Cellular Mechanisms for Amyloid β-Protein Activation of Rat Cholinergic Basal Forebrain Neurons

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Jhamandas, Jack H., Caroline Cho, Balvinder Jassar, Kim Harris, David MacTavish, and Jacob Easaw. Cellular mechanisms for amyloid β-protein activation of rat cholinergic basal forebrain neurons. J Neurophysiol 86: 1312–1320, 2001. The deposition of amyloid β-protein (Aβ) in the brain and the loss of cholinergic neurons in the basal forebrain are two pathological hallmarks of Alzheimer’s disease (AD). Although the mechanism of Aβ neurotoxicity is unknown, these cholinergic neurons display a selective vulnerability when exposed to this peptide. In this study, application of Aβ25–35 or Aβ1–40 to autolytically dissociated rat neurons from the basal forebrain nucleus diagonal band of Broca (DBB), caused a decrease in whole cell voltage-activated currents in a majority of cells. This reduction in whole cell currents occurs through a modulation of a suite of potassium conductances including calcium-activated potassium (IKCa), the delayed rectifier (IK), and transient outward potassium (Ito) conductances, but not calcium or sodium currents. Under current-clamp conditions, Aβ evoked an increase in excitability and a loss of accommodation in cholinergic DBB neurons. Using single-cell RT-PCR technique, we determined that Aβ actions were specific to cholinergic, but not GABAergic DBB neurons. Aβ effects on whole cell currents were occluded in the presence of membrane-permeable protein tyrosine kinase inhibitors, genistein and tyrphostin B-44. Our data indicate that the Aβ actions on specific potassium conductances are modulated through a protein tyrosine kinase pathway and that these effects are selective to cholinergic but not GABAergic cells. These observations provide a cellular basis for the selectivity of Aβ neurotoxicity toward cholinergic basal forebrain neurons that are at the epicenter of AD pathology.

INTRODUCTION

Deposition of amyloid protein in the form of diffuse and neuritic plaques is an important pathological hallmark of Alzheimer’s disease (AD) (Hardy 1997; Selkoe 1997). The major component of the neuritic amyloid plaques is amyloid β-protein (Aβ), a 39–43 amino acid peptide that is generated from a larger protein, the amyloid precursor protein (APP). There is considerable evidence to suggest that Aβ and other peptide fragments derived from APP influence cellular homeostasis and neuronal signaling through modulation of ion channel function (for review see Fraser et al. 1997).

The interactions of Aβ within the membrane occur either with preexisting ionophores or through the formation of de novo ion channels (Arispe et al. 1996; Fraser et al. 1997).

Aβ25–35, an 11 amino acid fragment considered to represent the neurotoxic domain of the parent Aβ peptide, activates large, nonselective cation currents in bullfrog sympathetic and rat hippocampal neurons (Furukawa et al. 1994; Simmons and Schneider 1993). Aβ25–35 and Aβ1–40 induce Ca2+ influx through voltage-gated channels in cortical and NIE-115 neuroblastoma cells, respectively (Davidson et al. 1994; Weiss et al. 1994). As yet no receptor for Aβ has been identified, but its neurotoxic effects have been postulated to be mediated via plasma membrane receptors for advanced glycation end products (RAGE), class A scavenger receptor (SR)-related proteins, and/or the 75 kDa-neurotrophin receptor (El Khoury et al. 1996; Kuner et al. 1998; Yan et al. 1996). Although Aβ modulation of ionic conductances has been studied in many neuronal and nonneuronal systems, the linkage of these observations to changes in neuronal excitability is less well understood.

It is now well accepted, that apart from Aβ deposition, certain chemically defined neurotransmitter systems, particularly the cholinergic basal forebrain neurons, display a selective vulnerability and degeneration in AD (Price 1986). Emerging data support a potential link between Aβ peptides and the basal forebrain cholinergic system. Aβ peptides can inhibit the release of endogenous acetylcholine and high-affinity choline uptake from the hippocampus and cortex (Kar et al. 1996, 1998). There is Aβ-mediated inhibition of acetylcholine synthesis in cultured cholinergic neurons (Pedersen et al. 1996). Single injection of Aβ into the basal forebrain, but not the striatum, induces damage to cholinergic neurons (Butcher et al. 1997). These observations indicate that the chemical phenotype of an individual cell is an important feature of Aβ toxicity, a notion that is strengthened by the finding that GABA-containing neurons of the hippocampus exhibit a relative resistance to Aβ in sharp contrast to the vulnerability of cholinergic neurons exposed to this peptide (Pike and Cotman 1993). However, whether the effects of Aβ are selective to cholinergic basal forebrain neurons, which are at the epicenter of AD pathology, is an important but unresolved question.

In this study, we investigated the actions of Aβ on acutely dissociated rat cholinergic basal forebrain neurons from the nucleus of the diagonal band of Broca (DBB) using a combination of whole cell patch-clamp and single-cell reverse tran-
scription polymerase chain reaction (RT-PCR) analysis. Our data show that the blockade of specific potassium conductances is a potential underlying mechanism for the action of Aβ on DBB neurons and may explain its effects in regulating their excitability. Finally, we identify tyrosine phosphorylation as an intracellular signal transduction pathway for these actions.

METHODS

Dissociation procedures

Details of the procedure for acute dissociation of neurons from the DBB are described in Jassar et al. (1999). Briefly, brains were quickly removed from decapitated male Sprague-Dawley rats (15–25 days postnatal) and placed in cold artificial cerebrospinal fluid (ACSF) that contained (in mM) 140 NaCl, 2.5 KCl, 1.5 CaCl2, 5 MgCl2, 10 HEPES, and 33 d-glucose 33 (pH 7.4). Brain slices (350 μm thick) were cut on a vibratome, and the area containing the DBB was dissected out. Although most of the tissue contained the horizontal limb of the DBB, some slices may have included a component of the vertical limb of the DBB. Acutely dissociated neurons were prepared by enzymatic treatment of slice with trypsin (0.65 mg/ml) at 30°C, followed by mechanical trituration for dispersion of individual cells. Cells were then plated on poly-l-lysine (0.005% wt/vol)–coated coverslips and viewed under an inverted microscope (Zeiss Axiovert 35). All solutions were kept oxygenated by continuous bubbling with pure oxygen.

Electrophysiological recordings

Whole cell patch-clamp recordings were performed at room temperature (20–22°C) using an Axopatch-1D amplifier. Series resistance compensation was continuously adjusted to >80% and monitored and readjusted as necessary during the course of each experiment. Junction potential was nullled with the pipette tip immersed in the bath. Internal patch pipette solution contained (in mM) 140 K-methylsulfonate, 10 EGTA, 5 MgCl2, 1 CaCl2, 10 HEPES, and 33 d-glucose 33 (pH 7.4). Brain slices (350 μm thick) were cut on a vibratome, and the area containing the DBB was dissected out. Although most of the tissue contained the horizontal limb of the DBB, some slices may have included a component of the vertical limb of the DBB. Acutely dissociated neurons were prepared by enzymatic treatment of slice with trypsin (0.65 mg/ml) at 30°C, followed by mechanical trituration for dispersion of individual cells. Cells were then plated on poly-l-lysine (0.005% wt/vol)–coated coverslips and viewed under an inverted microscope (Zeiss Axiovert 35). All solutions were kept oxygenated by continuous bubbling with pure oxygen.

Drugs and solutions

All chemicals were purchased from Sigma (St. Louis, MO) except the following: Aβ3-40, Aβ35-35, Aβ35-25 (QCB Biosource International, Camarillo, CA); genistein and daidzein (ICN, Costa Mesa, CA); and tyrophostin B-44 (Calbiochem, San Diego, CA). Stock solutions of Aβ peptides were prepared by dissolving the peptides at 1 mM in deionized water and stored in aliquots at −70°C (Ueda et al. 1997). On the day of the experiment, Aβ peptides were diluted in external perfusing medium just before the time of application. All drugs and chemicals were applied via bath perfusion at the rate of 3–5 μl per minute, which allowed complete exchange in less than half a minute. Data are presented as means ± SE. Student’s two-tailed t-test (paired when appropriate) was utilized for determining significance of effect.

Single-cell RT-PCR for chemical phenotyping

Where possible, neurons were harvested after electrophysiological recordings were completed and readied for RT-PCR according to a previously described protocol (Surmeier et al. 1996). In brief, contents of the electrode containing the cell and 5 μl of internal solution were expelled into a 0.2-ml PCR tube containing 5 μl sterile water (Sigma water W-4502), 0.5 μl dithiothreitol (0.1 M DTT), 0.5 μl RNasin (10 U/μl), and 1 μl oligo-dT (0.5 μg/μl). The tube was then placed on ice. Single-stranded cDNA was then synthesized from mRNA by adding a solution containing 1 μl SuperScript II RT (200 U/μl), 2 μl 10× PCR buffer, 2 μl 25 mM MgCl2, 1.5 μl 0.1 M DTT, 1 μl 10 mM dNTPs, and 0.5 μl RNasin (10 U/μl). The PCR tube was gently mixed and incubated in a Techne Progene thermal cycler at 42°C for 50 min. The process was then terminated by heating to 72°C for 15 min, and the tube cooled to 4°C. Subsequently 2 μl of the RT product was taken and combined with 5 μl 10× PCR buffer, 5 μl 25 mM MgCl2, 0.5 μl Taq polymerase (5 U/μl), 31.5 μl sterile water (Sigma water W-4502), 1 μl 25 mM dNTP mixture, and 1.5 μl of a specific set of primers (15 μM). All reagents were purchased from Gibco BRL. Primer sequences for choline acetyltransferase (ChAT) and for glu-
tamate decarboxylase (GAD) have been previously described (Surmeier et al. 1996; Tkatch et al. 1998), and that for β-actin was obtained from GenBank (the lower primer 5'-GAT AGA GCC ACC AAT CCA C, the upper primer 5'-CCA TGT ACG TAG CCA TCC A). All primers were synthesized at the University of Alberta Department of Biochemistry. The contents were mixed together and placed in the thermal cycler. The PCR amplification protocol was as follows: step 1: 94°C 4 min; step 2: 94°C 1 min, 53°C 1 min, 72°C 45 s (step 2 was repeated 35 times); step 3: 72°C 15 min; step 4: held at 4°C. A portion of the product was then run on a 2% TEA agarose gel, and the gel was then placed in a bath containing 2 μg/ml of ethidium bromide, after 10-min DNA bands were visualized with ultraviolet light box and photographed with a Polaroid camera.

RESULTS

Most of the acutely dissociated neurons from the DBB had neuronlike morphology (i.e., large cells with a conspicuous nucleus, nucleolus, and a few blunt processes that were truncated axon/dendrites). Under our recording conditions, the average input conductance measured from the slope of the current-voltage (I-V) relationships between −60 and −110 mV was 1.20 ± 0.16 nS (mean ± SE, n = 51).

Based on the previous observations (Jassar et al. 1999), we utilized a voltage-ramp protocol where the cells were held at −80 mV and subjected to voltage ramps from −110 to +30 mV at the rate of 20 mV/s after conditioning at −110 mV for 1 s.

Effects of Aβ on potassium currents

Whole cell potassium currents were recorded under control conditions and in the presence of Aβ (range of concentration 0.1–2.0 μM). The concentration of Aβ (1 μM) that we used in the present experiments is among the lowest used in previous electrophysiological studies of this peptide in acutely dissociated neurons (range 2–100 μM) (reviewed in Fraser et al. 1997) or in brain slices (range 200 nM to 2 μM) (Pettit et al. 2001; Wu et al. 1995). In 51 DBB neurons, the outward currents in the voltage range from −30 to +30 mV were decreased by both the longer isoform of the peptide (Aβ1–40) and the truncated active peptide fragment (Aβ25–35). Maximal effects of Aβ on whole cell currents were observed within 90 s application, and the response did not desensitize with repeated applications of the peptide. Figure 1, A and B, shows the reversible decrease in outward currents caused by application of Aβ25–35 (1 μM) and Aβ1–40 (1 μM), respectively. The amplitude of the currents at +30 mV was also similar with both peptides (Aβ25–35 = 5.72 ± 0.23 nA, Aβ1–40 = 7.52 ± 0.72 nA, n = 51, P < 0.001) or Aβ1–40 (control = 7.52 ± 0.72 nA, Aβ1–40 = 6.43 ± 0.70 nA, n = 12, P < 0.001). The percent reduction in amplitude of currents at +30 mV was also similar with both peptides (Aβ25–35 = 14.21 ± 1.34%, Aβ1–40 = 14.88 ± 4.16%, P = 0.8). The scrambled peptide fragment (reverse fragment, Aβ35–25) had no effect on the whole cell current-voltage relationships (not illustrated). Since the effects of Aβ1–40 and Aβ25–35 on whole cell currents were essentially identical, we therefore utilized active peptide fragment Aβ25–35 (1 μM) in all subsequent experiments. In 11 DBB neurons, Aβ25–35 did not evoke any change in whole cell current or caused an increase or decrease in whole cell current that was <5% from control values at +30 mV. An example of a cell that was nonresponsive to Aβ is shown in Fig. 1C.

The outward potassium currents are a mixture of calcium-activated and noncalcium-activated components. Calcium-activated currents include the voltage-sensitive conductances called maxi K(Ca) (I_C) and the voltage-insensitive ones that underlie action potential afterhyperpolarization (I_{AHP}). Noncalcium-activated conductances consist of the delayed rectifier, M- and A-conductances (I_K, I_M, and I_A, respectively) and sodium currents. In DBB neurons, there was little evidence for I_M relaxation in the currents evoked by hyperpolarizing com-
mmands (10 mV/step) to −110 mV from a holding potential of −30 mV. Therefore $I_{M}$ was not investigated in detail in this study. We studied the effects of Aβ on $I_{C}$, $I_{K}$, and $I_{A}$ as well as on calcium ($I_{Ca}$) and sodium ($I_{Na}$) currents.

EFFECTS OF Aβ ON CALCIUM-ACTIVATED POTASSIUM CURRENTS. Of the two main Ca$^{2+}$-activated potassium currents, under whole cell recording conditions, $I_{AHP}$ makes little contribution and majority of the currents flow through the voltage-sensitive Ca$^{2+}$-activated potassium channels, $I_{C}$ (Jassar et al. 1999). To elucidate the contributions of these conductances to Aβ effects, we examined Aβ actions under conditions where the external perfusion solution was replaced with 0 mM Ca$^{2+}$ and contained 50 μM Cd$^{2+}$. Figure 2A shows the average of current-voltage relationships obtained from 17 neurons under control conditions, with 0 mM Ca$^{2+}$ external medium, and with Aβ in the presence of 0 mM Ca$^{2+}$ external. Replacing the external solution with 0 mM Ca$^{2+}$ decreased the currents by 18.58 ± 2.46% (control = 6.94 ± 0.47 nA, 0 mM Ca$^{2+}$ = 5.62 ± 0.40 nA, $n = 17$). Application of Aβ under these conditions further reduced the currents by 8.77 ± 1.72% (0 mM Ca$^{2+}$ Aβ = 5.16 ± 0.41 nA, $n = 17$, $P < 0.001$). We also studied Aβ effects in the presence of charybdotoxin (CTX) and iberiotoxin, specific blockers of $I_{C}$ channels. Figure 2B shows the average of I-V relationships obtained from 19 neurons under control conditions, in the presence of CTX (25 nM) and Aβ application in the presence of CTX. CTX reduced the outward currents at +30 mV by 12.89 ± 2.56% (control = 6.62 ± 0.37 nA, CTX = 5.76 ± 0.37 nA, $n = 19$). Application of Aβ in the presence of CTX further reduced the currents by 7.81 ± 1.83% (CTX + Aβ = 5.31 ± 0.33 nA, $n = 19$, $P < 0.001$). The percent reduction of currents by Aβ in the presence of CTX was not significantly different from that obtained by omitting Ca$^{2+}$ from the external perfusate ($P = 0.09$).

In seven cells, we also examined the effects of Aβ in the presence of iberiotoxin (50 nM). Aβ reduction of whole cell currents with iberiotoxin was similar (10.2 ± 2.5%, not illustrated) to that observed with CTX.

![Figure 2](http://jn.physiology.org/)

**FIG. 2.** Effects of Aβ on whole cell potassium currents. A: I-V plots of mean whole cell currents from DBB neurons ($n = 17$) evoked under control conditions, in 0 mM Ca$^{2+}$, and in 1 μM Aβ$_{25-35}$ in 0 mM Ca$^{2+}$: B: I-V plots of mean whole cell currents from DBB neurons ($n = 19$) evoked under control conditions, in 25 nM charybdotoxin, and in 1 μM Aβ$_{25-35}$ in charybdotoxin. C: I-V relationships of mean barium currents ($I_{Ba}$) in DBB neurons ($n = 9$) under control conditions, in the presence of 1 μM Aβ$_{25-35}$, and on wash out of Aβ. D and E: the effects of Aβ$_{25-35}$ (1 μM) on $I_{k}$ and $I_{A}$ in a DBB neuron, respectively. D: voltage protocol for recording $I_{k}$ is depicted on the left with holding potential of −80 mV and a 150-ms conditioning pulse to −40 mV. In this protocol, outward currents are mediated through $I_{k}$ (delayed rectifier) and $I_{C}$ (calcium-activated potassium conductance). E: in the same neuron, Aβ causes a decrease in transient outward K$^{+}$ currents ($I_{A}$). $I_{A}$ was obtained as difference currents by subtracting the currents obtained by the voltage protocol shown in D from that obtained by applying the voltage protocol shown in E where cells were held at −80 mV and a 150-ms conditioning pulse to −120 mV was applied.
EFFECTS OF $\alpha\beta$ ON CALCIUM CURRENTS. Since $\alpha\beta$ reduction of whole cell currents is attenuated by approximately 50% in the presence of CTX or by the removal of external Ca$^{2+}$, Ca$^{2+}$-activated currents appear to play an important role in the response of DBB neurons to $\alpha\beta$ application. This can result from either an effect on Ca$^{2+}$-dependent conductances, i.e., $I_C$, or, a more upstream effect on Ca$^{2+}$ channels that in turn may activate potassium conductances. To examine this issue, we recorded barium currents ($I_{Ba}$) flowing through Ca$^{2+}$ channels. Figure 2C shows the average I-V relationships of $I_{Ba}$ recorded from nine neurons. $\alpha\beta$ did not significantly affect the $I_{Ba}$ (control = 3.32 ± 0.23 nA, $\alpha\beta$ = 3.19 ± 0.25 nA, n = 9, P = 0.12, at −10 mV).

EFFECTS OF $\alpha\beta$ ON TRANSIENT OUTWARD ($I_A$) AND THE DELAYED RECTIFIER ($I_K$) POTASSIUM CURRENTS. $I_A$ and $I_K$ are voltage-sensitive currents, and their activation and inactivation are strongly voltage dependent. $I_A$ requires the holding potential to be relatively hyperpolarized (approximately −110 mV) for removal of its inactivation, whereas it is inactivated at −40 mV. On the other hand, $I_K$ is not inactivated at −40 mV. These biophysical properties of $I_A$ and $I_K$ can thus be utilized to isolate these currents. Therefore a conditioning pulse to −40 mV will activate $I_K$ without any significant contamination by $I_A$ (Connor and Stevens 1971; Easaw et al. 1999). A conditioning pulse to −120 mV will activate both $I_A$ and $I_K$. The difference currents obtained by subtracting the currents evoked by depolarizing pulses following a conditioning pulse to −40 mV from those evoked following a conditioning pulse to −120 mV provide an accurate estimate of $I_A$ (Connor and Stevens 1971; Easaw et al. 1999). Figure 2D shows the currents recorded from a neuron with a conditioning pulse to −40 mV for 150 ms, representing mainly $I_K$, under control conditions, in the presence of $\alpha\beta$ and recovery on wash out of $\alpha\beta$. $\alpha\beta$ reduced $I_K$ by 10.93 ± 2.4% (control = 7.14 ± 0.42 nA, $\alpha\beta$ = 6.41 ± 0.45 nA, n = 34, P < 0.001 at +30 mV). Figure 2E shows the difference currents recorded from the same neuron representing mainly $I_A$, under control conditions, in the presence of $\alpha\beta$, and on wash out. $I_A$ was reduced significantly by $\alpha\beta$ by 16.96 ± 3.89% (control = 5.42 ± 0.58 nA, $\alpha\beta$ = 4.58 ± 0.55 nA, n = 34, P < 0.001). We have previously shown that the residual sustained current remaining at the end of the 100-ms test pulse (shown in Fig. 3B) consists mainly of $I_K$ and $I_C$ (Easaw et al. 1999), both of which are also reduced by $\alpha\beta$.

Tetraethylammonium (TEA) ions at a concentration of 5 mM block $I_K$ and $I_C$ (Jassar et al. 1999). Figure 3A shows the average I-V relationships obtained from seven neurons under control conditions, with 5 mM TEA alone, and $\alpha\beta$ in the presence of TEA. TEA blocked 87.25 ± 2.97% of the outward current at +30 mV (control = 6.30 ± 0.92 nA, TEA = 0.87 ± 0.26 nA, n = 7). $\alpha\beta$ failed to produce any significant effect on the remaining currents in the presence of TEA ($\alpha\beta$ = 0.80 ± 0.23 nA, n = 7, P = 0.22).

EFFECTS OF $\alpha\beta$ ON SODIUM CURRENTS. Sodium currents are involved in the fast depolarizing phase of the action potential. Enhancement of these currents can increase the excitability and vice versa. We recorded sodium currents in isolation to assess if $\alpha\beta$ has any effects on these currents. The sodium currents were TTX sensitive. Figure 3B shows the average I-V relationships obtained from 11 cells under control conditions, in the presence of Aβ, and recovery on wash out of Aβ. Aβ did not influence sodium currents in DBB neurons (control = −6.34 ± 0.59 nA, Aβ = −6.08 ± 0.57 nA, P = 0.12 at +20 mV).

**Effects of Aβ on excitability of DBB neurons**

Application of Aβ results in depolarization of the RMP, increase in excitability, and loss of accommodation. Figure 4A depicts a DBB neuron showing accommodation under control conditions. On application of Aβ, the number of spikes evoked by injecting the same amount of current as under control conditions in the same neuron was increased, indicating an increase in excitability (Fig. 4B). Under control conditions, the average RMP was −65.5 ± 2.2 mV, which depolarized to −56.4 ± 1.8 mV (n = 21, P < 0.001) on Aβ application and recovered to 72.0 ± 3.3 mV on wash out. The average number of spikes elicited by current injection was 8.9 ± 1.5 under control conditions, 13.9 ± 1.4 in the presence of Aβ, and 6.4 ±...
1.8 on wash out \((n = 21, P < 0.001)\). In addition to the increase in excitability, \(\alpha\beta\) also caused a loss of accommodation. The interspike interval between the first two and the last two action potentials provides a measure of accommodation. Under control conditions the first interspike interval was 46.5 ± 2.3 ms, and the last interspike interval was 90.1 ± 16.3 ms \((n = 6, \text{Fig. 4C})\). In the presence of \(\alpha\beta\), the first interspike interval was 33.3 ± 1.8 ms, and the last interspike interval was 42.1 ± 2.9 ms. On recovery the first interspike interval was 48.1 ± 1.5 ms, and the last interspike interval was 93.6 ± 18.1 ms.

A larger difference between the first and last interspike intervals indicates a greater degree of accommodation. \(\alpha\beta\) significantly reduced the difference between the first and the last interspike intervals consistent with a loss of accommodation \((P < 0.005)\).

**Chemical phenotype of the \(\alpha\beta\) responsive neurons**

There are two main chemical neurotransmitter phenotypes represented in the DBB neurons: GABAergic and cholinergic. Whole cell recordings were made from a heterogeneous population of DBB neurons. Definitive determination of the chemical phenotype was done by single-cell RT-PCR analysis. ChAT was used as a specific marker for cholinergic neurons, and GAD was used as a specific marker for GABAergic neurons. Figure 5 shows the photograph of a gel indicating RT-PCR products from an \(\alpha\beta\)-responsive cell shown in Fig. 1A and also an \(\alpha\beta\)-nonresponsive neuron (Fig. 1C). The \(\alpha\beta\) responsive cell on the left reveals a band corresponding to the molecular weight of the ChAT primer, and the \(\alpha\beta\) nonresponsive cell in the middle of the gel shows a band corresponding to the molecular weight of GAD primer. Results from 81 DBB neurons that were recorded in either voltage- or current-clamp modes and in which PT-PCR reaction was unequivocal are summarized in Fig. 5, bottom. All cells that responded \(\alpha\beta\) with a reduction in whole cell currents or an increase in excitability were ChAT positive \((n = 63)\) and GAD negative. On the other hand, all the \(\alpha\beta\)-nonresponsive neurons were GAD positive \((n = 18)\) and ChAT negative.

Previous studies have suggested that larger dissociated cells from the basal forebrain are more likely to be cholinergic (Griffith et al. 1994; Jassar et al. 1999). In the present study the average membrane capacitance estimated electronically was 16.7 ± 0.5 pF \((n = 171; \text{range 12–29 pF})\). ChAT-positive cells had an average membrane capacitance of 16.4 ± 0.7 pF \((\text{range, 13–21 pF})\), whereas GAD-positive cells were found to have a capacitance of 18.2 ± 1.4 pF \((\text{range, 14–22 pF})\). Therefore, on the basis of our results, it would seem that cell size correlates.
poorly with the chemical identity of a particular cell as determined by single cell RT-PCR.

**Involvement of protein tyrosine phosphorylation in Aβ response**

Protein tyrosine phosphorylation modulates voltage- and ligand-gated channels to influence neuronal function (Raymond et al. 1993; Wang and Salter 1994). In PC 12 cells and olfactory neuroblasts, application of Aβ_{1–40} and Aβ_{25–35} induces a rapid and dose-dependent tyrosine phosphorylation that is accompanied by a rise in cytosolic Ca^{2+} (Luo et al. 1995). The DBB cells are enriched with protein tyrosine kinase (PTK) activity, and we have previously shown that GABA responses in these cells are modulated by PTK phosphorylation (Jassar et al. 1997). We investigated whether PTK may play a role in Aβ-evoked responses in DBB neurons. Genistein and tyrphostin B-44 are relatively specific membrane-permeable blockers of PTK (Valenzuela et al. 1995; Wang and Salter 1994). Figure 6A shows the average I-V relationships from 7 neurons under control conditions, in the presence of genistein (100 μM), and Aβ in the presence of genistein. Figure 6B shows the average I-V relationships from six neurons under control conditions, in the presence of tyrphostin B-44 (50 μM), and Aβ in the presence of tyrphostin. These compounds decreased the whole cell outward currents by 60.3 ± 4.97% (control = 6.76 ± 0.55 nA, genistein = 2.66 ± 0.42 nA, n = 7) and 55.01 ± 3.04% (control = 5.87 ± 0.39 nA, tyrphostin = 2.63 ± 0.21 nA, n = 6), respectively. However, Aβ failed to affect these outward potassium currents in the presence of genistein or tyrphostin (genistein + Aβ = 2.44 ± 0.34 nA, 63.48 ± 4.06%, P > 0.05; tyrphostin + Aβ = 2.48 ± 0.18 nA, 57.54 ± 2.30%, P > 0.07). The response to Aβ, a reduction of 11.45 ± 0.82% of the outward currents at +30 mV (n = 6), was not significantly affected in the presence of daidzein (100 μM), an inactive analogue of genistein (Fig. 6C, control = 6.85 ± 0.32 nA, daidzein = 6.44 ± 0.36 nA, daidzein + Aβ = 5.71 ± 0.34 nA, n = 6, P < 0.001).

**DISCUSSION**

These experiments demonstrate four major findings. First, Aβ reduces a suite of potassium currents in basal forebrain neurons, including calcium-activated potassium (I_C), the delayed rectifier (I_K), and transient outward potassium (I_TO) conductances, but not sodium or calcium currents. Second, under current-clamp conditions, application of Aβ evoked an increase in excitability and a loss of accommodation in cholinergic DBB neurons. Third, using single-cell RT-PCR analysis, we show that Aβ actions are specific to cholinergic, but not GABAergic DBB neurons. Finally, the Aβ effects on cholinergic DBB neurons appear to be mediated via activation of protein tyrosine kinase signaling pathway.

**Aβ modulation of ionic conductances and neuronal excitability**

Aβ and related amyloidogenic metabolic fragments have been shown to alter cellular ionic conductances with existing channels or by de novo channel formation (Fraser et al. 1997). Such alteration in ionic homeostasis has been linked to the ability of Aβ to induce cell death and may provide a molecular mechanism for neurodegeneration seen in AD (Mattson et al. 1992; Yu et al. 1998). In hippocampal neurons, sAPP, a larger fragment of the parent APP, activates K^+ channels and reduces intracellular Ca^{2+} through cGMP production and protein dephosphorylation (Furukawa et al. 1996). Suppression of excitability and membrane hyperpolarization by such mechanisms have been advanced to support, in part, a “neuroprotective” role for sAPP. However, although Aβ has been shown to activate a wide variety of K^+ and Ca^{2+} conductances, there is limited information on its role in influencing neuronal excitability. Our data show that Aβ decreases I_C currents without influencing calcium currents and leads us to conclude that Aβ...
has a direct effect on $I_C$ channels. Since $I_C$ is responsible, in part, for the repolarization phase of the action potential and plays an important role in the process of spike frequency adaptation (accommodation) (Vergara et al. 1998), $A\beta$-induced blockade of $I_C$ that we have observed could explain the increase in excitability and loss of accommodation seen with $A\beta$. We have previously shown that in DBB neurons at RMP, inhibition of $I_C$ with either charybdotoxin or iberiotoxin results in an increase in excitability and loss of accommodation similar to that observed for $A\beta$ in the present study (Easaw et al. 1999; Jassar et al. 1999).

Fast-inactivating potassium channels ($I_A$) are also important in modulating neuronal excitability. $A\beta$-induced reduction of $I_A$ observed in our study is consistent with a similar effect observed in rat hippocampal neurons (Good et al. 1996). Blockade of $I_A$ by $A\beta$ could lead to increased duration of depolarization during an action potential, which in turn could increase $Ca^{2+}$ influx. However, unlike several previous reports, we did not observe an effect of $A\beta$ on $Ca^{2+}$ currents (Brorson et al. 1995; Mattson et al. 1992; Ueda et al. 1997) or $Na^+$ currents. Although $A\beta$ did not influence $Ca^{2+}$ currents, prolonged depolarization resulting from a blockade of $K^+$ currents (both $I_K$ and $I_{K1}$) could still lead to increased total $Ca^{2+}$ influx. In the present study we noted that $A\beta$ decreases the TEA-sensitive $I_K$ conductance, which is different from the observation in septal cell line (SN56) and murine cortical cultures where $A\beta$ exposure resulted in an enhancement of $I_K$ (Colom et al. 1998; Yu et al. 1998). However, in the latter studies, no acute effects of $A\beta$ on $I_K$ were noted, but 7–11 h after $A\beta$ exposure, the $I_K$ was enhanced, and this effect could be blocked by TEA. These delayed effects of $A\beta$ on potentiation of $I_K$ have been postulated to explain the ability of $A\beta$ to induce apoptotic cell death in cultures (Yu et al. 1998).

**Selective effects of $A\beta$ on cholinergic neurons**

Our data indicate that cell size is not a reliable index for distinguishing cholinergic from GABAergic neurons in the DBB. However, single-cell RT PCR offers an effective and reliable means to make a distinction between these two major chemical phenotype of cells in the basal forebrain. At present, the cause of preferential degeneration of forebrain cholinergic neurons remains unclear. The neurotoxic potential of $A\beta$ peptides could possibly mediate the degeneration of cholinergic neurons in AD brains. This notion is supported by data that a single injection of $A\beta$ peptide into the septal nucleus induces damage to cholinergic but not parvalbumin-containing (presumably GABAergic) neurons (Harkany et al. 1995). Cholinergic neurons in hippocampal cultures seem particularly susceptible to injury following exposure to $A\beta$, whereas GABA-containing neurons are relatively resistant to $A\beta$-induced neurotoxicity (Pike and Cotman 1993). Our experiments show that the effects of $A\beta$ in modulating $K^+$ channel conductances and neuronal excitability are specific to cholinergic and not GABAergic neurons. This finding apart from demonstrating, at a cellular level, a link between $A\beta$ peptides and cholinergic function, may help explain the selective vulnerability of cholinergic neurons to $A\beta$ that is observed in vivo and in cell cultures. $A\beta$’s ability to render cholinergic neurons hyperexcitable through loss of accommodation could result in prolonged depolarization and eventual cell death resulting from excessive $Ca^{2+}$ influx. Indeed $A\beta$ has previously been shown to markedly potentiate glutamate-induced cell death in human cortical neurons through an increase in $Ca^{2+}$ influx (Mattson et al. 1992).

**$A\beta$ and protein tyrosine phosphorylation**

In the present study we have observed that $A\beta$-mediated reduction in whole cell $K^+$-currents in DBB neurons is occluded by bath application of PTK inhibitors genistein and tyrphostin B-44 (–), but unaffected by daidzein, which is structurally similar to genistein but has no effect on PTK activity. It is possible that the effects of genistein, apart from its properties as a PTK inhibitor, could be also be attributed to its ability to directly block voltage-gated $K^+$ conductances (Ogata et al. 1997; Smirnov and Aaronson 1995). However, $A\beta$ effects were also blocked by tyrphostin B-44 (–), another PTK inhibitor, which has not been shown to block $K^+$ conductances. These results suggest that endogenous PTKs may play an important role in coupling the $A\beta$ effects on $K^+$ conductances in cholinergic forebrain neurons. There is considerable evidence that protein tyrosine kinases and phosphatases, acting on potassium channels, can regulate neuronal excitability (Jonas and Kaczmarek 1996).

**Conclusions**

The fact that $A\beta$ is constitutively produced in the brain (Shoji et al. 1992) and is capable of influencing ion channel function of cholinergic neurons as demonstrated in the present study suggests that this peptide may have a neuromodulatory role within the DBB in vivo apart from its neurotoxic effects in the context of AD. The underlying mechanism for the specificity of $A\beta$ response toward cholinergic, and not GABAergic, basal forebrain cells is an important question that remains unresolved. It is possible that the difference in responsiveness of cholinergic versus GABAergic cells to $A\beta$ that we have observed in the present study may be due to the presence of a receptor for $A\beta$, which has yet to be identified, on cholinergic but not GABAergic neurons. Further studies of the receptor and molecular mechanisms underlying the coupling of $A\beta$ effects to potassium channels through PTK signaling pathways in cholinergic basal forebrain neurons may shed additional important insights into cholinergic hypofunction seen in AD.

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