Inhibition of L-type Calcium Current by Tramadol and Enantiomers in Cardiac Myocytes from Rats

Emiliano Medei[2], Juliana M. Raimundo[1], José Hamilton M. Nascimento[2], Margarete M. Trachez[3], Roberto T. Sudo[1], Gisele Zapata-Sudo[1]

Programa de Desenvolvimento de Fármacos, Instituto de Ciências Biomédicas, Universidade Federal do Rio de Janeiro[1]; Instituto de Biofísica Carlos Chagas Filho, Universidade Federal do Rio de Janeiro[2], Rio de Janeiro, RJ; Serviço de Anestesiologia, Universidade Federal Fluminense[3], Niterói, RJ, Brazil

Abstract

Background: Tramadol is a centrally acting analgesic, whose mechanism of action involves opioid-receptor activation. Previously, we have shown that tramadol and its enantiomers had a negative inotropic effect on the papillary muscle in which the (+)-enantiomer is more potent than (-)- and (±)-tramadol.

Objective: In this study, we investigated the effects of tramadol and its enantiomers on L-type calcium current (ICa-L).

Methods: The experiments were carried out in isolated Wistar rat ventricular myocytes by using the whole cell patch clamp technique.

Results: Tramadol (200 μM) reduced the peak amplitude of ICa-L at potentials from 0 to +50 mV. At 0 mV, I_{Ca-L} was reduced by 33.7 ± 7.2%. (+)- and (-)-tramadol (200 μM) produced a similar inhibition of ICa-L, in which the peak amplitude was reduced by 64.4 ± 2.8% and 68.9 ± 5.8%, respectively at 0 mV ($p > 0.05$). Tramadol, (+)- and (-)-tramadol shifted the steady-state inactivation of ICa-L to more negative membrane potentials. Also, tramadol and (+)-tramadol markedly shifted the time-dependent recovery curve of I_{Ca-L} to the right and slowed down the recovery of I_{Ca-L} from inactivation. The time constant was increased from 175.6 ± 18.6 to 305.0 ± 32.9 ms ($p < 0.01$) for tramadol and from 248.1 ± 28.1 ms to 359.0 ± 23.8 ms ($p < 0.05$) for (+)-tramadol. The agonist of μ-opioid receptor DAMGO had no effect on the I_{Ca-L}.

Conclusions: The inhibition of ICa-L induced by tramadol and its enantiomers was unrelated to the activation of opioid receptors and could explain, at least in part, their negative cardiac inotropic effect. (Arq Bras Cardiol XXXX;XX(X):000-000)

Keywords: Tramadol; calcium channels, l-Type; myocytes, cardiac; rats.

Introduction

Tramadol (1RS, 2RS), -2- [(dimethylamino) - methyl] -1- (3-methoxyphenyl) – cyclohexanol hydrochloride, is a synthetic, centrally acting analgesic with effectiveness comparable to codeine, pentazocine or dextropropoxyphene when used for pain relief[1]. The mechanism of its analgesic action involves a combination of binding to μ-opioid receptors and the inhibition of the reuptake of serotonin and noradrenaline in pain pathways of the central nervous system (CNS). This dual mechanism of action has led to a description of tramadol as an "atypical" opioid agent[2]. Tramadol ((±)-tramadol) is formulated as a racemic mixture of (-)- and (+)-tramadol with different pharmacological actions. (+)-Tramadol has high affinity with μ-opioid receptors, inhibits preferentially serotonin uptake and promotes an increase of serotonin release. Nevertheless, (-)-tramadol has low affinity with μ-opioid receptors and inhibits norepinephrine uptake[3]. The complementary and synergistic actions of both enantiomers improve the analgesic profile of the racemate[4]. The main metabolite of tramadol, *O*-desmethyltramadol, seems to contribute to the analgesic effect because it has approximately a 300-fold greater affinity with μ-opioid receptors than tramadol[5]. Tramadol has been shown to affect 5-HT[6], muscarinic[7,8], nicotinic[9], NMDA and $GABA_A$[10] receptors. Also, voltage-dependent K^+ and Na^+ channels are involved in the antinociceptive and anesthetic effect of tramadol, respectively[11,12]. Limited information about the effects of tramadol on systems other than the central nervous system is available and few studies have compared the effects of tramadol and its enantiomers. In a previous study, we showed that tramadol and its enantiomers induced relaxation of precontracted rat aorta, which was stereoselective to (+)-tramadol[13]. Also, we have shown that tramadol reduced the contractility of rat cardiac muscle[13]. The possible mechanism involved in this phenomenon could be the inhibition of L-type calcium current (I_{Ca-L}), related to the activation of a receptor that modulates I_{Ca-L} and/or to a direct effect on the channel.

In order to evaluate the role of L-type Ca^{2+} channels on the negative cardiac inotropic action of tramadol and its enantiomers,

Mailing Address: Gisele Zapata-Sudo •
Programa de Desenvolvimento de Fármacos, Universidade Federal do Rio de Janeiro, Centro de Ciências da Saúde, Instituto de Ciências Biomédicas, Bloco J, Sala 14 - 21941-590 - Rio de Janeiro, Brazil
E-mail: gsudo@farmaco.ufrj.br
Manuscript received November 04, 2010, revised manuscript received November 04, 2010; accepted April 08, 2011.

we investigated their effects on the L-type Ca^{2+} currents (I_{Ca-L}) of rat ventricular myocytes. Comparisons among these compounds were done using the concentration (200 μM) that had induced negative inotropic effect on rat cardiac muscle[13].

Methods

The Animal Care and Use Committee at Universidade Federal do Rio de Janeiro approved the protocols used.

Isolation of Cardiomyocytes

Hearts from male Wistar rats (250-350 g) were rapidly removed and mounted on a modified Langendorff system. They were retrogressively perfused through the aorta for 5 min at 10 ml.min^{-1} with oxygenated Tyrode solution (in mM: 132.0 NaCl, 1.0 $CaCl_2$, 1.2 $MgCl_2$, 4.0 KCl, 10.0 HEPES, and 5.0 glucose; pH 7.3) at 33-35ºC. Ventricular myocytes were enzymatically isolated after 10 min perfusion with nominally Ca^{2+}-free solution containing collagenase type II (Worthington Biochemical, Lakewood, NJ; 150 U/ml). The enzyme was washed out by perfusion with Ca^{2+}-free Tyrode solution. Isolated myocytes were maintained in Kb solution (in mM: 1.0 $MgCl_2$, 30.0 KCl, 10.0 KH_2PO_4, 10.0 HEPES, 10.0 glucose, 70.0 glutamic acid, 0.3 EGTA, 20.0 taurine; pH 7.3) at room temperature until use. They were placed in the recording chamber mounted on the stage of an inverted microscope (Axiovert 40 CFL, Zeiss) and perfused at 5 ml.min^{-1} with Tyrode solution at 35ºC.

Electrophysiological studies

Recordings of Ca^{2+} currents were obtained by using the whole-cell configuration of the patch-clamp technique[14] through an Axopatch 200B amplifier (Axon Instruments, Foster City, CA). Voltage pulses were generated by pClamp software and a digital interface (Digidata 1200, Axon Instruments, Foster City, CA). Micropipettes were prepared with borosilicate glass capillaries and had a resistance of 4-7 MΩ when filled with pipette solution (in mM: 110.0 CsCl, 5.0 ATP-Mg, 0.1 GTP, 10.0 EGTA, 10.0 HEPES, and 30.0 TEA-Cl; pH 7.1). Currents were low-pass filtered at 1 KHz and digitized at 2 KHz. Current-voltage relationships were determined through 500ms voltage step from a holding potential of -40 mV to test potentials ranging from -50 to +60 mV, in 10 mV increments during 500 ms before and after 5 min of perfusion with 200 μM tramadol. Peak I_{Ca-L} value was expressed as relative to cell capacitance (pA/pF) and presented as mean ± SEM. Steady-state activation (*d*) and steady-state inactivation (*f*) curves were fitted with a Boltzmann function: d (or f) = $1/[1 + \exp(V_m - V_{0.5})/k]$, where V_m was the membrane potential, $V_{1/2}$ was the potential of half maximum activation/inactivation and k the slope factor.

Substances

Racemic tramadol and its enantiomers were generously donated by Cristália Produtos Químicos e Farmacêuticos (Itapira, São Paulo, Brazil) and were dissolved in distilled water at a stock concentration of 50 mM. Collagenase type II, taurine, tetraethylammonium chloride (TEA-Cl), HEPES, glutamic acid, EGTA, CsCl, MgATP, GTP and tetrodotoxin (TTX) were purchased from Sigma.

Statistical analysis

Data were presented as mean ± SEM. Intergroup comparisons were performed using ONE-WAY ANOVA with Bonferroni's multiple comparison test (selected pairs of column). While, intragroup comparisons were performed using repeated measures ONE-WAY ANOVA with Bonferroni's multiple comparison test (compare selected pairs of column). Statistical differences were considered significant when $p < 0.05$.

Results

Effect of tramadol and enantiomers on current-voltage relationship of I_{Ca-L}

Figure 1A shows a representative tracing of the inward Ca^{2+} currents recorded from rat ventricular myocytes at 0 mV in the absence and presence of 200 μM (±)-tramadol (Figure-1 top) and its enantiomers (Figure-1 middle and bottom). Mean values of I_{Ca-L} at different potentials (-50 to 60 mV) were plotted in I-V curves before and after treatment with (±)- (Figure-1B top), (+)- (Figure-1B middle) and (-)-tramadol (Figure-1B bottom). Upon perfusion of each enantiomer, the peak amplitude of I_{Ca-L} was significantly ($p < 0.05$) reduced at potentials more depolarized than -20 mV, while with (±)-tramadol the reduction was significant at potentials more positive t4han 0 mV ($p < 0.05$). (±)-Tramadol significantly inhibited the peak amplitude of I_{CaL} which was reduced by 33.7 ± 7.2% at 0 mV. There was no difference in the peak current inhibition induced by the enantiomers, (+)-tramadol (64.4 ± 2.8%) and (-)-tramadol (68.9 ± 5.8%). However, the inhibition of I_{Ca-L} induced by both enantiomers was significantly greater than that induced by (±)-tramadol ($p < 0.01$ vs (+)-tramadol; $p < 0.001$ (-)-tramadol). The reduction was partially reversed after a 10 min washout.

Effect of tramadol and enantiomers on activation of I_{Ca-L}

Activation curves were constructed from the current-voltage relationship by dividing the amplitude of I_{Ca-L} at each potential by the driving force (Figure 2). (±)-Tramadol decreased the $V_{1/2}$ of the steady-state activation curve of I_{Ca-L}, with no changes in k value. In the control condition, $V_{1/2}$ was -17.5 ± 2.1 mV and k was 4.2 ± 0.2 mV and in the presence of (±)-tramadol, $V_{1/2}$ was -21.0 ± 2.2 mV ($p < 0.05$) and k was 3.6 ± 0.3 mV ($p > 0.05$). On the other hand, the enantiomers did not change the steady-state activation curve of I_{Ca-L} (Table 1).

Effect of tramadol and enantiomers on inactivation of I_{Ca-L}

Steady-state inactivation of I_{Ca-L} was measured using a classic double-pulse protocol. Preconditioning steps of 1600 ms, from -60 to +60 mV, in 10 mV intervals from a holding potential of -40 mV were applied, followed by a 600ms test pulse to 0 mV. The peak current elicited by test pulses was normalized to maximum current and plotted relatively to the preconditioning potential. (±)-Tramadol and its enantiomers shifted the steady-

state inactivation curves to more negative potentials (Figure 3) and changed the $V_{1/2}$ of inactivation (Table 1).

In fact, the $V_{1/2}$ of inactivation of enantiomers was significant different when compared to (±)-Tramadol ((+)-tramadol $p < 0.01$ and (-)-tramadol $p < 0.001$ compared with (±)-tramadol). However, only (-)-tramadol altered the k value (control, 3.8 ± 0.1 mV; (-)-Tramadol, 4.9 ± 0.3 mV; $p < 0.01$). No differences were observed in the mean of the k value among groups.

Effect of tramadol and enantiomers on recovery of I_{Ca-L} from inactivation

The effects of (±)-tramadol and its enantiomers on the kinetics of recovery of I_{Ca-L} from inactivation were studied using a double-pulse protocol consisting of a 500 ms prepulse to 0 mV followed by a 500 ms test pulse to 0 mV, after a variable inter-pulse interval (0 to 2500 ms) from a holding potential of -40 mV, every 10 s. (±)- and (+)-tramadol shifted the time-dependent recovery curve to the right and slowed down the recovery from inactivation (Figure 4). The recovery from inactivation could be fitted by a single exponential, where the time constant (t) was increased from 175.6 ± 18.6 ms to 305.0 ± 32.9 ms ($p < 0.01$) for (±)-tramadol and from 248.1 ± 28.1 ms to 359.0 ± 23.8 ms ($p < 0.05$) for (+)-tramadol. (-)-Tramadol did not modify the time course for recovery of I_{Ca-L}. No differences were observed in the mean of the time constant (t) value among groups.

Effect of the agonist of μ-opioid receptor (DAMGO) on the I_{Ca-L} of rat cardiomyocites

In this study, our results have shown that tramadol and its enantiomers inhibit the I_{Ca-L}. The possible mechanism involved in this effect could be due to the activation of μ-opioid receptors. To verify this hypothesis, we tested whether the agonist of the μ-opioid receptor (DAMGO) modulates the I_{Ca-L} in rat cardiomyocytes. Thus, figure 5 shows that DAMGO has no effect on the calcium currents activated by depolarizing pulses to 0 mV (Control: -449.7 ± 4.4 pA vs DAMGO: -416.9 ± 2.7 pA; n=3; $p > 0.05$).

Discussion

In this study, our data have shown different effectiveness of tramadol enantiomers to inhibit I_{Ca-L} (~60%) when compared to racemic mixture (~30 %). In contrast, in our previous study, the negative inotropic effect on rat papillary muscles

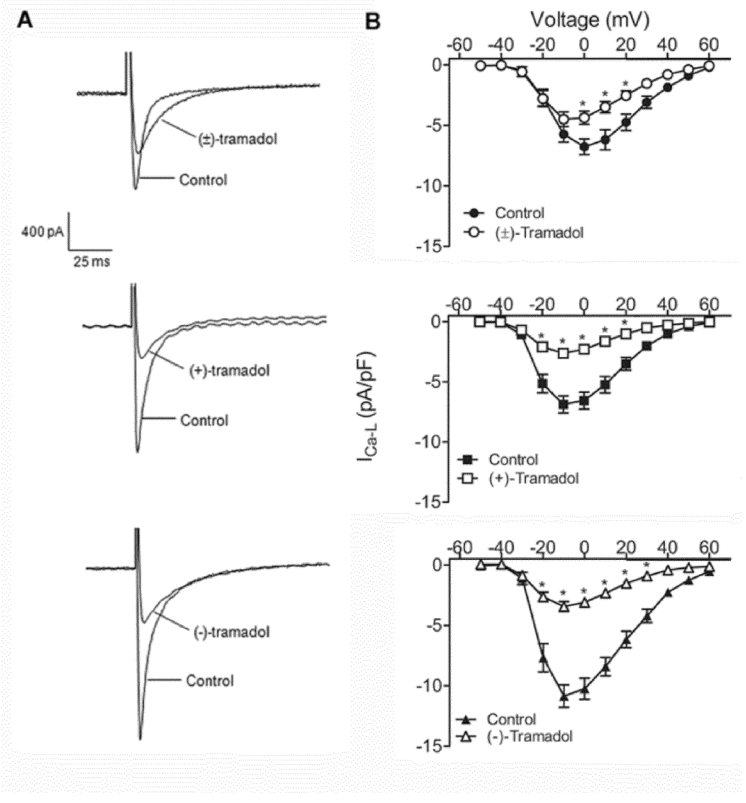

Figure 1 – *Effects of tramadol and enantiomers on L-type Ca2+ current (I_{Ca-L}) in isolated rat ventricular myocytes. In A, representative current traces following a depolarizing pulse from -40 to 0 mV, in the absence and presence of (±)- (top), (+)- (middle) and (-)-tramadol (bottom). In B, current-voltage relationships of I_{Ca-L} obtained before and after 5 min perfusion of (±)-(top), (+)- (middle) and (-)-tramadol (bottom) (200 μM) from -50 to 60 mV. *p < 0.05 vs. Control condition. Intragroup comparisons were performed using repeated measures ONE-WAY ANOVA with Bonferroni's multiple comparison test (selected pairs of column).*

observed at 200 μM presented the following order of potency (IC50): (+)-tramadol > (-)-tramadol > (±)-tramadol[13]. This difference between cell and tissue responses could be due to a possible inhibition of monoamine reuptake by tramadol and their enantiomers on the electrically-stimulated papillary muscle. It has been demonstrated that the electrically evoked

Table 1 – Effects of tramadol and its enantiomers on the kinetics of activation and inactivation of I_{Ca-L}

	Activation		Inactivation	
	$V_{1/2}$ (mV)	k (mV)	$V_{1/2}$ (mV)	k (mV)
Control	-17,5 ± 2,1	4,2 ± 0,2	-35,9 ± 1,4	3,8 ± 0,4
(±)-Tramadol	-21,0 ± 2,2*	3,6 ± 0,3	-39,8 ± 1,4*	4,0 ± 0,3
Control	-21,9 ± 1,7	3,8 ± 0,3	-42,3 ± 1,2	3,6 ± 0,4
(+)-Tramadol	-24,2 ± 1,3	3,8 ± 0,2	-47,3 ± 1,5***#	4,7 ± 0,4
Control	-20,9 ± 1,3	3,8 ± 0,3	-41,1 ± 1,3	3,8 ± 0,1
(-)-Tramadol	-22,9 ± 1,7	4,0 ± 0,3	-50,2 ± 1,5**&	4,9 ± 0,3**

**p <0.05, **p < 0.005, ***p < 0.0005 compared with control in the same group (ONE-WAY ANOVA repeated measures with Bonferroni's compared selected pairs of columns). #p<0.01, &p<0.001 compared with (±)-Tramadol, intergroup comparison (ONE-WAY ANOVA with Bonferroni's multiple comparison test compared all pairs of columns). V1/2 - potential of half maximum activation/inactivation; κ - slope factor.*

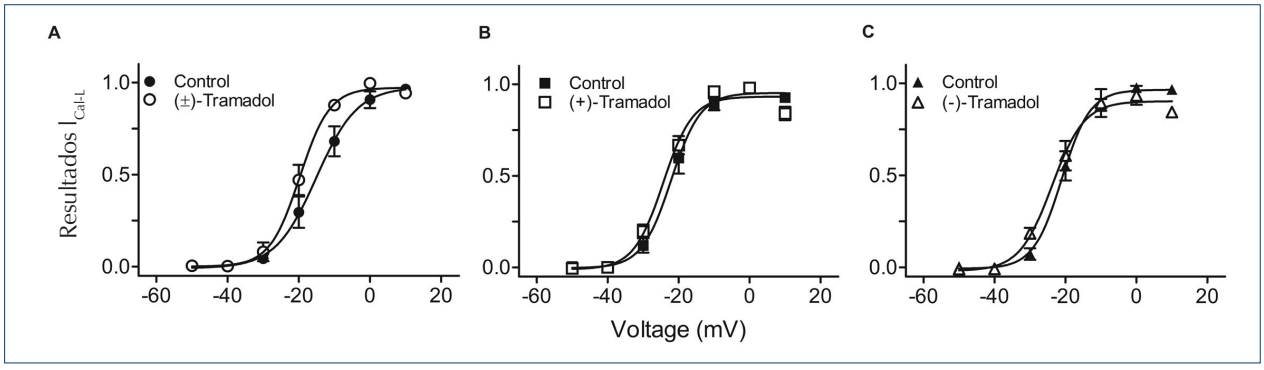

Figure 2 – *Effects of tramadol and enantiomers on the steady-state activation kinectics. Voltage-activation curves of ICa-L were obtained from the current-voltage relationship, in the absence and presence of 200 μM (±)-, (+)- and (-)-tramadol (A, B and C, respectively). Only (±)-tramadol shifted the voltage of half-activation curve. Intragroup comparisons were performed using repeated measures ONE-WAY ANOVA with Bonferroni's multiple comparison test (selected pairs of column).*

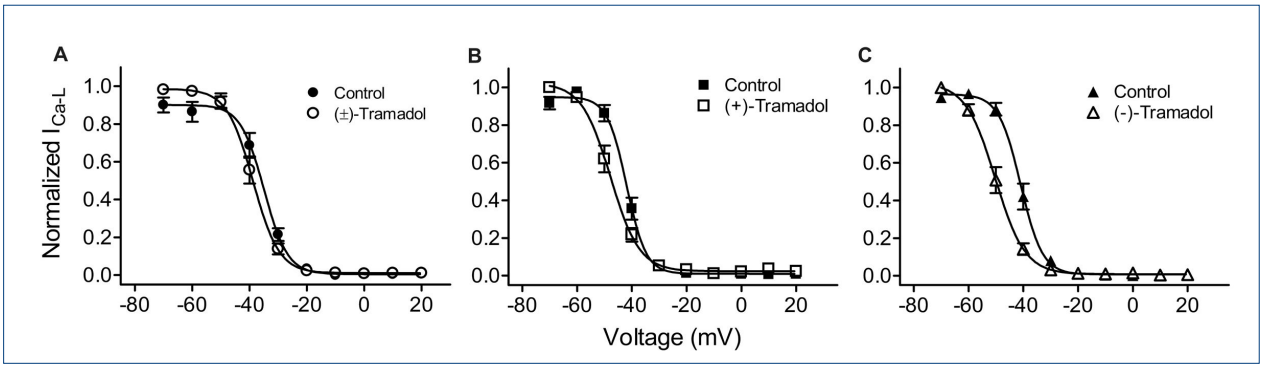

Figure 3 – *Effects of tramadol and enantiomers on steady-state inactivation kinectics. Voltage inactivation curves of I_{Ca-L} were obtained using a double pulse protocol, which consisted of conditioning voltage pulses to membrane potentials from –60 to + 60 mV followed by a test pulse to 0 mV. Test pulse currents were normalized to the maximum value. In A, B and C, steady-state inactivation curves of I_{Ca-L} were obtained in the absence and presence of (±)-, (+)- and (-)-tramadol (200 μM), respectively. Note the shift of the steady-state inactivation curve of I_{Ca-L} to negative potentials. Intragroup comparisons were performed using repeated measures ONE-WAY ANOVA with Bonferroni's multiple comparison test (selected pairs of column).*

release of noradrenaline by sympathetic nerve endings in isolated cardiac muscle and the amplitude of contraction are decreased by local anesthetics[15]. Different activities of tramadol have shown to be stereoselective, including opioid receptor activation[3,16], inhibition of monoamine reuptake[17,18], analgesic effect[3] and vascular relaxation[13,19]. However, this study demonstrated that tramadol enantiomers (200 μM) decreased $I_{Ca\text{-}L}$ (~60%), indicating a non-enantiomer-specific blockade of L-type Ca^{2+} channels. The racemic tramadol produced a minor inhibition of $I_{Ca\text{-}L}$ (~30%) when compared to its enantiomers which could be correlated to the small potency of racemic to reduce cardiac contractility[13]. Tramadol and enantiomers differently altered the kinectics of $I_{Ca\text{-}L}$. (±)- and (+)-tramadol shifted the steady-state inactivation curve for $I_{Ca\text{-}L}$ to more negative membrane potentials and markedly slowed the recovery of $I_{Ca\text{-}L}$ from inactivation. (-)-Tramadol significantly altered only the inactivation of $I_{Ca\text{-}L}$. The effects of tramadol on $I_{Ca\text{-}L}$ may be related to the activation of a receptor that modulates $I_{Ca\text{-}L}$ and/or to a direct effect on the channel. However, the effect of tramadol on $I_{Ca\text{-}L}$ seems not to be related to the activation of opioid receptors. Tramadol binds preferentially to m-receptors, which have been demonstrated not to be present in the mammalian heart[20-23]. Indeed, our results

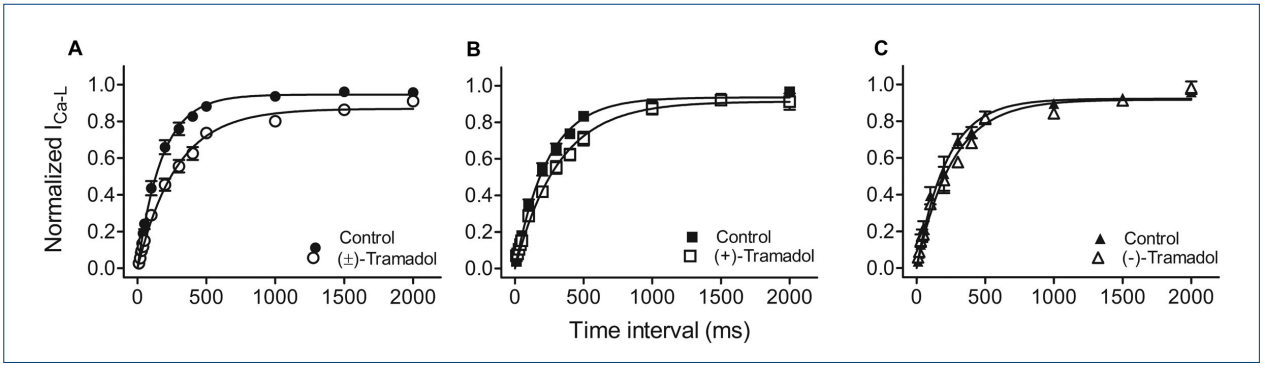

Figure 4 – *Effects of tramadol and enantiomers on ICa-L recovery from inactivation. The recovery of ICa-L from inactivation was determined using a double pulse protocol consisting of two pulses to 0 mV with a variable inter-pulse interval (0 – 2500 ms), in the absence and presence of 200 µM (±)-, (+)- and (-)-tramadol (A, B and C, respectively). The time course for recovery of ICa-L was significantly altered by (±)-, (+)-tramadol. Intragroup comparisons were performed using repeated measures ONE-WAY ANOVA with Bonferroni's multiple comparison test (selected pairs of column).*

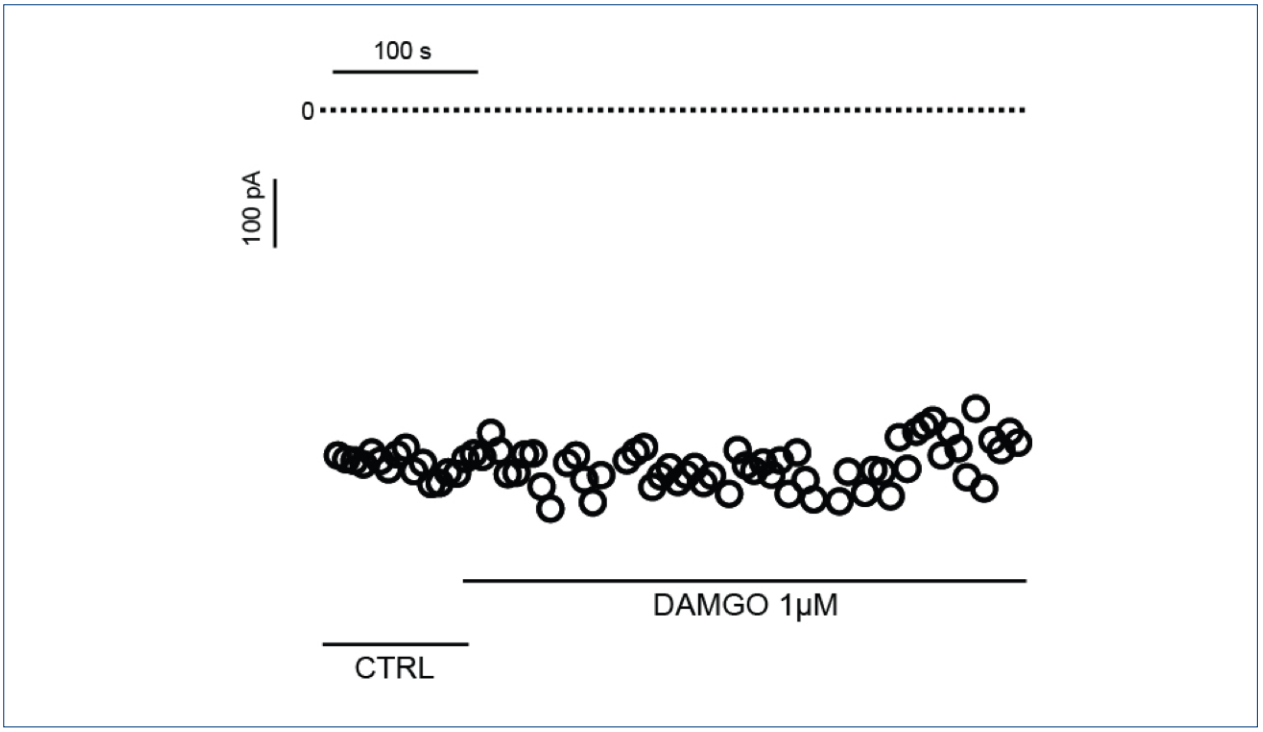

Figure 5 – *The agonist of µ-opioid receptor (DAMGO) had no effect on the $I_{Ca\text{-}L}$. Figure 5 shows a representative experiment where the $I_{Ca\text{-}L}$ time-course was recorded by depolarizing pulses at 0 mV in control condition and after DAMGO. DAMGO did not modify $I_{Ca\text{-}L}$.*

showed no effect of the m-receptor agonist DAMGO on the cardiac I_{Ca-L}. Moreover, it has been shown that the cardiac effects of opioids could be or not be dependent on opioid receptors. The effects of morphine on ionic currents in cardiac myocytes have shown not to depend on opioid receptor[24] or to be mediated via d- and k-receptors[25]. However, its cardioprotective effect is known to be due to the activation of d-receptors[26,27]. Studies have shown that opioids like dextropropoxiphene, pethidine and leucine enkephalin reduce I_{Ca-L} but only the inhibition induced by d-receptor agonist (leucine enkephalin) was blocked by naloxone[28,29]. The effects of tramadol on other ionic currents also suggest a direct effect on L-type Ca^{2+} channels. Haeseler et al[12] showed that tramadol, sufentanil and fentanil but not morphine blocked sodium currents of heterologously expressed $NaV_{1.2}$ neuronal Na^+ channels. It is important to note that the potency to block sodium current was irrespective of the relevant opioid receptor potency of the compound. Tramadol has also been reported to block delayed rectifier K^+ current ($I_{K(DR)}$) in NG108-15 neuronal cells in a concentration-dependent manner[30]. As observed in our study for I_{Ca-L}, tramadol shifted the steady-state inactivation curve of $I_{K(DR}$ to more negative potentials[30].

Conclusion

The effectiveness of tramadol enantiomers to inhibit I_{Ca-L} was twice the one observed with (±)-tramadol and such effect seems unrelated to the activation of opioid receptors.

Acknowledgements

The authors thank Cristália Produtos Químicos e Farmacêuticos Ltda. (BR), Conselho Nacional de Desenvolvimento Cientifico e Tecnológico (CNPq, BR), Coordenação de Aperfeiçoamento de Pessoal de Nível Superior (CAPES, BR), Fundação Universitária Jose Bonifácio (FUJB, BR), Fundação Carlos Chagas Filho de Amparo à Pesquisa do Estado do Rio de Janeiro (FAPERJ, BR) for finantial support and fellowships from CAPES (to JMR) and CNPq (to GZS, RTS). None of the authors have any conflict of interest or financial interest to disclose.

Potential Conflict of Interest

No potential conflict of interest relevant to this article was reported.

Sources of Funding

This study was partially funded by CNPq, CAPES and FAPERJ.

Study Association

This article is part of the thesis of Doctoral submitted by Juliana M. Raimundo, from Universidade Federal do Rio de Janeiro.

References

1. Raffa RB, Friderichs E, Reimann W, ShanK RP, Codd EE, Vaught JL. Opioid and non-opioid components independently contribute to the mechanism of action of tramadol, an atypical opioid analgesic. J Pharmacol Exp Ther. 1992;260(1):275-85.
2. Garrido MJ, Valle M, Campanero MA, Calvo R, Trocóniz IF. 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. 2000;295(1):352-9.
3. Raffa RB, Friderichs E, Reimann W, Shank RP, Codd EE, Vaught JL, et al. Complementary and synergistic antinociceptive interaction between the enantiomers of tramadol. J Pharmacol Exp Ther. 1993;267(1):331-40.
4. Grond S, Sablotzki A. Clinical pharmacology of tramadol. Clin Pharmacokinet. 2004;43(13):879-923.
5. Gillen C, Haurand M, Kobelt DJ, Wnendt S. Afinitty, potency and efficacy of tramadol and its metabolites at the cloned human m-opioid receptor. Naunyn-Schmiedeberg's Arch Pharmacol. 2000;362(2):116-21.
6. Ogata J, Minami K, Uezono Y, Okamoto T, Shiraishi M, Shigematsu A, et al. The inhibitory effects of tramadol on 5-hydroxytryptamine type 2c receptors expressed in Xenopus oocytes. Anesth Analg. 2004;98(5):1401-6.
7. Shiraishi M, Minami K, Uezono Y, Yanagihara N, Shigematsu A. Inhibition by tramadol of muscarinic receptor-induced responses in cultured adrenal medullary cells and in X*enopus laevis* oocytes expressing cloned M1 receptors. J Exp Pharmacol Ther. 2001;299(1):255-60.
8. Shiga Y, Minami K, Shiraishi M, Uezono Y, Murasaki O, Kaibara M, et al. The inhibitory effects of tramadol on muscarinic receptor-induced responses in X*enopus* oocytes expressing cloned M3 receptors. Anesth Analg. 2002;95(5):1269-73.
9. 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 a7 receptors. Br J Pharmacol. 2002;136(2):207-16.
10. Hara K, Minami K, Sata T. The effects of tramadol and its metabolite on glycine, g-aminobutyric $acid_A$, and N-methyl-D-aspartate receptors expressed in *Xenopus* oocytes. Anesth Analg. 2005;100(5):1400-5.
11. Yalcin I, Aksu F. Involvement of potassium channels and nitric oxide in tramadol antinociception. Pharmacol Biochem Behav. 2005;80(1):69-75.
12. Haeseler G, Foadi N, Ahrens J, Dengler R, Hecker H, Leuwer M. Tramadol, fentanyl and sufentanil but not morphine block voltage-operated sodium channels. Pain. 2006;126(1-3):234-44.
13. Raimundo JM, Sudo RT, Pontes LB, Antunes F, Trachez MM, Zapata-Sudo G. In vitro and in vivo vasodilator activity of racemic tramadol and its enantiomers in Wistar rats. Eur J Pharmacol. 2006;530(1-2):117-23.
14. Hamill OP, Marty A, Neher E, Sakmann B, Sigworth J. Improved patch-clamp techniques for high-resolution current recording from cells and cell-free membrane patches. Pflugers Arch. 1981;391(2):85-100.
15. Joseph A, Montiague R, Effendi AR, Urbanska RA, Vogel S, Winnie AP, et al. Effect of bupivacaine and levobupivacaine on exocytotic norepinephrine release from rat atria. Anesthesiology. 2005;102(5):977-84.
16. Lai J, Ma SW, Porreca F, Raffa RB. Tramadol, M1 metabolite and enantiomer affinities for cloned human opioid receptors expressed in transfected HN9.10 neuroblastoma cells. Eur J Pharmacol. 1996;316(2-3):369-72.
17. Bamigbade TA, Davidson C, Langford RM, Stamford JA. Actions of tramadol, its enantiomers and principal metabolite, O-desmethyltramadol, on serotonin (5-HT) efflux and uptake in the rat dorsal raphe nucleus. Br J Anaesth. 1997;79(3):352-56.

18. Halfpenny DM, Callado LF, Hopwood SE, Bamigbade TA, Langford RM, Stamford JA. Effects of tramadol stereoisomers on norepinephrine efflux and uptake in the rat locus coerulus measured by real time voltammetry. Br J Anaesth. 1999;83(6):909-15.

19. Shin IW, Sohn JT, Park KE, Chang KC, Choi JY, Lee HK, et al. A supraclinical dose of tramadol stereoselectively attenuates endothelium-dependent relaxation in isolated rat aorta. Anesth Analg. 2006;103(2):366-71.

20. Krumins SA, Faden AI, Feuerstein G. Opiate binding in rat hearts: modulation of binding after hemorrhagic shock. Biochem Biophys Res Commun. 1985;127(1):120-8.

21. Ventura C, Bastagli L, Bernardi P, Caldarera CM, Guarnieri C. Opioid receptors in rat cardiac sarcolemma: effect of phenylephrine and isoproterenol. Biochim Biophys Acta. 1989;987(1):69-74.

22. Zhang WM, Jin WQ, Wong TM. Multiplicity of kappa opioid receptor binding in the rat cardiac sarcolemma. J Mol Cell Cardiol. 1996;28(7):1547-54.

23. Wittert G, Hope P, Pyle D. Tissue distribution of opioid receptor gene expression in the rat. Biochem Biophys Res Commun. 1996;218(3):877-81.

24. Hung CF, Tsai CH, Su MJ. Opioid receptor independent effects of morphine on membrane currents in single cardiac myocytes. Br J Anaesth. 1998;81(6):925-31.

25. Xiao GS, Zhou JJ, Wang GY, Cao CM, Li GR, Wong TM. In vitro electrophysiologic effects of morphine in rabbit ventricular myocytes. Anesthesiology. 2005;103(2):280-6.

26. Ela C, Barg J, Vogel Z, Hasin Y, Eilam Y. Distinct components of morphine effects on cardiac myocytes are mediated by the k and d opioid receptors. J Mol Cell Cardiol. 1997;29(2):711-20.

27. McPherson BC, Yao Z. Signal transduction of opioid-induced cardioprotection in ischemia-reperfusion. Anesthesiology. 2001;94(6):1082-8.

28. Xiao RP, Spurgeon HA, Capogrossi MC, Lakatta EG. Stimulation of opioid receptors on cardiac ventricular myocytes reduces L type Ca^{2+} channel current. J Mol Cell Cardiol. 1993;25(6):661-6.

29. Wu C, Fry CH, Henry J. The mode of action of several opioids on cardiac muscle. Exp Physiol. 1997;82(2):261-72.

30. Tsai TY, Tsai YC, Wu SN, Liu YC. Tramadol-induced blockade of delayed rectifier potassium current in NG108-15 neuronal cells. Eur J Pain. 2006;10(7):597-601.