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THE ALZHEIMER'S PLAQUES, TANGLES AND MEMORY DEFICITS MAY HAVE A COMMON ORIGIN. PART V: WHY IS Ca^{2+} SIGNAL LOWER IN THE DISEASE?

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1. ABSTRACT

The state of intracellular Ca^{2+} in aging and in Alzheimer's disease (AD) is a key but highly controversial issue and direct measurement of the Ca^{2+} fluctuations in the living human brain has not been possible thus far. We therefore further considered this issue from a theoretical perspective. Ca^{2+} signaling mediates many life processes including: fertilization, gene expression, cell division, growth and differentiation, muscle contraction, neurotransmission and memory formation. It is common observation that these Ca^{2+}-mediated activities in human life are highest in young adulthood but diminish during aging, indicating that Ca^{2+} signaling potency (or intracellular Ca^{2+} levels) must be decreased in aging and AD. A potential explanation for this phenomenon could be that the Ca^{2+}-mediated processes are also energy-dependent processes, because they all utilize the free energy reserve of the body for "useful" work, and it is known that Ca^{2+} gradient formation and Ca^{2+} movement across cell membrane are driven by energy-dependent systems. This intimate relationship between energy and Ca^{2+} signaling implies that the potency of Ca^{2+} signaling would be affected by changes of energy levels, which would necessarily decline in aging. These may underlie the deficit of Ca^{2+} signaling in the presymptomatic stage of AD. These considerations also support our view that Aß and tau accumulation in AD is the result of inactivation of calcium-dependent enzymes, rather than overactivation of ß/γ-secretases and some tau kinases. This is because most enzyme activities should be diminished, rather than overactivated, during aging. Furthermore, since energy/Ca2+ deficit is a natural event in aging, it follows that the accumulation of Aß and tau would be initiated "spontaneously" as a result of "natural" aging, not necessarily by a "pathological" factor. Based on the analyses, we propose that intracellular Ca2+ deficit is most likely the primary and common cause (among the many contributing, secondary or individualized factors) for the plaque and tangle accumulation underlying sporadic AD. And we predict that this contention, though in contrast to many competing models, will be confirmed by the proposed experimentation in the future.

2. INTRODUCTION

Alzheimer's disease (AD) is manifested as an insidious and progressive memory loss leading eventually to dementia. In the brain of patients with AD, there are widespread synapse loss and neuronal death, which are the proximal causes for dementia. However, unlike many other neurological disorders which also lead to dementia, AD leaves behind two defining features that may serve as a starting point for unraveling the origin of the disease. These hallmarks are amyloid plaques and neurofibrillary tangles, which are mainly comprised of the abnormal degradation intermediates of ß-amyloid precursor protein (APP) and phosphorylated tau, respectively (1-5). Although many pathogenic factors have been proposed and multiple molecular pathways can lead to AD, a central issue is whether the actions of these pathogenic factors could be conceptualized to converge into a common pathway leading to AD (4). The fact that memory loss in AD patients by various pathogenic factors is invariably accompanied by the same plaques and tangles strongly suggests that such a pathway (or factor) exists.

3. COMMON FACTOR IN AD

While the identity of this putative common factor(s) is currently obscure, further analysis reveals that it should have following characteristics: (i) it occurs, to a certain extent, in every aged individual (plaques and tangles are a common feature of aging brains); (ii) it intensifies as age advances; (iii) it does not necessarily lead to dementia in most aged people; and (iv) it is probably a "regulatory" factor, because memory

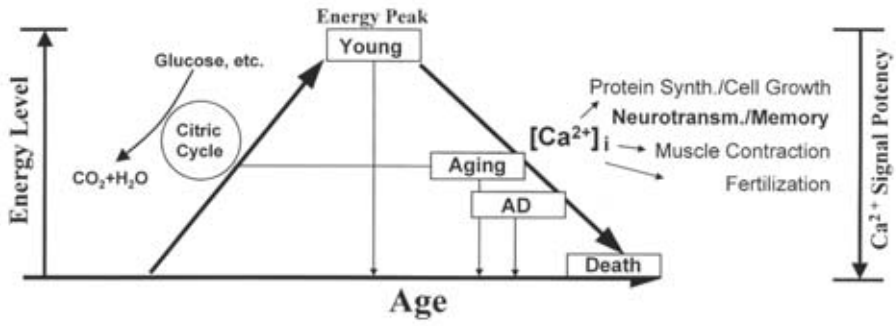

Figure 1. The relationships between energy and Ca^{2+}-mediated processes and their changes during life. Life is a continuous process of free energy accumulation and consumption, and energy levels in human life culminate at young adulthood followed by a diminution during aging. Because many Ca^{2+}-mediated processes are also energy-dependent, it is anticipated that the potency of these Ca^{2+}-mediated processes would be down-regulated in aging and, to a greater extent, in AD as a result of the energy level decline.

itself is a highly regulated process and is progressively diminished in AD. Among the many competing hypotheses regarding the mechanisms of AD origin (1-5), we have recently proposed that a mild but persistent "deficit" of intracellular calcium levels, or a down-regulation of the Ca^{2+} signaling potency, occurs in aging and in the development of AD. Such a deficit may underlie the accumulation of Aß and tau along with the progressive memory reduction (6-10). This hypothesis is in direct contrast to current views (11,12) and its logical predictions await experimental confirmation. But, it is apparently consistent with the aforementioned characteristics of the common factor and with several other basic AD features (7,8). It is also supported by a rapidly growing body of evidence emerging from various research areas (13-15).

Dynamic and subtle intracellular Ca^{2+} fluctuations in the living human brain cannot be measured directly by any current technologies, and data from indirect experiments on the Ca^{2+} state in aging and AD have been highly controversial (7,11,12). In this context, it may be helpful to undertake a theoretical approach to this key issue. We reasoned that Ca^{2+} signaling is a fundamental mechanism of life, hence any of its alterations would be expected to give rise to the concomitant changes of many other biochemical processes in the body. We therefore analyzed these other processes and proposed a mechanism that may underlie the Ca^{2+} deficit in AD.

4. RELATIONSHIPS BETWEEN Ca^{2+} SIGNALING AND ENERGY

Free intracellular calcium ion, or Ca^{2+} signal as a second messenger, mediates a wide variety of life processes. To our current knowledge, these processes include: fertilization, gene expression, protein synthesis, cell division, growth and differentiation, muscle contraction, neurotransmission and memory formation (for an excellent review, see ref. 16). Although the precise mechanism of Ca^{2+} involvement in these processes is complicated and not yet fully understood, it is generally recognized that Ca^{2+} signaling and Ca^{2+}-dependent enzymes mediate, for example, the effects of the growth factors in promoting cell division and growth. Neurotransmission and plasticity modulation in long-term potentiation would not occur in the absence of Ca^{2+} entry and activation of calcium-dependent enzymes (16-20).

Upon further examining these Ca^{2+}-mediated processes individually, it came to our attention that a unique feature that these processes have in common is that they represent those which utilize the free energy reserve of the body for the execution of "useful" (energetic) work. It is apparent that none of these Ca^{2+}-mediated processes, in concept, can take place unless the body has already acquired the necessary energy reserve and, when energy in the body is in short-supply (e.g., during prolonged starvation), the intensity of all these processes is reduced.

Yet, this intimate relationship between energy and Ca^{2+} signaling is not coincidental, because it is well-known that the formation of the steep Ca^{2+} gradient and rapid movements of Ca^{2+} across cell membrane are driven by energy-dependent systems (channels and pumps)(17). This indicates that free energy is required for Ca^{2+} signaling. In this regard, the above-listed Ca^{2+}-mediated processes may also be considered as "energy-dependent" (energy-consuming) processes. Indeed, the energy-dependent nature of neurotransmission and memory formation, the most active biochemical reactions in the brain, is particularly evident because brain accounts for as much as 60% of the total glucose consumption by the human body in the resting state (21).

It is worth noting that although Ca^{2+}-mediated processes account for a large part of life activities, Ca^{2+} signaling may not be responsible for all life processes (such as the citric acid cycle, the most important energy-generating process in eukaryotes)(21). It thus appears that Ca^{2+} signaling is preferentially involved in energy-consuming reactions (though not in all of them, e.g., many energy-consuming synthetic reactions may not be Ca^{2+}-mediated). Nevertheless, this intriguing relationship between energy and Ca^{2+}-mediated processes, which, to our knowledge, has not been explicitly documented, may reflect an important regulatory mechanism of calcium homeostasis in life. Although the cellular mechanism of this regulation has yet to be elucidated, it is obvious that Ca^{2+} signaling would be heavily influenced by the free energy state of the body.

5. CHANGES OF ENERGY AND Ca^{2+} IN AGING

In terms of bioenergetics, life can be considered as a continuous process of "energy accumulation and consumption", and the energy levels (the rate at which dynamic generation and utilization of energy occur) fluctuate throughout life. Since the energy levels in human body are highest at young adulthood and fully vanish at death, it is plausible to define aging, a necessary step in between, as a process during which the energy levels are progressively diminishing towards a complete disappearance (figure 1).

If free energy levels are necessarily diminished in aging, and if many Ca^{2+}-mediated processes are also energy-dependent, then it should follow that the potency (or efficiency) of Ca^{2+} signaling and Ca^{2+}-mediated processes during aging would be "down-regulated". And yet, this down-regulation would be deepening as a function of age (figure 1). In agreement with this reasoning, it is common observation that fertilization, cell growth, muscle contraction, neurotransmission and memory formation in humans all exhibit their maximal potency at young adulthood, followed by a slow reduction during aging. There is no exception to this rule. And also, this reduction is deepening in a progressive manner as aging advances.

These considerations indicate that intracellular Ca^{2+} levels, among other things, must be decreased in aging and in the early (presymptomatic) stage of AD, rather than "increased" as it is currently believed (11,12), although some interesting questions remain unsolved. For example, because Ca^{2+} gradient is maintained by energy-driven systems, it seems logical at a first glance that the reduced energy levels in aging should give rise to a reduced gradient, i.e., increased cytosolic Ca^{2+} (Ca^{2+} leaking), instead of further diminishing it (which might need more energy). Why does this not happen in aging cells?

While the underlying reasons for this perplexing question are unclear, we consider the following possibilities. Ca^{2+} gradient (10,000-fold lower in cytosol than in extracellular space and internal stores) is essential for life. The remarkable cross-membrane ion-driving force potentiated by such a steep gradient makes it possible for Ca^{2+} signaling to be extremely rapid (within a time scale of millisecond; note that no any other cell signaling system or ion transport has a comparable scale of gradient and sensitivity). As essential for life, any steady increase in the basal cytosolic Ca^{2+} levels would not be tolerated by the functioning, even though old, cells. If occurred, this would destroy the gradient and steadily activate calcium-dependent proteases, a destructive process which must lead to acute cell death (the functional enzyme activation is only transient).

Yet, if a steady decrease of the basal Ca^{2+} levels is energetically unfavorable in aging, the observed reduction of the Ca^{2+}-mediated processes can still be explained by at least one other mechanism, that is, a "functional" down-regulation of the frequency or altitude of the dynamic Ca^{2+} pulses. This down-regulation can occur as a result of the widespread age-related reduction of Ca^{2+} agonists (hormones, growth factors, excitatory neurotransmitters, etc.)(7,8). In the brain, these agonists would normally participate in cognition by "cooperating" with the electrical potentials to elicit a "full-scale" Ca^{2+} mobilization in neurons (this is why neurons possess both voltage-gated and ligand-gated Ca^{2+} channels)(14,17). Whereas the precise mechanism of this cooperation has yet to be fully understood, it is likely that the reduced agonist levels in aging would suffice to compromise the integrity of the mobilization process, leading to the long-term (chronic) and subtle diminution of the Ca^{2+} signaling potency (or efficiency). And understandably, such a diminution of the potency would be difficult to be directly measured in isolated cells, let alone in the living human brain (7).

This limitation of the methodology implies that the state of intracellular Ca^{2+} in AD patients can only be *deduced* by extrapolation from indirect experimental data and observations at the present time (this is in contrast to many other diseases where pathogenic factors can be directly demonstrated). This would also highlight the paramount importance of caution in the data interpretation and extrapolation in AD/Ca^{2+} research. It is important to note that the interpretation of a given set of experimental data should also take into account the overall disease features. For instance, many data seem to favor higher Ca^{2+} levels in AD cells (11,12)(note that massive Ca^{2+} rises are the only Ca^{2+} change that is readily measurable). However, if this were to be extrapolated to the presymptomatic phase of the disease, then, because of the fundamental roles of Ca^{2+} in life, the individuals would be expected to exhibit excessive activities of cell growth, muscle contraction and memory formation, etc. Apparently, this assumption, no matter bolstered by how many *in vitro* data, is inconsistent with the basic features of AD (for our alternative interpretations of some of these data, see 6-8). Moreover, cytosolic Ca^{2+} levels must arise in the late stage of AD because cells are damaged or dead at that stage. Cell damage or death, no matter by what causes, are *always* accompanied by the collapse of Ca^{2+} gradient (autolysis).

In addition to the reduction of Ca^{2+} agonists, there may be other potential mechanisms contributing to the functional Ca^{2+} down-regulation. For example, (i) reduction of electrical potentials (due to age-related inefficiency of Na^+ and K^+ mobilization systems) leading to a reduced potency of voltage-gated Ca^{2+} channels; and (ii) inefficiency of energy-dependent Ca^{2+} pumps and delayed Ca^{2+} extrusion in aging may result in a reduced frequency of Ca^{2+} pulses (Ca^{2+} mobilization is mainly by means of increasing pulse frequency)(17). Future investigations will prove, or disprove, these possibilities.

6. INITIATION OF PLAQUES AND TANGLES

AD is characterized by amyloid plaques and neurofibrillary tangles. It has long been recognized that mechanism of their formation is of key significance for understanding the origin of AD (1-5). However, despite extensive studies, continuing controversies persist over the mechanisms of their initiation. In the amyloid front, it is widely held that targeting ß- and γ-secretases should reduce the Aß levels and the underlying assumption is that these

proteases are overactivated in AD (10,22). In the tangle front, although an inactivation mechanism of protein phosphatases has been proposed (5), it has not been generally accepted and a competing model argues that the hyperphosphorylation of tau is due to overactivation of some protein kinases (23). Which of these models are theoretically more reasonable?

Our forgoing considerations of energy/Ca^{2+} changes should bring new insight into these controversies. First, it strengthens our view that overproduction of Aß in AD is primarily due to *inactivation* of calcium-dependent α-secretase, rather than overactivation of ß/γ-secretases (6,10); and similarly, the apparent hyperphosphorylation of tau is the result of *inactivation* of protein phosphatases including calcineurin (5,7), thus not of "overactivation" of protein kinases. Although the scenarios of enzyme overactivation are seemingly supported by some *in vitro* studies, it must be noted that, during the aging process, life activities are progressively *diminishing* towards a complete cessation. Therefore, it is much more likely that most if not all dynamic biochemical reactions in aged individuals should be down-regulated, rather than overactivated, particularly if they are calcium-dependent. Enzyme overactivation, though can be the underlying mechanism of many other diseases, is unlikely to occur in *aging* (otherwise the enzyme activities would culminate at death).

Second, can other aberrant factors besides these enzymes explain the plaques and tangles? It is now clear that the accumulation of Aß and tau is the "earliest" detectable histological lesion in the aging brain preceding the clinical manifestations of AD usually by decades (1-5). This important fact indicates that the accumulation of Aß and tau starts at a time when cells are intact but only memory, the most sensitive activity of the brain, starts to decline (as "forgetfulness" in normal aging). Since memory is a highly *regulated* process (at least by Ca^{2+} and cAMP) and its reduction slowly progresses in the normally-aged brain in which cells are apparently functioning, it is reasonable to assume that memory decline is mainly the result of the decline of a cellular *regulatory* system (not cell death, the proximal cause of advanced memory loss, dementia; a distinction between slow memory reduction and outright cell death must be made since one characterizes AD, the other is the common result of many diseases)(8). As such, the biochemical reactions that are intimately associated with memory reduction (i.e., Aß and tau accumulation) would most likely belong to the *regulated* processes, and thus would be the first to be affected along with memory in the intact cells.

By this reasoning, the Aß and tau accumulation should be initiated as a result of the down-regulation (subtle inefficiency of the functionality) of a signal transduction system (8,14), instead of a pathological destruction (collapse of the cell). According to the former scenario, the lesions would proceed as a "mild and insidious" event in the "functioning" brain that is progressively losing its function. In contrast, the "pathological" scheme would imply that many "unregulated" reactions would instead initiate the process. Following this scheme, it has been suggested that some non-calcium-dependent proteases may be responsible for Aß accumulation (10,22), and reactions such as glycosylation may initiate tau accumulation (24). However, it appears to us that while these reactions can be involved in the later stages of the process, but if they are not "regulated", then they perhaps would be unable to play a "primary and initial" role in the accumulation process (6,25). The reason for this is that such reactions, if they are not directly regulated by a signal transduction system, would then become "abnormal" (overactivated) probably only when cells are undergoing a "pathological destruction" process (such as membrane damage). However, if the process were destructive at the beginning, then it would not be expected to proceed "insidiously" for decades without severely damaging the brain functions in most normally-aged individuals.

In other words, we believe that the accumulation of Aß and tau is initiated most likely by a *spontaneous* down-regulation of energy/Ca^{2+} signaling as a result of *natural* aging, but not necessarily by a "pathological" factor. The deterioration of energy/Ca^{2+} signaling is a "physiological" (albeit unwanted) event in aging (which can be eventually attributed to a "genetic clock"), and can occur without a conventional pathological factor. This view, though unconventional, can actually explain a long-existing paradox: If a pathological factor is responsible for plaques and tangles, then why does it occur in every aged person and at approximately the same time?

By this view, the accumulation of Aß and tau, in essence, is similar to the natural accumulation of cholesterol (a physiological though undesirable event, a result of metabolic inefficiency in aging), whose impairments would not become clinically prominent unless surpassed a certain limit. We speculate that this may be why after extensive studies for decades, no *bona fide* pathological factor has been proved to be *causative* for the plaque and tangle formation (except for gene mutations in familial AD).

7. ROLES OF PATHOLOGICAL FACTORS

Numerous conventional pathological factors have been suggested to be potentially responsible for plaque and tangle formation and for sporadic AD (these include: disruptive Ca^{2+} rise, membrane damage, environmental toxins, aberrant gene activation, apoptosis and others). Our considerations, however, imply that these factors are unlikely to occur in normal aging to account for the plaques and tangles. And even if they occurred in some individuals, would need a preceding reason to be triggered (e.g., Ca^{2+} rise or apoptosis, the indicators of cell death, could not occur *spontaneously* but require a preceding condition that causes them). Thus, they themselves should not be viewed as the *initial* (or causal) factors in the process.

It should be emphasized that these pathological factors are proposed mostly on the basis that they are clearly present in postmortem AD brains as compared to age-matched controls. However, given that cells in AD brain are damaged or dead (as the end result of plaques/tangles and neurodegeneration), it is no surprise that many biochemical events in postmortem AD brains would be found altered (7). Although these factors are indisputably "altered" or "involved" in the disease, a long-lasting and critical question

that must be answered is whether they are the *cause* or *effect* of the cell damage (1-5). For a given factor to be considered "causal" in AD, we propose three criteria for discussion:

First, a causal factor should occur at *early stage* of AD progression (i.e., presymptomatic phase long before the symptomatic stage). Since an individual at that stage does not display remarkable clinical differences from normally-aged people, it is evident that such a factor should exist in the *normally-aged* brain (in which the absence of a factor has currently been considered a criterium for the factor to be "AD specific" contrary to our proposal). To our knowledge, the best confirmed such factors are plaques and tangles. Thus, if a lesion has only been demonstrated in the postmortem AD brain but not in the age-matched controls (e.g, Ca^{2+} rises or apoptosis), then this actually is a compelling piece of evidence showing that the lesion occurs at a time after plaques and tangles have been there for decades. Therefore, it is most likely the *end result* of plaques and tangles, but not the *cause* of them.

Second, since the plaques and tangles invariably accompany the progressive memory decline throughout the AD process (though their precise roles in the disease are debatable), it is necessary that any proposed causal factors for AD would need to offer a reasonable explanation for the mechanism of the plaque and tangle accumulation. Although the involvement of these factors at the present time cannot be entirely precluded in the early stages of AD, it is difficult to consider them as causal factors, because their primary and initial roles in the accumulation of Aß and tau have not been demonstrated and confirmed.

Third, the roles of many pathological factors in AD are proposed based on the fact that they are known to cause cell death in many other diseases. However, decades-long and accumulation of plaques and tangles are among the most striking features of AD, which are not shared by many other diseases. Therefore, the known actions of these pathological factors do not explain the unique AD process. Furthermore, any proposed causal factors also need to explain other basic disease features, e.g., AD incidence increases proportionally as a function of age. Notably, the age-correlated advance of energy/Ca^{2+} deficits is intrinsically consistent with this feature of the disease. On the other hand, if conventional pathological factors were primarily responsible for AD, then it would be difficult to explain why these factors can exhibit such a remarkable age discrimination (whereas they do not in many other diseases).

Nevertheless, our considerations do support the possibility that some of the pathological factors may occur in a way that parallels with (or accompanies) the accumulation process of plaques and tangles (such as free radicles), or occur in the later stages of the process. In both of these cases, they may still accelerate or exaggerate the adverse effects of the early lesions, thereby intensifying the clinical symptoms of dementia. In other words, they may play a contributing, secondary or individualized (only in some patients) role, rather than being the initial and common cause, for the disease.

8. EXPERIMENTAL TESTING OF THE HYPOTHESIS

Sporadic AD has long been considered as an abnormally accelerated process of aging. From the perspective of energy state, one may also consider AD as a case of an abnormally accelerated energy decline, which at the cellular level would exhibit a more prominent down-regulation of Ca^{2+} signaling than in normal aging (figure 1).

Given that energy levels are necessarily reduced in aging, it should follow that during the course of aging and AD, not only energy-consuming processes are down-regulated, but also the "energy-generating" reactions as well (if the latter were normal, then the former would not decline)(figure 1). As such, factors associated with energy-generating reactions would be expected to diminish with aging and, to a greater extent, with AD. These factors are many and may include the rate of dynamic ATP generation and hydrolysis, glucose consumption, insulin and cAMP signaling (which mediate glucose oxidative degradation), and particularly, the functional integrity of mitochondria (the primary site of energy generation in the cell)(21). In this respect, it has been well-documented that insulin system, glucose consumption and mitochondrial function are impaired in AD brain (26-28). And more importantly, the impairments of energy metabolism are closely correlated with the hallmarks of AD: abnormal processing of APP and tau (29,30).

Of particular importance, these theoretical considerations, coupled with experimental observations, would lead to the prediction that the strategies that target the glucose/energy system can be used as additional approaches both for AD prevention (via its activation) and for the development of the animal models for sporadic AD (via its inhibition). Thus, it is possible that prolonged application of the agents that can gradually inhibit energy metabolism in mitochondria should eventually induce the AD-like lesions in experimental animals. Indeed, significant progress in this respect has been reported. For example, Lannert and Hoyer (31) have recently found that injection of an insulin receptor inhibitor causes long-term diminutions in learning and memory abilities in rats.

In order to generate an animal model that mimics more typical and complete spectrum of sporadic AD lesions (i.e., severe and irreversible memory loss, appearance of plaque and tangle-like changes), here we propose the following two improvements over the current protocols. First, according to our contention that energy and Ca^{2+} are concomitantly diminished in AD, energy metabolism-inhibiting agents (such as streptozotocin, sodium azide and rotenone)(27,32,33) may be more effective if they are applied together with the Ca^{2+} antagonizing drugs [e.g., NMDA receptor inhibitors and Ca^{2+} channel blockers as discussed previously (9)]. The cooperative actions of these inhibitors may mimic more closely the conditions in the early and progressing phase of AD, in which an array of energy/Ca^{2+}-related or downstream factors, rather than a specific one of them, should be concomitantly dysfunctioning. Second, because AD lesions in humans occur slowly throughout the long aging process and culminate at the end stage of life, it is necessary that such experiments are conducted repeatedly and

extended to the advanced stage of the animal's lifespan in order for AD-like lesions to become prominent (i.e., to create an excessive energy/Ca^{2+} deficit condition over that in normal aging). In addition, due to the limited lifespan of rodents, manipulation in primates should hold a greater promise to generate histological and behavioral impairments which would have resemblance to those in the human patients with AD. This could be the most critical test of our overall hypothesis.

9. CONCLUSIONS

Ca^{2+} signaling is a fundamental mechanism of life and mediates many biochemical processes. And these Ca^{2+}-mediated processes may also be considered as energy-dependent. Since energy levels in human life culminate at young adulthood and slowly diminish during aging, it is reasonable to anticipate that the potency of Ca^{2+} signaling would be down-regulated in aging and in the early stages of AD. In fact, the Ca^{2+}-mediated processes are all reduced in the aged and AD individuals, indicating that the Ca^{2+} signaling potency is indeed diminished. This also implies that Aß and tau accumulation, in essence, is a natural event in aging, but not necessarily initiated by a pathological factor. Based on this and previous analyses (6-10), we propose that intracellular Ca^{2+} deficit most likely represents the primary and common cause for plaque and tangle accumulation underlying clinical AD (among the many contributing, secondary or individualized factors). And it is predict that this contention will be confirmed by prolonged and concomitant inhibition of energy metabolism and Ca^{2+} levels in the experimental animals.

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