Original Research

High-concentration tramadol-induced vasodilation in rabbit aorta is mediated by both endothelium-dependent and -independent mechanisms

Tijen KAYA[1], Sinan GURSOY[2], Baris KARADAS, Bulent SARAC, Haluk KAFALI[2], Ahmet Serdar SOYDAN

Department of Pharmacology; [2]Department of Anaesthesiology, Cumhuriyet University, Faculty of Medicine, Sivas 58140, Turkey

KEY WORDS tramadol; thoracic aorta; endothelium; vasodilation

ABSTRACT

AIM: The mechanism of tramadol-induced vasodilation was investigated using isolated rabbit thoracic aortic rings. **METHODS:** Aortic rings from 8 rabbits were placed in organ bath and precontracted with phenylephrine (10^{-5} mol/L) before addition of tramadol. Relaxation responses by tramadol were evaluated in the presence and absence of endothelium, indomethacin (an inhibitor of cyclooxygenase), N^G-nitro-*L*-arginine methyl ester (*L*-NAME, a specific inhibitor of nitric oxide synthase), glibenclamide (an inhibitor of ATP-sensitive potassium channels), tetraethylammonium chloride (TEA, an inhibitor of calcium-sensitive potassium channels), and naloxone (an antagonist of opioid receptors). **RESULTS:** Tramadol (10^{-4} mol/L and 3×10^{-4} mol/L) caused significant vasodilation in endothelium-intact and endothelium-denuded aortic rings ($P<0.05$). The relaxation response to tramadol was significantly greater in endothelium-intact rings than in endothelium-denuded rings. Pretreatment of aortic rings with indomethacin (10^{-5} mol/L), glibenclamide (10^{-5} mol/L), TEA (10^{-3} mol/L), and naloxone (10^{-4} mol/L) had no effect on the tramadol-induced relaxation. In endothelium-intact rings, *L*-NAME (10^{-4} mol/L) pretreatment caused marked inhibition of the relaxation induced by tramadol, but not endothelium-denuded rings. **CONCLUSION:** In the rabbit aorta, vascular relaxation induced by tramadol is due to both nitric oxide production from endothelium and a direct effect on smooth muscle.

INTRODUCTION

Tramadol, a combination of *R* and *L* enantiomers, is a novel analgesic that has a complex pharmacology[1]. Tramadol has a low affinity for opioid receptors, but its analgesic potency is only 5-10 times less than morphine, and in volunteer studies, only 30 % of its effect could be antagonized by naloxone[2,3]. In addition, tramadol has not been associated with clinically significant respiratory depression, and has a low potential for the development of tolerance, dependence and abuse[4]. The clinical observations implied that actions other than via opioid receptors should contribute to the effect of tramadol. This has led to a complete reevaluation of the pharmacology of tramadol and the recognition of both opioid and non-opioid mechanisms of action involving two different enantiomeric structures[5,6].

Hemodynamic and respiratory side effects were investigated in cardiovascular risk patients prior to surgery of the abdominal aorta[7]. Tramadol 1.65 mg/kg produced no significant changes in heart rate, total peripheral resistance, mean pulmonary artery or pulmo-

[1] Correspondence to Tijen KAYA, PhD. Phn 90-346-226-2367. Fax 90-346-222-4802. E-mail tkaya@cumhuriyet.edu.tr
Received 2002-06-21 Accepted 2003-03-11

nary capillary wedge pressure, stroke volume index, and arterial O_2 or CO_2 partial pressure. Tryba and Zenz[8] compared postoperative analgesia with a single intravenous dose of 50 mg tramadol after ortopaedic surgery. Heart rate decreased significantly in two patients and dropped below 60 /min. Karsch *et al*[9] measured the hemodynamic effects in 10 patients with coronary heart disease after intravenous application of 50 mg tramadol and found that tramadol decreased pulmonary artery resistance and had a distinct negative inotropic effect on left ventricular myocardium.

This study was designed to investigate the concentration-dependent effect and the mechanism of this effect of tramadol on isolated rabbit thoracic aorta.

MATERIALS AND METHODS

Experiments were performed on eight mature albino rabbits weighing 2.5 to 3 kg. The animals were anesthetized with sodium pentobarbital (50 mg/kg, iv) and their thoracic aorta were removed and immediately placed in oxygenated Krebs-Henseleit solution, and dissected free of fat and connective tissue. Rings approximately 3-mm width were suspended in a 10-mL organ bath containing a modified Krebs-Henseleit solution of the following composition in mmol/L; KCl 4.75, KH_2PO_4 1.19, $MgSO_4$ 1.19, $CaCl_2$ 2.54, NaCl 119, $NaHCO_3$ 25, glucose 11, pH 7.4. The solution was aerated continuously with a gas mixture of 95 % O_2 and 5 % CO_2 and maintained at 37 ºC. Aortic rings were equilibrated for 2 h with a resting tension of 2 g, determined to be the optimal resting tension in preliminary length-tension experiments. Isometric tensions were recorded with a Grass FT-03 force-displacement transducer and recorded on a four-channel Grass polygraph (model 79 E). When needed by protocol, endothelium was removed by gently rubbing the intimal surface with a wooden stick for 30-60 s.

After 2-h equilibration period, rings were contracted with phenylephrine (10^{-5} mol/L). Acetylcholine (10^{-6} mol/L) then was added to the bath to assess the integrity of the endothelium. No relaxation in response to acetylcholine in the denuded preparation indicated an effective functional removal of the endothelium. The rings with intact endothelium that produced less than 30 % relaxation in response to acetylcholine were discarded. After acetylcholine testings, the rings re-equilibrated for 60 min and then again contracted submaximally as before with phenylephrine. Tramadol (10^{-7}-3×10^{-4} mol/L) then was added to individual chambers by increasing concentrations cumulatively with each new dose added after reaching a steady state from the preceding dose. In some experiments *L*-NAME (10^{-4} mol/L), indomethacin (10^{-5} mol/L), naloxone (10^{-4} mol/L), glibenclamide (10^{-5} mol/L), and tetraethylammonium chloride (10^{-3} mol/L) were added to the organ bath 15 min before the precontraction in order to test the effects of nitric oxide, prostaglandins, opioid receptors, and potassium channels which could have a contribution to aortic smooth muscle relaxation induced by tramadol.

Drugs used in this study were phenylephrine hydrochloride, acetylcholine chloride, *L*-NAME, indomethacin, glibenclamide (Sigma, St Louis, Missouri, USA), tetraethylammonium chloride, and tramadol (ICN, Costa Mesa, CA, USA). All substances were dissolved in distilled water, except for indomethacin, which was dissolved in 1 % Na_2CO_3. All drugs were freshly prepared on the day of the experiments.

The relaxation responses recorded with increasing concentrations of tramadol were expressed as the percentage relaxation from the precontracted state by phenylephrine (10^{-5} mol/L). Five rings taken from the same aorta were studied in parallel, but for each type of experiment only one ring was used from each animal, thus the experimental number (n) of animals used was the same. All data were expressed as mean±SEM. Statistical analysis of the data was performed using a one-way analysis of variance (ANOVA) between experimental groups. When three groups of data were compared, statistical significance was determined further by the Newman Keuls test. $P<0.05$ was considered statistically significant.

RESULTS

The contraction induced by phenylephrine (10^{-5} mol/L) in aortic rings with intact endothelium (1.75 g±0.24 g, n=8) was not significantly different from endothelium-denuded rings (1.9 g±0.3 g, n=8). In functional studies, acetylcholine (10^{-6} mol/L), which induces endothelium-dependent relaxation, caused significant relaxation on the contraction induced by phenylephrine in rings with intact endothelium (67 %±4 %, n=8) but lacked any effect on denuded rings (4 %±3 %, n=8).

In preliminary experiments, tramadol showed no response at concentrations between 10^{-7} and 10^{-5} mol/L in precontracted with phenylephrine thoracic aortic rings

from rabbit, either with (n=4) or without (n=4) endothelium. At concentrations of 10^{-4} and 3×10^{-4} mol/L, tramadol produced significant relaxation on phenylephrine-induced contraction (P<0.05). Therefore, 10^{-4} and 3×10^{-4} mol/L concentrations were used for further studies.

Tramadol (10^{-4} and 3×10^{-4} mol/L) produced relaxation on aortic rings in both with and without endothelium. In addition, in endothelium intact rings, the relaxation responses induced by tramadol returned to control values by *L*-NAME (10^{-4} mol/L), but not in endothelium-denuded rings (Fig 1). The relaxation response to tramadol was greater in intact rings than in denuded and intact+*L*-NAME rings. There was no significant difference in terms of relaxation responses to tramadol between intact+*L*-NAME and denuded rings (Tab 1). In endothelium-denuded rings, the maximum relaxation response (E_{max}) induced by tramadol (3×10^{-4} mol/L) significantly reduced by 51 %±11 % compared to endothelium-intact rings (P<0.05) (n=8). Incubation of endothelium-intact rings with *L*-NAME significantly reduced the maximum relaxation induced by tramadol by 62.6 %±11.9 % (P<0.05) (n=8) (Tab 1).

Tab 1. Relaxation induced by tramadol in isolated thoracic aortic rings from rabbit. Relaxation is expressed as the percentage reduction in the contraction induced by phenylephrine (10^{-5} mol/L). n=8. Mean±SEM. ^bP<0.05 *vs* endothelium-intact group.

	% Relaxation	
	Tramadol (10^{-4} mol/L)	Tramadol (3×10^{-4} mol/L)
Endothelium-intact	36±5	65±9
Endothelium-denuded	12.8±2.6^b	32±10^b
Intact+*L*-NAME	9.5±1.9^b	24±6^b

Preincubation of aortic rings with and without endothelium, contracted by phenylephrine, with indomethacin (10^{-5} mol/L), glibenclamide (10^{-5} mol/L), TEA (10^{-3} mol/L), and naloxone (10^{-4} mol/L) had no significant effect on the tramadol-induced relaxation responses (Fig 2). At these concentrations, antagonists have no effect on phenylephrine-induced contractile response (data not shown).

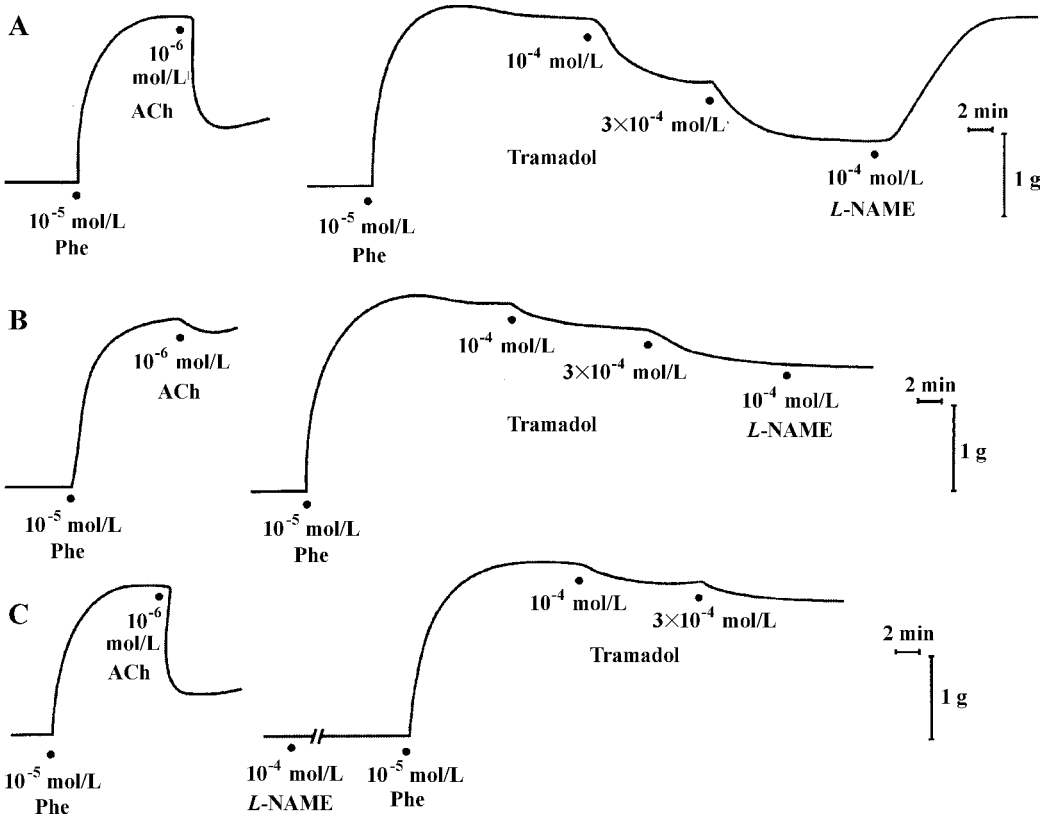

Fig 1. Recorder tracings indicating changes in relaxant effect of tramadol in endothelium-intact (A), endothelium-denuded (B), and endothelium-intact (C) aortic rings in presence of *L*-NAME. Initial contraction was induced by phenylephrine (Phe).

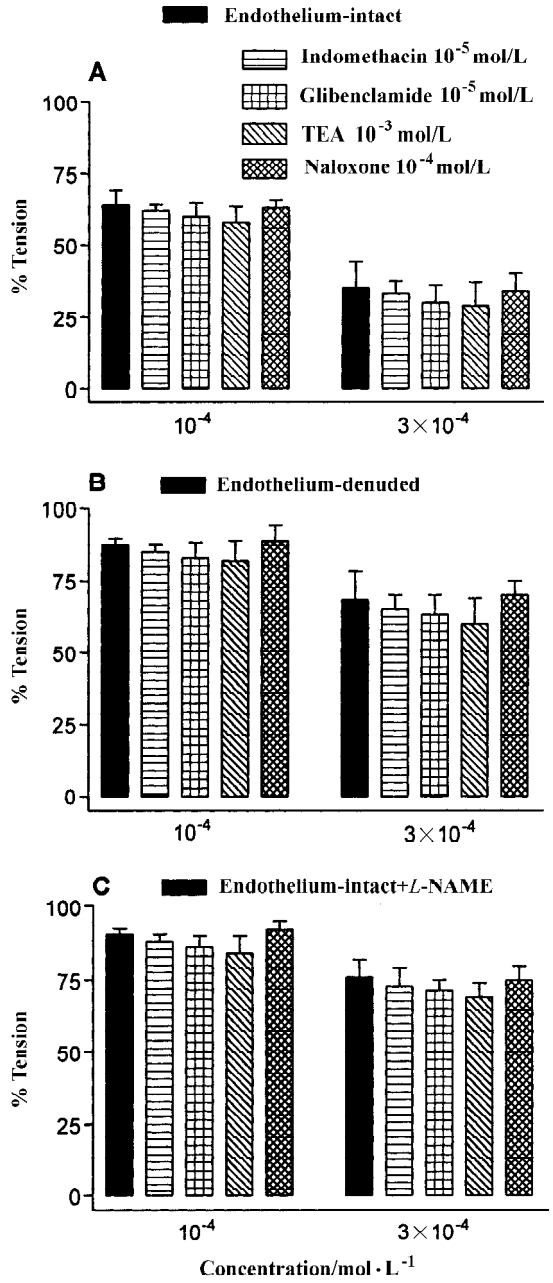

Fig 2. Relaxant effects of tramadol on phenylephrine-induced contractions in endothelium-intact (A), endothelium-denuded (B), and endothelium-intact+*L*-NAME (C) aortic rings in presence of indomethacin (10^{-5} mol/L), glibenclamide (10^{-5} mol/L), TEA (10^{-3} mol/L), and naloxone (10^{-4} mol/L). Relaxation is expressed as the percentage reduction in the contraction induced by phenylephrine (10^{-5} mol/L). *n*=8. Mean±SEM. None of these agents significantly changed the tramadol-induced relaxation.

DISCUSSION

The principal finding of this study is that tramadol produces vasodilation, which is, mainly, dependent on endothelium function in the *in vitro* rabbit aortic ring preparation precontracted by phenylephrine. In addition, neither indomethacin nor potassium channel inhibitors altered tramadol-induced relaxation of rabbit thoracic aorta. Naloxone did not antagonize the relaxant effect of tramadol.

Efficient analgesia may be major objective in the cardiovascular risk patients following myocardial infarction. Patients with acute myocardial infarction or unstable angina pectoris were treated with intravenous tramadol in an open study[10]. All patients reported sufficient pain relief after 50-100 mg tramadol. Respiratory and cardiovascular parameters were not changed, only one patient developed acute tachycardia and respiratory distress. Cats and dogs iv doses of tramadol up to 10 mg/kg have a slight, papaverine-like spasmolytic effect[11]. Karch *et al*[9] suggested that tramadol decreased pulmonary artery resistance and had a distinct negative inotropic effect on left ventricular myocardium. Authors did not investigate the mechanism of vasodilation induced by tramadol in isolated vessels. The current study indicates that tramadol relaxes rabbit aortic smooth muscle in isolated organ bath by both endothelium-dependent and independent mechanisms.

NO is a potent vasodilator produced by the endo thelium under basal conditions and in response to a variety of agonists. It diffuses from the endothelium to the underlying vascular smooth muscle, where it causes relaxation through the activation of soluble guanylate cyclase, causing an increase in 3,5-cyclic guanosine monophosphate[12]. NO is produced from *L*-arginine by the calcium-, calmodulin-, and NADPH-dependent enzyme, NO synthase, which is inhibited by specific analogues of *L*-arginine such as *L*-NAME, as used in this study[13]. In intact vessels precontracted with phenylephrine, tramadol produced concentration-dependent relaxation followed an increase in tension toward to preconstricted level by *L*-NAME-treatment. In addition, tramadol caused significant vasodilation in aortic rings with and without endothelium, but the relaxation responses to tramadol was significantly greater in endothelium-intact aortic rings than endothelium-denuded rings. The discrepancies in vasodilation between endothelium-intact and denuded rings may be explained by an increased NO production by tramadol, since endothelium-intact vessels pretreated with *L*-NAME resulted with decreased vasodilation to tramadol stimulation.

The vascular endothelium plays an important role in controlling the vascular tone via secretion of both

relaxant and contractile factors[14]. The most potent known are the vasodilators, endothelium-derived relaxing factor (EDRF), and prostacyclin (PGI_2). EDRF is now known to be NO or a closely related nitrosothiol[15]. The relaxing action of tramadol was attenuated in endothelium-denuded and endothelium-intact aorta pretreated with *L*-NAME. This suggested that the vasorelaxant effect of tramadol was, at least in part, dependent upon the endothelium. The vasorelaxation caused by tramadol in intact and denuded aorta was shown to persist in the presence of indomethacin (which blocks the formation of PGI_2 by inhibiting cyclooxygenase), TEA (which blocks Ca^{2+}-sensitive K^+ channels) and glibenclamide (which blocks ATP-sensitive K^+ channels), implying that this effect was not mediated by PGI_2 or K^+ channels. Interestingly, aortic rings, with or without endothelium, pre-contracted by phenylephrine were relaxed by tramadol, and this relaxation was not inhibited by the opiate receptor antagonist, naloxone. Since tramadol has a low affinity for opioid receptors, antagonistic effect of naloxone in our study, perhaps, was undetectable level. Therefore, it was concluded that the effect of opioid receptors on tramadol-induced relaxation does not seem to be important. However, *L*-NAME, a specific and competitive NO-synthase (NOS) inhibitor was able to partially inhibit the relaxant effect of tramadol on phenylephrine-induced contraction. Taken together, these results suggest that the relaxation of the rabbit aorta caused by tramadol may be mediated through the activation of the NOS-guanylate cyclase pathway.

Tramadol significantly reduced the contractile response induced by phenylephrine only at high concentrations (10^{-4} and 3×10^{-4} mol/L), whereas clinically relevant concentrations of tramadol have been reported less than 10^{-6} mol/L[7]. The mechanism of relaxant effect of tramadol may be related to nonspecific receptor systems since it seems that the affinity to effectors is very low. The present results suggest that tramadol, at clinically relevant concentrations, does not affect the contractile response of thoracic aorta. Therefore our results could be meaningful in terms of toxicology.

In conclusion, tramadol, at supraclinical concentrations, produces greater degree of vasodilation in endothelium-intact aorta, which can be explained by stimulation of NO production by tramadol in endothelium-intact vessel. Pretreatment of aortic rings either with or without endothelium with indomethacin, glibenclamide, TEA, and naloxone did not block the relaxation responses to tramadol, suggesting that cyclooxygenase pathway, potassium channels, and opioid receptors may not be involved in this relaxation.

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