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β-Amyloid-Related Peptides Inhibit Potassium-Evoked Acetylcholine Release from Rat Hippocampal Slices

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The 4 kDa β-amyloid (Aβ) protein, a major component of cerebral and cerebrovascular plaques in Alzheimer's disease (AD), is derived from the proteolytic cleavage of a larger, membrane-bound precursor, the Aβ precursor protein (APP). Until recently, it was assumed that an aberrant AD-specific proteolysis generated Aβ peptides, which subsequently could initiate and/or contribute to the pathological cascade leading to plaque formation and losses of selected neuronal populations, including basal forebrain cholinergic neurons that provide major inputs to the hippocampus and neocortex. However, the recent detection of soluble Aβ fragments in the plasma and CSF of normal individuals, as well as in the conditioned media of cultured brain cells, suggests a role for Aβ-related peptides in normal brain functions. Taking into consideration the reported toxic properties of Aβ and the preferential vulnerability of basal forebrain cholinergic neurons in AD, we investigated the possible effects of Aβ-related peptides on the release of endogenous acetylcholine (ACh) from rat brain slices. $A\beta_{1-28}$, in a concentration-dependent manner (10^{-12}–10^{-8} M), potently inhibited K^+-evoked ACh release from hippocampal slices. The inhibition of ACh release was fully reversible and was observed using other Aβ-related peptides such as $A\beta_{1-42}$, $A\beta_{1-40}$, and $A\beta_{25-35}$, but not with the scrambled, reverse, or all-D-isomer Aβ-peptide sequences, indicating that the effect of Aβ on ACh release is mediated via a stereoselective mechanism. Tetrodotoxin (10 μM) failed to alter the effect of $A\beta_{1-28}$ on ACh release, which suggests the lack of involvement of voltage-dependent Na^+ channels. Except for the hippocampal formation, the inhibitory effect of Aβ on K^+-evoked ACh release also was observed in the frontal cortex but not in the striatum. Taken together, our results demonstrate that APP-derived Aβ-related peptides can regulate the release of ACh potently by acting on cholinergic terminals. Additionally, the evidence that selected cholinergic neuronal populations are sensitive to Aβ suggests a potential mechanistic link between the deposition of Aβ and the preferential vulnerability of certain cholinergic projections in AD.

Key words: acetylcholine; β-amyloid; hippocampus; neuromodulation; transmitter release; Alzheimer's disease

Excessive extracellular deposition of β-amyloid (Aβ), a polypeptide of 39–43 amino acids, in brain parenchyma and cerebromeningeal blood vessels is a pathological hallmark of Alzheimer's disease (AD) (Mullan and Crawford, 1993; Cordell, 1994; Selkoe, 1994). This peptide is generated by the proteolytic cleavage of the Aβ precursor protein (APP), a transmembrane glycoprotein that exists as multiple isoforms resulting from the alternative splicing of a single transcript (Kang et al., 1987; Selkoe, 1993; Nitsch and Growdon, 1994). Several lines of evidence over the last decade have directly or indirectly implicated Aβ deposition as an initiating and/or contributing factor in the pathogenesis of AD (Mullan and Crawford, 1993; Cordell, 1994; Cotman and Pike, 1994; Hardy and Allsop, 1994; Selkoe, 1994; St George-Hyslop, 1994; Games et al., 1995; LaFerla et al., 1995). Because the normal processing of APP precludes intact Aβ formation (i.e., the extracellular domain of APP is cleaved at residue 16 within the Aβ region), it was assumed that assembly/deposition of Aβ protein in AD was caused by abnormal APP maturation (Esch et al., 1990; Sisodia et al., 1990; Cordell, 1994; Selkoe, 1994). However, the recent reports that Aβ peptides are secreted by cultured brain cells and can be detected in human plasma and CSF clearly indicate that Aβ can be secreted constitutively under normal conditions and, therefore, may have physiological functions (Haass et al., 1992; Seubert et al., 1992; Shoji et al., 1992). At present, neither the mechanisms underlying Aβ-mediated degenerative cascade nor the physiological functions of soluble Aβ, if any, are clearly established.

Accompanying Aβ deposition, certain brain regions, particularly the basal forebrain neurons that provide major cholinergic inputs to the hippocampus and neocortex, are affected most severely in AD (Price, 1986; Hohmann et al., 1988; Geula and Mesulam, 1994). In parallel with neuronal losses, choline acetyltransferase activity, high-affinity choline uptake, acetylcholinesterase (AChE) activity and, most importantly, the level of acetylcholine (ACh) are decreased significantly in cortical and hippocampal regions of the AD brain (Price, 1986; Hohmann et al., 1988; Wurtman, 1992; Quirion, 1993; Geula and Mesulam, 1994). It has been suggested that losses of hippocampal and cortical cholinergic innervation contribute to the progressive memory impairment associated with AD (Hohmann et al., 1988; Cordell, 1994; Geula and Mesulam, 1994; Selkoe, 1994). However, the cause of the rather early decimation of the basal forebrain cholinergic neurons in AD remains unclear.

Considering the preferential vulnerability of selected cholinergic neurons (Cordell, 1994; Geula and Mesulam, 1994; Selkoe,

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1994) and the neurotoxic potential of Aβ (Yankner et al., 1990; Mattson et al., 1993; Pike et al., 1993; Cotman and Pike, 1994), it is critical to establish whether functional inter-relationships exist between Aβ and these neurons, especially those affected in AD. Recent evidence suggested that the lesioning of basal forebrain cholinergic neurons elevates the *ex vivo* synthesis of cortical APP (Wallace et al., 1993) and that the activation of specific muscarinic receptor subtypes influences the normal maturation of APP (Buxbaum et al., 1992; Nitsch et al., 1992, Nitsch and Growdon, 1994). Application of Aβ also has been shown to induce the hypofunction of cholinergic neurons, although with delayed efficacy (Abe et al., 1994; Giovannelli et al., 1995). However, it is not known whether Aβ-related peptides can affect the functioning of cholinergic neurons. The present study reports that Aβ-related peptides can inhibit ACh release potently, likely by acting on cholinergic terminals of the rat hippocampus and cortex, whereas they are ineffective in the striatum. The finding that selected cholinergic neurons respond to Aβ may provide a functional link between the deposition of Aβ and the preferential vulnerability of certain cholinergic projections in AD.

MATERIALS AND METHODS

Materials. Adult male Sprague–Dawley rats (275–300 gm; Charles River Laboratories, St. Constant, Quebec, Canada), maintained under institutional and the Canadian Council for Animal Care guidelines, were used in the study. Human Aβ peptides including $A\beta_{1-42}$, $A\beta_{1-40}$, $A\beta_{1-28}$, $A\beta_{25-35}$, and $A\beta_{40-1}$ (reverse sequence of $A\beta_{1-40}$) were obtained from Bachem (Torrance, CA). Scrambled $A\beta_{25-35}$ was synthesized and purified in our laboratory, whereas the all-D-isomers of $A\beta_{25-35}$ were a gift from Dr. M. Staufenbiel (Sandoz Pharma, Basel, Switzerland). Peptides were stored lyophilized at −20°C and were dissolved in double distilled water, according to the manufacturer's instructions, immediately before use. Under these conditions, no evidence of oxidation, aggregation, or degradation was reported (Maggio et al., 1992). ACh chloride, physostigmine sulfate, choline kinase (ATP, choline phosphotransferase), AChE type V-S (ACh hydrolase), and tetrodotoxin (TTX) were obtained from Sigma (St. Louis, MO), and ATP and dithiothreitol (DTT) were purchased from Boehringer Mannheim (Laval, Quebec, Canada). Tetraphenylboron and butyronitrile were from Aldrich (Milwaukee, WI); AG 1-X8 Resin was from Bio-Rad (Hercules, CA), and [γ-^{32}P]ATP (2–10 Ci/mmol) was from DuPont NEN (Mississauga, Ontario, Canada). All other chemicals were purchased either from Sigma or from Fisher Scientific (Montreal, Quebec, Canada).

Tissue preparation and superfusion. Rats were killed by decapitation, and the selected brain regions i.e., hippocampus, striatum, or frontal cortex, were dissected out on ice and sliced to 400 μm with a McIlwain tissue chopper (Mickle Laboratory Engineering, Gomshall, Surrey, UK). Slices of one hemisphere were transferred to a superfusing chamber (Brandel Instruments, Gaithersburg, MD) and superfused continuously with oxygenated Krebs' buffer [(in mM) NaCl 120, KCl 4.6, $CaCl_2$ 2.4, KH_2PO_4 1.2, $MgSO_4$ 1.2, D-glucose 9.9, $NaHCO_3$ 25, adjusted to pH 7.4] at 37°C at a flow rate of 0.5 ml/min. The buffer also contained physostigmine (30 μM) to prevent degradation of ACh and choline chloride (10 μM) to support a constant supply of precursor for the synthesis of ACh. Effluents for the first 45 min were discarded to establish a stable basal efflux of ACh, and thereafter samples were collected every 20 min. After 1 hr of basal efflux, the tissues were stimulated with high-K^+ Krebs' buffer (25 mM KCl with equimolar reduction in NaCl to conserve isotonicity) in the presence or absence of regular or altered Aβ-related peptides. To determine whether the transient exposure to Aβ could modify the subsequent ACh release, some tissue slices after the typical 1 hr stimulation with Aβ peptides were rinsed with normal Krebs' buffer (40 min) and then restimulated for an additional 1 hr in the absence of the peptide. At the end of each experiment, tissue slices were removed and protein content was measured (Lowry et al., 1952). The superfusates collected every 20 min were spun (15,000 × *g*, 5 min, 4°C), and 1.5 ml of the supernatant then was stored at −70°C until further processing.

Radioenzymatic analysis of ACh. Superfusion samples, as described in detail previously (Hanisch et al., 1993), were processed in triplicate for ACh analysis using a radioenzymatic assay (Goldberg and McCaman, 1973). Briefly, ACh was extracted from the samples by mixing a 400 μl aliquot with an equal volume of tetraphenylboron (30 mM) in butyronitrile. After centrifugation, 300 μl of the organic phase was removed and shaken (4 min) with a half-volume of $AgNO_3$ solution (120 mM). The mixture was spun (15,000 × *g*, 4 min, 22°C), and the excess silver present in the aqueous phase was precipitated by the addition of 10 μl of $MgCl_2$ (1 M). Finally, after shaking (4 min) and spinning (15,000 × *g*, 4 min, 22°C), a 100 μl volume of supernatant was removed and lyophilized. The samples then were redissolved in 32 μl of a reaction medium containing ATP (0.8 mM), DTT (5 mM), $MgCl_2$ (12.5 mM), glycylglycine (25 mM, pH 8), and choline kinase (5 mU) and incubated at 30°C for 25 min to phosphorylate the choline in the sample. A solution (10 μl) containing AChE (2 U) and [γ-^{32}P]ATP (0.45 μCi) then was added to each sample, followed by another incubation of the samples for an additional 25 min at 30°C. During the second incubation, ACh was hydrolyzed and the choline that formed was phosphorylated to [^{32}P]phosphorylcholine. The reaction was terminated by the addition of 100 μl of NaOH (0.05 mM), and the radioactive phosphorylcholine then was separated from the radioactive ATP by ion-exchange chromatography on column of AG 1-X8 Resin. Phosphorylcholine, eluted with 3 ml of NaOH (0.05 mM), was mixed with scintillation cocktail, and the radioactivity was measured by liquid scintillation spectrometry. For each experiment, standard amounts of ACh dissolved in Krebs' buffer were processed in parallel to monitor recovery.

Statistical analysis. Evoked transmitter release was expressed as pmol ACh · min^{-1} · mg $protein^{-1}$ and considered to be net transmitter release over the basal efflux. The basal efflux was determined from the superfusate samples collected before, during, and after the K^+ stimulation, depending on the experimental paradigm. The data were analyzed statistically using one-way ANOVA followed by Fisher's post hoc test, and the level of significance was set at $p < 0.05$.

RESULTS

Effects of Aβ-related peptides on hippocampal ACh release

Stimulation of ACh release with 25 mM KCl is known to be submaximal and is appropriate to reveal both drug-dependent attenuation and augmentation of transmitter release (Pearce et al., 1991; Hanisch et al., 1993). For example, it is by this method that the existence of presynaptic muscarinic receptors first was proposed (Rodriguez de lores Arnaiz, 1988; Quirion, 1993). To assess whether Aβ-related peptides can affect the release of endogenous ACh acutely, hippocampal slices were superfused in 25 mM K^+ Krebs' buffer in either the absence or the presence of various concentrations of $A\beta_{1-28}$, a derivative that neither forms stable aggregate nor exhibits toxicity (Pike et al., 1993). The results clearly demonstrate that $A\beta_{1-28}$, in a concentration (10^{-12}–10^{-8} M)-dependent manner, potently inhibited endogenous ACh release (Fig. 1). The time dependence of the effects of $A\beta_{1-28}$ revealed that the potent inhibition of ACh release was apparent during the final 40 min at 10^{-8} M, whereas at lower concentrations (i.e., 10^{-12}–10^{-10} M), significant decreases in ACh were evident during the final 20 min of stimulation (Table 1). This likely is related to the relatively slow tissue penetration of $A\beta_{1-28}$. No significant alterations in K^+-evoked ACh release were observed at any time point from the tissue samples treated with 10^{-16}–10^{-14} M $A\beta_{1-28}$ (Fig. 1). The spontaneous release of ACh from hippocampal slices was not altered significantly by 10^{-8} M $A\beta_{1-28}$.

To explore further the structure–activity relationship of the inhibitory action of Aβ-related peptides, the effects of $A\beta_{1-42}$, $A\beta_{1-40}$, and $A\beta_{25-35}$ were evaluated under similar conditions (Fig. 2). All of these Aβ-related derivatives induced potent inhibition of K^+-evoked ACh release from rat hippocampal slices. The time-dependent response indicates that the selective inhibition of ACh release by various Aβ-related peptides, like $A\beta_{1-28}$, is observed primarily during the later periods of evoked release (Fig. 2).

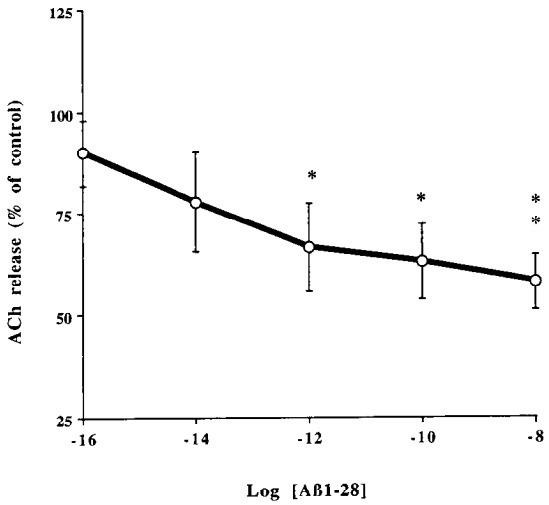


Figure 1. Effects of $A\beta_{1-28}$ on evoked ACh release from hippocampal slices. Slices were depolarized with 25 mM K^+ buffer in the presence or absence (control) of various concentrations of $A\beta_{1-28}$. Evoked release was inhibited significantly at concentrations ranging from 10^{-12} to 10^{-8} M. Results are expressed as mean ± SEM of three experiments, each performed five times for each concentration of peptide tested. $^*p < 0.05$; $^{**}p < 0.01$.

Specificity of Aβ effects on ACh release

A central issue relates to the specificity of the effects of Aβ-related peptides. Accordingly, hippocampal slices were superfused with a scrambled $A\beta_{25-35}$ sequence as well as the reverse sequence of $A\beta_{1-40}$ ($A\beta_{40-1}$). Unlike the normal derivatives, neither of the altered sequence of Aβ peptides affected the endogenous release of ACh from hippocampal slices (Fig. 3). Using an all-D-isomer $A\beta_{25-35}$, Frey et al. (1994) suggested recently that *in vitro* toxic effects of Aβ were mediated via some unknown mechanisms at the cellular membranes but not via specific "ligand-receptor-like" interactions, because both $A\beta_{25-35}$ and the all-D-isomer demonstrated similar effects. Unlike $A\beta_{25-35}$, the all-D-isomer peptide did not affect evoked release of ACh from hippocampal slices (Fig. 3), which suggests that the inhibition of ACh release is mediated via a stereospecific interaction. Moreover, preliminary results suggest that Aβ peptides (10^{-12}–10^{-8} M) fail to alter endogenous amino acid release (glutamate, aspartate, and GABA measured by HPLC) in rat hippocampal slices (D. Auld, S. Kar, and R. Quirion, unpublished observations).

Reversal of Aβ-mediated inhibition of ACh release

When hippocampal slices were stimulated twice for a period of 1 hr, each preceded by a 40 min rinse, the magnitude of ACh release induced by the first and second phases of stimulation was similar. To assess whether the presence of an Aβ peptide during the first phase of stimulation could alter subsequent ACh release, hippocampal slices were exposed to $A\beta_{1-28}$ (10^{-8} M) during the first stimulation, followed by a washout and a second stimulation. $A\beta_{1-28}$ inhibited ACh release during the first phase of stimulation but did not affect release after its washout (Fig. 4). This result clearly indicates that pre-exposure to Aβ did not modify the subsequent ACh release pattern, thus excluding acute toxic effects of Aβ for the duration of the experiment.

Effects of TTX on Aβ inhibition of ACh release

TTX is known to suppress neuronal depolarization and firing caused by fluxes through voltage-sensitive Na^+ channels (Narahashi, 1974). By itself, TTX (10 μM) does not alter evoked ACh release in the hippocampal slices (Araujo et al., 1990). Moreover, $A\beta_{1-28}$ (10^{-8} M)-mediated inhibition of ACh release was unaffected in the presence of 10 μM TTX (Fig. 5). This indicates that initiation of impulses distal to cholinergic terminals is not required to induce the inhibition of ACh release observed with Aβ peptides.

Regional differences in Aβ inhibition of ACh release

Except for the hippocampal formation, cholinergic terminals are most abundant in cerebral cortex and striatum (Hohmann et al., 1988; Geula and Mesulam, 1994). To investigate the regional specificity of Aβ-mediated inhibition of ACh release, slices of frontal cortex or striatum were superfused in the presence or absence of 10^{-8} M $A\beta_{1-28}$. Whereas $A\beta_{1-28}$ did not affect the K^+-evoked release of ACh from striatal slices (Fig. 6*A*), the responses of cortical ACh release were found to be decreased significantly (Fig. 6*B*). As in the hippocampal formation, the inhibition was apparent primarily during the later period of stimulation (Fig. 6*B*).

DISCUSSION

The present results clearly indicate that Aβ peptide with a unique potency can inhibit K^+-evoked ACh release from selected regions of the rat brain. The effect of the peptide is as follows: (1) it is concentration-dependent, with significant inhibition observed over a broad range of concentrations (i.e., 10^{-12}–10^{-8} M); (2) it is highly selective, because scrambled, reverse, or all-D-isomer Aβ peptides did not alter ACh release; and (3) it is region-specific—terminals of the hippocampal formation and frontal cortex are sensitive, whereas striatal intrinsic cholinergic neurons are unaffected. Furthermore, because Aβ-related peptides with (i.e., $A\beta_{1-42}$, $A\beta_{1-40}$, and $A\beta_{25-35}$) or without (i.e., $A\beta_{1-28}$) the hydrophobic 29–35 sequence believed to be essential for toxicity (Pike et al., 1993; Cotman and Pike, 1994) induced similar inhibition of ACh release, it is unlikely that the observed effect was caused by acute peptide toxicity. The lack of effect of the tissue pre-exposure to $A\beta_{1-28}$ on subsequent ACh release also supports this hypothesis.

The synaptic release of ACh is modulated by various agents, including cholinergic drugs themselves (i.e., autoregulation) (Quirion, 1993), other transmitters/modulators such as excitatory amino acids (Cai et al., 1991), and trophic factors such as the insulin-like growth factors (Araujo et al., 1989) and cytokines, in particular, interleukin-2 (Hanisch et al., 1993). However, the concentrations of these agents required to alter ACh release are usually much greater than for Aβ derivatives, often in the low micromolar range. For example, oxotremorine, a muscarinic M2-receptor antagonist, at 10 μM concentration inhibits only 45% of the evoked ACh release from rat hippocampal slices (Quirion, 1993). Accordingly, Aβ-related peptides are among the most potent, if not the most potent, regulators of brain ACh release characterized thus far. Of importance, Aβ derivatives likely act on cholinergic nerve terminals to inhibit ACh release, as demonstrated by the insensitivity to TTX. Moreover, the lack of effects of the reverse sequence $A\beta_{40-1}$, the scrambled $A\beta_{25-35}$ analog, and the all-D-isomer $A\beta_{25-35}$ homolog demonstrates further the specificity and stereoselective nature of the observed inhibitory response of Aβ-related peptides on ACh release. These findings, together with the evidence that APP-like immunoreactivity is present in neurons and fibers intrinsic to the hippocampal and cortical regions (Beeson et al., 1994; Ouimet et al., 1994), which are known to make contact with terminals originating from basal

Table 1. Effects of $A\beta_{1-28}$ on the evoked release of endogenous ACh from hippocampal slices

Time (min)	Concentrations of $A\beta_{1-28}$ (M)				
	10^{-16}	10^{-14}	10^{-12}	10^{-10}	10^{-8}
0–20	110.8 ± 10.5	91.1 ± 19.9	76.9 ± 19.1	71.1 ± 13.2	63.9 ± 20.1
20–40	86.6 ± 7.1	77.6 ± 17.7	74.9 ± 14.0	69.9 ± 11.9	56.5 ± 11.7**
40–60	82.4 ± 14.3	66.5 ± 10.9*	60.1 ± 17.0*	52.8 ± 13.6*	49.7 ± 15.7*
0–60 (Total)	90.0 ± 8.1	77.9 ± 12.3	66.6 ± 10.8*	62.9 ± 9.2*	57.9 ± 6.9**

Data represent evoked hippocampal ACh release, expressed as percent of control, determined for various periods of tissue exposure to different concentrations of $A\beta_{1-28}$ in the presence of 25 mM KCl. The data for a given concentration represent mean ± SEM of three experiments, each performed 5 times. *$p < 0.05$; **$p < 0.01$.

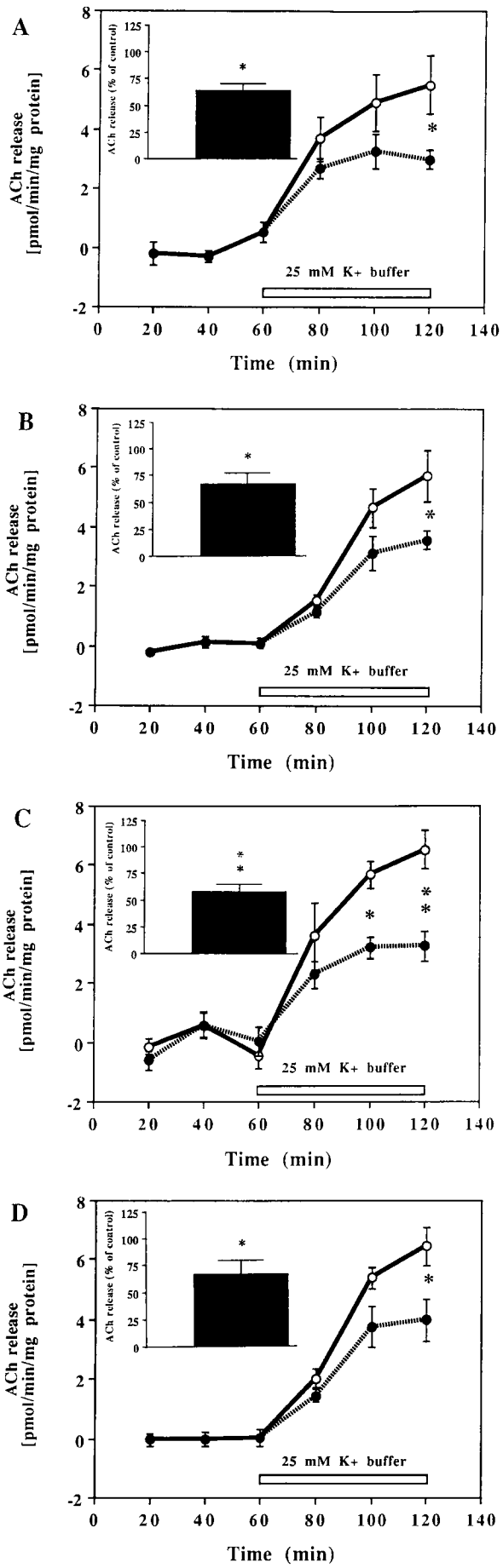
forebrain cholinergic neurons, provide a compelling anatomical substrate supporting a role for endogenous APP derivatives in the presynaptic regulation of ACh release in selected brain regions.

The underlying mechanism(s) associated with Aβ-induced inhibition of ACh release is unknown. It was suggested initially that biological actions of Aβ-related peptides, which exhibit some sequence homology with substance P, may involve tachykinin (Yankner et al., 1990) or serpin–enzyme complex-related receptors (Joslin et al., 1991). However, this hypothesis has been challenged recently by several studies that failed to observe any interactions between these two systems, thus indicating that Aβ derivatives likely act via other pathways (Mitsuhashi et al., 1991; Pike et al., 1993). Moreover, $A\beta_{1-28}$ was shown here to inhibit ACh release potently while having no sequence homology with substance P. It is unlikely, therefore, that tachykinin receptors are involved in mediating the observed response.

Plasma membrane-associated APP exhibits a receptor-like architecture, and a region of its cytoplasmic domain is capable of complexing with GTP-binding proteins (Nishimoto et al., 1993). Using a monoclonal antibody against the extracellular domain of APP, it was shown recently that APP can act as a functional G_o-coupled receptor (Okamoto et al., 1995). These results, together with findings that Aβ peptides can bind avidly to the N terminus of the APP molecule, suggest that homologous binding may be involved in targeting biological responses of Aβ-related peptides (Strittmatter et al., 1993) such as the one observed in the present study. Alternatively, given the evidence of undisplaceable Aβ-binding profile to normal tissue (Maggio et al., 1992) and its ability to interact with cell membranes (Chauhan et al., 1993), it is possible that Aβ-related peptides might act either via an as yet undefined association to alter membrane permeability or via the activity of some associated proteins. In that regard, it is of interest to note that soluble Aβ induces a dysfunction of K^+ channels in normal fibroblasts (Etcheberrigaray et al., 1993), which is a finding similar to those obtained in fibroblast and olfactory neuroblast studies of AD patients (Etcheberrigaray et al., 1994). In keeping with the purported role of K^+ channels in the acquisition of memory (Roberts, 1986; Hille, 1992), it has been shown that Aβ impairs post-training cognitive processing (Flood et al., 1991, 1994) and induces AD-like depletion of Cp20, a potent K^+ channel-regulating memory-associated GTP-binding protein, in control human fibroblasts (Kim et al., 1995). Because many K^+ channels play a critical role in the regulation of various cellular responses, including the release of neurotransmitters (Roberts, 1986; Hille, 1992; Etcheberrigaray et al., 1994), it is possible that interference with K^+ channels may be associated with the modulatory effects of Aβ-related peptides on ACh release. This possibility currently is under investigation in our laboratory.

The modulatory effect of Aβ on ACh release may be relevant to the well known impairments of cholinergic functions in AD. The selective inhibition of ACh release from cortical and hippocampal slices, but not in the striatum, suggests that only cholinergic target areas severely afflicted in AD (Price, 1986; Hohmann et al., 1988; Wurtman, 1992; Geula and Mesulam, 1994) are sensitive to the inhibitory action of Aβ-related peptides. Pharmacological evidence already has suggested that differences in the functional organization of the cholinergic synapse are observed between striatal interneurons and long cortical and hippocampal projections. For example, the nature of the autoreceptor regulating ACh release likely is different in these two groups of cholinergic neurons (Quirion, 1993). These findings, together with *in vitro* toxicity data (Yankner et al., 1990; Mattson et al., 1993; Pike et al., 1993; Cotman and Pike, 1994), provide a mechanistic framework supporting the idea that the preferential vulnerability of basal forebrain neurons and their projections in AD relate, at least in part, to their sensitivity to Aβ-related peptides.

Much evidence suggests that the APP can be processed either (1) via a nonamyloidogenic pathway in which the N-terminal portion of APP is released into the extracellular space, or (2) via an alternative pathway that generates Aβ-related peptides (Cordell, 1994; Nitsch and Growdon, 1994; Selkoe, 1994). Agonist-induced activation of muscarinic m_1 and m_3 receptor subtypes, which are known to be coupled to protein kinase C (PKC) transduction pathways, has been shown to increase the secretion of soluble APP derivatives and, concomitantly, to reduce the production of amyloidogenic Aβ peptides (Buxbaum et al., 1992; Nitsch et al., 1992; Nitsch and Growdon, 1994). It also has been reported that the lesioning of basal forebrain cholinergic neurons or a transient blockade of their transmission elevates the *ex vivo* synthesis of APP in cerebral cortex (Wallace et al., 1993). An alteration in normal APP processing pathway also has been reported after the destruction of selected neuronal populations, including cholinergic neurons (Iverfeldt et al., 1993). Collectively, these results point to a "protective role" of the normal cholinergic innervation that ensures the nonamyloidogenic maturation of APP (Iverfeldt et al., 1993; Wallace et al., 1993; Nitsch and Growdon, 1994). However, Aβ-related peptides under physiological conditions may be involved in the regulation of ACh release, which suggests the existence of reciprocal control mechanisms between cholinergic and Aβ-related systems in hippocampal and cortical regions. It now remains to be established how the normal biological functions of Aβ-related peptides are being compromised in AD, leading to neuronal degeneration and/or *in vivo* assembly of insoluble amyloid derivatives. One hypothesis is that the overproduction or malfunctioning of degradation/clearance mechanisms, as well as alterations of the local microenvironment, increases levels of Aβ, which subsequently inhibits cholinergic transmission and leads to further increments of the processing of APP via the amyloidogenic pathway (Wallace et al., 1993; Cordell, 1994; Nitsch and Growdon, 1994; Selkoe, 1994). Such a situation



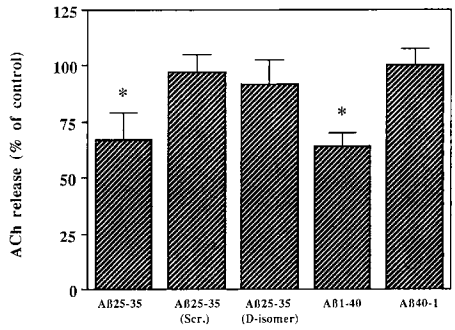


Figure 3. Comparative effects of $A\beta_{25-35}$, scrambled (*Scr.*) $A\beta_{25-35}$, all-D-isomer $A\beta_{25-35}$, $A\beta_{1-40}$, and reverse $A\beta_{1-40}$ (i.e., $A\beta_{40-1}$) on evoked hippocampal ACh release. Slices were depolarized with 25 mM K^+ buffer in the presence or absence (control) of 10^{-8} M of each of these peptides. Unlike the regular peptides, endogenous ACh release was not altered significantly in the presence of any of the unnatural peptides. Data for each peptide are expressed as mean ± SEM (n = 10–15). *$p < 0.05$.

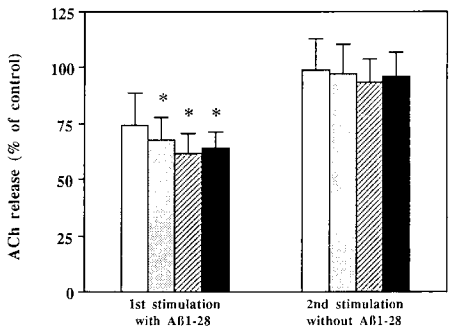


Figure 4. Evoked hippocampal ACh release after a pre-exposure of the tissue to 10^{-8} M $A\beta_{1-28}$. Slices were stimulated with 25 mM K^+ buffer for an initial period of 1 hr in the presence or absence of $A\beta_{1-28}$ peptide, rinsed for 40 min, and stimulated for a second time for an additional 1 hr without the peptide. Evoked release was inhibited in the first phase of stimulation but remained unaffected in the second period, demonstrating the lack of acute toxicity. Data are expressed as mean ± SEM (n = 12). *$p < 0.05$. *Open*, 0–20 min; *stippled*, 20–40 min; *hatched*, 40–60 min; *solid*, total release.

would create a vicious cycle causing the gradual degeneration of neurons sensitive to Aβ-related peptides. Alternatively, at-risk cholinergic neurons may be affected first by undefined insults, leading to altered PKC inputs and the activation of the amyloidogenic pathway and, finally, cell death.

In summary, the present study shows that Aβ-related peptides can inhibit K^+-evoked ACh release potently in rat hippocampal and cortical slices. The effect is TTX-insensitive and involves a stereoselective interaction. Taken together, these results suggest that endogenous Aβ-related peptides act as potent inhibitors of ACh release and may serve as a basis for the functional interrelationship between Aβ toxicity and the vulnerability of some cholinergic neuronal populations in AD.

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Figure 2. *A–D*, Comparative effects of various Aβ-related peptides on evoked hippocampal ACh release. Slices were depolarized with 25 mM K^+ buffer in the presence (*dotted line*) or absence (*solid line*) of 10^{-8} M $A\beta_{1-42}$ (*A*), $A\beta_{1-40}$ (*B*), $A\beta_{1-28}$ (*C*), or $A\beta_{25-35}$ (*D*) peptide, respectively. Endogenous ACh release was inhibited potently in the presence of each of these peptides during later periods of stimulation. The *insets* in *A–D* represent total release as percentage of control for the respective peptide over 60 min of KCl stimulation. Data for each peptide are expressed as mean ± SEM (n = 10–15). *$p < 0.05$; **$p < 0.01$.

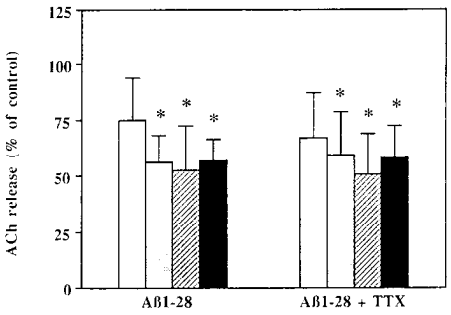

Figure 5. Effects of TTX on the $A\beta_{1-28}$-induced inhibition of evoked ACh release from hippocampal slices. Tissue slices were depolarized with 25 mM K^+ buffer in the presence or absence of 10^{-8} M $A\beta_{1-28}$ alone or with the peptide and 10 μM TTX. Evoked release was inhibited potently by the peptide with or without TTX. Data are expressed as mean ± SEM ($n = 12$). *$p < 0.05$. *Open*, 0–20 min; *stippled*, 20–40 min; *hatched*, 40–60 min; *solid*, total release.

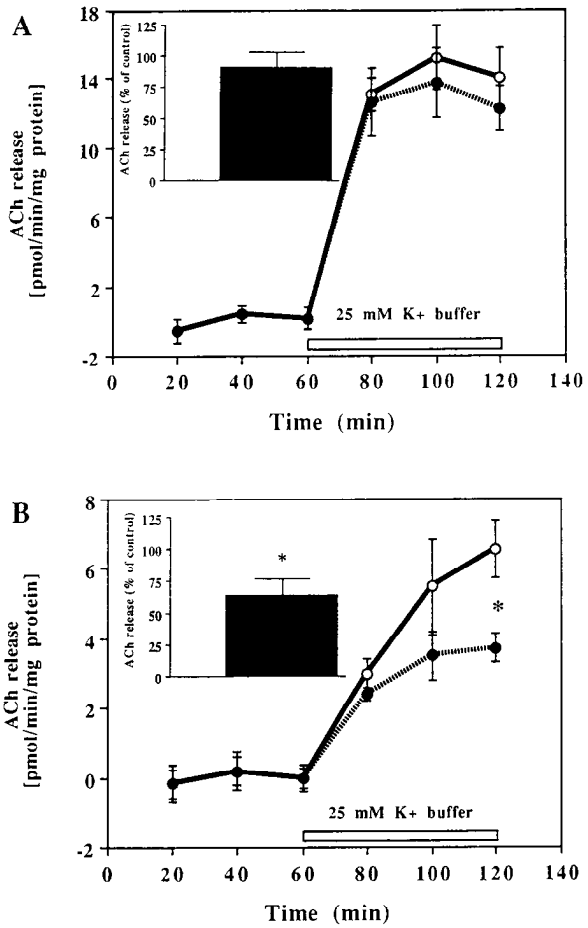

Figure 6. *A*, *B*, Time course effects of $A\beta_{1-28}$ on the evoked ACh release from slices of the striatum (*A*) and frontal cortex (*B*). Tissue slices were stimulated with 25 mM K^+ buffer in the presence (*dotted line*) or absence (*solid line*) of 10^{-8} M $A\beta_{1-28}$. Evoked ACh release, although remaining unaffected in the striatum, was inhibited significantly in the frontal cortex in the presence of $A\beta_{1-28}$. The *insets* in *A* and *B* represent total release as percentage of control for the respective tissues over 60 min of KCl stimulation. Data are expressed as mean ± SEM ($n = 10–12$). *$p < 0.05$.

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