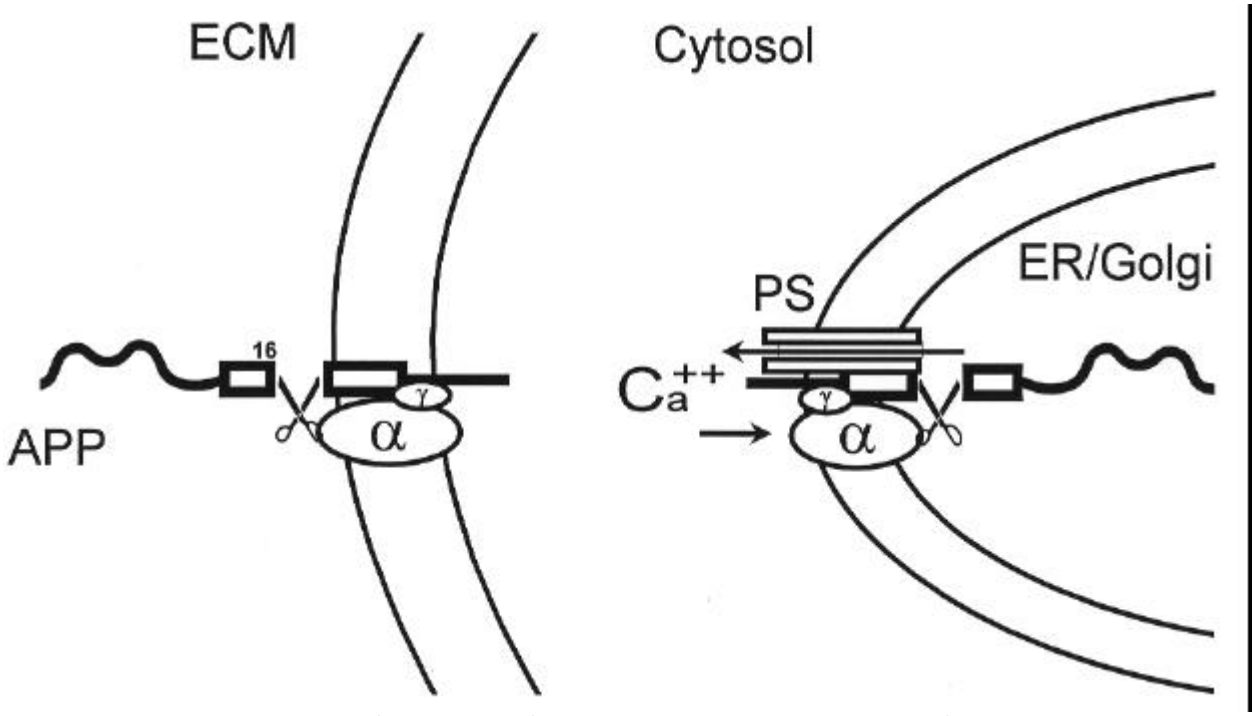

Figure 2. Schematic representation of the relations of presenilines to α- and γ-secretases. The figure shows that: (i), PSs (only one is shown) are located in the membranes of ER and Golgi (2,37), and in these organelles most secretory proteins are matured and processed including APP (6). ER and Golgi are the intracellular Ca^{2+} stores from which the cation is channeled to the cytosol for the α-secretase activation. The location of PSs in the plasma membranes has also been reported (22), where they may play a similar role (channeling Ca^{2+} from outside in; not shown). And (ii), PSs and γ-secretase are also in proximity; as such any conformational change in PSs would influence the binding of γ-secretase to APP, thereby generating longer Aß.

centered around Ca^{2+} signaling in relation to cognitive activities may be worth considering.

Synaptic activities are extremely sensitive to Ca^{2+} signaling. Neurotransmission oscillates by as much as four orders of magnitude within a fraction of a millisecond in response to Ca^{2+} fluctuations brought about by action potentials (9). Such knowledge about neurotransmission is mostly derived from the studies on neuromuscular systems or in primitive animals (9). There are reasons, however, to believe that the cortical synapses of human brain, the most exquisite creation of the nature, should be even more, perhaps the most, sensitive to Ca^{2+} signaling. Therefore, the cortical synapses would be the first, among any other biological structures of the body, to respond to a subtle Ca^{2+} imbalance. Probably, this is why a mild and systemic calcium deficit in AD only affects the most delicate intellectual activities of the brain, but not remarkably other functions of the body.

Brain neurons, by their non-proliferative nature, are known to be vulnerable to the protein turnover slowdown. The intracellularly accumulated tau or its degradation intermediates can disrupt the microtubule integrity, obstruct axonal transport, and perturb neurotransmitter release at synapses (4,17). These impairments, added by the deposition of Aß (which can be viewed as a degradation intermediate of APP) with its wide variety of cell-damaging effects (3), would lead to retrograde degeneration of neuronal processes and loss of synapses (4,17). Thus, synapse losses, which occur at the advanced stage of neurodegeneration and eliminate the structures essential for cognition, would be expected to be a close indicator for the severity of dementia (figure 1).

In comparison, plaques and tangles, which start accumulating early on and are present throughout aging and AD progression, would be anticipated to exert their cell-damaging effects through a "slow and incremental" mechanism. As such, during most part of the long course of neurodegeneration, they may not co-exist with, but rather precede, synapse losses and cell death. For these reasons, plaques and tangles should be considered, in our opinion, as prominent markers for calcium deficiency and neurodegeneration, but not as accurate indicators for dementia (figure 1). This scenario is consistent with the fact that most aged individuals, though carrying moderate amounts of plaques and tangles and some even having considerable such burdens, are however not demented (16,18) (perhaps not until synaptic and neuronal losses prevail).

In some cases, the long course of the cell death process can be abruptly accelerated by additional insults such as small strokes (18). They, if happened to the ailing cells already impaired by the plaques and tangles, may massively kill them, thereby severely intensifying the symptoms of dementia (figure 1).

3.3. Roles of presenilins (PSs)

Based on several reasons, we predicted that PSs may function as Ca^{2+} channels *in vivo* (or cation channels)(8). This prediction is supported by the report showing that the PS mutant human carriers develop excessive neurofibrillary tangles as well as amyloid plaques (19), suggesting that the mutations have affected a common pathway underlying both lesions (7) (figure 1). It may be argued: why must PSs be expected to interfere with Ca^{2+} signaling, knowing that they can cause, for example, apoptosis (20)? This is because if they cause AD only through apoptosis, then plaques and tangles, cardinal features that define AD, would not be explained in the PS mutant hosts. To this end, we believe that pathogenic factors that cause abnormal APP processing and tau accumulation should act, at least in part, by directly or indirectly interacting with Ca^{2+} signaling.

Several studies have shown that PSs bind to APP specifically *in vivo* (21-23). This important finding further implies that PSs would directly regulate the α-secretase activity (by supplying Ca^{2+} in proximity)(figure 2). Such a spatial association would ensure a sensitive regulation of the enzyme since Ca^{2+} ions once in the cytosol diffuse slowly (9). In proximity to PSs, α-secretase would be highly vulnerable to PS mutational distortions. This might be why the point mutations at almost any sites (more than 40 are known today) within or near the transmembrane domains of PSs would cause AD, and why mutations of other calcium channels have not been segregated with the disease (they may not be associated with APP).

Current studies have also shown that three APP secretases (α/ß/γ) are functioning in the same subcellular compartments (mostly ER and Golgi) in many, if not all, cells (24,25), and thus they are likely acting on the same APP pool (figure 2). As such, PSs binding to APP would also bring γ-secretase to their proximity. γ-Secretase exhibits flexible cleavage specificity probably via its membrane-association, which would loosen its interaction with APP, as we proposed (6). This model implies that γ-secretase, within such a "cluster" of several membrane proteins, would be sensitive to structural distortions of any members in the cluster, such as PS mutations [and APP717 mutations as well (6)]. It is possible that the PS mutations may somehow dislocate γ-secretase through a changed conformation of PS *per se* or by its consequent disturbance of the membrane integrity. Whereas the precise mechanisms involved are not yet clear, a consideration from this perspective might provide a clue for the following observations:

(i) the PS mutations in humans lead to excessive production of Aß42/43 (26)(inactivation of α-secretase caused by PS mutations would first make available of more intact APP, and the mutation's steric effects on γ-secretase would then generate longer Aß); (ii) some PS mutant carriers display the earliest onset ages of AD (no other type of AD involves such a permanent calcium imbalance and the double-defect on both α- and γ-secretases); and (iii) current PS animal models, generated by means of "mutant gene insertion" (27,28), though overproducing Aß42/43, have remained cognitively healthy (29) [Ca^{2+} levels are normal; and no excessive APP available; thus total Aß levels, tau dephosphorylation and degradation and neurotransmission would remain essentially normal; a successful PS model requires a "gene replacement" paradigm to create a "calcium deficit" condition (8)].

3.4. Targets of pharmacological intervention

AD is manifested by dementia (neuronal death); however, dementia is not the monopoly of AD since several other neurological disorders share this feature as common end results (e.g., cerebral ischemia, HIV infection, kidney dialysis-related dementia and prion diseases)(30). AD is perhaps only unique by the excessive plaques and tangles. This feature suggests that the pathological origin of AD-type dementia should be related to the mechanism of plaque and tangle formation and should be distinct to those of other disorders.

AD is often compared with ischemia for therapeutic rationales, perhaps because both disorders involve calcium rises (8). We nevertheless reasoned that because the steep calcium gradient across the membranes is maintained *in vivo* by energy-driven systems (channels and pumps)(9), and these systems must collapse during cell death process (due to energy failure). It then follows that intracellular calcium would inevitably arise during neuronal death caused by various reasons [e.g., obstruction of blood supply in ischemia, virus injuries in HIV infection, aluminum toxicity in kidney dialysis (30)]. Conceivably, the calcium rises in these cases would be similar to the conditions in the late stage of AD [this contention can be directly tested by measuring the activated forms of calpain by immunoreactivity in the postmortem brains of those patients, as it is done in AD (31)].

Thus, calcium rises are neither the initial cause nor the unique feature of these disorders (i.e., calcium would not arise without a reason), but rather the end result of any disorders in which neuronal death is involved. For these reasons, the calcium rises should not be considered as the primary target for the prevention of these disorders (though nimodipine is an effective therapy in ischemia, but not a prevention). Indeed, the respective initial causes have been successfully targeted as the primary preventive strategies for some disorders (e.g., preventing blood clots, blocking virus infection, and eliminating aluminum, respectively)(30).

If this consideration is correct, then in the case of AD, if a calcium deficit is involved in its early phase, it would be reasonable to propose that the protective attempts should target this feature of the disease. Even though calcium rises occur in the late stage of AD and nimodipine indeed can somewhat improve the conditions in the advanced AD patients (32), such a treatment, because it aims at a late-stage event, should not be considered as the preventive strategy for AD (although it might be used as an adjunct therapy for the advanced patients).

In this context, it may be necessary to distinguish the strategies for AD prevention from those for improving the conditions in the advanced patients. It should be noted however that it is the prevention or postponement of AD at onset that should be the emphasis of the disease research, because neurons once entered the late stage of AD may be

difficult to rescue due to the irreversible damages they suffer (8). This is in contrast to neurons in ischemia which might be rescued (by reducing the elevated calcium and restoring blood flow), because they are essentially intact (the cause is the blood supply, not the damaged neurons as in AD).

Our hypothesis also implies that the calcium deficit may not be the initial defect in AD (again, the deficit would not occur without a reason)(figure 1). However, given the complexity of the calcium maintenance systems (channels, pumps, phospholipids, buffering proteins and mobilization factors), the initial dysfunctioning elements may vary in the individuals and may involve intricate interactions among themselves, thus are difficult to be ascertained individually. But, if the Ca^{2+} imbalance can be considered as a conceptual "converging point" for the actions of those dysfunctioning elements (figure 1), then this would indicate a general target for the prevention or postponement of clinical AD, and would also highlight the direction for improving the efficacy of the current medications.

Calcium mobilization is essential for many life processes, therefore, the body would have a wide variety of ways to achieve it. In this context, the potential compounds for AD postponement, in theory, may fall into several categories: some hormones, growth factors and neurotrophic elements, cytokines, phospholipid regulators, bioactive peptides and stimulators of neurotransmitter receptors (e.g., glutamatergic, cholinergic, and their subclasses such as NMDA-, AMPA-, nicotinic and muscarinic; many of which are Ca^{2+} or cation channels)(9). It is encouraging to see that similar strategies have been proposed by other investigators (33-35) and progresses are being made in this field (36). Because calcium homeostasis *in vivo* is the sum of the actions of many calcium maintaining elements, it is also possible that a selective "cocktail" of such compounds may be more effective than a specific one in restoring the calcium balance *in vivo*.

In addition, many natural ingredients in foods and beverages also have similar effects (such as caffeine and folic acid, whose cellular actions include calcium activation; further discussed elsewhere). This last category underscores the importance of a concept of the balanced diet and active lifestyle in the prevention of AD in the aging population.

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