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