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

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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 (I_{Ca-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 I_{Ca-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 I_{Ca-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 I_{Ca-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 I_{Ca-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. 2011; [online].ahead print, PP0-0)

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. 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. 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. The complementary and synergistic actions of both enantiomers improve the analgesic profile of the racemate. 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. Tramadol has been shown to affect 5-HT₃, muscarinic, nicotinic, NMDA and GABA receptors. Also, voltage-dependent K⁺ and Na⁺ channels are involved in the antinociceptive and anesthetic effect of tramadol, respectively. 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. Also, we have shown that tramadol reduced the contractility of rat cardiac muscle. The possible mechanism involved in this phenomenon could be the inhibition of L-type calcium current, 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 Ca²⁺ channels on the negative cardiac inotropic action of tramadol and its enantiomers,
we investigated their effects on the L-type Ca\(^{2+}\) currents (I_{CaL}) 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 retrogradely 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 33-35°C. 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 K\(_b\) solution (in mM: 1.0 MgCl\(_2\), 30.0 KCl, 10.0 KH\(_2\)PO\(_4\), 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 35°C.

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_{CaL} value was expressed as relative to cell capacitance (pA/pF) and plotted relatively to the preconditioning 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_{CaL} 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: \(d (f) = 1/[1 + \exp (V_{m} - V_{1/2})/k]\), where \(V_{1/2}\) 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_{CaL}

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_{CaL} 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_{CaL} was significantly (\(p < 0.05\)) reduced at potentials more depolarized than -20 mV, while with (±)-tramadol the reduction was significant at potentials more positive than 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_{CaL} 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_{CaL}

Activation curves were constructed from the current-voltage relationship by dividing the amplitude of I_{CaL} at each potential by the driving force (Figure 2). (±)-Tramadol decreased the \(V_{1/2}\) of the steady-state activation curve of I_{CaL} 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 (\(p < 0.001\)). On the other hand, the enantiomers did not change the steady-state activation curve of I_{CaL} (Table 1).

Effect of tramadol and enantiomers on inactivation of I_{CaL}

Steady-state inactivation of I_{CaL} 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-
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Tramadol inhibit L-type calcium current

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 cardiomyocytes

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. Intrigroup 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 > (±)-tramadol13. 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}

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<th>Activation</th>
<th>Inactivation</th>
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<td></td>
<td>V_{1/2} (mV)</td>
<td>k (mV)</td>
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<td>Control</td>
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<td>4.2 ± 0.2</td>
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<tr>
<td>(±)-Tramadol</td>
<td>-21.0 ± 2.2*</td>
<td>3.8 ± 0.3</td>
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<td>Control</td>
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<tr>
<td>(-)-Tramadol</td>
<td>-22.9 ± 1.7</td>
<td>4.0 ± 0.3</td>
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*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; k - slope factor.

Figure 2 – Effects of tramadol and enantiomers on the steady-state activation kinetics. Voltage-activation curves of I_{Ca-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 kinetics. 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\textsuperscript{15}. Different activities of tramadol have shown to be stereoselective, including opioid receptor activation\textsuperscript{3,16}, inhibition of monoamine reuptake\textsuperscript{17,18} analgesic effect\textsuperscript{3} and vascular relaxation\textsuperscript{13,19}. However, this study demonstrated that tramadol enantiomers (200 µM) decreased $I_{\text{Ca,L}}$ (–60%), indicating a non-enantiomer-specific blockade of L-type Ca\textsuperscript{2+} channels. The racemic tramadol produced a minor inhibition of $I_{\text{Ca,L}}$ (–30%) when compared to its enantiomers which could be correlated to the small potency of racemic to reduce cardiac contractility\textsuperscript{13}. Tramadol and enantiomers differently altered the kinetics of $I_{\text{Ca,L}}$. (+)- and (-)-tramadol shifted the steady-state inactivation curve for $I_{\text{Ca,L}}$ to more negative membrane potentials and markedly slowed the recovery of $I_{\text{Ca,L}}$ from inactivation. (-)-Tramadol significantly altered only the inactivation of $I_{\text{Ca,L}}$. The effects of tramadol on $I_{\text{Ca,L}}$ may be related to the activation of a receptor that modulates $I_{\text{Ca,L}}$ and/or to a direct effect on the channel. However, the effect of tramadol on $I_{\text{Ca,L}}$ 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\textsuperscript{20-23}. Indeed, our results

Figure 4 – Effects of tramadol and enantiomers on $I_{\text{Ca-L}}$ recovery from inactivation. The recovery of $I_{\text{Ca-L}}$ 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 $I_{\text{Ca-L}}$ 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 $I_{\text{Ca-L}}$. Figure 5 shows a representative experiment where the $I_{\text{Ca-L}}$ time-course was recorded by depolarizing pulses at 0 mV in control condition and after DAMGO. DAMGO did not modify $I_{\text{Ca-L}}$.

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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 receptor24 or to be mediated via d- and k-receptors25. However, its cardioprotective effect is known to be due to the activation of d-receptors26,27. Studies have shown that opioids like dextropropoxyphene, phenethidine and leucine enkephalin reduce I_{Ca-L} but only the inhibition induced by d-receptor agonist (leucine enkephalin) was blocked by naloxone28,29. The effects of tramadol on other ionic currents also suggest a direct effect on L-type Ca^{2+} channels. Haeseler et al30 showed that tramadol, sufentanil and fentanyl 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_{KDR}) in NG108-15 neuronal cells in a concentration-dependent manner30. As observed in our study for I_{Ca-L}, tramadol shifted the steady-state inactivation curve of I_{KDR} to more negative potentials30.

**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.

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**Potential Conflict of Interest**

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

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**Study Association**

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