Neuroscience 135 (2005) 803–813

THE ROLE OF 5-HT$_{1A}$ RECEPTORS IN THE PROLIFERATION AND SURVIVAL OF PROGENITOR CELLS IN THE DENTATE GYRUS OF THE ADULT HIPPOCAMPUS AND THEIR REGULATION BY CORTICOIDS

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Abstract—These experiments explore the role of 5-HT$_{1A}$ receptors in the regulation of cell proliferation in the dentate gyrus of the intact and adrenalectomized adult rat. Depleting 5-HT with *p*-chlorophenylalanine (300 mg/kg initially followed by 100 mg/kg/day) or stimulating 5-HT$_{1A}$ receptors with 8-OH-DPAT (1 mg/kg or 2 mg/kg, s.c. injections twice daily) for 14 days had no effect on cell proliferation as measured by Ki-67 or BrdU (5-bromo-3-deoxyuridine) immunocytochemistry in the dentate gyrus. However, combined treatment with *p*-chlorophenylalanine followed by 8-OH-DPAT significantly increased cell proliferation compared with *p*-chlorophenylalanine alone. Micro-injection of the 5-HT neurotoxin 5,7-dihydroxytryptamine into the fimbria-fornix (3.0 μg/side) and the cingulate bundle (1.8 μg/side) depleted hippocampal 5-HT locally but did not change cell proliferation 3 weeks after the surgery. However, 8-OH-DPAT (1 mg/kg, twice daily) stimulated cell proliferation in the dentate gyrus of hippocampal 5-HT-depleted rats compared with controls. These results suggest that 5-HT$_{1A}$ modulates cell proliferation in the hippocampus by a direct post-synaptic effect. Previous studies demonstrate that adrenalectomy increases hippocampal 5-HT$_{1A}$ receptor expression and binding, and thus we investigated whether the effect of adrenalectomy on cell proliferation and survival was dependent on the activity of the 5-HT$_{1A}$ receptors. In contrast to the null effect following twice-daily s.c. injection, 8-OH-DPAT (2.0 mg/kg/day) delivered by s.c. osmotic pumps increased proliferation in intact rats. The 5-HT$_{1A}$ antagonist WAY-100635 (1.5 mg/kg/day also delivered by osmotic pump) by itself did not alter cell proliferation, confirming that reduced serotonin activity does not change proliferation, but blocked the effect of 8-OH-DPAT. However, WAY-100635 could not block the stimulating action of adrenalectomy cell proliferation. 5-HT$_{1A}$ mRNA expression was not altered in the hippocampus by adrenalectomy. Thus, the effect of adrenalectomy on cell proliferation and survival is not 5-HT$_{1A}$ dependent, despite the interaction between 5-HT$_{1A}$ and corticosterone. © 2005 Published by Elsevier Ltd on behalf of IBRO.

Key words: 5-HT$_{1A}$, corticosterone, neurogenesis, cell proliferation, hippocampus, dentate gyrus.

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Abbreviations: ADX, adrenalectomized/adrenalectomy; ANOVA, analysis of variance; BrdU, 5-brom-3-deoxyuridine; DCX, doublecortin; FF, fimbria-fornix; PCPA, *p*-chlorophenylalanine; 5,7-DHT, 5,7-dihydroxytryptamine.

0306-4522/05$30.00+0.00 © 2005 Published by Elsevier Ltd on behalf of IBRO.
doi:10.1016/j.neuroscience.2005.05.056

The formation of new cells, principally neurons, in the dentate gyrus of the adult hippocampus is regulated powerfully by adrenal corticoids (Cameron and Gould, 1994). Adrenalectomy (ADX) increases proliferation rates, as well as the proportion of new cells that survive 28 days after BrdU (5-bromo-3-deoxyuridine) labeling, whereas excess glucocorticoid (e.g. corticosterone) has the opposite effect (Wong and Herbert, 2004). Serotonin is a second potent factor known to regulate neurogenesis. New neurons have been postulated as being important in the anxiolytic action of antidepressants via 5-HT$_{1A}$ receptors (Malberg and Duman, 2003; Santarelli et al., 2003). However, there are conflicting accounts about whether lowering serotonin in the brain changes proliferation rates. Some report that this lowers mitotic activity (Brezun and Daszuta, 1999, 2000), but we have not been able to confirm this (Huang and Herbert, 2005). Nevertheless, there is general agreement that drugs increasing serotonin activity (e.g. SSRIs such as fluoxetine) also increase cell division in the dentate gyrus (Malberg et al., 2000). In particular, activation of 5-HT$_{1A}$ receptors by the agonist 8-OH-DPAT has been reported to increase cell proliferation (Santarelli et al., 2003; Banasr et al., 2004), whereas blockade of 5-HT$_{1A}$ receptors resulted in reduced cell proliferation (Radley and Jacobs, 2002).

There is much evidence to support multiple interactions between corticoids and 5-HT$_{1A}$ (Chaouloff, 1995; Gould, 1999). For example, corticosterone has been shown to decrease the expression of 5-HT$_{1A}$ receptors in the dentate gyrus (Meijer and de Kloet, 1994). ADX, which stimulates neurogenesis, has been reported to increase 5-HT$_{1A}$ receptor binding and mRNA expression in the hippocampus (Chalmers et al., 1993; Kuroda et al., 1994; Tejani-Butt and Labow, 1994). Stress, which decreases neurogenesis (Gould et al., 1997), has been shown to decrease 5-HT$_{1A}$ receptor mRNA expression (Lopez et al., 1998).

There is thus a significant question over whether regulation of the cell proliferation and survival by corticoids depends on 5-HT$_{1A}$ receptor activity. In this paper, we investigated the role of 5-HT$_{1A}$ receptors in the regulation of proliferation rates, and then determined whether these receptors are implicated in the effect of corticoid withdrawal (ADX) on cell division. First, we carried out a series of experiments to demonstrate the role of hippocampal 5-HT$_{1A}$ receptors on cell proliferation in both intact and 5-HT depleted rats. Then, we examined whether the ADX effect on cell proliferation and survival in the dentate gyrus is still apparent in the 5-HT$_{1A}$ receptor-blockaded animals

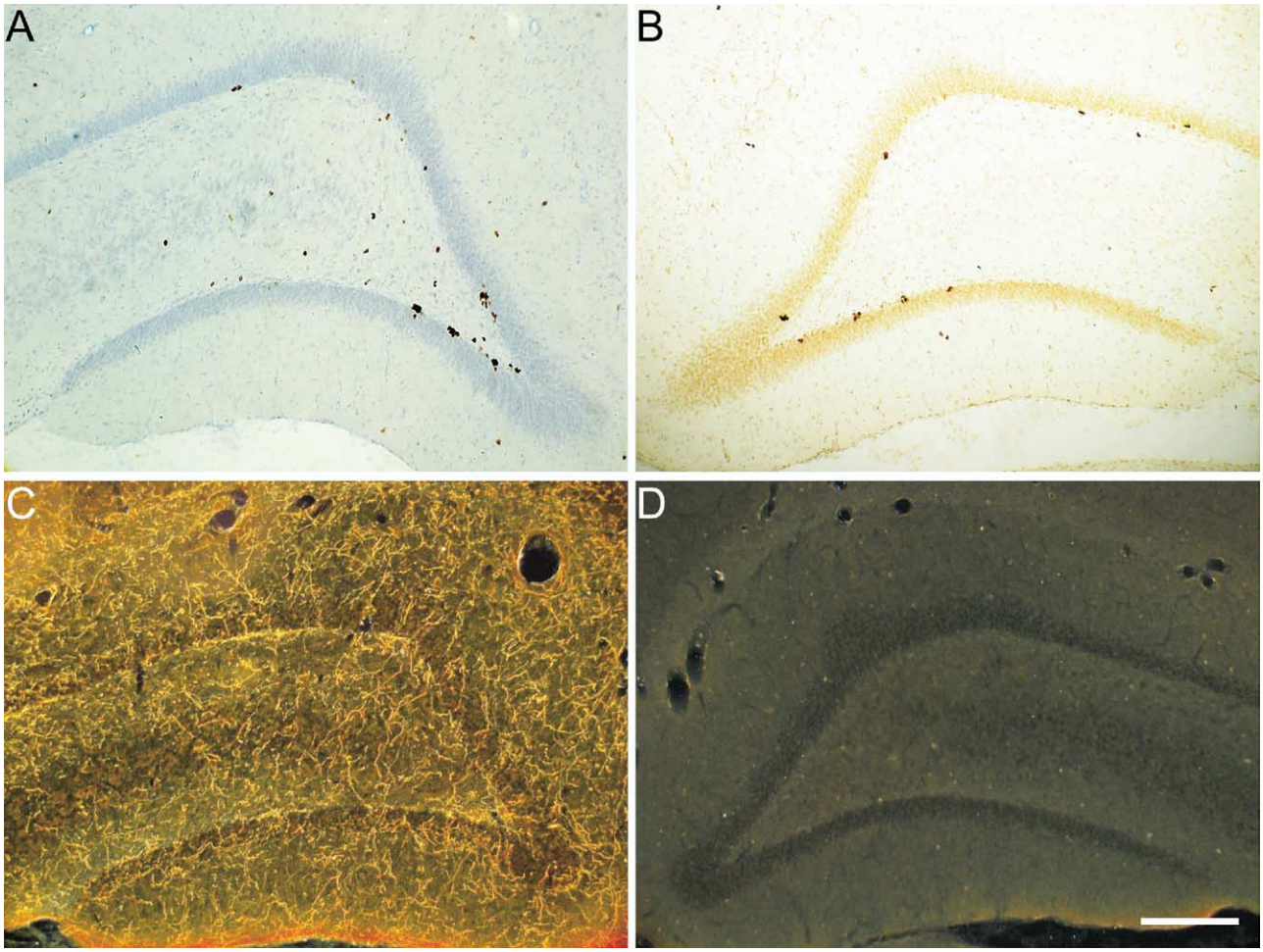

Fig. 1. (A, B) Image from the dentate gyrus stained for Ki-67 (A) and BrdU (B). (C, D) Immunostaining for 5-HT (dark field) in intact (C) and 14 days PCPA-treated (D) animals. Scale bar=0.2 mm.

to determine whether the effect of corticoid withdrawal is 5-HT_{1A} receptor dependent.

EXPERIMENTAL PROCEDURES

Animals

All procedures were carried out under Home Office (UK) licence and met the requirements to minimise numbers of animals and experimental suffering. Lister hooded male rats (Charles River, UK), weighing around 250–300 g (around 9–10 weeks old) at the beginning of the experiment, were used. Rats were housed three per cage on reversed 12-h light/dark cycles (lights off at 11:00 h). Ambient temperature was maintained at 21±2 °C. Food and water were available *ad libitum*. All ADX and sham surgery animals were given free access to both tap water and 0.9% saline.

Experiment 1. The effect of 5-HT_{1A} agonist (8-OH-DPAT) on cell proliferation in the dentate gyrus

Thirty-six intact male rats (n=6 in each final group) were used. Half of them (n=18) were depleted of 5-HT by *p*-chlorophenylalanine (PCPA) (Sigma, UK; once daily, initially 300 mg/kg, followed by 100 mg/kg, i.p.) for 13 days. The others received saline. The two groups were further subdivided into three: equal numbers of animals from 5-HT-depleted or control groups received 8-OH-DPAT (Sigma; twice daily, 0 mg/kg, 1 mg/kg, or 2 mg/kg s.c starting at 11:00 h, 12 h interval). The last PCPA injection was given 30 h and the last 8-OH-DPAT was given 6 h before killing. BrdU (Sigma; 200 mg/kg i.p) was given 24 h prior to killing.

Experiment 2a. The effect of depleting hippocampal 5-HT by local injections of 5,7-dihydroxytryptamine (5,7-DHT)

To explore the role of hippocampal 5-HT in cell proliferation, two groups of six intact rats were used. All animals were pre-treated with i.p. injections of 15 mg/kg nomifensine maleate (Sigma) and 20 mg/kg desipramine (Sigma) 20 min before surgery to prevent the effect of 5,7-DHT on tissue levels of dopamine (DA) and noradrenaline (NA). Surgery was performed under isoflurane anesthesia. The lesion group was infused with either 9.5 μg of 5,7-DHT (Fluka, UK; in 0.76 μL of saline containing 0.1% ascorbic acid) or its vehicle (0.8 μL) into two sites: the fimbria-fornix (FF, 0.24 μL) and cingulate bundle (CB, 0.14 μL). All injections were performed stereotaxically using a 1-μL Hamilton syringe. The cannula was inclined 10° from the vertical in the frontal plane. Coordinates from bregma (skull surface) were: 1.0 mm posterior, 1.4 mm lateral, 2.5 mm ventral for CB, 4.5 mm ventral for FF (Azmitia et al., 1978; Lehmann et al., 2002). On day 20 post-lesion, all rats were given a single injection of BrdU (200 mg/kg), and were killed 24 h later.