SHORT COMMUNICATION

Citalopram Inhibits L-type Calcium Channel Current in Rat Cardiomyocytes in Culture

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Summary
Selective serotonin reuptake inhibitors (SSRI) are believed to be less dangerous in the treatment of depressive disorder in comparison with tricyclic antidepressants (TCA) due to their relative lack of cardiotoxicity. Thus, we investigated the effect of citalopram (SSRI) on membrane electrophysiology in rat cardiomyocytes in tissue culture. The results were compared with those from amitriptyline (TCA). The whole-cell configuration patch-clamp technique was used. Both citalopram and amitriptyline exhibited the concentration-dependent inhibition of the L-type calcium channel current (ICa). Citalopram in concentrations of 3 µM and 10 µM inhibited peak calcium current by 2.7 % and 8 %, respectively. We demonstrated the same potency of citalopram and amitriptyline to inhibit ICa. These observations led us to conclude that citalopram and amitriptyline are drugs, which exhibit a similar potency for causing concentration-dependent inhibition of ICa.

Key words
Citalopram • Amitriptyline • L-type calcium channel current • Whole cell configuration patch-clamp

Citalopram is a widely used antidepressant drug belonging to the newer antidepressant group, selective serotonin reuptake inhibitors (SSRIs). SSRIs have proved to be very efficient in the treatment of depressive disorders and, in contrast to tricyclic antidepressants (TCA), they are believed to have more benign cardiovascular safety profile (Slavíček 1998). Tricyclic antidepressants (TCA) exhibit several side effects. The most dangerous and life-threatening side effect is their potential cardiotoxicity. They might exhibit conduction delays (Jo 2000), arrhythmias, orthostatic hypotension and sudden death. In contrast to TCAs, there are only occasional reports of severe bradycardia in association with an SSRI overdose (Ellison 1990, Óström 1996).

One of the hypotheses concerned the possibility that cardiotoxicity of TCAs might be caused by electrophysiological changes at the level of voltage-dependent ion channels of the cardiac membrane. Recent electrophysiological data demonstrated that both TCAs and SSRIs decrease the maximum upstroke velocity (Vmax) of the cardiac action potential (AP) (Rawling 1979, Delpon 1990) that is supposed to be an indirect
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index of the fast inward sodium current ($I_{Na}$). We earlier reported concentration-dependent inhibition of sodium current caused by citalopram and amitriptyline. Amitriptyline seemed to be more effective inhibitor of sodium channel than citalopram (Hamplová 2000). There is also evidence of reduced myocardial contraction force after application of fluoxetine, amitriptyline or other TCAs (Marshall 1982, Meledin 1997, Pacher 2000). The high threshold calcium current ($I_{Ca}$) appears to be the main current contributing to the activation of myocardial contraction (Maylie 1995, Hofmann 1999) and changes of this current might cause undesirable impairment of contractile function.

In our experiments, we compared the effect of citalopram, the most selective SSRI, and amitriptyline, one of the most widely used TCA, on L-type $Ca^{2+}$ current ($I_{Ca}$) in rat cardiac cells in tissue culture. The present study was undertaken to gain further insight into the ionic mechanism underlying cardiotoxic properties of antidepressants and to evaluate whether there are any differences at the ionic level between the effects of citalopram and amitriptyline according to their different cardiovascular side effects.

Experiments were performed on rat cardiomyocytes in tissue culture. Cells were isolated from 3 to 4-day-old rat hearts by trypsinization and fibroblasts were removed by preplating according to Mysliveček (1998). Cells used for the measurements were cultured for 2-5 days in the culture medium. During the experiments, cells were bathed in an extracellular solution containing (in mM): NaCl 140, KCl 5.4, CaCl$_2$ 2.0, MgCl$_2$ 1.0, HEPES 10, glucose 10, pH=7.3. For isolation of L-type $I_{Ca}$ the extracellular solution was replaced by (in mM): trishydroxymethylaminomethane 140 and BaCl$_2$ 10 (BaTRIS), pH=7.3. Whole cell membrane currents were measured by the whole-cell patch-clamp method (AXOPATCH 200A, Axon Instruments). Electrodes were pulled from 1.6 mm borosilicate glass tubes. To minimize cell dialysis for calcium current measurements, which are dependent on channel phosphorylation, a nystatin perforated patch was used. Patch electrodes were filled with a solution containing (in mM): Cs methanesulfonate 100, CsCl 30, CaCl$_2$ 0.5, MgCl$_2$ 1.2, EGTA 5, HEPES 10, pH=7.3. Nystatin (500 µg/ml) was added just before the experiment and solution was kept during the experiment on ice in darkness for 2-3 hours. Stock solution of nystatin (50 µg/µl DMSO) was kept in refrigerator for one day. To improve the dispersion of nystatin in aqueous solution, 500 µg/ml of pluronic F127 (stock solution 25 mg/ml in DMSO) was added. After perforation with nystatin (10-15 min) the access resistance was 15-20 MΩ. The drug-containing solutions were applied using a microcomputer controlled fast superfusion system (Mayer 1989). A complete change of the solution around the cell varied between 30-60 ms. Drugs tested were applied for at least 30 s before cell stimulation. Stimulation waveforms were generated and signals were digitised by an interface LABMASTER TL1 DMA (Axon Instruments) with PCLAMP-6 program package. Stimulating pulses started from holding potential −80 mV. Peak of $Ca^{2+}$ currents evoked by depolarization pulses were plotted in dependence on the test potential. Citalopram (Seropram) was purchased from Lundbeck, Amitriptyline from Léčiva CZ. All other chemicals were either from BDH Chemicals or from Sigma. Values are given as means ± S.E.M. Measurements from 21 cells were used for evaluation.

Fig. 1. Effect of citalopram on L-type calcium channel current of rat cardiocytes. (A) To measure L-type $I_{Ca}$ depolarizing test pulses from −70 mV to +70 mV were used. The holding potential used in experiments was −80 mV. The depolarizing pulse was applied for 180 ms. (B) Typical examples of current-voltage relationships shown in this figure were obtained by plotting maximal values of L-type $I_{Ca}$ against amplitude of depolarising pulse voltage. The L-type $I_{Ca}$ appeared at a threshold voltage of −30 to −40 mV and peaked at 0 mV to +10 mV. Concentration of 10 µM of citalopram caused partial and 130 µM of citalopram caused more pronounced inhibition of L-type calcium channel current.
To investigate I$_{\text{Ca}}$ characteristics, removing Na$^+$ from the external solution and substituting K$^+$ by Cs$^+$ in the internal solution eliminated Na$^+$ and K$^+$ currents. Ca$^{2+}$-activated currents were suppressed by adding EGTA into the internal solution. To measure I$_{\text{Ca}}$, the external solution contained only BaTRIS. Under these conditions, mainly Ba$^{2+}$ carried the current, as it is well known that in the presence of Ba$^{2+}$ only current carried through Ca$^{2+}$ channels can be detected. Furthermore, inward rectifying potassium current is blocked in the presence of Ba$^{2+}$ (Reuter, 1984). Superfusing cells with ECS, I$_{\text{Na}}$ was detected at the beginning of the pulse and a small Ca$^{2+}$ current was activated during longer depolarisation. When BaTRIS was substituted for ECS, I$_{\text{Na}}$ vanished and I$_{\text{Ca}}$ significantly increased. Due to these observations, it was confirmed that this particular current was the current through L-type Ca$^{2+}$ channels.

Citalopram was used in concentrations of 1, 3, 10, 130 µM and 1.3 mM and was compared with amitriptyline. Citalopram in concentration of 1 µM inhibited 6±3 % of L-type I$_{\text{Ca}}$. Concentration of 3 µM of citalopram caused 2.7±0.3 % inhibition of L-type I$_{\text{Ca}}$ peak. Concentration of 10µM of citalopram induced 8±5 % inhibition of L-type I$_{\text{Ca}}$ (Fig. 1). Amitriptyline in concentration of 3µM and 10µM induced 2.7±0.2 % and 11.3±1.3 % inhibition of L-type calcium channel. Higher concentrations induced significantly higher suppression of L-type calcium channel current peak. However, these effecting concentrations (30, 100, 130, 300, 1300 µM) are much higher than plasma concentrations in patients treated with these drugs. IC$_{50}$ for citalopram was 60.3±8.5 µM and IC$_{50}$ for amitriptyline was 71±2.3 µM. Neither citalopram nor amitriptyline altered the threshold potential for I$_{\text{Ca}}$ activation and the maximum I$_{\text{Ca}}$ peak potential. After washing out both citalopram and amitriptyline the currents mostly fully recovered.

These results suggest that both citalopram and amitriptyline directly decrease the peak of Ca$^{2+}$ current in concentration-dependent manner and that they have similar potency for inhibiting L-type I$_{\text{Ca}}$ (Fig. 2).

The holding potential, at which I$_{\text{Ca}}$ is maximal, differs from other observations (Maylie 1995, Park 1999). In our study, the I$_{\text{Ca}}$ peaked at +10 mV and very scarcely at 0mV. It might be due to the different methods used in the experiments; Ca$^{2+}$-channel conductivity for Ba$^{2+}$ ions is higher than for Ca$^{2+}$ ions (Brown 1986). It might be explained by the lower affinity of Ba$^{2+}$ to the Ca$^{2+}$-binding sites of the calcium channel (Pučelík 1990). Summing up all findings (threshold activation at –30 to 0mV and peak at 0 mV and +10 mV), we consider the observed current as a current passing through L-type Ca$^{2+}$ channels (Maylie 1995, Park 1999).

![Fig. 2. Concentration dependent inhibition of L-type calcium channel current caused by citalopram and amitriptyline. This figure shows the percentage of L-type I$_{\text{Ca}}$ inhibition after the cells were superfused for 30 s by various concentrations of citalopram and amitriptyline. Data are expressed as percentage inhibition of maximum peak. Citalopram seems to exhibit very similar potency in inhibiting of L-type I$_{\text{Ca}}$ as amitriptyline. Group data are from at least 3 cells.](image)

Concentration of citalopram and amitriptyline used in our experiments to inhibit I$_{\text{Ca}}$ were higher than the therapeutical plasma concentrations in vivo (0.57-1.07 µM) (Park et al. 1999). However, it is difficult to relate in vivo plasma concentration to those of drug superfusing isolated cardiac cells. Furthermore, both drugs exert a high lipophilicity (Baumann and Larsen 1995) and they tend to accumulate in tissue. Previous studies demonstrated that TCAs in the heart can reach concentrations up to 20-200 times higher than in plasma (Jandhyala et al. 1977, Elonen et al. 1975). Furthermore, metabolites such as didesmethylcitalopram and their possible efficacy to affect cardiac function also cannot be ruled out (van der Burgh, 1994). Negligible effect of therapeutical doses of both drugs together with the fact of equal potency of both drugs to block I$_{\text{Ca}}$ leads to the conclusion that cardiotoxic effect of prolonged TCA therapy is probably mediated by another mechanism.

It is generally accepted that calcium influx is the main factor eliciting cardiac contractions. Thus, decreased I$_{\text{Ca}}$ might play the most important role in
reducing cardiac contractility. Furthermore, $I_{Ca}$ in nodal cells is responsible for the upstroke of the AP (Carmeliet 1988). It means that $I_{Ca}$ is the most important current for spontaneous depolarization of sinoatrial and atrioventricular cells and for atrioventricular conduction (Carmeliet 1988). Inhibition of $I_{Ca}$ might lead to impaired atrioventricular conduction and induce PR prolongation and AV block on the ECG.

On the other hand and in contrast to TCAs, clinical studies have shown that the use of SSRIs is safe even when administered to depressed individuals with serious cardiac disease (Glassman 1998, Roose et al. 1998). There are very promising but still little clinical data to establish the safety of SSRIs in the depressed patients with serious heart disease including post-myocardial infarction period (Glassman 1998, Roose et al. 1998, Shapiro et al. 1998). Further clinical and theoretical studies are necessary for evaluating the safety of citalopram and other SSRIs administration.

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