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Chronic agomelatine and fluoxetine induce antidepressant-like effects in H/Rouen mice, a genetic mouse model of depression

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ABSTRACT

The novel antidepressant agomelatine behaves as an agonist at melatonergic MT_1 and MT_2 receptors and as an antagonist at serotonin 5-HT_{2C} receptors. This study investigated the effects of agomelatine and fluoxetine in a genetic model of depression called H/Rouen mice Male and female H/Rouen (helpless line) and NH/Rouen (nonhelpless line) mice, received once daily for 3 weeks agomelatine (10 and 50 mg/kg i.p.), fluoxetine (10 mg/kg i.p.) or vehicle. Immobility duration in the tail suspension test (TST) was assessed on day 1 (D1), day 8 (D8), day 15 (D15) and day 22 (D22). Locomotor activity in a novel environment was assessed on day 18 (D18) and anhedonia (2-bottle sucrose preference test) was considered after the end of chronic treatment, from days 22 to 25. Agomelatine (50 mg/kg) significantly reduced immobility at D15 ($p<0.01$), and D22 ($p<0.001$) in treated H/Rouen mice whereas agomelatine at 10 mg/kg did not induce a statistically significant change. Fluoxetine reduced immobility at D8 ($p<0.01$), D15 ($p<0.001$) and D22 ($p<0.001$). Locomotor activity was unchanged in all treated groups as compared to vehicle groups. In the sucrose test, there was a significant decrease in sucrose preference in H/Rouen mice compared with NH/Rouen mice receiving vehicle. Both agomelatine doses (10 mg/kg ($p=0.05$) and 50 mg/kg ($p<0.001$) as well as fluoxetine ($p<0.001$) significantly increased the sucrose preference in H/Rouen mice as compared with H/Rouen mice that had received vehicle. These data indicate that the novel antidepressant agomelatine has antidepressant-like properties in H/Rouen mice, a genetic model of depression.

1. Introduction

Major depressive disorder is extremely common. It is a leading cause of disability worldwide and has a marked impact on morbidity, mortality and health care costs. In a recent epidemiological study in Western Europe, almost 13% of the population reported a lifetime history of major depressive disorder, and approximately 4% had experienced major depression in the previous 12 months (Alonso et al., 2004). Most current antidepressants interact, more or less selectively, with the monoaminergic systems, in particular the noradrenergic and the serotoninergic systems. This interaction usually takes the form of inhibition of metabolizing enzymes (e.g., monoamine oxidase inhibitors) or inhibition of reuptake systems. Despite these different mechanisms of action, all antidepressants share a slow onset of activity in humans, resulting in a delay of several weeks before maximal antidepressant efficacy is attained. This occurs despite the fact that their interaction with their targets takes place immediately after the first administration. In addition to this slow onset of activity, an additional problem is that not all patients (only around 60–70%) respond to antidepressants. These two drawbacks of antidepressant therapy act synergistically, increasing the period during which symptoms persist (Belmaker and Agam, 2008; Fava and Kendler, 2000). The major challenges for the development of novel antidepressant agents are thus to reduce the delay to maximal effect and to increase the proportion of responders. Several agents have been combined with antidepressant treatment in an attempt to increase the rate of response, increase efficacy or increase the number of responders in line with the idea of multi-target strategies for improving the treatment of depression (Millan, 2006).

Agomelatine is a new antidepressant (de Bodinat et al., 2010) which acts as a potent agonist at the melatonergic receptors MT_1 and MT_2 (Audinot et al., 2003; Yous et al., 1992) and as an antagonist at the 5-HT_{2C} receptor (Millan et al., 2003). These properties have attracted considerable attention, as depressive disorders are worsened by a disturbance of daily rhythms and sleep patterns (Benca and Peterson, 2008; Germain et al., 2008; Kasper et al., 2007; Wirz-Justice

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and Campbell, 1982). Agomelatine modulates circadian rhythms in rodents (Redman and Francis, 1998; Tuma et al., 2001). Preclinical studies have shown antidepressant-like effects of agomelatine in several animal models, such as the learned helplessness test (Bertaina-Anglade et al., 2006), the chronic mild stress (Papp et al., 2003), the forced swimming test (Bourin et al., 2004) and in a transgenic mouse model of depression (Barden et al., 2005; Paizanis et al., 2009). Antidepressant activity has also been shown in patients with major depressive disorder (Goodwin, 2009; Goodwin et al., 2009; Kennedy, 2009; Kennedy and Emsley, 2006; Loo et al., 2002; Olie and Kasper, 2007).

Depression is a multifactorial illness and genetic factors play a role in its etiology. The understanding of its physiopathology relies on the availability of experimental models potentially mimicking the disease. We have previously reported on a new model developed by selective breeding of Swiss albino outbred CD1 mice with strikingly different responses in the tail suspension test (TST), a stress paradigm aimed at screening potential antidepressants. This selection in the TST has been done with the aim of obtaining two lines of mice called 'helpless' (H/Rouen) and 'non helpless' (NH/Rouen) diverging by their high (> 115 s) and low (< 35 s) immobility scores, respectively. Each mouse of any generation that entered the study was tested three times in the TST at weekly intervals. The helpless line (H/Rouen), which is much more immobile in the TST than the so-called non helpless (NH/Rouen) line, may correspond to a genetic model of depression (El Yacoubi et al., 2003). Thus this genetic model of depression shows great stability in terms of immobility scores and has several features associated with major depressive disorder, such as anhedonia and altered serotoninergic transmission. In the present study, H/Rouen and NH/Rouen mice (from generations S20–S25) were used to compare the effects of agomelatine and fluoxetine in this model of depression.

2. Materials and methods

2.1. Animals

Mice selectively bred in the laboratory facilities for high or low spontaneous "helplessness" in the TST were derived from an original stock of Swiss albino CD1 (Charles River, France) mice (El Yacoubi et al., 2003). The chosen selection criteria, which were the same for each generation, were a high immobility score (> 115 s) for 'helpless' (H/Rouen) and a low immobility score (< 35 s) for 'non-helpless' (NH/Rouen) in the TST. Each mouse of any generation that entered the study was tested 3 times at weekly intervals. They were kept on a 7 a.m.–7 p.m. light cycle with food and water *ad libitum*. Pups were weaned at 21 ± 2 days, and animals were subjected to the first TST at age 35–50 days (middle adolescence). Unless otherwise stated, all experiments were performed with mice from generations S20 to S25 aged 9–18 weeks. For breeding, male and female mice were housed together in pairs. When not under experimentation, they were kept in same-sex groups. Testing was performed between 9 a.m. and 5 p.m. and was in accordance with the European Community Council Directive of 24 November 1986 (86/609/EEC).

2.2. Behavioral studies

2.2.1. Tail Suspension Test (TST)

The TST was performed with a computerized device (ITEM-LABO, France) which allows 6 animals to be tested at one time (Steru et al., 1987). Mice were suspended by the tail with adhesive tape to a hook connected to a strain gauge. The latter transmitted movements to a computer which calculated the total duration of immobility during a 6-min test. Mice that climbed up their tail during the test session were withdrawn from the study. The test was done on D1 (30 min after injection), D8, D15 and D22 in the afternoon at around 14 h, 21 h after the last drug injection.

2.2.2. Locomotor activity

A Digiscan actometer (Omnitech Electronics Inc., Columbus, OH, USA) monitored the horizontal (locomotion) and vertical (rearing) movements of mice. The individual compartments (L = 20; W = 20; H = 30 cm) were put in a dimly lit and quiet room. The horizontal and vertical components of locomotor activity were expressed as number of beams crossed during a 15-min period. This test was done on D18 between 9 h and 14 h corresponding to 16 h to 21 h after the previous drug administration.

2.2.3. Anhedonia (sucrose preference test)

The sucrose test was performed at the end of the experiment because mice were kept in individual cages for this experiment. In the drinking test, mice were given access during 3 days (from D22 to D25) to two bottles, one containing water and the other containing a 2% sucrose solution. Bottles were weighed everyday and their position in the cage was switched daily to prevent possible effects of side-preference in drinking behavior. The preference for sucrose was calculated as a percentage of the consumed sucrose solution relative to the total amount of liquid drunk.

2.3. Drugs

Animals were treated during 3 weeks via the intraperitoneal (i.p.) route with vehicle (HEC 1%), agomelatine 10 or 50 mg/kg (obtained from Servier, France) or fluoxetine 10 mg/kg (kindly donated by Servier, France). For agomelatine, doses were chosen based on its activity over this range of doses in previously tested models of depression such as the forced swim test (Bourin et al., 2004), the chronic mild stress (Papp et al., 2003) or the GR-i mice model of depression (Barden et al., 2005; Païzanis et al., 2010; Barden et al., 2005). For fluoxetine, 10 mg/kg was the dose shown to be active in a previous paper (El Yacoubi et al., 2003).

Treatments were carried out between 4 pm and 5 pm (2–3 h before the dark phase, at 7 pm).

2.4. Statistics

Results are expressed as means ± S.E.M. ANOVAs with or without repeated measures were followed by multiple range test comparisons with the Student–Newman–Keuls *t* test for the difference between means. Significance levels were set at $p<0.05$.

3. Results

3.1. Effects of agomelatine and of fluoxetine in the TST in H/Rouen and NH/Rouen mice

The effects of chronic (3 weeks) administration of vehicle, agomelatine or fluoxetine on the duration of immobility of H/Rouen and NH/Rouen mice in the TST are shown in Fig. 1. No interaction between time and treatment was found with two way ANOVAs with repeated measures ($F_{2,660}=1.03$, $p>0.05$), no effects ($F_{2,660}=0.8$, $p>0.05$) of fluoxetine or agomelatine were observed in NH/Rouen mice as compared with administration of vehicle after 22 days of treatment and no effect of time ($F_{4,660}=2.1$, $p>0.05$) was found.

A two way ANOVA with repeated measures was performed and an interaction between treatment effect and time effect in H/Rouen [$F(12,328=2.48$; $p<0.01$) was found. The separate one way ANOVAs revealed a significant effect of fluoxetine from Day 8 as compared to the vehicle group ($p<0.01$). At Day 15 the effect of fluoxetine ($p<0.001$) and agomelatine 50 mg/kg ($p<0.01$) differed significantly from the vehicle group. At Day 22 both fluoxetine ($p<0.001$) and agomelatine 50 mg/kg ($p<0.001$) significantly decreased the duration of immobility compared to the vehicle group. At 10 mg/kg