The antinociceptive properties of reboxetine in acute pain

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Abstract

The antinociceptive effects of the selective noradrenaline reuptake inhibitor antidepressant reboxetine and its interaction with various opioid and noradrenaline receptor subtypes were evaluated. Reboxetine (i.p.) induced a weak dose-dependent antinociceptive effect in acute pain, using the hotplate model. The reboxetine-induced antinociception was significantly inhibited by the opioid receptor antagonists naloxone, nor-BNI, naltrindole and b-FNA, implying a non-selective role for the opioid receptors in the reboxetine's antinociceptive effect. The adrenergic antagonists yohimbine and phentolamine attenuated to some extent the reboxetine-induced antinociception, implying a minor adrenergic mechanism of antinociception. The addition of opioid or $\alpha_2$ agonists, did not potentiate the antinociception effect of reboxetine. Thus, it seems that reboxetine possesses a weak antinociceptive effect, mediated by non-selective opioid receptors and influenced somewhat by noradrenaline $\alpha_2$ receptors. These results suggest that reboxetine as monotherapy does not have sufficient efficacy in the management of acute pain. However, further research is needed in order to establish its possible use alone or in combination with other antidepressants or analgesics in the amelioration of chronic pain disorders.

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1. Introduction

Tricyclic antidepressants have been used for decades in the treatment of severe pain in non-depressed patients (Spiegel et al., 1983; Tura and Tura, 1990; Mattia et al., 2002). They have proven effective both for chronic pain syndromes such as post-herpetic neuralgia (Woodford et al., 1965; Kishore-Kumar, 1990), peripheral neuropathies (Taub and Collins, 1974;
Reboxetine inhibits noradrenaline reuptake in vitro to a similar extent to the tricyclic antidepressant desmethylimipramine. It does not affect dopamine or serotonin reuptake and it has low, both in vivo and in vitro, affinity for adrenergic, muscarinic cholinergic, histaminergic, dopaminergic and serotonergic receptors (Holm and Spencer, 1999). In the present study we have assessed reboxetine’s potential antinociceptive properties using the acute pain model of the hotplate analgesia meter in mice.

2. Materials and methods

Male ICR mice from Tel-Aviv University colony (Tel-Aviv, Israel), weighing 25–35 g were used. The mice were maintained on a 12 h light:12 h dark cycle with Purina rodent chow and water available ad libitum. Animals were housed five per cage in a room maintained at 22 °C ±0.5 °C. Mice were housed in groups of 5 until testing. Mice were used only once. The Sackler Faculty of Medicine Ethical Committee for Animal Experimentation approved the experimental protocol (M-08-076), which complied with the guidelines for animal experimentation of the National Institutes of Health [DHEW Publication (NIH) 85-23, revised, 1995].

2.1. Agents

Morphine was generously donated by Teva (Jerusalem, Israel), naloxone HCL (δ-FNA), naltrindole, nor-BNI, U50,488H, and DPDPE were obtained from the Research Technology Branch of NIDA. Clonidine, yohimbine, phentolamine and reboxetine were purchased from Sigma-Aldrich Israel Ltd. (Rehovot, Israel).

2.2. Analgesia/antinociception assessment

Mice were tested with the hotplate analgesia meter Model 35D, (IITC INC. Woodland Hills, CA, USA) as previously described (Schreiber et al., 2002a, b), to determine the nociceptive threshold. The device consists of a metal plate (40 × 35 cm) heated to a constant temperature, with a plastic cylinder placed on top. The analgesic meter was set to a plate temperature of 52.0 °C ±0.5 °C. The time of latency was recorded i.e., between the second the animal was placed on the hotplate surface till it licked its back paw or jerked it strongly or jumped out. Baseline latency was determined before experimental treatment for each mouse as the mean of two trials. All baselines were between 5 and 10 s. Post-treatment latencies were determined after 30 min. The analgesic/antinociceptive effect was defined quantitatively as doubling of the baseline value for each mouse. The quantitative (yes /no) definition of analgesia/antinociceptive effect was presented as percentage of effect in each treatment group. We used double baseline scores as a cut point value in our experiments, in order to minimize tissue damage, during the post treatment measurements.

2.3. Experimental procedures

The study was broken down into three experiments. In the first stage of the study groups of mice (n ≥ 15) were injected with increasing doses of reboxetine in order to determine the antinociceptive effect of the drug.

In the second experiment, the sensitivity of reboxetine to four selective opioid antagonists and two noradrenergic antagonists was examined: Five groups of mice (n ≥ 15) were treated with δ-FNA (40 mg/kg, δ antagonist) 24 h before the reboxetine challenge, or with one of the following drugs: naloxone (10 mg/kg, universal opioid antagonist), naltrindole (20 mg/kg, δ antagonist), nor-BNI (10 mg/kg, δ antagonist) or saline immediately before reboxetine (10 mg/kg) was injected. For comparison, the δ-FNA effect was tested against morphine, nor-BNI against the U50,488H antinociceptive effect and naltrindole against DPDPE, in separate groups of mice. In addition, two other groups of mice were injected with phentolamine (4 mg/kg α1 adrenergic antagonist) or with yohimbine (4 mg/kg α2 adrenergic antagonist) immediately before reboxetine (10 mg/kg) was injected.

In the third experiment, the influence of reboxetine on opioid and adrenergic antagonists was examined. The antinociceptive effect induced by a fixed sub-threshold dose of morphine (0.5 mg/kg, opioid receptor agonist) or a fixed sub-threshold dose of clonidine (0.1 mg /kg, adrenergic receptor agonist) was tested with increasing doses of reboxetine. All agonists and antagonists were chosen according to our previous studies (Schreiber et al., 2002b). None of the antagonists possesses any analgesic effect of their own, nor do they alter blood pressure. The agonists studied were used at sub-threshold doses, and did not manifest any effect on their own.

2.4. Statistic analysis

Dose–response curves were analyzed, using a SPSS computer program. This program maximizes the log-likelihood function to fit a parallel set of Gaussian normal sigmoid curves to the dose-response data. In the agonists–antagonists experiment significance was determined by using the Chi-square test.

3. Results

3.1. Reboxetine antinociceptive effect

Reboxetine induced a weak antinociceptive effect (Fig. 1). Reboxetine reached its maximal effect of 30% analgesia at 10 mg/kg.

Figure 1 Reboxetine antinociceptive effect: groups of mice (n ≥ 15) received an s.c. injection of reboxetine at the indicated dose and were tested in the hotplate test 30 min late. % Analgesia refers to the proportion of mice tolerating a doubling of their mean latency.

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3.2. Sensitivity of reboxetine-induced analgesia to selective antagonists

The antinociceptive effect of reboxetine (10 mg/kg) was antagonized by naloxone (10 mg/kg s.c.; \( P<0.05 \)), implying that there is an opioid mechanism of action involved in the reboxetine-induced antinociceptive effect (Fig. 2). In the next stage the involvement of the selective antagonists of \( \mu \), \( \delta \) and \( \kappa \) receptors was assessed to evaluate their potential involvement in the reboxetine’s antinociceptive effect, \( \beta \)-FNA (\( \mu \) antagonist), naltrexone (\( \delta \) antagonist) and Nor-BNI (\( \kappa \) antagonist) abolished completely reboxetine antinociceptive effect, suggesting a non-specific role for the opioid receptors in the reboxetine antinociceptive effect (\( P<0.05 \); Fig. 2).

In a different set of experiments the impact of the adrenergic antagonist phentolamine (\( \alpha_1-\alpha_2 \) adrenergic antagonist) and yohimbine (\( \alpha_2 \) adrenergic antagonist) on reboxetine’s antinociceptive effect was assessed. Both adrenergic substances did not abolish significantly reboxetine’s antinociception, indicating a minor role of adrenaline in this antinociception (Fig. 2).

3.3. Sensitivity of reboxetine antinociceptive effect to selective agonists

Groups of mice (\( n\geq15 \) in each group) were injected with an inactive dose of morphine (a non-selective opioid agonist, 0.5 mg/kg) or with an inactive dose of clonidine (\( \alpha_2 \) adrenergic agonist, 0.1 mg/kg) in addition with increasing doses of reboxetine (0.5–10 mg/kg). No significant increase in analgesia was found when a sub-threshold dose of morphine or clonidine was added to reboxetine (Fig. 2). When morphine was added to 0.5 or 4 mg/kg reboxetine a non-significant increase in analgesia was detected, and it disappeared with 10 mg/kg reboxetine.

Figure 2 Effects of various opioid and adrenergic antagonists on reboxetine antinociception: Groups of mice (\( n\geq15 \)) were treated with reboxetine alone (10 mg/kg) or were challenged in addition with one of the additional drugs. Naloxone (10 mg/kg), \( \beta \)-FNA (40 mg/kg), naltrexone (20 mg/kg), nor-BNI (10 mg/kg) or yohimbine (4 mg/kg). Naloxone, \( \beta \)-FNA, naltrexone, nor-BNI, significantly antagonized reboxetine antinociception (\( P<0.05 \)). All other drugs did not antagonize reboxetine antinociception.

Figure 3 Effects of various opioid and adrenergic agonists on reboxetine antinociception: Groups of mice (\( n\geq15 \)) were treated with increasing doses reboxetine alone or were challenged in addition with a fixed sub-threshold dose of morphine (an opioid agonist, 0.5 mg/kg) or with clonidine (an adrenergic agonist, 0.1 mg/kg). No changes in analgesia levels were found when the agonists were added.

Addition of clonidine did not affect significantly the analgesia induced by reboxetine at 4 and 10 mg/kg.

4. Discussion

In the present study we found reboxetine (i.p.) to induce very weak, dose-dependent antinociception in the mouse hotplate assay. This weak effect was fully antagonized by all (both selective and non-selective) opioid receptor antagonists, implying a clear, non-selective involvement of the opioid system in the antinociceptive properties of reboxetine. Phentolamine (\( \alpha_1-\alpha_2 \) adrenergic antagonist) and yohimbine (\( \alpha_2 \) adrenergic antagonist) did not significantly affect reboxetine antinociception, implying only a minor involvement of the \( \alpha_2 \) noradrenergic pathway in reboxetine’s antinociceptive properties. When administered together with an inactive dose of an opioid agonist, or an \( \alpha_2 \) adrenergic agonist, no significant potentiation in analgesia was noted.

In a series of previous studies we demonstrated the antinociceptive effect and mechanisms of action of several antidepressants of the selective serotonin reuptake inhibitors (SSRIs) group, and some “dual action” (the pre-synaptic venlafaxine and the post synaptic mianserin and mirtazapine) agents, and found them to induce various degrees of antinociceptive effects — some mediated through the opioid system, while others not involving the opioid system (Schreiber et al., 1998, 1999, 2002a,b; Schreiber and Pick, 2006). We would have expected a selective noradrenaline reuptake inhibitor like reboxetine to induce also a significant antinociceptive effect (either involving, or not involving the opioid system). Surprisingly, reboxetine induced a negligible analgesic effect in the current study. Furthermore, in all our previous studies, SSRIs, venlafaxine, mianserin and mirtazapine were found to enhance opioid analgesia when co-administered with opioid receptor agonists, while this was not the case with reboxetine.

One possible explanation of our findings may derive from the fact that noradrenaline normally has only very weak excitatory activities and does not induce either nociceptive response or hyperalgesia (Banik et al., 2004). However, a direct action of noradrenaline on sensory nerves is possible, since \( \alpha_2 \) adrenoceptors are expressed by some sensory neurons (Perl,
1994). In other cases, the effects of noradrenaline may be indirect, and it has been suggested that it stimulates the release of prostaglandins from sympathetic postganglionic neurons that then act on the sensory nerves (Malik, 1988).

Descending noradrenergic and serotoninergic pathways modulate nociceptive processing in the mature spinal cord (Millan, 2002). Studies suggest that spinal \( \alpha_2 \) noradrenergic receptors mediate the noradrenergic component of descending inhibition to the dorsal horn with particular importance attributed to the \( \alpha_2 \) receptor subtype (Millan, 2002). This mode of action may partially explain our findings: it is possible that serotonin and noradrenaline have different modulating effects on the conduction of pain sensation, with the noradrenergic component serving as an augmentor of the serotonergic mechanism—in part directly and in part through indirect involvement of the opioid system. A third possible explanation may derive from the fact that we have assessed the antinociceptive effect of reboxetine in an acute pain mouse model (the hotplate analgesia meter), and not in a chronic model of pain.

Nociceptive information is processed and integrated at the peripheral, spinal, and supraspinal levels (Ren and Dubner, 2002), and the noradrenergic system together with the serotoninergic system is involved in the efficacy of antidepressant in the treatment of chronic pain (Savynok et al., 2001). Our preclinical findings as well as the limited available clinical data regarding reboxetine usefulness in pain syndromes (Krell et al., 2005) indicate that this selective noradrenaline reuptake inhibitor is almost ineffective in the treatment of acute pain. Further studies are needed to evaluate its efficacy in subtypes of chronic pain and to address the involvement of specific adrenergic and opioid receptors in such putative analgesic activity.

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Contributors

Schreiber and Pick designed the experiment. Frishtick, Volis and Rubovitch performed the experiments. Schreiber, Pick and Weizman wrote the manuscript.

Conflict of interest

There is no conflict of interest by any of the authors.

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