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Involvement of potassium channels and nitric oxide in tramadol antinociception

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Abstract

It has been considered that tramadol, a centrally acting analgesic, shows its effect via opiatergic, noradrenergic, and serotonergic systems. It has a low affinity for opioid receptors, and its effect can be partly blocked by naloxone. Since the noradrenergic and serotonergic mechanisms are still unknown, other systems which are associated with pain and analgesia may have a role on the antinociceptive effect of tramadol.

The aim of this study was to evaluate the effects of K^+ channels and nitrergic systems on the antinociceptive action of tramadol. The antinociceptive effects of tramadol were determined in mice by the hot plate test. To examine the effects of K^+ channels and the nitrergic system nonspecific voltage-dependent K^+ channel blockers 4-aminopyridine (4-AP) and tetraethylammonium (TEA), nitric oxide (NO) precursor L-arginine, and the NO synthase (NOS) inhibitor N^G-nitro-L-arginine methyl ester (L-NAME) were used.

Our results indicated that 4-AP, TEA, and L-arginine reduced the antinociceptive effect of tramadol. However, L-NAME augmented the antinociceptive effect of tramadol. The reduction of the effects of tramadol by L-arginine was reversed by L-NAME.

The results of our study suggest that nonspecific voltage-dependent K^+ channels and nitrergic system have a role on the antinociceptive effect of tramadol in mice hot plate test.

Keywords: Tramadol; 4-Aminopyridine (4-AP); Tetraethylammonium (TEA); L-arginine; N^G-nitro-L-arginine methyl ester (L-NAME); Hot plate

1. Introduction

Tramadol is an orally active, clinically effective, centrally acting analgesic. The analgesic efficacy and potency of acutely administered tramadol is comparable to that of codeine, pentazocine, or dextropropoxyphene (Hennies et al., 1988), while its analgesic and antinociceptive potency is only 5- to 10-fold lower than that of morphine (Lehmann et al., 1990). It is believed that tramadol works by μ-opioid receptors (Raffa et al., 1992) despite its relatively low binding affinity (Hennies et al., 1988). In addition, its analgesic effect can only be antagonized 30% by naloxone (Collart et al., 1993). Unlike typical opioid analgesics, tramadol has not been associated with significant opioid side effects, such as respiratory depression, constipation, or sedation. Tramadol, as opposed to morphine, is not likely to induce tolerance and physical dependence (Raffa et al., 1992; Dayer et al., 1994; Besson and Vickers, 1994). Thus, it is speculated that nonopioid mechanisms are involved in tramadol analgesia. In accordance with the recognized implication of noradrenaline and serotonin in pain modulation, tramadol has been shown to inhibit the re-uptake of noradrenaline and serotonin, thereby increasing the concentration of these two neurotransmitters in selected brain areas, thus raising the pain threshold (Driessen and Reimann, 1992; Raffa et al., 1992). 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, and the noradrenergic and serotonergic involvement is still not completely understood (Oliva et al., 2002; Shiraishi et al., 2002).

It has been reported that K^+ channels and the NO-cGMP pathway have roles in the modulation of the antinociceptive effects of drugs, such as morphine

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(Rodrigues and Duarte, 2000), diclofenac (Ortiz et al., 2002), and ketorolac (Lazaro-Ibanez et al., 2001). Central K^+ channels appear to be involved in the modulation of pain perception. The opening of K^+ channels plays a role in opioid-mediated antinociception, since the specific ATP-dependent K^+ (K_{ATP}) channel blocker, glibenclamide, antagonizes the antinociceptive effect of morphine in a dose-dependent manner (Ocana et al., 1990). The K^+ channel activator pinacidil produces opposite effects (Vergoni et al., 1992).

On the other hand, it has also been reported that nitric oxide (NO) plays a role in the perception of pain at many levels of nociceptive neural pathways (Solodkin et al., 1992; Kitto et al., 1992). Using L-arginine/NO/cGMP cascade activators and/or inhibitors, it has been shown that NO plays both nociceptive and antinociceptive roles (Abacioglu et al., 2000). The role of NO in peripheral and spinal events in nociception is complex. N^G-nitro-L-arginine methyl ester (L-NAME) and N^G-monomethyl-L-arginine (L-NMMA), both NO synthase (NOS) inhibitors, and methylene blue, a soluble guanylate cyclase inhibitor, administered intracerebroventricularly (icv), produce opioid-independent antinociceptive effects in mice. These effects are blocked by coadministration of dibutyryl cyclic GMP, suggesting that the NO–cyclic GMP system potentiates supraspinal transmission of nociceptive information (Kawabata et al., 1994; Babbedge et al., 1993). In addition, oral and intraperitoneal (ip) administration of L-arginine dose-dependently and stereospecifically reduces the antinociceptive effect of morphine assessed in mice using the hot plate, tail-flick, and acetic acid-induced writhing tests (Brignola et al., 1994).

This study was undertaken to determine whether K^+ channels and the nitrergic system have any effect on the antinociception induced by tramadol. We therefore tested the effects of nonspecific voltage-dependent K^+ channel blockers 4-aminopyridine (4-AP) and tetraethylammonium (TEA), a nitric oxide precursor L-arginine and the NOS inhibitor L-NAME on the antinociceptive effects of tramadol.

2. Materials and methods

2.1. Animals

Male, albino, inbred Swiss mice weighing 25–30 g were obtained from the Cukurova University Experimental Research Center (TIBDAM). The mice were housed, five per cage, and kept at 24±1 °C, in a light–dark cycle. They received food and water ad libitium. All experiments were conducted between 0900 and 1100 h in a sound-attenuated laboratory and were performed according to the guidelines of the principles of laboratory animal care published by NIH. All protocols were approved by the Animal Ethics Committee of Cukurova University.

2.2. Hot plate test

The hot plate test was performed in mice as described by Woolfe and MacDonald (1944). Animals were placed on a hot plate apparatus (Ugo Basile) that was thermostatically maintaned at 55±1 °C. Animals were placed on the heated surface, and the time interval (seconds) between placement and a paw licking was recorded as the hot plate latency (HPL). Only one determination was performed for each animal. The cutoff time was of 30 s of exposure.

2.3. Drugs

We purchased 4-aminopyridine (A-0512), tetraethylammonium chloride (T-2265), N^G-nitro-L-arginine methyl ester dihydrochloride (N-5751), D-arginine hydrochloride (A-6757) and N^G-nitro-D-arginine methyl ester dihyrochloride (N-4770) from Sigma (Steinheim, Germany). L-arginine monohydrochloride was purchased from Merck (Darmstadt, Germany), and tramadol from Abdi Ibrahim (Istanbul, Turkey). All drugs were dissolved in 0.9% NaCl solutions.

4-AP and TEA were administered intracerebroventricularly (icv, 5 μl/mouse) 20 min after tramadol injection, and HPL was measured 10 min after the last injection. For all groups tramadol, L-arginine, D-arginine, L-NAME, and D-NAME were given intraperitoneally (ip). Intraperitoneal injections were given in a volume of 0.1 ml/10 g body weight. In tramadol+L-arginine, tramadol+D-arginine, tramadol+L-NAME, or tramadol+D-NAME groups, tramadol was given 5 min after the first drug, and HPL was measured 30 min after tramadol injection. In the tramadol+L-arginine+L-NAME group, L-NAME, L-arginine, and tramadol were administered in 5-min sequence. The HPL was measured 30 min after the last injection. Control groups received 0.9% NaCl.

2.4. Statistics

Results were expressed as the means±S.E.M. One-way analysis of variance (ANOVA) followed by a post hoc Student Newman–Keuls test was used for the comparison of multiple groups, and the unpaired Student's *t*-test was used for the comparison of two groups. Significance was set at $P<0.05$.

3. Results

3.1. Effect of tramadol on the hot plate latency

The dose–response curve for tramadol (10, 20, 40, 50, and 60 mg/kg, ip) in the hot plate test is shown in Fig. 1. At doses of 20, 40, 50, and 60 mg/kg, tramadol significantly and dose-dependently increased the HPL when compared to the control group ($P<0.05$, one-way

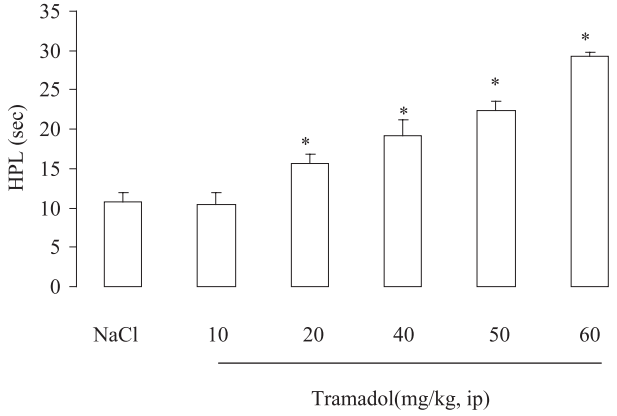

Fig. 1. Effect of tramadol (mg/kg, ip) on the hot plate latency (HPL).*P<0.05, significantly different when compared to the control (0.9% NaCl, ip) group, using one-way ANOVA, post hoc Student Newman–Keuls test. Eight mice were used in each group.

ANOVA, post hoc Student Newman–Keuls test). However, the lowest dose, 10 mg/kg, had no effect. As little as 20 mg/kg of tramadol produced a remarkable antinociceptive effect, and 40 mg/kg dose produced optimal antinociceptive effects. Thus, we chose these two doses for the remaining experiments.

3.2. Effect of 4-AP on the antinociceptive action of tramadol

The K^+ channel blocker 4-AP (0.05 μg/per mouse, icv) did not change the HPL of the mice when compared with control group. The HPL's of the mice that received saline (control) and 4-AP were 8.46±0.92 and 9.16±0.64, respectively. If we administered both 4-AP and tramadol, the effect of tramadol was significantly reduced. The HPL's of the mice that received 20 and 40 mg/kg tramadol alone and with 4-AP were 16.10±1.46, 20.10±1.43, 9.95±0.61, and 14.28±1.21, respectively. The differences were statistically significant when compared to the tramadol groups with related 4-AP+tramadol groups using two group comparisons in the unpaired Student's t-test (Fig. 2; P<0.05).

3.3. Effect of TEA on the antinociceptive action of tramadol

The K^+ channel blocker TEA (20 μg/per mouse, icv) did not change the HPL of the mice when compared with control group. The HPL's of the mice that received saline (control) and TEA were 9.25±0.90 and 7.91±0.46, respectively. However TEA significantly reduced the antinociceptive effect of tramadol. The HPL's of the mice that received 20 and 40 mg/kg tramadol alone were 16.08±1.46 and 20.06±1.43, respectively, and with TEA were 6.85±0.57 and 13.19±1.44, respectively. The differences were statistically significant when compared to the tramadol groups and the TEA+tramadol groups using two group comparisons in the unpaired Student's t-test (Fig. 3; P<0.05).

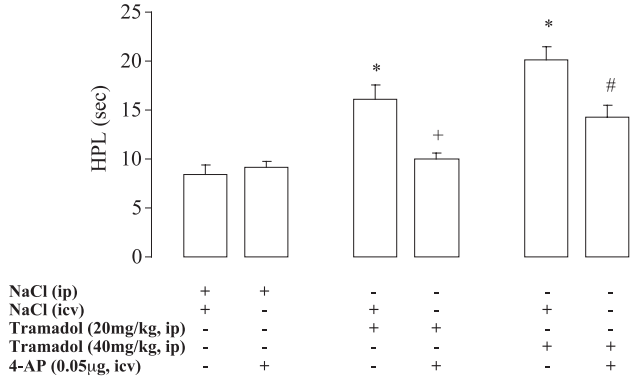

Fig. 2. Effect of 4-AP (0.05 μg/per mouse, icv) on the antinociceptive action of tramadol (20 and 40 mg/kg, ip) in the hot plate test. *P<0.05, significantly different when compared to the control (NaCl, ip+NaCl, icv) group, ^{+}P<0.05, significantly different when compared to the tramadol (20 mg/kg, ip)+NaCl (icv) group, and $^{\#}P$<0.05, significantly different when compared to the tramadol (40 mg/kg, ip)+NaCl (icv) group, using unpaired Student's t-test. Eight mice were used in each group.

3.4. Effect of L-arginine on the antinociceptive action of tramadol

The NO precursor L-arginine when used in the dose range of 0.25–50 mg/kg did not produce any nociceptive or antinociceptive effects (data not shown). Likewise low dose of L-arginine (0.25 mg/kg) did not modify the antinociceptive action of tramadol (data not shown). However, 50 mg/kg of L-arginine significantly decreased tramadol's antinociceptive action. The HPL's of the mice receiving 20 and 40 mg/kg tramadol were 15.45±0.83 and 20.21±1.38, respectively, and those receiving 20 and 40 mg/kg tramadol with L-arginine were 8.21±0.51 and 11.89±0.76, respectively. Significant differences were seen when compared the tramadol groups with related tramadol+L-arginine groups

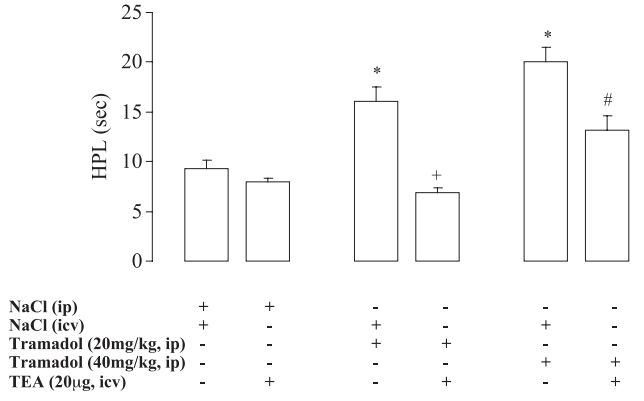

Fig. 3. Effect of TEA (20 μg/per mouse, icv) on the antinociceptive action of tramadol (20 and 40 mg/kg, ip) in the hot plate test. *P<0.05, significantly different when compared to the control (NaCl, ip+NaCl, icv) group, ^{+}P<0.05, significantly different when compared to the tramadol (20 mg/kg, ip)+NaCl (icv) group, and $^{\#}P$<0.05, significantly different when compared to the tramadol (40 mg/kg, ip)+NaCl (icv) group, using unpaired Student's t-test. Eight mice were used in each group.

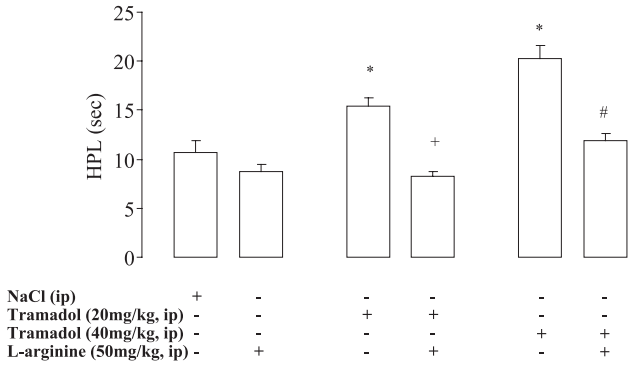

Fig. 4. Effect of L-arginine (50 mg/kg, ip) on the antinociceptive action of tramadol (20 and 40 mg/kg, ip) in the hot plate test. *P<0.05, significantly different when compared to the control (NaCl, ip) group, ^{+}P<0.05, significantly different when compared to the tramadol (20 mg/kg, ip) group, and $^{\#}P$<0.05, significantly different when compared to the tramadol (40 mg/kg, ip) group, using unpaired Student's t-test. Eight mice were used in each group.

using two group comparisons in the unpaired Student's t-test (Fig. 4; P<0.05).

3.5. Effect of L-NAME on the antinociceptive action of tramadol

The NO synthesis inhibitor L-NAME used in the dose range of 20–150 mg/kg did not produce any nociceptive or antinociceptive effects (data not shown). However, L-NAME (150 mg/kg) significantly increased the antinociceptive action of tramadol. The HPL's of mice receiving 20 and 40 mg/kg tramadol were 15.58±1.30 and 19.24±1.93, respectively, and those receiving 20 and 40 mg/kg tramadol with L-NAME were 20.11±1.63 and 28.28±0.86, respectively. These differences were significant using the two group comparison. (Fig. 5; P<0.05, unpaired Student's t-test).

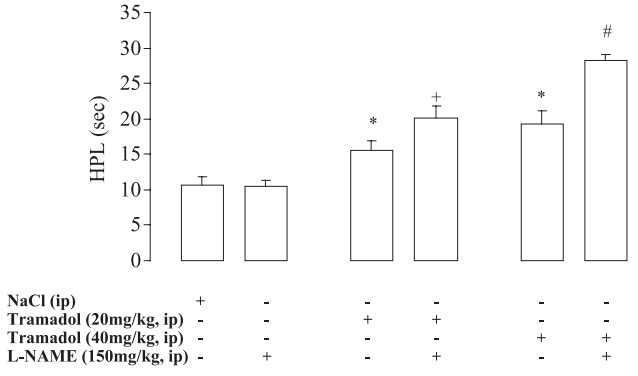

Fig. 5. Effect of L-NAME (150 mg/kg, ip) on the antinociceptive action of tramadol (20 and 40 mg/kg, ip) in the hot plate test. *P<0.05, significantly different when compared to the control (NaCl, ip) group, ^{+}P<0.05, significantly different when compared to the tramadol (20 mg/kg, ip) group, and $^{\#}P$<0.05, significantly different when compared to the tramadol (40 mg/kg, ip) group, using unpaired Student's t-test. Eight mice were used in each group.

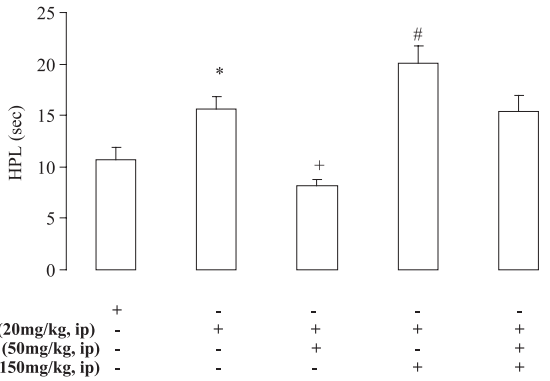

Fig. 6. Effects of L-NAME (150 mg/kg, ip)+L-arginine (50 mg/kg, ip) on the antinociceptive action of tramadol (20 mg/kg, ip). *P<0.05, significantly different when compared to the control (NaCl, ip) group, and ^{+}P<0.05, significantly different when compared to the tramadol (20 mg/kg, ip) group using unpaired Student's t-test. Eight mice were used in each group.

3.6. Antagonism of L-arginine+tramadol antinociception by L-NAME

L-NAME (150 mg/kg) reversed the effect of L-arginine (50 mg/kg) on tramadol (20 mg/kg) antinociception. The HPL's of the mice that received NaCl, tramadol, L-arginine+tramadol, L-NAME+tramadol, and L-arginine+tramadol+L-NAME were 10.71±1.19, 15.58±1.30, 8.21±0.51, 20.11±1.63, and 15.35±1.65, respectively. These differences were significant when compared using the two group comparison in the unpaired Student's t-test. No significant differences were seen between the tramadol+L-arginine+L-NAME and tramadol (20 mg/kg) groups (Fig. 6; P<0.05).

4. Discussion

In this study, we examined the effects of the K^{+} channel blockers 4-AP, TEA, and NO modulators on the antinociceptive action of tramadol. Tramadol at a dose of 10 mg/kg had no effect on HPL, whereas it had a dose-dependent antinociceptive effect at 20, 40, 50, and 60 mg/kg. This is in line with the studies of Rojas-Corrales et al. (2000), which showed that 20 and 40 mg/kg doses enhanced hot plate latency, but 10 mg/kg tramadol did not.

K^{+} channel blockers alone (4-AP and TEA) did not change the HPL, although they reduced the antinociceptive effect of 20 and 40 mg/kg tramadol. The lack of effect of the K^{+} channel blockers is consistent with the results of studies in which these compounds did not modify the nociceptive activity of thermal noxious stimuli and mechanical hyperalgesia (Rodrigues and Duarte, 2000; Ortiz et al., 2002). Thus, excluding the possibility that the prevention of tramadol antinociception is due to hyperalgesic or nociceptive effects of K^{+} channel blockers.

Many studies have focused on the role of K^+ channels in pain. It is known that K^+ channels play essential roles in setting the resting membrane potential and in controlling the excitability of neurons. The opening of K^+ channels leads to hyperpolarization of cell membranes, which results in a decrease in cell excitability. The opening of K^+ channels is important in opioid-mediated antinociception, since the specific ATP-dependent K^+ channel blockers glibenclamide and gliquidone dose-dependently antagonize the antinociceptive effect of morphine (Ocana et al., 1990; Rodrigues and Duarte, 2000), whereas the K^+ channel activators pinacidil (Vergoni et al., 1992) and cromakalim (Ocana et al., 1996) potentiate morphine-induced analgesia. On the contrary, the voltage-dependent K^+ channel blockers 4-AP and TEA do not modify the antinociceptive effects of morphine (Rodrigues and Duarte, 2000) and clonidine (Ocana and Bayens, 1993). However, the voltage-dependent K^+ channel blockers 4-AP and TEA significantly diminish the antinociceptive effects of diclofenac, baclofen, and resveratrol (Ortiz et al., 2002; Ocana and Bayens, 1993; Granados-Soto et al., 2002). In this study, they also decreased the antinociceptive effect of tramadol.

Raffa and Codd (1994) demonstrated that the K^+_{ATP} channel blocker glibenclamide or the nonspecific voltage-dependent K^+ channel blocker TEA could not bind directly to opioid receptors. This supported the hypothesis that antagonism of opioid-induced antinociception by glibenclamide or TEA occurs at the level of K^+ channels and not opioid receptors.

According to our results, K^+ channels are involved in the antinociceptive effects of tramadol. It is possible, however, that this effect of only these two nonselective K^+ channel blockers, 4-AP and TEA, is not enough to make satisfactory comments. It is quite clear that aminopyridines and TEA are not selective for any particular K^+ channels (Rudy, 1988; Schechter, 1997). Although both 4-AP and TEA have been shown to block voltage-dependent K^+ channels, some pharmacological studies have revealed that the affinities of these two blockers differ significantly among the voltage-gated K^+ channels (Rehm, 1991; Grissmer et al., 1994). TEA may also block a Ca^{++}-activated K^+ current (Farley and Rudy, 1988; Aranson, 1992). Additional experiments using different specific blockers, such as apamine or carybdotoxine (Aranson, 1992), which inhibit small-conductance Ca^{++}-activated and large-conductance Ca^{++}-activated K^+ currents, respectively, would help to clarify the role of tramadol antinociception and K^+ channels. Recent evidence has shown that TEA also blocks KCNQ-potassium channels (Hadley et al., 2000, 2003), a family of five voltage-gated K^+ channel subunits, four of which are present in the nervous system (Jentsch, 2000). This type of K^+ channels are important in the antihyperalgesic effect of some analgesic drugs (Dost et al., 2004; Passmore et al., 2003). KCNQ/M channels are present in nociceptive sensory systems, and IKM, a neuron-specific voltage-dependent K^+ current, plays a key role in controlling the excitability of nociceptors (Passmore et al., 2003). Retigabine, a specific activator of IKM, can alleviate some forms of chronic pain (Jensen et al., 2001). In addition, it has been shown that retigabine is as effective as tramadol in the L5 ligation model with thermal stimulation (Dost et al., 2004). The effect of retigabine was diminished by linopirdine, a selective KCNQ- K^+ channel blocker. Based on these results, it may be speculated that the effect of TEA on tramadol antinociception may result from the KCNQ- K^+ channel-blocking effect of TEA. Further experiments using specific KCNQ-K^+ channel blockers, such as linopiridin, or activators, such as retigabine, are necessary to suggest more precise mechanisms.

In this study, L-arginine and L-NAME alone did not alter the HPL, while L-arginine inhibited and L-NAME augmented the antinociceptive effect of tramadol. The inhibition of antinociceptive effect of tramadol by L-arginine was reversed by L-NAME.

There is increasing evidence indicating a role for NO in the development, maintenance and mechanisms that underlie mechanical, chemical, and thermal hyperalgesia (Meller et al., 1992; Moore et al., 1991). There are several studies indicating that NO plays a role in the perception of pain at many levels of nociceptive neural pathways (Solodkin et al., 1992; Kitto et al., 1992).

Abacioglu et al. (2000) showed that L-arginine at the doses 10–100 mg/kg has a dose-dependent triphasic pattern of nociception–antinociception–nociception. In contrast, L-NAME possesses antinociceptive activity at the doses of between 18.75 and 150 mg/kg. In addition, L-NAME has been shown antinociceptive effect on the acute nociceptive response induced by low concentrations of formalin (Sakurada et al., 2001). In contrast, NOS inhibitors have not been shown to have any effect by the modified tail-flick assay (Kitto et al., 1992). Ozbek et al. (2000) showed that L-arginine (300 and 500 mg/kg) and L-NA (4–16 mg/kg) had no analgesic effect, whereas L-NA, but not L-arginine, enhanced antinociception induced by morphine. On the contrary, Pataki and Telegdy (1998) reported that administration of L-NA diminished morphine-induced analgesia, whereas L-arginine pretreatment increased the analgesic effect of morphine, suggesting that increased NO synthesis potentiates morphine analgesia. As the results of many studies have not been consistent, Kawabata et al. (1993) aimed to clarify the role of L-arginine in supraspinal nociceptive processing mediated by kyotorphin–Met–enkephalin and NO–cyclic GMP systems. In this study, they concluded that L-arginine plays a dual role in the brain, exerting antinociceptive effects via the former system and nociceptive via the latter. For this reason, we used seven different doses of L-arginine (0.25–50 mg/kg). Any dose of L-arginine used in this study did not modify the HPL. To evaluate the effect of L-arginine on the antinociceptive action of tramadol, we used low (0.25 mg/kg) and high (50 mg/kg) doses of this drug. Only the higher dose changed the

antinociceptive action of tramadol. Based on the results of this study, it seems that NO has not a dual effect in the antinociceptive action of tramadol. NO can activate different types of K^+ channels in several tissues by increasing cyclic GMP (Armstead, 1996). Recently, Soares et al. (2000) suggested a link between the activation of the NO–cyclic GMP pathway and the opening of ATP-sensitive K^+ channels, as glibenclamide was able to block the antinociceptive effect of sodium nitroprusside. Lazaro-Ibanez et al. (2001) showed that the antinociceptive effect of ketorolac involves activation of the NO–cyclic GMP pathway followed by an opening of ATP-sensitive K^+ channels. We suggest that this mechanism has not been involved in the antinociceptive action of tramadol. The augmentation of NO synthases by L-arginine decreased the antinociceptive action of tramadol. In contrast, the inhibition of NO synthases by L-NAME increased this effect, suggesting that K^+ channels and nitrergic system act independently on tramadol analgesia.

In conclusion, our results show that nonspecific voltage-dependent K^+ channels and the nitrergic system have a role on the antinociceptive effect of tramadol in the mice hot plate test.

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References

Abacioglu N, Tunctan B, Akbulut E, Cakici İ. Participation of the components of L-arginine/nitric oxide/cGMP cascade by chemically-induced abdominal constriction in the mouse. Life Sci 2000;67(10):1127–37.

Aranson JK. Potassium channels in nervous tissue. Biochem Pharmacol 1992;43:11–4.

Armstead WM. Role of ATP-sensitive K^+ channels in cGMP-mediated pial artery vasodilation. Am J Physiol 1996;270:423–6.

Babbedge RC, Hart SL, Moore PK. Anti-nocieptive activity of nitric oxide synthase inhibitors in the mouse: dissociation between the effect of L-NAME and L-NMMA. J Pharm Pharmacol 1993;45(1):77–9.

Besson JM, Vickers MD. Tramadol analgesia Synergy in research and therapy. Drugs 1994;47:1–2.

Brignola G, Calignano A, Di Rosa M. Modulation of morphine antinociception in the mouse by endogenous nitric oxide. Br J Pharmacol 1994;113(4):1372–6.

Collart L, Luthy C, Dayer P. Partial inhibition of tramadol antinociceptive effect by naloxone in man. Br J Clin Pharmacol 1993;35:73.

Dayer P, Collart L, Desmeules J. The pharmacology of tramadol. Drugs 1994;47(1):3–7.

Dost R, Rostock A, Rundfeldt C. The anti-hyperalgesic activity of retigabine is mediated by KCNQ potassium channel activation. Naunyn Schmiedebergs Arch Pharmacol 2004;369:382–90.

Driessen B, Reimann W. Interaction of the central analgesic, tramadol, with the uptake and release of 5-hydroxytryptamine in the rat brain in vitro. Br J Pharmacol 1992;105(1):147–51.

Farley J, Rudy B. Multiple types of voltage-dependent Ca^{2+}-activated K^+ channels of large conductance in rat brain synaptosomal membranes. Biophys J 1988;53:919–34.

Granados-Soto V, Arguelles CF, Ortiz MI. The peripheral antinociceptive effect of resveratrol is associated with activation of potassium channels. Neuropharmacology 2002;43(5):917–23.

Grissmer S, Nguyen AN, Aiyar J, Hanson DC, Mather RJ, Gutman GA, et al. Pharmacological characterization of five cloned voltage-gated K^+ channels, types Kv1.1, 1.2, 1.3, 1.5 and 3.1, stably expressed in mammalian cell lines. Mol Pharmacol 1994;45:1227–34.

Hadley JK, Noda M, Selyanko AA, Wood IC, Abogadie FC, Brown DA. Differential tetraethylammonium sensitivity of KCNQ1-4 potassium channels. Br J Pharmacol 2000;129(3):413–5.

Hadley JK, Passmore GM, Tatulian L, Al-Qatari M, Ye F, Wickenden AD, et al. Stoichiometry of expressed KCNQ2/KCNQ3 potassium channels and subunit composition of native ganglionic M channels deduced from block by tetraethylammonium. J Neurosci 2003;23(12):5012–9.

Hennies HH, Friderichs E, Schneider J. Receptor binding, analgesic and antitussive potency of tramadol and other selected opiods. Arzneim-Forsch/Drug Res 1988;38(7):877–80.

Jensen TS, Gottrup H, Kasch H, Nikolajsen L, Terkelsen AJ, Witting N. Has basic research contributed to chronic pain treatment? Acta Anaesthesiol Scand 2001;45:1128–35.

Jentsch TJ. Neuronal KCNQ potassium channels: physiology and role in disease. Nat Rev Neurosci 2000;1:21–30.

Kawabata A, Umeda N, Takagi H. L-arginine exerts a dual role in nociceptive processing in the brain: involvement of the kyotorphin–Met–enkephalin pathway and NO–cyclic-GMP pathway. Br J Pharmacol 1993;109(1):73–9.

Kawabata A, Manabe S, Manabe Y, Takagi H. Effect of topical administration of L-arginine on formalin-induced nociception in the mouse: a dual role of peripherally formed NO in pain modulation. Br J Pharmacol 1994;112(2):547–50.

Kitto KF, Haley JE, Wilcox GL. Involvement of nitric oxide in spinally mediated hyperalgesia in the mouse. Neurosci Lett 1992;148(1–2):1–5.

Lazaro-Ibanez GG, Torres-Lopez JE, Granados-Soto V. Participation of the nitric oxide-cyclic GMP-ATP sensitive K^+ channel pathway in the antinociceptive action of keterolac. Eur J Pharmacol 2001;426:39–44.

Lehmann KA, Kratzenberg U, Schroeder-Bark B, Horrichs-Haermeyer G. Post-operative patient-controlled analgesia with tramadol: analgesic efficacy and minimum effective concentrations. Clin J Pain 1990;6(3):212–20.

Meller ST, Dykstra C, Gebhart GF. Production of endogenous nitric oxide and activation of soluble guanylate cyclase are required for *N*-methyl-D-aspartate produced facilitation of the nociceptive tail-flick reflex. Eur J Pharmacol 1992;214(1):93–6.

Moore PK, Oluyomi AO, Babbedge RC, Wallace P, Hart SL. L-N^G-nitro arginine methyl ester exhibit antinociceptive activity in mouse. Br J Pharmacol 1991;102(1):198–202.

Ocana M, Bayens JM. Differential effects of K^+ channel blockers on antinociception induced by α_2-adrenoceptor, $GABA_B$ and κ-opioid receptor agonists. Br J Pharmacol 1993;110(3):1049–54.

Ocana M, Del Pozo E, Barrios M, Robles LI, Baeyens JM. An ATP-dependent potassium channel blocker antagonizes morphine analgesia. Eur J Pharmacol 1990;186(2–3):377–8.

Ocana M, Barrios M, Bayens JM. Cromakalim differentially enhances antinociception induced by agonists of alpha$_2$ adrenoceptors, γ-amino-butyric acid (B), mu and kappa opioid receptors. J Pharmacol Exp Ther 1996;276(3):1136–42.

Oliva P, Aurilio C, Massimo F, Grella A, Maione S, Grella E, et al. The antinociceptive effect of tramadol in the formalin test is mediated by the serotonergic component. Eur J Pharmacol 2002;445(3):179–85.

Ortiz MI, Torres-Lopez JE, Castaneda-Hernandez G, Rosas R, Vidal-Cantu GC, Granados-Soto GC. Pharmacological evidence for the activation of K^+ channels by diclofenac. Eur J Pharmacol 2002;438(1–2):85–91.

Ozbek H, Karatas Y, Aksu F, Ozcengiz D, Inan SY, Isik G. The effects of L-arginine and *N*-nitro-L-arginine on the tail-flick response and morphine analgesia in the mice. Agri 2000;12(3):19–25.

Passmore GM, Selyanko AA, Mistry M, Al-Qatari M, Marsh SJ, Matthews EA, et al. KCNQ/M currents in sensory neurons: significance for pain therapy. J Neurosci 2003;23(18):7227–36.

Pataki I, Telegdy G. Further evidence that nitric oxide modifies acute and chronic morphine actions in mice. Eur J Pharmacol 1998; 357(2–3):157–62.

Raffa RB, Friderichs E, Reimann W, Shank RP, Codd EE, Vaught JL. Opioid and nonopioid components independently contribute to the mechanism of action of tramadol, an 'atypical' opioid analgesic. J Pharmacol Exp Ther 1992;260(1):275–85.

Raffa RB, Codd EE. Lack of glibenclamide or TEA affinity for opioid receptors: further evidence for in vivo modulation of antinociception at K^+ channels. Brain Res 1994;650(1):146–8.

Rehm H. Molecular aspects of neuronal voltage-dependent K^+ channels. Eur J Biochem 1991;202:701–13.

Rudy B. Diversity and ubiquity of K channels. Neuroscience 1988;25: 729–47.

Rodrigues AR, Duarte ID. The peripheral antinociceptive effect induced by morphine is associated with ATP-sensitive K^+ channels. Br J Pharmacol 2000;129(1):110–4.

Rojas-Corrales MO, Ortega-Alvaro A, Gibert-Rahola J, Roca-Vinardell JA, Mico JA. Pindolol, a beta-adrenoceptor blocker/5-hydroxytrptamine$_{1A/1B}$ antagonist, enhances the analgesic effect of tramadol. Pain 2000;88:119–24.

Sakurada C, Sugiyama A, Nakayama M, Yonezawo A, Sakurada S, Tan-No K, et al. Antinociceptive effect of spinally injected L-NAME on the acute nociceptive response induced by low concentrations of formalin. Neurochem Int 2001;38:417–23.

Schechter LE. The potassium channel blockers 4-aminopyridine and tetraethylammonium increase the spontaneous basal release of [3H]5-hydroxytryptamine in rat hippocampal slices. J Pharmacol Exp Ther 1997;282:262–70.

Shiraishi M, Minami K, Uezono Y, Yanagihara N, Shigematsu A, Shibuya I. Inhibitory effects of tramadol on nicotinic acetylcholine receptors in adrenal chromaffin cells and in Xenopus oocytes expressing α 7 receptors. Br J Pharmacol 2002;136(2):207–16.

Soares AC, Leite R, Tatsuo MA, Duarte ID. Activation of ATP-sensitive K^+ channels: mechanism of peripheral antinociceptive action of the nitric oxide donor, sodium nitroprusside. Eur J Pharmacol 2000;400(1):67–71.

Solodkin A, Traub RJ, Gebhart GF. Unilateral hindpaw inflammation produces a bilateral increase in NADPH-diaphorase histochemical staining in the rat lumbar spinal cord. Neuroscience 1992;51(3):495–9.

Vergoni AV, Scarano A, Bertolin A. Pinacidil potentiates morphine analgesia. Life Sci 1992;50(116):135–8.

Woolfe HG, MacDonald AD. The evaluation of the analgesic action of pethidine hydrochloride. J Pharmacol Exp Ther 1944;80:300.