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The antinociceptive effect of reversible monoamine oxidase-A inhibitors in a mouse neuropathic pain model

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ABSTRACT

Neuropathic pain is a debilitating condition that is often resistant to common analgesics, such as opioids, but is sensitive to some antidepressants, an effect that seems to be mediated by spinal cord 5-HT_3 receptors. Because the analgesic potential of monoamine oxidase-A (MAO-A) inhibitors is understudied, we evaluated the potential antinociceptive effect of the reversible MAO-A inhibitors moclobemide and 2-(3,4-dimethoxy-phenyl)-4,5-dihydro-1*H*-imidazole (2-DMPI) in a mouse neuropathic pain model induced by chronic constriction injury (CCI) of the sciatic nerve. Neuropathic mice showed a decreased mechanical paw withdrawal threshold (PWT) 7 days after lesion compared with the baseline PWT, characterizing the development of hyperalgesia. Moclobemide (100–300 μmol/kg, s.c.) and 2-DMPI (30–300 μmol/kg, s.c.) treatments were able to reverse the CCI-induced hyperalgesia, with 50% inhibitory dose (ID_{50}) values of 39 (18–84) and 11 (4–33) μmol/kg, and maximum inhibition (I_{max}) values of 88 ± 14 and 98 ± 15%, respectively, at the 300 μmol/kg dose. In addition, we observed a significant increase in the MAO-A activity in the lumbar spinal cord of CCI-submitted mice compared with sham-operated animals. Furthermore, the antihyperalgesic effects of both 2-DMPI and moclobemide were largely reversed by intrathecal injection of the 5-HT_3 receptor antagonist ondansetron (10 μg/site). These results suggest a possible involvement of MAO-A in the mechanisms of neuropathic pain and a potential utility of the reversible inhibitors of MAO-A in the development of new therapeutic approaches to treat it.

1. Introduction

Neuropathic pain is a pathological condition that frequently results from a lesion or disease of the somatosensory nervous system (Treede et al., 2008). Common complaints of neuropathic pain patients are spontaneous pain and painful hypersensitivity to mechanical, thermal and/or chemical noxious (hyperalgesia) or innocuous (allodynia) stimuli (Costigan et al., 2009). The management of neuropathic pain is still a major challenge to clinicians because it is unresponsive to most of the currently used analgesic drugs (Dworkin et al., 2007; Woolf and Mannion, 1999). In this regard, some antidepressants represent useful tools in neuropathic pain treatment (Sindrup et al., 2005). In fact, tricyclic antidepressants, together with anticonvulsants, are considered to be first-line drugs for the treatment of neuropathic pain (Micó et al., 2006).

Although antidepressants have been used for the treatment of chronic pain conditions for decades, the mechanism of this action is still unclear. Many believe that the increased availability of serotonin (5-hydroxytryptamine, 5-HT) and noradrenaline in the synaptic cleft is the main mechanism of the analgesic action of antidepressants (Micó et al., 2006). Furthermore, 5-HT_3 receptor activation has been implicated in the antinociceptive effect of spinal serotonin by increasing the activity of the descending inhibitory pathways (Bardin et al., 2000), which may be compromised in chronic pain conditions (Ardid et al., 1995).

Similar to tricyclic antidepressants, monoamine oxidase (MAO) inhibitors also increase the availability of monoamines in the central nervous system (Youdim et al., 2006). Although some authors have shown the analgesic properties of reversible MAO-A inhibitors, such as moclobemide, in the treatment of nociceptive and psychogenic

Abbreviations: ANOVA, analysis of variance; CCI, chronic constriction injury; 2-DMPI, 2-(3,4-dimethoxy-phenyl)-4,5-dihydro-1H-imidazole; GABA, γ-amino butyric acid; 4-HQ, 4-hydroxyquinoline; 5-HT, 5-hydroxytryptamine; ID50, 50% inhibitory dose; Imax, maximum inhibition; MAO-A, monoamine oxidase-A; MAO-B, monoamine oxidase-B; PBS, phosphate buffered saline; PWT, paw withdrawal threshold.

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pain (Coquoz et al., 1993; Pirildar et al., 2003; Schreiber et al., 1998), there are only a few preliminary studies evaluating the therapeutic potential of these drugs for neuropathic pain and their results are controversial (Apaydin et al., 2001; Menkes et al., 1995).

Because neuropathic pain is a debilitating condition of difficult treatment and antidepressants have long been used for the treatment of this type of pain (Sindrup et al., 2005), the aims of this study were to confirm the antinociceptive potential of the reversible MAO-A inhibitor moclobemide and evaluate the effect of 2-(3,4-dimethoxyphenyl)-4,5-dihydro-1*H*-imidazole (2-DPMI), a novel reversible and preferential MAO-A inhibitor with antidepressant properties (Villarinho et al., 2012a), in a mouse neuropathic pain model. We also investigated possible changes in monoamine oxidase activity in neuropathic mice, as well as the involvement of spinal 5-HT_3 receptors in the antinociceptive effect of reversible MAO-A inhibitors.

2. Materials and methods

2.1. Animals

Experiments were conducted using male Swiss mice (25–30 g) from our own colony. Mice were maintained in polycarbonate cages with free access to food and water on a 12 h alternating light–dark schedule in a temperature-controlled (22 ± 3 °C) room. Mice were kept and used in accordance with the guidelines of the National Council for Control of Animal Experiments (CONCEA) and the National Institutes of Health Guide for the Care and Use of Laboratory Animals. All experiments pertaining to this study were approved by the Ethics Committee of the Federal University of Santa Maria (process number 64/2011). The number of animals and intensity of the noxious stimuli used were the minimum necessary to demonstrate the consistent effects of drug treatments. For behavioral tests, drugs were administered in a random order and the behavioral measure was carried out by a blinded investigator. Animals were allowed to adapt to the test environment for 2 h before testing.

2.2. Drugs

The synthesis of 2-DMPI was carried out by the previously reported method of condensation involving 3,4-dimethoxybenzaldehyde and ethylenediamine in the presence of *N*-bromosuccinimide under ultrasound irradiation (Sant'Anna et al., 2009). Analysis of ^{1}H and ^{13}C nuclear magnetic resonance spectra showed analytical and spectroscopic data in full agreement with the assigned structure. Moclobemide (Aurorix®, Roche, Brazil) and 2-DMPI were dissolved in a vehicle solution (5% Tween 80, 20% polyethyleneglycol and 75% saline) and pregabalin (Lyrica®, Pfizer, Brazil) was dissolved in sterile saline. Moclobemide, 2-DMPI and pregabalin were administered to mice by the subcutaneous (s.c.) route, while ondansetron (Nausedron®, Cristália, Brazil) was intrathecally (i.t.) injected in a volume of 5 μL, according to the technique described by Hylden and Wilcox (1980). Kynuramine dihydrobromide and selegiline hydrochloride were purchased from Sigma Chemical Co. (St. Louis, USA) and dissolved in incubation buffer. All other reagents were of analytical grade and were purchased from local suppliers.

2.3. Measurement of mechanical hyperalgesia

In this study, we used mechanical hyperalgesia as a parameter of nociception, which was characterized by a significant decrease in the mechanical paw withdrawal threshold (PWT). The measurement of mechanical PWT was carried out using the up-and-down paradigm as previously described by Chaplan et al. (1994) for rats and adapted by Villarinho et al. (2012b) for mice. Briefly, mice were first acclimatized in individual clear Plexiglas boxes (9 × 7 × 11 cm) on an elevated wire mesh platform to allow the access to the plantar surface of the right hind paw. Seven calibrated von Frey filaments of increasing stiffness (0.02, 0.07, 0.16, 0.4, 1.4, 4, and 10 g) were chosen. Testing was initiated with the 0.4 g hair, and each hair was applied perpendicularly to the plantar surface of the right hind paw, with sufficient force to bend the filament, for about 5 s. Lifting of the paw indicated a positive response and prompted the use of the next weaker (i.e. lighter) filament. Absence of a paw withdrawal response prompted the use of the next stronger (i.e. heavier) filament. This paradigm continued until a total of six measurements or until four consecutive positive or four consecutive negative responses occurred. All measurements were carried out in the paw ipsilateral to the surgical or sham procedure, and the 50% mechanical PWT response was calculated from the resulting scores as previously described by Dixon (1980). The 50% PWT was expressed in grams (g) and was evaluated before (baseline) and several times after treatments or surgical procedures.

2.4. Neuropathic pain model

For induction of chronic mononeuropathy, mice were first anesthetized by intraperitoneal injection of 90 mg/kg of ketamine plus 3 mg/kg of xylazine hydrochloride. Neuropathy was induced by chronic constriction injury (CCI) of the sciatic nerve using a similar procedure to that previously described by Bennett and Xie (1988) for rats and adapted by Sommer et al. (1998) for mice. Three loosely constrictive ligatures were placed around the right sciatic nerve under anesthesia. In sham surgeries, animals were anesthetized and the sciatic nerve was exposed without performing constriction. Sham-operated animals were used as controls.

Seven days after the procedures (sham or CCI), the nociception test was carried out at time 0 (before drug injection). Then, mice were treated with 2-DMPI (300 μmol/kg, s.c.), moclobemide (300 μmol/kg, s.c.), vehicle (10 mL/kg, s.c.), pregabalin (100 μmol/kg, s.c.), or saline (10 mL/kg, s.c.), and mechanical sensitivity was measured 1, 2, 4, 6, and 24 h after treatment (time-course curve). For the dose–response curve, CCI-submitted animals received a single injection of 2-DMPI (3, 30, or 300 μmol/kg, s.c.), moclobemide (30, 100, or 300 μmol/kg, s.c.), or vehicle 2 h before the nociceptive test. To assess the involvement of the serotonergic system in the antinociceptive effect of reversible MAO-A inhibitors, mice received an intrathecal injection of the 5-HT_3 receptor antagonist ondansetron (10 μg/site) or phosphate buffered saline (PBS; 5 μL/site), 30 min before the peak effect of the drugs (90 min after subcutaneous injection of 2-DMPI or moclobemide). The mechanical paw withdrawal threshold was measured 30 min later. The dose of ondansetron was selected based on the results of a previous study (Dogrul et al., 2012).

2.5. Determination of MAO-A activity

For MAO-A activity measurement, mice submitted to surgical procedure (sham or CCI) were killed 7 days after the surgical procedure and different central nervous system structures (lumbar spinal cord, cerebral cortex, and striatum) were collected and homogenized in assay buffer (16.8 mM Na_2HPO_4, 10.6 mM KH_2PO_4, and 3.6 mM KCl, pH 7.4). The entire lumbar spinal cord (L1 to L6) was dissected by mechanical extrusion. The MAO-A activity was measured by a fluorometric method that detects the formation of the fluorescent product 4-hydroxyquinoline (4-HQ) from kynuramine, as previously described (Matsumoto et al., 1985; Villarinho et al., 2012b). Briefly, assays were performed in a reaction mix containing assay buffer, tissue homogenates of central nervous system structures (0.25 mg of protein), the selective monoamine oxidase-B (MAO-B) inhibitor selegiline (250 nM), and kynuramine (100 μM) and were incubated at 37 °C for 30 min. The results were expressed as nmol of 4-HQ/min/mg of protein.