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Blockade of the $GABA_B$ receptor increases neurogenesis in the ventral but not dorsal adult hippocampus: Relevance to antidepressant action

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ABSTRACT

$GABA_B$ receptor antagonists have been shown to have antidepressant-like properties in animal models and thus, could represent a novel approach for the treatment of depression. The neurobiological mechanisms underlying these effects are currently unknown. Adult hippocampal neurogenesis (the birth of new neurons) is thought to play a role in antidepressant drug action. However, the ability of $GABA_B$ receptors to modulate the proliferation and survival of newly-born cells in the adult hippocampus remains unexplored. Therefore, we investigated whether the $GABA_B$ receptor antagonist, CGP 52432, can induce antidepressant-like behaviour and increase hippocampal neurogenesis in the stress-sensitive mouse strain, BALB/c. Male mice were treated with CGP 52432 either acutely (one injection, 3; 10; 30 mg/kg, i.p.), subchronically (7 days, 3; 10 mg/kg, i.p.) or chronically (21 days, 3; 10 mg/kg, i.p.) and antidepressant-like behaviour was assessed using the forced swim test (FST). The effects of CGP 52432 on the proliferation and survival of newly-born cells in the hippocampus were assessed using BrdU immunohistochemistry. Acute, subchronic and chronic treatment with CGP 52432 induced antidepressant-like behavioural effects in the FST. Moreover, chronic but not acute or subchronic treatment with CGP 52432 increased hippocampal cell proliferation but had no effect on the survival of newly-born cells. This temporal effect is consistent with the time course for the therapeutic action of antidepressants. Interestingly, CGP 52432-induced increases in cell proliferation occurred in the ventral but not in the dorsal hippocampus. This topographical segregation concurs with the hypothesis that the ventral hippocampus is primarily involved in the regulation of stress and emotionality. Taken together, our data suggest that increased hippocampal cell proliferation is a plausible mechanism for the antidepressant-like effects of $GABA_B$ receptor antagonists following chronic but not acute treatments. Moreover, altered behavioural effects in the FST does not correlate with changes in neurogenesis.

1. Introduction

Depression is a serious disorder in today's society with the World Health Organization (WHO) predicting that by the year 2030, it will be the second leading cause of disease burden worldwide, preceded only by HIV (Mathers and Loncar, 2006). All marketed antidepressant agents are thought to induce their effects by modulation of monoaminergic neurotransmission (O'Leary et al., 2007; Wong and Licinio, 2001). Such therapies are burdened with a slow onset of action and a significantly high proportion of individuals are treatment-resistant (Nestler et al., 2002). Thus, there is a great impetus to develop non-monoaminergic based antidepressant drugs (Berton and Nestler, 2006; McKernan et al., 2009). However, drug development has been hampered because the neurobiology underlying the pathophysiology of depression and antidepressant drug action are not well understood (Berton and Nestler, 2006).

Accumulating evidence suggests an important role for the neurotransmitter γ-Aminobutyric acid (GABA) in both the pathophysiology and treatment of depression (Brambilla et al., 2003; Cryan and Kaupmann, 2005; Ghose et al., 2011; Lloyd et al., 1985; Pilc and Lloyd, 1984; Pilc and Nowak, 2005). GABA is the primary inhibitory neurotransmitter in the vertebrate CNS and hence GABAergic neurotransmission is fundamental for many physiological and psychological processes. More than a third of brain neurons use GABA for synaptic communication (Bloom and Iversen, 1971), probably

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more than any other neurotransmitter. In the brain, GABA acts at two main types of receptors: ionotropic ($GABA_A$ and $GABA_C$) and metabotropic ($GABA_B$) receptors. Clinical, preclinical and postmortem studies strongly implicate GABAergic dysfunction in depression (Mombereau et al., 2004). Proton magnetic resonance spectroscopy studies and cerebrospinal fluid measurements have reported reduced GABA levels in depressed patients (Brambilla et al., 2003; Sanacora et al., 2004). Furthermore, postmortem brain studies have demonstrated alterations in GABA synthesis and GABAergic neural circuitry (Bielau et al., 2007; Maciag et al., 2010; Rajkowska et al., 2007) in depression. Moreover, regional and specific modifications in $GABA_B$ receptor subunit expression have also been reported in postmortem brains from depressed individuals (Ghose et al., 2011). A multitude of studies have also demonstrated that $GABA_B$ receptors are modified by chronic antidepressant treatment (Cryan and Slattery, 2010; Ghose et al., 2011) and that pharmacological blockade or loss of function of the $GABA_B$ receptor results in antidepressant-like behaviour in rodent models (Mombereau et al., 2004, 2005; Nakagawa et al., 1996; Nakagawa et al., 1999; Nowak et al., 2006; Slattery et al., 2005). Taken together, this evidence suggests that the modulation of the $GABA_B$ receptor is an important target for the development of new antidepressants.

The precise mechanisms underlying the effects of antidepressants are still not well defined. However, increases in adult neurogenesis, the birth of new neurons, in the hippocampus may be one such mechanism. Chronic administration of several classes of antidepressant drugs increases the proliferation and survival of newly-born cells in the adult hippocampus (Malberg et al., 2000; Santarelli et al., 2003) and inhibition of hippocampal neurogenesis prevents the behavioural effects of chronic treatment with some antidepressant drugs (Santarelli et al., 2003). Moreover, there is some evidence that the effects of antidepressant treatments on adult hippocampal neurogenesis may be topographically segregated along the septo–temporal axis of the hippocampus (Boldrini et al., 2009; Jayatissa et al., 2006). Indeed, we recently reported that lithium, a mood stabilizer drug that is also used to augment antidepressant action increases hippocampal cell proliferation in the ventral but not dorsal hippocampus of stressed mice (O'Leary et al., 2012). This segregation of neurogenesis along the dorso–ventral axis of the hippocampus is in line with accumulating evidence that the ventral hippocampus is involved in the regulation of stress and emotionality, while the dorsal hippocampus is primarily recruited in the context of spatial learning and memory (Bannerman et al., 2004; Fanselow and Dong, 2010; Royer et al., 2010; Segal et al., 2010).

While a role for GABA in adult hippocampal neurogenesis has been well described (Ge et al., 2007; Markwardt and Overstreet-Wadiche, 2008), the role of the $GABA_B$ receptor in the modulation of neurogenesis is currently unknown. Therefore, the aim of the present study was to investigate whether the $GABA_B$ receptor antagonist, CGP 52432, affects cell proliferation and survival in the hippocampus at doses that produce antidepressant-like effects in the FST. Moreover, whether the effects of CGP 52432 on adult neurogenesis are segregated along the dorso–ventral axis of the hippocampus was also examined. Finally, it was important to test the behavioural activity of this specific $GABA_B$ receptor antagonist in the FST, the most widely used assay for assessing antidepressant-like activity, as no data exists on it.

2. Materials and methods

2.1. Animals

Male 8-week old BALB/cOLaHsd mice (Harlan Laboratories, United Kingdom) were housed in a humidity (55 ± 10%) and temperature (21 ± 1 °C) controlled room. Food and water were provided *ad libitum* on a 12/12 h light/dark cycle (lights on at 7:00 am). All experiments were conducted in accordance with the European Community Council Directive (86/609/EEC) and approved by the Animal Experimentation Ethics Committee of University College of Cork.

2.2. Experimental design

To assess the effects of acute administration of CGP 52432 on antidepressant-like behaviour (Fig. 1A), animals were injected with CGP 52432 (3 mg/kg; 10 mg/kg; 30 mg/kg; i.p.) or vehicle and tested in the forced swimming test (FST) 30 min later ($n = 10$ per each experimental group).

In the subchronic treatment ($n = 6$ per each experimental group), mice received daily injections of CGP 52432 (3 mg/kg; 10 mg/kg; i.p.) or vehicle for 7 days (Fig. 1B). On the 7th day of treatment, the mice were tested in the FST 30 min following the last drug injection (Fig. 1A).

In the chronic experiment ($n = 10$ per each experimental group), mice received daily injections of CGP 52432 (3 mg/kg; 10 mg/kg; i.p.) or vehicle for 21 days (Fig. 1B). On the 21st day of treatment, the behaviour of the mice was assessed in the FST 30 min following the last drug injection (Fig. 1A). The duration of the chronic treatment was chosen based on previous experiments (Mombereau et al., 2004).

To assess the effects of acute CGP 52432 administration on cell proliferation ($n = 6$ per each experimental group) (Fig. 1B), mice received one injection of CGP 52432 (3 mg/kg; 10 mg/kg; i.p.) or vehicle and were injected 30 min later with BrdU (5-bromo-2-deoxyuridine; Sigma-Aldrich; 75 mg/kg). Mice were deeply anaesthetized and perfused 2 h later the BrdU injection (O'Leary et al., 2012).

To assess the effects of subchronic ($n = 6$) and chronic ($n = 10$) CGP 52432 administration on cell proliferation mice received four injections of BrdU (4 × 75 mg/kg), 2 h apart to label proliferating cells as previously described (O'Leary et al., 2012). Mice were deeply anaesthetized and perfused 24 h following the last BrdU injection (O'Leary et al., 2012).

To assess the effects of chronic CGP 52432 treatment on the survival of newly-born cells (Fig. 1B), mice were injected with BrdU (4 × 75 mg/kg, every 2 h) one day prior to beginning drug treatment (day 0) and perfused 3 weeks later (O'Leary et al., 2012).

2.3. Drugs

CGP 52432 is a high affinity, commercially available specific $GABA_B$ receptor antagonist (low nanometre range), (Tocris Bioscience, Bristol, BS). CGP 52432 was freshly prepared before use by dissolving in Phosphate Buffer Saline (PBS) with brief sonication. CGP 52432 or vehicle (PBS) were administered intraperitoneally (IP) in a volume of 20 ml/kg. Doses of CGP 52432 were chosen based on their lack of effect on locomotor activity in BALB/c mice (Colombo et al., 2001). BrdU was freshly prepared by dissolving in 0.9% NaCl with brief sonication. BrdU was administered IP in a volume of 10 ml/kg.

2.4. Forced swim test (FST)

The antidepressant-like effects of acute or chronic treatment with CGP 52432 were evaluated using the FST (Fig. 1A). The test was performed in the light phase (09:00–15:00). Mice acutely treated with CGP 52432 (3 mg/kg; 10 mg/kg; 30 mg/kg) or vehicle (PBS) were habituated to the animal facility for one week prior to the

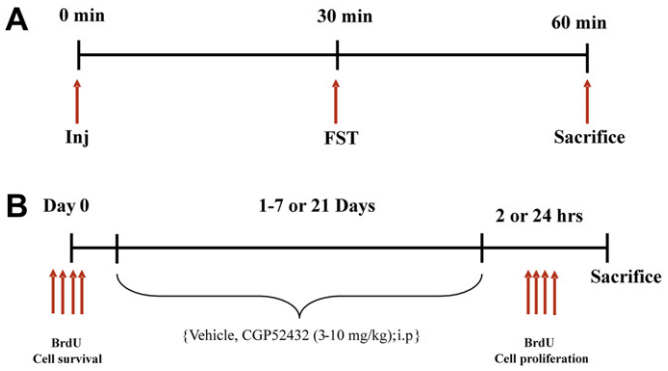

Fig. 1. Experimental design. (A) Schematic representation of FST experiment. Animals were injected with CGP 52432 30 min prior to the FST and were sacrificed 30 min following behavioural testing (B) Schematic representation of the acute, subchronic and chronic experiment. Animals were acutely (1 injection), subchronically (7 days) or chronically (21 days) treated with CGP 52432. Mice were injected with BrdU either at the end of drug treatment (cell proliferation) or one day prior to initiating drug treatment (cell survival).