Inhibitory effect of tramadol on vasorelaxation mediated by ATP-sensitive K^+ channels in rat aorta

[Effet inhibiteur du tramadol sur la vasorelaxation médiée par les canaux potassiques sensibles à l'ATP de l'aorte du rat]

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Purpose: Tramadol produces a conduction block similar to lidocaine by exerting a local anesthetic-like effect. The aims of this *in vitro* study were to determine the effects of tramadol on the vasorelaxant response induced by the adenosine triphosphate-sensitive K^+ (K_{ATP}) channel opener, levromakalim, in an endothelium-denuded rat aorta, and to determine whether this effect of tramadol is stereoselective.

Methods: The effects of tramadol (racemic, R(-) and S(+): 10^{-6} , 10^{-5} , 5×10^{-5} M), and glibenclamide on the levromakalim dose-response curve were assessed in aortic rings that had been pre-contracted with phenylephrine. In the rings pre-treated independently with naloxone, and glibenclamide, the levromakalim dose-response curves were generated in the presence or absence of tramadol. The effect of tramadol on the dose-response curve of diltiazem was assessed.

Results: Racemic, R(-) and S(+) tramadol (10^{-5} , 5×10^{-5} M) attenuated ($P < 0.0001$) levromakalim-induced relaxation in the ring with or without naloxone in a dose-dependent manner. The magnitude of the R(-)-tramadol-induced attenuation of vasorelaxant response induced by levromakalim was greater ($P < 0.05$) than that induced by S(+)-tramadol. Glibenclamide almost abolished the levromakalim-induced relaxation. Tramadol, 5×10^{-5} M, did not significantly alter the diltiazem-induced relaxation.

Conclusion: These results suggest that a supraclinical dose (10^{-5} M) of tramadol [racemic, R(-) and S(+)] attenuates the vasorelaxation mediated by the K_{ATP} channels in the rat aorta. The R(-) tramadol-induced attenuation of vasorelaxation induced by levromakalim was more potent than that induced by S(+) tramadol. This attenuation is independent of opioid receptor activation.

Objectif : Le tramadol produit un bloc de conduction similaire à la lidocaïne en exerçant un effet semblable à celui d'un anesthésique local. Les objectifs de cette étude *in vitro* étaient de déterminer les effets du tramadol sur la réaction de vasorelaxation induite par le levromakalim, une molécule provoquant l'ouverture des canaux potassiques (K_{ATP}) sensibles à l'adénosine triphosphate, sur l'endothélium dénudé de l'aorte de rat, et de déterminer si l'effet du tramadol est stéréosélectif.

Méthode : Les effets du tramadol (racémique, R(-) et S(+)) : 10^{-6} , 10^{-5} , 5×10^{-5} M), et du glibenclamide sur la courbe dose-réponse du levromakalim ont été évalués sur des anneaux aortiques pré-contractés avec de la phényléphrine. Sur les anneaux indépendamment prétraités au naloxone et au glibenclamide, les courbes de dose-réponse du levromakalim ont été générées en présence ou en l'absence de tramadol. L'effet du tramadol sur la courbe dose-réponse du diltiazem a été évalué.

Résultat : Le tramadol racémique R(-) et S(+) a atténué ($P < 0,0001$) la relaxation de l'anneau induite par le levromakalim, avec ou sans naloxone, dépendamment de la dose. L'atténuation induite par le tramadol R(-) sur la réaction de vasorelaxation induite par le levromakalim était plus grande ($P < 0,05$) que celle induite par le tramadol S(+). Le glibenclamide a pratiquement aboli la relaxation induite par le levromakalim. Le tramadol, 5×10^{-5} M, n'a pas modifié significativement la relaxation induite par le diltiazem.

Conclusion : Ces résultats suggèrent qu'une dose supraclinique (10^{-5} M) de tramadol [racémique, R(-) et S(+)] atténue la vasorelaxation médiée par les canaux K_{ATP} dans l'aorte du rat. L'atténuation induite par le tramadol R(-) de la vasorelaxation induite par le levromakalim était plus puissante que celle induite par le tramadol S(+). Cette atténuation est indépendante d'une activation des récepteurs opiacés.

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THE adenosine triphosphate (ATP)-sensitive K^+ (K_{ATP}) channels are likely to play an important role under normal and pathophysiological conditions in the cardiovascular system.¹ The K_{ATP} channels appear to be targets of a variety of synthetic and endogenous vasodilators.¹ The vasorelaxant effects of the K_{ATP} channel opener, cromakalim, in the rat aorta are mediated by the selective K_{ATP} channel antagonist glibenclamide-sensitive potassium channels.² The hyperpolarization induced by K_{ATP} channel openers in the sarcolemma of vascular smooth muscle cells prevents the entry of calcium (Ca^{2+}) through the voltage-operated Ca^{2+} channels, inhibits the agonist-induced mobilization of Ca^{2+} from the stores, and reduces the sensitivity of the contractile structures to Ca^{2+} , which leads to vasorelaxation.³

Tramadol hydrochloride is a combination of S(+) and R(-) enantiomers⁴ that produces a nerve conduction block similar to lidocaine by exerting a local anesthetic-like effect.⁵ Lidocaine, and bupivacaine enantiomers, which belong to amide-linked local anesthetics, inhibit the vasorelaxation mediated by K_{ATP} channel opener, levromakalim, in the rat aorta.^{6,7} Recently we found that tramadol stereoselectively attenuates endothelium-dependent relaxation in an isolated rat aorta.⁸ However, the effects of tramadol on the vasorelaxant response mediated by levromakalim have not been investigated previously. Therefore, the aims of this *in vitro* study were to examine the effects of tramadol on the vasorelaxant response induced by levromakalim in an isolated rat aorta, and to determine whether the effect of tramadol is stereoselective. Based on previous studies,⁵⁻⁷ we tested the hypothesis that tramadol would attenuate the vasorelaxant response induced by levromakalim in isolated rat aorta.

Methods

All the experimental procedures and protocols were approved by the Institutional Animal Care and Use Committee of Gyeongsang National University Hospital (Jinju, Gyeongnam, Republic of Korea).

Preparation of aortic rings for tension measurement

Male Sprague Dawley rats weighing 250–350 g were anesthetized with an ip injection of pentobarbital sodium (50 mg·kg⁻¹). The descending thoracic aorta was dissected free, and the surrounding connective tissue and fat were removed under a microscope while the vessel was bathed in a Krebs solution containing the following (in mM): 118 NaCl, 4.7 KCl, 1.2 MgSO₄, 1.2 KH₂PO₄, 2.4 CaCl₂, 25 NaHCO₃, 11

glucose, 0.03 EDTA. The aorta was then cut into 2.5-mm rings, which were suspended on Grass isometric transducers (FT-03, Grass Instrument, Quincy, MA, USA) at 2.0 g resting tension in 10 mL temperature-controlled baths (37°C) containing Krebs solution that was gassed continuously with 95% O₂ and 5% CO₂. The rings were equilibrated at a 2.0 g resting tension for 120 min during which the bathing solution was changed every 15 min. In all rings, the endothelium was intentionally removed by inserting a 25-G needle tip into the lumen of the ring and gently rolling the ring for a few seconds. Endothelial removal was confirmed by the absence of relaxation to acetylcholine (10⁻⁶ M). Only one concentration-response curve elicited by levromakalim or voltage-dependent Ca^{2+} channel antagonist diltiazem was made from each ring in all experiments.

Experimental protocols

The rings were pre-contracted with 10⁻⁶ M phenylephrine. The first series of this *in vitro* study was carried out to examine the effects of tramadol on the vasorelaxant response evoked by levromakalim. When the contractile response to phenylephrine (10⁻⁶ M) was stabilized, the incremental concentration of levromakalim (10⁻⁸ to 10⁻⁵ M) was added to the organ bath to generate a concentration-response curve in the endothelium-denuded rings. The effect of tramadol (10⁻⁶, 10⁻⁵, 5 × 10⁻⁵ M) on the concentration-response curve for levromakalim was assessed by comparing the vasorelaxant response in the presence or absence of tramadol. Tramadol was added directly to the organ bath 15 min before the phenylephrine (10⁻⁶ M)-induced contraction.

The second series of experiments was designed to determine whether the racemic tramadol-induced attenuation of vasorelaxant response evoked by levromakalim was stereoselective. The incubation period for R(-) tramadol or S(+) tramadol was 15 min before the phenylephrine (10⁻⁶ M)-induced contraction. The effect of the tramadol enantiomers (R(-) tramadol: 10⁻⁶, 10⁻⁵, 5 × 10⁻⁵ M, S(+) tramadol: 10⁻⁶, 10⁻⁵, 5 × 10⁻⁵ M) on the concentration-response curve for levromakalim was assessed by comparing each vasorelaxant response in the presence or absence of R(-) or S(+) tramadol.

In the third series of this *in vitro* study, the effect of glibenclamide (10⁻⁵ M) on the concentration-response curve for levromakalim was examined by comparing the vasorelaxant response in the presence or absence of glibenclamide (10⁻⁵ M). Glibenclamide was added directly to the organ bath 15 min before the phenylephrine (10⁻⁶ M)-induced contraction.

The fourth series of the study examined the effect of tramadol (5×10^{-5} M) on the vasorelaxant response evoked by levromakalim in the rings pre-treated with 10^{-5} M glibenclamide. In the rings pre-treated with 10^{-5} M glibenclamide, the effect of tramadol on the concentration-response curve for levromakalim was assessed by comparing the vasorelaxant response in the presence or absence of tramadol (5×10^{-5} M). The incubation period for glibenclamide (10^{-5} M) and tramadol (5×10^{-5} M) or glibenclamide (10^{-5} M) alone was 30 min before the phenylephrine (10^{-6} M)-induced contraction.

In the fifth series of the experiments, the effect of 5×10^{-5} M tramadol on the concentration (3×10^{-8} to 3×10^{-4} M)-response curve for diltiazem was examined by comparing the vasorelaxant response in the presence or absence of 5×10^{-5} M tramadol. Tramadol was added directly to the organ bath 15 min before the phenylephrine (10^{-6} M)-induced contraction.

Finally, participation of the opioid receptors in the tramadol-induced attenuation of the levromakalim-induced vasorelaxant response was investigated by assessing the levromakalim concentration-response curve 30 min after the non-specific opioid receptor antagonist, naloxone (10^{-6} M), had been added directly to the organ bath, either alone or after a combined pre-treatment with tramadol (10^{-6} , 10^{-5} , 5×10^{-5} M).

Drug and solutions

All the drugs were of the highest purity commercially available: levromakalim, phenylephrine HCl, acetylcholine, diltiazem, naloxone (Sigma Chemical, St. Louis, MO, USA), and racemic tramadol (Yuhan Co, Seoul, Republic of Korea). R(-) tramadol and S(+) tramadol were a kind gift from Grünenthal GmbH (Germany). All concentrations are expressed as the final molar concentration in the organ bath. Levromakalim was dissolved in 95% ethanol and subsequently diluted in distilled water (final organ bath ethanol concentration: 0.19% volume/volume). Glibenclamide was dissolved in dimethyl sulfoxide (DMSO) (final organ bath DMSO concentration: 0.1% volume/volume). The vehicle (DMSO) for glibenclamide did not affect the vasorelaxation in response to levromakalim (data not shown). Unless stated otherwise, all the other drugs were dissolved and diluted in distilled water.

Data analysis

Summary data are expressed as the mean $\pm$ SD. The vasorelaxant responses to levromakalim and diltiazem are expressed as the percentage relaxation of the pre-contraction induced by 10^{-6} M phenylephrine. The

FIGURE 1 Effect of tramadol on the levromakalim dose-response curve. Tramadol (10^{-6} M) did not significantly alter the levromakalim dose-response curve. Large doses (10^{-5} , 5×10^{-5} M) of tramadol produced a significant rightward shift in the levromakalim dose-response curve (ED_{50} = * $P < 0.05$ vs no drug; † $P < 0.05$ vs 10^{-6} M tramadol; ‡ $P < 0.05$ vs 10^{-5} M tramadol) in a dose-dependent manner. Tramadol, 5×10^{-5} M, attenuated (maximum relaxation: § $P < 0.05$ vs no drug or 10^{-6} M tramadol) the maximum relaxation induced by levromakalim compared with the rings not exposed to the drug or exposed to 10^{-6} M tramadol. The data are shown as the mean $\pm$ SD and expressed as the percentage relaxation of the pre-contraction induced by 10^{-6} M phenylephrine [pre-contraction induced by 10^{-6} M phenylephrine: 100% = 3.19 ± 0.45 g ($n = 14$), 100% = 3.30 ± 0.29 g ($n = 9$), 100% = 3.20 ± 0.49 g ($n = 10$) and 100% = 2.91 ± 0.31 g ($n = 7$) for the rings not treated with tramadol, the tramadol (10^{-6} M), (10^{-5} M) and (5×10^{-5} M) pre-treated rings, respectively].

logarithm of the drug concentration (ED_{50}) eliciting 50% of the maximum relaxation response (R_{max}) was calculated by non-linear regression analysis by fitting the dose-response relationship for each vasorelaxant to a sigmoidal curve using commercially available software (Prism version 3.02; Graph Pad Software, San Diego, CA, USA). The R_{max} was measured as the maximal response to each vasorelaxant, with $R_{max} = 100\%$ indicating the complete reversal of the phenylephrine (10^{-6} M)-induced contraction. The concentration ratio (CR) was calculated as the ratio of the ED_{50} for levromakalim in the presence or absence of the tramadol enantiomers, and was used to compare the magnitude of the tramadol enantiomer (R(-) and S(+)-tramadol)-induced attenuation of the vasorelaxant response evoked by levromakalim. Statistical analysis was performed using a Student's *t* test for paired samples for ED_{50} , pre-contraction, CR and maximum relaxation and a one-way analysis of variance (ANOVA) followed by a Tukey multiple comparison

FIGURE 2 A) Effect of R(-) tramadol on the levromakalim dose-response curve. R(-) tramadol (10⁻⁶ M) did not significantly alter the levromakalim dose-response curve. R(-) tramadol (10⁻⁵, 5 × 10⁻⁵ M) produced a significant rightward shift in the levromakalim dose-response curve (ED_{50} = * P < 0.05 *vs* no drug; † P < 0.05 *vs* R(-) 10⁻⁶ M tramadol; ‡ P < 0.05 *vs* R(-) 10⁻⁵ M tramadol) in a dose-dependent manner. R(-) tramadol, 5 × 10⁻⁵ M, attenuated (maximum relaxation: § P < 0.05 *vs* no drug) the maximum relaxation induced by levromakalim. The data are shown as the mean ± SD and expressed as the percentage relaxation of the pre-contraction induced by 10⁻⁶ M phenylephrine [pre-contraction induced by 10⁻⁶ M phenylephrine: 100% = 2.95 ± 0.37 g (n = 9), 100% = 3.15 ± 0.29 g (n = 6), 100% = 3.23 ± 0.38 g (n = 7) and 100% = 3.17 ± 0.35 g (n = 8) for the rings not treated with R(-) tramadol, the R(-) tramadol (10⁻⁶ M), (10⁻⁵ M) and (5 × 10⁻⁵ M) pre-treated rings, respectively]. B) Effect of S(+) tramadol on the levromakalim dose-response curve. S(+) tramadol (10⁻⁶ M) did not significantly alter the levromakalim dose-response curve. S(+) tramadol (10⁻⁵, 5 × 10⁻⁵ M) produced a significant rightward shift in the levromakalim dose-response curve (ED_{50} = * P < 0.05 *vs* no drug; † P < 0.05 *vs* S(+) 10⁻⁶ M tramadol; ‡ P < 0.05 *vs* S(+) 10⁻⁵ M tramadol) in a dose-dependent manner. The data are shown as the mean ± SD and expressed as the percentage relaxation of the pre-contraction induced by 10⁻⁶ M phenylephrine [pre-contraction induced by 10⁻⁶ M phenylephrine: 100% = 2.92 ± 0.43 g (n = 7), 100% = 3.26 ± 0.33 g (n = 5), 100% = 3.29 ± 0.36 g (n = 7) and 100% = 3.00 ± 0.28 g (n = 6) for the rings not treated with S(+) tramadol, the S(+) tramadol (10⁻⁶ M), (10⁻⁵ M) and (5 × 10⁻⁵ M) pre-treated rings, respectively].

for ED_{50} , pre-contraction and maximum relaxation. A P value < 0.05 was considered statistically significant; n refers to the number of rats whose descending thoracic aortic rings were used in each protocol. Each group contained at least two rings from the same rat.

Results

Tramadol (10⁻⁶ M) did not significantly alter the levromakalim concentration-response curve. However, large doses of tramadol (10⁻⁵, 5 × 10⁻⁵ M) significantly (P < 0.0001) attenuated the levromakalim-induced

TABLE Effect of tramadol enantiomers on the concentration ratio of ED_{50} for levromakalim in isolated endothelium-denuded aortic rings

	<i>n</i>	Concentration ratio of ED_{50}
R (-) Tramadol 10 ⁻⁵ M	7	1.72 ± 0.21*
S (+) Tramadol 10 ⁻⁵ M	7	1.41 ± 0.18
R (-) Tramadol 5 × 10 ⁻⁵ M	8	3.93 ± 0.97*
S (+) Tramadol 5 × 10 ⁻⁵ M	6	2.22 ± 0.47

n = number of rats. * P < 0.05 *vs* S (+) tramadol at the corresponding concentration. ED_{50} = the logarithm of drug concentration eliciting 50% of the levromakalim-induced maximum relaxation. Concentration ratio of ED_{50} = ratio of the ED_{50} for levromakalim in the presence or absence of the tramadol enantiomers.

FIGURE 3 A) Effect of glibenclamide on the levromakalim dose-response curve. Glibenclamide (10⁻⁵ M) almost abolished (maximum relaxation: * P < 0.00001 *vs* no drug) the levromakalim-induced relaxation. The data are shown as the mean ± SD and expressed as the percentage relaxation of the pre-contraction induced by 10⁻⁶ M phenylephrine [pre-contraction induced by 10⁻⁶ M phenylephrine: 100% = 3.16 ± 0.50 g (n = 6) and 100% = 2.90 ± 0.20 g (n = 6) for the rings not treated with glibenclamide and the glibenclamide (10⁻⁵ M) pre-treated rings, respectively]. B) Effect of tramadol on the levromakalim dose-response curve in rings pre-treated with 10⁻⁵ M glibenclamide. In the rings pre-treated with glibenclamide (10⁻⁵ M), tramadol (5 × 10⁻⁵ M) had no effect on the levromakalim dose-response curve. The data are shown as the mean ± SD and expressed as the percentage relaxation of the pre-contraction induced by 10⁻⁶ M phenylephrine [pre-contraction induced by 10⁻⁶ M phenylephrine: 100% = 2.90 ± 0.20 g (n = 6) and 100% = 2.87 ± 0.29 g (n = 6) for the rings not treated with tramadol and the tramadol (5 × 10⁻⁵ M) pre-treated rings, respectively].

FIGURE 4 Effect of tramadol on the diltiazem dose-response curve. Tramadol (5×10^{-5} M) did not significantly alter the diltiazem dose-response curve. The data are shown as the mean $\pm$ SD and expressed as the percentage relaxation of the pre-contraction induced by 10^{-6} M phenylephrine [pre-contraction induced by 10^{-6} M phenylephrine: $100\% = 3.07 \pm 0.45$ g ($n = 6$) and $100\% = 3.09 \pm 0.25$ g ($n = 6$) for the rings not treated with tramadol and the tramadol (5×10^{-5} M) pre-treated rings, respectively].

relaxation (ED_{50} ; no drug: -6.47 ± 0.13 vs 10^{-5} M tramadol: -6.25 ± 0.15 , 5×10^{-5} M tramadol: -5.89 ± 0.16) in a concentration-dependent manner (Figure 1). Tramadol (5×10^{-5} M) attenuated ($P = 0.0143$) the R_{max} evoked by levromakalim compared with the rings not exposed to the drug or exposed to 10^{-6} M tramadol (Figure 1).

R(-) and S(+) tramadol (10^{-5} , 5×10^{-5} M) attenuated ($P < 0.0001$) the levromakalim-induced relaxation (ED_{50} ; no drug: -6.45 ± 0.08 vs 10^{-5} M R(-) tramadol: -6.21 ± 0.12 , 5×10^{-5} M R(-) tramadol: -5.86 ± 0.17 , ED_{50} ; no drug: -6.42 ± 0.08 vs 10^{-5} M S(+) tramadol: -6.28 ± 0.11 , 5×10^{-5} M S(+) tramadol: -6.08 ± 0.10) in a concentration-dependent manner (Figures 2A and 2B). R(-) tramadol (5×10^{-5} M) significantly attenuated ($P = 0.0163$) the maximum vasorelaxant response evoked by levromakalim compared with the rings not exposed to the drug (Figure 2A). In addition, the magnitude of the tramadol enantiomer (10^{-5} , 5×10^{-5} M)-induced attenuation of the vasorelaxation evoked by levromakalim was greater (10^{-5} M tramadol: $P = 0.013$, 5×10^{-5} M tramadol: $P = 0.0019$) in the rings pre-treated with R(-) tramadol (10^{-5} , 5×10^{-5} M) than with S(+) tramadol (10^{-5} , 5×10^{-5} M) (Table, Figures 2A and 2B).

Glibenclamide almost abolished ($P < 0.00001$) the levromakalim-induced relaxation (Figure 3A). In the ring pre-treated with 10^{-5} M glibenclamide, the high-

FIGURE 5 Effect of tramadol on the levromakalim dose-response curve in the rings pre-treated with 10^{-6} M naloxone. Tramadol (10^{-6} M) did not significantly alter the levromakalim dose-response curve in the rings pre-treated with 10^{-6} M naloxone. Large doses (10^{-5} , 5×10^{-5} M) of tramadol produced a significant rightward shift in the levromakalim dose-response curve ($ED_{50} = *P < 0.05$ vs no drug; $\dagger P < 0.05$ vs 10^{-6} M tramadol; $\ddagger P < 0.05$ vs 10^{-5} M tramadol) in the rings pre-treated with 10^{-6} M naloxone in a dose-dependent manner. Tramadol, 5×10^{-5} M, attenuated (maximum relaxation: $\S P < 0.05$ vs no drug or 10^{-6} M tramadol) the maximum relaxation induced by levromakalim compared with the rings exposed to 10^{-6} M naloxone alone or to 10^{-6} M naloxone plus 10^{-6} M tramadol. The data are shown as the mean $\pm$ SD and expressed as the percentage relaxation of the pre-contraction induced by 10^{-6} M phenylephrine [pre-contraction induced by 10^{-6} M phenylephrine: $100\% = 3.39 \pm 0.50$ g ($n = 6$), $100\% = 3.44 \pm 0.36$ g ($n = 6$), $100\% = 3.48 \pm 0.57$ g ($n = 6$) and $100\% = 3.25 \pm 0.44$ g ($n = 6$) for the rings not treated with tramadol, the tramadol (10^{-6} M), (10^{-5} M) and (5×10^{-5} M) pre-treated rings, respectively].

est concentration (5×10^{-5} M) of tramadol had no effect on levromakalim-induced relaxation (Figure 3B).

The highest concentration (5×10^{-5} M) of tramadol did not significantly alter the diltiazem-induced relaxation (ED_{50} ; no drug: -4.16 ± 0.26 vs 5×10^{-5} M tramadol: -4.22 ± 0.09) (Figure 4).

Tramadol (10^{-6} M) did not significantly alter the levromakalim concentration-response curve in the rings pre-treated with 10^{-6} M naloxone. However, large doses (10^{-5} , 5×10^{-5} M) of tramadol significantly attenuated ($P < 0.0001$) the levromakalim-induced relaxation (ED_{50} ; no drug: -6.49 ± 0.05 vs 10^{-5} M tramadol: -6.24 ± 0.15 , 5×10^{-5} M tramadol: -5.79 ± 0.22) in the rings pre-treated with 10^{-6} M naloxone in a concentration-dependent manner (Figure 5). In the rings pre-treated with 10^{-6} M naloxone, tramadol ($5 \times$

10^{-5} M) attenuated ($P = 0.0025$) the $R_{\max}$ evoked by levromakalim compared with the rings not exposed to the drug or exposed to 10^{-6} M tramadol (Figure 5).

Discussion

This is the first study to demonstrate that racemic, R(-), and S(+) tramadol (10^{-5} , 5×10^{-5} M) attenuated the vasorelaxation induced by levromakalim in isolated rat aortic vascular smooth muscle. The R(-) tramadol-induced attenuation of vasorelaxant response induced by levromakalim was more potent than that induced by S(+) tramadol. This tramadol-induced attenuation of vasorelaxation induced by levromakalim was found to be independent of opioid receptor activation.

Tramadol produces a nerve conduction block similar to lidocaine by exerting local anesthetic-like effect, suggesting that tramadol may block the sodium channels, and block potassium channels more than lidocaine.⁵ Local anesthetics were reported to suppress the glibenclamide-sensitive K_{ATP} channels in *Xenopus* oocytes.⁹ Local anesthetic agents at clinically relevant concentrations are known to block the potassium channels in addition to sodium channels.¹⁰ Lidocaine and the R(+) bupivacaine enantiomer attenuate the vasorelaxation mediated by the K_{ATP} channels in rat aortic vascular smooth muscle.^{6,7} Tramadol produces a dose-dependent reversible inhibition of the transmembrane ion (sodium, potassium, calcium) currents and reduces the non-specific leak currents in the pond snail neuron.¹¹ Tramadol may be more effective in blocking the voltage-dependent potassium channels than lidocaine.¹² The results from previous studies^{5,6,9-11} suggest that the inhibitory effect of tramadol on levromakalim-induced relaxation might be associated with the direct effect of tramadol on the potassium channels. However, more study of the influence of tramadol on the hyperpolarization induced by levromakalim in vascular smooth muscle cells will be needed.

Tramadol hydrochloride produces its analgesic effect in humans through its weak affinity for μ -opioid receptors and the inhibition of norepinephrine and serotonin reuptake.⁴ R(-) tramadol (5×10^{-5} M) attenuates the acetylcholine-induced relaxation in rat aorta, whereas S(+) tramadol (5×10^{-5} M) has no effect.⁸ High doses (2×10^{-4} to 10^{-3} M) of (+)-tramadol, but not (-)-tramadol, produce a concentration-dependent relaxation of rat aorta pre-contracted with phenylephrine.¹³ In accordance with previous stereoselectivity studies,^{4,8,13} R(-) tramadol produced the rather augmented inhibitory effect on the levromakalim-induced relaxation, compared with the S(+) tramadol enantiomer, even though both R(-) and S(+)

tramadol (10^{-5} , 5×10^{-5} M) attenuated the vasorelaxation mediated by levromakalim in a concentration-dependent manner.

Tramadol (10^{-5} , 5×10^{-5} M) attenuated the levromakalim-induced relaxation, whereas the highest dose (5×10^{-5} M) of tramadol had no effect on the vasorelaxation induced by diltiazem. This suggests that the inhibitory effect of tramadol is not caused by the non-specific reduction in the vasorelaxant response to levromakalim. The K_{ATP} channel is composed of at least two subunits: an inwardly rectifying K^+ channel six family that forms an ion conducting pore, and a modulatory sulfonylurea receptor that accounts for the pharmacologic properties of several drugs such as levromakalim and glibenclamide.¹⁴ In agreement with previous studies,^{2,6,7} glibenclamide almost abolished the vasorelaxation mediated by levromakalim in the rat aorta. A pretreatment with glibenclamide completely abolished the tramadol-induced attenuation of the vasorelaxant response induced by levromakalim. These results suggest that tramadol attenuates the vasorelaxation mediated by levromakalim through its effect on some of the components of the sulfonylurea receptor¹⁴ of the K_{ATP} channels. S(+) tramadol and the metabolite (+)-O-desmethytramadol (M1) are agonists of the μ -opioid receptors.⁴ Naloxone inhibits the cardioprotective effect that opioids provide through the activation of mitochondrial K_{ATP} channels.¹⁵ In contrast, in this study, tramadol attenuated the levromakalim-induced relaxation in rings pre-treated with 10^{-6} M naloxone, which suggests that the tramadol-induced attenuation is caused by a direct action on the pathway for levromakalim-induced relaxation.

Previous studies have demonstrated that activation of the K_{ATP} channels appears to contribute to the dilation of the cerebral vessel in response to hypoxia,¹⁶ acidosis,¹⁷ and the relaxation of subarachnoid hemorrhage-induced vasospasm,¹⁸ suggesting that activation of the K_{ATP} channel channels in these pathophysiologic states may partly contribute to a beneficial vasodilator response. Therefore, a supraclinical dose of tramadol enantiomers, particularly R(-) tramadol, may attenuate the beneficial vasodilator responses induced by K_{ATP} channel activation. Bolus administration ($2 \text{ mg} \cdot \text{kg}^{-1}$) of tramadol causes a transient increase in blood pressure with a concomitant increase of serum norepinephrine.¹⁹ In isolated guinea-pig atria and papillary muscle, tramadol (10^{-6} to 10^{-4} M) shows a concentration-dependent positive inotropic effect.²⁰ High doses (10^{-4} and 3×10^{-4} M) of tramadol produce vasodilation by both nitric oxide production and a direct effect on smooth muscle in the rabbit aorta.²¹ Thus, the net hemodynamic effects of tramadol *in*

vivo are a composite of vascular, myocardial, and neural effects. Any clinical implication of tramadol on the activation of the vascular K_{ATP} channels involved in the regional hemodynamics must be tempered by the fact that a large conduit artery, the aorta, was used in this *in vitro* experiment, whereas the resistance vessels²² with a diameter of 100–300 µm control blood flow to organs. However, the K_{ATP} channels have important roles in physiologic and pathophysiologic vasodilation.^{1,16–18} Even with this limitation, this finding may help provide a pharmacologic basis for the interaction between tramadol and the vascular smooth muscle K_{ATP} channel stimulated by levromakalim.

Tramadol (10⁻⁵, 5 × 10⁻⁵ M) at a supraclinical concentration significantly attenuated the levromakalim-induced relaxation, whereas 10⁻⁶ M tramadol,²³ which is the peak plasma concentration after the oral administration of 100 mg tramadol, had no effect on vasorelaxation. This suggests that the clinically relevant concentration of tramadol does not impair the beneficial vasodilator response induced by K_{ATP} channel activation. The peak plasma concentration increases in elderly healthy patients as well as in patients with a hepatic or renal insufficiency.²⁴ Because 20% of the tramadol is bound to the plasma protein,²⁴ changes in the amount or binding capacity of a protein under certain pathologic conditions (e.g., liver disease, hemodilution, hypoproteinemia) can increase the free fraction of tramadol. Considering above factors, 10⁻⁵ M tramadol may be a concentration encountered in clinical settings such as during a tramadol overdose or severe hepatic and renal dysfunction.

In conclusion, these results suggest that a supraclinical dose (10⁻⁵ M) of tramadol (racemic, R(-), and S(+)) attenuates the vasorelaxation mediated by the K_{ATP} channels in a rat aorta. The R(-) tramadol-induced attenuation of vasorelaxation mediated by the K_{ATP} channels is more potent than S(+) tramadol-induced attenuation. This attenuation does not occur via opioid receptor activation.

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