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Beta-amyloid peptides undergo regulated co-secretion with neuropeptide and catecholamine neurotransmitters

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Abstract

Beta-amyloid (Aβ) peptides are secreted from neurons, resulting in extracellular accumulation of Aβ and neurodegeneration of Alzheimer's disease. Because neuronal secretion is fundamental for the release of neurotransmitters, this study assessed the hypothesis that Aβ undergoes co-release with neurotransmitters. Model neuronal-like chromaffin cells were investigated, and results illustrate regulated, co-secretion of Aβ(1–40) and Aβ(1–42) with peptide neurotransmitters (galanin, enkephalin, and NPY) and catecholamine neurotransmitters (dopamine, norepinephrine, and epinephrine). Regulated secretion from chromaffin cells was stimulated by KCl depolarization and nicotine. Forskolin, stimulating cAMP, also induced co-secretion of Aβ peptides with peptide and catecholamine neurotransmitters. These data suggested the co-localization of Aβ with neurotransmitters in dense core secretory vesicles (DCSV) that store and secrete such chemical messengers. Indeed, Aβ was demonstrated to be present in DCSV with neuropeptide and catecholamine transmitters. Furthermore, the DCSV organelle contains APP and its processing proteases, β- and γ-secretases, that are necessary for production of Aβ. Thus, Aβ can be generated in neurotransmitter-containing DCSV. Human IMR32 neuroblastoma cells also displayed regulated secretion of Aβ(1–40) and Aβ(1–42) with the galanin neurotransmitter. These findings illustrate that Aβ peptides are present in neurotransmitter-containing DCSV, and undergo co-secretion with neuropeptide and catecholamine neurotransmitters that regulate brain functions.

Keywords

β-Amyloid; Regulated secretion; Dense core secretory vesicles; Neuropeptides; Catecholamines; Neurotransmitters

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1. Introduction

Beta-amyloid peptides (Aβ) are a major factor involved in the development of neurodegeneration of Alzheimer's disease (AD), resulting in severe loss of memory and cognitive functions [6,13,18,51,53]. Secretion of Aβ peptides from brain neurons provides accumulation of extracellular Aβ peptides to result in loss of neurons in hippocampal and cortical brain regions that are responsible for memory function.

Studies in the field have illustrated regulated secretion of Aβ that represents electrical activity-dependent neuronal secretion [9,19,20,22,26,37,38]. More recently, evidence has emerged indicating that brain neuronal activity is related to Aβ deposition [3]. However, the secretory organelle mechanisms for regulated Aβ secretion have not yet been examined in detail.

Brain function requires regulated secretion of neurotransmitters from secretory vesicles [52,55], the fundamental mechanism for synaptic neurotransmission. The property of regulated secretion of neurotransmitters from neurons, combined with evidence for regulated activity-dependent secretion of Aβ, suggests the hypothesis that Aβ and neurotransmitters undergo co-secretion. To test this hypothesis, the goal of this study was to assess regulated, co-secretion of Aβ with neuropeptide and catecholamine neurotransmitters from model neuronal-like chromaffin cells of the sympathetic nervous system. Chromaffin cells have been utilized extensively for studies of regulated secretion of neurotransmitters from dense core secretory vesicles (DCSV), and have advanced knowledge of enzymes that produce such neurotransmitters in brain [5,39].

Results of this study show that Aβ(1–40) and Aβ(1–42) peptides undergo regulated secretion with neuropeptide and catecholamine neurotransmitters upon stimulation of regulated secretion by KCl depolarization and nicotine. Regulated co-secretion of Aβ and neurotransmitters was also stimulated by forskolin that stimulates cAMP production and secretion [11,49,50]. The co-localization of Aβ with enkephalin and NPY neurotransmitters in secretory vesicles of chromaffin cells was demonstrated. Because these neurotransmitters are secreted from DCSV of neurons, the content of Aβ peptides with such neurotransmitters in isolated DCSV was quantitated. Notably, the DCSV contain β- and γ-secretases that convert APP, present in DCSV, into neurotoxic Aβ peptides. Thus, the DCSV organelle contains the APP processing components that produce Aβ peptides, which are co-secreted with neuropeptide and catecholamine neurotransmitters. In addition, human IMR32 neuroblastoma cells display regulated co-secretion of Aβ with the galanin neurotransmitter. These findings illustrate the co-secretion of Aβ peptides with neuropeptides and catecholamines, suggesting that Aβ functions with multiple neurotransmitter systems.

2. Materials and methods

2.1. Secretion of Aβ with peptide and catecholamine neurotransmitters from neuronal-like chromaffin cells in primary culture

Primary cultures of chromaffin cells were prepared from fresh bovine adrenal medulla (from Sierra Medical Sciences, Whittier, CA), as we have described previously [40,66]. Cells were plated in 6-well plates at 1.5×10^6 cells/well. Cells were subjected to regulated secretion achieved by stimulation by depolarization with high KCl (50 mM) in the medium for 15 min at 37 °C. Controls included incubation of cells without KCl for basal, constitutive secretion. The media was collected for measurements of Aβ(1–40) and Aβ(1–42) by ELISA assays (ELISA kits #27718 and #27711, respectively, IBL International, Toronto, Canada). The peptide neurotransmitters of galanin, NPY, and (Met)enkephalin were measured by radioimmunoassays (Phoenix Pharmaceuticals, Burlingame, CA). The catecholamines

dopamine, norepinephrine, and epinephrine were also measured in the media, conducted as described [67]. In addition, nicotine stimulation of regulated secretion from chromaffin cells was also conducted by incubating cells with nicotine (10 μM for 15 min.), and without nicotine as control; the secretion media was subjected to measurements of Aβ(1–40) and Aβ(1–42), peptides, and catecholamines as described for media from KCl secretion experiments. Furthermore, regulated secretion was induced by treating cells with forskolin (50 μM, 2 hrs incubation) which elevates cAMP [49] that induces regulated secretion [11,50]. Each secretion experiment (with control, KCl, nicotine, or forskolin conditions) was conducted in triplicate, and each experiment was repeated at least three times. Statistical analyses of Aβ peptides, neuropeptides, and catecholamine levels in secretion media from stimulated and unstimulated control cells were conducted by the Student's *t*-test, with significance level of $p < 0.05$. Results are expressed as the mean ± s.e.m. (standard error of the mean).

2.2. Immunofluorescence deconvolution microscopy of Aβ-related forms and peptide neurotransmitters

Immunofluorescence histochemistry of Aβ and peptide neurotransmitters was assessed by deconvolution microscopy, as we have described [12]. Briefly, chromaffin cells were fixed in 4% paraformaldehyde (PFA), permeabilized with 0.1% Triton X-100, and incubated with anti-APP 6E10 (1:100, mouse, Covance, San Diego, CA), anti-galanin (1:200, rabbit, Bachem, Torrance, CA), anti-NPY (1:300, rabbit, Chemicon/Bioscience Research Reagents/ Millipore, Temecula, CA), or anti-(Met)enkephalin (1:50, mouse, Abcam, Cambridge, MA) in PBS containing 3% bovine serum albumin (PBS-BSA 3%) for 2 h at room temperature. After washing with PBS, cells were then incubated with secondary goat anti-rabbit Alexa Fluor 488 and goat anti-mouse Alexa Fluor 594 (green and red fluorescence, respectively, 1:300, Molecular Probes/Life Technologies, Grand Island, NY), respectively, in PBS-3% BSA. Immunofluorescent co-localization of Aβ-related forms (anti-APP 6E10 detects Aβ and its APP precursor forms) with peptide neurotransmitters was examined with the Delta Vision Spectris Image Deconvolution System on an Olympus IX70 microscope using Softworx Explorer software from Applied Precision. As control, incubation with only secondary antibodies (no primary antibodies) was performed, resulting in a lack of immunofluorescence thus indicating specific immunofluorescence signals resulting from the primary antisera.

2.3. Purification of dense core secretory vesicles (DCSV) and analyses of Aβ and neurotransmitters

Secretory vesicles from fresh bovine adrenal medulla tissue, specifically the dense core secretory vesicles (DCSV), was purified by sucrose density gradient centrifugation as we have described previously [63]. The high purity of the isolated secretory vesicles has been established by enzyme markers of subcellular organelles [1,54,62,63,65]. The homogeneity and integrity of the purified DCSV was confirmed by electron microscopy, conducted as we have described previously [66].

The purified DCSV were lysed by freeze–thawing in buffer (50 mM Na-acetate, pH 6.0, 150 mM NaCl, 1 mM EDTA) containing a cocktail of protease inhibitors consisting of 10 μM pepstatin A, leupeptin, and chymostatin, and E64c, and 500 μM AEBSF (4-(2-aminoethyl) benzenesulfonyl fluoride hydrochloride). An acid extract was prepared from the lysed DCSV by bringing the sample to 1.0 N acetic acid, heating at 95 °C for 10 min, centrifuging at 13,000 × g for 10 min, and collecting the supernatant for measurement of Aβ(1–40), Aβ(1–42), galanin, NPY, (Met)enkephalin, and the catecholamines dopamine, norepinephrine, and epinephrine. Assays for these components by ELISAs and RIAs are described above for secretion media in this methods section. VIP and somatostatin

neuropeptides were also measured in DCSV by radioimmunoassays (from Phoenix Pharmaceuticals, Burlingame, CA and Bachem, Torrance, CA). Protein content of the purified DCSV was measured by the Bio-Rad DC protein assay kit (Bio-Rad, Hercules, CA). Aβ and neurotransmitter contents in DCSV were expressed as pg per μg protein.

2.4. Western blot analyses of DCSV for β- and γ-secretase components with APP and Aβ

Purified DCSV were subjected to western blots analyses to assess the presence of β- and γ-secretase components utilized for processing APP, as well as APP and Aβ in DCSV [45,48]. Western blots were conducted as we have described previously [57,66]. Antibodies to peptide domains of Aβ and APP were used in western blots to assess the presence of Aβ, APP, as well as APP-derived fragments in DCSV. Custom-generated antisera were produced to peptide antigens consisting of Aβ(17–28), and the N-terminus of APP of residues 1–18 (APP1-18) (these antisera were produced by Immuno-Dynamics, La Jolla, CA). The R7 and R1 antisera to APP were gifts from the laboratory of Professor Nikolaos Robakis (Mt. Sinai Univ., New York [57]). The 22C11 antibody detecting an N-domain region of APP (APP66-81) was obtained from Millipore Chemicals (Billerica, MA).

Antisera utilized for western blots were directed to the BACE1 β-secretase (Sigma, St. Louis, MO) and the wild-type β-secretase recently identified as cathepsin B [13,17,25] (Athens Research & Technology, Athens, GA). Western blots of γ-secretase components were conducted using antisera to presenilins 1 and 2 (Millipore, Billerica, MA and Cell Signaling Technology/Millipore, Temecula, CA), nicastrin (Sigma-Aldrich, St. Louis, MO), Pen-2 (Sigma, St. Louis, MO), and Aph-1 (Covance, San Diego, CA). Western blot of ADAM10, representing α-secretase, was conducted with anti-ADAM10 (rabbit anti-ADAM10 from abcam, Cambridge, MA).

2.5. Human IMR32 neuroblastoma cells: secretion of Aβ with the galanin neurotransmitter

Human IMR32 neuroblastoma cells were obtained from ATCC (American Type Culture Collection, Manasssus, VA) and cultured in MEM/10% FBS (fetal bovine serum) (Invitrogen, Carlsbad, CA). Cells were plated at 5×10^6 cells per 10 cm petri dish. Activity-dependent secretion was stimulated by high KCl (50 mM) in the culture media for 90 min. The media was collected for measurement of Aβ(1–40) and Aβ(1–42) (ELISA kits) (#27718 and #27711, respectively, IBL International, Toronto, Canada), as well as galanin (Phoenix Pharmaceuticals, Burlingame, CA). Each secretion condition (with or without KCl) was conducted in triplicate in each experiment, and experiments were repeated two to three times.

3. Results

3.1. Regulated secretion of Aβ with neurotransmitters from neuronal chromaffin cells

Neuronal-like chromaffin cells of the sympathetic nervous system produce peptide and catecholamine neurotransmitters and have been utilized as a model for biosynthesis and secretion of such neurotransmitters [5,16,39]. These cells also produce Aβ peptides [15]. But the question of whether Aβ undergoes co-secretion with neuropeptide and catecholamine neurotransmitters has not yet been determined, and was, therefore, assessed in this study.

Regulated secretion from chromaffin neuronal cells was stimulated by KCl depolarization (50 mM KCl in the medium for 15 min in this study). KCl treatment stimulated the regulated secretion of Aβ(1–40) and Aβ(1–42) by several-fold above basal constitutive secretion (no KCl) (Fig. 1a and b). Similarly, KCl depolarization stimulated the regulated secretion of the neuropeptide transmitters galanin, NPY, and (Met)enkephalin (Fig. 2a–c) from chromaffin cells. Regulated secretion was also observed for the catecholamines dopamine,

norepinephrine, and epinephrine (Fig. 2d–f) from these cells. These data demonstrate KCl stimulation of regulated co-secretion of Aβ peptides with neuropeptide and catecholamine neurotransmitters.

Regulated secretion can also be stimulated by nicotine via the nicotinic acetylcholine receptor of chromaffin cells [33,47]. Nicotine induced the regulated co-secretion of Aβ(1–40) and Aβ(1–42) peptides (Fig. 1a and b), the peptides galanin, NPY, and (Met)enkephalin (Fig. 2a–c), as well as the catecholamines dopamine, norepinephrine, and epinephrine (Fig. 2d–f). Nicotine stimulates secretion above basal secretion (no nicotine) by several-fold.

Regulated secretion is known to be stimulated by activation of protein kinase A [11,50]. Forskolin activates adenylyl cyclase that produces cAMP, activating cAMP-dependent protein kinase A (PKA) [49]. Treatment of chromaffin cells with forskolin stimulated the regulated co-secretion of Aβ(1–40) and Aβ(1–42) (Fig. 3a and b) with the peptide neurotransmitters galanin, NPY, and (Met)enkephalin (Fig. 4a–c). Forskolin also stimulated the regulated secretion of the catecholamines dopamine, norepinephrine, and epinephrine (Fig. 4d–f).

These combined secretion experiments demonstrate that Aβ(1–40) and Aβ(1–42) peptides undergo regulated co-secretion with neuropeptide and catecholamine neurotransmitters.

3.2. Cellular co-localization of Aβ-related forms with peptide neurotransmitters in dense core secretory vesicles (DCSV)

The co-secretion of Aβ with neurotransmitters suggests their joint localization in dense core secretory vesicles (DCSV) which store neurotransmitters for their secretion. Thus, the localization of Aβ with neurotransmitters in DCSV was assessed by immunofluorescence deconvolution microscopy. Cellular Aβ-related immunofluorescence detected with the 6E10 antibody was co-localized with galanin and NPY that are present in dense core secretory vesicles (DCSV) (Fig. 5) of chromaffin cells and neurons. The 6E10 antibody detects Aβ and APP precursor forms of Aβ-related immunoreactivity. These data suggest the presence of Aβ- and APP-related forms in neurotransmitter-containing dense core secretory vesicles.

3.3. Aβ, APP, and neurotransmitters in dense core secretory vesicles (DCSV) isolated from chromaffin cells

To determine the content of Aβ peptides with neurotransmitters in DCSV, the DCSV were purified to homogeneity for measurements of Aβ peptides, neuropeptides, and catecholamines. The integrity and homogeneity of the purified DCSV was demonstrated by electron microscopy (Fig. 6a). Quantitation of Aβ peptides and neurotransmitter components indicates their presence in purified DCSV (Table 1). The DCSV contain Aβ(1–40) and Aβ(1–42), with the more neurotoxic Aβ(1–42) present at a lower level than Aβ(1–40). Many peptide neurotransmitters are present in DCSV, including galanin, NPY, (Met)enkephalin, VIP, somatostatin, and β-endorphin. Moreover, the catecholamines dopamine (DA), norepinephrine (NE), and epinephrine (Epi) are present in DCSV (Table 1).

Aβ and APP forms were also assessed by western blots of the purified DCSV conducted with epitope-specific antisera to APP domains consisting of Aβ (antisera 1659 directed to Aβ17–28), the N-terminal region of APP (antisera 1656), an N-domain region of APP (22C11 antibody), and the R7 and R1 antisera that recognize the KPI and C-domains of APP (illustrated in Fig. 6b). Western blots detected endogenous Aβ, present at ~ 10pg/ml of DCSV extract, observed as a band of ~4.5 kDa by anti-Aβ(17–28) (Fig. 6c), representing monomeric Aβ. This Aβ band was consistently observed in replicate western blots. The anti-Aβ prepared in rabbits for this study (described in methods) is capable of detecting endogenous levels of Aβ of DCSV at the pg level, which is more sensitive than many other

antisera which detect ng (rather than pg) amounts of Aβ [28,36,45,61]. Furthermore, APP, the precursor of Aβ, was observed by western blots as a band of ~100–110 kDa detected by the antisera 1656, 22C11, and R7 (Fig. 6d). In addition, bands of ~87–90 kDa were detected by these antisera, consistent with the presence of sAPPα and sAPPβ.

These data illustrate the presence of Aβ, generated from the APP precursor, with multiple neuropeptide and catecholamine neurotransmitters in DCSV that store such components for their regulated secretion.

3.4. APP processing secretase components are present in dense core secretory vesicles (DCSV) that contain Aβ, APP, and neurotransmitters

Western blots indicate that APP processing secretases are present in the DCSV Beta-secretases are present in DCSV, consisting of the recently identified cathepsin B [15,17,25] and the well-known aspartyl protease BACE 1 [6,18,21,59] (Fig. 7a and b). The γ-secretase complex components – presenilins 1 and 2, nicastrin, Aph-1, and Pen-2 [22,23,29] – are present in DCSV (Fig. 7c–g). Moreover, the DCSV contain the ADAM10 protease (Fig. 8) that represents α-secretase that cleaves within Aβ [27,42,48,58]. These data demonstrate that the DCSV organelle contains the full repertoire of β-secretase and γ-secretase components for processing APP into Aβ peptides that undergo regulated secretion.

3.5. Human neuroblastoma cells display regulated co-secretion of Aβ with peptide neurotransmitters

To demonstrate regulated co-secretion of Aβ from a human model of neurons, the human IMR32 neuroblastoma cell line was assessed for Aβ co-secretion with the galanin neurotransmitter. Regulated secretion of Aβ(1–40) and Aβ(1–42) was observed upon KCl depolarization (Fig. 9a and b). Furthermore, these Aβ peptides were co-secreted with galanin, a peptide neurotransmitter (Fig. 9c). Overall, results from these experiments demonstrate that Aβ peptides are co-secreted with neurotransmitter systems in a regulated manner.

4. Discussion

This study demonstrates that Aβ peptides undergo regulated co-secretion with neuropeptide and catecholamine neurotransmitters from the dense core secretory vesicle (DCSV) organelle of neuronal-like chromaffin cells, a widely studied model for neurotransmitter secretion. Regulated secretion was stimulated by KCl depolarization of chromaffin cells, resulting in co-secretion of Aβ(1–40) and Aβ(1–42) with the peptide neurotransmitters galanin, NPY, and enkephalin, as well as with the catecholamines dopamine, norepinephrine, and epinephrine. Nicotine and forskolin also stimulated regulated co-secretion of Aβ peptides with neuropeptides and catecholamines. Cellular immunofluorescence microscopy demonstrated the co-localization of Aβ-related forms with galanin and NPY peptide neurotransmitters that are present in DCSV. Quantitation of Aβ peptides with neurotransmitters in isolated DCSV demonstrated the presence of Aβ(1–40) and Aβ(1–42) with neuropeptides and catecholamine neurotransmitters, consistent with their co-secretion from chromaffin cells. Notably, the DCSV organelle contains APP with the APP processing enzymes consisting of β- and γ-secretase components that produce Aβ, as well as α-secretase that cleaves within Aβ (precluding Aβ formation). Additionally, human IMR32 neuroblastoma cells display regulated secretion of Aβ with the galanin neurotransmitter. These new findings demonstrate that Aβ undergoes regulated co-secretion with neuropeptide and catecholamine neurotransmitters from dense core secretory vesicles, indicating that Aβ functions with multiple neurotransmitter systems.

Deposition of extracellular Aβ in brain is a significant factor in the development of Alzheimer's disease. However, details of the secretory organelle mechanism for neuronal Aβ release have been limited. Results from the present study demonstrate that Aβ is secreted from dense core secretory vesicles (DCSV) of neurons in a regulated, activity-dependent manner. The DCSV represents the key secretory organelle for secretion of Aβ and the neuropeptide and catecholamine type of neurotransmitters.

Of notable significance is the finding that the DCSV organelle contains the β- and γ-secretase amyloidogenic components utilized for processing endogenous APP into Aβ peptides. Alpha-secretase is also present in DCSV. The presence of APP processing components in DCSV indicates that Aβ production is under the control of the neurotransmitter secretory system. Indeed, the APP processing system in DCSV is sensitive to neuronal activity regulation of Aβ formation and secretion [20]. Activity-dependent secretion of neurotransmitters is known to drive biosynthesis of neurotransmitters to replenish stores of these molecules for neurotransmission. Thus, regulated neuronal activity participates in the release of both Aβ and neurotransmitters.

Regulated co-secretion of Aβ with peptide and catecholamine neurotransmitters implicates involvement of neurotransmitter systems with Aβ functions. The co-secretion of Aβ suggests involvement of those neurotransmitters that modulate cognition and memory. For example, this study illustrates the co-secretion of Aβ with galanin, a neurotransmitter that impairs memory function [46,56]. Other neurotransmitters co-secreted with Aβ may participate in the development of memory and cognitive dysfunction in Alzheimer's disease.

Activity-dependent secretion of neurotransmitters utilizes the regulated secretory pathway of neurons, involving secretory vesicles that respond to electrical depolarization of neurons [40]. Secretory vesicles of the regulated secretory pathway are distinct from those of the constitutive, basal secretory pathway [4,31]. Studies of Aβ secretion into conditioned medium from unstimulated neurons utilize the basal constitutive, unregulated secretory pathway. Results here illustrate the regulated secretory pathway as a major source of extracellular Aβ.

Regulated secretion of neurotransmitters from neurons is controlled by neurotransmitter receptors that control the electrical activity of neurons. Thus, receptors of neurotransmitters are involved in controlling activity-dependent secretion of Aβ. This study demonstrates that nicotinic cholinergic receptor activation of chromaffin cells results in co-secretion of Aβ with neurotransmitters. Earlier studies show that activation of muscarinic acetylcholine receptors [29] and glutamate receptors [19] release Aβ-related APP products. Recent studies of the involvement of glutamate receptors in regulating Aβ secretion [20,22] indicates, as example, roles for neurotransmitter modulation of neuronal activity and Aβ. Clearly, receptor-mediated control of neuronal electrical activity can, in turn, regulate activity-dependent secretion of Aβ.

The DCSV at nerve terminals provide chemical messengers for neurotransmission. The DCSV contain peptide neurotransmitters and catecholamines [5,7,41]. In addition, the synaptic vesicles of nerve terminals contain the classical neurotransmitters composed of glutamate, acetylcholine, GABA, glycine and others [52]. Regulated, activity-dependent neurotransmitter secretion results in release of classical neurotransmitters from synaptic vesicles, and release of neuropeptides and catecholamines with Aβ peptides from DCSV [41].

The co-secretion of endogenous Aβ with neurotransmitters suggest that Aβ has normal physiological functions. Indeed, studies in the field have demonstrated endogenous roles for Aβ in synaptic functions and plasticity [30,43,64], metal homeostasis of Cu and Zn [35,44],

mitochondrial function [8,34], and others. The data of this study suggest that Aβ functions may include coordination with neurotransmitter functions, since Aβ undergoes co-secretion with catecholamine and peptide neurotransmitters. Moreover, it will of interest in future studies to understand how Aβ from secretory vesicles may undergo oligomerization [2,10,14] to neurotoxic Aβ forms that result in neurodegeneration of Alzheimer's disease.

In summary, regulated co-secretion of Aβ with neurotransmitters from dense core secretory vesicles, containing the APP processing machinery for Aβ production, indicates the key role of regulated neuronal activity for regulating Aβ deposition and memory dysfunction of Alzheimer's disease.

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Abbreviations

Aβ	β-amyloid
APP	amyloid precursor protein
BSA	bovine serum albumin
DCSV	dense core secretory vesicles
NPY	neuropeptide Y
PBS	phosphate-buffered saline

Glossary

Amyloid precursor protein

Aph-1

BACE 1

β-amyloid

cathepsin B

chromaffin cells

dense core

secretory vesicles

dopamine

enkephalin

epinephrine

forskolin

galanin

neuroblastoma

neurons

neuropeptide Y

nicastrin

nicotine

norepinephrine

PEN-2

potassium chloride

presenilin

regulated secretion

secretases

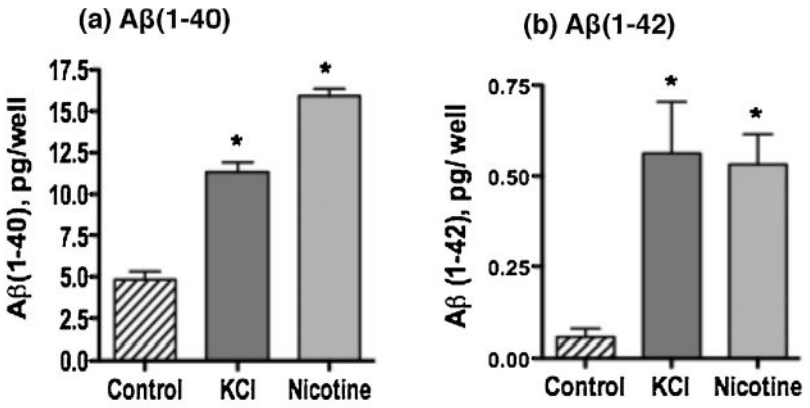

Fig. 1.

Regulated secretion of Aβ peptides from neuronal chromaffin cells stimulated by KCl depolarization and nicotine. Regulated secretion from neuronal-like chromaffin cells in primary culture was stimulated by KCl depolarization (50 mM) and nicotine (10 μM) (15 min incubation). At the end of this treatment period, secretion media was collected and analyzed for concentrations of the Aβ(1–40) and Aβ(1–42) peptides (panels (a) and (b), respectively). *$p < 0.05$ (Student's *t*-test).

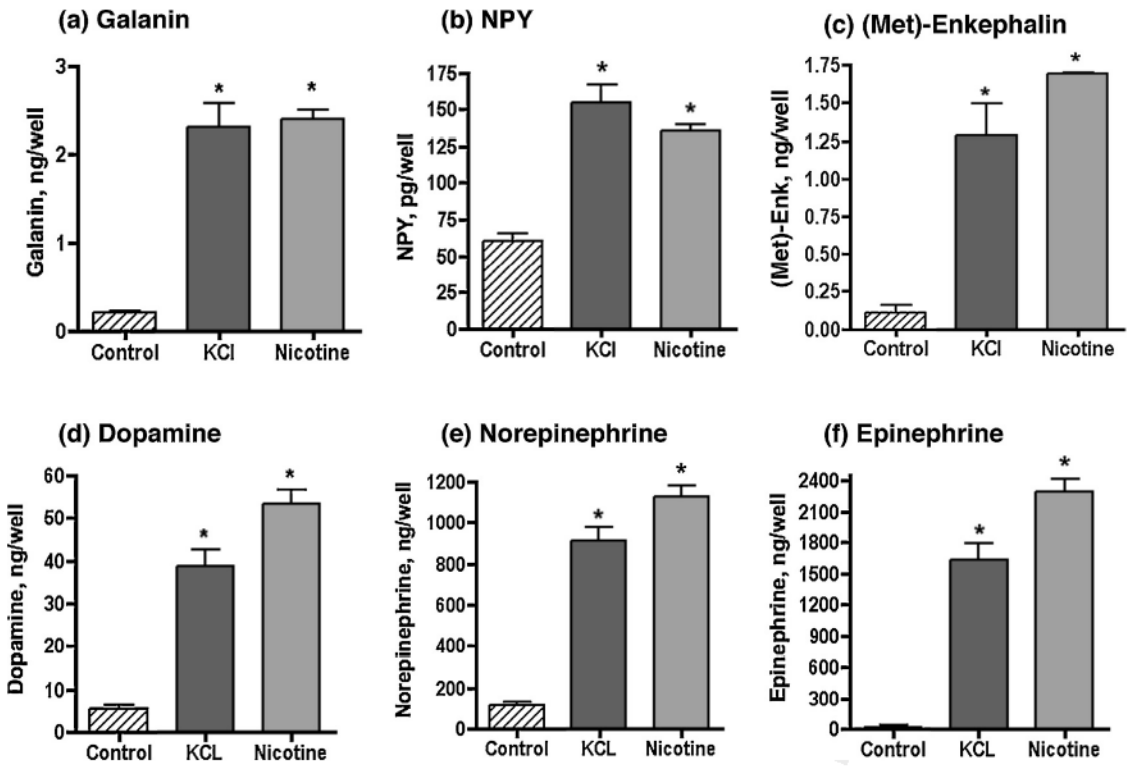

Fig. 2.

Regulated co-secretion of peptide and catecholamine neurotransmitters from neuronal chromaffin cells with Aβ peptides. Regulated secretion from chromaffin cells in primary culture was stimulated by KCl depolarization (50 mM) and nicotine (10 μM) (15 min incubation), as described in Fig. 1. The secretion media was measured for Aβ peptides (illustrated in Fig. 1). Data in this Fig. 2 show co-secretion of the peptide neurotransmitters galanin, NPY, and (Met)enkephalin (panels (a)–(c), respectively), and co-secretion with the catecholamine neurotransmitters dopamine, norepinephrine, and epinephrine (panels (d)–(f), respectively). *$p < 0.05$ (Student's *t*-test).

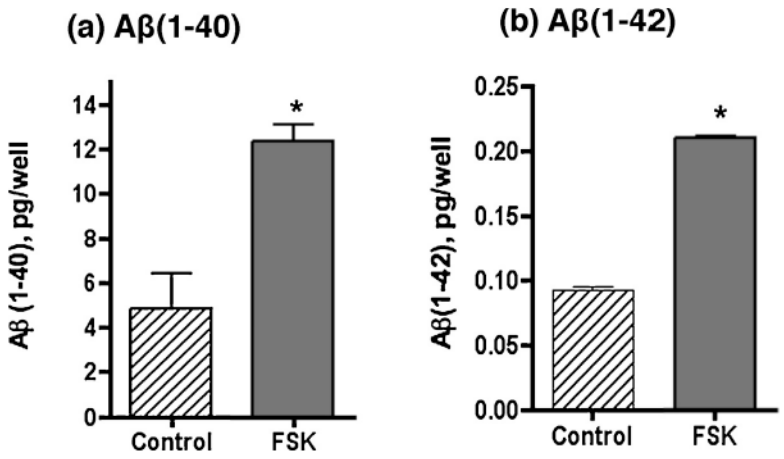

Fig. 3.

Forskolin stimulates secretion of Aβ peptides from chromaffin cells. Chromaffin cells were treated with forskolin (2 h) to induce regulated secretion. The secretion media was collected and levels of Aβ peptides were measured. Forskolin induced the regulated, co-secretion of Aβ(1–40) and Aβ(1–42) (panels (a) and (b)). *$p < 0.05$ (Student's *t*-test).

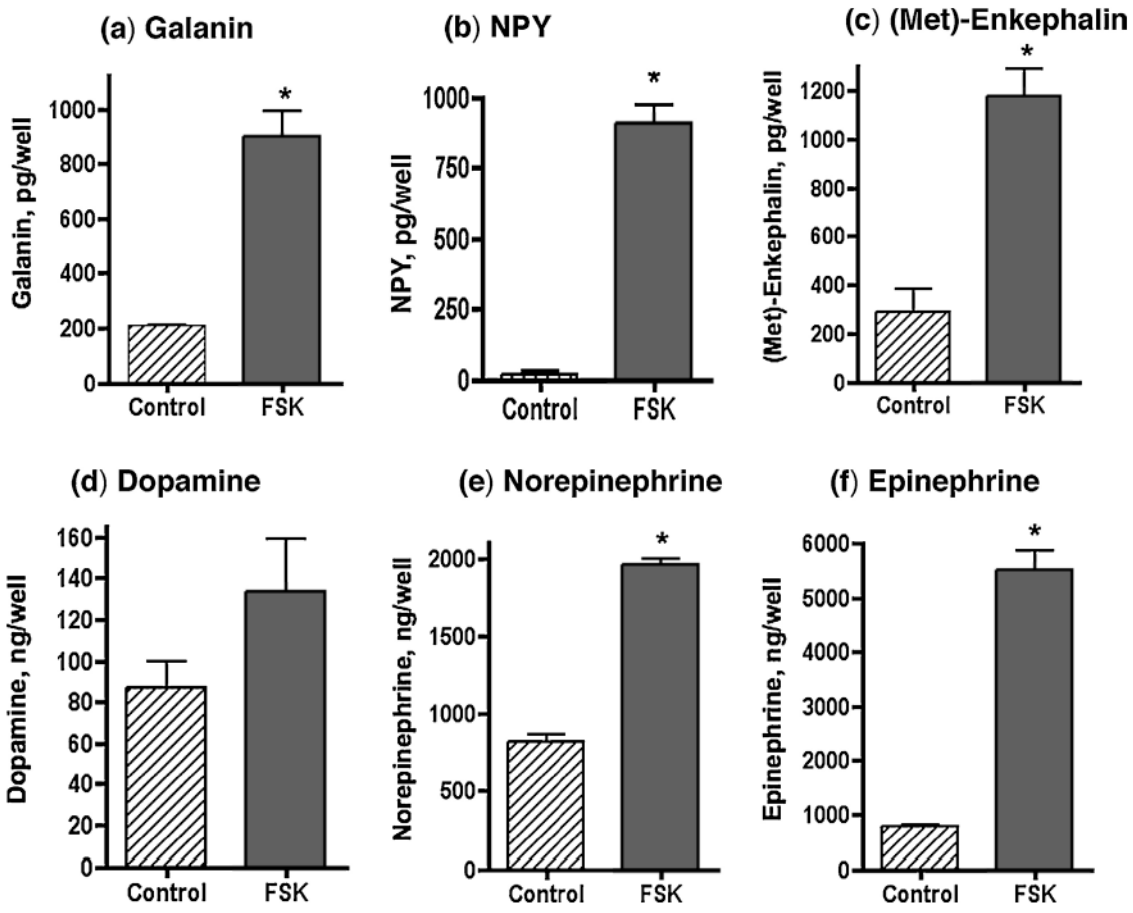

Fig. 4.

Forskolin stimulates the co-secretion of peptide and catecholamine neurotransmitters with Aβ peptides from chromaffin cells. As described in Fig. 3, chromaffin cells were treated with forskolin (2 h) to stimulate regulated secretion. The secretion media was collected after the forskolin treatment, and levels of peptide and catecholamine neurotransmitters were measured, to evaluate their secretion with Aβ peptides (data shown in Fig. 3). Results of this Fig. 4 show that the peptide neurotransmitters galanin, NPY, and (Met)enkephalin (panels (a)–(c)) and the catecholamine neurotransmitters dopamine, norepinephrine, and epinephrine (panels (d)–(f)) are co-secreted with Aβ peptides (Fig. 3) from chromaffin cells,. Data show that forskolin stimulates the regulated co-secretion of Aβ peptides with neurotransmitters. *$p < 0.05$ (Student's *t*-test).

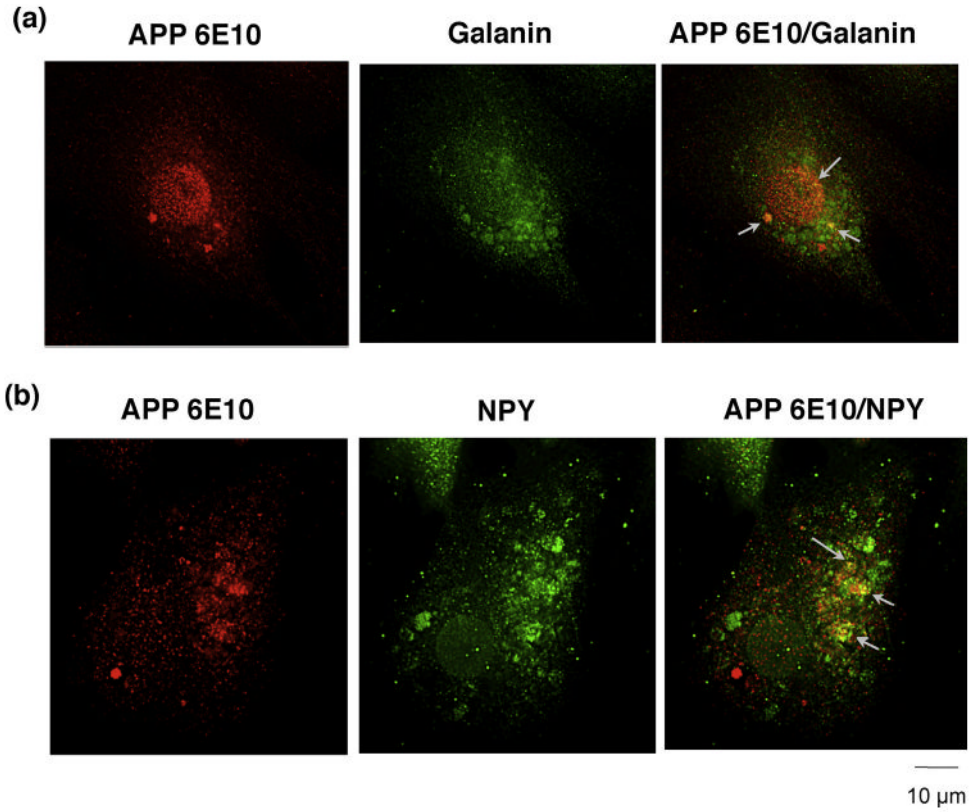

Fig. 5.

Cellular localization of Aβ/APP immunoreactivity with galanin and NPY neurotransmitters. (a) Aβ/APP localization with galanin. Localization of Aβ and APP precursor forms (detected by 6E10 immunoreactivity, red fluorescence) with the galanin peptide neurotransmitter (green fluorescence) was assessed by immunofluorescence deconvolution microscopy. The co-localization of Aβ/APP with galanin was assessed by their merged images, with areas of co-localization illustrated by yellow fluorescence (shown by arrows). The 6E10 antibody detects Aβ peptides and its APP precursor protein. (b) Aβ/APP localization with NPY in chromaffin cells. Localization of Aβ and APP precursor forms (assessed by 6E10 immunoreactivity, red fluorescence) with the NPY peptide neurotransmitter (green fluorescence) was evaluated by immunofluorescence deconvolution microscopy. The co-localization of Aβ/APP with NPY was assessed by their merged images, with areas of co-localization shown by yellow fluorescence (see arrows). Controls conducted with only secondary antibody (labeled with Alexa Fluor) showed no fluorescence, thus indicating that the immunofluorescence was due to the primary antisera to Aβ/APP, galanin, and NPY. (For interpretation of the references to color in this figure legend, the reader is referred to the web version of the article.)

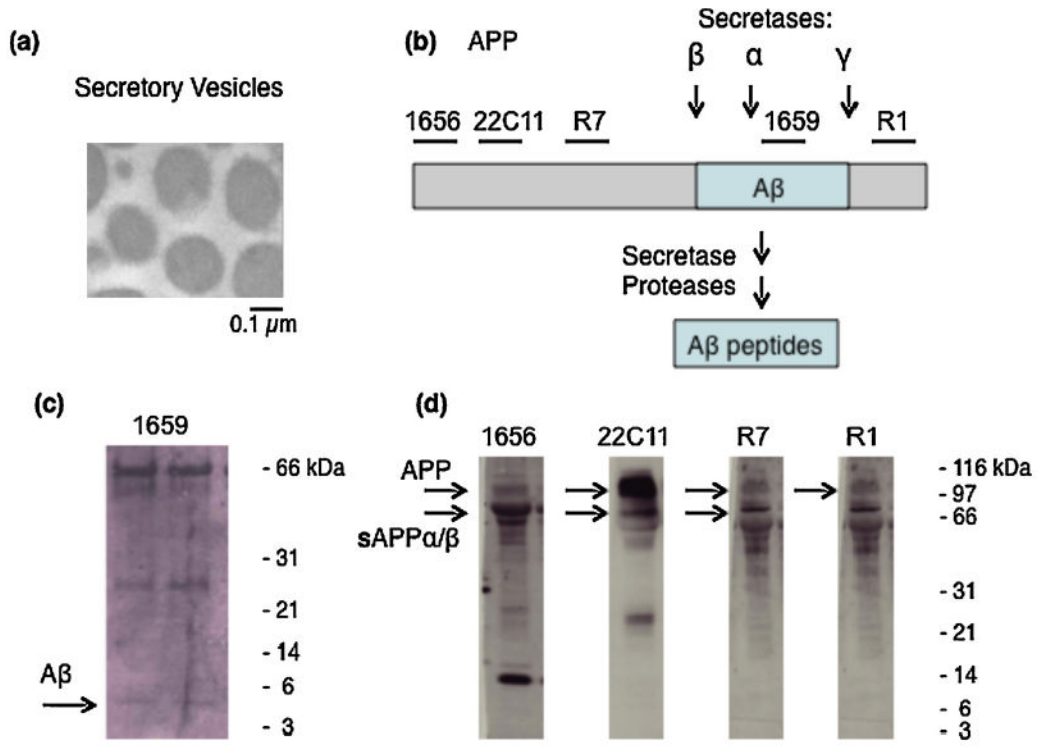

Fig. 6.

Neurotransmitter dense core secretory vesicles (DCSV) contain Aβ and APP. (a) Electron microscopy of dense core secretory vesicles (DCSV) isolated from chromaffin cells. The homogeneity and integrity of DCSV purified from adrenal medullary chromaffin cells was illustrated by electron microscopy, conducted as described in the methods. (b) APP and Aβ in DCSV: antibody epitopes. Endogenous full-length APP (amyloid precursor protein) is the precursor of Aβ, present in DCSV [45,48]. Antisera to different epitopes of APP are illustrated for antibodies 1656, 22C11, R7, 1659, and R1, with peptide antigen sequences described in the methods and procedures. The presence of Aβ with APP in DCSV indicates the presence of β- and γ-secretase components in the DCSV organelle. (c) Aβ in DCSV. Western blot with antisera 1656 directed to Aβ(17–28) detected a band of ~4.5 kDa, consistent with the presence of monomeric Aβ. Replicate lanes (two lanes) are shown. (d) APP in DCSV. APP was observed in DCSV by western blots with antisera 1656, 22C11, R7, and R1 directed to epitope regions of APP (shown in Fig. 6b). APP of ~100–110 kDa on SDS-PAGE gels of western blots was observed with these antisera. These antisera also detected bands of ~ 87–90 kDa that are consistent in apparent molecular weight to sAPPα and sAPPβ.

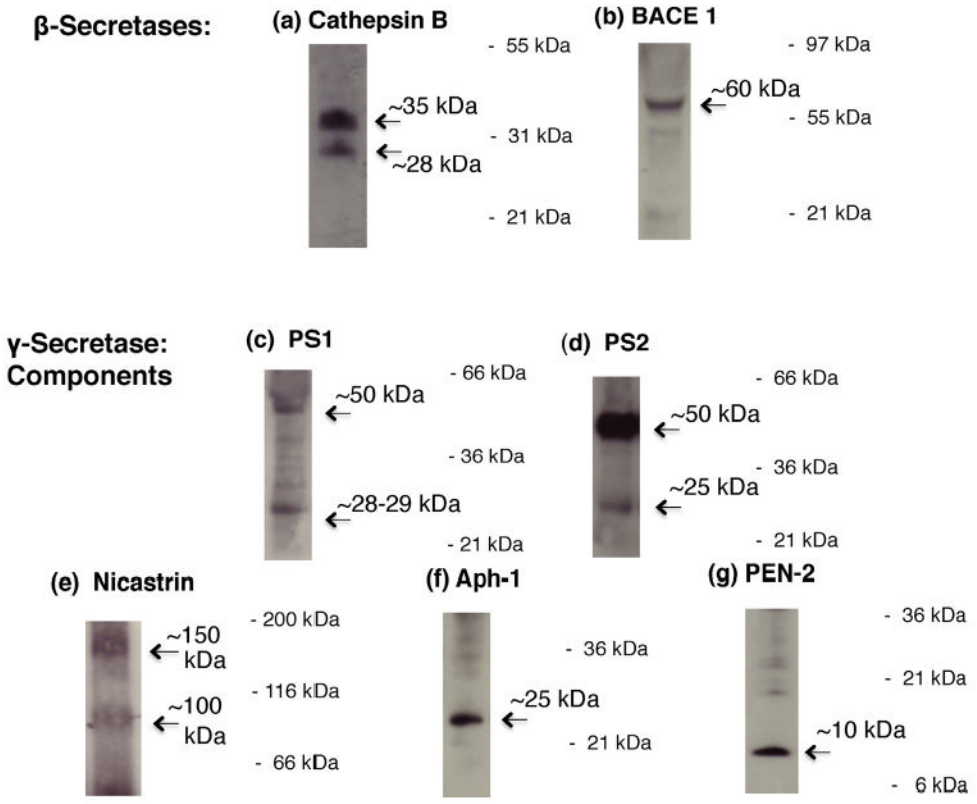

Fig. 7.

Amyloidogenic β- and γ-secretase components are present in neurotransmitter-containing dense core secretory vesicles (DCSV). Western blots assessed the presence of β-secretase and γ-secretase components in dense core sescretory vesicles (DCSV) purified from chromaffin cells. These DCSV contain the β-secretase components of the recently identified cathepsin B (~28 kDa mature form, and ~35 kDa proenzyme) [15] and the well known BACE1 (~ 60–70 kDa) [21,59], illustrated by western blots (panels (a) and (b), respectively). Western blots illustrated that γ-secretase complex components are present, consisting of presenilin 1 (panel (c))of ~50 kDa and ~28 kDa representing full-length and cleaved forms [9,56,58], presenilin 2 (panel d) of ~50 kDa and ~25 kDa bands representing full-length and cleaved forms [24], nicastrin (panel (e)) of ~150 kDa and ~100 kDa bands [23], Aph-1 (panel (f)) of ~25 kDa [29], and PEN-2 (panel (g)) of ~10–12 kDa band [23,32]. Arrows indicate the bands observed as secretase components, with their apparent molecular weights observed.

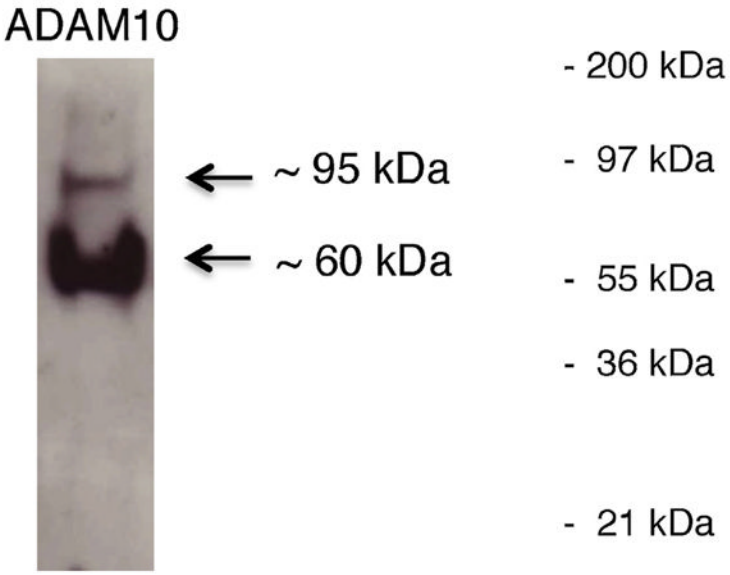

Fig. 8.

ADAM10, representing α-secretase, is present in neurotransmitter secretory vesicles of the DCSV type. The presence of ADAM10 in DCSV isolated from neuronal chromaffin cells was assessed by anti-ADAM10 western blots. The western blots illustrate the presence of ~95 kDa and ~60 kDa forms of ADAM10, representing proform and mature forms of ADAM10, respectively [27,42].

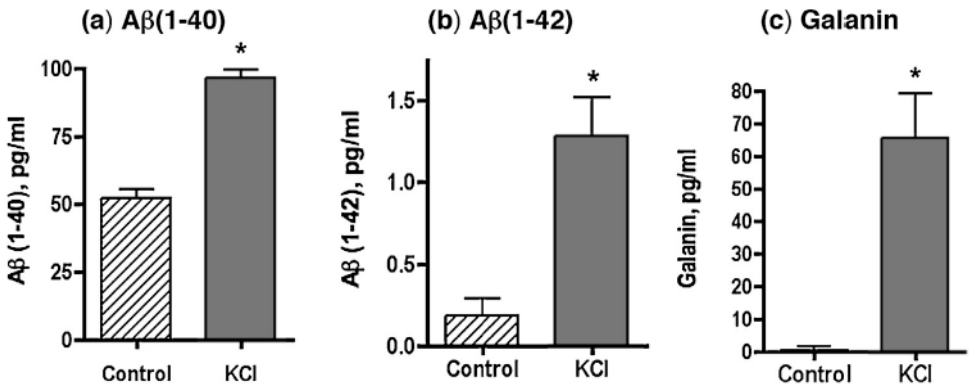

Fig. 9.

Human IMR32 neuroblastoma cells display regulated co-secretion of Aβ peptides with the galanin neurotransmitter. Regulated secretion was stimulated by KCl depolarization (50 mM in media, for 90 min.) of human IMR32 neuroblastoma cells. Controls include cells that were not treated with KCl. The secretion media was collected for measurements of Aβ(1–40), Aβ(1–42), and the galanin neurotransmitter (panels (a), (b), and (c), respectively). *$p <$ 0.05 (Student's *t*-test).

Table 1

Beta-amyloid and neurotransmitter content in purified dense core secretory vesicles (DCSV).

Component	Content in chromaffin secretory vesicles
Beta-amyloid peptides	Content (pg/mg protein)
Aβ(1–40)	0.22 ± 0.01
Aβ(1–42)	0.085 ± 0.004
Peptide neurotransmitters	Content (pg/mg protein) × 10^3
(Met)enkephalin	215 ± 14.4
NPY	269 ± 27.8
Galanin	0.408 ± 0.015
VIP	0.013 ± 0.003
Somatostatin	0.008 ± 0.002
β-Endorphin	0.053 ± 0.005
Catecholamines:	Content (pg/mg protein) × 10^3
Dopamine	1.15 ± 0.13
Norepinephrine	4.56 ± 0.34
Epinephrine	6.49 ± 1.02

The concentrations of Aβ peptides, peptide neurotransmitters (neuropeptides), and catecholamines in purified chromaffin secretory vesicles were measured as described in the methods.