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Ionic and Signal Transduction Alterations in Alzheimer's Disease

Relevance of Studies on Peripheral Cells

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Abstract

Several lines of evidence indicate that Alzheimer's disease (AD) has systemic expression. Systemic changes are manifested as alterations in a number of molecular and cellular processes. Although, these alterations appear to have little or no consequence in peripheral systems, their parallel expression in the central nervous system (CNS) could account for the principal clinical manifestations of the disease. Recent research seems to indicate that alterations in ion channels, calcium homeostasis, and protein kinase C (PKC) can be linked and thereby constitute a model of pathophysiological relevance. Considering the difficulties of studying dynamic pathophysiological processes in the disease-ridden postmortem AD brain, peripheral tissues such as fibroblasts provide a suitable model to study molecular and cellular aspects of the disease.

Index Entries: Alzheimer; ion channels; calcium; PKC; fibroblasts; peripheral cells.

Introduction

Over the last two decades, research on cellular and molecular aspects of Alzheimer's disease (AD) has resulted in a significantly improved understanding of this disease. Particular achievements have occurred in the areas of molecular genetics and the biology of the amyloid precursor protein (APP), including the potential role of the latter as a central player in AD pathophysiology *(1–6)*. Despite the progress and the data accumulated, the understanding of the pathophysiological framework remains incipient. Part of the difficulties in studying the pathophysiology of AD arise from the fact that dynamic processes are difficult to

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assess in the brain as a number of signal transduction mechanisms are altered by postmortem events (7). Furthermore, AD brain tissue usually becomes available after a long period of disease, i.e., at an advanced pathological state. Therefore, significant effort has been devoted to alternative approaches (7–11). Skin fibroblasts and blood cells can be obtained easily from patients and at any stage of the disease. These cells, specifically fibroblasts, can be used multiple times, stored, shared with other researchers, and so forth (7,12). In addition, there is precedent for the use of fibroblasts in the study of the pathophysiology of other neurological disorders (7,13,14). Many of the molecular genetics studies of AD have also been done using fibroblasts (2). The fact that fibroblasts are not the primary target and are remote to the site of injury may constitute another advantage. Pathological changes may be limited in fibroblasts and perhaps represent early events, allowing the study of the pathological process in a more pure form, before the occurrence of a variety of nonspecific generalized changes (15,16). Despite the many advantages peripheral cells have, including the fact that some express neuronal markers (such as platelets), many assumptions are made when we try to understand brain pathology by looking at fibroblasts or lymphocytes. Peripheral cells do not constitute a perfect model and certainly not all observed changes would have equivalent expression in the brain. Peripheral cells are, however, useful (if not the only alternative) to study complex disease processes such AD, before the post-mortem stage.

A host of cellular and molecular changes ranging from nucleic-acid defects to second-messenger alterations have been described in peripheral cells of AD patients (7–9,11,12). Some alterations have also been observed in neuronal animal cells and brain (17–20). Although some degree of controversy regarding these changes has limited their acceptance (7), there are a number of isolated findings that begin to suggest that such changes might be both related and relevant. This review will summarize and discuss primarily the findings in peripheral cells, concentrating on ionic and signal transduction events, and will attempt to identify those that are more likely to be relevant in a pathophysiological context.

Ion Channels

Ion channels play a fundamental role in the physiology of all types of cells (21,22). They are also targets of various pathological processes (22,23). Their study in the context of AD is recent and more limited than calcium studies. Potassium channels seem particularly interesting to consider given their implication in memory and learning processes as suggested by many invertebrate and some vertebrate studies (24–28). The ion channel-forming properties of β-amyloid and its modulatory role on K^+ channels may also shed light onto AD pathophysiological mechanisms (29).

Potassium Channels

The first direct potassium channel study related to AD was conducted on fibroblasts (there was an earlier apamin-binding study on brain tissue, discussed in forthcoming paragraphs) using the patch clamp technique (30). The basic finding was the functional absence of an approx 113 pS K^+ channel in fibroblasts from AD patients (31). It was also established that this was not a generalized defect because other K^+ channels were functional in both AD and control cells. The patch clamp findings were confirmed in a large number of cells and cell lines by indirectly assessing K^+ channel function by measuring intracellular calcium changes (31). Because the 113 pS channel is sensitive to external blockade by tetra-ethyl ammonium (TEA), the resulting depolarization opens voltage-dependent calcium channels, causing rapid and transient calcium elevations in control cells. Because the 113 pS channel is not present (at least, functionally absent) in AD cells, there is no significant depolarization and the calcium elevation is minimal (compared to control cells) or entirely absent. It was determined that this calcium ele-

vation depends on the presence of extracellular calcium because the response was not observed in calcium-free medium *(31)*. Depolarization induced with elevated external KCl (50 mM), caused comparable $[Ca^{2+}]_i$ elevations in cells from control and AD patients, suggesting that voltage-activated calcium channels are functional in fibroblasts from AD patients and are not the primary site of defect *(31)*. Thus, the TEA-induced calcium response, or lack of it, constitutes an additional measurement or assessment of the functional state of the 113 K^+ channel. This method, although indirect, has the advantage over patch clamp of allowing simultaneous testing of a larger number of cells. In addition, it measures the combined activity of all 113 K^+ channels in a given cell, not just the channel contained in the patch. Of pathophysiological significance is the observation that β-amyloid (Aβ), the main component of neuritic plaques and likely to play a critical role in the pathophysiology of AD *(1,3,6,32,33)*, was capable of inducing a similar K^+ channel defect in cells from normal donors *(34)*. Fibroblasts from control individuals, known to have functional 113 pS channels and normal TEA-induced $[Ca^{2+}]_i$ elevations, were treated for 48h with low concentrations (10 nM) of β-amyloid 1–40. This concentration of β-amyloid does not cause an increase in basal Ca^{2+} levels. The electrophysiological analysis revealed, however, that the 113 pS channel was missing in patches from β-amyloid treated normal cells. The 166 pS channel, not affected in AD fibroblasts, was also not affected by the treatment. Thus, the β-amyloid treated fibroblasts presented an AD-like phenotype for potassium channel function. Moreover, only the 113 pS channel was affected, indicating specificity and not a generalized toxic effect of soluble β-amyloid. Accordingly, TEA responses were also abolished or greatly reduced in β-amyloid treated cells, confirming that the treatment induces a K^+ channel dysfunction similar to that naturally observed in AD fibroblasts *(34)*. This alteration could have resulted from external actions or intracellular accumulation of the peptide, which has since been shown to undergo internalization by cells in culture *(6,35)*. The effect also appears to be because of soluble β-amyloid. The overall conditions and type of β-amyloid (1–40) do not seem to favor fibril formation. Because such an event was not strictly monitored, a contribution of the fibrillary form to the phenomenon cannot be completely ruled out. More recently, Bhagavan et al. reported that a novel PKC activator, with improved isozyme selectivity, induces restoration of K^+ channel function in fibroblasts from AD patients *(36)*. Interestingly, the same compound enhances the α-processing of amyloid precursor protein (APP), thus reducing the proportion of amyloidogenic fragments *(37)*.

The potential involvement of K^+ channels or K^+ channel dysfunction has also been documented in blood cells of AD patients. It had been previously established that TEA (70 m*M*) produced a >50% reduction of the phytohemagglutinin (PHA)-induced calcium response in lymphocytes. Depolarization by elevated KCl produced similar effects *(38,39)*. Bondy *(40)* has recently reported a significant reduction of the TEA inhibition of the PHA-induced calcium response in lymphocytes from AD patients. This treatment allowed an almost perfect separation between controls and AD patients. All controls save one (n = 23) exhibited more than 30% inhibition, whereas only three of the AD (n = 20) patients had inhibitions >30%. In a comparable study de Silva, et al. *(41)* using platelets demonstrated a lack of/depressed Rb^+ efflux inhibition (induced by either thrombin or ionomycin) by a cocktail of the K^+ channel blockers, apamin, and charybdotoxin. Nine out of twelve AD patients had abnormal inhibition (<15%), whereas only 3 out of 11 controls had <15% inhibition. Therefore, these reports support the notion of defective K^+ channel function in AD with pathophysiological and diagnostic implications. It also suggests that these channel defects may be present in various cell types and not only in fibroblasts. A few reports, however, have failed to fully reproduce the TEA effect in fibroblasts. Failli et al. correctly identi-

fied most of the controls and AD but there was significant overlap *(42)*. This study, compared to the original report, contains some important methodological differences. These include serum deprivation, longer post-seeding interval, uncontrolled cell density, lower concentration of agonist, and perhaps idiosyncratic differences of some of the familial cases. These methodological differences between studies could account for most of the discrepancies in findings. They did reproduce the effects of β-amyloid (the 25–35 form) in rendering defective potassium channel function in control fibroblasts *(42)*. Matsuyama et al. reported no differences between AD and controls using photon emission technology, which measures integrated responses of cell populations *(43)*. It has been shown that single-cell analysis is required to distinguish between AD and controls *(31,70,71)*. Therefore, it is not surprising that a method that integrates the fluorescence of cell populations (many of which do not exhibit responses) failed to separate the experimental groups. Insufficient methodological details makes it impossible to determine if additional technical differences contribute to the discrepancies observed.

The effect of β-amyloid and related peptides originally observed in fibroblasts has also been found in neuronal cells. Good et al. *(17)* reported that acute application (delivered close to the cell) of β-amyloid 1–40 (also 1–28 and 1–39 fragments) caused a marked decrease in rat hippocampal currents, particularly the fast-inactivating (I_A) component. Noticeable changes were observed at an estimated concentration of 10 μ*M* for the I_A. The observed effects were reversible upon removal of the peptide. Other ion channels (Na^+, Ca^{2+}) and other K^+ channels were unaffected. The concentration of β-amyloid used in this study was significantly higher than in the original *(34)* and the Failli *(42)* reports. In this context, it is also worth noting that instead of incubation for a prolonged period, Good et al. *(17)* used acute application. Therefore, they achieve the effect with a higher concentration but by exposing the cells for a significantly shorter period of time. It is also important to note that given the application method, the exact effective concentration remains undetermined. Despite these differences, the selective effect of β-amyloid on specific K^+ channels was a common occurrence in fibroblasts and culture neurons. A related study *(44)* explored the effects of soluble (secreted) APPs on K^+. In concordance with the previously mentioned studies, the effect was opposite to those for β-amyloid. The K^+ currents were enhanced after bath application of APPs in the nM range in a dose-dependent manner. Single K^+ channel measurements confirmed the effect by showing a dramatic increase in the open probability of the channel after APPs application. The effects of APP 695 and APP 751 were similar. The use of pharmacological tools suggested that cGMP and protein dephosphorylation mediate the effects of APPs on K^+ channels. One report using freshly isolated lymphocytes *(45)* did not find significant differences in K^+ currents (measured by whole-cell patch clamp) after bath treatment with β-amyloid 25–35. Five days exposure to the 1–40 peptide also was without effect on maximal K^+ conductance. Cell-specific differences and cell type could explain this apparent discordant result in lymphocytes. More importantly, it should be emphasized that the previously reported effects are not generalized for all K^+ channels and/or currents. For instance, Etcheberrigaray et al. *(34)* reported a selective effect on the 113 pS channel, whereas a 166 pS was unaffected. Similarly, in the report by Good et al. *(17)*, only the I_A current was clearly and most significantly affected by the treatment. Therefore, the lack of effect on the K_V current in lymphocytes might be equivalent to the lack of effect on the 166 pS channels or on other K^+ currents (different than I_A) in hippocampal rat neurons. Direct electrophysiological assessment of potassium channel function in human brain from AD patients remains not only impractical on technical grounds, but also unwarranted from a bioethical standpoint. Reports assessing K^+ channels in post-mortem human brain have been limited to binding studies using K^+ channel toxins. Ikeda et al.

(46) reported a selective reduction of apamin (a bee-venom toxin that blocks small K^+–Ca^{2+} channels) binding sites in the hippocampus of AD patients, particularly in subiculum and CA1 areas. The same authors *(47)* later reported increased apamin binding in temporal cortex (layers I–II, III–IV, V–VI), decreased glibenclamide (blocker of ATP-sensitive K^+ channels) binding in the same area (layers I–III, IV–VI) and a small decrease in L-type calcium channel binding. In the occipital cortex, only apamin binding was increased in layers I–II and V–VI. These intriguing results are complex and cannot receive a straightforward and unique explanation. Many factors, including the intrinsic difficulties of working with post-mortem tissues and the relatively small sample, may play a role. Interestingly though, these studies do suggest alterations of specific K^+ channels in selected areas. In addition, the later electrophysiological studies in fibroblasts and animal neurons directly identified specific K^+ channels that were affected by the disease process *(17,34)*. In summary, different groups, employing a variety of techniques and cell types have provided strong evidence that some types of K^+ channels may be affected by AD.

Other Membrane Conductances

An early report *(48)* showed that sympathetic bullfrog neurons treated with relatively high concentrations of β-amyloid 25–35 (10–100 μ*M*) exhibited a nonspecific membrane conductance increase within 3–4 min after drug application. This increase in membrane conductance partially reverted toward basal levels upon removal of the peptide. The authors also reported a disruption of the time-dependent Ca^{2+} and K^+ currents normally present in these cells. However, no specific channel was identified as the main current carrier. Another report *(49)* also showed the induction of irreversible nonspecific inward currents in rat cortical neurons after application of 10 nM β-amyloid. Galdizcki et al. initially reported a dose dependent (4.6–46 μ*M*) passive membrane conductance increase in pc12 cells treated for 12 h with β-amyloid 1–40 *(50)*. In an expanded communication *(51)*, the authors reported that there is a choline conductance increase in pc12 cells after β-amyloid treatment. There is also a report showing that the C-terminal fragment (CT105 or C100), which contains the β-amyloid fragment, induced nonselective currents in Xenopus oocytes *(52)*. These currents were blocked by an antibody against β-amyloid. A relatively recent review focusing on ionic effects of β-amyloid is available elsewhere *(29)*. In addition, a couple of articles have shown a modulatory effect of β-amyloid on calcium channels. Ueda et al. *(53)* reported that (10 μ*M*) β-amyloid potentiates calcium influx through L-type calcium channels in rat cortical and hippocampal neurons. Consistent results were obtained using patch clamp and $^{45}Ca^{2+}$ uptake techniques. Based on pharmacological manipulations with nimodipine and vitamin E (a free-radical scavenger), the authors concluded that free radicals were involved in mediating the effects of β-amyloid on calcium currents. Activation of calcium permeable (cationic nonselective) channels by β-amyloid (1 μM) was reported by Ye et al. in rat hippocampal neurons using patch clamp *(54)*. Experiments with HEK293 cells showed that only those transfected with calcium-sensing receptors (CaR) exhibit the effect observed in rat hippocampal cells. Furthermore, the effect was not observed in neurons from mice CaR −/−, whereas it was present in wild-type mice that express endogenous CaR. Therefore, it can be concluded that β-amyloid exerts its effect by acting on the G protein calcium-sensing receptors *(54)*.

Artificial Lipid Bilayer Studies

Using this technique, it has been shown that β-amyloid itself can form large conductance, cationic selective, multi-level ion channels with high calcium permeability. These properties of forming ion channels may arise from the formation of a complex of peptides *(55)*. Improved channel forming properties appear

to occur when the peptide is "aged," presumably because there is an increase in β-pleaded sheet conformation and even fibril formation. The authors of the original studies *(55)* have also proposed that the calcium-carrying capacity of these channels might constitute a significant pathophysiological event in AD. A separate study using the 25–35 β-amyloid fragment also showed ion channel-forming capabilities for β-amyloid with some differences in terms of the biophysical properties of the channels as compared to the original studies *(56)*. These studies provided novel and intriguing pathophysiological mechanisms for β-amyloid toxicity. A related study showing attenuation of β-amyloid toxicity by Ca^{2+} blockers also indirectly suggests that ion channels (calcium) and intracellular calcium may be part of the β-amyloid-induced alterations *(57)*. Ionophore-like properties were shown in erythrocytes, indicating that Aβ can interact with natural membranes *(58)*. More recently, Kawahara et al. *(59)* showed that Aβ (1–40) was capable of forming channels in natural membranes (silent excised patches of hypothalamic cells) similar to those seen in artificial bilayers. The channels showed properties comparable to those observed in artificial bilayers. The allegedly more toxic Aβ1–42 was also shown to form calcium-permeable channels in phospholipid vesicles *(60)*.

Intracellular Calcium

Alteration in calcium regulation has been thought to play a role in many disease processes and AD is no exception *(61–63)*. Measurements of resting calcium levels and alterations in response to pharmacological challenges in fibroblasts and blood cells have been the subject of numerous studies. Most studies report significant differences between cells from AD compared to controls. However, controversies and lack of agreement between different groups have been a constant in this area *(7)*. Still, when careful attention is paid to account for different methodologies and experimental procedures, a clearer picture emerges as will be discussed in the following paragraphs.

Fibroblasts Studies

An early study reported that basal (nonstimulated fibroblasts) free-calcium levels were decreased in cells from AD patients *(64)*. This finding, however, was not reproduced in subsequent studies. In fact, most authors report no significant differences in basal calcium levels between AD and control cells *(31,42,43,65,66)*. There is also a complex picture when cells have been challenged with various pharmacological agents. A report by Peterson et al. *(67)* showed depressed responses in AD and aged controls to fetal bovine serum (FBS) and 3,4 - diaminopyridine compared to young controls. AD and aged cells did show calcium increases in response to N-formyl-methionyl-leucyl-phenylalanine and bradykinin but the responses were significantly smaller than those in young controls. Bradykinin, at the same concentration (25 nM), was used in a separate study that found no statistical differences between young controls and AD *(65)*. A third study *(68)* found higher responses (group averages) in controls compared to AD cell lines, upon stimulation with bradykinin (10 and 100 nM) and serum. There was, however, significant overlap between cell lines of both groups. It appears that the significant difference between the group's averages is the result of a few rather high values in controls *(68)*. A more recent report *(69)* using 100 nM bradykinin showed no differences between controls and AD (4 sporadic and 2 members of Japanese pedigree OS-3). For another four members of Japanese families OS-1 and OS-2, the authors reported a variable, cell cycle-dependent depressed response *(69)*. Another approach to assess the responses to bradykinin has been not only to measure the magnitude of the responses (individual or group averages), but also to assess the proportion of responding vs nonresponding cells. Using low concentrations of bradykinin (0.1 nM), Hirashima et al. *(70)* showed that AD cell lines exhibited measur-

able responses in a significantly larger proportion compared to controls. In fact, responses were virtually absent in all but one of the control cell lines examined. Interestingly, higher concentrations of bradykinin (1 and 10 nM) elicited comparable proportion of responders in AD and controls *(70)*. The magnitude of the responses was also similar. Therefore, stimulation with concentrations of bradykinin higher than the sub-nanomolar range induced similar responses in AD and controls by most of the data reported. In a minority of the cases there was a somewhat lower average response in AD with significant data overlap *(68)*. On the other hand, very low concentration of bradykinin elicited characteristic responses in AD cells only *(70)*. A more recent study from a different laboratory confirmed this finding. Gibson el al. *(71)* reported that low bradykinin (0.1 nM) caused calcium increases exclusively in AD (PS1 bearing cells) compared to controls. Cells bearing the Swedish mutation on average had higher responsiveness to bradykinin *(71)*. Individually, however, only 2 out of 6 cell lines exhibited increased proportion of responders comparable to sporadic and PS1 carrying cell lines. The inclusion of two cell lines from asymptomatic carriers may have been a factor. This report also repeated a previous findings *(66)* that bombesin (a peptide that also induces calcium release via IP_3) caused exaggerated (in magnitude) responses in PS1-AD compared to control. Interestingly, the opposite pattern (decreased responses to bombesin) was observed in the Swedish AD lines *(71)*. All of these studies used fura 2 as the calcium indicator. McCoy et al. *(68)* also used aqueorin in their study. Despite the basic similarities between studies, it seems clear careful attention must paid to technical details, experimental procedures, and analyses methods. Consideration of these details, therefore, allows appropriate comparisons between studies that explain most of the apparent contradictory results. Findings of increased IP_3 production correlated to increased numbers of bradykinin receptors *(72)* further supports the hypothesis of defective calcium handling in AD. Recent research also indicates that the calcium-handling defects (and perhaps others), although prevalent in the majority of AD cases studied (sporadic and PS1 mutations), may not occur to the same extent in specific cases like the Swedish mutation carriers *(71,73)*. This issue, in part, also may contribute to some of the controversies and disparity of the results. Furthermore, in some familial cases changes can be observed in the pre-symptomatic state *(71,74)*.

Unlike the effect on K^+ channels, Aβ (10 nM) did not produce any noticeable effect on the bradykinin-induced calcium response *(34)*. A more recent report *(75)*, however, showed that higher concentration of Aβ (0.1 μ*M*) resulted in increased IP_3 accumulation and enhanced calcium elevation upon bradykinin stimulation.

Blood Cells Studies

Calcium studies conducted in blood cells have also generated discordant results. Moreover, only a few can be directly compared to studies in fibroblasts. Adunsky et al. *(74)* reported elevated resting calcium levels (group averages) in AD compared to healthy controls and vascular dementia. Stimulation with phytohemagglutinin (100 μg/mL) induced a striking calcium rise in AD cells (ca. eight fold increase), whereas a very modest increase was observed in controls and multiple infarct dementia (MID). More interestingly, a comparison between individual cases showed that very high calcium elevations (>650 nM), were only observed in AD cells (16 of 22). Cell lines from all controls and MID individuals had modest (<650 nM) elevations. Six of the 22 AD individuals had responses in the control range. In general, therefore, this study *(76)* is in agreement with those studies in fibroblasts showing enhanced responses to agents that mobilize calcium from intracellular stores. Elevated basal calcium levels were also reported by Ibarreta et al. *(77)* in lymphoblasts (Epstein-Barr transformed lymphocytes) from AD patients. In serum-free medium, AD lymphoblasts exhibited higher calcium elevations

upon stimulation with αIgM and Aβ25–35. A third report *(78)*, however, did not find significant differences between AD and controls, in both basal and after PHA (25 μg/mL) stimulation. The same authors did find significant differences between AD and controls when cells were pre-treated with TEA (as discussed in previous section). An additional report *(79)* failed to show differences in polymorphonuclear granulocytes after stimulation with formyl-methyonil-leucyl-phenylalanine, a substance linked to the IP_3 pathway. It is important to keep in mind that, unlike fibroblasts, lymphocytes can be significantly and acutely affected by environmental factors that influence the general state of the donor *(6)*. Moreover, lymphocytes in some cases may correspond to a mixture of various types of white cells. In some studies lymphocytes have been transformed by viruses, which may also influence their calcium homeostasis. It is also important to consider that fibroblasts studies have demonstrated that only low levels of agonists allow a clear distinction between AD and controls *(70,71)*. Another key factor is that the measurements in blood cells are population responses, which may obscure small but true differences. These issues may help explain discordant results from different groups.

Elevated basal calcium levels were also reported in platelets of AD and in control males >50 yr old. Female control subjects presented no significant changes in calcium levels with increased age *(80)*. In conclusion, despite the additional variables in blood cells, most accounts report elevated basal calcium levels in AD, and in some instances, enhanced responses to various agents that promote intracellular calcium release. Therefore, there is some degree of agreement between fibroblasts and blood cells studies indicating altered calcium homeostasis in AD.

Protein Kinase C

Protein kinase C (PKC) is a ubiquitous family of enzymes involved in a number of cellular processes including (but not limited to) growth and differentiation, ion channel regulation, neuronal plasticity, and memory storage *(81–85)*. Some of the functions may be related to specific isozymes and/or their differential expression in different tissues and organs. PKC has also been the subject of attention in a wide range of pathological processes from cancer to nervous systems disorders *(81,85–87)*. A number of reports have identified alterations in PKC in brains and fibroblasts of AD patients. Reduced levels of the particulate fraction in AD brains as measured by radioactive phorbol ester binding and in vitro phosphorylation of histone H 1 was originally reported by Cole et al. *(18)*. Phosphorylation of P86 was reduced in the cytosolic fraction *(18)*. Additional reports have indicated that, in particular, the βII isoform was significantly lower in the particulate fraction from AD hippocampal and cortical tissues *(19)*. The opposite was true for the cytosolic fraction from cortex. The isoforms βI and α were also reduced in the particulate fraction from hippocampus of AD patients *(19)*. Shimoama et al. confirmed a predominant involvement of the β isoform in AD brains *(20)*. A more recent report found attenuated PKC activity and translocation in AD brains *(88)*. However, the immunoreactivity (membranous fraction) of the α and γ isozymes was increased in the frontal cortex. This finding was attributed to reduced proteolytic activity and/or a compensatory response in AD *(88)*. Some authors have suggested that PKC alterations may be an early event in AD *(89)*. Levels of mRNA for the α isozyme were found somewhat reduced in AD brains, although there was enough overlap so the differences did not reach statistical significance *(90)*. Other reports have not found salient differences in brain distribution of PKC in AD compared to controls *(91,92)*.

Studies in fibroblasts have, in general, paralleled those in brain tissues. Both immunoreactivity and protein phosphorylation were reduced in familial and sporadic AD fibroblasts *(92)*. More recently, it was reported that the affinity (phorbol ester binding) and the

phosphorylating activity (histone) were lower in cells from AD patients *(93)*. Immunoblotting analyses revealed that the α isoform was primarily affected in AD fibroblasts *(95–97)*. At the present time, is impossible to determine whether the brain PKC changes are an initial or early alteration or a random consequence of other events in AD pathology. The parallel findings in fibroblasts, the PKC relation to K^+ channels and to APP processing (*see* below) suggest, however, that PKC alterations may indeed be an integral event in the pathophysiological cascade.

PKC may also play a significant role in APP processing. APP is the precursor protein from which β-amyloid originates and it possesses phosphorylation sites *(1,32)*. PKC activators influence the type of APP products secreted. The APP ectodomain can be constitutively secreted by cleavage within the β-amyloid sequence (α-secretase), thus resulting in nonamyloidogenic processing. APP can also be internalized and degraded in endosomal/lysosomal compartment generating fragments that contain the intact β-amyloid sequence, therefore resulting in potentially amyloidogenic (and possibly pathologic) processing *(1,6,33,97)*. It has been shown that PKC activation can increase the rate of the nonamyloidogenic processing in cellular models. In addition, some studies have directly shown a reduction of the secretion of the β-amyloid peptide after phorbol treatment or activation of PKC *(32,97–99)*. There are, however, two reports *(100,101)* that show an increase in sAPP without changes in Aβ. These apparently contradictory results could be owing to tissue specific differences. In addition, Savage et al. *(102)* reported phorbol-induced *decrease* in Aβ species without a noticeable *increase* in sAPP secretion in mouse brain. Therefore, the general finding of reduced PKC amounts and/or activity in AD (brain and fibroblasts) is consistent with the apparently normal regulatory role of PKC favoring nonamyloidogenic processing of APP. It is worth noting that some of the Italian cell lines that showed the K^+ channel defect and enhanced IP_3 mediated calcium releases were the same ones in which defective PKC activity and immunoreactivity was demonstrated *(70,94–97)*. Interestingly, in these AD fibroblasts, the reduced PKC activity has been correlated with a reduced ability to secrete soluble APP *(97)*. More recently, it has been shown that novel PKC activators *(103)*, with improved sensitivity for the α, β, and γ isozymes, significantly increase the amount of sAPP in human fibroblasts (AD and controls) and in PC12 cells *(37)*.

Other Findings in Peripheral Tissues

In addition to the studies mentioned previously, a variety of reports cover a number of other aspects on cellular and molecular biology of peripheral tissues. A brief summary of studies in human tissues follows.

β-*Amyloid Secretion*

Strong and direct evidence that peripheral tissues may parallel events in the brain of AD patients, was the demonstration that fibroblasts from carriers of the Swedish double mutation (K670N/M671L) secrete elevated amounts of all forms of Aβ. Elevated levels were also observed in fibroblasts from presymptomatic carriers *(104,105)*. This finding in the Swedish family was later shown for various PS1 and PS2 mutations in particular, for the amount of the longer Aβ 1–42 form *(106)*. An increase βAPP mRNA transcription has also been reported *(107)* in fibroblasts of the Canadian kindred (PS1). A significant increase in the ratio of mRNA for APP751 and APP770 relative to APP695 was also reported in a relatively small sample of sporadic cases from Japan *(108)*.

DNA and Related Alterations

Defects in DNA repair have been reported in peripheral cells of individuals with neurodegenerative disorders. Li and Kaminskas *(109)* reported that DNA repair after treatment with

N-methyl-N-nitro-N-nitrosoguanidine was significantly slower in fibroblasts from familial AD cases. Hypersensitivity to the same chemical was later reported for sporadic AD *(110)*. A subsequent study showed the same DNA repair deficiency in lymphoblasts from familial AD *(111)*. However, a more recent report *(112)* stated that the DNA repair mechanism was normal in young and AD but that aged donors and Werner's cells showed lower telomeric repair efficiency. It is important to note that the latest report used only one cell line from an old donor, one from Werner's and the other from AD. It is also curious that this AD patient being older than the oldest aged donor does not show any deficiency *(112)*. Because the earlier reports had larger samples and showed consistent results, it would be reasonable to conclude that DNA repair deficiencies do occur in AD. A decrease in levels of interferon-induced 2′, 5′-oligoadenylate synthetase mRNA has also been reported *(113)*. Studies of the enzyme itself revealed differences in activation requirements and kinetics between extracts from AD and controls *(114)*. Whether or not these nucleic acid alterations have any pathophysiological significance remains to be determined.

Cytoskeletal Proteins and Adhesiveness

Early studies reported that fibroblasts from AD patients adhered less well to plastic culture dishes *(115)*. This was interpreted as related to reduced levels of mRNA for APP in familial AD *(115)*. The same basic observation was repeated by a different group *(116)*. In this case, aberrant fiber distribution of vimentin (a cytoskeletal protein) was linked to the decreased adhesion efficiency of fibroblasts from familial cases. Vimentin fibers were also found to regrow at a slower rate in AD after treatment with colchicine *(117)*. In related studies using Western blot analyses, Takeda et al. *(118)* reported however that all major cytoskeletal proteins (actin, tubulin, vimentin) were not altered. Only fodrin was found altered, i.e., nondegraded in AD. Alterations in the patterns of vimentin were attributed to potential interactions with other molecules and not to a primary defect of the protein itself *(118)*. In addition to the published reports, many researchers routinely observe this deficient adhesiveness in AD fibroblasts. The significance of this highly prevalent finding, however, has not yet been established.

Miscellaneous Enzymatic and Related Alterations

Increased proteolytic activity (presumably linked to altered calcium regulation) has been reported in AD and aged donors *(119)*. This increased proteolytic activity has been linked to transketolase abnormalities observed in AD fibroblasts *(120–123)*. Reduction in hexokinase activity was found in familial AD cases, whereas it was not altered in sporadic AD *(124)*. Reduced activity of thiamine-dependent enzymes was reported in brains and blood cells of AD patients *(125)*. A number of additional biochemical and metabolic alterations are reported in peripheral tissues of AD patients. These are summarized in various review articles, including some recently published *(1,2,7,9–12,123,126)*.

Concluding Remarks

There is obviously a plethora of observations and findings made in fibroblasts and other peripheral cells pertaining to a wide range of molecular and cellular processes. A number of these findings have been isolated reports or else interest in the observations has faded. In other cases, despite repeated and consistent observations, the pathophysiological relevance of the findings remains unclear. Although some controversies still remain, there is increased acceptance and agreement that calcium homeostasis alterations are a key event in AD. The involvement of potassium channels has now been documented by independent groups and it includes observations on blood cells. The finding that Aβ is oversecreted in

familial AD fibroblasts lends additional support to the hypothesis that relevant changes do occur in peripheral cells. Furthermore, Aβ mimics the effects of the disease in otherwise normal fibroblasts *(34)*. More recently, it has been shown that Aβ also induces increased responses to BK in PC12 cells *(75)*, resembling the observations in AD fibroblasts. A comparable effect has been observed with the C100 fragment of APP *(127)*. Therefore, Aβ is not only present in peripheral tissues but also plays a key role in rendering altered calcium and ionic homeostasis in these cells. Furthermore, PKC involvement has been linked to both K^+ channel dysfunction and Aβ. Bhagavan et al. *(36)* demonstrated that AD fibroblast, documented to have altered K^+ channel function, recover such K^+ channel function after treatment with a novel PKC activator. This is particularly relevant because PKC has been found altered in brains and fibroblasts of AD patients. Moreover, the same compound enhances the α-secretase pathway reducing the relative amount of Aβ *(37)*. Therefore, some of the alterations (highlighted in this review) present in AD fibroblasts can be mechanistically connected and perhaps in a pathophysiological context. Based in part on the accumulated evidence and our own research, we postulate that PKC might be one of the principal molecules targeted by the disease process, perhaps directly by APP metabolites such as Aβ and C-100. K^+ channels can be altered by Aβ directly, mediated by PKC, or both. Because PKC is known to regulate ion channels *(128–131)*, it is plausible that abnormalities of this molecule would have consequences for K^+ channel function. In fact, restoring or upregulating PKC function in AD fibroblasts results in restoring the normal potassium channel function in AD cells. Aβ both induces potassium channel defects and selectively alters some isozymes of PKC mimicking the effects of the disease process in fibroblasts. In addition, Aβ and C100 mimic the altered response to BK in PC12. Because K^+ channels, PKC, and calcium-dependent processes have a significant role in memory (for reviews, *see* refs. *24–28,82)*, one could also speculate that this chain of events may ultimately result in the clinical manifestations of AD, memory loss in particular. In summary, the peripheral cell model offers enormous advantages, and in some instances the only option, to further study of the AD disease mechanisms and for the design of therapeutic interventions.

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