Melatonin exerts an antidepressant-like effect in the tail suspension test in mice: evidence for involvement of \( N \)-methyl-\( d \)-aspartate receptors and the \( L \)-arginine-nitric oxide pathway

Michela Mantovani\(^a\), Roberto Pértile\(^a\), João B. Calixto\(^b\), Adair R.S. Santos\(^c\), Ana Lúcia S. Rodrigues\(^a,\ast\)

\(^a\)Departamento de Bioquímica, Centro de Ciências Biológicas, Universidade Federal de Santa Catarina, Florianópolis, SC 88040-900, Brazil
\(^b\)Departamento de Farmacologia, Centro de Ciências Biológicas, Universidade Federal de Santa Catarina, Florianópolis, SC 88040-900, Brazil
\(^c\)Departamento de Ciências Fisiológicas, Centro de Ciências Biológicas, Universidade Federal de Santa Catarina, Florianópolis, SC 88040-900, Brazil

Received 9 December 2002; received in revised form 27 January 2003; accepted 11 February 2003

Abstract

This study investigated the effect of melatonin in the mouse tail suspension test (TST), and the contribution of \( N \)-methyl-\( d \)-aspartate (NMDA) receptors and the \( L \)-arginine-nitric oxide (NO) pathway to its antidepressant-like effect. The immobility time in the TST was reduced by melatonin given either by intraperitoneal (0.1–30 mg/kg) or intracebroventricular (0.001–0.1 nmol/site) route. The anti-immobility effect of melatonin (1 mg/kg, intraperitoneal, i.p.) was prevented by pre-treatment with guanosine 5\(^0\)-monophosphate (GMP), ascorbic acid, \( L \)-arginine or \( S \)-nitroso-\( N \)-acetyl-penicillamine, but not with \( D \)-arginine. Pre-treatment with melatonin (100 mg/kg, i.p.) prevented the anti-immobility effect of MK-801, ketamine or zinc chloride, but did not alter the effect of imipramine. Furthermore, a sub-effective dose of melatonin (0.001 mg/kg, i.p.) produced a synergistic antidepressant-like effect with MK-801, ketamine, zinc chloride and imipramine in the TST. Taken together these data indicate that the effect of melatonin in the TST seems to be mediated through an interaction with NMDA receptors and the \( L \)-arginine-NO pathway.

© 2003 Elsevier Science Ireland Ltd. All rights reserved.

Keywords: Depression; Melatonin; NMDA; \( L \)-Arginine; Nitric oxide; Tail suspension test

Melatonin is a neurohormone produced mainly by the pineal gland from the amino acid precursor \( L \)-tryptophan in most vertebrate species, including humans. Despite a great number of studies, the physiological significance of melatonin is not yet fully understood [11]. Apart from its known involvement in circadian rhythms, melatonin is thought to influence physiological and behavioural processes, as well as neuroendocrine function [9]. Melatonin has been implicated in several pathological states, including psychiatric disorders. The majority of the studies dealing with the secretory pattern of melatonin in depressed patients have shown that the daily secretion of this hormone is reduced [11]. Moreover, preclinical studies indicate that melatonin possesses putative antidepressant properties. For example, it has been shown that melatonin exerts an antidepressant-like action when assessed in the tail suspension test (TST) and forced swimming test (FST), which are animal models predictive of antidepressant action of drugs in humans [13,14,18]. However, the mechanisms underlying the antidepressant effect of melatonin remain unclear.

Recent evidence indicates that the \( N \)-methyl-\( d \)-aspartate (NMDA) receptor complex is involved in the pathogenesis of depression, as shown by the fact that NMDA receptor antagonists exert antidepressant-like effects both in preclinical and clinical tests [16]. Moreover, there are findings indicating that the \( L \)-arginine-nitric oxide pathway is also involved in the modulation of depression [3,19]. Considering that melatonin possesses an antidepressant-like effect in animal models, and since it has been shown to block some of the excitatory effects elicited by glutamate itself and its receptor agonists [5,8] as well as inhibiting cerebellar nitric...
oxide synthase (NOS) activity [12], the present study sought to investigate the involvement of NMDA receptors and the L-arginine-nitric oxide pathway in the antidepressant-like action of melatonin in the TST in mice.

Male Swiss mice (35–45 g) were maintained at 22–27 °C with free access to water and food, under a 12:12 h light:dark cycle (lights on at 07:00 h). All manipulations were carried out between 11:00 and 16:00 h, with each animal used only once. The experiments were performed after approval of the protocol by the institutional Ethics Committee and all efforts were made to minimise animals suffering. Melatonin or vehicle was administered by the intraperitoneal (i.p.) or the intracerebroventricular (i.c.v.) route 30 or 15 min, respectively, before the TST or open-field test. In the experiments designed to verify the involvement of the glutamatergic system and the L-arginine-nitric oxide pathway in the mechanisms underlying the antidepressant-like effect of melatonin in the TST, mice were pre-treated with vehicle (10 ml/kg, i.p., control group) or with GMP (250 mg/kg, i.p.), ascorbic acid (100 mg/kg, i.p.), L-arginine (750 mg/kg, i.p.), D-arginine (750 mg/kg, i.p.) or with S-nitroso-N-acetyl-penicillamine (SNAP; 25 μg/site, i.c.v.). Thirty min after GMP, ascorbic acid, L-arginine, D-arginine, or 15 min after SNAP administration, melatonin (1 mg/kg, i.p.) or vehicle was injected, and 30 min later the TST was carried out.

To test the hypothesis that the antidepressant-like effect of melatonin is mediated through the inhibition of NMDA receptors and the modulation of NOS activity, animals received a dose of melatonin which is inactive in the TST (100 mg/kg, i.p.) 20 min before the administration of active doses of MK-801 (0.01 mg/kg, i.p.), ketamine (1 mg/kg, i.p.), zinc chloride (5 mg/kg, i.p.) or imipramine (15 mg/kg, i.p.); a further 30 min were allowed to elapse before the animals were tested in the TST.

In another set of experiments, we also investigated the synergistic effect of melatonin (0.001 mg/kg, i.p., a sub-effective dose) with sub-effective doses of MK-801 (0.001 mg/kg, i.p.), ketamine (0.1 mg/kg, i.p.), zinc chloride (5 mg/kg, i.p.) or imipramine (0.1 mg/kg, i.p.). Melatonin or saline (control) was administered 20 min before the drugs, except that melatonin was co-administered with MK-801. A further 30 min was allowed to elapse before the animals were tested in the TST.

The total duration of immobility induced by tail suspension was measured according to the method described by Steru et al. [17]. Mice both acoustically and visually isolated were suspended 50 cm above the floor by adhesive tape placed approximately 1 cm from the tip of the tail. Immobility time was recorded during a 6-min period.

The ambulatory behaviour was assessed in an open-field test as described previously [3,4,20]. The apparatus consisted of a wooden box measuring 40×60×50 cm. The floor of the arena was divided into 12 equal squares. The number of squares crossed with all paws (crossing) was counted in a 6-min session. Comparisons between experimental and control groups were performed by ANOVA followed by Duncan’s test when appropriate. A value of $P < 0.05$ was considered to be significant.

Melatonin (dose range 0.1–30 mg/kg, i.p.) significantly decreased ($P < 0.01$) the duration of immobility in comparison to the control group when mice were tested in the TST (Fig. 1A), but it did not produce any change in ambulation in mice tested in an open-field in a separate experiment (data not shown). Melatonin administered by the i.c.v. route (0.001–0.1 nmol/site) also significantly reduced ($P < 0.01$) the duration of immobility in the TST (Fig. 1B), without causing any significant change in open-field ambulation (data not shown).

The results depicted in Figs. 2A–D show that the pre-treatment of mice with GMP (250 mg/kg, i.p., a nucleotide known to block some of the actions of NMDA), ascorbic acid (100 mg/kg, i.p., a putative neuromodulator that antagonises NMDA), L-arginine (750 mg/kg, i.p., a nitric oxide precursor) or with SNAP (25 μg/site, i.c.v., a nitric oxide donor), but not D-arginine (750 mg/kg, i.p., an inactive isomer of L-arginine), significantly ($P < 0.01$) inhibited the decrease in immobility caused by melatonin (1 mg/kg, i.p.) in the TST.

Fig. 3A shows that the pre-treatment of animals with an inactive dose of melatonin (100 mg/kg, i.p.) completely prevented ($P < 0.01$) the antidepressant-like effect of MK-801 (0.01 mg/kg, i.p. a non-competitive NMDA antagonist), ketamine (1 mg/kg, i.p., a non-competitive NMDA antagonist) and zinc chloride (10 mg/kg, i.p., an inhibitor of NMDA receptors). However, the same treatment with melatonin did not alter the anti-immobility effect of imipramine (15 mg/kg, i.p., a tricyclic antidepressant). Furthermore, administration of a sub-effective dose of melatonin (0.001 mg/kg, i.p.) produced a synergistic antidepressant-like effect with MK-801, ketamine, zinc chloride and imipramine in the TST (Fig. 3B).

In the present study, we demonstrate that melatonin given systemically (i.p. route) or centrally (i.c.v. route), is effective in producing significant antidepressant-like effects when assessed in the TST. The TST is a widely accepted stress model of depression used to screen new antidepressant drugs. A wide variety of antidepressants, and compounds with potential antidepressant activity reduce the...
duration of immobility in the TST [3,4,17,20]. An antidepressant effect of melatonin in animal models was previously shown after its acute administration to mice [13, 18], and upon sub-chronic exposure to the swimming test in mice [14]. Confirming these data, S20304, a melatonin receptor agonist, produced an antidepressant-like effect when assessed in the FST in Flinders Sensitive Line rats, which have been proposed as a genetic animal model of depression [10]. Our data show that the antidepressant-like effect of melatonin is not due to a psychostimulant action of this hormone, as no alteration in the locomotor activity was found in the open-field test. In addition, the result observed in the TST after the i.c.v. administration of melatonin shows that its central antidepressant-like effect occurs at very low doses, suggesting that melatonin may be an endogenous modulator of depression.

The mechanisms underlying the antidepressant-like effect of melatonin are not well established. This study investigated the possible involvement of NMDA receptors in its action in the TST. NMDA receptor antagonists have been proposed as potential antidepressants. It is important to note that even conventional antidepressants that act through the monoaminergic system seem to ultimately affect NMDA receptors, leading to the same functional effects as NMDA antagonists [16].

The mechanism by which melatonin exerts its anti-immobility effect is probably dependent directly or indirectly on the inhibition of NMDA receptors. The following evidence substantiates this affirmation. First, the pre-treatment of mice with GMP, at a dose that produced no effect by itself in the TST [4], completely blocked the melatonin antidepressant-like effect in the same test. GMP is an endogenous guanine nucleotide that has been shown to antagonise some actions elicited by NMDA receptor activation [15]. Eckeli et al. [4] have demonstrated that acute treatments with GMP, depending on its dose, produce an antidepressant-like effect in the FST and TST, probably through the inhibition of NMDA receptors. We have recently demonstrated that GMP can reverse the antidepressant-like effect of agmatine in the forced swimming test, probably by preventing the blockade of NMDA receptors by this amine [20]. The second piece of evidence is the fact that the pre-treatment of mice with ascorbic acid also significantly prevented the antidepressant-like effect of melatonin in the TST. Ascorbic acid is thought to act as a neuromodulator in the brain, where it may alter the redox state of the NMDA receptor and, thus, block its function [6]. In fact, many reports indicate that compounds that reduce the activation of NMDA receptors have antidepressant actions. Furthermore, chronic antidepressant treatment can, in turn, impact on NMDA receptor function [16]. Some reports in the literature indicate that melatonin blocks some of the effects of glutamate and its receptor agonists, including NMDA [5,8].

The involvement of the l-arginine-nitric oxide pathway in the antidepressant-like effect of melatonin in the TST was indicated by the results showing that pre-treatment of mice with the substrate for NOS, l-arginine, at a dose previously shown not to produce any effect in the TST [3], or with SNAP, a nitric oxide donor, but not with the inactive isomer d-arginine, significantly inhibited the anti-immobility effect of melatonin. We have previously demonstrated that l-arginine, but not d-arginine, at the same dose, prevented the antidepressant-like effect of l-NNA, a NOS inhibitor [3]. This result may indicate that, at least in part, the antidepressant-like effect of melatonin could be due to the inhibition of NOS. It is well established that the activation of the NMDA receptor causes activation of NOS by an increased influx of calcium through the NMDA receptor [2]. In addition, it has been shown that distinct NOS inhibitors exert antidepressant-like effects in animal models of...
Melatonin has been shown to attenuate the neuronal NADPH-d/NOS expression in the nodose ganglion [1] and to inhibit neuronal NOS activity, possibly by acting as an antagonist of calmodulin [12].

In addition, we showed that the antidepressant-like effects of MK-801 and ketamine, two non-competitive NMDA receptor antagonists, and of zinc, which binds to an inhibitory site on the NMDA receptor were reversed by the pre-treatment of mice with an inactive dose of melatonin without affecting the action of the tricyclic antidepressant imipramine; these findings reinforce the hypothesis that this neurohormone may have a modulatory role in depression, mediated by its action on NMDA receptors. Our data suggest that the pre-treatment of animals with melatonin could block the activation of NMDA receptors, thus preventing the action on these receptors of MK-801, ketamine and zinc chloride. It is now well known that MK-801 preferentially binds to the activated NMDA receptor complex [7]. Finally, on the basis of the present results it may be speculated that melatonin might usefully supplement antidepressant therapy with imipramine.

In conclusion, our study presents some evidence for involvement of NMDA receptors and the L-arginine-NO pathway in the antidepressant-like effect of melatonin in the TST. However, the exact mechanism underlying melatonin’s antidepressant-like effect needs further exploration.

**Acknowledgements**

This study was supported by CNPq (479309/01-9 and 471271/01-2), CAPES, FUNPESQUISA-UFS and PRO-NEX.

**References**


