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# Lipid Raft Domains: the Probable Place Where Proteins of the Cholinergic System Meet, Accumulate and Communicate

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## Letter to Editor

Acetylcholine (ACh), a classical neurotransmitter of the central and peripheral nervous systems, triggers signal transduction events. The availability of ACh in cells and body fluids relies in the actions of choline acetyltransferase (ChAT), the enzyme that makes ACh from acetyl-coenzyme A and choline [1], and the cholinesterases acetyl- (AChE) and butyrylcholinesterase (BChE) [2]. Owing to their high ACh-hydrolyzing capacity, AChE and BChE allow sustain temporal and spatial control of the great variety of down-stream cellular events that follows activation of nicotinic (nAChRs) and muscarinic receptors (mAChRs). Down-regulation of ChAT decreases the level of available ACh, this ACh deficiency being especially intense in Alzheimer's disease (AD) owing to the progressive loss of cholinergic neurons in the brain areas involved in cognitive functions [3]. The attempts to overcome this deficiency of ACh by prescribing ChE inhibitors (ChE-I) have been unsuccessful. Over 100 million AD patients have been treated with ChE-I since 1986 and, despite clinical stabilization for up to one year in 20% of them an efficient therapy against cognitive troubles has not been found yet, which has prompted clinicians to pursuit new therapeutic approaches [4]. While at the muscular level the deficiency of AChE is causally related with myasthenic disorders [5], AChE down-expression and the subsequent risen level of ACh likely facilitate intense and long lasting cholinergic effects, which may even favour tumour growth [6-9].

ACh occurs in the blood [10] and many types of non-neuronal cells, including epithelial, endothelial and immune cells along with tumour-derived cells, can produce and release ACh [9]. This non-neuronal ACh likely functions like a local signalling molecule, which, acting through autocrine and paracrine routes, may regulate basic cell functions. The amount of ACh which arises from non-neuronal cells can impair cell cycle regulation and the same may take place with nicotine and other exogenous chemicals capable of occupying the site and mimicking the actions elicited by agonists of nAChRs and mAChRs. An excess of available ACh, and the concurrent over-activation of AChRs, may collaborate to malignancy in intestine, lung, liver and kidney

[11]. Inactivation or persistent inhibition of AChE (and BChE) by nerve agents, pesticides or some other xenobiotic may also stimulate cell proliferation and tumour growth [12].

While choline transporters, vesicular transporters of ACh and all classes of nAChRs and mAChRs consist of proteins that span the membrane several times, the full range of AChE proteins are devoid of multiple transmembrane regions. This does not mean that the whole range of AChE variants behave as water-soluble proteins. We shall see below that several classes of AChE proteins occur in cells and tissues as amphiphilic (detergent-interacting, lipid-binding) proteins. Thus, while water-soluble (hydrophilic) AChE species occur in the blood serum, saliva and cerebrospinal fluid [13], tissue-specific mixtures of hydrophilic and amphiphilic AChE species occur in the mouse brain [14], muscle [15], nerve [16], thymus [17] and other visceral organs [18].

The 3'-splicing of the AChE pre-mRNA leads to R (readthrough), H (hydrophobic) and T (tailed) mRNAs, along with their corresponding proteins. Before processing at the rough endoplasmic reticulum (RER), AChE<sub>R</sub>, AChE<sub>H</sub> and AChE<sub>T</sub> proteins exhibit hydrophilic properties, but addition of glycosylphosphatidylinositol (GPI) supplies amphiphilicity to AChE<sub>H</sub> subunits [19], and linkage of PRiMA (proline-rich membrane-anchor) does so to tetrameric AChE<sub>T</sub> [20]. The variety of AChE proteins is expanded by including the E1e exon in the 5'-end of the mRNA. The E1e-including AChE mRNAs translates into N-terminally extended N-AChE<sub>R</sub>, N-AChE<sub>H</sub> and N-AChE<sub>T</sub> variants [21]. The protection lent by the N-extension prevents the signal peptide from being cleaved off in the RER. As a result, the transmembrane signal peptide allows linkage to membranes of N-AChE proteins, which, later on move and remain at the cell membrane as ecto-proteins [22]. Summarising, current information indicates that AChE mRNAs and proteins have changed along evolution to generate an ample range of monomeric and oligomeric AChE variants made of N-extended N-AChE<sub>R</sub>, N-AChE<sub>H</sub> and N-AChE<sub>T</sub> and non-extended AChE<sub>R</sub>, AChE<sub>H</sub> and AChE<sub>T</sub>.

Thus, N-extension, glypiation and PRiMA addition likely represent evolutionary strategies to solve the difficult task of immobilizing in the cell membrane a series of catalytically competent (and/or not competent?) AChE species with hydrophilic faces exposed outwards. As for the membrane-bound AChE species, although speculative, it is tempting to think that physiological reasons may be behind the choice of GPI or the transmembrane peptide which include PRiMA and the N-extension: the release of AChE in a catalytically competent state by the actions of phospholipases and proteases [23]. Thus, while the release of catalytic AChE may be useful for withdrawing ACh in interstitial fluids, this loss of AChE may form part of the ways needed for achieving ACh homeostasis in body fluids, tissues and cells.

The widely accepted idea of glypiation as a one of the motifs that favour protein targeting to lipid rafts prompted us to test the presence in rafts of GPI-linked AChE<sub>H</sub>. The observation of glypiated AChE in rafts of mouse muscle and liver [24] lends weight to our proposal and gives sense to the specific splicing that leads to AChE<sub>H</sub> protein. But if glypiation allowed directing AChE to raft domains, what about PRiMA? The observation of PRiMA-bound AChE in rafts of brain [25] demonstrated that PRiMA linkage was another means of targeting AChE to rafts. As this point, we wondered whether the N-extension was a third manner of directing AChE to rafts. The observation of distinct N-AChE proteins in hepatic rafts [24] allowed us substantiate the proposal. Therefore, N-extension, glypiation and PRiMA addition are not only strategies used by AChE variants for membrane linkage, but also the means by which the variants are hosted in membrane patches that possess high levels of sphingolipid and cholesterol. The targeting to rafts of AChE variants explains previous observations regarding the capacity of AChE to interact with diverse protein partners, including prion protein, aurora B kinase, glycogen synthase kinase-3, the death receptor FAS, the membrane integrin receptor and other proteins that temporally or permanently reside in lipid rafts [26]. The capacity of AChE to interact with an ample range of proteins, the fact that several AChE variants localize to rafts, the relevance of cholesterol for activating M3 mAChR in smooth muscle, and the role of caveolae in M2/M3 mAChR-evoked airways constriction [27] place lipid rafts in the cholinergic scenario. In addition, the co-localization of M3 receptors and AChE variants in hepatic rafts [24] indicates that in liver cells at least both AChRs and AChE variants share the same raft domain. This co-existence makes it possible that the surface membrane of ACh-sensitive cells, regardless of their neural and non-neural origin, can be decorated with raft patches holding AChRs, AChE variants and other proteins of the cholinergic system. Each raft with embedded ACh-related proteins may represent the molecular device used by cells to speed-up spatial and temporal control of cholinergic pulses, but further experiments are needed to validate this possibility.

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