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Pindolol, a beta-adrenoceptor blocker/5-hydroxytryptamine$_{1A/1B}$ antagonist, enhances the analgesic effect of tramadol

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Abstract

The ability of pindolol, a beta-adrenoceptor blocker/5-hydroxytryptamine$_{1A/1B}$ antagonist, to enhance the clinical antidepressant response to selective serotonin re-uptake inhibitors is generally attributed to a blocking of the feedback that inhibits the serotoninergic neuronal activity mediated by somatodendritic 5-hydroxytryptamine (5-HT)$_{1A}$ autoreceptors. The current study examined the ability of pindolol to enhance the analgesic effect of tramadol, an atypical centrally-acting analgesic agent with relatively weak opioid receptor affinity and which, like some antidepressants, is able to inhibit the re-uptake of 5-HT in the raphe nuclei. Racemic pindolol (2 mg/kg, s.c.), rendered analgesic a non-effective acute dose of tramadol (10–40 mg/kg, i.p.) in two nociceptive tests: a hot plate test in mice and a plantar test in rats. Moreover, (±)8-OH-DPAT (0.125–1 mg/kg, s.c.), a selective 5-HT$_{1A}$ agonist, reduces the analgesic effect of tramadol in the same tests. These results suggest an implication of the somatodendritic 5-HT$_{1A}$ receptors in the analgesic effect of tramadol and open a new adjuvant analgesic strategy for the use of this compound.

Keywords: Tramadol; Pindolol; 5-Hydroxytryptamine; Serotonin; Pain; Antinociception; 5-Hydroxytryptamine$_{1A}$ autoreceptors; 8-Hydroxy-2-(di-*n*-propylamino)tetralin; Rat; Mice

1. Introduction

Tramadol is a centrally-acting, clinically-effective analgesic (Moore and McQuay, 1997; Sindrup et al., 1999) with several modes of action, particularly the activation of opioid receptors (Hennies et al., 1988) preferentially of the μ-subtype (Raffa et al., 1992), and enhancement of the extraneuronal concentration of the monoamine neurotransmitters noradrenaline (NA) and serotonin (5-HT) by interference with the uptake and release mechanisms (Driessen and Reimann, 1992; Raffa et al., 1992; Driessen et al., 1993; Bamigbade et al., 1997). Tramadol, therefore, causes the activation of both the main systems involved in the inhibition of pain: the opioid and the descending monoaminergic system. In respect of 5-HT, tramadol has been shown to reduce the synaptosomal uptake of [^{3}H]5-HT (Raffa et al., 1992). This mechanism of action of tramadol is very similar to that of some antidepressants (Markowitz and Patrick, 1998). Indeed, tramadol has been shown to induce antidepressant-like properties in mice (Rojas-Corrales et al., 1998; Micó et al., 1999) and has been shown to be effective clinically as an adjunct treatment for antidepressant drug potentiation (Fanelli and Montgomery, 1998).

Serotonin pathways within the CNS arise from a series of nuclei situated in the midline of the brain stem, the raphe nuclei, which represent the richest source of neuronal 5-HT synthesized in the mammalian brain (for review, see Baumgarten and Grozdanovic, 1997). The dorsal raphe nucleus (DRN) has been implicated in the regulatory effect of antidepressants on mood disorders (Artigas et al., 1996) as well as in pain modulation (Wang and Nakai, 1994) and the magnus raphe nucleus (MRN) is probably the most important serotoninergic nucleus modulating the descending control of pain transmission.

A specific feature of 5-HT-containing neurons in the DRN and MRN is the abundance of dendrodendritic, dendrosomatic and axodendritic contacts derived from recurrent axon collaterals (Felten and Harrigan, 1980; Chazal and Ralston, 1987). This type of connectivity may be the substrate of the unique electrophysiology of these neurons: the rhythmicity of these spontaneously and regularly discharging neurons which is under inhibitory control of 5-HT acting on somatodendritic 5-HT$_{1A}$ autoreceptors coupled to Gi protein. In this sense, the use of selective 5-

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HT_{1A} receptor agonists and antagonists has clearly demonstrated the importance of 5-HT_{1A} receptors, located in the raphe nuclei (Pazos and Palacios, 1985; Weissman-Nanopoulos et al., 1985), in the regulation of 5-HT neuronal firing, synthesis, metabolism, and release (Hjorth et al., 1982; Hutson et al., 1987; Sprouse and Aghajanian, 1987; Sinton and Fallon, 1988; Fletcher et al., 1996).

Acute administration of selective serotonin re-uptake inhibitors (SSRIs) (Bel and Artigas, 1992) and tramadol (Bamigbade et al., 1997) leads to an increase in the extracellular 5-HT concentrations in the vicinity of the cell body and the dendrites of 5-HT neurons of the raphe nuclei. Thus, an explanation for the limited acute effects of SSRIs on extracellular 5-HT concentrations appears to be related to the fact that SSRIs (and probably tramadol) indirectly activate somatodendritic 5-HT_{1A} autoreceptors in the raphe region. The 5-HT excess induced by the specific action of SSRIs and tramadol, essentially a blockade of 5-HT transport in this area, would activate somatodendritic 5-HT_{1A} autoreceptors. A negative feedback control is thus brought into action, leading to a hypoactivity of these neurons, i.e. a fall in the electrical activity of raphe neurons, as well as reduced 5-HT synthesis and release by nerve endings. Therefore, the efficacy of SSRIs as antidepressants (Romero and Artigas, 1997) and probably tramadol as an analgesic could be limited by this negative feedback. In accordance with this, the preventive blocking of somatodendritic 5-HT_{1A} autoreceptors by specific antagonists prevents the reduction of 5-HT release in terminal areas caused by administration of 5-HT uptake blockers and potentiates the effects of systemic SSRIs in these terminal areas (Artigas, 1995; Romero and Artigas, 1997).

This process accounts for ascending 5-HT projections from the DRN and probably for descending serotoninergic projections from the MRN to the dorsal horn. Therefore, the aim of our study has been to explore whether the blockade or stimulation of somatodendritic 5-HT_{1A} autoreceptors by pindolol (a relatively somatodendritic 5-HT_{1A} autoreceptor blocker) or 8-hydroxy-2-(di-*n*-propylamino)tetralin (a selective 5-HT_{1A} agonist), respectively, modify the antinociceptive effects of tramadol.

2. Methods

2.1. Experimental animals

The experimental protocols were reviewed and approved by the Local Committee for Animal Experimentation of the Faculty of Medicine at the University of Cádiz (License number 079604). Animal care complied with that stipulated by the local committee and conformed to the guidelines on the study of pain in awake animals established by the International Association for the Study of Pain (Zimmermann, 1983). Experiments were performed using both albino male mice of the CDI strain (25–30 g) and albino male Wistar rats (225–250 g). All the animals were provided by the Servicio de Experimentación y Producción Animal (SEPA) at the University of Cádiz. Animals were maintained under standard conditions: 12 h light/dark schedule (lights on at 08:00 h) with ad libitum food and water and at constant temperature (21 ± 1°C).

2.2. Drug preparation and administration

All substances, except pindolol, were dissolved in saline (ClNa 0.9%). Tramadol (Grünenthal, Spain) was intraperitoneally (i.p.) administered at 10 and 40 mg/kg in mice and at 40 mg/kg in rats. 8-Hydroxy-2-(di-*n*-propylamino)tetralin (8-OH-DPAT) (Sigma, St. Louis, MO) was s.c. injected at 0.125 and 1 mg/kg in mice and rats, respectively. (±)Pindolol (Sigma, St. Louis, MO) was suspended in gum arabic (1%) and s.c. injected at doses of 2 mg/kg in all the experiments. Control animals received saline. Drugs were injected at a volume of 0.1 ml/10 g of body weight in mice, and 0.1 ml/100 g of body weight in rats.

2.3. Hot plate test

The hot plate test was performed in mice basically as described by Woolfe and Macdonald (1944). Animals were placed on a hot plate apparatus (Digital DS-37 Socrel model). It was thermostatically maintained at 55 ± 1°C. The latency to the first response of the animal (either paw licking or jumping) was recorded as the pain response latency in seconds. Only one determination was performed for each animal and 30 s of exposure to the hot plate was established as the cut-off time. In experiments using this test, pindolol or 8-OH-DPAT were administered 10 min before the tramadol, which was administered 30 min before the test.

2.4. Plantar test

A plantar test apparatus (Ugo Basile no. 7370) (Hargreaves et al., 1988) was used to assess nociceptive responses to thermal stimuli in rats. In each trial, rats were placed in a clear plexiglass box on an elevated plexiglass floor. Animals were allowed to acclimate for approximately 5 min. A constant intensity radiant heat source was aimed at the midplantar area of both hind paws. The time, in seconds, from initial heat source activation until paw withdrawal was recorded. One latency measurement for each paw was recorded per rat. The mean of two measures was used for each experimental animal as the paw withdrawal latency. In order to avoid excessive suffering of animals, a cut-off was set at 30 s. A basal withdrawal latency was determined in order to select those animals which showed a basal latency between 6 and 10 s. After this, pindolol or 8-OH-DPAT were administered 10 min before the tramadol, which was administered 20 min before the test.

2.5. Statistical analysis

The results are expressed as the mean ± SEM of the pain

response latency in the hot plate test and the paw withdrawal latency in the plantar test measured in seconds. Percentages of maximal possible effect (%MPE) induced by the drugs related to control animals were also calculated (%MPE = ((latency of test group − latency of control group)/(cut-off − latency of control group)) × 100). Raw data were analyzed using one-way analysis of variance followed by the Student–Newman–Keuls test. A P value of <0.05 was considered to be significant.

3. Results

3.1. Hot plate test in mice

The effect of administration of pindolol and tramadol was evaluated in the hot plate (Fig. 1A). One-way ANOVA showed a significant effect of treatment ($F_{(3,36)} = 3.47$; $P < 0.05$). Injection of tramadol (10 mg/kg i.p.) (10.94 ± 1.02 s; $n = 10$; %MPE = +1.74) or pindolol (2 mg/kg s.c.) (10.56 ± 0.83 s; $n = 10$; %MPE = −0.22) had no effect on pain response latency compared to saline control (10.60 ± 2.10 s; $n = 10$). Animals receiving pindolol and tramadol (16.44 ± 1.84 s; $n = 10$; %MPE = +30.13) showed a significantly enhanced pain response latency compared to tramadol and saline control ($P < 0.05$). The antinociception obtained with tramadol plus pindolol was similar to that induced by 20 mg/kg of tramadol given alone (15.90 ± 2.39 s; $n = 10$; %MPE = +27.33).

The effect of administration of 8-OH-DPAT and tramadol was studied using the hot plate model (Fig. 1B). One-way ANOVA showed a significant effect of treatment ($F_{(3,36)} = 4.77$; $P < 0.01$). Administration of tramadol (40 mg/kg i.p.) (20.51 ± 2.91 s; $n = 10$; %MPE = +48.45) induced a significant increase in pain response latency from saline control (11.60 ± 1.16 s; $n = 10$; $P < 0.05$). 8-OH-DPAT (12.21 ± 1.48 s; $n = 10$; %MPE = +3.30) treatment did not significantly modify pain response compared to saline-treated mice. Mice receiving 8-OH-DPAT and tramadol (15.06 ± 1.36 s; $n = 10$; %MPE = +18.80) showed a significant reduction in pain response latency compared to tramadol alone ($P < 0.05$). Co-administration of 8-OH-DPAT and 40 mg/kg of tramadol reduced the latency to a value similar to that obtained with administration of 20 mg/kg of tramadol given alone (15.90 ± 2.39 s; $n = 10$; %MPE = +27.33).

3.2. Plantar test in rats

The effect of administration of pindolol and tramadol was evaluated in the plantar test (Fig. 2A). One-way ANOVA showed a significant effect of treatment ($F_{(3,28)} = 8.72$; $P < 0.01$). Injection of tramadol (40 mg/kg i.p.) (11.99 ± 1.82 s; $n = 8$; %MPE = +16.70) induced an increase in withdrawal latency, but this was not statistically significant compared to saline control (8.38 ± 1.18 s; $n = 8$). Pindolol (2 mg/kg s.c.) (6.16 ± 0.89 s; $n = 8$; %MPE = −10.27) had no effect on withdrawal latency compared to saline control. Animals receiving pindolol and tramadol (20.91 ± 3.71 s; $n = 8$; %MPE = +57.96) showed a significantly enhanced paw withdrawal latency compared to tramadol and saline control ($P < 0.05$). The effect induced by 40 mg/kg of tramadol plus pindolol was near that induced by 80 mg/kg of tramadol given alone (27.89 ± 1.27 s; $n = 8$; %MPE = +90.24) (Fig. 2A).

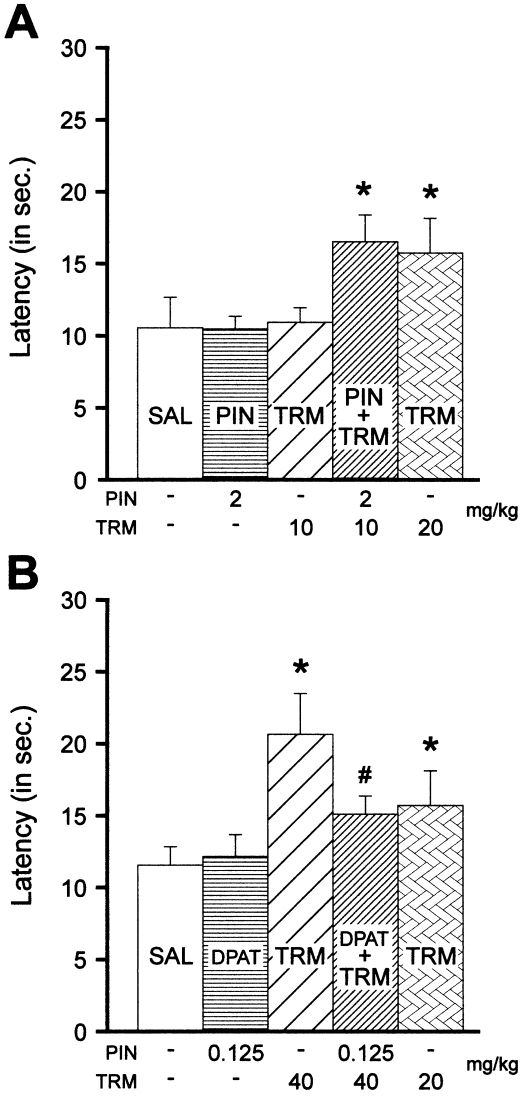

Fig. 1. Variation in latency measured by time elapsed up to the first response in the hot plate test in mice (in seconds) by the injection of pindolol (2 mg/kg s.c.) (A) or 8-OH-DPAT (0.125 mg/kg s.c.) (B) and tramadol. Tramadol (10 and 20 mg/kg in (A); 20 and 40 mg/kg in (B)) was intraperitoneally administered 30 min before the test. Pindolol (A) and 8-OH-DPAT (B) were administered 10 min before tramadol. The figure shows the mean ± SEM based on results of ten animals per group. (A) Tramadol shows an antinociceptive effect only at 20 mg/kg. The pindolol–tramadol association induces a significant increase in response latency compared to saline control, pindolol and tramadol (10 mg/kg; not antinociceptive dose) (Student–Newman–Keuls test: $P < 0.05$). (B) The antinociceptive effect of tramadol (40 mg/kg) (*$P < 0.05$ versus saline control) is significantly antagonized by 8-OH-DPAT (#$P < 0.05$ versus tramadol 40 mg/kg).

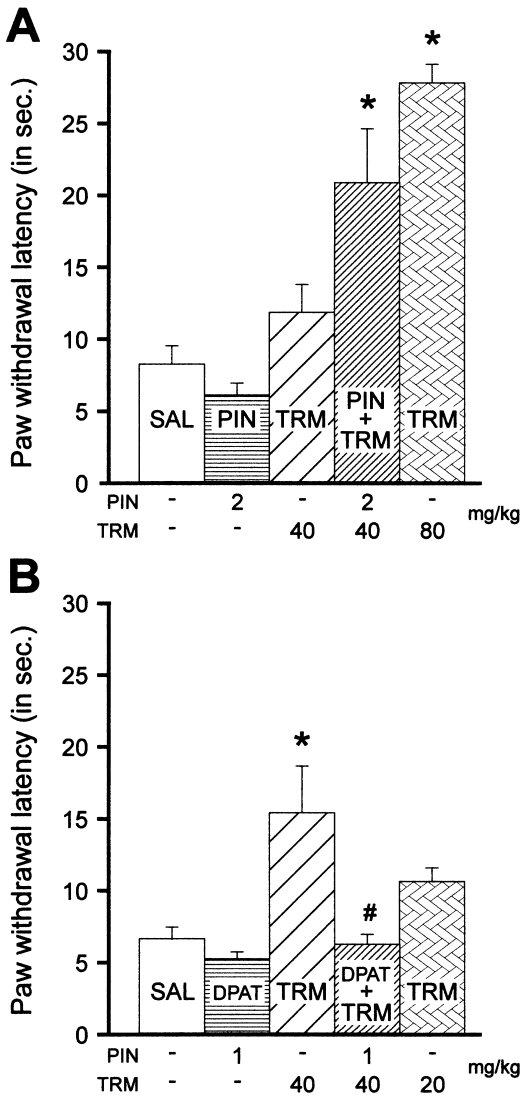

Fig. 2. Influence on paw withdrawal latency measured in the plantar test in rats (in seconds) produced by the injection of tramadol (40 and 80 mg/kg i.p.) and pindolol (2 mg/kg s.c.) (A) or tramadol (20 and 40 mg/kg i.p.) and 8-OH-DPAT (1 mg/kg s.c.) (B). Tramadol (20, 40 and 80 mg/kg) was intraperitoneally administered 20 min before the test. Pindolol (A) and 8-OH-DPAT (B) were administered 10 min before tramadol. The figure shows the mean ± SEM based on results of eight to ten animals per group. (A) Tramadol (40 mg/kg) induces a non-significant increase in paw withdrawal latency compared to the saline control group. Pindolol is ineffective alone but its association with tramadol (40 mg/kg) induces a significant increase in latency compared to tramadol (40 mg/kg) and saline control groups (Student–Newman–Keuls test: $^*P < 0.05$). (B) A clear anti-nociceptive effect of tramadol (40 mg/kg) ($^*P < 0.05$ versus saline control group) is antagonized by association with an ineffective dose of 8-OH-DPAT ($\#P < 0.05$ versus tramadol).

The effect of administration of 8-OH-DPAT and tramadol was studied using the plantar test (Fig. 2B). One-way ANOVA showed a significant effect of treatment ($F_{(3,34)} = 6.90$; $P < 0.01$). Administration of tramadol (40 mg/kg i.p.) (15.43 ± 3.26 s; $n = 10$; %MPE = +37.68) induced a significant increase in paw withdrawal latency compared to saline control (6.62 ± 0.85 s; $n = 9$; $P < 0.05$). 8-OH-DPAT (5.23 ± 0.58 s; $n = 9$; %MPE = −5.95) treatment did not modify withdrawal latency. Rats receiving 8-OH-DPAT and tramadol (6.29 ± 0.69 s; $n = 10$; %MPE = −1.41) showed a significant reduction in paw withdrawal latency compared to tramadol ($P < 0.05$). The paw withdrawal latency obtained with this association was comparable to that induced with half of the dose of tramadol (20 mg/kg) given alone (10.78 ± 0.95 s; $n = 8$; %MPE = +11.10).

4. Discussion

The present study clearly demonstrates that (±)pindolol, a beta-adrenoceptor blocker/putative 5-$HT_{1A/1B}$ antagonist, rendered analgesic a non-effective acute dose of tramadol in two nociceptive tests in mice and rats. Moreover, (±)8-OH-DPAT, a selective 5-HT_{1A} agonist (Hamon et al., 1984) reduced its analgesic effect in the same tests. These results suggest an implication of the somatodendritic 5-HT_{1A} receptors in the analgesic effect of tramadol.

Tramadol is a centrally-acting analgesic which inhibits the synaptosomal uptake of 5-HT (Raffa et al., 1992; Giusti et al., 1997), a mechanism similar to that of the SSRIs.

Recent studies indicate that pindolol can also enhance the experimental (Redrobe et al., 1996) and therapeutic responses to SSRIs (Artigas et al., 1994; Pérez et al., 1997). This action is generally attributed to the ability of pindolol to block putative 5-HT_{1A} autoreceptors located on the cell bodies and dendrites of central serotoninergic neurons (Artigas, 1995; Romero and Artigas, 1997). In fact, these receptors are part of a negative feedback mechanism whereby the local release of 5-HT in the raphe region acts to decrease the discharge activity of serotoninergic neurons. The indirect activation of somatodendritic 5-HT_{1A} autoreceptors by SSRIs, and probably by tramadol, leads to an inhibition of neuronal activity and neurotransmitter release, which counteracts the ability of these compounds to potentiate the action of endogenous 5-HT at post-synaptic target sites (i.e. the induction of analgesia).

It is well known that 5-HT plays a multifaceted role in the regulation of nociceptive transmission. Complexity arises from actions at multiple sites within the pain transmission system (periphery, spinal cord, and supraspinal sites), and from actions by multiple 5-HT receptor subtypes. The 5-HT_{1A} receptors apparently play a modulatory role in nociception (Hamon, 1997). In the spinal cord, these receptors are especially concentrated within the superficial layers of the dorsal horn where primary afferent fibers that convey nociceptive signals terminate (Daval et al., 1987). Furthermore, some of these receptors are located on the terminals of primary afferent fibers (Laporte et al., 1995) and may therefore mediate some pre-synaptic modulatory action of 5-HT on the release of their neurotransmitters. In addition, it is clear that 5-HT_{1A} receptors are located both post-synaptic to 5-HT neurons and on the 5-HT neurons themselves at the level of the soma and dendrites in the mesencephalic and

medullary raphe nuclei (Weissman-Nanopoulos et al., 1985) that are implicated in descending and ascending pain projection pathways (Wang and Nakai, 1994).

On the other hand, given that pindolol is able to block the 5-HT_{1B} receptors effectively, its participation in the facilitation of the tramadol analgesia cannot be excluded. Recently, Bourin et al. (1998) have demonstrated that pindolol acting at pre-synaptic 5-HT_{1B} serotoninergic receptors, in addition to the 5-HT_{1A} receptors, was able to enhance the activity of some antidepressants in an experimental model of depression. This receptor has consistently been implicated in pain processes, but at the spinal level (Eide et al., 1990; Alhaider and Wilcox, 1993). However, 5-HT_{1B} autoreceptors are also present on 5-HT nerve terminals in many brain regions, including the DRN. These receptors have been shown to regulate 5-HT release within the DRN where their location is unclear (i.e. whether on dendrites or on recurrent collaterals) (Davidson and Stamford, 1995).

Finally, in addition to its 5-HT re-uptake inhibiting properties, tramadol, like morphine but to a lesser extent, is an agonist to μ-opioid receptors (Hennies et al., 1988). A marked decrease in morphine-induced nociception has been reported in rats treated with 8-OH-DPAT (Millan and Colpaert, 1991; Alhaider and Wilcox, 1993), a result similar to that obtained in our study with tramadol. Therefore, an interrelationship between μ-opioid and 5-HT_{1A} receptors cannot be ruled out in our study. Indeed, morphine stimulates 5-HT release via a supraspinal action (Bineau-Thourottes et al., 1984) and 5-HT depletion in the CNS decreases the analgesic effect of morphine (Bodnar et al., 1981). Moreover, immunohistochemistry has revealed the existence of enkephalinergic neurons in the raphe nuclei (Moss et al., 1981) and μ-opioid receptors exist in this area (Bowker and Dilts, 1988). In this context, Peckys and Landwehrmeyer (1999) recently found signals for μ-opioid receptor mRNA over neurons in the DRN in humans. Thus, it can be assumed that morphine, like tramadol, exerts its analgesic effect, at least in part, through the serotoninergic system (Yang et al., 1994), although the participation of 5-HT neurons in the MRN in the analgesic effect of morphine has recently been questioned (Gao et al., 1998). However, a more recent study (Hain et al., 1999) emphasizes the importance of 5-HT_{1B} receptors in the mediation of morphine antinociceptive sensitivity. In the cited study, the 5-HT_{1A} agonist 8-OH-DPAT always antagonized the analgesic effect induced by morphine.

In conclusion, it is presumed that the association of an inhibitor of serotonin re-uptake, such as tramadol, with a somatodendritic 5-$HT_{1A/1B}$ receptor blocker, such as pindolol, may facilitate the effect of 5-HT at the level of the nerve terminal, leading to a more effective analgesia. Similar studies should be extended to agents acting more selectively on 5-HT_{1A} or 5-HT_{1B} receptors at different levels of the serotoninergic pathways regulating nociception.

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