Cholinergic agonists stimulate secretion of soluble full-length amyloid precursor protein in neuroendocrine cells

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ABSTRACT The A β peptide of Alzheimer disease is derived from the proteolytic processing of the amyloid precursor proteins (APP), which are considered type I transmembrane glycoproteins. Recently, however, soluble forms of full-length APP were also detected in several systems including chromaffin granules. In this report we used antisera specific for the cytoplasmic sequence of APP to show that primary bovine chromaffin cells secrete a soluble APP, termed solAPPcyt, of an apparent molecular mass of 130 kDa. This APP was oversecreted from Chinese hamster ovary cells transfected with a full-length APP cDNA indicating that solAPPcyt contained both the transmembrane and A β sequence. Deglycosylation of solAPPcyt showed that it contained both N- and O-linked sugars, suggesting that this APP was transported through the endoplasmic reticulum–Golgi pathway. Secretion of solAPPcyt from primary chromaffin cells was temperature-, time-, and energy-dependent and was stimulated by cell depolarization in a Ca²⁺-dependent manner. Cholinergic receptor agonists, including acetylcholine, nicotine, or carbachol, stimulated the rapid secretion of solAPPcyt, a process that was inhibited by cholinergic antagonists. Stimulation of solAPPcyt secretion was paralleled by a stimulation of secretion in catecholamines and chromogranin A, indicating that secretion of solAPPcyt was mediated by chromaffin granule vesicles. Taken together, our results show that release of the potentially amyloidogenic solAPPcyt is an active cellular process mediated by both the constitutive and regulated pathways. solAPPcyt was also detected in human cerebrospinal fluid. Combined with the neuronal physiology of chromaffin cells, our data suggest that cholinergic agonists may stimulate the release of this APP in neuronal synapses where it may exert its biological function(s). Moreover, vesicular or secreted solAPPcyt may serve as a soluble precursor of A β .

The A β peptide, the main proteinaceous component of the amyloid depositions of the Alzheimer disease (AD) brains, is derived from the proteolytic processing of the amyloid precursor proteins (APPs) which display the structural characteristics of type I transmembrane glycoproteins. APPs contain a large extracytoplasmic region, a single transmembrane sequence of about 24 residues, and a cytoplasmic (carboxyl-terminal) domain of 47 aa (for review, see ref. 1). Several APP isoforms, resulting from alternative exon splicing, have been identified including APP751 which contains a 56-aa insert with high homology to the Kunitz-type serine protease inhibitors (2). The A β sequence includes the last 28 aa of the extracytoplasmic region and about 12 to 15 residues of the transmembrane sequence of APP. It has been suggested that A β is produced after membrane full-length APP is cleaved initially by β -secretase at the N terminus of the A β sequence, followed by a cleavage of the resultant transmembrane APP fragment

by γ -secretase at the C terminus of A β (3, 4). However, in the transmembrane topology of APP, the peptide bond cleaved by γ -secretase is located within the lipid bilayer and may not be easily accessible to proteases. It is therefore possible that A β is derived from soluble nontransmembrane precursors where the peptide bond cleaved by γ -secretase is not protected by the lipid bilayer (5, 6).

Soluble truncated APPs (solAPPtrunc) are secreted as proteolytic derivatives of APP that do not contain the cytoplasmic sequence. They have most or all of the extracytoplasmic sequence and are produced after full-length APP is cleaved by secretases (3, 7, 8). Recently, however, we showed that the lumen of isolated bovine adrenal medullary chromaffin granules (CG), secretory vesicles used as a model for the study of neuronal secretion (9), contained a soluble APP with an intact cytoplasmic domain, and an apparent molecular mass similar to that of full-length APP. This protein, termed here solAPPcyt, contained both the transmembrane and the A β sequence of APP (6). A similar APP was detected in the culture media of pheochromocytoma PC12 cells and was released from membrane preparations *in vitro* (5, 6). In addition, soluble full-length APP species, as well as soluble truncated potentially amyloidogenic APP fragments with an intact cytoplasmic domain, have been detected in several cell culture systems (6, 10–12). It has been suggested that the latter species are derived from solAPPcyt and may be further degraded to produce A β (6). Although several functions, including cell growth (13), neurite outgrowth (14, 15), and stimulation of potassium channels (16), have been proposed for solAPPtrunc, the function of the soluble full-length APP is not known. The presence of this APP species in neuroendocrine secretory vesicles, however, suggested that it may be secreted in response to neuronal stimulation. Here we report that primary chromaffin cell cultures secrete a glycosylated solAPPcyt species of about 130 kDa. The secretion of this potentially amyloidogenic APP was temperature-, time-, and energy-dependent and was regulated by cell depolarization and cholinergic agonists.

MATERIALS AND METHODS

Materials. Penicillin/streptomycin and L-glutamine were obtained from GIBCO/BRL. Collagenase was obtained from Worthington, [³⁵S]methionine/cysteine from Amersham, and

Abbreviations: APP, amyloid precursor protein; AD, Alzheimer disease; CHO, Chinese hamster ovary; FBS, fetal bovine serum; CG, chromaffin granules; SRM, standard release medium; MTT, 3-[4,5-dimethylthiazole-2-yl]-2,5-diphenyl tetrazolium bromide; ER, endoplasmic reticulum; CSF, cerebrospinal fluid; solAPPcyt, soluble cytoplasmic APP; solAPPtrunc, soluble truncated APP.

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N-glycanase, *O*-glycanase, and neuraminidase from Genzyme. All other materials, including RPMI 1640 medium without methionine and cysteine, McCoy's 5A culture medium, and fetal bovine serum (FBS), were purchased from Sigma. Anti-cytoplasmic domain APP antisera R1, CT15, and C8 directed against APP751 sequences 729–751, 732–751, and 737–751, respectively, were obtained as described (5, 17, 18). Anti-chromogranin A antiserum was obtained from Incstar (Stillwater, MN).

Cell Cultures. Chinese hamster ovary (CHO) cells were obtained from the American Type Culture Collection and were transfected with APP751 as described (19). Primary cultures of bovine chromaffin cells were prepared from adrenal medulla as described (20). Cells were plated onto poly-L-lysine-coated flat-bottom 24-well plates (Costar) at a density of 5×10^5 cells per well in Dulbecco's modified Eagle's medium (DMEM) supplemented with 2 mM L-glutamine, 0.01 mM sodium pyruvate, 10% heat-inactivated FBS, 10 μ M cytosine arabinoside, 100 units of penicillin per ml, and 100 μ g of streptomycin per ml. Three days after plating, chromaffin cell cultures were metabolically labeled with 150 μ Ci of [³⁵S]methionine/cysteine per ml in DMEM (final methionine-cysteine concentration 2 μ M; 1 Ci = 37 GBq) plus 10% heat-inactivated dialysed FBS (1000 cut off). CHO cells were labeled in methionine-cysteine free RPMI 1640 medium plus supplements. For the depolarization experiments, labeled chromaffin cells were washed once with standard release medium (SRM) containing 118 mM NaCl, 4.6 mM KCl, 10 mM glucose, 25 mM Hepes, 2.2 mM CaCl₂, 1.2 mM MgCl₂, 100 units of penicillin per ml, and 100 μ g of streptomycin per ml, and then incubated for 10 min with either SRM, SRM containing 56 mM KCl, SRM containing 2 mM BaCl₂ instead of CaCl₂, or SRM containing cholinergic receptor agonists. To maintain isosmotic conditions during KCl depolarization, NaCl concentration was reduced accordingly. To study the effect of extracellular calcium on the secretion of solAPPcyt, cells were washed and stimulated in Ca²⁺-free buffer containing 2 mM EGTA and 2.2 mM MgCl₂. In experiments with inhibitors of cellular energy, cultures were chased for 2 h in the presence or absence of rotenone. In all cases, labeled media were centrifuged first at $1000 \times g$ for 10 min and then at $200,000 \times g$ for 90 min to remove floating cells and membrane fragments. Secreted full-length APP and chromogranin A were assayed 10 min after depolarization or cholinergic agonist treatment by immunoprecipitation using antisera R1 and anti-chromogranin A respectively. The release of catecholamines was determined fluorometrically (21), and cell viability was assayed using 3-[4,5-dimethylthiazol-2-yl]-2,5-diphenyl tetrazolium bromide (MTT) as described (22).

Immunoprecipitation and Glycosidase Treatment. Immunoprecipitation of conditioned media or cell extracts, $\approx 3 \times 10^7$ or 1×10^7 trichloroacetic acid-precipitable counts, respectively, was performed as described (19). To remove N- and O-linked sugars, immunocomplexes were sequentially treated with *N*-glycanase, neuraminidase, or *O*-glycanase according to manufacturers directions and amended as described (23). After deglycosylation samples were boiled in 1 $\times$ Laemmli buffer for 5 min and analyzed on 6% SDS/PAGE.

RESULTS

Adrenal medullary chromaffin cells have a common ontogenetic origin with sympathetic neurons and display neuronal physiology. As a result, CG-mediated secretion has been used extensively as a model for the regulation of neuronal secretion (9). In addition, primary chromaffin cell cultures express various APP isoforms in a manner similar to neurons (24), and CGs have been shown to contain solAPPcyt (6). We used this system to study secretion of the potentially amyloidogenic solAPPcyt. Primary chromaffin cell cultures were labeled

overnight with [³⁵S]methionine/cysteine, and conditioned media from these cultures were prepared and centrifuged at high speed as described (19). Supernatants were then immunoprecipitated using antisera R1, CT15, or C8, each directed against the cytoplasmic domain of APP (6, 17, 18). As shown in Fig. 1A, each of these antisera recognized a protein of ≈ 130 kDa in the culture medium. This protein is not APLP2 (25) because the latter is not recognized by the APP-specific antisera R1 and CT15 (5, 6). A similar APP was detected in the conditioned medium of CHO cell cultures (Fig. 1B). The apparent molecular mass of the solAPPcyt detected in the media of either CHO or primary chromaffin cell cultures was intermediate between the molecular mass of the immature, endoplasmic reticulum (ER), and mature forms of cellular full-length APP (26, 27). To exclude the possibility that solAPPcyt is derived from an alternatively spliced mRNA, we transfected CHO cells with the full-length APP751 cDNA, which encodes both the A β and transmembrane sequence (2). It can be seen in Fig. 1B that the isolated transfected clones overexpressed secreted solAPPcyt, indicating that alternative splicing is not a prerequisite for the production of solAPPcyt. Similar to the solAPPcyt detected in primary chromaffin or untransfected CHO cell cultures, the apparent molecular mass of the solAPPcyt secreted from transfected cells was also intermediate between the molecular mass of the cellular mature and immature forms of full-length APP.

To examine whether secreted solAPPcyt is glycosylated, labeled solAPPcyt from chromaffin cell culture medium was treated with *N*- or *O*-glycanases, specific for the removal of N- or O-linked sugars, respectively. It can be seen in Fig. 2 that these treatments lowered the apparent molecular mass of the solAPPcyt, showing that this protein is posttranslationally modified by the addition of both N- and O-linked sugars.

To determine whether production of solAPPcyt was due to a nonspecific release from damaged membranes or dead cells, we studied the time, temperature, and energy dependence of solAPPcyt secretion. It can be seen in Fig. 3A that solAPPcyt accumulated into the culture media as a function of time. To examine the temperature effect, labeled primary chromaffin cell cultures were chased for 3 h at 4, 25, or 37°C. As shown in Fig. 3B, cultures chased at 37°C secreted more solAPPcyt than cultures kept at 4°C. At 25°C, the released solAPPcyt was intermediate between the 37 and 4°C levels. As expected, inhibition of APP metabolism at 4°C resulted in the accumu-

FIG. 1. Primary chromaffin (A) or CHO (B) cell cultures were labeled overnight with [³⁵S]methionine/cysteine, and cell extracts or conditioned media were then immunoprecipitated using anti-cytoplasmic APP antisera. Samples were analyzed on a 6% SDS/PAGE. (A) Lanes 1, 5, and 7, chromaffin cell extracts immunoprecipitated with R1, CT15, or C8 antisera, respectively. Lanes 3, 6, and 8, conditioned media from chromaffin cell cultures were immunoprecipitated with R1, CT15, or C8 antisera, respectively. Lanes 2 and 4, chromaffin cell extracts or conditioned media, respectively, immunoprecipitated with R1 antiserum preabsorbed with 10 μ g/ml R1 peptide. (B) Lanes 1 and 4, nontransfected CHO cell extract or conditioned media, respectively. Lanes 2 and 3 or 5 and 6, cell extract or conditioned media, respectively, derived from two distinct CHO clones expressing different levels of APP751. All samples were immunoprecipitated with R1 antiserum. Arrows show cellular full-length APP species, and arrowheads indicate solAPPcyt.

FIG. 2. Primary chromaffin cell cultures were labeled overnight with [35 S]methionine/cysteine. Conditioned media samples were immunoprecipitated with R1 antiserum and the immunoprecipitates were treated with neuraminidase, *N*-glycanase, and *O*-glycanase (lane 1); *O*-glycanase (lane 3); or *N*-glycanase (lane 4). Lane 2 represents nontreated control. Samples were analyzed on a 6% SDS/PAGE.

lation of cellular full-length APP (Fig. 3*B*, lanes 4–6). To examine whether secretion of solAPPcyt was energy-dependent, labeled chromaffin cell cultures were treated with either glucose or rotenone, an inhibitor of oxidative phosphorylation (28). In parallel, we assayed for cell death and compromised cell function by the MTT cell viability assay, which measures mitochondrial dehydrogenase activity in living cells (22). Treatment with 1 μ M rotenone reduced the activity of mitochondrial dehydrogenases by $\approx 35\%$ (data not shown). However, it can be seen in Fig. 3*C* that this treatment decreased secretion of solAPPcyt by $\approx 50\%$. Inhibition of APP metabolism during rotenone treatment resulted in the accumulation of cellular full-length APP (Fig. 3*C*, lanes 4–6). Taken together, these data suggest that secretion of solAPPcyt is not a result of cell death, but rather an active cellular process.

Chromaffin cells possess functional cholinergic receptors and can be stimulated to secrete the contents of CGs by depolarization or treatment with cholinergic agonists in a calcium-dependent manner (9). Recently it was reported that CGs contain soluble full-length APP (6). We took advantage of the primary chromaffin cell culture system to study the regulation of solAPPcyt secretion. As shown in Fig. 4, depolarization by 56 mM KCl in the presence of Ca^{2+} resulted in a 4-fold stimulation of solAPPcyt secretion. KCl stimulation of solAPPcyt secretion was significantly reduced in the absence of Ca^{2+} (Fig. 4). BaCl_2 , which stimulates CG-mediated exocytosis, induced a 11-fold increase in solAPPcyt secretion (Fig. 4). Secretion of solAPPcyt was also stimulated by cholinergic agonists, both nicotinic and muscarinic. Thus, treatment of our cultures with 1 mM acetylcholine, 1 mM carbachol, or 10 μ M nicotine stimulated the secretion of solAPPcyt by ≈ 4 -fold. The effect of carbachol was eliminated when 100 μ M atropine, a

FIG. 3. (A) Conditioned media from a primary chromaffin cell culture labeled with [35 S]methionine/cysteine for 3 h (lane 1), 6 h (lane 2), 12 h (lane 3), or 24 h (lane 4) were immunoprecipitated with R1 antiserum and then analyzed on 6% SDS/PAGE. (B) Cell cultures were labeled as above overnight and then chased for 3 h at 4°C (lanes 1 and 4), 25°C (lanes 2 and 5), or 37°C (lanes 3 and 6). At the end of each chase period, conditioned media (lanes 1–3) or cell extracts (lanes 4–6) were immunoprecipitated with R1 antiserum. (C) Cultures were labeled as above overnight and chased in the absence (lanes 1 and 4) or presence of either 0.5 μ M (lanes 2 and 5) or 1 μ M (lanes 3 and 6) rotenone. Conditioned media (lanes 1–3) or cell extracts (lanes 4–6) were immunoprecipitated as above. All samples were analyzed on a 6% SDS gel. Arrows show membrane-bound full-length APP species and arrowheads show solAPPcyt.

FIG. 4. Primary chromaffin cells were labeled overnight with [35 S]methionine/cysteine, washed with SRM without calcium, and then treated with 56 mM KCl in the presence of 2 mM CaCl_2 (lane 2), 56 mM KCl in the absence of CaCl_2 (lane 3), 2 mM BaCl_2 (lane 4), 1 mM acetylcholine (lane 5), 10 μ M nicotine (lane 6), 1 mM carbachol (lane 7), or 1 mM carbachol and 100 μ M atropine (lane 8). Lane 1 corresponds to untreated cultures incubated in SRM. Total catecholamines were assayed fluorometrically as described (21). solAPPcyt and chromogranin A were detected by immunoprecipitation using R1 and anti-chromogranin A antisera, respectively. Immunoprecipitates were analyzed on 6% SDS/PAGE and quantitated by densitometry. Bars represent the mean $\pm$ SD of four independent experiments. *, Significance of difference from nontreated cultures, $P < 0.01$; **, significance of difference from cultures treated with 56 mM KCl in the presence of 2 mM CaCl_2 , $P < 0.05$; ***, significance of difference from cultures treated with 1 mM carbachol, $P < 0.02$.

muscarinic receptor antagonist, was included (Fig. 4). The increase in secretion of solAPPcyt was paralleled by an increase in the secretion of both chromogranin A and total catecholamines. Both classes of molecules are found in the lumen of CGs and are used as markers for CG-mediated secretion.

Our results indicated that solAPPcyt is stored in secretory vesicles from which it was secreted both constitutively and through the regulated secretory pathway. This observation suggested that solAPPcyt may be released *in vivo* through a similar mechanism and may be present in physiological fluids. To examine whether solAPPcyt is present in human serum, we used a heparin-agarose column to bind serum APP as described (23, 29). Bound APP was then eluted with 0.25 M NaCl, dialyzed, and then concentrated before it was electrophoresed on 7% SDS gels. Fig. 5*A* shows that CT15 antiserum, which is specific for the cytoplasmic sequence of APP, detected a protein of about 130 kDa in the eluted fraction. R1 antiserum

FIG. 5. (A) solAPPcyt concentrated from 0.5 ml of human serum on a heparin-agarose column (see Results) was loaded in lanes 1 and 2 of a 7% SDS gel. Following electrophoresis and Western blotting, filters were probed with CT15 antiserum (lane 1) or CT15 preabsorbed with the corresponding peptide (lane 2). R1 antiserum gave similar results. (B) Lanes were loaded with 30 μ l human CSF, electrophoresed on a 6% SDS gel, and the resultant Western blots were probed with either R1 antiserum (lane 1) or R1 preabsorbed with the corresponding peptide (lane 2).

gave similar results (data not shown). Our results strongly suggest that serum contains potentially amyloidogenic solAPPcyt. The serum levels of this APP species are rather low because we were unable to detect solAPPcyt without prior concentration on heparin. In contrast, we were able to detect solAPPcyt in human cerebrospinal fluid (CSF) without prior binding to heparin (Fig. 5B), suggesting that the concentration of this APP in CSF is significantly higher than in serum. CSF contained an additional solAPPcyt of about 110 kDa. It is possible that this APP is a glycosylation or proteolytic variant of the 130-kDa solAPPcyt.

DISCUSSION

Both theoretical and experimental considerations suggest that APP is a type I transmembrane protein. Recently, however, several groups presented evidence that a fraction of full-length APP, termed here solAPPcyt, is found in soluble form (5, 6, 11, 12). Other type I transmembrane proteins have also been found intact in soluble form. Olsen *et al.* (30), showed that functional forms of the neural cell adhesion molecule (N-CAM) with intact transmembrane sequence, are present in CSF and plasma (31). Similarly, a form of the insulin receptor, a type I transmembrane protein, was secreted with its transmembrane sequence from cultured cells and was also detected in human plasma (32). Several other type I transmembrane proteins including insulin-like growth factor I receptor, c-Kit receptor, and recombinant epidermal growth factor precursor (32–34) are also secreted with intact transmembrane sequences. These observations suggest the existence of cellular mechanisms for the secretion of intact transmembrane proteins in soluble forms.

The results presented here show that a solAPPcyt species of an apparent molecular mass between that of the immature (ER) and mature cellular forms of full-length APP is constitutively secreted from both primary adrenal chromaffin and CHO cells. Since the APP gene contains a large number of exons, it was conceivable that solAPPcyt was derived from an alternatively spliced mRNA without the exon that encodes the transmembrane sequence used to anchor APP in the lipid bilayer (35). However, transfection of CHO cells with full-length APP751 cDNA, which encodes both the transmembrane and A β sequence of APP, resulted in oversecretion of solAPPcyt. These results indicate that solAPPcyt is not the product of an alternatively spliced mRNA and it contains both the transmembrane and A β sequence of APP. This conclusion is in agreement with recent immunoblot evidence from several groups, suggesting that soluble secreted full length APP contains both of these sequences (5, 6, 11, 12). solAPPcyt had a higher apparent molecular mass than the immature cellular full-length APP (26, 27), suggesting that the former is a glycosylation variant of full-length APP which contains both N- and O-linked sugars (23, 27). Treatment of solAPPcyt with glycanases confirmed this suggestion indicating that like the mature cellular full-length APP, solAPPcyt is inserted into the ER and follows the ER–Golgi pathway after which it is packaged in secretory vesicles and secreted.

Our experiments indicate that secretion of potentially amyloidogenic solAPPcyt is an active functional process. Constitutive secretion of solAPPcyt was time-, energy-, and temperature-dependent. Energy deprivation which compromised cell viability inhibited release of solAPPcyt suggesting that this process requires energy and is not the result of cell death. Recently, it was reported that the lumen of CGs, the secretory vesicles of chromaffin cells, contained soluble full-length APP (6). Chromaffin cells contain cholinergic receptors coupled to CG-mediated exocytosis, a system often used to study neuronal secretion (9). These observations prompted us to examine whether secretion of full-length APP was stimulated by depolarization or treatment with cholinergic agonists. Indeed, we

found that secretion of solAPPcyt was stimulated within 10 min of depolarization with either KCl in a Ca²⁺-dependent manner, or BaCl₂. The rapid stimulation of secretion suggested that preexisting vesicular solAPPcyt was secreted in response to our treatments. Furthermore, several cholinergic agonists including carbachol, nicotine, and acetylcholine, stimulated the rapid secretion of solAPPcyt at concentrations used to induce granule-mediated secretion (9). Carbachol stimulation of solAPPcyt secretion was inhibited by the muscarinic receptor antagonist atropine providing further support for the suggestion that secretion of this APP was mediated by cholinergic stimulation. Secretion of solAPPcyt was paralleled by the secretion of catecholamines and chromogranins, both of which are used as markers for CG-mediated secretion.

Combined with the presence of solAPPcyt in the soluble fraction of CGs (6), our data indicate that this APP is stored in the lumen of neuroendocrine secretory vesicles from where it is secreted through the regulated pathway in response to depolarization or to stimulation by muscarinic or nicotinic cholinergic agonists. Because chromaffin cells display neuronal physiology, our observations suggest that solAPPcyt may also be secreted in response to neuronal depolarization in synaptic clefts of the brain, where it may function in neurotransmission. Several lines of evidence provide further support for this suggestion. First, solAPPcyt is present in CSF at relatively high concentrations (see *Results*), suggesting that this potentially amyloidogenic APP is secreted in the central nervous system, presumably through a mechanism similar to that described in the primary chromaffin cells. Second, APP has been shown to be transported to and concentrate in nerve terminals (36, 37), and third, solAPPcyt was secreted by a depolarization-dependent mechanism from mouse brain synaptosomal preparations, indicating that it is stored in and released from neuronal presynaptic vesicles (S.E. and N.K.R., unpublished data). Secretion of the nonamyloidogenic solAPPtrunc has also been found to be stimulated by a number of different agents, including cholinergic agonists. However, several lines of evidence suggest that the regulation of secretion of these two soluble APPs may differ. First, stimulation of solAPPtrunc secretion was Ca²⁺-independent (38). Second, cholinergic agonist-stimulated secretion of solAPPcyt in chromaffin cells was completed in less than 10 min (S.E. and N.K.R., unpublished observations). In contrast, stimulated secretion of solAPPtrunc is much slower (38), and third, secretion of solAPPtrunc in AtT20, a model cell line for neuroendocrine secretion, was not mediated by the regulated pathway (39).

Secreted solAPPcyt may subserve biological functions distinct from those of the transmembrane APP. It has been proposed that an alteration in the biological function(s) of APP may be involved in the neurodegeneration and synapse destruction observed in some forms of AD (40–42). Elucidation of the exact nature of the synaptic function of solAPPcyt is an important step in understanding the involvement of this molecule in the development of AD. It has been suggested that secreted solAPPtrunc promotes neurite outgrowth (14, 15) and regulates neuronal excitability (16). Since solAPPcyt contains all amino acid sequence of solAPPtrunc, these two secreted APP forms may have similar or even complementary neuronal functions.

Secretion of the potentially amyloidogenic solAPPcyt has important implications for the production of A β , a peptide with neurotoxic activities. A β plays an important role in the development of AD neuropathology, although its role as the primary cause of this disease remains controversial. Production of A β requires that potentially amyloidogenic APP is cleaved by both β - and γ -secretases. However, in the transmembrane topology of APP, the peptide bond cleaved by γ -secretase is located within the lipid bilayer (1, 35) and should be inaccessible to proteases. Since production of A β is the

result of normal cellular metabolism and requires no obvious membrane damage (43, 44), it was proposed that this peptide may derive from solAPPcyt where the cleavage site of γ -secretase is not protected by the lipid bilayer (5, 6). Several lines of evidence indicate that the secretory pathway is involved in the production of A β (45, 46). The presence of the potentially amyloidogenic solAPPcyt in secretory vesicles, where also secretase activities including β -secretase have been located (46, 47), provides additional support for this theory, although the exact site of A β production is not known. We detected A β in the conditioned media of our primary chromaffin cells and in CGs (S.E., N.T., and N.K.R., unpublished observations). It is tempting to speculate that A β is produced from solAPPcyt in secretory vesicles followed by secretion. Alternatively, A β may be produced extracellularly following secretion of solAPPcyt, in agreement with the colocalization of both molecules in CSF and serum (43). In either case, neuronal depolarization by cholinergic agonists would be expected to cause an accumulation of A β in neuronal synapses. It remains an interesting question whether the stimulation of solAPPcyt secretion by cholinergic agonists is related to the selective vulnerability of the cholinergic system observed in AD.

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