Another weapon against amyloid

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Alzheimer's disease (AD) is one of those conditions that, like cancer in earlier generations, inspires particular terror among the general public. Therapeutic advances have lagged behind insights into genetics and pathophysiology, and although β -amyloid ($A\beta$), the product of amyloid precursor protein (APP) cleavage, remains the leading suspect among proposed pathogenic factors, its causal role in AD has not been established with certainty.

At present, patients with AD have access to two approved specific treatments, acetylcholinesterase inhibitors and the glutamate receptor antagonist memantine, neither of which is dramatically effective. Several experimental approaches are also under study, including $A\beta$ vaccines, metal chelators, and derivatives of Congo red dye, which bind $A\beta$. In this issue of PNAS, Robert Messing and colleagues (1) report a finding that suggests a new strategy for stimulating the enzymatic breakdown of $A\beta$, which could produce benefit in AD.

This strategy is based on the enzyme PKC, a serine/threonine kinase that catalyzes the calcium- and phospholipid-dependent phosphorylation of protein substrates and is also a receptor for phorbol esters. Messing and colleagues (1) have been studying PKC for many years, especially the neurological effects of PKC isozymes. For example, one recent study (2) implicated neutrophil PKC δ in reperfusion injury after experimental stroke, consistent with work from Mochly-Rosen and colleagues (3) showing that a PKC δ inhibitor reduced infarct size. The present study (1) builds on previous findings that phorbol esters reduce $A\beta$ levels and the number of amyloid plaques in AD-transgenic mice (4) and that PKC ϵ decreases $A\beta$ levels in cell culture (5). These and other threads that link PKC and $A\beta$ are discussed in a recent review (6).

Messing and colleagues (1) crossed two lines of transgenic mice, one that overexpresses PKC ϵ in neurons and another that expresses a mutant form of APP (the Indiana or V717F mutation) found in some patients with the rare familial form of AD. The latter mice exhibit some features of clinical AD, including the age-dependent deposition of extracellular amyloid plaques in selected brain regions. Plaque density, astrogliosis, and alterations in neurites were reduced in PKC ϵ -transgenic/

Fig. 1. Proposed scheme for regulation of $A\beta$ degradation by PKC ϵ via ECE. PKC ϵ activates the transcription factor Ets-1, which promotes ECE expression. ECE cleaves isoforms of big ET (themselves the products of pre-pro-ET processing by furin) to produce active ET isoforms (ET-1, -2, and -3). ECE also degrades $A\beta$ (including both $A\beta_{1-40}$ and $A\beta_{1-42}$), produced through the action of α -, β -, and γ -secretases, into smaller fragments; ECE cleavage sites occur between $A\beta$ residues 16 and 17, 17 and 18, and 19 and 20 (7). Molecules outlined in black (PKC ϵ , ECE, $A\beta$, and $A\beta$ fragments) were studied directly in ref. 1.

APP^{V717F} compared with APP^{V717F} mutant mice. Similar findings were obtained with other PKC ϵ and APP transgenics. Contrary to expectations, however, $A\beta$ synthesis was unaffected by PKC ϵ overexpression. Instead, PKC ϵ seemed to act by stimulating the degradation of $A\beta$.

$A\beta$ is a substrate for several enzymes, including insulin-degrading enzyme and neprilysin, the activities of which were unaltered in PKC ϵ transgenics. However, the activity of another $A\beta$ -degrading enzyme, endothelin-converting enzyme (ECE) (7), was increased in hippocampus and cerebral neocortex of these mice. Previous cell-culture studies suggested that the effect of phorbol esters on ECE expression was mediated through the transcription factor Ets-1. Messing and colleagues (1) concluded that amyloid deposition was decreased in PKC ϵ -overexpressing mice because of increased ECE-mediated breakdown of $A\beta$ (Fig. 1). On the other hand, amyloid plaques were not increased in APP^{V717F}/PKC ϵ -null mice, presumably because of the redundancy of $A\beta$ -degrading enzyme systems.

As its name implies, ECE is involved in the processing not only of $A\beta$ but also of endothelins (ETs), a family of 21-aa peptides with potent vasoconstrictor action (8). These peptides are structurally and functionally related to sarafotoxins isolated from the venom of the burrowing asp, *Atractaspis engaddensis*. ET-1 was first isolated from porcine aortic endothelial cell cultures and shown to constrict blood vessels with picomolar affinity. ET-2 and ET-3 have

subsequently been identified, as have two G protein-coupled receptors, ET_A and ET_B. ET-1 and its receptors are expressed on cerebral neurons, glia, and blood vessels, with highest brain ET-1 levels in cerebral cortex, striatum, hippocampus, and hypothalamus. The ETs are synthesized from pre-pro-ETs, which are cleaved by a furin-like endopeptidase to form big ETs, and then by either of three ECE isozyme families (ECE-1, ECE-2, or ECE-3) to generate mature ET peptides (9). Alternative synthetic pathways seem to exist in some cells. Like ETs and ET receptors, ECEs are associated with neurons as well as endothelial cells. ECE-1 hydrolyzes not only ETs (and $A\beta$, as discussed above), but also bradykinin, substance P, angiotensin, and insulin.

Alterations in ET signaling have been implicated in a variety of pathological conditions, including systemic hypertension, cerebral vasospasm after aneurysmal subarachnoid hemorrhage, atherosclerosis, myocardial infarction, congestive heart failure, pulmonary hypertension, chronic airway inflammation, and chronic renal failure (8). For this reason, there has been considerable interest in the ET system as a therapeutic target. For example, a variety of ECE inhibitors and ET receptor antagonists have been developed as potential treatments for some of the conditions listed above (9, 10).

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What is the significance of the findings reported by Messing and colleagues (1)? The most important implication is that both PKC ϵ and ECE may be therapeutic targets for AD, a disease for which more effective treatment is desperately needed. This is an important insight, notwithstanding that some caveats are in order. The present study (1) did not show that overexpression of PKC ϵ produced functional improvement in AD-transgenic mice or that less than lifetime treatment was effective in reducing plaques. As mentioned previously, a causal connection between plaques and disease symptoms in AD is also still unproven. It would be espe-

cially interesting to know whether electrophysiological defects in hippocampal synaptic function, synapse loss, and behavioral deficiencies are alleviated in APP/PKC ϵ compared with APP-transgenic mice. It is encouraging that plaques were not increased in PKC ϵ -null mice because, as noted above, ECE inhibitors are under consideration as drugs for treating hypertension and other disorders.

Considering that PKC ϵ is proposed to affect A β breakdown through an effect on ECE, might therapeutic stimulation of PKC ϵ in AD lead to adverse effects mediated by increased ET production? The direct vasoconstrictor

effect of ET as well as its central hypertensive action raise concerns about undesirable effects on blood pressure, although these complications would likely be amenable to antihypertensive therapy and might be an acceptable tradeoff for improved cognitive function. Although ET does not seem to be directly neurotoxic (11), its intracerebral injection causes ischemic brain lesions (12), to which patients with AD, many of whom have concurrent cerebrovascular disease, might be especially susceptible. These are issues that may need to be addressed if the exciting observations reported by Messing and colleagues (1) progress toward clinical application.

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