Membrane alterations as causes of impaired signal transduction in Alzheimer's disease and aging

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Changes in cell-membrane composition in normal aging and in Alzheimer's and other age-related diseases appear to result in impaired neurotransmitter-triggered signal transduction. The impaired signal transduction seems to be related to dysfunctions in the coupling of G proteins to their receptors and effectors. Direct demonstration of altered physiochemical properties of brain tissue of patients with Alzheimer's disease has been achieved by small-angle X-ray diffraction. In this disease, thinner membranes correlate with a 30% decrease in moles of cholesterol : phospholipid. Such changes can affect directly the coupling and uncoupling properties of G proteins, and can account for signal transduction deficits. These findings offer a complementary alternative to the β-amyloid hypothesis, and an opportunity to consider new types of therapeutic interventions.

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RESEARCH ON ALZHEIMER'S disease (AD) has become increasingly focussed on β-amyloid and the role of this substance in the etiology of the disease (for review, see Ref. 1). Examples include analyses of mutations in the β-amyloid precursor-protein (APP) gene of AD patients[2], transgenic mice with altered forms of the gene[3–5], and administration of β amyloid in attempts to mimic the pathophysiology of the disease[6]. These studies might eventually be of great value in devising therapeutic strategies to ameliorate some of the debilitating aspects of AD. However, for the present, they have not provided a great deal of information concerning the failure of ACh-replacement therapy to be effective in the treatment of AD. Attempts to restore cognitive function in age-related memory dysfunctions and AD through replacement of ACh might be of some use but have had minimal success[7–9]. Conversely, this therapy has been relatively successful in young organisms in which cholinergic function has been compromised pharmacologically or surgically. However, these model systems have not proven to have a great deal of validity when applied to the study of aging or AD patients. Our hypothesis is that the muscarinic ACh receptor (mAChR) systems that would be most affected by ACh-replacement therapy lose their sensitivity to stimulation by ACh in both aging and AD (Refs 10–12). We suggest a complementary alternative to the β-amyloid hypothesis, based on the idea that changes in cell-membrane composition might constitute a major molecular mechanism of AD. Although not excluding a possible role for β amyloid, these membrane alterations result in impaired signal transduction in a manner similar to that of normal aging.

Mechanisms of altered signal transduction in aging and age-related diseases

Extrapolations from numerous systems suggest that a variety of mechanisms of altered signal transduction are involved. These include changes in high-affinity forms of receptors[10], reduced sensitivity to shifts that are mediated by GDP and GTP (Refs 12 and 13), reductions in formation of phosphoinositide 4,5-bisphosphate (PIP_2)[11], APP–G_0 signalling[14] and diminished neuronal staining for protein kinase C (PKC) (βII) in frontal cortex[15]. Other changes include decreased β-adrenoceptor-mediated stimulation of production of cAMP in AD fibroblasts[16] and granulocytes[17]. These last two changes suggest that the biological mechanisms that are responsible for the disease might be much more fundamental, and manifest in tissues other than the CNS. Additional examples in AD patients include altered sensitivity to mitogens in lymphocytes[18], altered Ca^{2+} dynamics and properties of signal transduction[19,20] in both lymphocytes and fibroblasts and decreased platelet microviscosity[21,22]. It is clear that such ubiquitous changes could reduce drastically the effectiveness of pharmacological agents that are designed to increase the availability of ACh. In fact, Fowler and colleagues[23] have suggested that a number of components and events of signal transduction might be altered in AD (Ref. 23), and some groups have now reported impaired signal transduction mechanisms that are coupled to ACh receptors in brain regions in AD that are strikingly similar to those that are observed for a variety of hormones and neurotransmitters in various tissues during normal aging and in other age-associated diseases (Table 1).

Table 1 lists some representative changes in coupling and affinity properties of G-protein-coupled receptors that occur in aging and age-related diseases. These include a variety of receptor types, tissues, cells, and species that are typical of and complementary to, the kinds of studies reported[10–23]. These dysfunctions appear to be related to changes in the capacity of G-protein-coupled receptors to alter their affinity states. Initial binding studies of

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TABLE I. Reports of changes in coupling and affinity properties of G-protein-coupled receptors in aging and age-related diseases

Receptor	Tissue or cell	Species	Condition	Change	Refs
Muscarinic	Heart	Rat	Aging	Decreased ability to form high-affinity state	24, 25
	Cortex	Human	Alzheimer's disease	Decreased ability to form high-affinity state, or decreased PIP_2 hydrolysis, or both (but see Ref. 8)	26–28
	Hippocampus, striatum	Rat	Aging	Decreased ability to shift from high- to low-affinity state	29
D_1	Putamen	Human	Huntington's disease	Decreased ability to shift from high- to low-affinity state	30
	Striatum	Rat	Aging	Decreased activation of adenylate cyclase	31
D_2	Striatum	Mouse	Aging	Decreased amount of receptors in high-affinity state	32
α_1-Adrenoceptor	Parotid	Rat	Aging	Decreased ability to shift from high- to low-affinity state	33
α_2-Adrenoceptor	Cortex	Rat	Aging	Decreased coupling of G proteins to adenylate cyclase	34
	Platelets	Human	Aging	Decreased amount of receptors in high-affinity state	35
β-Adrenoceptor	Lymphocyte	Human	Aging	Decreased amount of receptors in high-affinity state	36
	Lymphocyte	Human	Mitral-valve prolapse	Decreased ability to shift from high- to low-affinity state	37
	Neutrophil	Human	Aging	Decreased ability to form high-affinity state	38

muscarinic-receptor agonists suggested that mechanisms of G-protein–receptor coupling or uncoupling, or both, might be disturbed in AD (Refs 26–28). However, some studies have failed to detect changes in such parameters during AD. For example, Pearce and Potter[39] assessed the coupling of M_1 receptors to their respective G proteins in cortex of AD patients, and reported no differences in agonist affinities for high- and low-affinity receptors, while Wallace and Claro[8] showed that carbachol-stimulated phospholipase C (PLC) activity (leading to hydrolysis of PIP_2) in cortex was not reduced in AD samples obtained from Brodman's area nine compared with age-matched controls, and appears to be somewhat increased. Similar findings were reported by Shimohama and colleagues[12]. There are, no doubt, many differences (for example, neuroanatomical, population and experimental) that are responsible for the disparate findings among these studies. In addition, there could be interpretational differences. For example, Ferrari-Di Leo and Flynn[28] report only a small additional effect of carbachol on hydrolysis of PIP_2, whereas Wallace and Claro[8] concluded that the sensitivity of PLC to GTPγ*S* increased in the presence of carbachol, during AD. However, the latter data suggest that the increased sensitivity occurred only at a concentration of 0.1 μM GTPγ*S*. At the two higher concentrations of GTPγ*S*, the PLC response appears to be lower in AD than in control subjects. Some of the disparate findings among the studies of signal transduction in AD also might be explained by differences in the duration of disease. This parameter is not generally examined in studies that show no changes in indices of signal transduction in AD (for example, see Refs 8, 39 and 40), presumably because the small number of patients in these studies probably prevented these analyses from being carried out. A recent experiment from our laboratory has suggested that at least one important parameter of signal transduction, that is, carbachol-stimulated low-K_m GTPase activity (an index of G-protein uncoupling from the muscarinic receptor) is impaired markedly in AD basal ganglia[41]. Moreover, significant inverse correlations were found between the degree of carbachol-stimulated low-K_m GTPase activity and duration of disease in three of four brain regions examined (that is, basal ganglia, hippocampus and superior frontal gyrus). Although we cannot exclude the effects of possible peri- or post-mortem changes in these tissues, the fact that these results show such a strong relationship to duration of disease would suggest that any differences in the former were minimized. Thus, it is possible that indices of malfunction of signal transduction, other than low-K_m GTPase activity, might emerge as the disease progresses.

Although one report has suggested that APP can form a complex with at least one of the G proteins[42], it seems likely from the above studies that some common mechanism(s) apart from, or in addition to, deposition of β amyloid might play an important role in the etiology of the disease. In addition, the possible role of G_0 binding to APP is still controversial and needs to be confirmed independently. A logical site for a deficit that involves coupling and uncoupling of receptors and G proteins might be within the neuronal membranes, since essentially all of the initial processes (for example, stimulation of receptors, and uncoupling of receptor and G protein), as well as cleavage of APP, might be localized at this site. Moreover, we have shown recently that both generalized and specific perturbation of the cell membrane by dietary manipulation[43] or by the *in vitro* application of agents that alter its composition, fluidity and other physiochemical properties, such as cholesterol and S-adenosyl-methionine (SAM), can mimic the changes in signal transduction that are observed in both AD and normal aging[44]. Pre-incubation of cross-cut striatal slices from young (6 months) rats with cholesterol or old (24 months) rats with SAM resulted, respectively, in decreased carbachol-stimulated low-K_m GTPase activity in the young, and increased activity in the old. Since membrane parameters, such as shape, permeability and osmotic fragility, are determined by membrane phospholipids, it is not difficult to see how these membrane factors might prove to be of extreme importance in the regulation of parameters of signal transduction in AD and aging.

Membrane change in Alzheimer's disease

It thus became critical to demonstrate alterations in membrane physiochemical parameters in AD that could be responsible directly for impaired signal transduction. Small-angle X-ray-diffraction technology has now been applied to characterize changes in the membrane structure of AD and aging brain directly. Reconstituted lipid membranes from cortical gray matter of AD brain samples were significantly thinner (that is, had less microviscosity) than corresponding age-matched controls[45]. This change in membrane width correlated with a 30% decrease in the moles of cholesterol : phospholipid[45]. Addition of cholesterol restored the membrane width to that of the age-matched control samples. Alterations in other membrane components of AD brains have also been reported[46,47]. Changes in the membrane composition and structure could alter the conformation and function of transmembrane ion channels, as well as affect the interaction of receptors and effectors, leading to altered signal transduction, handling of Ca^{2+}, and response to exogenous stimuli[48–50]. Moreover, thinner membranes might expose abnormal protease cleavage sites on the transmembrane APP, resulting in accumulation of β amyloid, and associated cellular damage.

A similar possibility was suggested recently for cholinergic neurons that might deplete their own membrane stores of choline under certain conditions[51], although such membrane alterations might be much more widespread in terms of both neuronal types and lipid and protein components. For example, the primary determinants of the width of the membrane lipid bilayer are cholesterol and phospholipid-acyl chain composition. As observed directly with X-ray diffraction, using brain and model membrane preparations, even modest changes in the cholesterol content can alter membrane width significantly[45,52]. In addition, the length of the phospholipid-acyl chain, and the degree of saturation, is an important determinant of the membrane dimensions[52]. Only to a lesser extent would changes in phospholipid headgroups be expected to alter the membrane-bilayer width, perhaps by perturbing inter-phospholipid packing constraints. Small-angle X-ray diffraction has also demonstrated that the free β-amyloid peptide is lipophilic, and resides deep in the membrane hydrocarbon core at equilibrium[45]. These findings suggest that β amyloid remains in the membrane following cleavage from the precursor protein where it might subsequently disrupt the function of membrane-bound ion channels, as has been shown recently[53].

Future directions

It is now essential to test the hypothesis that altered membrane composition might be a primary cause of impaired signal transduction in both AD and normal aging with a variety of approaches, ranging from the genetic to the behavioral. In light of our recent observations that membrane perturbations[43,44] might mimic the alterations in signal transduction that are observed in AD and aging, it might be possible to develop models at both the cellular and whole-animal levels. One approach might be to explore further the role of oxidative stress in these putative membrane alterations. Recent findings suggest that increased lipid peroxidation might occur in degenerative disorders such as AD (Ref. 54) and Parkinson's disease[55,56]. It is clear that one of the most attractive targets of free radicals is membrane lipids. Thus, oxidative damage incurring in these membranes might be expressed as deficits in signal transduction.

From a therapeutic perspective, this multifaceted approach should prove ultimately to be even more valuable than those paradigms that deal exclusively with damage that is associated with β amyloid. For example, some studies suggest that reduced cholinergic tone in AD (that results from altered signal transduction) might accelerate deposition of β amyloid[57–59]. Thus, the nature of the interactions among these various indices of neurological dysfunction should be examined. If we may draw an ecological analogy to the neurological problem, building rupture-proof tankers will be more efficient than finding new ways to clean up oil spills.

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PERSPECTIVES

Lodewijk Bolk and the comparative anatomy of the cerebellum

Mitchell Glickstein and Jan Voogd

The cerebellum of mammals is histologically uniform, but it varies greatly in the relative size of its different parts. The Dutch anatomist Lodewijk Bolk studied a large series of mammalian cerebella, and put forward a general scheme of organization that can be applied to all mammals. Bolk also speculated about the functional role of different regions of the cerebellum, based on the idea that there might be a single somatotopically organized representation of the body surface on the cerebellar cortex. Although his idea of a single map is wrong, Bolk's anatomical descriptions are thorough, and his insights are profound. These descriptions formed the basis for much subsequent thinking about the structure of the cerebellum.

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> '...[but the Spirits inhabiting the cerebel perform unperceivedly and silently their Work of Nature without our Knowledge or Care.]'[1]
> Thomas Willis (1681).

FROM THE FIRST anatomical studies, the cerebellum has been recognized as a distinct subdivision of the brain. The narrow size of its folia in contrast to the much wider gyri of the cerebral cortex suggested that the cerebellum must differ in function from the cerebrum. Since there was little experimental evidence, until the 19th century, opinions about cerebellar function were often based on comparisons between species. Galen[2,3] reproached Erasistratus for suggesting that the cerebellum might have some relation to intelligence, since the human cerebellum appeared to be so highly convoluted. Galen wrote:

> '...[it does not appear to me that he is reasoning correctly, since even asses have a very complicated cerebellum although their imbecile character demands a very simple and unvariegated cerebrum]...'.

Galen's text reflects two recurrent themes in the study of the structure and function of the cerebellum. From the earliest descriptions, species have been compared. Galen also began a long tradition, that is still alive in the study of the cerebellum, of beginning your own work by attacking that of your predecessors.

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Vesalius[2,4] carried on both traditions. He emphasized that:

> '[In men the cerebellum does not extend at all into the occiput, as perhaps in oxen – which Galen seems only to have used for the study of the brain]...'.

Anatomical descriptions of the human cerebellum were extended and improved in the 16th and 17th centuries. By the middle of the 18th century, the gross structure of the cerebellum was well known but there was no systematic description of its major subdivisions. Malacarne[5] published the first monograph that was devoted entirely to the cerebellum. He gave names to many subdivisions of the cerebellum on the basis of their resemblance to a common object, or to some other anatomical structure. Several of the names that Malacarne used have remained. Ugola (Latin: uvula) and tonsilla were so named because of their similarity to those structures. The most rostral tip of the cerebellum looked like a cat's tongue; hence linguetta (Latin: lingula).

Functions of the cerebellum

By the beginning of the 19th century, the gross anatomy of the cerebellum was well known but there was no knowledge about its possible functions. Volta had discovered that electricity is produced by an alternating series of dissimilar metals. Because of the alteration of its grey and white layers, Reil