Aβ₁–₄₂ stimulates adult SVZ neurogenesis through the p75 neurotrophin receptor

Areechun Sotthibundhu a,b, Qiao-Xin Li c, Wipawan Thangnipon b, Elizabeth J. Coulson a,∗

a Queensland Brain Institute, The University of Queensland, Brisbane, QLD 4072, Australia
b Neuro-Behavioural Biology Centre, Institute of Science and Technology for Research and Development, Mahidol University, Salaya, Thailand
c Department of Pathology, The University of Melbourne, Melbourne, VIC 3052, Australia

Received 13 December 2007; received in revised form 6 February 2008; accepted 12 February 2008

Abstract

The generation of amyloid-beta peptide (Aβ) and its accumulation in amyloid plaques are generally recognized as key characteristics of Alzheimer’s disease. A number of reports have indicated that Aβ can regulate the proliferation of neural precursor cells and adult neurogenesis, suggesting that this may underpin the cognitive decline and compromised olfaction also associated with the condition. Here we report that Aβ₁–₄₂ treatment both in vitro and in vivo, as well as endogenous generation of Aβ in C100 and APP/PS1 transgenic models of Alzheimer’s disease, stimulate neurogenesis of young adult subventricular zone precursors. The neurogenic effect of Aβ₁–₄₂ was found to require expression of the p75 neurotrophin receptor (p75NTR) by the precursor cells, and activation of p75NTR by metalloprotease cleavage. However, precursors from 12-month-old APP/PS1 mice failed to respond to Aβ₁–₄₂. Our results suggest that overstimulation of p75NTR-positive progenitors during early life might result in depletion of the stem cell pool and thus a more rapid decline in basal neurogenesis. This, in turn, could lead to impaired neurogenic function in later life.

© 2008 Elsevier Inc. All rights reserved.

Keywords: p75NTR; Alzheimer’s disease; Subventricular zone; Olfaction; Amyloid; Neurosphere; Proteolysis

1. Introduction

Alzheimer’s disease is characterized by cognitive decline and the deposition of amyloid plaques, a core component of which is a peptide known as amyloid beta (Aβ). This peptide is derived from a larger type I membrane receptor, the amyloid protein precursor (APP), following proteolytic processing by β- and γ-secretases (Selkoe, 1994). Two major forms of Aβ exist, with the 40-amino acid peptide (Aβ₁–₄₀) being the predominant form produced during normal metabolism of APP. The longer Aβ₁–₄₂ species is usually a minor product, but is more cytotoxic and prone to aggregation than Aβ₁–₄₀ (Li et al., 1999). Mutations in both APP and a component of the γ-secretase complex, presenilin, can result in increased production of Aβ₁–₄₂ and early-onset Alzheimer’s disease (Golde et al., 2000), implicating the accumulation of Aβ in the brain as a major factor in the pathogenesis of this condition. In line with this, many studies have demonstrated the ability of Aβ to cause synaptic dysfunction and death of mature neurons (Yankner, 2000; Hardy and Selkoe, 2002). However, there are also an increasing number of reports that misregulation of adult neurogenesis might underlie features of Alzheimer’s disease (Kuhn et al., 2007).

Under normal conditions, adult neurogenesis occurs predominantly in two brain areas: the subventricular zone (SVZ) of the lateral ventricle, and the subgranular layer of the dentate gyrus in the hippocampal formation. Given the importance of the hippocampus in learning and memory, most studies have focused on this area of the brain, revealing
that hippocampal neurogenesis is decreased in a variety of mouse models of Alzheimer’s disease displaying a pathological plaque burden (Dong et al., 2004; Donovan et al., 2006; Zhang et al., 2007; Gan et al., 2008). However, hippocampal neurogenesis and/or proliferation is increased in younger Alzheimer’s model animals and human patients (Jin et al., 2004a,b; Donovan et al., 2006; Gan et al., 2008). Similarly, neurogenesis of SVZ-derived cells from developing or young adult animals is promoted by Aβ, or more specifically by Aβ1–42, and not by Aβ1–40, or Aβ23–35 (Jin et al., 2004b; Lopez-Toledano and Shelanski, 2004; Calafiore et al., 2006; Heo et al., 2007).

It is still unclear how the involvement of Aβ in neurogenesis is regulated. We and others have reported that mitotically active neurogenic precursors within the SVZ of adult rodents are marked by expression of the p75 neurotrophin receptor (p75NTR; Calza et al., 1998; Giuliani et al., 2004; Young et al., 2007). We have also recently demonstrated that ligand activation of p75NTR by brain derived neurotrophic factor (BDNF) in vitro, and by unknown ligands in vivo, promotes SVZ neurogenesis, which is required for generation of neuroblasts and maintenance of normal olfactory bulb size (Young et al., 2007). As Aβ has been demonstrated to be a ligand for p75NTR (Yaar et al., 1997, 2002), activating signaling pathways independently shown to regulate cell cycle genes (Fradec, 2000; Coulson, 2006), we hypothesized that it may act on p75NTR-expressing SVZ progenitors to regulate neurogenesis.

2. Materials and methods

2.1. Aβ preparation and analysis

Human Aβ1–42, and Aβ1–40 peptides and a control Aβ1–16 peptide were supplied by Dr. J. Elliot (Yale University, New Haven, CT). Peptides were dissolved in sterile water at a concentration of 200 µM, and stored at 4 °C for 24 h. Peptide solutions were incubated at 37 °C for 1 h prior to experimental use and were found to occur in a predominantly oligomeric form as determined by Western blotting. We have found that a 5 µM concentration of Aβ1–42 prepared in this way is only marginally toxic to embryonic hippocampal neurons, inducing not more than 10–20% cell death over 24 h (data not shown). Fibrillar Aβ1–42 was prepared as described by Dahlgren et al. (2002).

2.2. Mouse strains

Wildtype and p75NTR-deficient C57Bl6 mice (Lee et al., 1992), or C100 and APP/PS1 transgenic mice producing increased levels of Aβ and their wildtype littermates (C57Bl6/DBA and C57Bl6/SJL, respectively), were used in these experiments. C100 transgenic mice overexpress the human APP β-carboxyl-terminal 100 amino acid fragment (C100 (Li et al., 1999). APP/PS1 transgenic mice express the chimeric mouse/human APP gene containing the Swedish mutation near the β-secretase cleavage site and the presenilin gene containing an exon-9 deleted variant (Jankowsky et al., 2001). These mice were obtained from Jackson Laboratory (#004462; Bar Harbor, ME). All animal experiments were approved by the University of Queensland Animal Ethics Committee. When examining the effects of genotype or age, dissections and experimental manipulations of tissues from mice of different genotypes or ages were performed in parallel.

2.3. Neurosphere proliferation assay

Adult neural precursors were derived from the SVZ of the lateral ventricle as previously described (Young et al., 2007). Briefly, SVZ tissue was dissociated in 0.05% trypsin (Gibco/Invitrogen, Melbourne, Australia), plated in 24-well plates at a density of 1800 cells per well and cultured at 37 °C in 5% CO2 for 7 days in 0.5 ml of serum-free neurosphere medium (NeuroCult; Stem Cell Technologies, Vancouver, Canada) containing 10 ng/ml recombinant basic fibroblast growth factor (bFGF; Roche, Mannheim, Germany) and 20 ng/ml epidermal growth factor (EGF; BD Bio Sciences, Sydney, Australia). Aβ (to a final concentration of either 2 or 5 µM) was added to SVZ cultures at day 0, 1, 3, 5 or at the time of differentiation (day 7). In some experiments, SVZ cultures derived from p75NTR+/−, p75NTR−/− or C100 mice were also treated with 20 µM of the p75NTR metalloprotease inhibitor TAPI-2 (Peptides International, Louisville, KY) which was added together with Aβ or 50 ng/ml insulin-like growth factor (IGF1; R&D Systems, Minneapolis, MN) on day 0. The number of neurospheres per well (6–14 wells per condition per animal) was determined at the end of the 7-day proliferation culture period. Neurosphere size was measured using a microscope graticule.

2.4. Neurosphere differentiation assay

After the 7-day culture period as outlined above, neuronal differentiation was induced by transferring a subset of medium sized (80–120 µm in diameter) neurospheres onto 12 mm diameter glass coverslips coated with poly-1-ornithine hydrobromide (Sigma–Aldrich, Sydney, Australia) within 24-well tissue culture plates containing growth factor-free medium and 1% fetal bovine serum (FBS; Sigma–Aldrich). Six or seven neurospheres were transferred onto each coverslip, with at least three coverslips established per condition per experiment. Neurospheres were differentiated for a further 5 days, before assessment by immunocytochemistry.

2.5. Immunocytochemistry

Neurospheres grown on coated glass coverslips were fixed with 4% paraformaldehyde (PFA) for 10 min at 37 °C and rinsed with phosphate-buffered saline (PBS) three times...
before being blocked for 30 min at 37 °C in 10% FBS/PBS.

The blocking solution was then removed and cultures were treated with a permeabilizing solution of 0.1% Triton X-100/2% normal goat serum/2% FBS in PBS, for 30 min at 37 °C. To determine the number of neurons per neurosphere, the differentiated neurospheres were labeled with mouse anti-β-III-tubulin (1:2000; Promega, Sydney, Australia) diluted in PBS. The cultures were then incubated for 30 min at 37 °C and washed, after which the coverslips were mounted onto glass slides using Fluoromount (DakoCytomation). Neurospheres of similar size (i.e., containing approximately the same number of DAPI-positive nuclei; C57Bl6: 594 ± 57, p75NTR−/−: 529 ± 50 nuclei per sphere, mean ± S.E.M., n = 12 spheres per genotype) were counted and compared between experimental conditions for statistical analysis.

2.6. Assessment of p75NTR cleavage in HEK293 cells

The effect of Aβ on p75NTR proteolytic processing was determined using HEK293 cells as previously described (Underwood et al., 2008). Briefly, HEK293 cells were transfected with a p75NTR expression plasmid and 48 h later they were incubated overnight in fresh serum-free medium containing Aβ and the metalloprotease inhibitor 20 μM TAPI-2 (Peptides International) and 5 μM β-clasto-lactacystin (proteasome inhibitor; Calbiochem, Sydney, Australia). Cell lysates were analyzed following SDS-PAGE by western blotting as described previously (Underwood et al., 2008). p75NTR was detected using a 1:2000 dilution of rabbit anti-human p75NTR intracellular domain (Promega).

2.7. In vivo subventricular zone injection of Aβ

Eight-week-old wildtype and p75NTR-deficient C57Bl6 mice were anesthetized with 80 mg/kg ketamine/8 mg/kg xylazine by intraperitoneal injection and mounted in a stereotaxic frame (David Kopf Instruments, Tujunga, CA). The skull was exposed and a burr hole made at stereotaxic coordinates measured from the Bregma (AP, 0.3 mm; ML, 1 mm; V, 2.3 mm), through which 0.5 μl of saline solution containing 11 pmol of either Aβ1–42 or Aβ1–16 (100 μg/ml), was injected into the lateral ventricle region of the SVZ over the course of a minute. The needle was kept in this configuration for 2 min to prevent reflux of the injected material along the injection track. Three or 14 days after surgery, the SVZ tissue was harvested and neurosphere cultures established.

2.8. Aβ ELISA

The amount of Aβ present in the soluble supernatants of whole brain samples of 4-, 7- and 12-month-old APP/PS1 mice was analyzed by ELISA using G210 (for Aβ1–40) and G211 (for Aβ1–42) as capture antibodies (Ida et al., 1996) and WO2 as the detection antibody as previously described (George et al., 2004).

2.9. Statistical analysis

The data were analyzed by two-tailed Student’s t-test or ANOVA, with Bonferroni post-tests. Values of p < 0.05 were considered significant.

3. Results

3.1. Aβ1–42 increases proliferation and differentiation of progenitor cells from the SVZ

To determine the effect of Aβ peptides on neural precursor cell proliferation and differentiation, in vitro neurosphere cultures, established with cells isolated from the SVZ of wildtype adult (2-month-old) mice, were treated with either Aβ1–16, Aβ1–40 or Aβ1–42 peptides. The total number of neurospheres in each culture was counted after 7 days as an assessment of precursor proliferation. Consistent with previous reports, treatment with 5 μM oligomeric Aβ1–42 resulted in a significant increase in the overall number of neurospheres, as did fibrillar Aβ1–42 treatment (Fig. 1A and B). There was also a marked difference in the ratio of large to small neurospheres in the presence of Aβ1–42; the number of large neurospheres increased significantly, whereas there was a concomitant significant decrease in the number of small neurospheres (Fig. 1C; Supplementary Fig. 1). The number of neurospheres generated in Aβ1–16-or Aβ1–40-treated cultures was not significantly different from that in untreated cultures (Fig. 1A).

The effect of Aβ on neuronal differentiation was determined by counting the number of neurons per medium sized (80–120 μm) neurosphere of SVZ cell cultures treated with Aβ1–42 at the time of establishment (day 0), and transferred into differentiating conditions after a 7-day proliferation period. Under these conditions, 5 μM Aβ1–42 treatment was found to significantly increase the number of neurons per neurosphere compared with control conditions (Fig. 1D).

3.2. The neurogenic effect of Aβ requires p75NTR

Given that p75NTR ligands can stimulate neurogenesis in vitro (Young et al., 2007) and Aβ has been reported to bind to p75NTR (Yaar et al., 2002), we hypothesized that the p75NTR-positive precursor cells within the SVZ may be the cells responding neurogenically to Aβ. To test whether p75NTR was required for Aβ to mediate its effects, cells were cultured from the SVZ of p75NTR-deficient mice. Neither the number nor the size of neurospheres generated from p75NTR−/− mouse SVZ tissue was significantly different from that in

Please cite this article in press as: Sotthibundhu, A., et al., Aβ1–42 stimulates adult SVZ neurogenesis through the p75 neurotrophin receptor, Neurobiol Aging (2008), doi:10.1016/j.neurobiolaging.2008.02.004
wildtype cultures under control conditions (Fig. 2A and B). However, in contrast to the results obtained using wildtype cultures, Aβ<sub>1-42</sub> failed to induce neurogenesis of p75<sub>NTR</sub>-deficient neural precursors as measured by the neurosphere proliferation and differentiation assays (Fig. 2A and C). This demonstrates that p75<sub>NTR</sub> is required for Aβ-induced neurogenesis in vitro.

We have previously observed that p75<sub>NTR</sub> is rapidly down-regulated in SVZ cells once they are placed into culture conditions (Young et al., 2007) and, as a result, predicted that if Aβ were acting through p75<sub>NTR</sub>, it would only exert an effect early in the neurosphere culture period. Therefore, we next determined at which stage of neurosphere formation cells were most responsive to Aβ<sub>1-42</sub>. Aβ<sub>1-42</sub> was added to the neurosphere culture medium on day 0, 1, 3, or 5 of the culture period, and the number of neurospheres was assessed on day 7. This revealed that the number of neurospheres was increased most dramatically when Aβ<sub>1-42</sub> was added at day 0, with a significant increase in neurosphere number also occurring when the peptide was added on day 1 (Fig. 2D). The number of neurons per neurosphere was also significantly increased when Aβ<sub>1-42</sub> treatment took place on the first day of the proliferation culture period (Fig. 2E). Surprisingly, if Aβ<sub>1-42</sub> was added to the cultures immediately following transfer to differentiation conditions, the vast majority of neurosphere cells died (data not shown). The number of neurons per surviving neurosphere was not significantly different from that in untreated cultures (Fig. 2E). This suggested that Aβ was acting through p75<sub>NTR</sub>, as opposed to stimulating a particular cell population that could have been absent in p75<sub>NTR</sub><sup>−/−</sup> mice.

3.3. The neurogenic effect of Aβ is mediated through cleaved forms of p75<sub>NTR</sub>

Signaling of cell death and inhibition of neurite outgrowth by p75<sub>NTR</sub> have been shown to require proteolytic processing of the full-length receptor into receptor fragments with independent signaling properties (see Fig. 3A; Domeniconi et al., 2005; Kenchappa et al., 2006; Underwood et al., 2008). Therefore, we next tested whether cleavage of p75<sub>NTR</sub> was required to mediate the neurogenic actions of
A. Sotthibundhu et al. / Neurobiology of Aging xxx (2008) xxx–xxx

Fig. 2. Aβ1–42 increases proliferation and differentiation of p75NTR-positive progenitor cells from the SVZ. (A) Number of neurospheres per well derived from dissociated SVZ cells of p75NTR-deficient mice, and plated in the presence of 2 or 5 μM Aβ1–42. (B) Number of small (<40 μm), medium (40–120 μm) and large (>120 μm) neurospheres generated in control or Aβ1–42-treated p75NTR−/− cultures (mean ± S.E.M.). Treatment with Aβ1–42 had no significant effect on the number or size of neurospheres generated. Mean ± S.E.M., N = 3 experiments. (C) Number of β-III-tubulin-positive neurons per neurosphere generated following differentiation of the cultures shown in (C). In this case, day 7 indicates that 5 μM Aβ1–42 was added at the time of differentiation. Mean ± S.E.M., N = 3 experiments. *p < 0.05, **p < 0.01 and ***p < 0.001.

Aβ. We found that Aβ1–42 treatment of p75NTR-transfected HEK293 cells regulated p75NTR cleavage (Supplementary Fig. 2), and that an inhibitor of the p75NTR metallopeptase (TAPI-2; Kanning et al., 2003; Underwood et al., 2008) prevented the Aβ-stimulated proteolysis (Fig. 3A, Supplementary Fig. 2). Moreover, TAPI-2 treatment of wild-type SVZ cultures completely inhibited the ability of Aβ1–42 to stimulate increased neurosphere formation (Fig. 3B), or neuronal production (Fig. 3C). In contrast, TAPI-2 had no effect on neurogenesis of p75NTR-deficient cells, demonstrating that it had no p75NTR-independent effect on the SVZ progenitors (Fig. 3B and C). To determine whether TAPI-2 could inhibit neurogenesis stimulated by other factors, neurosphere cultures were treated with IGF1. TAPI-2 had no effect on the ability of IGF1 to stimulate increased progenitor proliferation or neuronal generation in wildtype neurosphere cultures (Fig. 3D and E), suggesting that TAPI-2 was specifically inhibiting Aβ-stimulated p75NTR-mediated neurogenesis. Together, these data indicate that Aβ1–42 can regulate the proteolysis of p75NTR, thereby stimulating proliferation and neuronal differentiation of SVZ precursor cells.

3.4. Aβ-induced increase in neurosphere proliferation and differentiation in vivo requires p75NTR

To examine the effect of Aβ on neurogenesis in vivo, a solution of either Aβ1–42 or Aβ1–16 was injected into the lateral ventricles of wildtype mice. Three or 14 days after the injection, cells from the SVZ were collected and cultured to assess their neurogenic activity. Consistent with the in vitro assays, in vivo treatment with Aβ1–42, but not Aβ1–16, 3 days prior to the establishment of cultures, resulted in a significant increase in both the number of neurospheres generated (Fig. 4A), and the number of neurons per neurosphere (Fig. 4B). However this phenomenon was not observed when the SVZ tissue was harvested 14 days after the in vivo treatment with Aβ1–42 (Fig. 4C and D).

The role of p75NTR in mediating the neurogenic effects of Aβ in vivo was then tested by injecting Aβ1–16 or Aβ1–42 peptide solution into the lateral ventricles of p75NTR-deficient mice. Three days after the injection, cells from the SVZ of these mice were collected and cultured. Supporting a requirement for p75NTR in mediating the Aβ1–42 response, the number of neurospheres (Fig. 4E) and the number of neu-
A. Sotthibundhu et al. / Neurobiology of Aging xxx (2008) xxx–xxx

3.5. Endogenous Aβ increases proliferation and differentiation via p75NTR

To further examine the in vivo neurogenic effects of Aβ, the C100 strain of transgenic mice, genetically engineered to produce increased levels of Aβ in the brain, was used. The β-carboxyl-terminal APP protein transcribed from the C100 transgene is constitutively processed by γ-secretase to generate soluble forms of Aβ. Mice have approximately 30 pmol/g of soluble Aβ in their brains, which includes both Aβ_{1-40} and Aβ_{1-42} (Li et al., 1999).

Consistent with the above findings, neurogenesis in 7-month-old C100 transgenic mice was found to be increased compared to that in wildtype controls, as assessed by quantifying neurosphere formation (Fig. 5A) and neuronal differentiation (Fig. 5B) of SVZ cultures. In order to test whether p75NTR was mediating the effects of endogenously produced Aβ, proteolytic processing of p75NTR was inhibited by applying TAPI-2 to the C100 neurosphere cultures. Under these conditions, the number of neurospheres per well formed from SVZ cells isolated from C100 mice was significantly inhibited (C100: 12.8 ± 1.9, C100 + TAPI-2: 7.8 ± 2.3; mean ± S.D., N = 3 animals, p < 0.05), implicating direct p75NTR involvement in endogenous Aβ-stimulated neurogenesis.

3.6. Endogenous Aβ-stimulated proliferation and differentiation are age dependent

A second strain of transgenic mice (APP/PS1), genetically engineered to produce human Aβ, was also examined. These mice produce increased levels of Aβ, including significant levels of Aβ_{1-42} (27 nmol/g of brain tissue at 8 months of age; Jankowsky et al., 2005; see also Supplementary Fig. 3). This results in amyloid plaque deposition and behavioral changes by 4–6 months of age (Jankowsky et al., 2005). Similar to the results obtained with C100 SVZ cultures, neurogenesis of SVZ cells isolated from 5-month-old APP/PS1 animals was significantly increased above that of their age-matched wildtype littermates (Fig. 5A and B). Surprisingly, however, no significant difference in the number of neurospheres or neurons per neurosphere (Fig. 4F) of p75NTR-deficient mice did not vary with treatment condition.
neuronal progeny was found in cultures derived from 12-month-old APP/PS1 mice (Fig. 5A and B).

Although the loss of neurogenic potential seen in aged APP/PS1 SVZ cultures could be due to a change in the ratio of soluble to plaque-bound Aβ1–42, we had found that fibrillar Aβ1–42 resulted in a significant pro-neurogenic response (Fig. 1A). Furthermore, measurement of the levels of Aβ in the soluble protein fractions of the brains of APP/PS1 mice at 4, 7, and 12 months of age revealed an increased burden of soluble as well as insoluble Aβ with age (Supplementary Fig. 3). Our results therefore suggested that, rather than the endogenous Aβ being present in a non-neurogenic form, the precursor cells of the aged mice were unable to respond to the Aβ.

It has previously been reported that the rate of neurogenesis declines with age (see Kuhn et al., 2007). In line with this, we have found that the number of p75NTR-positive precursor cells in the SVZ of adult rats significantly decreases between 8 and 14 months of age (K.M. Young and EJC, unpublished observations). To examine whether aged wildtype mice retain the ability to respond to Aβ, neurosphere cultures were assessed. Despite a reduced neurogenic response compared to cultures established from younger mice, SVZ precursor cells from 8- to 12-month-old mice also produced significantly more neurospheres when treated with Aβ1–42 than cells from untreated cultures (Fig. 5C and D). Therefore, it is possible that aged APP/PS1 mice have a more significant reduction in precursor cells capable of responding...
Fig. 5. Endogenous Aβ-stimulated neurogenesis is mediated by p75NTR and is age dependent. The number of neurospheres per well (A) or neurons per neurosphere (B) generated in SVZ cultures of 7-month-old C100 wildtype (B6/DBA) or transgenic (C100 B6/DBA) mice (N = 6 animals per genotype), and 5- and 12-month-old APP/PS1 wildtype [B6/SJL(5) and B6/SJL(12), respectively] and transgenic [APP/PS1(5)B6/SJL and APP/PS1(12)B6/SJL, respectively] mice (N = 4 animals per genotype and per age). Only young transgenic mice had significantly increased neurogenesis. Mean ± S.E.M., ***p < 0.001. The number of neurospheres per well of SVZ cells isolated from different aged wildtype (C57BL6, C; B6/DBA, D) mice, and plated in the presence of 5 μM Aβ1–42. Although not as dramatic as the effect seen in SVZ cultures derived from young animals, Aβ1–42 treatment also significantly increased the number of neurospheres generated from older animals. Mean ± S.E.M., N ≥ 3 animals per age group *p < 0.05 and ***p < 0.001.

to neurogenic stimuli than their aged wildtype counterparts.

4. Discussion

We have shown that both exogenous and endogenous stimulation of SVZ precursor cells by Aβ promote neurogenesis. This effect is dependent on p75NTR expression by the precursors and can be blocked by preventing the proteolytic processing of p75NTR by TAPI-2 sensitive metalloproteases. Furthermore, the neurogenic effect of Aβ is also dramatically reduced with age.

Aβ1–42 was shown to increase both the proliferation of precursors to form neurospheres and the neuronal differentiation of daughter cells within neurospheres, the end result being increased neurogenesis. Our findings in this regard, as well as the length and aggregation state of Aβ that promotes neurogenesis, are consistent with those of previous studies in which embryonic and adult SVZ precursors were assayed (Lopez-Toledano and Shelanski, 2004; Calafiore et al., 2006; Heo et al., 2007). They also lend support to other reports in which neurogenesis was measured in vivo in another Alzheimer’s disease mouse model (PDGF-APPSw.Ind; Jin et al., 2004b; Gan et al., 2008).

Regardless of the method of Aβ1–42 stimulation – in vitro application, in vivo application, or endogenous production through transgene expression – our experiments resulted in an increase in both neurosphere and neuron number. Although it is difficult to compare the concentration of Aβ applied in vitro and in vivo with that endogenously produced in vivo, taken together our results indicate that the observed neurogenic effect of Aβ was produced by lower concentrations of Aβ1–42 than those which cause synaptic dysfunction and neurotoxicity (cf. Heo et al., 2007). Furthermore, our findings, together with those of others (Jin et al., 2004b; Gan et al., 2008), suggest that the source of the Aβ1–42 mediating this effect is not the insoluble pool, but one that can be dynamically regulated (Morgan, 2003; Tabaton and Piccini, 2005). Indeed, our finding that neurogenesis is increased in cultures derived from C100 mice, in which Aβ1–42 is a minor product and fails to form insoluble aggregates, suggests that non-
pathogenic concentrations of Aβ1-42 are sufficient to elicit neurogenic effects and thus may contribute to basal levels of neurogenesis. Support for this concept comes from the demonstration that APP is expressed by stem-like cells in the SVZ (Caille et al., 2004; Yasuoka et al., 2004), where Aβ could be locally generated to act cell autonomously or on neighboring cells.

The present study also provides compelling evidence that the neurogenic effect of Aβ is mediated through p75NTR expressed by precursor cells. Such p75NTR-positive cells are found in the wall of the lateral ventricle in a similar pattern to APP and are required for normal SVZ neurogenesis (Giuliani et al., 2004; Young et al., 2007). Although p75NTR-positive cells make up less than 1% of SVZ cells, we have previously found all adult neurosphere activity to involve the p75NTR-positive cell population (Young et al., 2007). The fact that both the proliferation and neuronal generation effects were greatest when Aβ1-42 was applied early in the culture period, at a time when p75NTR remains highly expressed by cultured precursor cells (Young et al., 2007), suggests that both neurogenic stages can be regulated by the one action of Aβ on the p75NTR-expressing cell. Moreover, since both neurosphere number and subsequent production of daughter neurons were prevented by blocking p75NTR expression/cleavage, including that of cells from the C100 mouse in which Aβ is endogenously produced, the effect of Aβ is likely to be primarily regulated through p75NTR-mediated signals.

The results presented here further demonstrate that proteolytic processing of p75NTR by metalloproteases is important for the neurogenic effects stimulated by Aβ, but not those mediated by IGF1. Investigations into p75NTR signaling of other functional outcomes – cell death and inhibition of neurite outgrowth – have similarly demonstrated that metalloprotease cleavage is a necessary first step for signal transduction (Domeniconi et al., 2005; Kenchappa et al., 2006; Underwood et al., 2008), and have shown that this cleavage can be activated by ligand binding (Domeniconi et al., 2005; Kenchappa et al., 2006). Thus, it is possible that Aβ acts directly as a ligand for p75NTR, regulating the cell cycle of neural progenitors (Frade, 2000; Yaar et al., 2002; Coulson, 2006). Interestingly, the in vitro response of SVZ progenitors to Aβ was different from that observed with the traditional ligand BDNF, which stimulates neuronal differentiation of daughter neuroblasts but has no effect on the numbers of neurospheres generated from SVZ cultures (Young et al., 2007). This suggests that neurotrophins and Aβ might result in the activation of alternative downstream pathways.

p75NTR interacts with a number of intracellular protein partners known to control different aspects of the cell cycle, promoting proliferation and/or differentiation (Chittka and Chao, 1999; Kendall et al., 2002; Chittka et al., 2004; Vilar et al., 2006). In order to explain our results, signaling through p75NTR would need to result in increased mitosis of progenitors on the first day(s) of culture, with the subsequent generation of increased numbers of daughter neuroblasts. The ability of Aβ to regulate cleavage and thereby the nuclear translocation of the intracellular fragment of p75NTR and/or its binding partners (Kanning et al., 2003; Kenchappa et al., 2006) provides a further mechanism for regulation of these signal transduction pathways. Although both neurogenic outcomes could be mediated by one signal transduction pathway, it remains possible that each process is induced by a specific p75NTR ligand, regulating the binding of a certain signaling molecule to the intracellular domain of p75NTR. This in turn could promote either proliferation or differentiation. For example, stimulation of p75NTR-positive progenitor cells by Aβ could directly promote proliferation but at the same time could also cause secretion of neurogenic factors such as BDNF, stimulating neighboring and/or daughter neural precursors to commit to a neuronal fate (Morrison, 2001; Young et al., 2007).

In agreement with other studies, our results demonstrate that the pro-neurogenic effect of Aβ is absent in Alzheimer’s disease mice at an age at which cognitive and pathologic hallmarks of the disease are present (Dong et al., 2004; Donovan et al., 2006; Zhang et al., 2007; Gan et al., 2008). This could not be accounted for by the age of the animals alone, or by a decrease in the concentration of soluble Aβ1-42 due to its aggregation. Rather, our results suggest that the response of the precursor cells is lost. It has previously been reported that the number of neural stem cells, responsible for replenishing neurogenic precursors in the SVZ, decline during the course of an animal’s life (Luo et al., 2006; Kuhn et al., 2007). Importantly, however, the absolute number of stem cells can also be influenced by intrinsic and extrinsic factors, leading to premature or delayed loss of stem cells (Kippin et al., 2005; Beausejour and Campisi, 2006; Molofsky et al., 2006). Although p75NTR is expressed by both the stem cell and neural progenitor populations (Young et al., 2007), it does not appear to intrinsically regulate stem cell number, as the loss of neurosphere-forming cells in aged p75NTR-deficient mice is equivalent to that of age-matched wildtype mice (K.M. Young and EJC, unpublished observations). Overstimulation of p75NTR-positive neural progenitor cell division by Aβ during early life might, therefore, result in premature depletion of the stem cell pool and thus a more rapid decline in basal neurogenesis later in life.

In the case of the SVZ, committed neuroblasts migrate to the olfactory bulb where they terminally differentiate into neurons (Lois and Alvarez-Buylla, 1994). Ongoing olfactory bulb neurogenesis has been shown to be directly related to olfaction (Rochefort and Lledo, 2005), with a loss of progenitor production impacting on olfactory function, as it does for hippocampal function (see Lledo et al., 2006). Interestingly, a number of reports have shown that compromised olfaction is an early feature of Alzheimer’s disease (Serby et al., 1991; Moore and Murphy, 1999; Murphy et al., 2002). Our findings that exogenous and endogenously produced Aβ1-42 stimulates neurogenesis via p75NTR in younger animals, but that this effect is arrested with age, suggest that SVZ neurogenesis could be differentially regulated over the course of...
Alzheimer’s disease, an effect that may contribute to disease manifestation.

Conflict of interest

None.

Acknowledgements

The National Health and Medical Research Council of Australia supported this work. AS is the recipient of a Development and Promotion of Science and Technology Talent Scholarship, Mahidol University, Thailand. We would like to thank Briony Fox for performing surgical procedures and Rowan Tweedale for editorial assistance.

Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at doi:10.1016/j.neurobiolaging.2008.02.004.

References


ity NGF receptor p75 leads to deficits in the peripheral sensory nervous system. Cell 69, 737–744.


