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Involvement of L-arginine–nitric oxide–cyclic guanosine monophosphate pathway in the antidepressant-like effect of tramadol in the rat forced swimming test

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ABSTRACT

Tramadol is a centrally acting analgesic which is used mainly for the treatment of moderate or severe pain. It is a synthetic opioid in the aminocyclohexanol group that binds weakly to μ-opioid receptors. Since it has been suggested that both opioid and monoaminergic systems play a role in depressive disorders, tramadol has been studied in the forced swimming test (FST). The present study was designed to explore the antidepressant activity of tramadol in rat FST and its possible mechanisms of action. The involvement of L-arginine–nitric oxide (NO)–cyclic guanosine monophosphate (cGMP) signaling pathway in the antidepressant action of tramadol was investigated. Treatment with tramadol, given (30 min earlier) by oral route (p.o.) at the doses of 10, 20 and 40 mg/kg, decreased immobility time in the FST. Pretreatment of rats with L-arginine (250 mg/kg, intraperitoneal, i.p., a nitric oxide precursor) or sildenafil (5 mg/kg, i.p., a phosphodiesterase 5 inhibitor, PDE5) significantly reversed the reduction in immobility time elicited by tramadol (20 mg/kg, p.o.) in the FST. Treatment of animals with a sub-effective dose of tramadol (5 mg/kg, p.o.) produced a synergistic antidepressant-like effect with N^G-nitro-L-arginine (L-NNA, 3 mg/kg, i.p., an inhibitor of nitric oxide synthase) or with 7-nitroindazole (7-NI, 9 mg/kg i.p., a specific neuronal nitric oxide synthase inhibitor) in the FST. Pretreatment of animals with methylene blue (3.75 mg/kg i.p., an inhibitor of NO synthase and soluble guanylate cyclase — sGC) or (1*H*-[1,2,4] oxadiazolo[4,3-a]quinoxalin-1-one) (ODQ, 2 mg/kg, i.p., a specific inhibitor of sGC) significantly caused a synergistic effect with a sub-effective dose of tramadol (5 mg/kg, p.o.) in the FST. In the present study, different doses of tramadol and the combination with the L-arginine–NO–cGMP pathway modulators had no effect on the locomotor activity of rats in the open-field test. Thus, our findings suggest that the acute administration of tramadol produces antidepressant-like effect in the rat FST by a mechanism that involves the inhibition of L-arginine–NO–cGMP pathway.

1. Introduction

Tramadol, (1RS, 2RS)-2-[(dimethylamine)-methyl]-1-(3-methoxyphenyl)-cyclohexanol hydrochloride, is a centrally acting analgesic which is used mainly for the treatment of moderate or severe pain. It is a synthetic opioid in the aminocyclohexanol group that binds weakly to μ-opioid receptors (Hennies et al., 1988). However, it has been shown that tramadol possesses a non-opioid mechanism that contributes to its pharmacological actions (Rojas-Corrales et al., 2005). Clinically active tramadol is a racemic mixture of two enantiomers that have two distinct but complementary mechanisms of action: the (+) tramadol is a selective agonist for μ-opioid receptor, it preferentially inhibits serotonin reuptake and enhances serotonin efflux in the brain, whereas the (−) enantiomer mainly inhibits noradrenaline reuptake (Frink et al., 1996).

Since it has been suggested that both opioidergic and monoaminergic systems play a role in depressive disorders, tramadol has been studied in the forced swimming test (FST) in mice, a test developed to predict the antidepressant action of drugs. Several case reports have illustrated a clinically effective antidepressant effect of tramadol in various depressive states (Spencer, 2000), including resistant depression (Shapira et al., 2001). In addition, the mechanism of action and structure of tramadol is very similar to that of some antidepressants such as venlafaxine. The study of Rojas-Corrales et al. (1998) has shown that tramadol displays an antidepressant-like effect in mice mediated by the noradrenergic system rather than serotonergic or opioidergic pathways. Thus, it appears probable that tramadol, in addition to its well known analgesic effect, could have a direct action on the emotional component

Abbreviations: ANOVA, analysis of variance; FST, forced swimming test; i.p., intraperitoneal; p.o., per oral; L-NNA, N^G-nitro-L-arginine; 7-NI, 7-Nitroindazole; NO, nitric oxide; sGC, soluble guanylate cyclase; ODQ, 1*H*-[1,2,4] oxadiazolo[4,3-a] quinoxalin-1-one; cGMP, cyclic guanosine monophosphate; PDE5, phosphodiesterase 5; NOS, nitric oxide synthase; SNAP, *S*-nitroso-*N*-acetylpenicillamine; DMSO, dimethylsulfoxide; L-NAME, N^G-nitro-L-arginine methyl ester; L-NPA, N^ω-propyl-L-arginine.

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of chronic pain, such as decreased affectivity and helplessness (Tejedor-Real et al., 1995; Stoll and Rueter, 1999).

L-arginine–nitric oxide (NO)–cyclic guanosine monophosphate (cGMP) is an important signaling pathway that is reported to be involved in depression (Mantovani et al., 2003). Nitric oxide, a messenger molecule in the brain, is synthesized from L-arginine by nitric oxide synthase (NOS) and has been implicated in neurotransmission, synaptic plasticity, learning, perception of pain, aggression and depression (Esplugues, 2002). In fact, some studies have also shown that NOS inhibitors display an antidepressant-like behavioural profile in the mouse FST (Harkin et al., 1999, 2004). NO donors and inhibitors have been shown to affect serotonin release in a dose-dependent manner in rodents (Lorrain and Hull, 1993; Kaehler et al., 1999). Recent studies have shown the possibility that the inhibition of NOS could be used as a strategy to enhance the clinical efficacy of serotonergic antidepressants (Harkin et al., 2004).

Therefore, based on the considerations above, the present study was performed to investigate whether tramadol causes antidepressant-like effect, employing the FST in rats. In addition, the present study attempts to investigate the participation of L-arginine–NO–cGMP pathway in the antidepressant activity of tramadol in the FST in rats.

2. Materials and methods

2.1. Animals

The behavioural experiments were conducted using male Wistar rats (180–250 g) maintained at 22–25 °C with free access to water and food, under a 12:12 h light/dark cycle, with lights on at 6:00 a.m. All manipulations were carried out between 08.00 a.m. and 04.00 p.m. All experiments were performed on separate groups of animals and each animal was used only once in each test. The animals were used according to the guidelines of the Committee on Care and Use of Experimental Animal Resources, the Federal University of Santa Maria, Brazil. All efforts were made to minimize animal suffering and to reduce the number of animals used in the experiments.

2.2. Behavioral analysis

2.2.1. Forced swimming test (FST)

The FST was performed as described by Porsolt et al. (1977) and was carried out over 2 days, i.e. a day for the pre-swimming session and a day for the test session. Briefly, in the pre-swimming session, rats were individually placed for 15 min in open cylinders (45 cm height×20 cm diameter) containing 23 cm of filled water at 25±1 °C. Twenty four hours later, rats underwent the test session. In the test session, rats were again placed in cylinders filled with water, and the duration of immobility was recorded for 5 min. Each rat was recorded as immobile when floating motionless or making only those movements necessary to keep its head above water.

2.2.2. Open-field task

To assess the possible effects of tramadol on the locomotor and exploratory activities, rats were evaluated in the open-field test. The open field was made of polywood and surrounded by walls 30 cm in height. The floor of the open field, 45 cm in length and 45 cm in width, was divided by masking tape markers into 9 squares (3 rows of 3). Each animal was placed individually at the center of the apparatus and observed for 6 min to record the locomotor (number of segments crossed with the four paws) and exploratory activities (expressed by the number of time rearing on the hind limbs) (Walsh and Cummins, 1976).

2.3. Experimental procedure

In order to assess the antidepressant-like effect of tramadol in the FST, this compound was orally administered (dose range: 5–40 mg/kg, p.o.) 30 min before the open-field and the FST tests. The locomotor activity was evaluated in rats and immediately after that the same rats were assessed in the FST.

2.4. Drugs and treatment

Tramadol,(1RS,2RS)-2-[(dimethylamine)-methyl]-1-(3-methoxyphenyl) cyclohexanol hydrochloride, was a gift from Cristália (São Paulo, Brazil). L-arginine, methylene blue, 7-nitroindazole (7-NI), sildenafil, (1*H*-[1,2,4] oxadiazolo[4,3-a]quinoxalin-1-one) (ODQ) and N^G-nitro-L-arginine (L-NNA) and all other chemicals were purchased from Sigma (St. Louis, USA). All the drugs were dissolved in saline solution (0.9% NaCl), except ODQ and 7-NI that were dissolved in 15% dimethylsulfoxide (DMSO) and were made up to final volume by addition of 0.9% NaCl. All drugs were freshly prepared. Drugs were injected in a volume of 1 ml/kg of body weight to the experimental groups. Control animals received injection of saline (0.9% NaCl) or DMSO (15% saline).

To address the role played by the L-arginine–NO–soluble guanylate cyclase pathway in the antidepressant-like effect caused by tramadol in the FST, distinct groups of animals were treated with different classes of drugs. For this purpose, rats were pretreated with L-arginine, a precursor of NO (250 mg/kg, i.p., a dose that produces no effect on the FST) (Heiberg et al., 2002) or sildenafil, a specific type 5 phosphodiesterase (PDE5) inhibitor, (5 mg/kg, i.p. a dose that produces no effect on the FST) (Kaster et al., 2005). Thirty minutes after L-arginine or sildenafil, tramadol (20 mg/kg, p.o., a dose active in the FST) or saline was injected, and 30 min later the FST was carried out.

In another set of experiments, the synergistic effect of tramadol (5 mg/kg, p.o., a sub-effective dose) with a sub-effective dose of L-NNA (3 mg/kg, i.p., an inhibitor of NOS) (Harkin et al., 2003), 7-NI (9 mg/kg, i.p., a specific neuronal NOS inhibitor) (Harkin et al., 2003), methylene blue (3.75 mg/kg, i.p., an inhibitor of NOS and sGC) (Eroglu and Caglayan, 1997) or ODQ (2 mg/kg, i.p., a specific sGC inhibitor) (Heiberg et al., 2002) were investigated. Tramadol (5 mg/kg, p.o., a sub-effective dose) or vehicle (saline) was administered 30 min before the drugs. After 30 min of the i.p. administration of L-NNA, 7-NI, methylene blue or ODQ the FST was carried out. To study the synergistic effect of tramadol and 7-NI, the control group received saline (p.o.) followed by 15% DMSO/saline, i.p.

2.5. Statistical analysis

All experimental results are given as the mean (sec) ±S.E.M. Comparisons between experimental and control groups were performed by one-way (tramadol) or two-way ANOVA (inhibitors of NOS and/or sGC×tramadol) followed by Newman–Keuls test for post hoc comparison when appropriate. A value of $P<0.05$ was considered to be significant. Main effects are presented only when the higher second order interaction was non-significant.

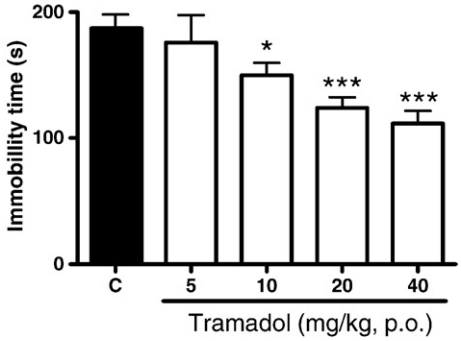

Fig. 1. Effect of tramadol on the forced swimming test in rats. Tramadol (5–40 mg/kg) was orally administered 30 min before the test. Values are expressed as mean±S.E.M. of 5–7 animals.*$P<0.05$ and ***$P<0.001$ when compared to vehicle treated group (control; C).