Available online at www.sciencedirect.com
SCIENCE DIRECT
European Journal of Pharmacology 530 (2006) 117–123
www.elsevier.com/locate/ejphar

In vitro and in vivo vasodilator activity of racemic tramadol and its enantiomers in Wistar rats

Juliana Montani Raimundo [a], Roberto Takashi Sudo [a], Luana Braga Pontes [a], Fernanda Antunes, Margarete Manhães Trachez [b], Gisele Zapata-Sudo [a,*]

[a] *Departamento de Farmacologia Basica e Clinica, Universidade Federal do Rio de Janeiro, Centro de Ciencias da Saude, Instituto de Ciencias Biomedicas, Bloco J, Sala 14, Rio de Janeiro 21941-590, Brazil*
[b] *Serviço de Anestesiologia, Universidade Federal Fluminense, Rio de Janeiro, Brazil*

Received 11 July 2005; received in revised form 10 November 2005; accepted 14 November 2005
Available online 20 December 2005

Abstract

Tramadol ((±)-tramadol) is an analgesic agent formulated as a racemic mixture (1 : 1) of (−)- and (+)-tramadol, which differ in their potency to bind to μ-opioid receptors and to inhibit monoamine-reuptake. We investigated the stereoselectivity of in vitro tramadol-induced vasodilatation of aortic rings and its effect on the arterial blood pressure measured in conscious Wistar rats. (+)-Tramadol, but not (−)-tramadol, produced a concentration-dependent relaxation of aorta precontracted with phenylephrine. The concentration–response curve was significantly altered by the removal of endothelium. Vascular relaxation was also inhibited by pre-incubation of endothelium-intact aorta with naloxone, suggesting the involvement of opioid receptors. The vasodilatation produced by tramadol was stereoselective, and the (+)-tramadol-induced vasodilatation was mediated by μ-opioid receptors and partially dependent on endothelium integrity. The hypotensive response induced by (+)-tramadol was also observed after bolus injection of 5.0 and 10.0 mg/kg. The results indicate that only high doses of tramadol cause cardiac depression and hypotension, indicating that it can be used safely.

Keywords: Tramadol; Enantiomers; Aorta; Vasodilatation; Papillary muscle; Naloxone

1. Introduction

Tramadol, (1RS, 2RS)-2-[(dimethylamino)-methyl]-1-(3-methoxyphenyl)-cyclohexanol hydrochoride, is a synthetic, centrally acting analgesic with an efficacy comparable to that of codeine, pentazocine or dextropropoxyphene when used for pain relief (Raffa et al., 1992). The mechanism of analgesic action involves a combination of tramadol binding to μ-opioid receptors and inhibition of the reuptake of serotonin and noradrenaline in the pain pathways of the central nervous system (CNS). Its affinity for μ-opioid receptors is approximately 10-fold less than that of codeine, 60-fold less than that of dextropropoxyphen and 6000-fold less than that of morphine (Raffa et al., 1992). Despite this fact, tramadol is only 5- to 10-fold less potent than morphine as an analgesic agent (Eggers and Power, 1995). Thus, the affinity of tramadol for opioid receptors appears not to be the most important factor responsible for its efficacy and potency. The inhibition of serotonin and noradrenaline reuptake by tramadol plays a significant role in its analgesic action, since endogenous norepinephrine and serotonin are known to be involved in pain modulation (Close, 2005; Lewis and Han, 1997). This dual mechanism of action makes tramadol an "atypical" opioid (Garrido et al., 2000). Tramadol ((±)-tramadol) is formulated as a racemic mixture (1 : 1) of (−)- and (+)-tramadol, which differ in their potency to bind to μ-opioid receptors and to inhibit monoamine-reuptake. (+)-Tramadol has a higher affinity for μ-opioid receptors and preferentially inhibits serotonin uptake and enhances serotonin release, whereas (−)-tramadol inhibits norepinephrine uptake (Raffa et al., 1993). The complementary and synergistic actions of the two enantiomers improve the analgesic efficacy profile of the racemate (Grond and Sablotzki, 2004). Unlike typical opioid analgesics, tramadol has not been

* Corresponding author. Tel./fax: +55 21 25626505.
E-mail address: gsudo@farmaco.ufrj.br (G. Zapata-Sudo).

0014-2999/$ - see front matter © 2005 Elsevier B.V. All rights reserved.
doi:10.1016/j.ejphar.2005.11.028

associated with significant side effects, such as respiratory depression or constipation. In addition, it has low potential for the development of tolerance, dependence and abuse (Yalcin and Aksu, 2005; Vickers et al., 1992). Although tramadol does not affect hemodynamics (Mildh et al., 1999), there have been some reports of orthostatic hypotension, especially after intravenous administration (Close, 2005), and of a transient rise in arterial blood pressure after intravenous injection (Müller et al., 1982). A high dose of tramadol has been found to cause myocardial depression (Close, 2005). Recently, Kaya et al. (2003) described that a racemic mixture of tramadol induced vascular relaxation of rabbit aorta as a result of nitric oxide production and a direct effect on smooth muscle. However, it has not been elucidated whether the vasodilator activity of (±)-tramadol is dependent on one or both enantiomers. The present work investigated the stereoselectivity of (±)-tramadol for inducing vasodilatation of aorta from Wistar rats. Experiments in vivo were used to investigate the interference of (±)-tramadol and its enantiomers with hemodynamic parameters. Additionally, comparative effects on cardiac contractility were investigated by measuring the amplitude of muscular twitches in papillary muscles from Wistar rats.

2. Materials and methods

The following protocols used in this randomized study were approved by Animal Care and Use Committee at Universidade Federal do Rio de Janeiro.

2.1. Preparation of aortic rings

The thoracic aorta was dissected from male Wistar rats (200–280 g), cleaned of connective tissue and prepared for isometric tension recording. The aorta was cut into 2–3 mm rings and was placed in a vertical chamber (internal volume 10 ml) filled with saline solution composed in millimolar of NaCl, 120; KCl, 5.9; $MgCl_2$, 1.2; NaH_2PO_4, 1.2; $NaHCO_3$, 18; $CaCl_2$, 2.5; glucose, 11 (pH 7.4) and oxygenated with carbogen gas at 37.0±0.5 °C. Each aorta ring was mounted between two hooks, one of which was attached to a force transducer (Grass Mod. FT-03) whose signal was conditioned by a Cyberamp (Axon Instruments, Inc.) and then displayed and stored on a computer for future analysis, using Axoscope software (Axon Instruments, Inc.). Preparations were stabilized under 1g resting tension for 2 h before the experimental protocol was started. The contractile response to a single concentration of phenylephrine (10 μM) was measured before and after exposure of aortic rings to increasing concentrations of tramadol (0.1–1.0 mM). Phenylephrine-induced contracture was also observed at the beginning and end of each experiment, followed by exposure to acetylcholine (10 μM) to test the integrity of endothelium. Endothelium was considered intact if the acetylcholine-induced relaxation of precontracted aorta was greater than 80%. In some experiments, (+)-tramadol was tested in aorta in which the endothelium has been mechanically removed. The removal of functional endothelium was confirmed by the lack of relaxation (<10%) in response to acetylcholine (10 μM). In other experiments, naloxone (100 μM) was added 15 min before the precontraction with phenylephrine in order to test the involvement of opioid receptors in the vascular effect of (+)-tramadol.

2.2. Experiments in vivo

Male Wistar rats (240–300 g) were anesthetized with ether and the right carotid artery was dissected for arterial blood pressure measurement using a calibrated pressure transducer (Statham, P022). A pair of external electrodes was placed on the chest for recording the electrocardiogram. Both blood pressure and electrocardiogram were recorded with a polygraph (Astro-Med Grass Physiological Recorder, Mod. 7400). Also, a catheter was placed in the jugular vein for intravenous injection of tramadol and its enantiomers. One hour after surgical procedures, rats were randomly allocated to three experimental groups. Each group was treated with a bolus injection of tramadol or its enantiomers at doses of 1.0, 5.0 and 10.0 mg/kg administered at 30-min intervals. Blood pressure and electrocardiogram were continuously recorded before and during administration of drugs.

2.3. Preparation of papillary muscles

Left papillary muscles were dissected from male Wistar rats (240–280 g) for isometric tension recording. Muscles were mounted in vertical chambers in which one end of each muscle was attached to a force transducer (Grass, FT 03) and the other end fixed to the bottom. Chambers were filled with Tyrode solution composed in millimolar of NaCl 130; KCl 5; $MgCl_2$ 1; NaH_2PO_4 0.5; $NaHCO_3$ 24; dextrose 5.6; $CaCl_2$ 2.5 and were oxygenated with a mixture of 95% O_2 plus 5% CO_2 at 37.0±0.5 °C. Muscular twitches were obtained by electrical stimulation at a rate of 1 Hz and 2 ms duration and stored on a computer as described above for smooth muscle. Increasing concentrations of racemic tramadol or its enantiomers (0.001–1.0 mM) were added to the solution and twitches were recorded before and after drug exposure. Recovery was observed 30 min after the solution containing tramadol was replaced with a drug-free solution.

2.4. Compounds

Tramadol hydrochloride and its enantiomers were synthesized and kindly donated by Cristália Produtos Quımicos e Farmacêuticos Ltda. (São Paulo, SP, Brazil). Phenylephrine and acetylcholine were purchased from Sigma Chemical Co. (St. Louis, MO, USA). All compounds were dissolved in distilled water.

2.5. Statistics

The relaxation induced by tramadol and its enantiomers is expressed as a percentage of maximal tension. All data are expressed as means±S.E.M. The difference between the effect of different concentrations was considered statistically

significant when $P<0.05$, using one-way analysis of variance (ANOVA) followed by a post hoc Dunnett's test. For the comparison of multiple groups, ANOVA was used, followed by Newman Keuls test. Paired Student's test was used for comparison between two groups (experiments in vivo). The concentration–response curves for the effect of tramadol and its enantiomers on papillary muscle were fitted to the equation:

$y = y_0 + a/(1 + e^{-x-xo/b})$ where $y_0 = y_{min}$; $a = y_{max} - y_{min}$; b = slope and $x_0 = IC_{50}$.

3. Results

3.1. Effects of tramadol and its enantiomers on vascular smooth muscle

The stereoselectivity of vasodilator activity was investigated in aortic rings pre-contracted with phenylephrine. Fig. 1A shows a typical recording of the maximal contractile response of aorta with intact endothelium induced by 10 μM phenylephrine, followed by exposure to cumulative concentrations of (+)-tramadol. (+)-Tramadol produced a concentration-dependent relaxation of precontracted aorta. At 0.5 and 1.0 mM, (+)-tramadol significantly reduced the amplitude of the contracture to 44.3±5.9% ($n=6$, $P<0.05$) and 22.2±9.4% of control ($n=6$, $P<0.05$), respectively (Fig. 1B). The concentration necessary to reduce by 50% the maximal contraction induced by phenylephrine (IC_{50}) in aorta with intact endothelium was 0.41±0.08 mM. A similar effect on vascular muscle was observed with (±)-tramadol. At 1.0 mM, (±)-tramadol reduced the phenylephrine-induced contracture to 80.7±4.5% of control (Fig. 1B). In contrast, (−)-tramadol did not affect the contractile response of aortic rings. The phenylephrine-induced contractures were 104.2±7.3 and 90.9±7.8% of control in the presence of 0.5 and 1.0 mM of (−)-tramadol. In order to determine the role of vascular endothelium integrity in the vasodilator action of (+)-tramadol, additional experiments were performed with aortic rings in which the endothelium had been mechanically removed. Removal of the endothelium was confirmed by the lack of relaxation in the presence of acethylcholine (10 μM). Vascular relaxation was significantly smaller in endothelium-free than in endothelium-intact aortic rings (Fig. 1C). The mechanical removal of endothelium significantly altered the concentration–contractile response curve for (+)-tramadol (Fig. 1C). In the presence of 0.5 and 1.0 mM of (+)-tramadol, the phenylephrine-induced contracture of aorta without endothelium was reduced to 66.3±1.9% and 50.9±1.5% of control, respectively. These data indicated that the relaxation of smooth muscle induced by (+)-tramadol was partially dependent on the presence of intact endothelium. The involvement of opioid receptors in the vascular effect of (+)-tramadol was investigated in aortic rings pretreated with naloxone, an opioid receptor antagonist. Fig. 2A shows a representative tracing of the vasodilatation induced by (+)-tramadol in aorta with endothelium pretreated with naloxone (0.1 mM). The vascular relaxation induced by (+)-tramadol was significantly and partially inhibited by pre-incubation of endothelium-intact aorta with naloxone (Fig. 2B). The inhibitory effect induced by 0.5 mM of (+)-tramadol was significantly reduced from ~56% to ~20% in the presence of naloxone (Fig. 2B). Endothelium-denuded preparations exhibited only a slight concentration-dependent relaxation after pretreatment with naloxone (Fig. 2C). These results indicated that the vasodilator

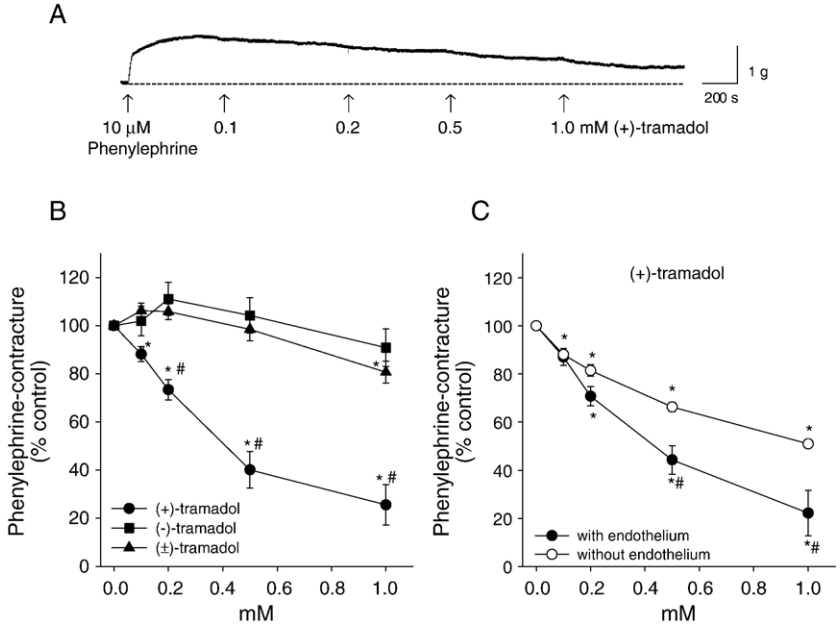

Fig. 1. Effects of tramadol and its enantiomers on the contracture induced by phenylephrine in rat aortic rings. (A) Representative tracing of isometric tension in response to 10 μM of phenylephrine followed by exposure to increasing concentrations of (+)-tramadol (0.1–1.0 mM). (B) Concentration–response curves for (±)-, (+)- and (−)-tramadol in aorta with intact endothelium. (C) Concentration–response curves for (+)-tramadol in aorta with and without intact endothelium. Data represent means±S.E.M. of 6 experiments. *$P<0.05$, compared with control; #$P<0.05$, compared with (±)-tramadol in (B) or without endothelium in (C).

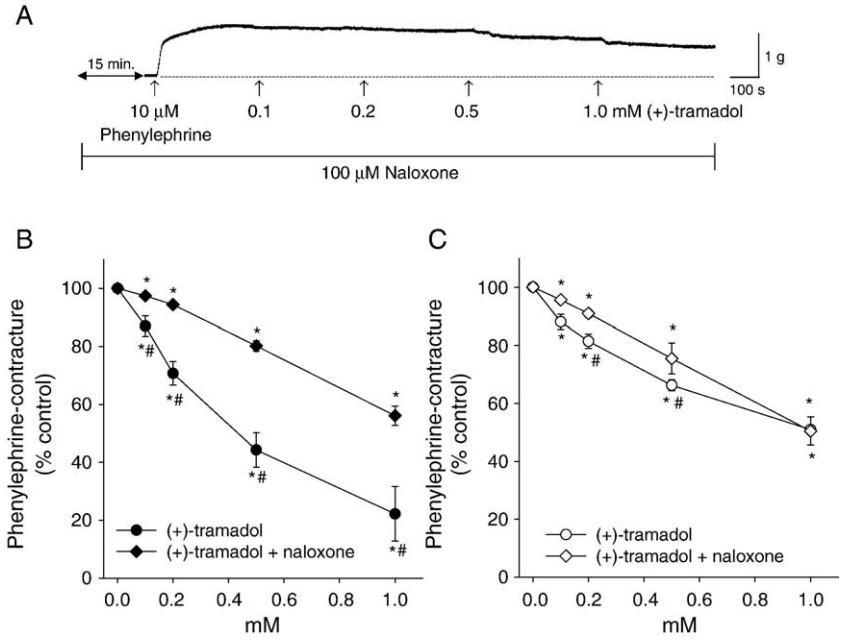

Fig. 2. Effects of (+)-tramadol in rat aortic rings with and without endothelium precontracted with phenylephrine in the presence of naloxone. (A) Representative tracing of isometric tension in response to phenylephrine (10 μM) followed by exposure to increasing concentrations of (+)-tramadol (0.1–1.0 mM), after pretreatment with 100 μM of naloxone. (B) and (C) Concentration–response curves for (+)-tramadol in aorta with and without endothelium in the absence or presence of naloxone. Data represent means ± S.E.M. of 6 experiments. *$P<0.05$, compared with control; #$P<0.05$, compared with (+)-tramadol in the presence of naloxone.

action was stereoselective for (+)-tramadol and was partially dependent on activation of μ-opioid receptors.

3.2. Effects of tramadol and its enantiomers on blood pressure

As (+)-tramadol had an in vitro vasodilator effect, we investigated whether it would interfere with the arterial blood pressure of Wistar rats. No changes in hemodynamic parameters were observed after bolus injection of 1.0 mg/kg of (+)-tramadol. The control heart rate of 410.0 ± 10.0 beats/min was not significantly altered by (+)-tramadol (1.0 mg/kg). However, rapid and transitory reductions of systolic and diastolic pressures were observed in association with alterations in electrocardiogram pattern at doses higher than 1.0 mg/kg. Fig. 3 shows a typical recording of arterial blood pressure and electrocardiogram before and after intravenous injection of 5.0 and 10.0 mg/kg of (+)-tramadol. Systolic and diastolic pressures were significantly reduced from 107.1 ± 7.3 and 79.4 ± 4.8 to 93.6 ± 8.6 ($P<0.05$) and 57.1 ± 6.7 mm Hg ($P<0.05$), respectively, after injection of 5.0 mg/kg of (+)-tramadol (Table 1). Mean arterial pressure was reduced from 88.6 ± 4.6 and 82.1 ± 6.2 (control) to 69.5 ± 7.0 ($n=6$, $P<0.5$) and 55.1 ± 7.2 mm Hg ($n=6$, $P<0.05$) after administration of (+)-tramadol, 5.0 and 10.0 mg/kg, respectively. In contrast, injection of (−)-tramadol caused an increase in systolic and diastolic pressures, resulting in an increase in mean arterial pressure (Table 1). Mean arterial pressure increased from 103.0 ± 3.6 (control) to 117.3 ± 2.8 mm Hg ($P<0.05$) and 100.7 ± 3.0 to 126.0 ± 6.9 ($P<0.05$) after intravenous injection of 5.0 and 10.0 mg/kg of (−)-tramadol, respectively. Also, as shown in Table 1, (±)-tramadol significantly increased systolic and mean arterial pressures with no effect on diastolic pressure.

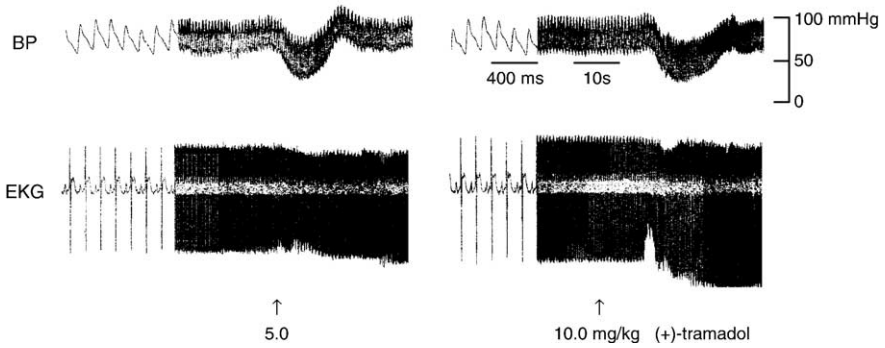

Fig. 3. Typical recording of arterial blood pressure (upper panel, BP) and electrocardiogram (lower panel, EKG) before and after bolus injection of 5.0 and 10.0 mg kg^{-1} of (+)-tramadol in rats.

Table 1
Changes in blood pressure after intravenous injection of tramadol and its enantiomers

Dose (mg kg^{-1})	(+)-tramadol		(±)-tramadol		(−)-tramadol	
	SP (mm Hg)	DP (mm Hg)	SP (mm Hg)	DP (mm Hg)	SP (mm Hg)	DP (mm Hg)
0	104.3±7.3	76.1±7.5	126.0±6.2	94.0±3.7	125.0±3.4	95.0±4.7
1.0	96.4±9.4	67.9±7.6	135.0±7.1[a]	99.0±3.7	139.2±6.4[a]	107.5±6.7[a]
0	107.1±7.3	79.4±4.8	127.0±6.4	95.0±3.9	123.3±3.6	94.2±4.0
5.0	93.6±8.6[a]	57.1±6.7[a]	142.0±4.9[a]	102.0±4.4	140.0±2.6[a]	106.7±3.1[a]
0	99.0±7.0	73.6±6.1	123.0±5.4	91.0±3.7	120.8±3.3	90.8±2.4
10.0	72.9±8.8[a]	46.3±7.4[a]	119.0±9.1	77.0±10.8	155.0±6.8[a]	113.3±5.4[a]

SP, systolic pressure; DP, diastolic pressure. [a]$P<0.05$ compared to control.

3.3. Effects of tramadol and its enantiomers on cardiac muscle

Whether tramadol and its enantiomers have a direct effect on cardiac muscle was investigated in isolated papillary muscles. Fig. 4A shows representative tracings of twitches of electrically stimulated papillary muscles in the absence and presence of increasing concentrations of (+)-tramadol. The isometric tension of papillary muscle was reduced in a concentration-dependent manner and partially recovered after a 30-min washout period (Fig. 4A). Concentration–response curves showed that (+)- and (−)-tramadol promoted complete inhibition of cardiac contractility at 1.0 mM, the highest dose tested (Fig. 4B). At 1.0 mM, (±)-tramadol was less effective than (+)-tramadol because it reduced the amplitude of twitches to 26.9±9.6% of control ($n=8$, $P<0.05$) compared to complete inhibition (100%) in the presence of same concentration of (+)-tramadol (Fig. 4B). At concentrations higher than 0.5 mM, both enantiomers induced spontaneous contractile activity of papillary muscle. These extra contractions were more frequent in the presence of (+)-Tramadol and were totally reversed after washout. (+)-tramadol was more potent in reducing the amplitude of twitches. The IC_{50} for (+)-tramadol to inhibit cardiac contractility was 0.22±0.05 mM ($n=8$, $P<0.05$), whereas for (−)- and (±)-tramadol the IC_{50} values were 0.54±0.10 ($n=8$) and 0.71±0.09 mM ($n=8$), respectively.

4. Discussion

Tramadol is purchased as a racemic (1:1) mixture of two enantiomers that act in a complementary manner to induce analgesia. In this study, we demonstrated a clear difference in the pharmacological properties of the tramadol enantiomers in the cardiovascular system in vitro and in vivo experiments. (+)-Tramadol significantly reduced phenylephrine-evoked contractions of rat aorta in a concentration-dependent manner. In contrast, (−)-tramadol produced a small, but not significant, increase in the contractile response of aortic rings with functional endothelium. The vascular relaxation induced by (+)-tramadol was partially dependent on vascular endothelium integrity, since its activity was greater in arteries with an intact

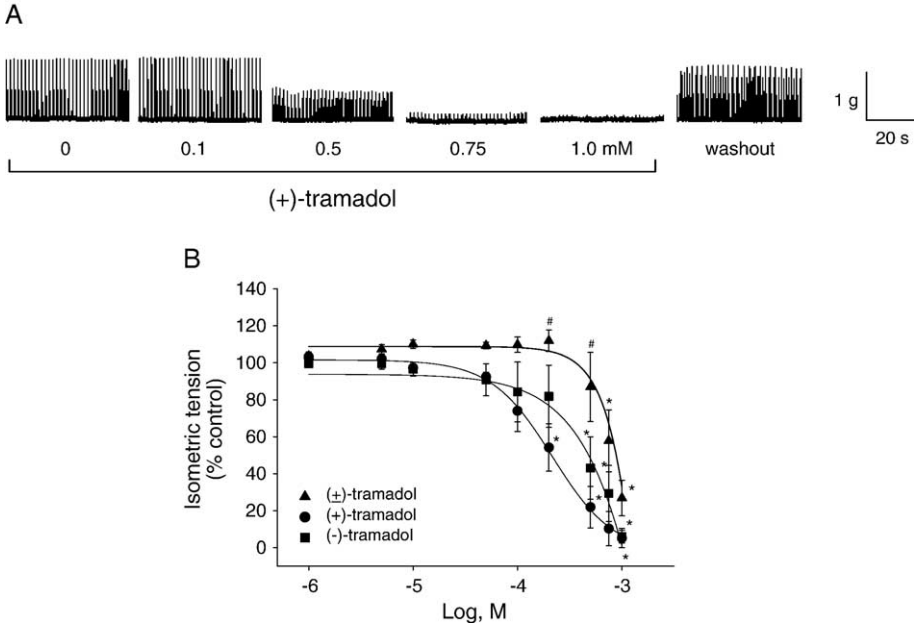

Fig. 4. Effects of tramadol and enantiomers on the contractile response of isolated cardiac muscle. (A) Typical tracings of twitches in rat papillary muscle induced by electrical stimulation at 1 Hz before and after exposure to (+)-tramadol (0.1 and 1.0 mM) and 30 min after washout. (B) Concentration–response curves for (±)-, (+)- and (−)-tramadol in papillary muscle. Data represent means±S.E.M. of 8 experiments. *$P<0.05$, compared with control; #$P<0.05$, compared with (+)-tramadol.

endothelium than in those without endothelium. These findings indicate that the vasorelaxant effect of (+)-tramadol is, at least in part, mediated by nitric oxide synthesis/release by endothelial cells. (±)-Tramadol caused a significant vasodilatation only at 1.0 mM, in contrast to the findings of Kaya et al. (2003), who reported that racemic tramadol induced vasodilatation in rabbit aortic rings at 0.1 and 0.3 mM. The reasons for these contradictory results are not clear, but it is known that the effect of opioids on the vascular tone of isolated vessels varies between species, and even between different vascular beds in the same species (Parra et al., 1995). The vasodilator activity of (+)-tramadol was also responsive to naloxone antagonism in aorta with endothelium. The activation of opioid receptors, μ_3, present in endothelial cells could be involved in the vasodilatation induced by (+)-tramadol. Stefano et al. (1995) showed that μ-opioid receptors on vascular endothelium are coupled to nitric oxide release. This hypothesis is reinforced by the observation that removal of the endothelium and pretreatment with naloxone both caused a similar inhibition of the (+)-tramadol-induced vascular effect (49.0±1.5% and 43.9±2.6% of inhibition). As naloxone inhibited the relaxant response in aorta without endothelium, opioid receptors might also be present in smooth muscle cells on rat aorta. This has already been proposed in a study with morphine, in which morphine produced a concentration-dependent relaxation in endothelium-intact as well as in endothelium-denuded rat aortic rings. The relaxation of smooth muscle produced by morphine was partially inhibited by pretreatment of the tissues with naloxone, with no differences between endothelium-intact and endothelium-denuded preparations (Sahin et al., 2002). Besides the activation of opioid receptors and the release of nitric oxide, the opening of potassium channels also appears to be a possible mechanism for the vasodilatation induced by (+)-tramadol. Activation of these channels results in hyperpolarization of the cell membrane and consequently in relaxation of vascular smooth muscle. Recently, Yalcin and Aksu (2005) described that a nonspecific voltage-dependent potassium channel could participate in the antinociceptive effect of tramadol in mice.

A decrease in both diastolic and systolic pressures was observed after tramadol injection at doses of 5.0 and 10.0 mg/kg. The hypotensive response to tramadol could be mediated not only by a central effect but also by a direct effect on the vessels. The reduction in systolic pressure observed after injection of (+)-tramadol might be caused by the strong decrease in diastolic pressure and not by a direct effect on cardiac muscle because serum concentrations of tramadol do not reach 0.1 mM even after intravenous injection of 30 mg/kg in rats (Matthiesen et al., 1998). In the experiments in which the isometric tension of papillary muscle was measured, (+)-tramadol had a negative inotropic effect, being more potent than (−)- and (±)-tramadol. The negative inotropic action observed with (±)-tramadol and its enantiomers seems not to be mediated by the activation of opioid receptors because these receptors are not involved in the cardiac depression induced by opioid agonists (Llobell and Laorden, 1995; Alarcón et al., 1995). In contrast to (+)-tramadol, (−)-tramadol increased systolic, diastolic and mean arterial pressures. These findings could be explained by adrenergic nerve stimulation because (−)-tramadol inhibits noradrenaline re-uptake and increases its efflux (Halfpenny et al., 1999). Thus, the increase in mean arterial pressure observed after (−)-tramadol injection could be related to an increase in noradrenaline serum levels. Nagaoka and co-workers (2002) demonstrated that injection of tramadol increased mean arterial blood pressure and serum noradrenaline levels and suggested that tramadol activated the sympathetic nervous system. Similar results were observed in this study: (±)-tramadol increased systolic pressure at 1.0 and 5.0 mg/kg. However, (±)-tramadol reduced mean arterial pressure at 10.0 mg/kg. It has already been reported that tramadol at 1.0 and 4.64 mg/kg i.v. slightly increase arterial blood pressure and heart rate but at 10.0 mg/kg i.v. transiently decrease blood pressure in anesthetized rabbits (Müller and Wilsmann, 1984).

In conclusion, we showed stereoselectivity of the vasodilator activity of (+)-tramadol. Our results suggest that (+)-tramadol probably is responsible for the vasodilator action of the racemic mixture of tramadol. (+)-Tramadol-induced vasodilatation is mediated by both endothelium-dependent and -independent processes, and opioid receptors are also involved in this response, possibly through the activation of nitric oxide production. The recommended dose for intravenous administration of tramadol ranges from 1 to 3 mg/kg (Radbruch et al., 1996) and the blood concentration is less than 1 μM after injection of 100 mg (Lintz et al., 1986). Tramadol and (+)-tramadol promote cardiac depression and vasodilatation only at high concentrations (100–1000 μM), suggesting that at clinically relevant concentrations tramadol does not exert toxic effects on the cardiovascular system.

Acknowledgements

Tramadol and its enantiomers used in this study were kindly donated by Cristália Produtos Químicos e Farmacêuticos Ltda., Itapira, SP, Brazil. The authors thank to CRISTALIA (BR), CAPES (BR.), CNPq (BR.), PRONEX (BR.), FAPERJ (BR.) and FUJB (BR.) for financial support and fellowships (JMR, LBP).

References

Alarcón, S., Hernández, J., Laorden, M.L., 1995. Electrophysiological effects of opioid receptor selective agonists on guinea-pig papillary muscle. Regul. Pept. 55, 149–154.

Close, B.R., 2005. Tramadol: does it have a role in emergency medicine? Emerg. Med. Australas 17, 73–83.

Eggers, K.A., Power, I., 1995. Tramadol. Br. J. Anaesth. 74, 247–249.

Garrido, M.J., Valle, M., Campanero, M.A., Calvo, R., Trocóniz, I.F., 2000. Modeling of the in vivo antinociceptive interaction between an opioid agonist, (+)-O-desmethyltramadol, and a monoamine reuptake inhibitor, (−)-O-desmethyltramadol, in rats. J. Pharmacol. Exp. Ther. 295, 352–359.

Grond, S., Sablotzki, A., 2004. Clinical pharmacology of tramadol. Clin. Pharmacokinet. 43, 879–923.

Halfpenny, D.M., Callado, L.F., Hopwood, S.E., Bamigbade, T.A., Langford, R.M., Stamford, J.A., 1999. Effects of tramadol stereoisomers on norepinephrine efflux and uptake in the rat locus coeruleus measured by real time voltammetry. Br. J. Anaesth. 83, 909–915.

Kaya, T., Gursoy, S., Karadas, B., Sarac, B., Kafali, H., Soydan, A.S., 2003. High-concentration tramadol-induced vasodilation in rabbit aorta is

mediated by both endothelium-dependent and -independent mechanisms. Acta Pharmacol. Sin. 5, 385–389.

Lewis, K.S., Han, N.H., 1997. Tramadol: a new centrally acting analgesic. Am. J. Health-Syst. Pharm. 54, 643–652.

Lintz, W., Barth, H., Osterloh, G., Schmidt-Bothelt, E., 1986. Bioavailability of enteral tramadol formulations: 1st communication-capsules. Arzneimittelforschung 36, 1278–1283.

Llobell, F., Laorden, M.L., 1995. Characterization of the opioid receptor subtypes mediating the negative inotropic effects of DAMGO, DPDPE and U-50,488H in isolated human right atria strips. Neuropeptides 29, 115–119.

Matthiesen, T., Wöhrmann, T., Coogan, T.P., Uragg, H., 1998. The experimental toxicology of tramadol: an overview. Toxicol. Lett. 95, 63–71.

Mildh, L.H., Leino, K.A., Kiverlä, O.A., 1999. Effects of tramadol and meperidine on respiration, plasma catecholamine concentrations, and hemodynamics. J. Clin. Anesth. 11, 310–316.

Müller, B., Wilsmann, K., 1984. Cardiac and hemodynamic effects of the centrally acting analgesics tramadol and pentazocine in anaesthetized rabbits and isolated guinea-pig atria and papillary muscles. Arzneimittelforschung 34, 430–433.

Müller, H., Stoyanov, M., Brahler, A., Hempelmann, G., 1982. Haemodynamic and respiratory effects of tramadol during nitrous oxide oxygen artificial respiration and in the postoperative period. Anaesthesist 31, 604–610.

Nagaoka, E., Minami, K., Shiga, Y., Uezono, Y., Shiraishi, M., Aoyama, K., Shigematsu, A., 2002. Tramadol has no effect on cortical renal blood flow – despite increased serum catecholamine levels – in anesthetized rats: implications for analgesia in renal insufficiency. Anesth. Analg. 94, 619–625.

Parra, I., Perez-Vincaino, F., Alsasua, A., Martin, M.I., Tamargo, J., 1995. Mu- and delta-opioid receptor-mediated contractile effects on rat aortic vascular smooth muscle. Eur. J. Pharmacol. 227, 99–105.

Radbruch, L., Grond, S., Lehmann, K.A., 1996. A risk–benefit assessment of tramadol in the management of pain. Drug Safety 15, 8–29.

Raffa, R.B., Friderichs, E., Reimann, W., Shank, R.P., Codd, E.E., Vaught, J.L., 1992. Opioid and non-opioid components independently contribute to the mechanism of action of tramadol, an atypical opioid analgesic. J. Pharmacol. Exp. Ther. 260, 275–285.

Raffa, R.B., Friderichs, E., Reimann, W., Shank, R.P., Codd, E.E., Vaught, J.L., Jacoby, H.I., Selve, N., 1993. Complementary and synergistic antinociceptive interaction between the enantiomers of tramadol. J. Pharmacol. Exp. Ther. 267, 331–340.

Sahin, N., Özden, S., Sadan, G., 2002. The effect of nitric synthase blockade on responses to morphine in rat aortic rings. Auton. Autacoid Pharmacol. 22, 103–107.

Stefano, G.B., Hartman, A., Bilfinger, T.V., Magazine, H.I., Liu, Y., Casares, F., Goligorsky, M.S., 1995. Presence of μ3 opiate receptor in endothelial cells. J. Biol. Chem. 270, 30290–30293.

Vickers, M.D., O'Flaherty, D., Szekely, S.M., Read, M., Yoshizumi, J., 1992. Tramadol: pain relief by an opioid without depression of respiration. Anaesthesia 47, 291–296.

Yalcin, I., Aksu, F., 2005. Involvement of potassium channels and nitric oxide in tramadol antinociception. Pharmacol. Biochem. Behav. 80, 69–75.