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Apomorphine and Alzheimer's A β : Roles for Regulated α Cleavage, Autophagy, Antioxidation?

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In this issue, Himeno and colleagues¹ describe a new, exciting – and potentially therapeutically important – regulatory event in the life cycle of the Alzheimer's amyloid precursor protein (APP) and the fragments of APP generated by proteolytic processing. At the outset, before diving deeply into the cell biology of APP and A β metabolism, it should be noted that, if confirmed, the effect identified by Himeno *et al*¹ would constitute an important addition to the experimental A β -lowering armamentarium currently being martialed against the cerebral amyloidosis of Alzheimer's disease (AD). About half of the over 65 population has measurable cerebral accumulation of fibrillar A β as defined by PET scans employing Pittsburgh compound B (PiB) as tracer². By extension, at the time of diagnosis, patients with clinically apparent mild cognitive impairment (MCI) or early AD are bound to have a substantial cerebral burden of both fibrillar (detectable on PiB scan) and oligomeric A β (undetectable on PiB scan). All current experimental therapies are more effective as prophylaxis than as treatment^{3,4,5}. The potential breakthrough from Himeno *et al* is the suggestion that something as simple and drug-like as apomorphine – through some pathway that is not immediately obvious – can activate clearance of A β from the brain. Because of this potential clinical implication, it is important to try to make sense of the new data in light of the current conventional wisdom about APP metabolism.

Each newly synthesized APP molecule is co-translationally inserted across the membrane of the endoplasmic reticulum (ER) with its large hydrophilic amino terminal ectodomain (~600-700 amino acids in length) oriented into the ER lumen and its relatively short hydrophilic carboxyl terminal domain (~50 amino acids in length) oriented into the cytoplasm. As with any type I transmembrane protein, a single hydrophobic membrane-spanning domain of 20-22 amino acids connects the APP ectodomain to its cytoplasmic domain.

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Note added in proof: While this paper was in press, we learned of data reported by Necula *et al*³⁷ showing that apomorphine has anti-oligomerization activity. Since it is clear that aggregated A β is much longer-lived and protease-resistant, this provides another potential explanation for the results of Himeno *et al*¹.

The nascent APP holoprotein matures through the central vacuolar pathway of the cell, acquiring along the way a series of posttranslational modifications including N- and O-glycosylation, sialylation, phosphorylation, and tyrosyl sulfation (for review see6). This last modification is a *trans*-Golgi network (TGN)-specific modification, and molecules so modified face a critical decision point in heading toward one of two alternate proteolytic fates (Figure 1). The proteases catalyzing the first of these reactions (known informally as the β - and α -secretases) are spatially segregated into separate post-TGN compartments. The A β -generating (a/k/a “potentially amyloidogenic”) pathway begins with proteolysis of mature APP molecules within the TGN and early endosomes by β -secretase, more formally known as the aspartyl protease BACE-1 (for β -site APP cleaving enzyme-1). Like APP, BACE-1 is also a type-I transmembrane protein, and BACE-1 catalytic activity has an acidic pH optimum well suited to the TGN and endosomes where the enzyme is concentrated. Cleavage by BACE-1 occurs within the APP ectodomain precisely at the amino terminus of the A β domain, liberating a soluble N-terminal APP fragment (sAPP β) fragment and leaving behind a cell-associated 99-amino acid membrane-bound C-terminal fragment (known as C99 or the β -CTF). The sorting of APP from the TGN to the endosome is antagonized by an important organelle known as the retromer which retrieves APP from the endosome and conveys the protein retrogradely back to the TGN and away from A β generation^{7,8}. More on this below.

The alternative fate of mature, TGN-localized APP molecules involves traversing the constitutive secretory pathway to arrive at the plasma membrane, where metalloprotein α -secretases are concentrated. Total cellular α -secretase activity is comprised of several proteases of the ADAM (or adamalysin) class, wherein ADAM is the acronym for “a disintegrin and metalloproteinase”. ADAM10 is responsible for basal α -secretase activity while ADAM17 (also known as TACE, for pro-TGF α or pro-TNF α converting enzyme) is responsible for a phenomenon known as “regulated α -secretase cleavage”. Note that, unfortunately, the term “regulation” and especially the term “secretion” acquire ambiguous meaning in the APP processing narrative since *regulated* α -secretase cleavage (a/k/a “*regulated* ectodomain shedding”) is distinct from conventional *regulated* secretion of neurotransmitters and peptides and is also distinct from *regulated* intramembranous proteolysis (or RIP). More on these terms and events below.

The ADAM10 and ADAM17 α -secretases cleave APP between K16 and L17 of the A β domain, thereby destroying any potential for any fragments so cleaved to contribute to cerebral amyloidosis. Both α -secretases in this “non-amyloidogenic” pathway cause the shedding from the cell surface of the amino terminal ectodomain (known as soluble APP α or sAPP α) and retention by the cell of an 83 amino acid membrane-bound CTF (C83 or the α -CTF). The C99/ β -CTFs and the C83/ α -CTFs are the substrates for γ -secretase in the RIP reaction, and those cleavage events liberate either the potentially amyloidogenic A β peptide (from γ -secretase processing of C99) or the nonamyloidogenic p3 peptide (from the γ -secretase processing of C83; see Figure 1). Notably, APP proteases (ADAM10, γ -secretase¹⁰) and APP trafficking molecules (e.g., LR11/SorL1/SorLA¹¹, SorCS1^{12,13}, PICALM¹⁴, Vps35¹⁵) can be tied to genetic mechanisms that either cause or increase the risk for AD, thereby establishing these fine details of APP processing as inarguably having clinical relevance. The consistent linkage of these AD risk genes to pro-amyloidogenic events constitutes the strongest evidence that at least some AD begins with an imbalance between A β -generating and A β -degrading pathways¹⁶.

Despite use of the term “secretion” to describe release of APP derivatives, pre-processed APP fragments are not stored in secretory vesicles and released in response to depolarization as is typical for neurotransmitters and neuropeptides handled by the conventional regulated secretory pathway. However, APP *does* undergo regulated α -secretase cleavage wherein

depolarization and numerous first messenger neurotransmitters and hormones activate the consumption of mature APP molecules by ADAM17¹⁷. Notably, if these signaling systems are employed to cause hyperactivation of the α -secretase pathway, one can shunt an excess of APP molecules into the α -secretase pathway and out of the β -secretase pathway, and this can virtually abolish A β generation^{18,19}. Hyperactivation of the α -secretase pathway can also occur at the level of ADAM10 transcription as linked to caloric restriction and sirtuins by several research groups, beginning with Qin *et al* in 2006²⁰⁻²¹.

The canonical APP processing pathway does not provide a neat explanation for the accumulation of the intraneuronal A β -like-immunoreactive (A β -LIR) material that is featured in the Himeno *et al* paper; yet we and others have encountered this enigmatic substance colocalizing with rab5-LIR in dense multivesicular bodies that resemble terminal lysosomes or autophagic vacuoles (see Figure 2)²². Where might that A β -LIR material have originated? The most likely origin is from holoAPP and APP β -CTFs that are targeted to clathrin-coated vesicles and on to endosomes by the cytoplasmic (YE)NPTY motif. Under unstimulated conditions, only about 15% of the moles of newly synthesized APP molecules are converted into discrete released fragments²³. The other 85% of newly synthesized APP molecules are terminally degraded by lysosomes. If, however, α -secretase cleavage is activated by, e.g., phorbol esters, the stoichiometry can be shifted to 45% of the moles of new holoAPP converted to sAPP α and only 55% of moles terminally degraded²³. Thus, since apomorphine has some agonist activities at neurotransmitter receptors (see below for discussion), regulated α -secretase activation is one plausible conventional pathway for diminishing the fraction of APP targeted to lysosomes, and, presumably, reducing the amount of A β -LIR material generated there. Consistent with this model, Gunnar Gouras has recently shown that depolarization can reduce intraneuronal A β -LIR material²⁴. It is worth noting that the reactions involved in generating intra-lysosomal A β -LIR, however, may be more akin to limited digestion than to conventional generation of A β by the successive action of β - and γ -secretases. Indeed, we and others have demonstrated that C99/ β CTF can aggregate and be digested to an A β -like species²⁵. Some investigators have gone so far as to propose that indigestible A β -like fragments such as these may be extruded from neurons and that this could be how amyloid plaques are initiated. Supporting such a model is the milinary nature of AD amyloid as compared to the wholesale replacement of liver and kidney by fibrils in systemic and light chain amyloidoses, respectively.

Apomorphine is a rather promiscuous compound, with nanomolar agonist affinities for dopamine receptors (D₁, D_{2S}, D_{2L}, D₃, D₄, and D₅), serotonin receptors (5HT_{1A}, 5HT_{2A}, 5HT_{2B}, and 5HT_{2C}) and α -adrenergic receptors (α_{1B} , α_{1D} , α_{2A} , α_{2B} , and α_{2C})²⁶. However, Himeno *et al* found that 3xTg-AD mice that were treated with pramipexole (another dopamine receptor agonist) showed no improvement in memory behavior, indicating that the A β -degrading efficacy of apomorphine in this system is probably not derived from dopaminergic activity¹. This problem of a plethora of receptor targets and an unexpected effect on A β is highly similar to the situation we faced in our recent studies of latrepirdine (DimebonTM)²⁷. As with latrepirdine, when contemplating the linkage of neurotransmitter activation to APP catabolism, our first thoughts were drawn toward the regulated α -secretase phenomenon as a likely mechanism¹⁷. Himeno *et al* have not directly tested the possibility that apomorphine stimulates α -secretase metabolism of APP in their system, so one cannot determine whether this mechanism plays a role, although the signal transduction cascades downstream of several members of the aforementioned roster of apomorphine receptors have been linked to activation of α -secretase cleavage. Rather than linking apomorphine to neurotransmission, Himeno *et al* investigated the possibility that apomorphine acted through extracellular A β degrading proteases. Levels of insulin degrading enzyme (IDE) rise following apomorphine treatment, but levels of the most important A β protease in brain,

neprilysin (NEP), were unchanged. Intracellular catabolism of A β by IDE has not been described, so the importance of the IDE changes is unclear.

In contrast to the enzymes and pathways discussed so far in this editorial, Himeno *et al* propose that apomorphine readily stimulates catabolism of intracellular A β via activation of the ubiquitin-proteasome pathway. However, there is a topology problem with this formulation, since intraneuronal A β is intraluminal while the ubiquitin-proteasomal system is cytoplasmic. One possible link between the two compartments involves the role of ubiquitin in modulating autophagic degradation of protein aggregates within multivesicular bodies or autophagosomes (for review, see^{28,29}). Of possible relevance is the recent finding that pharmacologically modulating the mTOR pathway (a pathway known to regulate autophagy) with rapamycin also rescues cognitive deficit and reduces intracellular accumulation of A β via induction of autophagy in 3xTg-AD mice³⁰. These findings were recently supported by Spilman *et al*³¹, indicating that inhibition of mTOR signaling by rapamycin abolishes cognitive deficit and reduces A β levels in the PDAPP mouse model of AD. In this latter instance, mTOR-related changes in A β levels were not associated with activation of either NEP or IDE and were attributed to clearance of intraneuronal A β via autophagy³¹. When the findings by Caccamo *et al* and those of Spilman *et al* are taken together with the current results from Himeno *et al*, there would appear to be good reason to conclude that intracellular A β accumulation might well be a relevant and druggable target for novel AD therapeutics, especially in light of the associated behavioral benefits.

Himeno *et al* argue for a link between apomorphine's A β -clearance activation and its antioxidant activity. This mechanistic claim is based on the ability of the drug to protect against the toxicities of MPTP, H₂O₂ and 6-hydroxydopamine^{32,33}. Here the authors use p53 as a marker for "oxidative stress", but it is difficult to know how the acceleration of A β clearance by apomorphine might be related to the drug's antioxidant activities. Oxidative stress is an imprecise term that, as usually applied, roughly equates to "insufficient buffering of H₂O₂, superoxide, and/or hydroxyl radical". Oxidative stress has been invoked as a possible cause for sporadic AD, and, *in vitro*, peroxides do indeed promote aggregation of synthetic A β ³⁴. However, upon close scrutiny at the molecular level, the usual articulation of the oxidative stress hypothesis of AD is shaky on details of: (1) whether the problem might be an imbalance caused by either radical excess or scavenger deficiency; (2) where in the cell this radical imbalance might be localized; (3) why the imbalance might occur; and (4) how the imbalance might lead to amyloidosis. Himeno *et al* do not address any of these issues, but we and others have demonstrated that the oxidative stress induced by thiamine deficiency (TD) exacerbates amyloid³⁵ establishing that a cause and effect relationship may exist between some forms of oxidative stress and amyloidosis. Himeno *et al* may yet be proven correct about the relevance of antioxidant mechanism, even if the molecular and subcellular details are not immediately evident.

To be clear, the possibility that apomorphine stimulates brain A β catabolism is an exciting premise. One of the chief obstacles to successful anti-amyloid therapy for AD is believed to lie in the fact that patients have accumulated A β oligomers and plaques in their brains and cerebral blood vessels for perhaps 10-20 years before the first cognitive changes are apparent. With the exception of immunotherapy, only the PAI-1 pathway³⁶ has been suggested to be a therapeutically accessible pathway for clearing existing cerebral amyloidosis. The expansion of our armamentarium to include a druggable pathway for accelerating A β catabolism as exemplified by Himeno *et al*¹, Caccamo *et al*³⁰, Spilman *et al*³¹, and Tampelini *et al*²⁴ would be more than welcome, even if some of the mechanistic underpinnings reported here are not directly contributory. The first order of business is an independent replication of the apomorphine effect. If the basic observation is confirmed,

then the next step will be an elucidation of the specific subcellular and molecular mechanism(s) that are relevant.

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Figure 1. Schematic diagram of APP metabolism by cognate enzymes

Mature APP (center) is metabolized by two competing pathways, which generate “non-amyloidogenic” (left) and “amyloidogenic” (right) byproducts. Primary APP metabolism by α -secretase destroys A β and therefore precludes A β generation, aggregation, and related neurodegeneration. Processing of APP by β -secretase generates the C99 CTF, a γ -secretase substrate from which the neurotoxic 37-44 aminoacid A β peptide is derived. A β aggregates into low-n multimers (dimers, trimers, etc) known as oligomers, which have been associated with potent mnemonotoxicity. The current focus on oligomer toxicity predict that the senile plaque may be a relatively inert, endstage structure. (Figure¹⁶ is reprinted from with permission of *The Journal of Clinical Investigation*).

Figure 2. Immunoperoxidase and immunoelectron microscopy reveals APP/A β -LIR in intraneuronal organelles in the cerebral neurons of APP^{E693Q} overexpressing transgenic mice (Left and center) Intraneuronal vesicles are identifiable with monoclonal antibodies against two different A β epitopes. (Right) Immunoelectron microscopy using anti-rab5 shows that APP/A β -LIR is associated with small-, medium-, and large-sized cytoplasmic vesicular structures (including multivesicular bodies, dense lysosomes and lipofuscin). Interestingly, despite the absence of plaques, TgAPP^{E693Q} accumulate oligomeric A β assemblies, intraneuronal APP/A β -LIR in the hippocampi and cerebral cortices, and impaired performance of the Morris water maze task²² (Figure²² is reprinted with permission from *Annals of Neurology*).