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The antinociceptive effect of tramadol in the formalin test is mediated by the serotonergic component

Patrizia Oliva [a], Caterina Aurilio [b], Francesco Massimo [b], Antonio Grella [b], Sabatino Maione [a], Elisa Grella [b], Mariantonietta Scafuro [b], Francesco Rossi [a], Liberato Berrino [a,*]

[a] *Department of Experimental Medicine, Section of Pharmacology "L. Donatelli", Second University of Naples, Via Costantinopoli, 16, 80138 Naples, Italy*
[b] *Department of Anaesthesiological and Surgical Sciences and Intensive Care, Faculty of Medicine and Surgery, Second University of Naples, Via Costantinopoli, 16, 80138 Naples, Italy*

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Abstract

The aim of this study was to investigate the neurotransmissions involved in the antinociceptive effect of tramadol in the formalin test, which is an animal model of acute and tonic pain. A subcutaneous injection of formalin produces a biphasic nociceptive response: phase 1 (0–10 min—acute pain) and phase 2 (21–60 min—tonic pain). Nociceptive activity is reduced greatly during the 10 min between these two phases. We measured in mice the effects of (±)-tramadol, and of (+)- and (−)-tramadol administered before the induction of pain by formalin, in the presence and absence of drugs that act on the opioidergic, serotonergic and noradrenergic systems (naloxone, ketanserin, fluoxetine, maprotiline). With respect to animals treated with formalin alone, (±)-tramadol and its enantiomers significantly reduced the duration of nociceptive behaviours (lifting, licking, favouring, shaking, and flinching of the formalin-treated paw) during phase 2. This effect was prevented by the 5-HT_2 receptor antagonist ketanserin, but not by naloxone which, on the contrary, was able to prevent the antinociceptive effect of morphine. Naloxone and ketanserin did not affect the duration of nociceptive behaviour in animals not treated with tramadol. Fluoxetine (a selective 5-hydroxytryptamine (5-HT) reuptake inhibitor), but not maprotiline (a selective norepinephrine reuptake inhibitor), potentiated the antinociceptive effect of (±)-tramadol. In conclusion, we demonstrate that the serotonergic pathway is responsible for the antinociceptive effect of tramadol in phase 2 of the formalin test, and that this effect is mediated by 5-HT_2 receptors.

Keywords: Formalin test; Tramadol; 5-HT (5-hydroxytryptamine, serotonin); Norepinephrine; Opioid

1. Introduction

The serotonergic and adrenergic pathways and endogenous opioids are involved in the descending inhibition of nociception that originates in the spinal cord (Mayer et al., 1971; Zemlan et al., 1980; Cannon et al., 1982; Aimone et al., 1987; Kwiat and Basbaum, 1992). Stimulation of the serotonergic system activates endogenous opioids (Vaz et al., 1996), and the analgesic effect of opioids is proportional to serotonergic tone (Proudfit and Andersson, 1975). Therefore, enhanced serotonergic tone may increase the analgesic effect of morphine and similar drugs.

Unlike other opioid receptor agonists, tramadol (1 *RS*, 2 *RS*)-2-[(dimethylamino)-methyl]-1-(3-methoxyphenyl)-cyclohexanol hydrochloride, a racemic mixture of two enantiomers, has affinity for μ-opioid receptors (Raffa et al., 1992) and inhibits neuronal reuptake of serotonin (5-hydroxytryptamine (5-HT)) and norepinephrine (Driessen et al., 1993; Rhoda Lee et al., 1993). However, the mechanism of action of tramadol remains unclear because its binding affinity for opioid receptors appears to be too low to account for the antinociceptive effect via this system (Rhoda Lee et al., 1993), and the noradrenergic and serotonergic involvement is still not completely understood. The formalin test is an experimental model by which to assess an animal's response to moderate, continuous pain generated by damaged tissue. The response to formalin is typically biphasic. The early phase of intense pain, which starts immediately after formalin injection, seems to be caused predominantly by activation of C-fibres subsequent to peripheral stimula-

* Corresponding author. Tel.: +39-081-5665890; fax: +39-081-5667531.
E-mail address: liberato.berrino@unina2.it (L. Berrino).

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tion. Then, there is a period (about 10 min) of reduced nociceptive activity. The late phase of moderate pain, which starts about 20 min after formalin injection and lasts about 40 min, appears to be caused by tissue and functional changes in the dorsal horn of the spinal cord (Dickenson and Sullivan, 1987; Coderre et al., 1990).

The aim of this study was to clarify the mechanisms underlying the antinociceptive effect of (±)-tramadol and its enantiomers in the formalin test. To this aim, we evaluated the effects of (±)-tramadol and its enantiomers in mice before subcutaneous (sc) formalin injection either in the presence or absence of drugs that interfere with serotonergic, noradrenergic and opioid neurotransmitter systems.

2. Materials and methods

Male Swiss Webster (40–45 g) mice were housed at a constant temperature (21±1 °C) and relative humidity (60%), under a regular light/dark schedule (light 7:00–19:00). Food and water were freely available. Animal care was in compliance with Italian (D.L. 116/92) and EU (O.J. of E.C.L. 358/1 18/12/1986) regulations governing the protection of laboratory animals. All experimental procedures and protocols used in this study were reviewed and approved by the Animal Care and Use Committee of the Medical College of Naples.

2.1. Formalin test

Each mouse was randomly assigned to 1 of the 27 experimental groups, placed in a plexiglass cage and allowed to move freely for 15–20 min. A mirror was placed under the cage at an angle of 45° to allow full view of the hind paws. Fifteen minutes after intraperitoneal (i.p.) injection of vehicle, tramadol or morphine, each mouse was given a formalin (1.25%, 50 μl) injection into the dorsal skin of one side of the hind paw. Lifting, favouring, licking, shaking and flinching of the injected paw were recorded as nociceptive responses. Recording of the nociceptive behaviour began immediately after formalin injection and lasted 60 min. The recording time was divided into 12 blocks of 5 min and the nociceptive response was determined for each block, i.e. we recorded the duration of the lifting, favouring, licking, shaking and flinching of the affected limb; 7–10 animals per treatment were used.

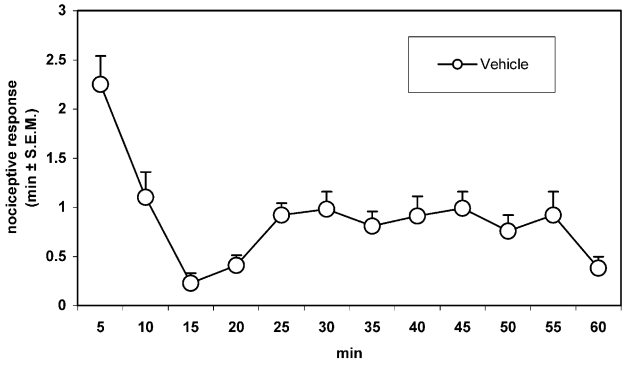

Fig. 1. Time course of nociceptive behaviour induced by formalin (1.25%, 50 μl) in mice treated with saline (0.3 ml). The data represent the total time of nociceptive responses (mean±S.E.M. of 7–10 mice per group) measured every 5 min.

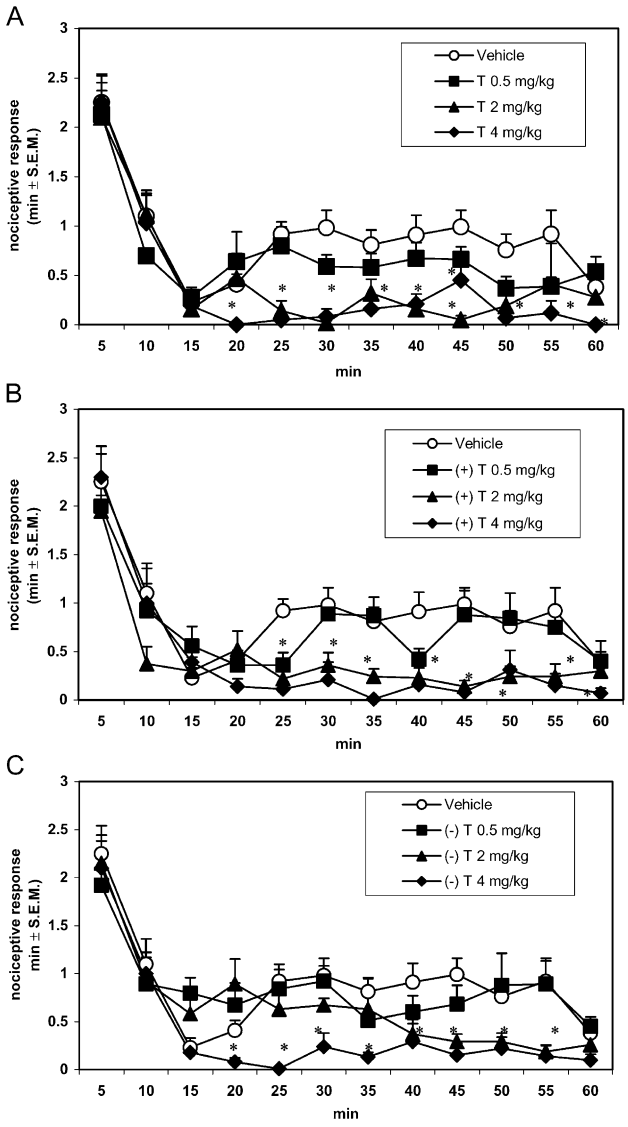

Fig. 2. Time course of nociceptive behaviour induced by formalin (1.25%, 50 μl) in mice treated with (±)-tramadol (T) [A], (+)-tramadol [(+) T] [B], (−)-tramadol [(−) T] [C] (0.5, 2 and 4 mg/kg, i.p.) 15 min before formalin. The data represent the total time of nociceptive responses (mean±S.E.M. of 7–10 mice per group) measured every 5 min. *P<0.05 vs. vehicle.

2.2. Treatment

We used the following experimental protocol: (±)-tramadol, (+)-tramadol, (−)-tramadol or morphine (control) were injected i.p. 15 min before formalin. The antagonists of opioid and 5-HT_2 receptors (naloxone and ketanserin,

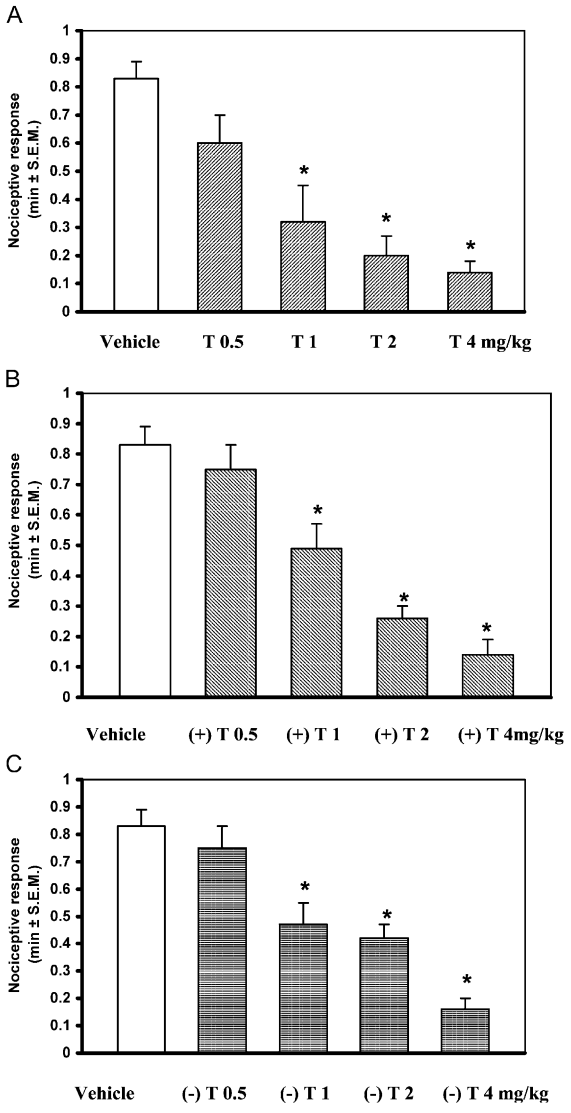

Fig. 3. Duration of nociceptive behaviour during phase 2 of the formalin test (1.25%, 50 μl) in mice treated with (±)-tramadol (T) [A], (+)-tramadol [(+) T] [B], (−)-tramadol [(−) T] [C] (0.5, 1, 2 and 4 mg/kg, i.p.) 15 min before formalin. The data represent the total time of nociceptive responses (mean±S.E.M. of 7–10 mice per group) measured every 5 min. *P<0.05 vs. vehicle.

respectively) and the inhibitors of 5-HT and norepinephrine reuptake (fluoxetine and maprotiline, respectively) were injected i.p. 15 min before and after formalin administration. Doses were: (±)-tramadol and its enantiomers 0.5, 1, 2 and 4 mg/kg; morphine 1 mg/kg; naloxone 2 mg/kg; ketanserin 0.5 mg/kg; fluoxetine 5 mg/kg and maprotiline 5 mg/kg. A vehicle/control consisting of saline 0.3 ml was also used in all experiments.

2.3. Drug preparation

The following drugs, dissolved in NaCl 0.9%, were used: a stock solution of formalin (aqueous solution of 40% p/v, formaldehyde) (Sigma, St. Louis, MO, USA), naloxone HCl (Biologici Italia Laboratori, Italy), (±)-tramadol (Prodotti Formenti, Italy), (+)-tramadol hydrochloride, (−)-tramadol hydrochloride (Grunenthal, Germany), morphine chloride (Iacopo Monaco, Italy), ketanserin tartrate, maprotiline hydrochloride and fluoxetine hydrochloride (Tocris Cookson, Bristol, UK).

2.4. Data analysis

Statistical analysis of the data (mean±S.E.M.) was performed by the t-test, the one-way unpaired analysis of

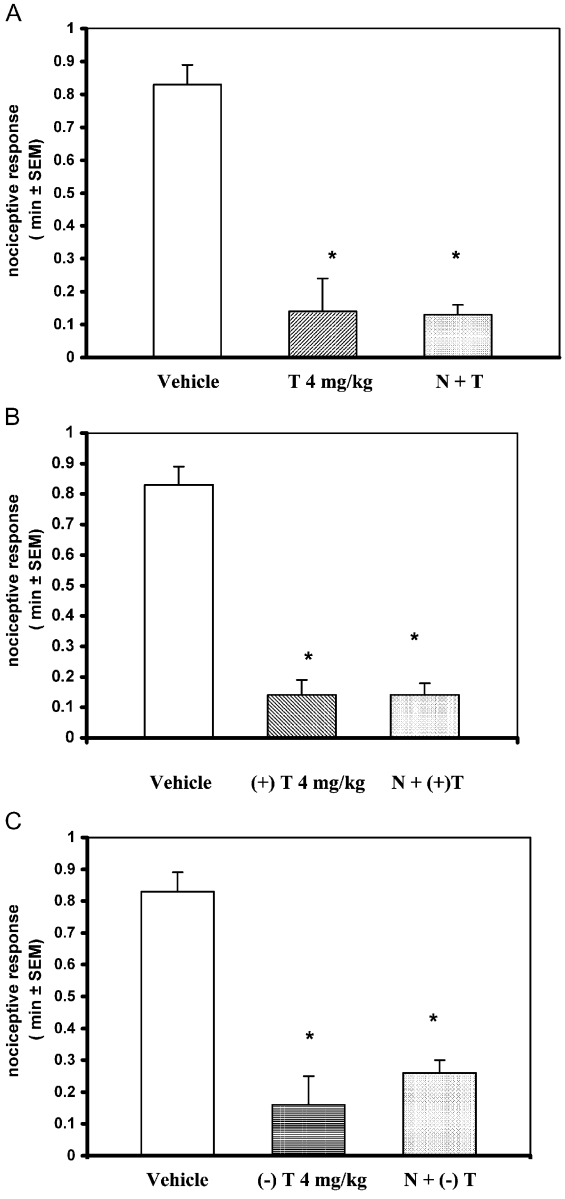

Fig. 4. Duration of nociceptive behaviour during phase 2 of the formalin test (1.25%, 50 μl) in mice treated with (±)-tramadol T [A], (+)-tramadol [(+) T] [B], (−)-tramadol [(−) T] [C] (4 mg/kg, i.p.) and naloxone (N) (2 mg/kg, i.p.) 15 min before formalin. The data represent the total time of nociceptive responses (mean±S.E.M. of 7–10 mice per group) measured during phase 2. *P<0.05 vs. vehicle.

variance (ANOVA) test followed by the Dunnett or Bonferroni post-test as appropriate. ED_{50} values were determined using Probit analysis.

3. Results

Injection into the dorsal skin of the hind paw of 50 μl of formalin (1.25%) generated a classical biphasic nociceptive response (Fig. 1).

The administration of (±)-tramadol and its (+)- and (−)-enantiomers (at doses of 0.5, 1, 2 and 4 mg/kg) 15 min before formalin significantly and dose-dependently ($P<0.05$) reduced phase 2 of the formalin test, without interfering with phase 1 of the test (Figs. 2 and 3). There were no differences in the effects of tramadol on the various nociceptive behaviours. The rank order of potency of the

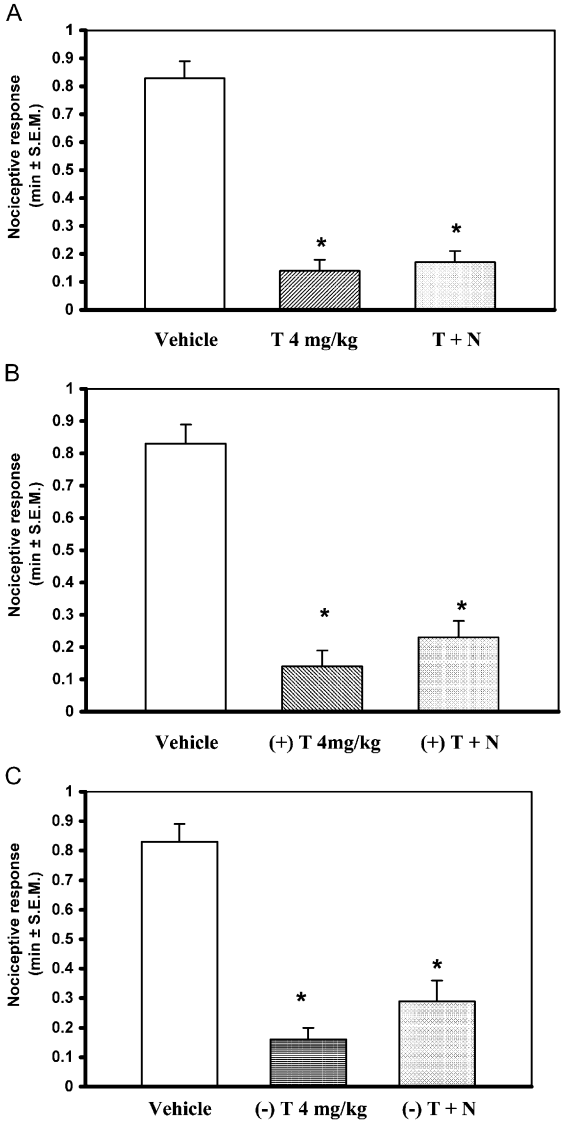

Fig. 5. Duration of nociceptive behaviour during phase 2 of the formalin test (1.25%, 50 μl) in mice treated with (±)-tramadol T [A], (+)-tramadol [(+) T] [B], (−)-tramadol [(−) T] [C] (4 mg/kg, i.p.) 15 min before formalin, and with naloxone (N) (2 mg/kg, i.p.) 15 min after formalin. The data represent the total time of nociceptive responses (mean±S.E.M. of 7–10 mice per group) measured during phase 2. *$P<0.05$ vs. vehicle.

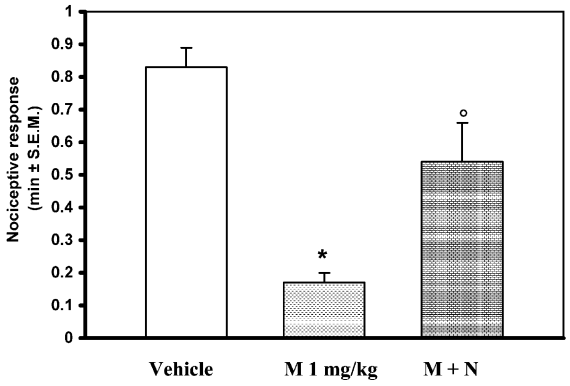

Fig. 6. Duration of nociceptive behaviour during phase 2 of the formalin test (1.25%, 50 μl) in mice treated with morphine (M) (1 mg/kg, i.p.) 15 min before formalin and naloxone (N) (2 mg/kg, i.p.) 15 min after formalin. The data represent the total time of nociceptive responses (mean±S.E.M. of 7–10 mice per group) measured during phase 2. *$P<0.05$ vs. vehicle; °$P<0.05$ vs. morphine.

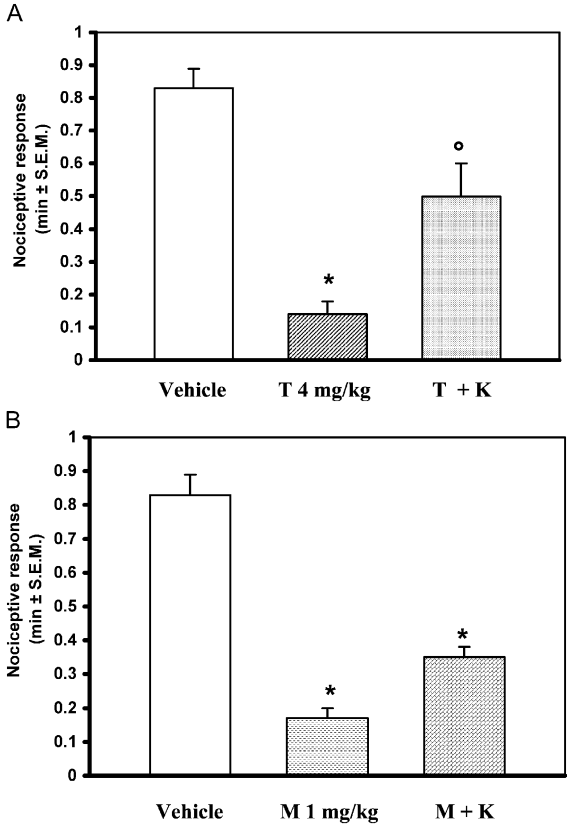

Fig. 7. Duration of nociceptive behaviour during phase 2 of formalin test (1.25%, 50 μl) in mice treated with (±)-tramadol (T) [A] (4 mg/kg, i.p.) or morphine (M) [B] (1 mg/kg) 15 min before formalin and with ketanserin (K) (0.5 mg/kg, i.p.) 15 min after formalin. The data represent the total time of the nociceptive responses (mean±S.E.M. of 7–10 mice per group) measured during phase 2. *$P<0.05$ vs. vehicle; °$P<0.05$ vs. tramadol.

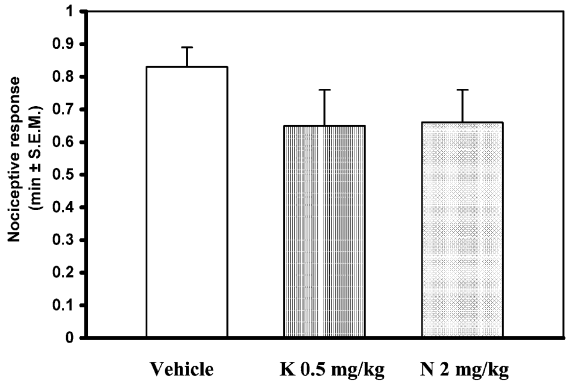

Fig. 8. Duration of nociceptive behaviour during phase 2 of the formalin test (1.25%, 50 μl) in mice treated with ketanserin (K) (0.5 mg/kg, i.p.) and naloxone (2 mg/kg, i.p.) 15 min before formalin. The data represent the total time of nociceptive responses (mean±S.E.M. of 7–10 mice per group) measured during phase 2.

antinociceptive activity of (±)-tramadol and its enantiomers was: (±)-tramadol [ED_{50} 0.87 (0.67–1.06)] > (+)-tramadol [ED_{50} 1.44 (1.20–1.72)]>(−)-tramadol [ED_{50} 1.65 (0.74–3.70)]. Naloxone (2 mg/kg), a non-selective opioid receptor antagonist, administered 15 min before and 15 min after formalin, did not inhibit the antinociceptive effect of the highest dose of (±)-tramadol and its enantiomers (Figs. 4 and 5). On the contrary, naloxone prevented the antinociceptive effect of morphine (1 mg/kg) during phase 2 of the formalin test (Fig. 6). The 5-HT_2 receptor antagonist ketanserin (0.5 mg/kg), administered 15 min after-formalin, significantly reduced the effect of the highest dose of (±)-tramadol (Fig. 7A), but not inhibit the antinociceptive effect of morphine (Fig. 7B). Ketanserin (0.5 mg/kg) and naloxone (2 mg/kg), administered 15 min before formalin, did not change the pattern of the early and late nociceptive behaviours induced by formalin (Fig. 8). Fluoxetine (5 mg/kg), a selective 5-HT reuptake inhibitor, administered 15 min

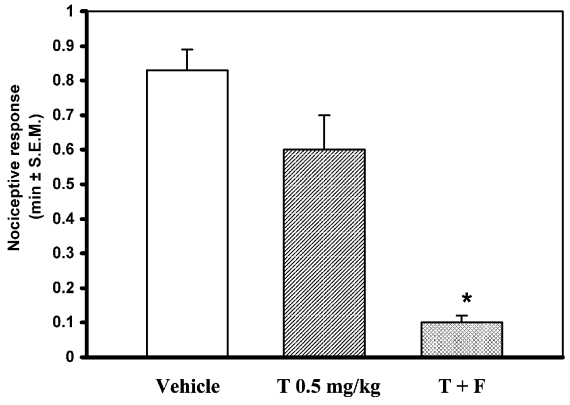

Fig. 9. Duration of nociceptive behaviour during phase 2 of the formalin test (1.25%, 50 μl) in mice treated with (±)-tramadol (T) (0.5 mg/kg, i.p.) 15 min before formalin and fluoxetine (F) (5 mg/kg, i.p.) 15 min after formalin. The data represent the total time of nociceptive responses (mean±S.E.M. of 7–10 mice per group) measured during phase 2. *P<0.05 vs. tramadol.

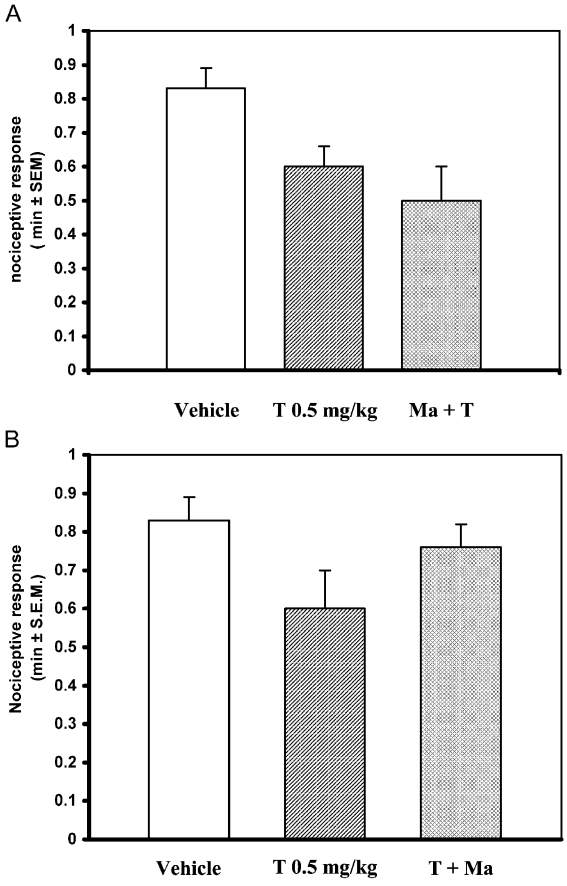

Fig. 10. Duration of nociceptive behaviour during phase 2 of the formalin test (1.25%, 50 μl) in mice treated with (±)-tramadol (T) (0.5 mg/kg, i.p.) 15 min before formalin and maprotiline (Ma) (5 mg/kg, i.p.) 15 min before [A] and after [B] formalin. The data represent the total time of nociceptive responses (mean±S.E.M. of 7–10 mice per group) measured during phase 2. *P<0.05 vs. tramadol.

after-formalin, modified the effect of the lowest dose (0.5 mg/kg) of (±)-tramadol that significantly (P<0.05) reduced phase 2 of the formalin test (Fig. 9). On the contrary maprotiline (5 mg/kg), a selective norepinephrine reuptake inhibitor, administered 15 min before and 15 min after formalin, did not modify the effect of the lowest dose of (±)-tramadol (Fig. 10 A,B). Fluoxetine and maprotiline (5 mg/kg) administered 15 min before and after formalin, did not modify the duration of phase 2 of the formalin test (data not shown).

4. Discussion

The opioid analgesic tramadol, which has long been used in clinical practice, has a distinct pharmacodynamic profile that has yet to be characterised. Similarly, the role played by monoamines and opioids in the antinociceptive effect exerted by tramadol is still not completely known. Therefore, the aim of this study was to evaluate the involvement of the serotonergic, adrenergic and opioidergic neurotrans-

missions in the antinociceptive effect of tramadol in the formalin test. Although there is evidence that a drug-induced circulatory change may contribute to the antinociceptive effect of some drugs (Hole and Tjolsen, 1993), to our knowledge there is no evidence that tramadol is able to modify cutaneous circulation or the release of vascular mediators. At the doses used in our study, tramadol did not induce motor impairment. However, at higher doses, tramadol has been found to cause motor impairment (Raffa et al., 1993).

Our findings indicate that (±)-tramadol and its enantiomers reduced nociceptive behaviour during phase 2 of the formalin test without affecting phase 1 of the test. The rank order potency of this antinociceptive effect, i.e. (±)-tramadol>(+)-tramadol>(−)-tramadol, confirms the greater activity of the racemic form. The enantiomers of tramadol have distinct pharmacologic profiles. The (+)-enantiomer had Ki values of only 1.33, 62.4 and 54.0 μM at the μ_1, δ and κ receptors, respectively. The (−)-enantiomer had a lower affinity at the μ and δ sites (Ki=24.8, 213 and 53.5 μM, respectively). The (+)-enantiomer preferentially inhibited 5-HT uptake and enhanced 5-HT release, whereas the (−)-enantiomer preferentially inhibited NE uptake. The finding that racemic tramadol is more potent than the theoretical additive effect of the enantiomers suggests there is a synergistic and complementary antinociceptive interaction between the enantiomers (antinociceptive synergy) (Raffa et al., 1993). Since the 5-HT reuptake inhibition resides mainly in the (+)-enantiomer (Driessen and Reimann, 1992), the stronger activity shown in our study by this enantiomer suggests that the antinociceptive effect of tramadol in the formalin test is mainly due to serotonergic neurotransmission. The importance of serotonin in the descending pathway that modulates pain is well established (Fields et al., 1977; Yaksh and Elde, 1981). A key site of the serotonergic pathway is the nucleus raphe magnus, which is part of the ventral midbrain periaqueductal gray, and it is connected to the spinal cord (Fields and Basbaum, 1978; Yaksh and Wilson, 1979). At this level, 5HT inhibits spinal cord neurons that participate in the integration of nociceptive stimuli (Fields and Basbaum, 1978; Aimone et al., 1987).

The descending inhibitory system includes a noradrenergic pathway that originates in the locus coerulus and inhibits the nociceptive response at the level of the dorsal horn via activation of α_2 adrenoreceptors in the upper cellular layers. There is evidence that the descending inhibitory pathways are important sites for opioid analgesics (Heinricher et al., 1992).

Our experiments with a 5-HT selective reuptake inhibitor confirm the main involvement of serotonergic neurotransmission. In fact, fluoxetine significantly increased the antinociceptive activity of (±)-tramadol. These findings are broadly consistent with literature (Bamigbade et al., 1997). In fact, this synergic effect may be due to the capacity of (±)-tramadol and its (+)-enantiomer to inhibit 5-HT reuptake and to increase 5-HT efflux. On the contrary, maprotiline, a selective norepinephrine reuptake inhibitor, did not modify the tramadol effect, thus excluding the involvement of noradrenergic neurotransmission. Furthermore, the 5-HT_2 antagonist ketanserin reduced the antinociceptive effect of tramadol, thus confirming the involvement in this effect of 5-HT via 5-HT_2 receptors. Recently, Sasaki et al. (2001) demonstrated that 5-HT_2 and 5-HT_3 receptors were involved at spinal level in the modulation of pain induced by formalin. On the other hand, the incapacity of naloxone to modify the effect of tramadol rules out the involvement of opioid neurotransmission. It has been demonstrated that tramadol not only inhibits 5-HT reuptake, but also induces 5-HT release in the raphe dorsal nucleus (Bamigbade et al., 1997). Consequently, we speculate that tramadol reduces formalin-induced tonic pain by increasing 5-HT concentration at spinal cord level. This hypothesis is consistent with the data of Nayebi and Ahmadiani (1999) that intrathecal injection of 5-HT inhibits phase 2 of the formalin test. The possibility that opioids or noradrenaline might exert their effects mainly during the induction phase seems unlikely because the double administration (before and after induction) of drugs interfering with these systems did not affect the appearance of nociceptive behaviours.

In conclusion, this study demonstrates that the antinociceptive effect of tramadol in the formalin test seems to be mediated by serotonergic transmission and that the opioid and noradrenergic systems play a minor role in this process. Moreover, these results indirectly show that 5-HT, as well as such other neurotransmitters as glutamate, substance P and CGRP (Coderre et al., 1993; Hudspith, 1997; Furst, 1999) may play a role in the maintenance of tonic pain.

Acknowledgements

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