Calcium Hypothesis of Alzheimer’s Disease and Brain Aging

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The purpose of this volume, which represents the proceedings of a workshop with the same title, was to reevaluate the “Calcium Hypothesis of Brain Aging and Alzheimer’s Disease.” This exercise is warranted in light of new evidence that might support or refute the proposition that cellular mechanisms for maintaining the homeostasis of cytosolic calcium concentration ([Ca²⁺]) play a key role in aging and that sustained changes in [Ca²⁺] homeostasis could provide the final common pathway for the neuropathological changes associated with Alzheimer’s disease (AD).

The conference was held on December 15-17, 1993 in Bethesda Maryland. This was the second meeting on the same topic organized by the National Institute on Aging (NIA); the first one was held in October 1988 and published by the New York Academy of Sciences.¹ The calcium hypothesis, first formulated in 1982 and later published (1984),² was primarily aimed at stimulating research and redirecting the focus of studies towards cellular mechanisms of brain aging and Alzheimer’s disease. In 1989, the hypothesis was revised to accommodate new data and the increasing knowledge about the aging brain and Alzheimer’s disease.³ The proceedings of the present conference will likely result in a further refinement of the hypothesis.

Since 1984, the calcium hypothesis has flourished by gathering stronger evidence in its support as well as several articulate proponents.⁴ The evolution of the hypothesis has been an integral part of the recent history of the neurobiology of aging which has matured and emerged as a distinct area of study within the larger field of neuroscience. Now, there are approximately 3,000 scientists worldwide who are interested in research on the aging brain, dementia, and neurodegenerative diseases. The scientific questions being pursued currently are at the cutting edge of neuroscience; they are more concerned with the molecular mechanisms rather than descriptive studies of normal and pathological change in the aging brain. However, the neurobiology of Alzheimer’s disease, now more than ever, needs an infusion of new talent and expertise; also, new concepts, and perspectives. There are a number of compelling reasons for expanding the scope and improving the vigor and the quality of research on Alzheimer’s disease.

¹ Some of the discussions in this article are excerpts from previous public speeches, presentations, or publications. See references 3, 13, and 22.
Several studies have indicated that "aging" is one of the most important and consistent risk factors for AD. As an increasing proportion of the population survives beyond the age of 85 years, increasing numbers of individuals will be at risk for developing a dementia. It is estimated that in the United States, by the year 2050, between 7 and 14 million individuals will be affected by some form of dementia requiring care and institutionalization. The challenge of reducing the numbers of individuals with AD is made particularly difficult by the demographic trends in the age distribution of the U.S. population. The U.S. Bureau of Census data indicate that in 1980-1990 the "65 and over age group" increased at a substantially higher rate than did any other age group.

In recent years, with the "graying of America," it has become apparent that AD as a public policy issue is very complex. The rising cost of long-term care in recent years has made the economics of health care one of the most important issues in the array of major public health concerns. Alzheimer's disease, with its long clinical course, is perhaps the most significant disability requiring various forms of long-term care services in different settings ranging from home care to special nursing homes. It is estimated that the total annual direct and indirect cost of care for AD to the country as a whole is between 90 and 100 billion dollars. The cost of disability most immediately affects the families of AD patients, but ultimately has major economic impact on society at large by reducing or impairing potential productivity.

The burden of care to the families of AD patients is not just emotional stress and physical hardship but financial considerations as well. The long clinical course of the disease, its devastating effects on the ability of the patient to function independently, the psychosocial stress on the families, and the heavy economic burden associated with care have combined to raise public awareness and concern about the lack of any means to cope with this national problem. The discovery of safe and effective treatments is an urgent public health challenge.

Strategies of developing symptomatic treatments and care programs designed to delay the disabling symptoms and postpone institutionalization will not be adequate to overcome the demographic forces increasing the total number of affected individuals. Although it might appear premature, there is a need to develop strategies for determining preventive measures. To address this problem there is an urgent need for planned systematic expansion of basic research programs on the neurobiology of AD that are already underway, such as studies designed to identify selective risk factors, the search for the cause(s), and treatments designed to halt or reverse the clinical course of the disease. The papers in this volume provide new insights into the systems regulating [Ca^{2+}], as potential targets for developing treatments for AD.

One of the persistent scientific challenges facing AD research is the discovery of the cascade of events leading to AD and the establishment of a causal relationship between the disease and such variables as aging, genetics, clinical symptoms of dementia, neuropathological hallmarks, molecular lesions, loss of synapses, mechanisms of cell dysfunction, and mechanisms of the specificity of neuronal loss. To accommodate the rapid progress of research in AD there is a need to synthesize all the divergent pieces of data into a coherent story. The calcium hypothesis of aging and dementia as it evolves and gathers more supporting evidence has the potential of unifying and accounting for most of the available data on AD.
PARAMETERS FOR A UNIFYING HYPOTHESIS

This volume represents a compilation of recent studies buttressing the calcium hypothesis. However, the relative merits of the hypothesis and its ultimate utility need to be evaluated in light of the following essential parameters for a unifying hypothesis for AD.

First, a unifying hypothesis for AD must be parsimonious. It must provide simple explanations that are plausible on the basis of well established biological processes.

Second, the hypothesis must account for the clinical symptoms of Alzheimer's disease including cognitive and other behavioral changes on the basis of neural mechanisms of cell dysfunction and cell death. An attempt to explain the cause(s) of AD needs to address not only the biological mechanism of neural dysfunction but also the cause(s) of the behavioral manifestations of the disease. A number of the noncognitive behavioral symptoms associated with the disease, which often cause great stress to care providers, may or may not have a direct relationship to the primary cause(s) of the disease. Still, there is a need to determine the neurobiological basis of secondary behavioral and psychiatric symptoms associated with the disease. Therefore, a unifying hypothesis must seek to explain these secondary changes.

Third, the hypothesis must provide an explanation for the specificity of neuronal dysfunction, synaptic loss, and neuronal death. One of the important characteristics of AD is that specific neural systems, such as the cholinergic system, are affected. The hypothesis must provide answers to such critical questions as: why the cholinergic system is particularly vulnerable; why only specific types of cells are affected; and why particular regions of the brain and specific structures, such as synapses, and dendrites are affected?

Fourth, the hypothesis must explain the heterogeneity of AD. At present, no one can say with assurance if AD is a single disease or a complex syndrome with many subtypes and varieties of patterns in its manifestations or if it is many different diseases with similar clusters of symptoms. The heterogeneity of the disease is typified by the many aspects of its presentation including age of onset, duration, clinical course, types and patterns of neurological and psychiatric symptoms, response to treatments, and neuropathological lesions. A number of the critical scientific problems facing the field of AD research are directly associated with heterogeneity in the expression of this disease. Although during the last 14 years significant progress has been made in identifying and describing the different manifestations of AD, the underlying biological mechanisms of the heterogeneity still remain to be uncovered. The general problem of heterogeneity provides an unusually rich array of scientific opportunities for further research directions.14

The biological basis of heterogeneity most probably will be found in the interaction between genetic and other factors. The search for genes associated with various brain metabolic dysfunctions and abnormal processing of cytoskeletal proteins promises to be one of the most productive lines of research in uncovering the cause(s) of this disease. The recent findings of mutations in the amyloid precursor protein (APP) gene have created great excitement and given special impetus to the search for other loci and other mutations.15 Unfortunately, identifying the locus and the nature of the mutation will not be sufficient; this field still needs to determine the functional consequences of these mutations on protein synthesis, structure, function, and behav-
ior, that is, protein-protein or protein-membrane interactions. Ultimately, we need to determine if, and if so how, these mutations lead to cell dysfunction and/or cell death.

Presently, it is not clear if mutations in genes are a necessary and sufficient condition to cause the disease or if one or more additional biological insults are necessary to trigger the degenerative processes of AD. If a relationship exists between genetic predisposition for AD and environmental factors or systemic metabolic dysfunctions, the mechanism for the interaction between genes and such triggering factors is not well studied. We in the field need to know how changes in metabolic functions, the immune system, neuroendocrine factors, infectious agents, and exposure to toxins influence the expression of the disease or modulate its course.\textsuperscript{14}

Fifth, the hypothesis must demonstrate a relationship between known risk factors for AD and the neurobiology of the disease. Although there is no evidence that aging per se causes AD, it is strongly associated as one of the major risk factors for this disease. A history of severe head trauma which leads to loss of consciousness has also been found to increase the risk for AD. A third risk factor is a family history of AD in a first degree relative, which increases the odds of developing AD three to four times. These three risk factors—aging, head trauma, and genetic predisposition—meet the generally accepted epidemiological criteria for causal factors, because they provide a plausible biological explanation and their effects are strong and consistent. A recently discovered gene linked to AD is the apolipoprotein E (ApoE) gene on chromosome 19, which has been associated with many late onset familial cases of AD as well as sporadic cases in the over-60 age group.\textsuperscript{16} The ApoE gene not only appears to have a strong and consistent relationship with AD but also offers a plausible biological explanation for its role in the pathological processes of AD. It is very likely that AD is caused by complex interactions among biological variables such as genetic predisposition and environmental, cultural, or educational factors.

Cross-cultural epidemiological studies of risk factors as extensions of the search for cause(s) are essential for teasing out the complex interactions of genetic and epigenetic factors in causing AD. Recent epidemiological investigations have suggested that lack of education might be a risk factor for AD.\textsuperscript{17} At the present it is not clear if educational attainment may be a surrogate marker for synaptic density or some other neural developmental state. If these observations are confirmed, they may provide clues to possible mechanisms of heterogeneity by linking risk factors and other life experiences to changes in synaptic density or synaptic reserve. Further systematic epidemiological investigations of comorbidity of AD with other neurodegenerative diseases, systemic metabolic disorders, vascular diseases, health history, dietary habits, occupation, exposure to toxins, and life experiences such as education may provide clues for testable hypotheses concerning the cascade of events leading to the disease.\textsuperscript{14}

Sixth, the hypothesis must relate the mechanisms of cell dysfunction and cell death not only to the clinical symptoms of AD, but also to the key pathognomonic features of the disease and the subtle molecular lesions associated with structural and functional changes in the AD brain. The precise reasons for and the cascade of events preceding cell dysfunction and death are not known. However, it is well established that the synthesis and release of several neurotransmitters, particularly acetylcholine, are compromised in AD.\textsuperscript{18} It is also well established that several structural and functional changes are found in AD brains including loss of synapses,
pruning of dendrites, changes in membrane constituents, regulation of intracellular ions, processing of transmembrane proteins (eg, amyloid precursor protein), abnormal processing of various other cytoskeletal proteins (eg, Tau), and metabolic changes affecting cellular energy utilization. At present it is not clear which one or combination of these events initiates the process of cell dysfunction and cell death.\textsuperscript{14}

In summary, the following observations have been linked to AD. Although some of these linkages may not be as strong as others, all should be incorporated into any hypothesis concerning AD:

- aging, head trauma, and lack of education as risk factors and ApoE as a major susceptibility gene;
- clinical, neuropathological, and genetic heterogeneity of the disease;
- specificity of cell dysfunction and loss;
- neuropathological changes including abnormal synthesis/processing/accumulation of cytoskeletal proteins; neurotransmitter deficits (particularly cholinergic); dysfunction of glucose metabolism; loss of dendrites-synapses-neurons; extra-neuronal changes involving microvessels, glia, glucose transporters, and neuroendocrine and neuroimmune systems;
- the 5-20-year clinical course of the disease;
- symptoms of the disease including cognitive and other behavioral changes.

Any useful and testable hypothesis of AD causation needs to accommodate and account for these observations. If it cannot explain the role of any of these observations, then it must provide convincing arguments that the observation in question is an epiphenomenon.

**ESSENTIAL FEATURES OF THE CALCIUM HYPOTHESIS AND CHALLENGES IT MUST MEET**

At present, the fundamental biological underpinning of AD is thought to be a gradual dysfunction of neurons, eventually leading to cell death. One of the major challenges remaining in the search for etiology(ies) of AD is the proximal cause of cell dysfunction and cell death. During the last 14 years, a rich array of ideas and scientific leads has emerged, including endogenous and exogenous toxins, infectious agents, abnormal proteins, deficits in growth-promoting factors, corticosteroids, membrane changes, calcium homeostasis, and deregulation of proteolysis. Clearly there are many mechanisms each involving several steps in the process of cell dysfunction and death. Although each of these various insults or abnormal cellular processes potentially could influence cell functioning and survival, it is important to determine which ones are critical in causing the degenerative process of AD. Some of the crucial scientific questions that need to be answered are:

- What initiates the process of cell dysfunction, when does it start, and what are the key steps in the cascade of events leading to cell death?
• What factors determine or regulate the specificity of cell dysfunction and neuron death?

• What are the key interactions between various cellular components, such as changes in membrane structure affecting the behavior of membrane-bound proteins; changes in the primary or secondary structure of proteins affecting their tertiary structure and interactions with other proteins; or abnormally cleaved protein fragments that form potentially toxic aggregates?

KEY ELEMENTS OF THE CALCIUM HYPOTHESIS

The current version of the calcium hypothesis proposes six interrelated postulates. First, it proposes that cellular mechanisms that regulate the homeostasis of cytosolic free calcium ion \([\text{Ca}^{2+}]\) play a critical role in brain aging and the neuropathology of AD, and that altered \([\text{Ca}^{2+}]\) might account for a number of age-related changes in neural function and AD-associated neural dysfunctions.\(^{2,4}\)

Second, it suggests that the cellular mechanisms underlying AD neuropathologies are part of a continuum of molecular processes associated with the developing nervous system and aging-related changes. The neural dysfunction, dendritic pruning, loss of synapses, and neural loss observed in AD essentially involve the same molecular mechanisms as those associated with programmed cell death in the developing nervous system, regulation of neurite elongation and growth cone motility, regulation of neuroplasticity in the adult brain, and synaptic turnover/synaptogenesis in the normal aging brain.\(^{13}\)

Third, it postulates that the plasticity of neuroarchitecture is regulated by a functional equilibrium between molecular mechanisms promoting growth/regeneration and those processes that control regression/degeneration. The intracellular calcium concentrations play a central role in modulating the direction of this equilibrium governing regeneration and degeneration. The \([\text{Ca}^{2+}]\), in particular cellular compartments, through various intermediary regulators, governs dendritic arborization, stability, regression, pruning, or complete elimination. A parsimonious explanation is that the neuronal dysfunction, dendritic regression, and loss of synapses associated with AD reflect disregulation of normal processes that are essential for the development or normal function of the adult brain.\(^{13}\) The disruption of this equilibrium between growth and regression could be the consequence of the breakdown of systems that control and regulate the homeostasis of \([\text{Ca}^{2+}]\); thus, a small but chronic elevation in \([\text{Ca}^{2+}]\), could shift the equilibrium in favor of degeneration.

Fourth, it proposes a systematic interaction between the amount of the perturbation in the \((\Delta[\text{Ca}^{2+}]\)) and the duration \((\Delta T)\) of the deregulation in the calcium homeostasis, so that the product of the two variables is a constant:

\[
K = \Delta[\text{Ca}^{2+}] \times \Delta T.
\]

This relationship suggests that a small change in \(\Delta[\text{Ca}^{2+}]\), that is sustained over a prolonged period \(\Delta T\) will result in similar cellular damage as will a large change in \(\Delta[\text{Ca}^{2+}]\), over a short period \(\Delta T\), or;
A calcium channel that is leaky or an extrusion pump that loses its efficiency over the course of several years could potentially cause as much damage chronically as could a massive but acute insult, such as one that occurs in stroke.\(^3\)

Fifth, it promotes the concept that the calcium-mediated signaling system and regulation of \([\text{Ca}^{2+}]\), homeostasis are part of the final common pathway for the cellular changes leading to neuronal dysfunction and cell death. The final common pathway concept accounts for several alternative mechanisms through which the regulation of \([\text{Ca}^{2+}]\), can be disrupted. These include changes in ion channel functioning or formation of new channels; changes in membrane structure altering the functioning of transmembrane proteins; and alterations in the behavior of calcium binding proteins, extrusion pumps, buffers, and sequestration.\(^{19,20}\) Also it explicitly suggests that there could be many different antecedent factors, each involving a separate cascade of events, but all leading to disruptions in calcium homeostasis. The downstream consequences of destabilizing calcium homeostasis could also result in different outcomes depending on the particular cellular compartment involved and the amount and duration of the changes in the \([\text{Ca}^{2+}]\).\(^{21}\) Some of the possible variables in the alternative cascade of events leading to neuronal dysfunction and death are listed in Figure 1 under the headings of: I. Antecedent Variables; II. Mechanisms for Maintaining \(\text{Ca}^{2+}\) Homeostasis; and III. Subsequent Potential Degenerative Processes.

Sixth, it proposes that age-related and AD-associated changes in the brain may not be due to a single event or insult, but are brought about by a series of different antecedent events occurring, in combination or sequence, over a long period. The concept of the “AND gate” allows for several postulated etiologic factors working in combination and sequentially to provide various plausible paths or cascades of events all leading to the final common result of dis-regulating calcium homeostasis.\(^22\)

In discussing the cause(s) and effect relationship of this disorder, it might be useful to use the analogy of an “AND gate” (a digital logic element) to conceptualize the cascade of events or the process of timing and interaction of multiple potential etiological factors. As is well known, an “AND gate” as a Boolean logic element can have two or more inputs (switch) and one output. Each input as a switch can be only in one of two states, “ON” or “OFF”; the output also can be only in the “ON” or “OFF” state at any given time. To get an output it is necessary that certain well-specified rules (a truth table) be followed. That is, a discrete event has to take place at each of the inputs (all switches must be “ON”) of the “AND gate” for an output to occur. The timing of the events at the various inputs can vary widely, but once an event takes place, its effect must linger on. The basic rule is that an event has to be present at all inputs at the same time for an output to occur. Now, transposing this model to AD, one can conceptualize an “AND gate” with the number of the inputs ranging between 2 and N. The simplest gate would have only two inputs, that is, a gene mutation and another event to trigger the gene. A more complex hypothetical model “AND gate” for AD could have as inputs or switches events such as:

- genetic mutation(s) predisposing one for various metabolic disorders, pathologies, or biochemical abnormalities, such as APP processing, ApoE functioning, glucose transporter, or mitochondria defect;
FIGURE 1. This illustrates the flow of the possible cascades of events leading to cell dysfunction and death through the final common pathway of disrupting Ca^{2+} homeostasis.
• cerebral microvascular pathology which affects the transport of glucose and/or O₂, thus creating a chronic deficiency in perfusion;
• changes in oxidative metabolism due to enzymatic deficiencies; exposure to exogenous and/or endogenous toxins, such as glutamate;
• changes in the structure and function of membranes and/or proteins in systems that regulate [Ca²⁺], or maintain homeostasis;
• other changes in brain chemistry that affect glucose metabolism, neurotransmitter synthesis, neuroendocrine functioning, and neuroimmune system.

According to the "AND gate" model, these events do not have to occur at the same time, but each could produce a lingering effect that eventually might lead to cell loss and clinical symptoms. Therefore, to understand the etiology of AD it might be important to examine evidence showing a relationship between a particular variable, such as toxins and AD, in the context of other preceding critical physiological events during the lifetime of the patient which may have predisposed the vulnerability of the brain to the disease. For example, chronic low level hypoglycemia could modulate the toxicity of glutamate.

CONCLUSION

The Calcium Hypothesis of Aging and Dementia conference and this volume have focused on the scientific issues concerning postulated molecular mechanisms underlying the disease and potential targets for treatments. The conference and this volume have both used the calcium hypothesis as a convenient heuristic tool to help conceptualize the neurodegenerative processes in brain aging and dementia.

The calcium hypothesis has been revised twice; no doubt the proceedings of this conference and this volume will lead to a further revision. Any hypothesis, to maintain its utility, must be tested constantly and revised in light of new data. The best statement about the value of scientific speculation is attributed to Henri Poincare who, in a commentary about the value of science, is to have said, "There is a value in developing an hypothesis... There is a hierarchy of facts. Some have no reach. They teach us nothing but themselves. The scientist who has ascertained them has learned nothing but a fact and has not become more capable of foreseeing new facts. Such facts, it seems, come once but are not destined to reappear. There are, on the other hand, facts of great yield. Each of them teaches of a new law. And since a choice must be made, it is to these that the scientist should devote himself.''

It is our hope that this volume will tempt many to look for the whole story beyond the immediately available data and speculate about how the data might best fit together in solving the puzzle that is Alzheimer's disease.

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