European Neuropsychopharmacology (2006) **16**, 538–545

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Melatonin affects the immobility time of rats in the forced swim test: The role of serotonin neurotransmission

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Received 25 August 2005; received in revised form 11 January 2006; accepted 17 January 2006

KEYWORDS
Melatonin;
Forced swim test;
Antidepressant activity;
5-HT;
Luzindole

Abstract The efficacy of melatonin or its derivatives in depressive patients has been recently considered for clinical application. However, the evidence for its effect on experimental models of depression is not consolidated. Here, the effects of melatonin on the model of forced swim test (FST) paradigm were studied in male rats of the Wistar strain after acute intraperitoneal (i.p.) administration of 0.1, 0.5 or 1 mg/kg of the hormone. Melatonin at doses of 0.5 and 1 mg/kg, but not of 0.1 mg/kg, decreased the immobility of rats in the FST paradigm suggesting a possible antidepressant-like activity. The dose of 0.5 mg/kg appeared to be as potent as clomipramine 50 mg/kg in reducing the immobility time of rats in the FST paradigm. The effect of melatonin on immobility time of rats in the FST paradigm was abolished by the simultaneous injection of the non-selective melatonin antagonist, luzindole (0.25 mg/kg, subcutaneously). Similarly, administration of small quantities of serotonin (5-HT, 5 ng/1 μl) or of the 5-HT_{2A}/5-HT_{2C} receptor agonist 1-(2,5-dimethoxy-4-iodophenyl)-2-aminopropane (2 ng/1 μl) injected into the amygdale totally suppressed the reduction of immobility time in the FST paradigm induced by melatonin 0.5 mg/kg. These results may suggest that effects of melatonin on the behavioral reaction of rats in the FST paradigm are due to an interaction of the hormone with central 5-HT neurotransmission.

1. Introduction

Melatonin, produced rhythmically by the pineal gland, is a hormone involved in the regulation of several physiological and behavioral processes, including the sleep-inducing effect and the activation of male and female sexual activity (Golombek et al., 1996; Drago et al., 1999). The synthesis of

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doi:10.1016/j.euroneuro.2006.01.005

this hormone is regulated by environmental light. As a result, high levels of melatonin are produced in vertebrates during the dark phase of the daily light/dark cycle (Coon et al., 1995). In rats, the hormone may influence explorative behaviors (Sampson and Bigelow, 1971; Chuang and Lin, 1994), stimulates sexual activity (Drago et al., 1999; Drago and Busà, 2000), antagonizes barbiturate-induced sleep (Drago et al., 2001a) and stimulates memory processes (Argyriou, 1998) with an action similar to that of antidepressant drugs (Golombek et al., 1993). Furthermore, melatonin has been reported to attenuate stress-induced gastric ulcers (Kato et al., 1997), hypothalamus—pituitary—adrenal axis dysregulation (Konakchieva et al., 1998) and stress induced sexual dysfunction (Brotto et al., 2001). These effects seem to be consistent with the physiological action of melatonin, e.g. regulation of behavioral activity of vertebrates during the dark phase of daily light/dark cycle and neutralization of the biological effects of stress (Kopp et al., 1999; Brotto et al., 2000).

In men, melatonin has been studied for a possible application in mood disorders as disturbances were evidenced in melatonin circadian profile of depressed patients (Arendt et al., 1979; Wetterberg et al., 1979). It has been suggested that the hormone possesses therapeutic benefits to individuals suffering from depression (Halbreich, 1997), although extensive controlled clinical trials are still needed to confirm these data. In patients with depressive disorders, seasonal affective disorders, sleep disorders and manic-depressive illness serum melatonin levels at night time have been reported to be greatly reduced compared to non-psychiatric patients (Beck-Friis et al., 1984; Reiter, 1991; Wetterberg et al., 1992; Costa et al., 1998; Fountoulakis et al., 2001; Pacchierotti et al., 2001). Other studies have shown that some patients with major depression have elevated melatonin concentration levels during day time and night time hours, and this melatonin secretion pattern was independent on the severity of depression (Rabe-Jablonska and Szymanska, 2001; Szymanska et al., 2001). Thus, it is still unclear which pattern of melatonin secretion may be associated to depressive symptoms. Moreover, preclinical studies have shown that melatonin exerts antidepressant-like action as assessed by the tail suspension test (TST) and the forced swim test (FST) procedures, which are considered predictive tests of antidepressant efficacy in humans (Overstreet et al., 1998; Shaji and Kulkarni, 1998; Mantovani et al., 2003). However, it is not known whether the antidepressant effects of melatonin are mediated by central neurotransmission. Indeed, the serotonin (5-HT), γ-aminobutiric acid (GABA), glutamate and nitric oxide (NO) pathways have been often considered as possible mediators of brain melatonin effects (Gaffori and Van Ree, 1985; Eison et al., 1995; Raghavendra et al., 2000; Hill et al., 2003).

It is well known that 5-HT neurotransmission plays a crucial role in depression. In particular, the 5-$HT_{1A/2A}$, 5-$HT_{1B/1D}$, 5-HT_{2C} and 5-HT_3 receptors are most involved in the disease (Cryan et al., 2005). High density of 5-HT_{2A} receptors in the brain has been implicated in the etiology and exacerbation of depression (Arora and Meltzer, 1989; Arango et al., 1990). A highly selective 5-HT_{2A} receptor antagonist, EMD 281014, produces effects in the FST resembling those of antidepressants and the pre-treatment with the 5-HT_{2A}/5-HT_{2C} receptor antagonist, ritanserin, enhances the anti-immobility effect of several tricyclic antidepressants (Redrobe and Bourin, 1997; Patel et al., 2004). Thus, the antidepressant effects of melatonin may be mediated by 5-HT_{2A} receptors as the hormone has been shown to act as a 5-HT_{2A} antagonist (Eison et al., 1995; Gorzalka et al., 1999; Raghavendra and Kulkarni, 2000). The evidence that the atypical antidepressant mianserin possesses high affinity for the 5-HT_{2C} receptors supports the concept that these receptors are also involved in the action of antidepressant drugs (Pazos et al., 1984). Consistently, the potent agonist at melatonin receptors, agomelatine, acts as an antagonist at 5-HT_{2C} receptors and induces antidepressant-like effects in several animal models such as the learned helplessness test (Bertaina-Anglade et al., 2002), chronic mild stress (Papp et al., 2003), FST (Bourin et al., 2004) and in a transgenic mouse model of depression (Barden et al., 2002). Furthermore, it has been shown that melatonin per se regulates both spontaneous efflux and evoked release of 5-HT in the hippocampus, a major target for serotonergic antidepressants (Monnet, 2002).

The present experiments were designed to study the effects of different doses of melatonin on the behavioral response of rats in the FST paradigm. Among various experimental models, this test was selected as judged to highly fulfil face, construct and predictive criteria established for experimental models of mental diseases and is widely used for pre-clinical studies on novel antidepressant drugs (Porsolt et al., 1978; Willner, 1984). In particular, the possible involvement of 5-HT neurotransmission in melatonin effects in the FST paradigm was studied. To evaluate the interaction of melatonin with 5-HT_{2A}/5-HT_{2C} receptors, drugs acting as non-selective or selective antagonists on these receptors were used.

2. Materials and methods

2.1. Animals

Male rats of the Wistar strain (purchased from Charles River, Italy) weighing 220—240 g were used throughout all experiments. For at least 1 week prior to experiments, the rats were housed four to a cage at a constant temperature (21 °C), and under a 12-h light/dark cycle (lights on between 8.00 and 20.00), with food and water available ad libitum. Animals were randomly assigned to any treatment group and were used only once in the behavioral experiments.

All experiments were carried out according to the European Community Council Directive 86/609/EEC and efforts were made to minimize animal suffering and to reduce the number of animals used. The rationale, design and methods of this study have been approved by the Ethical Committee for Animal Research, University of Catania.

2.2. Surgery

A group of male rats were subjected to the implantation of stainless steel cannulas (external diameter: 0.5 mm) into the amygdale (coordinates P3456, König and Klippel, 1963), bilaterally. The operation was performed under a ketamine/xylazine general anesthesia. All animals were sacrificed at the end of behavioral procedure. The correct insertion of the cannulas was checked in post-mortem examination injecting small quantities of Alcyan blue