sAPPα Rescues Age-Linked Decline in Neural Progenitor Cell Proliferation

Michael P. Demars¹, Carolyn Hollands¹, Kai Da (Tommy) Zhao¹, and Orly Lazarov¹,#
¹Department of Anatomy and Cell Biology, College of Medicine, The University of Illinois at Chicago, Chicago, IL 60612

Abstract

Neurogenesis is thought to play a role in cognitive function and hippocampal plasticity. Previous studies suggest that neurogenesis declines with aging. However, the onset and mechanism of declined neurogenesis are not fully elucidated. Here we show that the major decline in neurogenesis takes place during adulthood, prior to aging. Decline in neurogenesis takes place in both the subgranular layer of the dentate gyrus and in the subventricular zone, and is primarily due to reduced number of fast-proliferating neural progenitor cells. Importantly, this decline can be rescued by intraventricular injection of recombinant soluble amyloid precursor protein (sAPPα) that regulates neural progenitor cell proliferation in the adult brain. The counterpart sAPPβ, a product of the amyloidogenic cleavage pathway of APP, fails to exhibit a proliferative effect in vitro and in vivo, in equimolar concentration to sAPPα. These observations suggest that adulthood is an appropriate time window for an intervention that upregulates neurogenesis, such as enhancement of sAPPα levels, for the prevention of declining brain plasticity and cognitive function.

Keywords

Neurogenesis; amyloid precursor protein; adulthood; aging; cognition; learning and memory; Alzheimer’s disease

Introduction

Amyloid precursor protein (APP) can undergo metabolism through two distinct pathways leading to the production of a number of intra- and extracellular metabolites. The amyloidogenic pathway of APP cleavage, so termed for its involvement in the formation of β-amyloid (Aβ), begins with β-secretase, beta-site APP cleaving enzyme 1 (BACE1), and releases soluble amyloid precursor protein β (sAPPβ) to the lumen [for review (Vassar, 2012)]. In the non-amyloidogenic APP cleavage pathway, initial cleavage occurs through α-secretase enzymes, primarily composed of a disintegrin and matrix-metalloproteinase proteins (ADAM), leading to the release of sAPPα (Buxbaum et al., 1998; Lammich et al., 1999; Asai et al., 2003; Jorissen et al., 2010). It has been previously reported that sAPPα has...
trophic properties in a number of different cell types including fibroblasts (Saitoh et al., 1989), thyroid epithelial cells (Pietrzik et al., 1998), embryonic stem cells (Ohsawa et al., 1999) and carcinoma cells (Ko et al., 2004). With respect to adult neural progenitor cells (NPC), sAPPα has been shown to bind to and stimulate the proliferation of NPC both in vitro and in vivo (Caille et al., 2004; Demars et al., 2011).

In the aging brain, there is a marked decline in neurogenesis in both the subventricular zone (SVZ) (Mirich et al., 2002; Shook et al., 2012) and subgranular layer (SGL) (Kuhn et al., 1996; Cameron and McKay, 1999; Bernal and Peterson, 2004; Bondolfi et al., 2004; Kronenberg et al., 2006; Ben Abdallah et al., 2010; Encinas et al., 2011; Miranda et al., 2012). This decline in neurogenesis has been shown to manifest in deficits in olfactory function and hippocampal dependent learning and memory (Bizon et al., 2004; Enwere et al., 2004; Dupret et al., 2008). However, the cause of neurogenic decline in the aging brain remains somewhat controversial. Some studies argue that the proliferation of NPC and their maturation declines with age (Kuhn et al., 1996; Heine et al., 2004; Rao et al., 2005; Morgenstern et al., 2008). Olariu and colleagues suggest that this decline is not due to alterations in the length of the cell cycle of NPC in the SGL (Olariu et al., 2007). Encinas and colleagues suggest that neurogenic decline is due to the disappearance of NSC by their conversion into mature hippocampal astrocytes (Encinas et al., 2011). Bonaguidi et al. (2008) suggest that upregulation of bone morphogenetic protein (BMP) signaling or downregulation of its antagonists may underlie age-linked reduced self-renewal of NSC leading to the decline in neurogenesis (Bonaguidi et al., 2008). Other groups have argued that the rate of proliferation does not decline with aging, but the cells may exhibit increased quiescence that may be attributed to a decline in the vascular niche (Hattiangady and Shetty, 2008). Although this debate is still ongoing, it is apparent that the neurogenic niche undergoes changes associated with aging that have the potential to underlie neurogenic decline (Ahlenius et al., 2009; Villeda et al., 2011; Miranda et al., 2012). For instance, it has been well established that the expression of many growth factors and their receptors that are integral to neurogenic processes peak during development and decline in expression thereafter [for review (Klempin and Kempermann, 2007)]. Neurogenesis in the adult central nervous system (CNS) plays a role in hippocampus- and olfaction-dependent learning and memory [for review (Kempermann and Gage, 2000; Bernal and Peterson, 2004; Lie et al., 2004)]. These findings raise the possibility that reduced neurogenesis may, at least in part, account for impaired learning and memory and cognitive deterioration in the elderly (Seki and Arai, 1995; Kuhn et al., 1996; Tropepe et al., 1997; Kempermann et al., 1998; Kempermann et al., 2002) and may enhance vulnerability to Alzheimer’s disease [(AD) for review see (Lazarov et al., 2010)].

Aging remains the predominant risk factor for the development of sporadic forms of AD. Alterations in the cleavage pattern of APP are causative of familial forms of the disease [for review (Selkoe, 2001)]. We have previously reported that deficits in proliferation and neurogenesis occur long before the appearance of pathological hallmarks or cognitive impairments in a mouse model of familial Alzheimer’s disease (Demars et al., 2010). Taken together with our observation that sAPPα is a proliferation factor of adult NPC, this may suggest the hypothesis that increasing levels of sAPPα in the neurogenic niches would rescue neurogenic deficits.

Here we show that a dramatic decline in neurogenesis takes place at the age of 7–9 months, compared to mice at 2 months of age, and only a minor further decrease takes place at age of 20 months. This suggests that the significant decline in neurogenesis takes place during adulthood, rather than during aging. This decline is characterized by reduced numbers of fast proliferating NPC (type II cells in the SGL and type C cells in the SVZ), but no decline in the number of NSC. Intracerebroventricular (ICV) injection of sAPPα ameliorates
proliferation deficits in adult mice. In support of that, adding sAPPα to a neurosphere culture derived from 7–9 month old or 20 month old mice enhances NPC proliferation. Additionally, we show that at similar concentration range, sAPPβ does not evoke the same proliferative effect as sAPPα. This study suggests that therapy aimed at rescuing decline in neurogenesis should be applied during adulthood rather than aging, and that enhancement of sAPPα levels may rescue this decline.

Materials and methods

Animals

Wild type mice 2 months, 7–9 months and 20 months of age are on a C57/Bl6XC3H background and were maintained in our colony. Our colony is maintained via group housing (<5 mice per cage) in a barrier facility under a 14:10 light:dark cycle with free access to food and water. Mice were euthanized using isofluorane and cervical dislocation. Animal care and procedures were conducted according to the National Institutes of Health Guide for the Care and Use of Laboratory Animals.

Recombinant sAPPα and sAPPβ

sAPPα (Sigma-Aldrich) was used at 10 nM concentrations unless otherwise indicated (dissolved in phosphate-buffered saline, or PBS). sAPPβ (Sigma-Aldrich) was also dissolved in PBS and is used in the indicated concentrations.

Intracerebroventricular injections

A PBS vehicle or recombinant sAPPα or sAPPβ (Sigma-Aldrich) at a concentration of 1 μM (1 μl/mouse; 0.25 μl/min.) were stereotaxically injected into the left lateral ventricle of 7–9 month old C57BL/6 mice using the following coordinates: (anteroposterior, 0 mm; mediolateral, −0.8 mm; dorsoventral, −2.0 mm from bregma). Mice were anesthetized using a mixture of ketamine (100 mg/kg) and xylazine (10 mg/kg). The heads of the mice were then shaved and wiped with 70% ethanol. Animals were placed into a stereotaxic frame and a centimeter incision was made in the midline of the scalp to reveal the bregma. The scalp was rinsed with 30% hydrogen peroxide and a small hole was drilled at the coordinate site as measured from bregma according to the mouse atlas (Franklin and Paxinos, 2008). Animals then received unilateral injection of sAPP to the lateral ventricle. The sAPP was delivered through a 5 μl Hamilton syringe connected to a hydraulic injection system set to inject at a rate of 0.25 μl/min. The injection needle was then left in place for an additional minute to ensure distribution on the solution. The needle was slowly removed and the incision closed using EZ-clips from Stoelting. Following 6 hrs of recovery, mice were given a single IP dose of 100 mg/kg BrdU solution. 24 hours after the BrdU injection, mice were transcardially perfused and the brains processed for immunohistochemistry as described on subsequent pages.

Neural progenitor cell culture

Mice were euthanized and their brains were removed and placed into sterile Dulbecco’s modified Eagle’s medium/F12 (DMEM/F12). A coronal slice (approximately 1 mm) was dissected starting 1 to 2 mm posterior to the olfactory bulb. The region occupying the lateral wall and anterior horn of the lateral ventricles was removed with the aid of a dissecting microscope and diced with a sterile scalpel. Neurosphere culture was prepared as previously described (Demars et al., 2011). Briefly, tissue pieces were collected in a mixture of Papain and DNase in Earl’s balanced salt solution and incubated at 37°C for 40 minutes. Then, tissue pieces were pelleted by centrifugation and dissociated to a single-cell suspension, and cells were plated in NPC media that includes complete medium-water, DMEM/F12 (Gibco,
now part of Invitrogen Corporation, Carlsbad, CA, USA), glucose (Sigma-Aldrich, St. Louis, MO, USA), NaHCO$_3$ (Sigma-Aldrich), HEPES (Sigma-Aldrich), L-glutamine (Invitrogen Corporation), penicillin/streptomycin (Invitrogen Corporation), putrescine (9.6 µg/mL; Sigma-Aldrich), apotransferrin (0.1 mg/mL; Sigma-Aldrich), insulin (0.025 mg/mL; Roche, Indianapolis, IN, USA), selenium (5.2 ng/mL; Sigma-Aldrich), progesterone (6.3 ng/mL; Sigma-Aldrich), bovine serum albumin (BSA) (2 mg/mL; Sigma-Aldrich), heparin (4 µg/mL; Sigma-Aldrich), EGF (20 ng/mL; PeproTech Rocky Hill, NJ, USA), and bFGF (10 ng/mL; Pepro-Tech)-and passaged after 10 days.

**Clonogenic assay**

Briefly, neurospheres were singly dissociated by mechanical dissociation and plated at 1,000 cells per well onto 96-well plates. Cells were then treated with the indicated molar concentration of GM6001 or GM6001 negative control (Millipore Corporation), β-secretase inhibitor IV (EMD/Millipore) and the indicated molar concentrations of sAPP. Cells were treated every day for 7 days. After 7 days in culture, neurospheres were counted under an inverted light microscope, and the average neurosphere diameter was calculated for each sphere by measuring 4 diameters for each to control for irregularly shaped neurospheres using a Zeiss AX10 microscope (Carl Zeiss Ltd., Hertfordshire, UK) and StereoInvestigator software (MBF Bioscience, Williston, VT, USA). After sphere size determination, cells were singly dissociated with a p200 pipette and counted with a hemocytometer. Each experiment was repeated 5 times using neurosphere cultures derived from 5 different animals.

**Immunoprecipitation and Western blotting**

The detection of sAPP in NPC culture was performed by conditioning media using 5 – 10$^5$ cells plated for 2 hours in fresh NPC media. Media was then spun (1000 X g for 10 minutes) to remove any cells and precleared with protein A agarose beads (Pierce). Next, media was incubated overnight with 22C11 antibodies against the N-terminus of APP. The next day, media was incubated for 30 min in protein A agarose beads, spun and the pellet was resuspended in sample buffer. Samples were heated at 100°C for 5 minutes and run on a 6% Tris-glycine gel. For the extraction of protein from the neurospheres used to condition media a lysis buffer containing 150 mM NaCl, 50 mM Tris-Cl, 5 mM EDTA, 1% Triton-X 100, 0.5% Sodium Deoxycholate, Protease inhibitor cocktail (mammalian protease inhibitor cocktail, Sigma) and 250 µM PMSF was used. Quantification of protein was performed using the BCA-method (Pierce) and equal amounts of protein were subjected to direct immunoblotting. For quantification N ≥3 was used.

**Brain tissue processing**

For *in vivo* immunohistochemical staining, all mice were anesthetized with a mixture of ketamine (100 mg/kg) and xylazine (10 mg/kg) and transcardially perfused with 100 mL of iced PBS. The brains were then removed and halved in the sagittal plane. The left half was immediately placed into 4% paraformaldehyde on ice.

**Immunohistochemistry**

Left hemibrains from PBS-perfused mice were post-fixed in 4% paraformaldehyde for 3 days and stored in 30% sucrose at 4°C. Hemibrains were sectioned sagittally at 50 µm by using a freezing stage microtome and placed into cryopreservent (47.6% PBS, 28.57% ethylene glycol, and 25% glycerin vol/vol). Sections were blocked by using a solution containing 0.25% vol/vol Triton-X 100 (Sigma-Aldrich) and 5% vol/vol Normal Donkey Serum (Jackson ImmunoResearch Laboratories, Inc.) in TBS. The following antibodies were used: BrdU (1:400; Accurate Chemicals, Westbury, New York), nestin (1:100; Millipore...
Corporation), doublecortin (DCX, 1:400; Santa Cruz Biotechnology, Inc.) and GFAP (1:500 Millipore Corporation). Floating sections were incubated in primary antibodies for 72 hours at 4°C before continuing with blocking, biotin conjugation (Jackson ImmunoResearch Laboratories, Inc.), and secondary antibody incubation (cy2 Streptavidin, anti-mouse cy3, anti-goat cy5, and anti-rabbit cy5; Jackson ImmunoResearch Laboratories, Inc.).

Stereological quantification

The number of positively stained cells in sagittal brain sections was quantified using designbased stereology (StereoInvestigator version 8, MBF Bioscience, Williston, VT, USA). For the analysis, every sixth section of brain tissue was quantified by applying Nv × VRef method. The following parameters were used; for SVZ, sections were traced using a Zeiss AX10 microscope (Carl Zeiss Ltd., Hertfordshire, England) in low magnification (5X) and counting was performed at high magnification (63X), counting frame= 100 µm X 100 µm, grid size 100 µm X 100 µm and all sections were counted using 25µm top and bottom guard zones. For the dentate gyrus the counting frame was set equal to the grid size (100 µm X100 µm) in order to count the entirety of the DG due to the relative paucity of cells. All other parameters remained the same.

Results

To examine the course of decline in neurogenesis with age we first assessed the extent of neurogenesis in the SGL and SVZ of 2 (young), 7–9 (adult) and 20 (aging) months old mice (N=4). For this purpose, we quantified the number of type B (nestin+GFAP+), total fast proliferating cells (BrdU+), type C (BrdU+nestin+, BrdU+DCX+), and type A (BrdU-DCX +) cells. Unbiased stereology revealed a severe reduction in the number of total proliferating cells (Figure 1A), and the number of type C and A cells by 7–9 months of age in the SVZ (Figure 1B–D). Notably, the number of fast proliferating cells (BrdU+) and NPC (nestin +BrdU+) is significantly reduced by 7–9 months of age, and stays reduced without further significant reduction by 20 months of age (Figure 1A,B). This may suggest that the reduced proliferation of NPC in the SVZ by 7–9 months of age might play a major role in reduced extent of neurogenesis. The number of neuroblasts and immature neurons is significantly reduced by 7–9 months, and this number is further reduced by 20 months (Figure 1C,D,Kiii–v). The number of type B cells (GFAP+nestin+) was not significantly changed in any of the age groups (Figure 1J), suggesting that the pool of NSC in the SVZ does not decline with age.

Examination of the cell population in the SGL of the different ages revealed similar observations. We quantified the number of type I (nestin+GFAP+), total fast proliferating cells (BrdU+), type II (BrdU+nestin+, BrdU+DCX+), and type III (BrdU-DCX+) cells. The number of total proliferating cells (Figure 1F), and the number of type II and III cells (Figure 1G–I) was dramatically reduced by 7–9 months of age in the SGL. The number of type II and III cells remains reduced. The number of proliferating neuroblasts, BrdU+DCX +, is reduced severely by 7–9 months of age, with a further significant reduction in their number between middle and old age (Figure 1H,Kiii,iv). The same is true for the total number of post-mitotic immature neurons (BrdU-DCX+). A dramatic reduction in this population occurs between 2 and 7–9 months of age with a smaller but significant reduction taking place thereafter up to 20 months of age (Figure 1I). Of note, the number of total proliferating BrdU+ cells and of fast proliferating NPC type II (BrdU+nestin+, BrdU+DCX +) was dramatically reduced. Notably, there is no change in the number of NSC (Figure 1J), suggesting that the pool of NSC in the SGL does not decline with age. We and others have shown that sAPPα is a proliferation factor of NPC in the adult brain (Caille et al., 2004; Baratchi et al., 2011; Demars et al., 2011). Thus, in light of the dramatic reduction in proliferation of NPC in the SVZ and the SGL of mice at age of 7–9 months, we asked
whether addition of sAPPα into the neurogenic areas can ameliorate deficits in proliferative cells in the brains of 7–9 months old mice *in vivo*. To this end, we performed intracerebroventricular (ICV) injection of recombinant sAPPα or PBS (supplementary Figure 1) in mice at 7–9 months of age followed 6 hours later with a single i.p. dose of BrdU and sacrifice 24 hours later (N=4). Single ICV injection of sAPPα in 7–9 month old mice dramatically increased the number of proliferating (BrdU+) cells in both the SVZ (Figure 2A,I,ii) and SGL (Figure 2E,iii,iv). To assess the impact of ICV sAPPα injection on NPC proliferation, we quantified the number of cells co-labeled with BrdU and nestin. We show that sAPPα injection rapidly and significantly increased the number of proliferating NPC in both the SVZ (Figure 2B,Ivi) and SGL (Figure 2F) of 7–9 month old mice. Notably, in the SVZ, the number of proliferating neuroblasts was increased following sAPPα injection (Figure 2C,Iv), suggesting that enhanced proliferation had a direct effect on the number of neuronally-committed NPC. In the SGL, the number of neuroblasts appeared albeit reduced but not significantly changed (Figure 2G). Interestingly, there was a statistically insignificant reduction in the number of NSC (GFAP+/Nestin+) in the SVZ (Figure 2D). In the SGL, reduction in the number of NSC was significant (Figure 2H). This may raise the possibility that sAPPα induces NSC asymmetric division resulting in more NPC in *in vivo*. Alternatively, sAPPα may facilitate their commitment to neuronal lineage. Taken together, these results suggest that sAPPα can enhance aging-linked reduced proliferation in both the SVZ and SGL through stimulating NPC proliferation.

To further establish that the addition of sAPPα stimulates NPC proliferation, we isolated NPC from the SVZ of mice at age of 2, 7–9 or 20 months old, and treated the neurosphere cultures with either recombinant sAPPα or vehicle. Consistent with previous reports suggesting proliferation deficits could be masked by culture conditions (Ahlenius et al., 2009; Bouab et al., 2011), we saw no significant difference in neurosphere diameter or total NPC number in neurosphere cultures derived from mice that are 2, 7–9 or 20 months old (Figure 3). However, we could stimulate proliferation with the addition of 10nM sAPPα in neurospheres derived from 2, 7–9 month and even 20 months old mice as indicated by increased neurosphere diameter (Figure 3A,C) and increased total NPC numbers (Figure 3B). This result suggests that NPC derived from mice at various ages retain the ability to proliferate in response to sAPPα *in vitro*.

The cleavage of APP can produce either sAPPα or sAPPβ. Therefore, we sought to determine if NPC respond differentially to the two sAPP species. We assayed sphere diameter of neurospheres derived from single-dissociated NPC treated with varying concentrations of either sAPPα or sAPPβ. Treatment with sAPPα increased neurosphere diameter at 10nM concentrations with a trend toward significance at 1nM. However, sAPPβ treatment failed to significantly increase proliferation at any of the concentrations tested (Figure 4A, representative images C). In our previous work, we showed that sAPPα is able to ameliorate deficits in NPC proliferation caused by matrix-metalloproteinase inhibition that inhibits enzymes exhibiting α-secretase activity that yields sAPPα production (Demars et al., 2011). The use of the inhibitor is meant to inhibit endogenous production of sAPPα, thus preventing a masking of the proliferative effect exerted by recombinant sAPPα. Therefore, we sought to determine whether sAPPβ would enhance proliferation in the absence of endogenous production of sAPPα. As previously reported, 1 µM GM6001 MMP inhibitor treatment impairs proliferation, while sAPPα ameliorates these proliferation deficits (Figure 4B, (Demars et al., 2011)). At 1 nM concentrations, sAPPβ enhances proliferation to the same extent as sAPPα. However, at concentrations above 10 nM, sAPPβ treatment has no effect on rescue of MMP inhibitor induced proliferation deficits (Figure 4B).
Treatment with MMP inhibitor likely exclusively reduces sAPPα. Thus, we next asked whether endogenous production of sAPPβ masks the proliferative effect of recombinant sAPPβ. For this purpose, we inhibited endogenous production of sAPPβ by treating NPC culture with β-secretase inhibitor IV. We show that treatment of NPC with β-secretase inhibitor reduces proliferation dramatically as assayed by neurosphere diameter and total cell number (Figure 5A). While sAPPα ameliorates this deficit at 10 nM concentrations, 1 nM sAPPβ, the most effective dose in previous experiments, does not enhance proliferation following β-secretase inhibition (Figure 5A, B). To further exclude the possibility that the presence of either endogenous sAPPα or sAPPβ mask the proliferative effect of recombinant sAPPβ, NPC cultures were treated with both MMP and β-secretase inhibitor. Treatment of NPC with GM6001 or β-secretase inhibitor IV reduced neurosphere diameter (Figure 6A) and total cell number (Figure 6B) to the same extent following 7DIV. To assess the ability of sAPPα or sAPPβ to stimulate proliferation under conditions of minimal endogenous sAPP, we added exogenous recombinant sAPPα or sAPPβ to NPC under dual secretase inhibition. sAPPα rescued proliferation deficits to control levels. Conversely, sAPPβ failed to ameliorate the proliferation deficits incurred from dual secretase inhibition (Figure 6A,B). These results suggest that sAPPα is a more potent proliferation factor for NPC than sAPPβ.

Finally, we sought to address whether, similarly to sAPPα, sAPPβ would rescue age-dependent proliferation deficits of NPC. Because age-dependent proliferation deficits are not apparent in vitro, we examined the effect of sAPPβ administration on proliferation in 7–9 month old mice compared to the young in vivo (N=4). We show that ICV injection of sAPPβ led to a markedly reduced population of proliferating cells in both the SVZ (Figure 7A–C) and SGL (Figure 7D–F), including a dramatically reduced population of BrdU+/DCX+ proliferating neuroblasts (Figure 7B SVZ, Figure 7E SGL). Interestingly, similarly to the sAPPα injected cohorts, sAPPβ injected animals showed a decrease in GFAP+/nestin+ NSC in the SGL (Figure 7F) and a trend in the SVZ, albeit not significant (Figure 7C). These results indicate that at equimolar concentrations, sAPPα and sAPPβ have significantly different effects on proliferating cells in the two neurogenic niches. While sAPPα is able to ameliorate deficits in proliferation, sAPPβ reduces the number of proliferating cells in the already depleted brains of 7–9 month old animals.

Discussion

This study reports several important observations. First, there is a dramatic decline in neurogenesis in adulthood, prior to aging. This is consistent with previous reports on neurogenesis in the aging brain (Cameron and McKay, 1999; Bernal and Peterson, 2004; Kronenberg et al., 2006). Second, the number of proliferating cells is dramatically reduced by 7 months of age in both the SVZ and SGL, with no further significant decline. We further show that the decline in proliferation is due, in large part, to a reduction in the number of proliferating NPC without any change in the number of NSC in either region. The molecular mechanism underlying this decline in proliferation is not fully elucidated. It is possible that the level of essential proliferation factors is declined with age. Notably, binding sites for sAPP are localized to rapidly proliferating-C cells in the adult brain (Caille et al., 2004). However, our results cannot rule out the possibility that the age-linked decline in proliferating cells is due to increased quiescence of NSC and a reduced NPC pool. As NSC proliferate very slowly (Zheng et al., 2004), our paradigm of single-pulse BrdU injection likely will not capture this population. However, our results do indicate that the total number of NSC is steady across all ages and suggest that a waning number of NSC is not the cause of neurogenic decline.
Third, we show that a single ICV injection of recombinant sAPPα is sufficient to significantly ameliorate age-linked deficits in the number of proliferative NPC. It should be noted that while the final concentration of sAPP following injection is uncertain, it was designed to be diluted approximately 100 fold in the cerebrospinal fluid of the ventricular space. Given the injected dose of 1 µM, this would result in a final concentration of approximately 10 nM, the most effective concentration of sAPPα in our culture conditions. The injection of sAPPα significantly increased the number of BrdU/nestin co-labeled NPC in both the SGL and SVZ. A single injection of sAPPα into the lateral ventricle enhanced the number of neuroblasts in the SVZ but not in the SGL. One possibility is that because of the relatively low number of neuroblasts in the SGL compared to the SVZ, a single injection is not sufficient to evoke an increase in neuroblasts within 24 hours. It is reasonable to assume that some of the detected BrdU+DCX+ started to proliferate prior to the stimulation of recombinant sAPPα. Thus, more studies are warranted in order to determine whether injection of sAPPα leads to increased number of new neurons and to their survival and functional incorporation in the olfactory bulb and granular layer of the dentate gyrus.

Fourth, we show that sAPPβ is a less potent proliferation factor than sAPPα in vitro and may only function to stimulate proliferation in a relatively small concentration range. Several of the mutations in APP, that are causative of FAD, lie in close proximity to the β-secretase cleavage site at the N-terminal portion of the Aβ region. These mutations cause a shift in the metabolism of APP toward the amyloidogenic pathway, increasing sAPPβ production at the expense of sAPPα (Thinakaran et al., 1996). FAD-linked transgenic mice display impaired proliferation of NPC prior to the onset of pathological hallmarks or the presentation of memory deficits. In vitro, NPC derived from these mice have impaired proliferation, suggesting a potential intrinsic mechanism caused by the mutations (Demars et al., 2010). The differential activity between sAPPα and sAPPβ has been previously reported with respect to other trophic properties such as neuroprotection against glutamatergic or β-amyloid toxicity (Furukawa et al., 1996), promotion of axonal elongation and primary dendritic length (Chasseigneaux et al., 2011), hippocampal long-term potentiation (LTP) (Taylor et al., 2008) and rescue of prenatal lethality in an APP/APLP2 knockout mouse (Li et al., 2010; Weyer et al., 2011). However, the cause of this functional divergence between two peptides that differ by only 16 amino acids has yet to be elucidated. The C-terminal portion of sAPPα alone is unable to stimulate proliferation in embryonic stem cells (Ohsawa et al., 1999). Whatever the cause of these differences, it is feasible that a shift in the homeostatic balance of APP processing favoring the amyloidogenic pathway would impair neurogenesis. Not only would this result in the production of the less trophic sAPPβ but it would also lead to increases in the transcriptionally active form of APP intracellular domain (AICD), which has been shown to be preferentially produced by this pathway (Goodger et al., 2009; Belyaev et al., 2010) and to be a negative regulator of proliferation (Ma et al., 2008; Ghosal et al., 2010). Therefore a shift in the balance of these cleavage pathways could underlie, at least in part, neurogenic deficits in aging and AD.

Finally, we show that ICV injection of sAPPβ at similiar/equimolar concentrations to sAPPα does not enhance the number of proliferating cells in either the SVZ or SGL of the aging brain. Conversely, sAPPβ injection reduced the number of proliferating cells. There is evidence from peripheral neurons that during times of reduced growth factor support, sAPPβ is released and binds to DR6 inducing neurodegeneration (Nikolaev et al., 2009). The mechanism underlying this suppression of proliferation is not known. Future experiments will be aimed at unraveling the significance of sAPPβ regulated decline in proliferation. It should be noted that the concentration of sAPPβ used was designed to be an equimolar dose to sAPPα injections. As we have shown in our in vitro assays the sAPPs may have different optimal concentrations thus we may have not captured the optimal dose in our experiments.
Alternatively, a difference in the half-life of sAPPα versus sAPPβ or their rate of metabolism may underlie this outcome.

In summary, this study shows that extent of neurogenesis declines well prior to aging, that the decline in neurogenesis as a function of age is largely due to a decline in the number of proliferating NPC and that this reduction can be reversed by a single-dose ICV injection of sAPPα, while the same dose of sAPPβ resulted in further impairment of proliferation. These findings highlight the differential activity of the sAPPs with respect to proliferation of NPC. Together with previous studies, this work provides evidence that the regulation of APP processing plays a major role in the regulation of NPC proliferation in the adult brain. This paves the way for therapeutic intervention with an emphasis on maintaining a homeostatic balance in APP processing both in AD and physiological aging. Though aging is the predominant risk factor for sporadic forms of AD, little is known about the expression of APP and APP metabolites during normal aging. One study of note did examine APP maturation and processing during cellular aging in a human lung fibroblast cell line and showed a decreasing metabolism with increasing cellular age including a decline in sAPPα (Kern et al., 2006). This group further showed that increasing membrane cholesterol levels correlated with increasing cellular age. Intriguingly, APP, BACE1 and presenilin1 have been shown to associate increasingly with detergent resistant, cholesterol rich, membranes or “lipid rafts” with increasing cellular age (Kang et al., 2006). Evidence suggests that amyloidogenic processing of APP is sequestered in these detergent resistant membrane micro-domains (Simons et al., 1998; Schneider et al., 2008). Thus the possibility exists that in both physiological aging and AD there is a shift in the metabolic pathway or cleavage pattern of APP favoring the amyloidogenic pathway over the seemingly more trophic non-amyloidogenic pathway.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

Acknowledgments

The work was supported by the NIA AG033570, NIA 1RC1AG036208-01 ARRA, The Brain Research Foundation and The Alzheimer’s Association Young Investigator Award (OL). The authors declare no competing financial interests.

References


APP and APLP2 are essential at PNS and CNS synapses for transmission, spatial learning and LTP. Embo J. 2011; 30:2266–2280. [PubMed: 21522131]

Figure 1. Aging-linked decline in proliferating neural progenitor cells and immature neurons in the SVZ and SGL.
Quantification of proliferating cells [BrdU+ (A,F)], proliferating NPC [BrdU+/nestin+ (B,G)], proliferating neuroblasts [BrdU+/DCX+ (C,H)], immature neurons [BrdU-/DCX+ (D,I)], and NSC [GFAP+/nestin+ (E,J)] in the SVZ (A–E) and SGL (F–J) of 2, 7–9 and 20 month old mice. K, Representative images of (i) 2 month SVZ (BrdU green; DCX red), (ii) 20 month old SVZ, (iii) 2 month old SGL, (iv) 20 month old SGL, (v) representative image of GFAP (green) / nestin (red) staining. N=4, *p<0.05, ANOVA with post-hoc analysis.
Figure 2. Intracerebroventricular injection of sAPPα ameliorates aging-linked proliferation deficits in the SGL and SVZ

Quantification of proliferating cells [BrdU+, SVZ (A) and SGL (E)], proliferating NPC [BrdU+/nestin+, SVZ (B) and SGL (E)], neuroblasts [BrdU+/DCX+, SVZ (C) and SGL (G)] and NSC [GFAP+/nestin+, SVZ (D) and SGL (H)] in 7–9 month old mice injected ICV with either PBS or 1 µl of 1 µM sAPPα. I. Representative images of (i) PBS injected SVZ (BrdU green), (ii) sAPPα injected SVZ (BrdU green), (iii) PBS injected SGL (BrdU green), (iv) sAPPα injected SGL (BrdU green), (v) high magnification of BrdU+(green)/DCX+(red) staining, (vi) representative image of BrdU+(green)/nestin+(red) staining. N=4, *p<0.05, student’s t-test.
Figure 3. NPC from aged mice retain the ability to proliferate in response to sAPP
A. Neurosphere diameter following 7 DIV single-dissociated NPC of 2 month, 7–9 month
and 20 month old mice as well as NPC supplemented daily with 10nM sAPPα. B. Total cell
number of dissociated neurospheres quantified in A. C. Representative images of
neurospheres in clonogenic analysis following 7 DIV with the indicated ages and treatments.
N=5, * p < 0.05, students t-test or ANOVA with post-hoc analysis.
Figure 4. sAPPα and sAPPβ differentially affect proliferation of NPC and proliferation rescue following matrix-metalloproteinase inhibition

A. Clonogenic assay following treatment with varying concentrations of sAPPα (green) or sAPPβ (purple) compared with PBS treated controls (red). B. Total cell number from dissociated neurospheres treated with GM6001 (blue), GM6001 and sAPPα (green) or GM6001 and sAPPβ (purple) compared with GM6001 negative control treated controls (red). C. Representative images of neurospheres from 3A. N=5, *p < 0.05, ANOVA with post-hoc analysis.
Figure 5. β-secretase inhibitor induced proliferation deficits are ameliorated by sAPPα but not sAPPβ
A. Neurosphere diameter in a clonogenic assay following 7 DIV treatment with β- secretase inhibitor IV (blue), β-secretase inhibitor IV and sAPPα (green) or β-secretase inhibitor IV and sAPPβ (purple) compared with DMSO treated controls (red). B. Total cell number of dissociated neurospheres from 4B. C. Representative images of neurospheres in clonogenic assay. N=5, *p < 0.05, ANOVA with post-hoc analysis.
Figure 6. sAPPα, but not sAPPβ, can rescue proliferation deficits incurred by dual secretase inhibition
A. Neurosphere diameter in a clonogenic assay following 7 DIV GM6001 (blue), β-secretase inhibitor IV (gold), dual secretase inhibition (light purple), dual secretase inhibition and sAPPα (green) or dual secretase and sAPPβ (dark purple) compared with DMSO (red) treated controls. B. Total cell number of dissociated neurosphere from 5A. C. Representative images of neurospheres following 7 DIV with the indicated treatments. N=5, * p < 0.05, ANOVA with post-hoc analysis.
Figure 7. Intracerebroventricular injection of sAPPβ exacerbates aging-linked deficits in proliferating cell numbers in the SVZ and SGL.

Quantification of proliferating cells [BrdU+ (SVZ A, SGL D)], proliferating neuroblasts [BrdU+DCX+ (SVZ B, SGL E)] and NSC [GFAP+/nestin+ (SVZ C, SGL F)] in 7–9 month old mice injected ICV with either PBS or 1 µl of 1 µM sAPPα. N=4, * p < 0.05, student’s t-test.