Development/Plasticity/Repair

# Neurogenic Effect of $\beta$-Amyloid Peptide in the Development of Neural Stem Cells

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The adult mammalian brain contains neural stem cells (NSCs) with self-renewal and multilineage potential in the hippocampus and subventricular zone. However, neurogenesis from these areas does not compensate for neuronal loss in age-related neurodegenerative disorders such as Alzheimer's disease (AD). To test whether an impairment of neurogenesis could contribute to the pathogenesis of AD, we examined the effects of amyloid-$\beta$ peptide (A$\beta$) on the survival and neuronal differentiation of cultured NSCs from striatum and hippocampus. We show that A$\beta$ peptide does not impair the neurogenic rate in NSC progeny, but that it increases the total number of neurons *in vitro* in a dose-dependent manner. The neurogenic effect of A$\beta$ peptide is not dependent on soluble factors released from the NSC progeny. Neurogenesis is induced by A$\beta$42 and not A$\beta$40 or A$\beta$25–35, and the activity appears to be a property of A$\beta$ oligomers and not fibrils.

These results suggest that A$\beta$ may have positive as well as deleterious actions, and that a knowledge of the mechanisms involved in the former could be valuable in exploiting the regenerative and plastic potential of the brain in preventing and treating Alzheimer's disease.

*Key words:* Alzheimer; differentiation; hippocampus; stem cells; neurogenesis; amyloid $\beta$ peptide

## Introduction

Over the past decade, it has become clear that new neurons are generated in a number of areas in the postnatal mammalian brain. In the dentate gyrus of the hippocampus, neurogenesis is still detectable in old age, although the rate of neurogenesis decreases during normal aging (Kuhn et al., 1996). This decrease in neurogenesis could be involved in the age-related decline in hippocampus-dependent learning (Kempermann et al., 1998). It has been suggested that neurogenesis is required for some types of memory and learning (Gould et al., 1999; Shors et al., 2001). It also has been shown that several pathological conditions (ischemia, epilepsy, and trauma) seem to upregulate neural stem cell (NSC) activity in the subventricular zone (SVZ) and dentate gyrus (for review, see Kuhn et al., 2001). These findings suggest that neural stem cell populations might be affected in Alzheimer's disease (AD).

To approach this question, we studied the effect of the $\beta$-amyloid peptide (A$\beta$) on the proliferation, survival, and differentiation of NSCs in culture. The increased production of A$\beta$, or its decreased clearance, seems to play a major role in the development of AD (Yankner, 2000). A$\beta$ is a self-aggregating protein that promotes synaptic dysfunction and death of mature neurons in AD (for review, see Mattson, 2000). Chronic infusion

of A$\beta$ into the lateral ventricles impairs spatial learning in mice (Nitta et al., 1997), and amyloid precursor protein (APP) mutant mice with amyloid deposition show impaired learning and memory loss (Hsiao et al., 1996). NSCs have the ability to grow in the presence of growth factors like epidermal growth factor (EGF) or basic FGF (bFGF) and the potential to differentiate into neurons, astrocytes, and oligodendrocytes. These cells have been isolated *in vitro* from several regions of embryonic and adult CNS, including the SVZ (Reynolds and Weiss, 1992; Gritti et al., 1999), cortex (Davis and Temple, 1994), spinal cord (Ray and Gage, 1994; Weiss et al., 1996), or midbrain (Bazan et al., 1998). However, there are only two regions where *in vivo* neurogenesis in the adult brain has been widely accepted: the SVZ, where cells migrate through the rostral migratory pathway and become neurons in the olfactory bulb (Altman and Das, 1965; Luskin, 1993; Lois and Alvarez-Buylla, 1994; Lois et al., 1996), and the subgranular layer of the dentate gyrus in the hippocampus, where cells migrate a short distance into the granular cell layer and differentiate into hippocampal granule cells (Altman and Das, 1965; Bayer et al., 1982; Kaplan and Bell, 1984; Stanfield and Trice, 1988; Kuhn et al., 1996).

In the present study, we find that treatment of hippocampal NSC progeny with A$\beta$ peptide induces an increase in the number of newborn neurons with no changes in the rate of cell death or proliferation, suggesting that A$\beta$ peptide could act on neuronal progenitors and promote their differentiation into neurons.

## Materials and Methods

*Neural stem cell cultures.* Striata from embryonic day (E) 15 Sprague Dawley rat embryos or hippocampi from postnatal day (P) 0 Bl6 mice were dissected and mechanically dissociated. Cell suspensions were grown in a defined medium (DF12) composed of DMEM/F12 (1:1), 2

mM L-glutamine, 1 mM sodium pyruvate, antibiotic-antimycotic (Invitrogen, Grand Island, NY), 0.6% glucose, 25 μg/ml insulin, 20 nM progesterone, 60 μM putrescine, 30 nM sodium selenite (all from Sigma, St. Louis, MO), 100 μg/ml human transferrin (Roche, Indianapolis, IN), 20 ng/ml human recombinant EGF (Roche or Invitrogen, Chicago, IL) and bFGF (Upstate Biotechnology, Lake Placid, NY). The cells grew as free-floating aggregates (neurospheres) and were passaged by mechanical dissociation every 3–4 d. After a minimum of four passages, cells were plated at a density of 18,000 cells/cm$^2$ on 15 μg/ml poly-L-lysine (Sigma) into coated 12 mm glass coverslips (VWR Scientific, Rochester, NY) or eight-well glass slide chambers (Nalge Nunc International, Naperville, IL). Cultures were maintained in DF12 and EGF or EGF plus bFGF for 3 d and then switched to DF12 without growth factors for longer culture periods. Immunocytochemical studies were performed at different time points between 3 and 10 d postplating (dpp). To analyze the effects of Aβ treatment, cells were treated with 1, 5, or 10 μM of Aβ (American Peptide, Sunnyvale, CA). Parallel wells were maintained in DF12 without addition of peptide (untreated group). Immunocytochemical analyses were performed 1, 4, or 7 d after treatment.

*Treatments with inhibitors.* Genistein was obtained from Sigma. LY294002 was obtained from Biomol (Plymouth Meeting, PA), U0126 was obtained from Upstate Biotechnology, and the mouse monoclonal anti-human amyloid-β protein (6E10) was obtained from Signet (Dedham, MA). Lyophilized, HPLC-purified Aβ1–42 or 1–40 peptide was purchased from American Peptide, and Aβ 25–35 was purchased from Bachem (Torrance, CA). Peptides were reconstituted in sterile water at a concentration of 400 μM and incubated at 37°C for 3 d or 24 hr to form aggregated Aβ or maintained at 4°C in soluble or nonaggregated form. Oligomeric and fibrillar Aβ were prepared using the method of Dahlgren et al. (2002).

*NSC conditioned medium.* Cells were treated with Aβ peptide beginning 3 dpp for 4 d. At 7 dpp, medium was removed and cells were fixed for immunostaining. This pretreated NSC conditioned medium (CM) was added to nontreated cells at 7 dpp for a period of 24 hr in presence or absence of 6E10 antibody to test whether the neurogenic effect was mediated through a soluble factor released to the medium for the cells in culture.

*Indirect immunocytochemistry.* Cells were fixed with 4% paraformaldehyde for 20 min, permeabilized with an ethanol acetic acid solution (19:1) at −20°C for 20 min, blocked with 10% fetal bovine serum, and incubated with primary antibodies overnight at 4°C. Sister cultures served as negative controls and were similarly processed, except for incubation without the primary antibody in each case. Immunofluorescence was used for detection of all antigens.

*Primary antibodies.* Monoclonal anti-nestin (clone Rat 401; 1:200) was obtained from Developmental Studies Hybridoma Bank (University of Iowa, Iowa City, IA). Polyclonal anti-glial fibrillary acid protein (1:500) was purchased from Dakopatts (Glostrup, Denmark). Monoclonal anti-β-tubulin isotype III (1:2000) and polyclonal anti-β-tubulin isotype III (1:2000) were both from Covance (Richmond, CA). Polyclonal anti-O1 (1:5) was obtained from a hybridoma purchased from American Type Culture Collection (Manassas, VA). Monoclonal anti-bromodeoxyuridine (BrdU; 1:50) was obtained from Dako (High Wycombe, UK), and monoclonal anti-neuronal nuclei (NeuN) was obtained from Chemicon (Temecula, CA).

*Secondary antibodies.* For single labeling of neural antigens, goat anti mouse IgG (H+L) or goat anti-rabbit IgG (H+L) labeled with either AlexaFluor 568 or 488 were purchased from Molecular Probes (Eugene, OR).
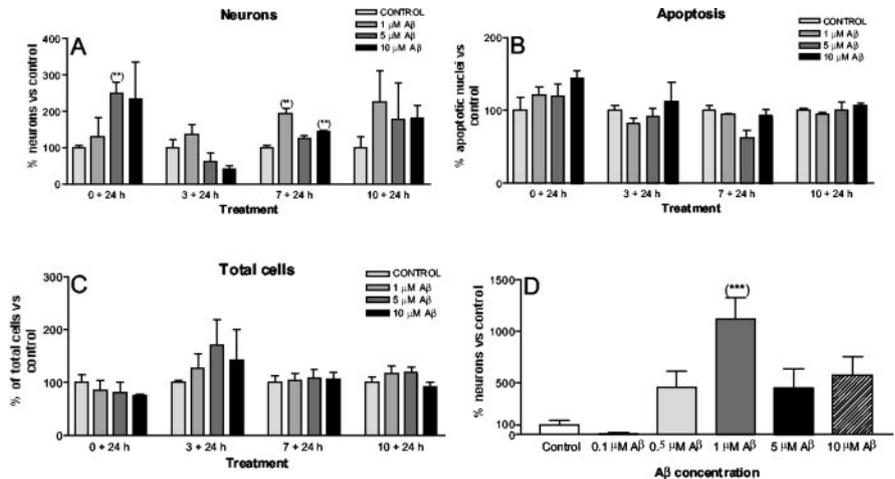


**Figure 1.** Neurogenic effect of Aβ peptide. Neural stem cells from hippocampus (P0) were treated with different concentrations of aggregated Aβ1–42 peptide for a period of 24 hr at 0, 3, 7, and 10 dpp. Neurons (*A, D*), apoptotic nuclei (*B*), and total number of cells (*D*) were counted. No changes in the total number of cells (*C*) or apoptotic nuclei (*B*) were founded at any of the times or concentrations studied, but statistically significant increases in the percentage of neurons versus control were found (*A*). Dose–response experiments with different concentrations of Aβ peptide for 24 hr at 7 dpp (*D*) show that the most effective concentration in inducing neurogenesis in NSC progeny is 1 μM. Data represent mean ± SEM of normalized data versus control from one representative experiment of a total of four experiments done in quadruplicate. Statistical significance of ANOVA followed the Bonferroni post-test; **p < 0.01; ***p < 0.001.

*Proliferation studies.* To enable the detection of proliferating cells, 100 μM BrdU, an analog of thymidine, was added 24 hr before cell culture fixation. After permeabilization with ethanol acetic solution (19:1), cells were treated with 2N HCl for 30 min at 4°C to denature DNA. Primary monoclonal antibody against BrdU (1:20; Dakopatts) was added for 1 hr at room temperature and recognized by fluorescent secondary goat anti-mouse IgG (H+L) labeled with AlexaFluor 488. This method allows identification of cells that have duplicated their DNA in the last 24 hr.

*Data analysis.* Results are expressed as mean ± SEM of direct counts of positive cells for each antibody from several independent experiments done in triplicates or quadruplicates. Where indicated, the data were normalized in relation to their own control group. In each culture, 25 predetermined visual fields were counted under fluorescence microscopy. The number of positive cells was corrected for total cells in the same area and counted with nuclear staining with Hoechst. Statistical analyses were performed using ANOVA and the Bonferroni post-test or Student's *t* test. The difference was considered significant when *p* ≤ 0.05. The statistical analysis was conducted using the Prism3 program from Graph-Pad Software (San Diego, CA).

## Results

### β-Amyloid peptide increases the number of neurons in the progeny of hippocampal stem cells

To test the effect of β-amyloid peptide on the fates of neural stem cells, NSC progeny from striatum and hippocampus were plated on poly-L-lysine, and varying concentrations of aggregated Aβ1–42 peptide were added at 2 hr and 3, 7, and 10 dpp for a period of 24 hr. Under all of these conditions, Aβ peptide increased the number of neurons, as identified by immunostaining with β-tubulin III antibody (Fig. 1*A*) with no changes in the number of apoptotic nuclei (Fig. 1*B*) or total cells in the culture (Fig. 1*C*). The increases varied from experiment to experiment, averaging a threefold increase in neurons compared with the controls. Results were similar in both hippocampal and striatal NSC progeny. The cells treated at 0 dpp required higher concentrations of aggregated Aβ1–42 for an optimal response, whereas "older" cells showed a peak response at 1 μM (Fig. 1*A*). Based on these results, we focused on 7 dpp hippocampal NSCs treated for 24 hr with 1 μM Aβ. At 7 dpp, NSC cultures contained a mix of
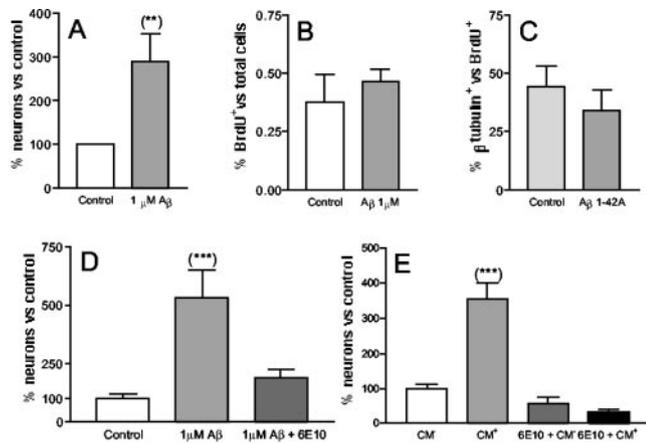
**Figure 2.** β-Amyloid peptide (1 μM) induces neurogenesis with no changes in proliferation, and its neurogenic effect is abolished by the 6E10 Aβ antibody. In A, we show mean ± SEM of a total of 15 experiments in quadruplicate. In B, we can see that the increase in the number of neurons does not correlate with an increase in proliferative cells as measured by incorporation of BrdU. C, BrdU-positive cells differentiated into neurons at similar rates in treated and untreated cultures. D, The effect of treatment with 1 μM aggregated Aβ1–42 peptide was completely abolished by the 6E10 anti-Aβ antibody (1:400 dilution). In E, we show the effect of exposure to conditioned medium for 24 hr at 7 dpp. To obtain the CM, NSC was treated 1 μM Aβ or maintained untreated (control) at 3 dpp. Four days later, medium was removed and added for 24 hr to untreated cells at 7 dpp with or without 6E10 antibody. The antibody abolished completely the Aβ-induced neurogenesis and the effect of CM. Results show mean ± SEM of one representative experiment of between two and four done in quadruplicate. Statistical significance of ANOVA followed by the Bonferroni post-test; **p < 0.01; ***p < 0.001.



**Figure 3.** The neurogenic effect of Aβ peptide is dependent of its state of aggregation. To test the effect of Aβ aggregation in neurogenesis, we treated cells (A) with 1 μM Aβ1–40 and 1–42 aggregated (1–40A or 1–42A) or nonaggregated (1–40NA or 1–42NA) and Aβ25–35 aggregated (25–35A) for 24 hr at 7 dpp. Only the Aβ1–42 aggregated peptide had a neurogenic effect on the NSC progeny. In B, Aβ produced by the method of Dahlgren et al. (2002) was used. Only the oligomeric peptide (Aβo) and 1–42 aggregated had a neurogenic effect. C, Fibrillar Aβ (Aβf) caused a statistically insignificant decrease in the total number of cells. In D, we show the increase of neurons with the Aβo counting NeuN-positive cells, another marker of neurons. The graphs represent mean ± SEM of four (A) or three (B–D) different experiments done in quadruplicate. ANOVA followed by Bonferroni post-test; *p < 0.05; **p < 0.01.

progenitors and mature cells composed of 25–30% neurons, 40–50% astrocytes, 1–2% oligodendrocytes, and a variety of progenitors, mimicking, in part, the mature hippocampus. A dose–response study showed that 1 μM Aβ was optimal in increasing neurogenesis (Fig. 1D). In 15 experiments, each done in quadruplicate, the increase in number of neurons averaged threefold (290 ± 65%) (Fig. 2A).

### Aβ appears to act directly on neurogenic precursors

The increase in the number of neurons was not attributable to an increase in the proliferating cells in the culture, because 24 hr treatment with BrdU showed that the low number of proliferating cells in control cultures (0.3 ± 0.06%, of total cells n = 3) did not change with 1 μM Aβ treatment (Fig. 2B) nor did the number of βIII-tubulin-positive neurons that incorporated BrdU (Fig. 2C). This suggests that whether or not the cell was in the cell cycle within the preceding 24 hr did not affect differentiation into neurons. The neurogenic effect of Aβ peptide was totally and specifically abolished in the presence of the anti-human β-amyloid antibody (6E10; 1:400) (Fig. 2D) and not in the presence of other similar IgG1 antibodies such as anti-NeuN (data not shown).

Many of the studies of Aβ peptide actions have used pure cultures of neurons or neuronal cell lines. In our experimental model, there is a complex mixture of cell types and stages of differentiation. Thus, it is possible that the observed increase in neurogenesis could result from Aβ action on cells other than the neuronal progenitors that, in turn, could produce an agent that induces neurogenesis. It is known that glial cells produce a variety of soluble factors (Chen et al., 1991; Engele et al., 1991; Kilpatrick et al., 1993; Dugan et al., 1995). To determine whether this occurs in our system, we tested the ability of CM from NSC progeny to induce neurogenesis. We obtained NSC conditioned medium
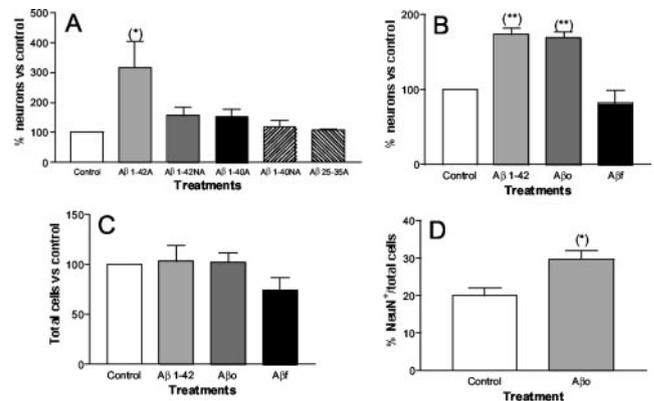
from cells treated with Aβ for 4 d starting at 3 dpp. In this condition, 1 μM Aβ peptide induced an increase of 360 ± 117% versus control (n = 4 independent experiments in quadruplicates). We then used the CM to replace the medium on 7 dpp cultures that had not been exposed to Aβ. An increase in neurogenesis was seen in the cells exposed to the conditioned media from Aβ-treated cultures but not in those exposed to CM from control cultures (Fig. 2E). However, addition of anti-Aβ antibody blocked this increase, suggesting that the CM-induced neurogenesis was caused by residual Aβ in the CM and that the neurogenic effect of Aβ peptide was directly on the progenitor cells (Fig. 2E). In two experiments, CM were centrifuged at high speed (100,000 × g for 1 hr) before use. The centrifuged CM maintained neurogenic activity, suggesting that smaller rather than larger Aβ aggregates are the active component.

### Aβ form and aggregation state are critical for the neurogenic effect of Aβ peptide

The persistence of Aβ-induced neurogenic activity in CM from which larger Aβ aggregates had been removed by centrifugation suggested that the aggregation state of Aβ might play a significant role in its effects. Cleavage of the APP produces two major forms of the Aβ peptide, Aβ1–40 and Aβ1–42. The experiments in the previous sections were conducted using Aβ1–42, which had been aggregated for 3 d. In a series of experiments described in this section, we examined the ability of Aβ1–40 and Aβ1–42 peptides either aggregated or freshly prepared (unaggregated) to induce neurogenesis. Only aggregated Aβ1–42 (1 μM) was able to induce neurogenesis. Neither freshly prepared Aβ1–42 nor Aβ1–40 (aggregated or not) increased the percentage of neurons (Fig. 3A). To test the effect of Aβ25–35 in our system and compare it with the results of Haughey et al. (2002a), we treated cultures at 7 dpp for 24 hr with aggregated Aβ25–35 with no increase in the number of β-tubulin III-positive cells (Fig. 3A) and no change in the total number of cells or apoptotic nuclei (data not shown). We then prepared Aβ1–42 using a protocol that allows enrichment for either oligomers or fibrils (Dahlgren et al., 2002). The treatment of

NSCs with 1 μM oligomeric Aβ$_{1-42}$ caused a similar increase in neurogenesis than seen with Aβ$_{1-42}$ that had been aggregated for 3 d, with no increase in apoptotic nuclei or change in total cell number (Fig. 3 B,C).

To exclude the possibility that β-tubulin III was detecting non-neuronal cells, we also stained parallel experiments with the neuronal marker NeuN. As with β-tubulin III, we saw a significant increase in the total number of neurons in response to Aβ. A typical experiment is shown in Figure 3D.

### The neurogenic effect of Aβ is mediated through tyrosine-kinase phosphorylation and the MAPK (mitogen-activated protein kinase) pathway

To evaluate the possible signal transduction pathways involved in Aβ-induced neurogenesis, we used several inhibitors of pathways known to be involved in neurogenesis in other systems. These included Genistein, an inhibitor of tyrosine kinases (TyrK) and a competitive inhibitor of ATP in other protein kinase reactions. At 7 dpp, NSC progeny were treated for 24 hr with 1, 5, and 10 μM Genistein. At these concentrations, the inhibitor did not increase the number of apoptotic nuclei nor did it change the number of neurons observed versus controls. When 1 μM Aβ was added in the presence of Genistein, the increase of neurons mediated by Aβ was abolished (Fig. 4A). Similarly, treatment with U0126, a selective inhibitor of MEK (MAP kinase kinase), did not induce any change in total neurons in the culture (Fig. 4B) but was able to inhibit the Aβ-induced increase in neurons. In contrast, LY294002, a selective inhibitor of PI3K (phosphatidylinositol 3-kinase), was unable to inhibit Aβ-induced neurogenesis (Fig. 4C).

### Discussion

Our working hypothesis in initiating this study was that Aβ would inhibit neurogenesis and prevent reparative processes. The results presented here suggest that, at least in cell culture, the opposite occurs. Our results are at variance with two previous studies (Haughey et al., 2002a,b) that show that Aβ treatment impairs the proliferation and differentiation of NSCs to neurons. Our experiments differ from theirs in the form of Aβ used, in the type of NSC culture used, and in the concentrations of Aβ. Any or all of these could explain the differences in results. For example, in one report, the Aβ peptide used was aggregated Aβ$_{25-35}$ (Haughey et al., 2002a). The Aβ$_{25-35}$ peptide does not occur naturally but has been shown to mimic the adverse effects of Aβ$_{1-42}$ in several studies. In fact, our results show that aggregated Aβ$_{25-35}$ does not affect the neurogenic rate in our system when added for 24 hr at 7 dpp. In the study by Haughey et al. (2002b), 5 μM Aβ decreased the area of radial outgrowth of cells from neurospheres because of the reduced adhesion to the substrate. In our system, neither cells plated onto Aβ peptide nor treatment with 1, 5, or 10 μM aggregated Aβ$_{1-42}$ affected the radial outgrowth (data not shown). However, when our hippocampal NSCs were plated onto the peptide, no change in the number of neurons was observed, but apoptosis increased in a concentration-dependent manner, showing a toxic effect on NSCs in the culture similar to that obtained by Haughey et al. (2002) (data not shown). The previous studies also show a three-fold increase in the number of apoptotic cells, many of which are
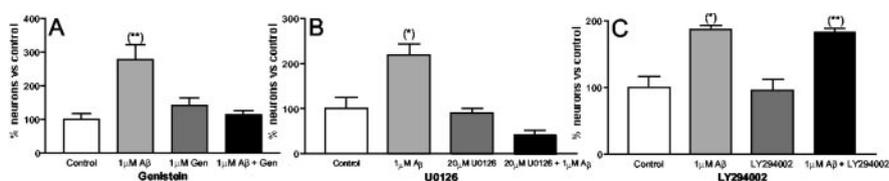


**Figure 4.** Possible pathways involved in the neurogenic effect of Aβ. NSC progeny were treated at 7 dpp for 24 hr with Aβ aggregated peptide in the presence or absence of 1 μM Genistein (A), 20 μM U0126 (B), or 20 μM LY294002 (C). Our results show a blockade of neurogenesis by Genistein, an inhibitor of TyrK, and with U0126, an inhibitor of MEKK, and no blockade by the PI3K inhibitor LY294002. The graphs show one representative experiment of between two and four different experiments done in quadruplicate. ANOVA followed by Bonferroni test; *$p < 0.05$; **$p < 0.01$.

E-NCAM (also known as polysialylated form of neurol cell adhesion molecule)-positive neurons (Haughey et al., 2002b), whereas there is no significant apoptosis in our cultures. Haughey et al. (2002b) performed their studies on neurospheres from 8- to 10-week-old cortical tissues from human fetuses, and we obtained ours from P1 mouse hippocampus. Active neurogenesis is limited to discrete regions of CNS of adult mammals. In fact, NSCs from hippocampus or spinal cord give rise to neurons after grafting to the dentate gyrus but not after graft to other CNS regions (Shihabuddin et al., 2000). The regional differences could be a function of hippocampal astrocytes that may provide a unique niche for adult neurogenesis (Haydon, 2001; Seri et al., 2001; Cheng et al., 2002; Kempermann et al., 2002; Song et al., 2002a,b; Monje and Palmer, 2003). However, our data with NSC conditioned medium demonstrate that soluble factors released into the medium by NSC progeny are not responsible for neurogenic effect of Aβ peptide.

Apoptosis is a common feature in regions of neurogenesis (Biebl et al., 2000). Cell death is several-fold higher in neurogenic areas than in other areas of the brain, and cells die during all phases of neurogenesis: stem cell proliferation, progenitor migration, and neuronal differentiation. Despite these neuronal losses, neuronal cell numbers in the dentate gyrus increase continuously, suggesting that production outweighs loss over the life of the animal (Kaplan and Hinds, 1977; Bayer et al., 1982). It has been proposed that cell death or damage releases signals that trigger neurogenesis and cell replacement in vivo (Snyder et al., 1997; Kokaia and Lindvall, 2003). These lesions could create a permissive microenvironment for neuronal differentiation through re-expression of developmental signals ordinarily available only during embryonic neurogenesis. In our system, we did not observe any significant loss of the number of total cells, and the number of apoptotic nuclei did not increase after Aβ treatment. However, we cannot completely discard the possibility that a small increase in the apoptotic or necrotic death of mature neurons, perhaps concealed by the variation in our experiments, could start a cascade of events that would increase neurogenesis. The fact that when undifferentiated NSCs were grafted into 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine damaged brains, the majority of new dopaminergic neurons were "rescued" host cells (Ourednik et al., 2002) opens the possibility that undifferentiated progenitors might spontaneously express neuroprotective signals, and that NSCs might have an inherent capacity to alter the host environment, reactivating or preserving dysfunctional endogenous neurons. Previous studies (Ciaroni et al., 2002) describe a correlation between proliferation and cell survival. If proliferation is inhibited, the rate of survival increases. This suggests a possible link between neurogenesis and regulation of cell number. We see very low numbers of proliferative cells in our cultures (~0.3% of total cells) (Fig. 2 B,C) with no significant

differences between control and Aβ-treated cultures. These data and the inability of conditioned medium to induce an increase in neurogenesis suggest that the effect of Aβ is directly on postmitotic neuronal progenitor cells driving their differentiation toward neurons.

The dependence of the neurogenic effect of Aβ on the state of aggregation of Aβ suggests that the formation of new neurons is more likely to be induced by the "soluble" forms of Aβ than by the Aβ that has been organized into senile plaques. One possible interpretation of these data is that in earlier stages of Alzheimer's disease, when the excess of Aβ is enough to form oligomeric but not fibrillar aggregates, the oligomers (Dahlgren et al., 2002; Kim et al., 2003) could activate a compensatory mechanism to replace lost or damaged neurons by increasing the differentiation of neuronal progenitors into new neurons. As time passes and senile plaques are formed, the balance could shift to fibrillar Aβ that could be more neurotoxic.

The inhibition of neurogenesis by Genistein suggests that tyrosine kinases are involved in this process. These kinases include the classical neurotrophin receptors that are essential in nervous system development (for review, see Chao et al., 2003). The experiments with conditioned media point to a direct effect of the Aβ peptide on the target cell and not to the release of a growth factor that would then act on these receptors. However, it is possible that the interaction of $A\beta_o$ with another surface protein (Gong et al., 2003) could activate TyrK and drive neurogenesis. We examined two signaling pathways downstream of TyrK. Inhibition of the PI3K system did not affect neurogenesis. PI3K plays an important role in the protection from apoptosis (Carpenter and Cantley, 1996), but its inhibition did not increase apoptosis. In contrast, inhibition of the ERK (extracellular signal-regulated kinase) pathway blocked neurogenesis. The ERK pathway has been implicated in the regulation of cell growth and proliferation, in differentiation, and in apoptosis (Chang and Karin, 2001). Additional examination of its role in neurogenesis in this system is needed to understand the events downstream of TyrK activation. A better understanding of Aβ on these pathways has the potential to provide targets for therapeutic interventions to prevent Aβ-induced neuronal loss and encourage neuronal replacement.

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