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A diacylglycerol lipase-CB2 cannabinoid pathway regulates adult subventricular zone neurogenesis in an age-dependent manner

Maria Beatriz Goncalves [a], Philipp Suetterlin [a], Ping Yip [a], Francisco Molina-Holgado [a], Deborah J. Walker [a], Madeleine J. Oudin [a], Marc P. Zentar [a], Steven Pollard [b], Rafael J. Yáñez-Muñoz [c], Gareth Williams [a], Frank S. Walsh [d], Menelas N. Pangalos [d], Patrick Doherty [a,*]

[a] Wolfson Centre for Age-Related Diseases, King's College, London SE1 9RT, UK
[b] Wellcome Trust Centre for Stem Cell Research, University of Cambridge, Tennis Court Road, Cambridge, CB2 1QR, UK
[c] School of Biological Sciences, Royal Holloway University of London, Egham TW20 0EX, UK
[d] Neuroscience Discovery, Wyeth Research, Princeton, NJ 08543, USA

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ABSTRACT

The subventricular zone (SVZ) is a major site of neurogenesis in the adult. We now show that ependymal and proliferating cells in the adult mouse SVZ express diacylglycerol lipases (DAGLs), enzymes that synthesise a CB1/CB2 cannabinoid receptor ligand. DAGL and CB2 antagonists inhibit the proliferation of cultured neural stem cells, and the proliferation of progenitor cells in young animals. Furthermore, CB2 agonists stimulate progenitor cell proliferation in vivo, with this effect being more pronounced in older animals. A similar response was seen with a fatty acid amide hydrolase (FAAH) inhibitor that limits degradation of endocannabinoids. The effects on proliferation were mirrored in changes in the number of neuroblasts migrating from the SVZ to the olfactory bulb (OB). In this context, CB2 antagonists reduced the number of newborn neurons appearing in the OB in the young adult animals while CB2 agonists stimulated this in older animals. These data identify CB2 receptor agonists and FAAH inhibitors as agents that can counteract the naturally observed decline in adult neurogenesis that is associated with ageing.

Introduction

Determining where ligands for cannabinoid receptors are made is important for understanding the function of the endocannabinoid signalling system. 2-arachidonylglycerol (2-AG) is a ligand for the CB1/CB2 cannabinoid receptors (Stella et al., 1997; Sugiura et al., 1999) and two closely related diacylglycerol lipases (DAGLα and DAGLβ) that synthesise 2-AG have now been cloned (Bisogno et al., 2003). During development DAGL and CB1 transcripts can be found in newly born neurons (Begbie et al., 2004; Watson et al., 2008) with their protein products expressed in growing axons (Bisogno et al., 2003). Here DAGL and CB1 receptor function are required for the normal development of fasciculated axonal tracts (Brittis et al., 1996; Watson et al., 2008), a phenotype that is consistent with adhesion molecules promoting axonal growth by activating an FGF receptor/DAGL/CB1 signalling cascade in neuronal growth cones (Archer et al., 1999; Walsh and Doherty, 1996; Williams et al., 1994, 2003).

In the adult brain there is a requirement for the post-synaptic synthesis of an endocannabinoid that can function as a retrograde synaptic messenger (Wilson and Nicoll, 2002). Here, DAGLα and DAGLβ expression in neurons is restricted to dendrites, suggesting that they contribute to this endocannabinoid function (Bisogno et al., 2003). In support, DAGLα is enriched in dendritic spines at CB1-positive synapses throughout the adult nervous system (Lafourcade et al., 2007; Uchigashima et al., 2007; Yoshida et al., 2006). Thus, a switch in the expression for of the DAGLs from growing axons to dendrites allows for two fundamentally different endocannabinoid functions.

We now report the expression of DAGLs by ependymal and proliferating cells in the adult subventricular zone (SVZ). Here, neural stem cells generate rapidly dividing progenitors that migrate along the rostral migratory stream (RMS) and populate the olfactory bulb (OB) with new neurons (Altman, 1969; Kornack and Rakic, 2001; Lois and Alvarez-Buylla, 1994). SVZ neurogenesis is of additional interest in that it is modulated in neurodegenerative disease states and can act as a source of new neurons that can migrate to sites of injury (Alvarez-Buylla et al., 2000; Arvidsson et al., 2002; Curtis et al., 2007).

CB1 and CB2 receptors can modulate neural stem cell proliferation in culture (Molina-Holgado et al., 2007; Rueda et al., 2002) and/or in adult mice (Aguado et al., 2005; Jiang et al., 2005; Jin et al., 2004; Palazuelos et al., 2006). However, the role of DAGL activity and CB2 function has not been investigated in the SVZ. We now show that in the young adult, DAGL and CB2 antagonists inhibit cell proliferation in the SVZ, and that this is associated with a reduction in the appearance

* Corresponding author. Fax: +44 207 848 6816.
E-mail address: patrick.Doherty@kcl.ac.uk (P. Doherty).

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of new neurons in the OB. Furthermore, CB2 agonists stimulate cell proliferation in the SVZ of older animals and the appearance of new neurons in the OB. A similar effect on cell proliferation can be induced by inhibiting the activity of fatty acid amide hydrolase (FAAH), an enzyme involved in the breakdown of endocannabinoids (McKinney and Cravatt, 2005). This effect is also inhibited by a CB2 receptor antagonist. In contrast to the key role for the CB2 receptor, we find no evidence to support a role for the CB1 receptor in adult SVZ neurogenesis. Overall these data suggest that a rundown in endocannabinoid tone might be responsible for the reduced SVZ neurogenesis that is seen in older animals, and provide proof-of-concept evidence for CB2 receptors and FAAH as potential therapeutic targets to counteract this age-related phenomenon.

Results

DAGL expression in the lateral wall of the ventricle in the SVZ and cultured neural stem cells

Recent evidence suggests that endocannabinoid signalling plays a role in neurogenesis in the adult SVZ (see Introduction), however little is known concerning the ability of the various cell types within the region to make ligands for cannabinoid receptors. In order to determine where 2-AG might be made in the lateral ventricle we labelled coronal sections of adult mouse brain with affinity purified antibodies to DAGLα. We found that DAGLα is highly expressed in essentially all of the ependymal cells that line the walls of the ventricle (Fig. 1A). Interestingly, whereas expression was restricted to a single cell layer in one wall of the ventricle (single arrow in Fig. 1A), expression was seen in adjacent cell layers in the lateral wall of the ventricle (double arrow in Fig. 1A). Proliferating progenitor cells in the SVZ can be identified by use of markers such as Ki-67 or BrdU (Kee et al., 2002). In young (~6-week) and old (~6-month) mice we found that essentially all of the Ki-67 positive cells in the lateral wall of the SVZ clearly expressed DAGLα (e.g. in 6 week old mice, out of a total of 327 Ki-67 positive cells counted in 3 animals 323 clearly expressed DAGLα — also see Fig. 1B). We also treated young mice for 7 consecutive days with BrdU to label slowly proliferating cells within the

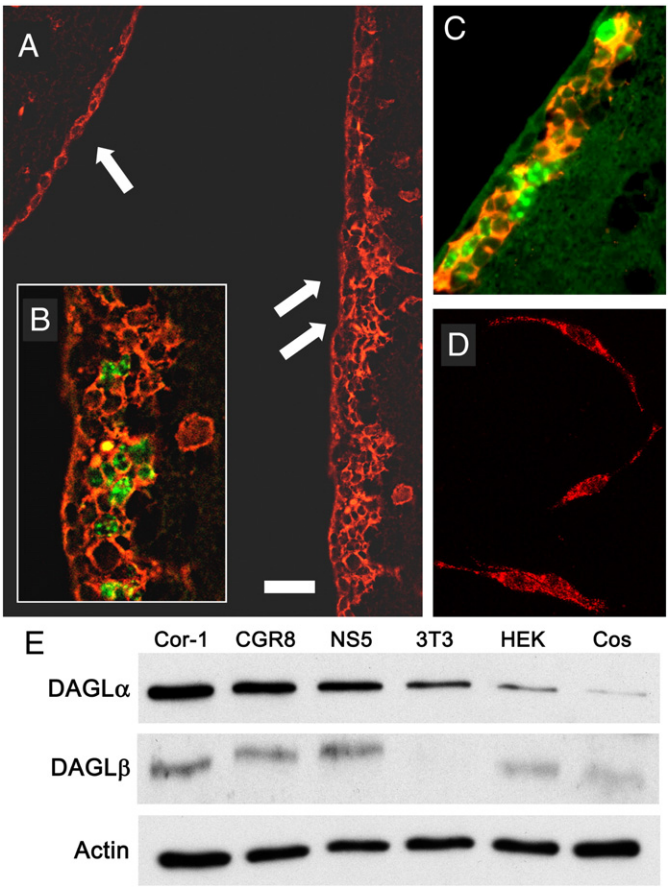

Fig. 1. Expression of DAGLα in the SVZ and in cultured cells. In (A) DAGLα localisation is shown in the SVZ region in a 6-month-old mouse. Single arrow shows the ependymal cells lining the ventricle, and the double arrow shows the lateral wall of the ventricle. The inset (B) shows a higher magnification region of the lateral wall double stained for DAGLα (red) and Ki-67 (green). A representative example of a double stain for DCX (red) and Ki-67 (green) is shown in (C). In (D) DAGLα expression is shown in the COR1 neural stem cell line. In (E) western blot analysis for DAGLα, DAGLβ and actin is shown (as indicated) for three independent neural stem cells lines, and in 3T3, HEK and COS-7 cells. Scale bar is 40 μm.

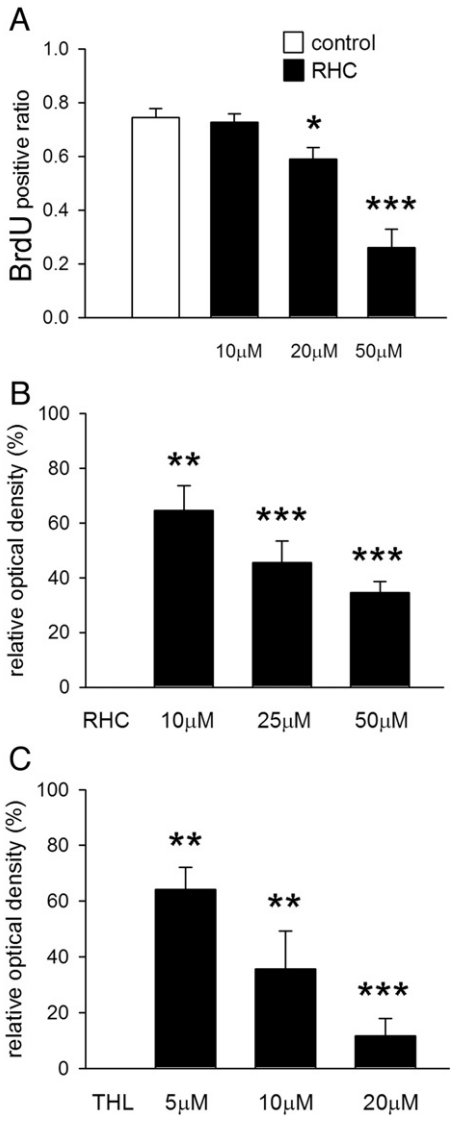

Fig. 2. DAGL activity is required for stem cell proliferation in culture. In (A) COR-1 cells were cultured in gelatin-coated 24-well plates at a density of 30,000 cells/well as described in the methods. After allowing cells to attach overnight, the DAGL inhibitor RHC-80276 (10, 20 or 50 μM) was added for a further 24 h period of culture. Proliferation was measured as the number of cells incorporating BrdU (10 μM, 20 h pulse) as a percentage of the number of cells labelling with Hoechst 33342 (10 μM, $t = 15$ min). This was measured using automated software in an InCell Analyser 1000 (GE Healthcare, UK). Each value is the mean from three independent cultures in a single representative experiment. In (B) and (C) RHC-80267 or THL were added as indicated to COR-1 cells (allowed to attach overnight) in individual wells of a 96-well microtitre plate. After 48 h cell number was indexed using the MTS assay and plotted as the mean relative to cultures maintained in control media (taken as 100%). Each value is the mean determined from a minimum of three independent experiments. Bars show SEM.