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Agomelatine suppresses locomotor hyperactivity in olfactory bulbectomised rats: A comparison to melatonin and to the 5-HT_{2c} antagonist, S32006

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ABSTRACT

The novel melatonergic agonist/5-HT_{2C} antagonist agomelatine displays robust antidepressant properties in humans and is active in pre-clinical models predictive of antidepressant effects. In this study, we investigated its potential influence on the locomotor hyperactivity displayed by olfactory bulbectomised rats, a putative measure of potential antidepressant activity. In addition, we compared the actions of agomelatine to those of melatonin and S32006, a selective antagonist at 5-HT_{2C} receptors. Vehicle, agomelatine (10 and 50 mg/kg), melatonin (10 and 50 mg/kg), S32006 (0.16 mg/kg to 10 mg/kg) and the prototypical tricyclic antidepressant, imipramine (10 mg/kg), were administered by intraperitoneal injection for 14 days to male, Sprague–Dawley sham-operated and bulbectomised rats. In agreement with previous studies, imipramine was active in the model and both the lower and higher doses of agomelatine also significantly and markedly reversed the bulbectomy-induced hyperactivity to a level comparable to that seen in sham operated animals, in which agomelatine exerted no effect. Similarly the 5-HT_{2C} antagonist, S32006, dose-dependently and significantly attenuated hyperactivity of bulbectomised animals, albeit with a maximal effect somewhat less marked than that of agomelatine. On the other hand, melatonin did not affect the locomotor behaviour of bulbectomised rats. The activity of agomelatine in the model is consistent with its known antidepressant properties in the clinic.

1. Introduction

Depression is a chronic, recurrent disorder (Hirschfeld and Schatzberg, 1994; Millan, 2006). While two thirds of depressed patients may respond to initial antidepressant therapy, 10-15% will remain resistant to current treatments (Burrows et al., 1994). Non-compliance with treatment is a short coming of current medications (Zajecka, 2000). Most pharmacological interventions focus on altering serotonergic and noradrenergic neurotransmission (Norman and Olver, 2010; Olver et al., 2001). Novel approaches to the treatment of depression are needed to address some of the apparent problems of current agents (Möller, 2008; Norman, 2006).

Disruption to circadian rhythms has been proposed as an aetiological factor in depressive disorders (Bunney and Potkin, 2008; Monteleone and Maj, 2008; Norman, 2010). Cardinal features of depression (e.g., sleep disturbances, alterations in diurnal body temperature, motor activity) favour a circadian hypothesis (Duncan, 1996; Parry et al., 1989). This is supported by a positive association between the degree of disruption in the timing of sleep-wake cycles and severity of depressive symptoms (Emens et al., 2009). Re-entrainment of disturbed circadian rhythms has been found to be useful in the treatment of some depressive states (Lewy and Sack, 1989; Lewy et al., 2006).

Agomelatine is an agonist at the melatonergic MT_1 and MT_2 receptor (Audinot et al., 2003), and an antagonist at 5 HT_{2C} receptors (Millan et al., 2003, 2010). It can re-synchronise experimentally disrupted circadian rhythms (Redman et al., 1995). Agomelatine restores the circadian rest-activity cycle in depressed patients (Kasper et al., 2010). Agomelatine increases noradrenaline and dopamine in the frontal cortex, without modifications of serotonin levels (Millan et al., 2003, 2005).

In pre-clinical tests agomelatine displays activity indicative of antidepressant potential under chronic conditions. Thus in the forced swim test (FST) (Bourin et al., 2004), the learned helplessness task (Bertaina-Anglade et al., 2006), the chronic mild stress paradigm (Papp et al., 2003) and in a transgenic mouse model (Barden et al., 2005; Païzanis et al., 2010) agomelatine is active. Clinical trials have confirmed antidepressant efficacy superior to placebo (Goodwin et al., 2009; Loo et al., 2002) and at least comparable to other antidepressants (e.g., Hale et al., 2010; Kasper et al., 2010). Preclinical studies have suggested a potential synergy between the melatonin agonist and the 5-HT_{2C} antagonist properties contributing to the antidepressant activity

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(Bertaina-Anglade et al., 2006; De Bodinat et al., 2010; Papp et al., 2003).

This study examined the potential antidepressant activity of agomelatine compared to S32006, a 5-HT_{2C} antagonist (Dekeyne et al., 2008) and melatonin using the olfactory bulbectomised rat model of depression. The bulbectomy model is recognised to possess high face and predictive validity. Removal of the bulbs results in behavioural changes (e.g., irritability, impaired learning, hyperactivity in an open field) (Cairncross et al., 1979; Kelly et al., 1996)), relevant to several major clinical dimensions of depression (Marazziti et al., 2010; Millan, 2006; Song and Leonard, 1995). Changes in immune function have also been reported (Kelly et al., 1997). Behavioural changes can be reversed by chronic treatment with antidepressants (Jancsar and Leonard, 1984a,b; McGrath and Norman, 1998).

2. Materials and methods

2.1. Animals

Male Sprague Dawley rats (SPF Laboratories, Perth, Western Australia.) weighing 200-250 g at the start of the experiments were used for all studies described. Rats were housed two per cage in a 12:12 light dark cycle (lights on 7 AM, lights off 7 PM). Food and water were available *ad libitum*. All experimental procedures were performed in accordance with guidelines set down by the National Health and Medical Research Council of Australia. The studies were approved by the Austin Hospital Animal Ethics Committee (Approval Number A2000/00888 and A2005/02254).

2.2. Surgery

Following a one week acclimatisation period, during which rats were handled daily, bilateral olfactory bulbectomy was performed on rats anaesthetised with a mixture of ketamine (90 mg/kg) and xylazine (10 mg/kg). Surgery was performed as described by Cairncross et al. (1979). The head was shaved and a midline sagittal incision made extending 1 cm rostral to bregma. Two drill holes of 2 mm diameter were made in the skull, 5 mm rostral to bregma and 2 mm lateral to the midline. For sham operated animals, the dura was pierced and the wound closed. For bulbectomised animals, the olfactory bulbs were aspirated using a water suction pump, care being taken not to damage the frontal cortex. The wound was sealed with haemostatic sponge, sprinkled with oxytetracycline dusting powder (to prevent infection) and closed with Michel wound clips. (In previous studies carried out in our laboratory using this technique no infections have been observed following surgical intervention). The integrity of the surgery was confirmed at the end of the study when all animals were euthanased and the brain examined.

2.2.1. Post-operative care

Following surgery the animals were placed in clean bedding in their home cages and closely monitored until recovery from anaesthesia (usually within about 30 minutes of the surgery). The animals were maintained at approximately 35 °C for 1 hour during the recovery period to ensure no loss of body heat and a reduction in mortality from the procedure. Animals were handled daily during a two week recovery period prior to treatment with test substances.

2.3. Drugs and chemicals

Agomelatine and S32006 (N-pyridin-3-yl-1,2-dihydro-3H-benzo[e] indole-carboxamide) used in this study were supplied by Institut de Recherches Internationales Servier (I.R.I.S) and were used as received. Melatonin, imipramine hydrochloride and hydroxy ethyl cellulose (HEC) were purchased from Sigma-Aldrich (Sydney, Australia).

2.4. Drug administration

Two weeks after surgery the animals were randomly assigned to their treatment groups: Agomelatine (10 mg/kg and 50 mg/kg), imipramine (10 mg/kg) and vehicle in the first experiment; melatonin (10 mg/kg and 50 mg/kg), imipramine (10 mg/kg) and vehicle in the second experiment; S32006 (0.16 mg/kg, 0.63 mg/kg, 2.5 mg/kg and 10 mg/kg), imipramine (10 mg/kg) and vehicle in the third experiment. Drugs were suspended in 1% HEC in an injection volume of 1 ml/kg and administered by intraperitoneal (i.p.) injection daily 2 h before lights off (i.e., at 5 PM) using 26 G X ½" (0.45 X 13 mm) needles for 14 days. The choice of agomelatine, melatonin and S32006 doses was made on the basis of their activity at these doses in other animal models of depression and anxiety (Dekeyne et al., 2008; Papp et al., 2003, 2006).

2.5. Behavioural testing

The open field is essentially similar to that described by Gray and Lalljee (1974). It consists of a white circular base (90 cm diameter) which is divided into 10 cm squares by black lines. The wall (75 cm in height) surrounding the base is made of aluminium sheeting. The sole source of illumination for the field was provided by a 60 W bulb positioned 90 cm above the floor of the open field apparatus. In order to prevent shadows falling across the apparatus all testing was performed with the normal room lighting turned off. Behaviour in the field was assessed in the morning (15-17 h after the last drug administration on day 14). The test apparatus was carefully cleaned with a damp cloth after each animal was tested.

Each animal was placed in the centre of the open field and the following parameters were measured over a 3-minute testing period: Ambulation (the number of squares crossed by each rat); Rearing (the number of times a rat simultaneously raised both forepaws off the floor of the apparatus); Grooming (the number of times the animal stopped and cleaned itself); Defecation (the number of faecal boli).

2.6. Statistical analysis

Statistical tests were performed using the PRISM program (Version 5, GraphPAD Software Inc, USA). Each behavioural measure was analysed by two-way ANOVA with "Surgery" and "Drug" as between factors, followed by Student-Newman-Keuls tests.

A crude calculation of 'relative antidepressant activity' was estimated from the effect of the drugs on ambulation scores. Thus, if X is the difference in ambulation scores between mean bulbectomised vehicle and mean sham vehicle treated animals and Y is the difference between individual bulbectomised drug and mean sham drug scores then crude antidepressant activity can be obtained from (1-Y/X) * 100%. These data were analysed by one-way ANOVA followed by Dunnett's test

3. Results

3.1. Agomelatine experiment

As shown in Fig. 1, bulbectomised / vehicle rats displayed significantly higher ambulation score than sham / vehicle rats. In bulbectomised rats, 14-days chronic administration of agomelatine at the doses of 10 and 50 mg/kg significantly reduced locomotor hyperactivity to mean scores comparable to their sham treated counterparts. Imipramine (10 mg/kg) also elicited a reduction of ambulation score in bulbectomised rats. Neither agomelatine nor imipramine exerted an effect on ambulation in sham animals different from vehicle treated animals.

There was no overall effect of surgery or drug treatment or a significant interaction on rearing and grooming behaviour (data not shown). Defecation scores were significantly higher in bulbectomised