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Amyloid β precursor protein regulates neuron survival and maturation in the adult mouse brain

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ABSTRACT

The amyloid-β precursor protein (APP) is a transmembrane protein that is widely expressed within the central nervous system (CNS). While the pathogenic dysfunction of this protein has been extensively studied in the context of Alzheimer's disease, its normal function is poorly understood, and reports have often appeared contradictory. In this study we have examined the role of APP in regulating neurogenesis in the adult mouse brain by comparing neural stem cell proliferation, as well as new neuron number and morphology between *APP* knockout mice and C57bl6 controls. Short-term EdU administration revealed that the number of proliferating EdU^+ neural progenitor cells and the number of PSA-$NCAM^+$ neuroblasts produced in the SVZ and dentate gyrus were not affected by the life-long absence of APP. However, by labelling newborn cells with EdU and then following their fate over-time, we determined that ~48% more newly generated EdU^+ $NeuN^+$ neurons accumulated in the granule cell layer of the olfactory bulb and ~57% more in the dentate gyrus of young adult *APP* knockout mice relative to C57bl6 controls. Furthermore, proportionally fewer of the adult-born olfactory bulb granule neurons were $calretinin^+$. To determine whether APP was having an effect on neuronal maturation, we administered tamoxifen to young adult *Nestin-CreER^{T2}::Rosa26-YFP* and *Nestin-CreER^{T2}::Rosa26-YFP::APP-knockout* mice, fluorescently labelling ~80% of newborn (EdU^+) $NeuN^+$ dentate granule neurons formed between P75 and P105. Our analysis of their morphology revealed that neurons added to the hippocampus of *APP* knockout mice have shorter dendritic arbors and only half the number of branch points as those generated in C57bl6 mice. We conclude that APP reduces the survival of newborn neurons in the olfactory bulb and hippocampus, but that it does not influence all neuronal subtypes equally. Additionally, APP influences dentate granule neuron maturation, acting as a robust regulator of dendritic extension and arborisation.

1. Introduction

The amyloid-β precursor protein (APP) is a 110–130 kDa type I transmembrane protein (Dawkins and Small, 2014; Patterson et al., 1988) that belongs to a highly conserved family of proteins which includes APP-like protein (APLP)-1 and -2 (Slunt et al., 1994; Wasco et al., 1992). APP is expressed throughout the central nervous system (CNS) (Arai et al., 1991; Selkoe et al., 1988), and has been implicated in a wide variety of functions including cellular proliferation and differentiation (Bolós et al., 2014; Caille et al., 2004; Hu et al., 2013; Small et al., 2014), cell-fate specification (Dinet et al., 2011), and neurite outgrowth (Billnitzer et al., 2013; Milward et al., 1992; Small et al., 1994; Young-Pearse et al., 2008). As many of these processes are integral to neurogenesis, APP may in fact be an important regulator of neuronal production and maturation in the adult CNS.

In the adult mouse brain, neurogenesis continues in two niches: the subventricular zone (SVZ) of the lateral ventricles, and the subgranular zone of the hippocampal dentate gyrus. Each niche contains neural stem cells that are relatively quiescent, but divide to generate progenitor cells, which proliferate rapidly to produce neuroblasts (reviewed by Bonds et al., 2015). Neural stem cells in the SVZ are heterogeneous (Young et al., 2007; Merkle et al., 2007), but the neuroblasts born here collectively migrate through the rostral migratory stream to the olfactory bulb, and differentiate into deep and superficial granule neurons, as well as calretinin, calbindin and tyrosine hydroxylase-positive periglomerular interneurons (Sakamoto et al., 2014). By contrast neural stem cells in the subgranular zone of the dentate gyrus give rise to neuroblasts which mature locally, moving only as far as the granular cell layer, before they elaborate dendrites into the molecular layer and project an axon through the hilus to synapse with neurons in CA3 of the hippocampus (Deng et al., 2010). In both CNS regions the immature

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neurons are highly plastic, and are important for learning and memory, as well as mood and pattern separation (reviewed by Cameron and Glover, 2015).

There is significant evidence to suggest that APP (or its cleavage products) plays a role in regulating adult neurogenesis. For example, adult mice carrying a human mutant form of APP ($APP_{Indiana}$), initially have a normal number of new cells born in the dentate gyrus, but by one year of age proliferation is reduced relative to wildtype mice (Donovan et al., 2006). However, since a similar proportion of newly generated cells survive in $APP_{Indiana}$ and wildtype mice, this mutation has no impact on cell survival (Donovan et al., 2006). By contrast, mice carrying an alternative human mutant form of APP ($APP_{Swedish}$) have fewer proliferating ($BrdU^+$) cells in the dentate gyrus (Dong et al., 2004; Haughey et al., 2002b) and subventricular zone (Haughey et al., 2002a) relative to wildtype mice, but more of the new cells survive (Ermini et al., 2008; Mirochnic et al., 2009), such that $APP_{Swedish}$ mice ultimately accumulate more new dentate granule neurons than wildtype mice (Ermini et al., 2008). Mice carrying both of these mutations ($APP_{Swedish/Indiana}$) have a different phenotype again, with neural stem and progenitor cell proliferation becoming abnormally elevated by middle age (Gan et al., 2008; Jin et al., 2004). While these findings appear contradictory, each transgenic mouse line expresses a different mutant form of human APP and has a different amyloidogenic profile and potential gain of function phenotypes.

The influence that mutant forms of APP have on neurogenesis may be highly relevant to Alzheimer's dementia, but may not bear any resemblance to the normal physiological function of APP in the CNS. To address this, our experiments instead examine neurogenesis in *APP*-knockout (*APPKO*) and C57bl6 control mice, and reveal that the lifelong absence of *APP* influences adult neurogenesis by regulating neuronal survival and maturation.

2. Materials and methods

2.1. Transgenic mice

APPKO (Zheng et al., 1995; stock number 004133) and C57bl6 wildtype mice were purchased from the Jackson Laboratory (Bar Harbor, ME). The *APPKO* mice were initially bred with the C57bl6 mice before being inbred to produce *APPKO* homozygous mice for experiments. *Nestin-CreER^{T2}* transgenic mice were a kind gift from Ryoichiro Kageyama (Imayoshi et al., 2006). *Nestin-CreER^{T2}* mice were crossed with a Cre-sensitive reporter strain, *Rosa26-yellow fluorescent protein* (*Rosa26-YFP*; Srinivas et al., 2001; Jackson Laboratory) to generate *Nestin-CreER^{T2}::Rosa26-YFP* offspring, and further mated to generate *Nestin-CreER^{T2}::Rosa26-YFP::APPKO* transgenic mice. All mice are on a C57bl6 background, and were housed in individually ventilated cages at 22 °C on a 12 h light-dark cycle. Animals of the same sex were housed in groups of 2–4 to avoid any effect of social isolation. Food and water were available *ad libitum*. All experiments using animals were approved by the University of Tasmania Animal Ethics Committee.

2.2. Genotyping

Ear biopsies were processed to extract genomic DNA as previously described (Auderset et al., 2016). The PCR was performed as a 25 μL reaction containing 50–100 ng DNA, 0.5 μL of each primer (100 nmol/mL; GeneWorks), 12.5 μL GoTaq® green master mix (Promega) in MilliQ water. The *APPKO* PCR amplified a 250 bp product corresponding to wildtype *APP* and a 470 bp product indicating the absence of *APP*, and was carried out using the following primers: APP forward CTGCT GCAGG TGGCT CTGCA; APP reverse CAGCT CTATA CAAGC AAACA AG; APP knockout forward CCATT GCTCA GCGGT GCTG under the following conditions: 94 °C for 4′, followed by 34 cycles of 94 °C for 30″, 62 °C for 45″, and 72 °C for 60″, and a final 10 min at 72 °C. To genotype mice expressing the *Rosa26-YFP* transgene we used three primers: Rosa26 wildtype forward AAAGTC GCTCT GAGTT GTTAT, Rosa26 wildtype reverse GGAGC GGGAG AAATG GATATG and Rosa26 YFP forward GCGAA GAGTTT GTCCT CAACC in a program of: 94 °C4′, and 37 cycles of 94 °C for 30″, 60 °C for 45″, and 72 °C for 60″, followed by 72 °C for 10 min. Expression of the *Nestin-CreER^{T2}* transgene was determined using the PCR program detailed for *APPKO* genotyping and the following primers: Nestin-Cre forward TTCCG CTGGG TCACT GTCGC CGCTAC and Nestin-Cre reverse TAATC GCGAA CATCT TCAGG TTCTGC.

2.3. Primary neurosphere culture

Primary neurosphere cultures were derived from the SVZ of adult mice as previously described (Young et al., 2007). Briefly, the SVZ was dissected and incubated for 10 min in 3 mL of digest buffer [HBSS/30% (v/v) TrypLE Express containing EDTA (Gibco, 12604-013)/100 units/mL of penicillin and streptomycin (Gibco, 15140-122)/0.01 ng/mL DNase (Sigma, D5025)]. The trypsin was inactivated by the addition of 3 mL of trypsin inhibitor [HBSS/0.14 mg/mL Trypsin inhibitor (IC10111380, ICN Biomedicals)/1% (v/v) DNAse solution], the sample centrifuged at 100*g* for 10 min, and the medium removed. The tissue was triturated in 1 mL of HBSS, passed through a 40 μm cell strainer (BD Biosciences, North Ryde, Australia), and then centrifuged at 100*g* for 10 min. The supernatant was removed, and the pellet resuspended in proliferation medium [DMEM/F12 containing 2% (v/v) B27 supplement (Gibco, 17504-044), 100 units/mL penicillin/streptomycin, 20 ng/mL human basic fibroblast growth factor (Abcam, Ab9569) and 20 ng/mL human epidermal growth factor (Peprotech, AF-100-15)]. The resulting cell suspension was plated across a 6 well plate. All cultures were incubated in a humidified incubator at 37 °C/5% CO_2 for 7 days until the neurospheres were fully grown (~100 μm in diameter).

2.4. EdU administration

5-Ethynyl-2′-deoxyuridine (EdU; Invitrogen) is a thymidine analogue which is incorporated into the DNA of dividing cells during S phase of the cell cycle. EdU was reconstituted at 5 mg/mL in phosphate-buffered saline (PBS) and filter sterilised before being administered to mice at a dose of 25 mg/kg by intraperitoneal injection. To acutely label dividing cells, mice received three consecutive injections, 2 h apart, and were perfused with 4% paraformaldehyde (Sigma) (w/v) in PBS, 2 h after the final injection. To trace newly generated cells over a longer time period, EdU was instead administered *via* the drinking water *ad libitum* as previously described (Clarke et al., 2012; 0.2 mg/mL).

2.5. Tamoxifen administration

Tamoxifen (Sigma) was reconstituted to 40 mg/mL in corn oil and sonicated for 1 h until dissolved. Mice received a dose of 300 mg/kg, daily for 4 consecutive days, starting from postnatal day 75. Mice were perfusion fixed with 4% paraformaldehyde (w/v) in PBS 30 days after the initial dose (P75 + 30).

2.6. Tissue preparation for histology

After perfusion fixation the brains were removed, sliced into 2 mm thick coronal slices using a brain matrix, and immersion fixed overnight at 4 °C. Brain tissue was cryopreserved in 20% sucrose (w/v) in PBS overnight at 4 °C prior to embedding in optimal cutting temperature (OCT) cryomatrix (Thermo Scientific). Tissue was stored at −80 °C until use.

2.7. Immunohistochemistry

Coronal cryosections (30 μm) were collected as floating sections from brain bregma levels: 3.8 mm (olfactory bulb), 2.2 mm (rostral migratory stream), 0.8 mm (subventricular zone), and −1.8 mm