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THE ALZHEIMER'S PLAQUES, TANGLES AND MEMORY DEFICITS MAY HAVE A COMMON ORIGIN - PART III: ANIMAL MODEL

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TABLE OF CONTENTS

1. ABSTRACT

We have hypothesized that an intracellular calcium deficit may occur in the early phase of Alzheimer's disease (AD). This hypothesis has two important corollaries. First, it predicts that elevation of calcium levels by many calcium agonists, in principle, would have protective effects in the individuals at-risk to AD. Second, it implies that an artificial decrease of the calcium levels by the use of calcium antagonists might mimic the AD pathologies in the experimental animals. Obviously, the latter prediction not only would allow a direct testing of the hypothesis, but also might offer a "new" route for developing an animal model for sporadic AD. In fact, a number of the existing models that target various neurotransmitter receptors and calcium channels has manifested memory deficits. This suggests that a fully successful animal model for AD might be developed by improving the current paradigms. Furthermore, we discuss a potential relationship between AD and schizophrenia in terms of intracellular calcium imbalance.

2. INTRODUCTION

Alzheimer's disease (AD) is characterized by memory loss, the presence of amyloid plaques, neurofibrillary tangles, synapse losses and neuronal death as hallmark lesions (1-4). Despite that enormous research efforts have been devoted to the disease, the mechanism of its pathological origin has remained elusive. A major obstacle that has limited the progress of AD research and drug development is the absence of animal models that mimic the full spectrum of the disease pathologies. Several strategies have been attempted to create animal models, including overexpression of the wild-type gene of ß-amyloid precursor protein (APP), infusion of ß-amyloid protein (Aß) and other components of the amyloid plaques, and insertion of mutant APP and presenilin genes (for reviews, see Refs. 1,5,6). Despite considerable efforts devoted in this area and current models have developed some AD lesions, a fully successful animal model, especially for sporadic AD, the vast majority of all AD cases, has yet to be developed (1,5,6). In this context, it is necessary to explore other avenues to achieve this goal.

3. DISCUSSION

Rational design of the strategy for animal model development for AD depends upon the identification of the primary pathogenic defect(s) particularly in the early phase of the disease process. Whereas AD pathogenesis remains elusive, we have recently proposed that abnormally low concentrations of intracellular calcium, or a functional down-regulation of calcium mobilization, could occur early in sporadic AD. This defect may underlie or contribute to the formation of plaques, tangles and memory deficits (7-9).

Two important corollaries, among other things, can be inferred from this hypothesis. The first corollary is that administration of many calcium agonists, in principle, would have protective effects in the individuals at-risk to AD, as discussed (8,9). The second corollary is that if the calcium deficit plays a key role in AD pathogenesis, then an artificial diminution of intracellular calcium levels in the experimental animals by the use of calcium antagonists would be expected to induce, to a certain extent, the appearance of amyloid plagues, neurofibrillary tangles and memory deficits, defining lesions of the disease. Obviously, this latter prediction is not only a direct test for the hypothesis, but if proven, might also offer a promise for creating a much-needed animal model for sporadic AD.

Could this goal be approached through such a

direct route? Here, based on a review of the literature, we consider the characteristics of some existing models and the known effects of three groups of calcium antagonists which might be used as potential agents for animal model development.

3.1. Neurotransmitter receptor inhibitors

The first group of the agents includes various inhibitors of neurotransmitter receptors. Several neurotransmitter receptors are known to be essential for long-term potentiation (LTP), a process that is believed to be responsible for at least some types of memory formation (10). For example, it has been established that N-methyl-D-aspartate (NMDA) receptor, expressed in high density in cortical and hippocampal regions, is crucial in the initiation steps of learning and memory, performance of spatial, working and passive avoidance memory tasks of LTP (10-12). It is well-known over the past decades that NMDA receptor inhibitors (such as AP5, MK-801 and CGP37949) can block LTP and cause learning impairments in the animals (13-15). Although the mechanism of actions of these inhibitors has mostly been attributed to their disruption of specific ligand binding, it is clear that NMDA receptor is a major Ca^{2+} channel in the cells (10). As such, the mode of actions of its inhibitors should necessarily involve the disruption of Ca^{2+} channeling, that is, directly or indirectly as "calcium antagonists". Indeed, the calcium antagonizing effects of some of the inhibitors have been experimentally observed (16,17).

In addition to NMDA receptor, inhibition of acetylcholine receptor and AMPA receptor (both are channels for cations including Ca^{2+}) has also been found to result in cognitive impairments similar to NMDA receptor in murine models (18-20). Again, the mode of actions of the inhibitors has mostly been attributed to the specific receptors. We however consider these findings are consistent with the knowledge that intracellular calcium *in vivo* is supplied by many channels (10), and thus blocking any one of them would perhaps be expected to give rise to a "calcium deficit" condition in the animal's brain.

3.2. Calcium channel blockers

The second group of the agents includes a large number of calcium-channel blockers, such as nimodipine and nifedipine (21). But, their proposed use for animal model would be controversial because nimodipine has been shown to somewhat improve the conditions in advanced AD patients and in aged rodents (22,23). However, as we proposed (9), calcium rises are a late-stage event in AD progression, therefore, such effects of nimodipine would not necessarily conflict with our proposal (i.e., calcium deficits in the early phase of AD; the time difference between the early and late phases can be as long as twenty years in humans). In supporting of our contention, it is important to note that Maurice *et al.* (24,25) and others (26) have repeatedly reported that nimodipine, in young animals, can induce learning impairments. Nifedipine has also been found to have similar effects (27).

Though continuing controversies exist, we believe that these observations are in line with the indispensable and even sufficient roles of calcium in LTP, as demonstrated by Lynch *et al.* (28) and Malenka *et al.* (29). This role of calcium predicts that inhibition of calcium entry, through a variety of routes, would result in memory deficits. Because inhibition of these receptors all leads to the similar consequences in memory, and because calcium can be considered as a common effect that all of these inhibitors manifest, it should be acceptable that it is the calcium imbalance, rather than the distinctive role of a specific receptor, that more consistently explains the mechanism of actions of these receptor/channel inhibitors.

3.3. Plaques and tangles

While these results appear to support our view, a crucial question remains unanswered. Should the cognitive impairments, mostly short-term and reversible events in these young animals, be considered to represent those of AD, a chronic, age-related and irreversible disorder? The answer to this question would largely depend upon, in our opinion, whether or not the memory impairments in these animals would be accompanied by the appearance of plaques and tangles, hallmark lesions for diagnosis of AD (1-3). Because the reported impairments are mostly observed after an acute treatment, it seems unlikely that the impairments in the current models are directly associated with the plaques and tangles.

Here we propose two modifications for improving the current paradigms to further test this approach. First, because the formation of plaques and tangles is a slow process in humans, it is plausible to speculate that the histological lesions similar to plaques and tangles might eventually appear as a result of a "repetitive and prolonged" application of the calcium antagonists. Second, because calcium in cells is supplied by many calcium channels, it is likely that a "cocktail" of several inhibitors of calcium channels and/or neurotransmitter receptors may be more effective than a specific one in inducing the AD-like lesions in the animals. It remains to be tested whether a combination of these modifications, possibly aided by other fine-tuned improvements, would lead to more prominent manifestations of AD pathologies in the animals.

It is noteworthy that the functional integrity of certain calcium channels may be more important than others in AD (perhaps relevant to their cell-type and subcellular specificity; for example, presenilins)(9). Given the paucity of the available information today regarding the target specificity of many calcium antagonists, considerable efforts by trials and errors may be needed before the most effective ones can be identified. Conceivably, the histological lesions, if appearing in these animals, should be expected to be at most "similar" to the early stages of the accumulating process of Aß and tau. It might be unpractical to expect that fully matured plaques and tangles, precisely as those in advanced AD patients, would be reproduced within a moderate length of time in rodents, because of the extraordinary time frame it takes for those lesions to develop in humans. Furthermore, the amino acid

differences between the human and rodent Aß sequences (30) may give rise to a distinctive structure and morphology of the amyloid lesions in the animals.

3.4. Anti-schizophrenia drugs

The third group of potential agents for animal model development might be, somewhat unexpectedly, certain anti-schizophrenia drugs. We first consider a potential relationship between AD and schizophrenia.

Intracellular calcium, or Ca^{2+} signaling pathway, is a central factor in brain function and is most tightly maintained in the body. As such, its alterations in either direction (high or low) would be expected to be pathological. On this background, we reasoned that if a calcium deficit is involved in AD which is manifested by cognitive deficits, then an "excess" of calcium levels, if occurs in human brain, might be expected to cause a condition somewhat opposite to that in AD, that is, perhaps an "overactivation" of cognitive function. Such a condition would be reminiscent of some clinical symptoms of schizophrenia (paranoid)(SP). SP is characterized by excessive neurotransmitter release (particularly dopaminergic system)(10).

Several years ago, Etienne and Baudry (31) noted an inverse relationship between these two disorders, in part because symptoms of SP patients usually improve with age, and SP population exhibits lower incidence of developing AD in late life. Here we consider additional reports in the literature which seem to strengthen this view. First, neurotransmitter release (dopaminergic as well as other systems affected in SP) is quantitatively controlled by intracellular calcium levels (10). It is thus reasonable to assume that the excessive neurotransmitter release in SP should be related, at least in part, to an excess in calcium levels. Second, amphetamine, a drug that induces paranoid psychosis indistinguishable with symptoms of SP, appears to exert its effects via calcium elevation (32). Third, it has been reported that there is an increase of intracellular Ca^{2+} levels in the peripheral cells from SP patients (33), and that calcium channel blockers (nimodipine and verapamil) can alleviate the symptoms in some patients (34,35).

Furthermore, by analogy to the proposed role of hormones in AD (8), higher calcium levels in SP may be partly due to an excessive calcium mobilization, which in turn may be related to higher hormone levels. In accord with this possibility, SP is a disorder typical of late adolescent or young adult (36) [when their hormone levels reach the peak (37)]. In contrast, AD is a disorder in aging [when the hormone levels drop to the lowest (37); it is also possible that other calcium agonists such as growth factors may fluctuate similarly during life].

If this consideration is correct, then it may be inferred from it that the pathophysiology of SP may involve, among other things, an abnormal "up-regulation" of intracellular calcium levels or its mobilization, although some important questions remain unanswered [e.g., why dopaminergic and cholinergic systems are particularly vulnerable in SP and AD, respectively (12)]. If further confirmed, it would be of interest to consider these two disorders representing, in our view, the pathological consequences in humans that are partly related to intracellular calcium imbalance, but in opposite directions. Furthermore, it should be emphasized here that the concept of higher calcium levels in SP is fundamentally different from the calcium rises in the late stage of AD. The former, in theory, should be mild, non-destructive and reversible (functional up-regulation), whereas the latter is dramatic, destructive and irreversible (entire system collapse)(8,9).

To our interest, the calcium states in SP would suggest that some medications that can improve SP symptoms of the patients might exert their effects in part by diminishing calcium levels. Indeed, it has been documented that some anti-SP drugs can reduce calcium levels in cultured cells (38). Yet, many anti-SP drugs cause side-effects in humans such as hypotension, a condition that is related to calcium imbalance (21).

Could these anti-SP drugs in the body induce histological lesions characteristic of AD? In this regard, Wisniewski *et al.* (39) have reported that frequent users of chlorpromazine and trifluoperazine (two widely used neuroleptics) display a higher tendency of developing neurofibrillary tangles and AD-like symptoms in their late years. It has also been found that these drugs can induce hyperphosphorylation of tau similar to that in the neurofibrillary tangles (40). These two drugs are calmodulin inhibitors (40), and calmodulin mediates the activity of calcineurin (41), as well as many other calcium-dependent enzymes. Together, these observations raise the possibility that these drugs, perhaps many more in the category, may indirectly interfere with the calcium-dependent processes essential for cognition. Therefore, they might be considered as potential agents to induce AD-like lesions in the experimental animals

4. FINAL REMARKS

Several lines of evidence reviewed here suggest that a possible route to create an animal model for sporadic AD might be by decreasing the intracellular calcium levels. Despite the controversies and unanswered questions, this route is worth considering. It also appears to us that a successful model for sporadic AD might be created by improving the existing ones that target various neurotransmitter receptors and calcium channels.

Compared to this route, some other current approaches may have intrinsic limitations. For example, APP mutant-based models have not developed neurofibrillary tangles; and a solely ApoE4-based model may not develop full AD pathologies within the animal's lifespan [late onset in humans (42)]. A successful presenilin mutant-based model, though will exhibit early and severe AD pathologies, would however be difficult to develop (requiring a "gene replacement" paradigm)(8,9), and may not fully represent the sporadic AD conditions (e.g., its overproduction of Aß42/43).

AD is manifested by cognitive impairments, hence this lesion should be considered as the primary criterium for model development and evaluation. It is of interest to note that while severe cognitive impairments have not been reported in most of the current "AD models" (1,5,6), some other models in which cognitive impairments are prominent (13-15,24-26), have not however been generally accepted as "the models for AD".

It is important to note that the animal model experiments can also be used to examine the current "calcium hypothesis", which proposes that elevated calcium levels are responsible for neurodegeneration in AD (23). This hypothesis can be tested by the administration of calcium agonists such as glutamate, estrogen, calcium channel activators (as a matter of fact, it is tempting to speculate that such models might mimic some conditions of SP). Presumably, these experiments can help to resolve the controversies around this key issue of AD research in the near future.

It should be kept in mind that the AD-type cognitive impairments, unlike many other human diseases which can be perfectly mimicked in animals, will not be fully and exactly reproduced in the models, because the higher intellectual function affected by AD is the monopoly of humans. For example, the loss of some long-term memories (e.g., the patient's own name) and logical thinking will be difficult to reproduce in rodents. Despite these limitations, an animal model that targets the early pathological lesion would allow hands-on studies on the biochemical and other aspects of AD in its dynamic progression. The model will also allow a screening of pharmaceutical compounds that may be able to compensate, perhaps more effectively than the current ones, for the deficient element, thereby offering a promise for the prevention or postponement of clinical AD in the aging population.

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