β-Amyloid Peptide Blocks the Fast-Inactivating K^+ Current in Rat Hippocampal Neurons

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ABSTRACT Deposition of β-amyloid peptide (Aβ) in senile plaques is a hallmark of Alzheimer disease neuropathology. Chronic exposure of neuronal cultures to synthetic Aβ is directly toxic, or enhances neuronal susceptibility to excitotoxins. Exposure to Aβ may cause a loss of cellular calcium homeostasis, but the mechanism by which this occurs is uncertain. In this work, the acute response of rat hippocampal neurons to applications of synthetic Aβ was measured using whole-cell voltage-clamp techniques. Pulse application of Aβ caused a reversible voltage-dependent decrease in membrane conductance. Aβ selectively blocked the voltage-gated fast-inactivating K^+ current, with an estimated $K_i < 10$ μM. Aβ also blocked the delayed rectifying current, but only at the highest concentration tested. The response was independent of aggregation state or peptide length. The dynamic response of the fast-inactivating current to a voltage jump was consistent with a model whereby Aβ binds reversibly to closed channels and prevents their opening. Blockage of fast-inactivating K^+ channels by Aβ could lead to prolonged cell depolarization, thereby increasing Ca^{2+} influx.

INTRODUCTION

Deposition of β-amyloid peptide (Aβ) in fibrillar form into senile plaques is a primary pathological feature of patients with Alzheimer's disease. Transgenic mice have been developed that overexpress human Aβ precursor protein, secrete Aβ, develop Aβ amyloid deposits, and demonstrate neuronal pathology and learning deficits (Games et al., 1995; Moran et al., 1995). Neurotoxicity of Aβ to cortical cultures has been demonstrated (Yankner et al., 1990). Aβ may be directly toxic or may increase the vulnerability of neurons to external insults (Koh et al., 1990; Cotman et al., 1992; Loo et al., 1993; Busciglio et al., 1993). The mechanism(s) by which Aβ mediates neurotoxicity is uncertain. Several hypotheses have been put forth, including the following: Aβ generates free radicals, which oxidize membrane proteins and lipids, thereby increasing membrane permeability (Hensley et al., 1994); Aβ forms a cation-selective ion pore (Arispe et al., 1993); or Aβ causes a Ca^{2+} influx or release of intracellular stores of Ca^{2+} (Mattson et al., 1993). Incubation of human cortical cultures with Aβ led to increased intracellular Ca^{2+} and generalized loss of Ca^{2+} homeostasis (Mattson et al., 1992). Ca^{2+} channel blockers reduced toxicity of Aβ in one study (Weiss et al., 1994), but not in another (Whitson and Appel, 1995). Aβ also caused functional loss of 113-pS K^+ channels in fibroblasts (Etcheberrigaray et al., 1994).

Typically, chronic incubation of Aβ with neurons for several hours or days elicited the neurotoxic responses described above. Measurements of cellular properties after long-term incubation of neurons with Aβ may reflect a cascade of responses of the cell to Aβ, including new protein synthesis or altered gene expression. Therefore, these experiments do not reveal the immediate, acute effect of Aβ interaction with neurons, which could then trigger subsequent changes in neuronal functioning.

In work reported here, the acute response of rat hippocampal neurons to Aβ was assessed to elucidate the initial steps in Aβ interaction with neurons. Whole-cell voltage-clamp recordings were obtained from rat hippocampal neurons before and after pulse application of Aβ. Aβ caused a voltage-dependent decrease in membrane conductance, indicating that the peptide affected functioning of a voltage-gated channel. By evoking specific ion currents, we show that Aβ selectively blocks the fast-inactivating K^+ current.

MATERIALS AND METHODS

Materials

Peptides homologous to the first 39 and 40 residues of Aβ, β(1–39) and β(1–40), respectively, were purchased from Anaspec (San Jose, CA). Purity was assessed by amino acid analysis, mass spectrometry, and reverse-phase chromatography. A peptide homologous to the first 28 residues of Aβ, β(1–28), was synthesized and purified as described (Shen et al., 1993). The N-terminal sequence Gln^{11}-β(1–16), the reverse sequence peptide β(40–1), and tetraethylammonium chloride (TEA) were purchased from Sigma (St. Louis, MO). β_2-microglobulin, 4-aminopyridine (4-AP), and α-dendrotoxin (DTX) were purchased from Calbiochem (La Jolla, CA).

Hippocampal neuronal cultures

Hippocampal cultures were prepared from Sprague-Dawley rats at postnatal day 0 or 1 as described (Trussell and Jackson, 1987) with minor modifications. Briefly, animals were decapitated, and hippocampi were removed quickly. Tissue was cut into small pieces, treated for 1 h at 32°C

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in L-15 medium containing 0.5 mM EDTA, 1 mM cysteine, and 40 U/ml papain at pH 7.2, then washed twice in papain-free L-15 solution containing 1 mg/ml ovomucoid and bovine serum albumin. Using fine-tipped Pasteur pipettes, tissue was slowly triturated, and cells were spread onto collagen-coated plates (Collaborative Biomedical Products, Bedford, MA). Cells were maintained in minimum essential medium containing 6 mg/ml glucose, 10% horse serum, 10% fetal calf serum, and antibiotics at 37°C and 5% CO_2. After glial cells covered the bottom of the culture dish (3–5 days), 80 μM 5-fluoro-2-deoxyuridine and 200 μM uridine were added to the culture dish to prevent further glia proliferation. Hippocampal neurons were cultured for a total of 3–10 days before use. Animals were handled according to institutional guidelines under the university animal welfare assurance program.

Voltage-clamp experiments

Whole-cell voltage-clamp recordings were obtained from rat hippocampal neurons before and after pulse application of Aβ. An Axopatch 200A amplifier (Axon Instruments, Foster City, CA) was used for whole-cell recordings. Currents were filtered at 5 kHz and digitized using a Digidata 1200 interface (Axon Instruments). For recordings, cells were bathed in a room temperature (22°C) Ringer's solution (140 mM NaCl, 5 mM KCl, 3 mM $CaCl_2$, 1 mM $MgCl_2$, 20 mM glucose, and 10 mM HEPES, pH 7.3). The recording pipette solution consisted of 130 mM KCl, 2 mM $MgCl_2$, 2 mM ATP, 1 mM GTP, 5 mM EGTA, and 10 mM HEPES, pH 7.3. Data were acquired and analyzed using pClamp (Axon Instruments).

Unless otherwise indicated, lyophilized peptides were dissolved at 10 mg/ml in 0.1% trifluoroacetic acid (TFA) for 1 h and then stored at −70°C until use. Peptide stock solutions were thawed 24 h before use and diluted to 10^{-4} M in Ringer's solution, and the pH was adjusted if required. Blue food coloring dye (5–10% by volume) was added as a visible indicator. Aβ was applied using a Picospritzer II from General Valve Corporation (Fairfield, NJ). An initial control record was measured, and then β(1–39) was applied for 380 ms via a pressure pulse with a micropipette placed close (30–50 μm) to the clamped neuron. Delivery of the peptide to the clamped neuron was confirmed by the presence of a pool of dye-colored fluid surrounding the cell. The pool persisted for approximately 0.5–1 min. The second current measurement was taken beginning 25 ms after pressure ejection of Aβ.

Current-voltage curves were measured from a holding potential of −100 mV using a linear voltage ramp from −100 mV to +60 mV over 1.5 s. Specific ion currents were evoked as follows. The total K^+ current was evoked by holding the neuron at −100 mV for 380 ms and then stepping to 0 mV for 50 ms. The delayed rectifying potassium current (I_K) was then evoked by stepping to 0 mV for 50 ms from a holding potential of −40 mV. In both cases 2 μM tetrodotoxin and 200 μM $CdCl_2$ were added extracellularly to block Na^+ and Ca^{2+} currents, respectively. Control experiments showed that Ca^{2+} currents were completely blocked under these conditions (n = 5). The fast inactivating potassium current (I_A) was obtained by subtracting I_K from the total K^+ current (Ficker and Heinemann, 1992). Whether fast inactivating currents are functional in rat hippocampal cells depends on a number of factors, including time in culture (Ficker and Heinemann, 1992); only cells with both fast inactivating and delayed rectifying components of the K^+ current were included in the data analysis of potassium current effects. In some experiments, voltage-dependent K^+ channel blockers TEA (10 mM), 4-AP (4 mM), or DTX (100 nM) were added to the extracellular bath. In other experiments, the voltage command paradigm was altered: the voltage was held at −100 mV for 380 ms, then stepped to −40, −30, −20, −10, 0, 10, 20, 40, or 60 mV to evoke the total K^+ current. To evoke I_K, a 50-ms voltage step to −40 mV was inserted after the activating voltage command of −100 mV. Na^+ currents were evoked by holding the cell at −70 mV for 380 ms and then stepping to −25 mV for 50 ms, with K^+ currents blocked by substituting 130 mM CsCl for the KCl in the intracellular solution. Ca^{2+} currents were evoked by stepping the cell from a holding potential of −80 mV to 10 mV for 50 ms, with K^+ currents blocked by 130 mM intracellular CsCl and Na^+ currents blocked either by 2 μM extracellular tetrodotoxin or by replacing NaCl in the extracellular solution with 140 mM choline chloride. Leak currents were typically 1–4% of the total measured current and were not subtracted.

RESULTS

A decrease in membrane conductance was observed for 61% of neurons (n = 18) in response to β(1–39) application, as measured in voltage-ramp experiments (Fig. 1). A neuron was considered to respond if the percentage change in conductance after Aβ application exceeded that of the largest percentage change in conductance observed when solvent alone was applied. The membrane conductance was calculated from the linear portion of the current-voltage curve. In responding neurons, a 45 ± 10% decrease in the membrane conductance was observed upon application of β(1–39), compared to a 5 ± 5% decrease observed after application of the solvent vehicle (0.1% TFA diluted into the recording bath (Ringer's) solution, n = 10). The membrane conductance after application of Aβ was voltage dependent, suggesting that Aβ alters the functioning of a voltage-gated ion channel. The effect was reversible: after diffusion of Aβ away from the clamped cell, as assessed by disappearance of the blue dye indicator, current-voltage curves returned to the original (data not shown).

To identify the ion currents affected by Aβ application, specific ion currents were evoked by voltage steps in the presence of ion channel blockers. With Na^+, K^+, and Ca^{2+} currents blocked, only a small leak current was observed, and the size of this current did not change with β(1–39) application (n = 3). When K^+ currents were blocked, Na^+ (n = 5) and Ca^{2+} (n = 6) currents with the expected time and voltage dependencies were observed; these currents were unchanged by β(1–39) application (data not shown). With Na^+ and Ca^{2+} currents blocked, application of β(1–

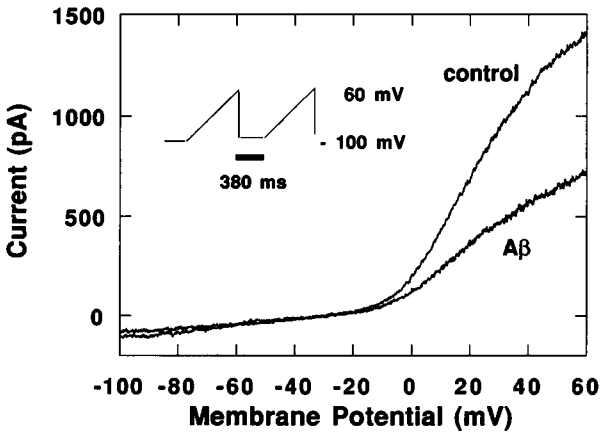

FIGURE 1 Representative measured current as a function of membrane potential. Peptide concentration was 100 μM in the application micropipette. The voltage ramp used to elicit currents is shown; the bar indicates Aβ pulse application. The Aβ pool persists for approximately 30 to 60 s after pulse application.

39) resulted in significant decreases (60 ± 20%, mean ± SD) in the fast-inactivating K^+ current (I_A) in 66% of cells tested (n = 32). Fifty-nine percent of cells (n = 32) displayed a smaller but still significant decrease (40 ± 10%) in the delayed rectifying K^+ current (I_K). Representative recordings that illustrate these effects in a responder cell are shown in Fig. 2. The mean decrease in current relative to solvent alone reached statistical significance for I_A ($p < 0.0025$) and for I_K ($p < 0.08$) (Fig. 3). Both K^+ currents returned to their control levels after diffusion of Aβ away from the cell (data not shown).

To determine the voltage dependence of the effect of Aβ on I_A and I_K, K^+ currents were evoked at depolarizing command potentials from −40 to +60 mV with Na^+ and Ca^{2+} currents blocked. I_A was activated at slightly lower membrane potential than I_K, consistent with literature reports (Ficker and Heinemann, 1992). The activation curves for both I_A and I_K were unaffected by Aβ application (Fig. 4, *A* and *B*). The fractional reduction in I_A and I_K upon Aβ application was not a function of the depolarizing potential (Fig. 4 *C*), indicating that Aβ does not sense the electric field in the pore.

The delayed rectifying current was sensitive to TEA but insensitive to 4-AP and DTX at the concentrations tested, whereas the fast-inactivating current was sensitive to 4-AP and DTX but not to TEA. All tested cells were

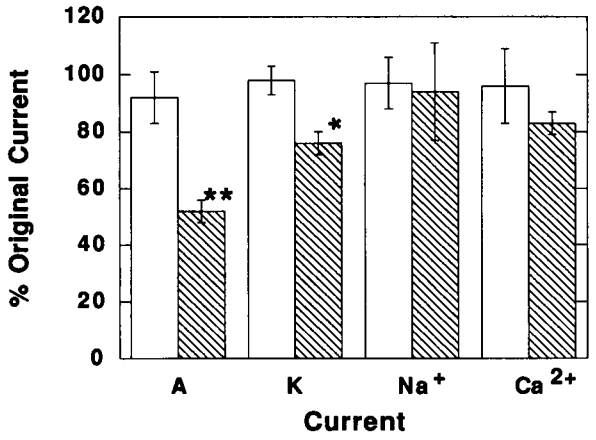

FIGURE 3 Mean change in specific ion currents after pulse application of solvent (0.1% TFA diluted into Ringer's solution) or 100 μM β(1–39). Open bars, solvent; hatched bars, peptide. For I_A and I_{Na+}, the ratio of the peak current before Aβ or solvent application to peak current after application was reported; for I_K and I_{Ca2+}, the ratio of steady-state values was used. The error bars represent the standard error of the mean (n = 32 for I_A and I_K, n = 5 for all others) of the ratios of currents before and after Aβ or solvent application. *, indicates the change in current is significantly different from the change in current with solvent application ($p < 0.08$). **, indicates current is significantly different from the change in current with solvent application ($p < 0.0025$).

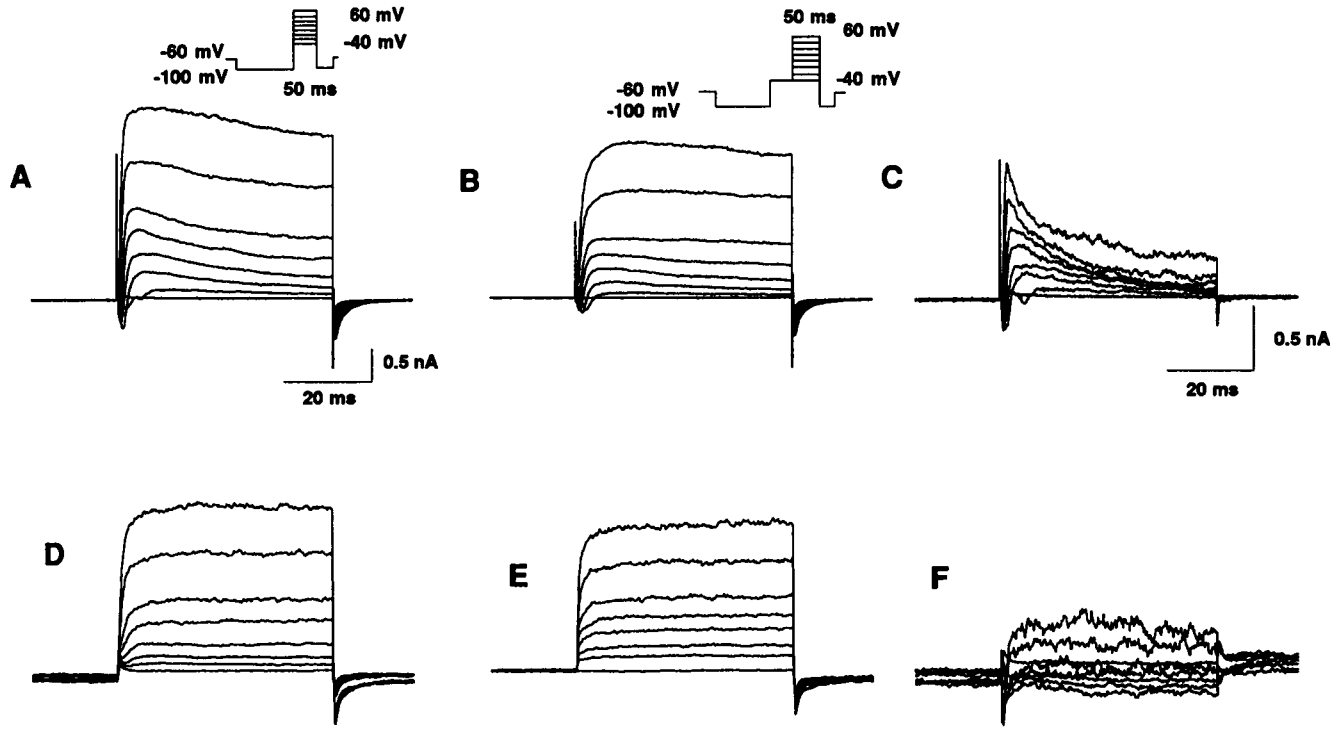

FIGURE 2 Effect of Aβ on voltage-dependent K^+ currents. (*A, B, C*) Representative recording from a responding cell of total (*A*), delayed rectifying (*B*), and fast-inactivating (*C*) K^+ current before Aβ application. (*D, E, F*) Representative recording from a responding cell of total (*D*), delayed rectifying (*E*), and fast-inactivating (*F*) K^+ current after Aβ application. The fast-inactivating current was obtained by subtraction of the delayed rectifying from the total K^+ current, as described in Materials and Methods. The command potentials used to evoke total and delayed rectifying currents are shown. For recordings from the cell in the presence of Aβ, the peptide was applied for 380 ms at the beginning of the −100 mV activating voltage command. The Aβ pool persists for approximately 30 to 60 s after pulse application. Leak currents were not subtracted.

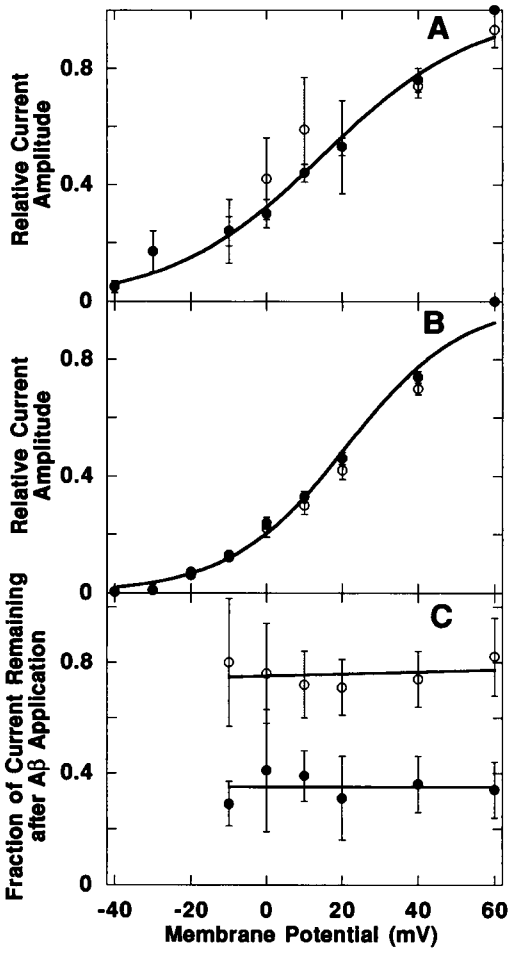

FIGURE 4 Voltage dependence of activation and of Aβ block of K^+ currents. (*A*) Relative amplitude of fast-inactivating current as a function of depolarizing command potential for cells before (●) and after (○) Aβ application. (*B*) Relative amplitude of delayed rectifying current as a function of depolarizing potential for cells before (●) and after (○) Aβ application. (*C*) Fractional reduction in I_A (●) and I_K (○) caused by Aβ application as a function of depolarizing potential. Results are mean ± SE for seven cells.

sensitive to TEA or 4-AP, but only four of seven tested cells responded to DTX. These results are in qualitative agreement with previous reports on rat hippocampal CA1 neurons (Ficker and Heinemann, 1992; Halliwell et al., 1986). TEA (10 mM) caused a 50 ± 30% decrease in I_K (n = 7). 4-AP (4 mM) caused a 70 ± 20% decrease in I_A (n = 7). In responding cells, DTX (100 nM) caused a 80 ± 10% decrease in I_A. For DTX-sensitive cells, the total K^+ current dropped to 50 ± 10% of its original value after Aβ application without DTX, but in the presence of DTX Aβ had a less pronounced effect, decreasing the total K^+ current to 70 ± 10% of its original value ($p < 0.05$). TEA and 4-AP had no statistically significant effect on the reduction in total K^+ current due to Aβ application. Recordings from TEA- or DTX-treated cells are shown in Fig. 5. The recordings illustrate the effects observed on cells sensitive to both toxin and Aβ. TEA reduced the delayed rectifying current but did not affect the fast-inactivating current (Fig. 5 *A*). Aβ application to this cell caused a similar loss of the transient K^+ current whether or not the cell was incubated with TEA. In contrast, DTX caused a loss of the transient current in the cell shown in Fig. 5 *B*; Aβ had little additional effect.

The effect of Aβ on I_A and I_K was concentration dependent (Fig. 6 *A*). At concentrations of 10 μM or below in the application micropipette, there was no effect of Aβ on I_K. Some reduction in I_A was observed with pulse application of Aβ down to 1 μM in the micropipette. To test the effect of peptide aggregation on neuronal response, β(1–39) was dissolved in dimethyl sulfoxide (DMSO), diluted into Ringer's solution, and applied to the neuron within 2 h. We have previously shown that under these conditions, the peptide is less aggregated than when using our standard protocol of predissolving in 0.1% TFA, diluting into Ringer's solution, and incubating for 24 h before application (Shen and Murphy, 1995). Response of the K^+ current was independent of the solvent used to predissolve β(1–39) (Fig. 6 *A*). There was no effect of diluted DMSO on evoked currents (data not shown).

The concentration dependence was fit assuming a simple inhibition expression

$$\frac{I}{I_0} = (1 - f_{NR})\left(\frac{K_I}{K_I + [A\beta]}\right) + f_{NR} \qquad (1)$$

where I or I_0 is the current after or before Aβ application, respectively, K_I is the inhibition constant, [Aβ] is the concentration of peptide in the application micropipette (expressed in units of peptide monomer), and f_{NR} is the fraction of nonresponding channels. For the fast-inactivating K^+ current, K_I and f_{NR} were estimated to be 10 ± 4 μM and 0.4 ± 0.1, respectively. The actual concentration detected by the neuron is lower than the concentration in the micropipette. Therefore, the calculated K_I represents an upper limit of the true constant.

β(1–28) and β(1–40) evoked effects on I_A and I_K similar to those of β(1–39) (Fig. 6 *B*). Application of the reverse sequence peptide β(40–1) caused a 40 ± 10% decrease in I_A but did not affect I_K. In control experiments, the N-terminal Aβ fragment Gln^{11}β(1–16), β_2-microglobulin (a 12 kDa amyloidogenic protein with high β-sheet content; Gorevic et al., 1985), or bovine serum albumin (data not shown) did not decrease evoked currents significantly.

We considered several alternative mechanisms by which Aβ could reduce the fast-inactivating K^+ current, including: 1) Aβ binds reversibly to closed channels to prevent them from opening, 2) Aβ binds reversibly to open channels and increases their rate of deactivation, and 3) Aβ irreversibly blocks open channels. To evaluate these alternatives, the response of fast-inactivating K^+ channels to a depolarizing step in the absence of Aβ application was modeled assuming C ⇒ O ⇒ D, where

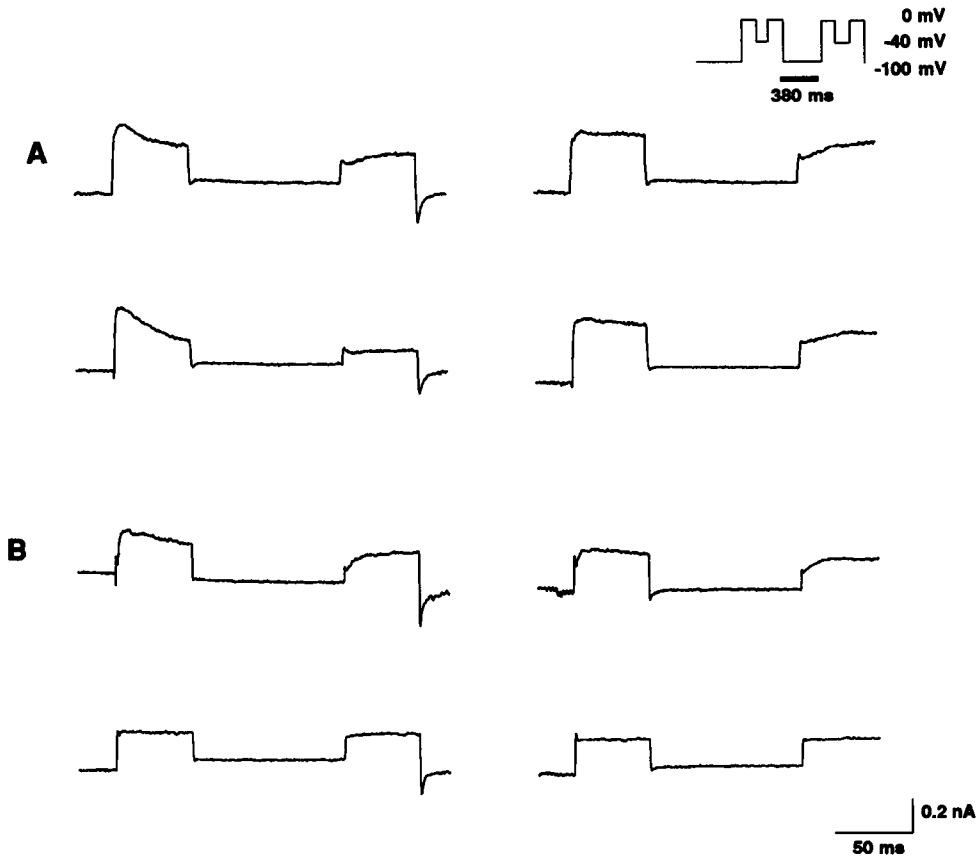

FIGURE 5 Effect of pharmacological agents on Aβ-mediated block of K^+ currents. (*A*) Recording from a cell treated with TEA. The initial portion of the top recording is of the evoked total and delayed rectifying current before Aβ or TEA application. The break in the recording indicates the time during which Aβ was applied to the cell. No change in measured current was observed during this period. The second portion of the top recording is of the evoked total and delayed rectifying current after Aβ application. The lower recording was made in an identical fashion from the same cell, taken 15–20 min after addition of TEA to the extracellular medium. (*B*) Recording from a DTX-sensitive cell. The top recording is from a cell before DTX addition, before (*left*) and after (*right*) Aβ application. The lower recording is taken from the same cell 15–20 min after addition of DTX to the extracellular medium.

C, O, and D are the number of closed (and activated), open, or deactivated channels, respectively. For simplicity, all closed but activated states were lumped together. The current was assumed to be directly proportional to the number of open channels. A kinetic equation was derived using first-order rate constants k_o and k_d for opening and deactivating steps, respectively. For the base case (no Aβ applied),

$$\frac{I_A}{I_{max}} = \left(\frac{k_o}{k_d - k_o}\right)(\exp(-k_o t) - \exp(-k_d t)) \qquad (2)$$

where I_A is the transient current through fast-inactivating K^+ channels and I_{max} is the maximum current if all channels are open.

For case (1), an additional equilibrium binding step of C + Aβ ⇔ B, where B is a blocked channel, was incorporated into the model, or,

$$\frac{I_A}{I_{max}} = \left(\frac{K_I}{K_I + [A\beta]}\right)\left(\frac{k_o}{k_d - k_o}\right)(\exp(-k_o t) - \exp(-k_d t)) \qquad (3)$$

where K_I is the dissociation equilibrium constant. For case (2), binding of Aβ to an open channel was assumed to be at equilibrium at all times and deactivation was modeled as a first-order rate process. This was expressed as O + Aβ ⇔ O*Aβ ⇔ D + Aβ, where O*Aβ is an open channel with bound Aβ. The kinetic equation becomes

$$\frac{I_A}{I_{max}} = \left(\frac{K_I + [A\beta]}{K_I}\right)\left(\frac{k_o}{k_d + k_d^*\left(\frac{[A\beta]}{K_I}\right) - k_o\left(\frac{K_I + [A\beta]}{K_I}\right)}\right) \qquad (4)$$

$$\times \left(\exp(-k_o t) - \exp\left(-\left(\frac{K_I}{K_I + [A\beta]}\right)\left(k_d + k_d^*\left(\frac{[A\beta]}{K_I}\right)\right)t\right)\right)$$

where k_d^* is the deactivation rate constant for the open channel with Aβ bound. For case (3), blocking was modeled as O + Aβ ⇒ B, yielding

$$\frac{I_A}{I_{max}} = \left(\frac{k_o}{k_d + k_b[A\beta] - k_o}\right)$$

$$\times (\exp(-k_o t) - \exp(-(k_d + k_b[A\beta])t)) \qquad (5)$$

where k_b is the rate constant for blocking of open channels and Aβ is assumed to be in excess.

k_o and k_d were obtained by nonlinear regression fit of Eq. 2 to a representative recording before β(1–39) application and used for all cases. For cases (1) and (2), K_I was obtained

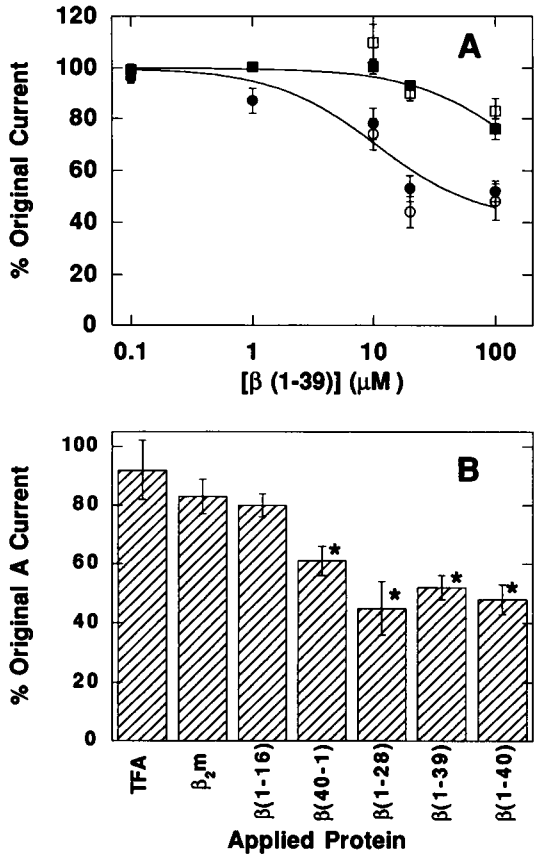

FIGURE 6 Concentration and sequence dependence of current inhibition by Aβ. Current ratios were calculated as described in Fig. 3. Data include both responder and nonresponder cells. (*A*) β(1–39) was prepared either by dissolving lyophilized peptide in 0.1% TFA, diluting into Ringer's solution, and incubating for 24 h (*filled symbols*), or by dissolving lyophilized peptide in DMSO, diluting into Ringer's solution, and using within 2 h (*open symbols*). Whole cell recordings of I_K (■ or □) and I_A (● or ○) were made from five to eight cells at each concentration, except at 100 μM, where 32 cells were tested. The concentration reported is the concentration in the application pipette. Curves were fit by nonlinear regression to Eq. 1. (*B*) Whole cell recordings of potassium currents were made from six to nine cells before and after application of a 100 μM solution of each of the test proteins or peptides, except for β_2-microglobulin (β_2m), which was at 50 μM. The exact concentration of β(40–1) is ambiguous because of solubility limitations of the peptide in Ringer's solution. *, indicates that the change in current is significantly different from the change in current with solvent application ($p < 0.01$).

from the fit of concentration data to Eq. 1. k_d^* (case 2) or k_b (case 3) was obtained by nonlinear regression fit of the data at 10 μM β(1–39) to Eq. 4 or 5, respectively. Response curves were calculated for cases (1), (2), and (3) as a function of Aβ concentration and compared to experimental data (Fig. 7). In the experimental data, the peak height decreases with increasing Aβ concentration, but the peak position is unaffected by Aβ. The shape of the experimental curves, the peak positions, and the dependence of peak height on Aβ concentration are most closely captured by the case 1) model.

DISCUSSION

Pulse application of Aβ caused a voltage-dependent decrease in membrane conductance of hippocampal neurons, indicating that the peptide affected functioning of a voltage-gated ion channel. Aβ did not form a pore in the membrane. Our results are contrary to reports of Aβ-mediated pore formation in lipid bilayers (Arispe et al., 1993), or Aβ-mediated increases in membrane conductance and loss of voltage-gating characteristics in bullfrog sympathetic ganglia (Simmons and Schneider, 1993). The reasons for these differences are unknown, but we note that hippocampal neurons comprise a different model system than artificial lipid bilayer membranes or sympathetic ganglia, and that the latter investigators used the short peptide β(25–35) rather than the full-length Aβ peptide. In addition, using our experimental protocol, cells were exposed to Aβ for a shorter period of time and current measurements were taken within several milliseconds after Aβ application.

Specific ion current experiments isolated this effect to blockage of a voltage-dependent K^+ current. The fractions of responding cells in experiments using the voltage-ramp and voltage-step paradigms were similar, as were the fractional decreases in membrane conductance and total K^+ current upon Aβ application, further indicating that the decrease in the membrane conductance is attributable primarily to a decrease in K^+ outward flux. The decrease in the fast-inactivating current was greater, and persisted to lower concentrations, than the decrease in the delayed rectifying current. The effect is unlikely to be due to Ca^{2+}-activated K^+ channels, because Cd^{2+} effectively blocked Ca^{2+} entry, EGTA was added in the recording pipette as a divalent ion chelating agent, and almost all Ca^{2+}-activated K^+ channels are noninactivating. Pharmacological assays suggested that the channel(s) affected by Aβ are DTX sensitive. Taken together, these data indicate that the primary acute effect of Aβ on ion channels in rat hippocampal neurons is blockage of voltage-gated fast-inactivating DTX-sensitive K^+ channels.

Blocking efficacy was unaffected by changes in the depolarizing potential, implying that Aβ does not sense the electric field. Therefore, if Aβ blocks via binding to channel proteins, it likely binds to an external site rather than inserting into the pore. DTX, which binds to a negatively charged region near the external mouth of the K^+ channel pore, also blocks in a voltage-independent manner (Hurst et al., 1991).

The decrease in I_A was concentration dependent, with a fitted $K_I \approx 10$ μM. The actual concentration detected by the neuron is estimated to be approximately tenfold lower than the concentration in the micropipette. Thus, the calculated K_I represents an upper limit of the true constant. For comparison, in vitro neurotoxic effects are generally detectable at Aβ concentrations of 1–20 μM (Cotman et al., 1992; Loo et al., 1993; Busciglio et al., 1993; Mattson et al., 1993).

The independence of the effect on aggregation state was unexpected because in vitro measurements of peptide tox-

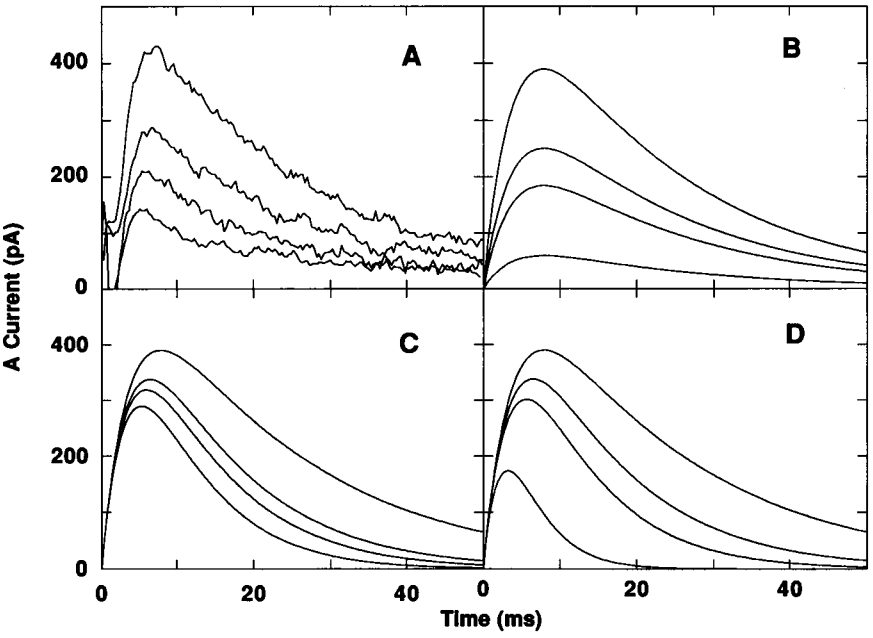

FIGURE 7 Model predictions of effect of Aβ on fast inactivating potassium current. The uppermost curve represents the current before Aβ application. Curves of decreasing amplitudes represent currents upon application of 10 μM, 20 μM, and 100 μM β(1–39), respectively. (*A*) Representative recordings from responding cells. (*B*) Calculated response curve for case (1): Aβ binds reversibly to a closed channel and blocks it. (*C*) Calculated response curve for case (2): Aβ binds reversibly to an open channel and increases the rate of deactivation. (*D*) Calculated response curve for case (3): Aβ binds irreversibly to an open channel and blocks it.

icity correlate with increased peptide aggregation (Pike et al., 1993). Dependence of toxicity on aggregation state in long-term cultures could be influenced by such factors as persistence of aggregated peptide because of increased resistance to proteolysis (Tennent et al., 1995), or enhanced association of aggregated peptide with cellular membrane components (Good and Murphy, 1995). Thus, the acute response that we observe may be necessary but not sufficient for neurotoxicity. Cellular dysfunction and death may require additional factors, such as formation of high concentrations of stable fibrillar aggregates.

β(1–28) and β(40–1) were similar in effectiveness to β(1–39) and β(1–40) in blocking K^+ currents. β(1–28), the N-terminal fragment of Aβ that is extracellular in the precursor protein, is considerably less toxic to cultured neurons than β(1–40) (Yankner et al., 1990). β(1–28) aggregates into amyloid fibrils (Kirschner et al., 1987), albeit at higher concentrations than the longer Aβ forms (Burdick et al., 1992; Shen et al., 1993). The reverse sequence peptide β(40–1) was as toxic as β(1–40) in one study (Giordano et al., 1994), although two other studies reported that β(40–1) was not neurotoxic (Busciglio et al., 1993; May et al., 1993). β(40–1) retains the amphiphilic and β-sheet character of β(1–40) but forms amorphous rather than fibrillar aggregates (Fraser et al., 1992).

Antiparallel β-sheet structure is a common denominator for many K^+ channel toxins, including DTX (Skarzynski, 1992; Garcia et al., 1994; Johnson and Sugg, 1992). Electrostatic interactions between basic residues (Lys and Arg) in the toxins with acidic residues in the channel mouth appear to mediate binding of many K^+ channel blockers (Hurst et al., 1991; Park and Miller, 1992; Smith et al., 1993). All Aβ peptides that blocked the fast inactivating K^+ current have the same basic residues (Arg^5, Lys^{16}, Lys^{28}), and all can form β-sheet containing aggregates. Gln^{11}β(1–16), which had no effect on K^+ currents, is predominantly random coil and lacks the important 14–28 sequence, which confers amyloid fibril-forming capability (Gorevic et al., 1987). β_2-microglobulin, which is predominantly β-sheet but has a different size and charge distribution, also had no effect on K^+ currents. The results observed here may suggest that β-sheet structure and basic residues are important in blocking the transient K^+ current, but that neurotoxicity after chronic exposure to Aβ may require other factors in addition, such as fibrillar structure.

Our interpretation of these results is that Aβ acts selectively, rapidly, and reversibly to block fast-inactivating K^+ channels. Aβ blocks the delayed rectifying K^+ channel but with less potency. Aβ does not alter the gating properties of the channel (Fig. 4) or the kinetics of channel opening (Fig. 7). Rather, our modeling suggests that Aβ reversibly binds to closed channels and prevents them from opening.

These results are the first indication of an acute response of neurons to Aβ by blockage of K^+ channels. Fast-inactivating potassium channels are important in modulating neuronal excitability. Blockage of K^+ channels in hippocampal neurons causes Ca^{2+} influx and accumulation of intracellular Ca^{2+} during synaptic stimulation (Jaffe et al., 1994). Blocking the fast-inactivating K^+ channel with DTX facilitates Ca^{2+} entry, increases cell excitability, causes convulsions, and increases neurotransmitter release (Halliwell et al., 1986; Bagetta et al., 1994). Very recently it has been shown that Aβ increases cell excitability and causes a rise in intracellular Ca^{2+} in hippocampal neurons (Brorson et al., 1995) and that anticonvulsants reduce Aβ neurotoxicity (Mark et al., 1995). Elevated intracellular Ca^{2+} can alter cell

function and lead to cell damage by multiple pathways, including induction of apoptosis, abnormal membrane permeability, damage to cytoskeleton, protein phosphorylation, and production of free radicals (Siesjo, 1994; Franklin and Johnson, 1994). Thus, we hypothesize that Aβ-mediated neurotoxicity is initiated by blockage of fast-inactivating K^+ channels, leading to increased Ca^{2+} influx, intracellular Ca^{2+} accumulation, and neurotransmitter release, thus beginning a cascade of subsequent cellular responses that eventually result in neuronal dysfunction and death.

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