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ORIGINAL ARTICLE

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Effects of norfluoxetine on the action potential and transmembrane ion currents in canine ventricular cardiomyocytes

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Abstract Norfluoxetine is the most important active metabolite of the widely used antidepressant compound fluoxetine. Although the cellular electrophysiological actions of fluoxetine are well characterized in cardiac cells, little is known about the effects of its metabolite. In this study, therefore, the effects of norfluoxetine on action potential (AP) configuration and transmembrane ion currents were studied in isolated canine cardiomyocytes using the whole cell configuration of patch clamp techniques. Micromolar concentrations of norfluoxetine (1–10 μM) modified AP configuration: amplitude and duration of the AP and maximum velocity of depolarization were decreased in addition to depression of the plateau and elimination of the incisura of AP. Voltage clamp experiments revealed a concentration-dependent suppression of both L-type Ca^{2+} current, I_{Ca} (EC_{50}=1.13 ±0.08 μM) and transient outward K^+ current, I_{to} (EC_{50}=1.19±0.17 μM) having Hill coefficients close to unity. The midpoint potential of the steady-state inactivation of I_{Ca} was shifted from −20.9±0.75 mV to −27.7 ±1.35 mV by 3 μM norfluoxetine (P<0.05, n=7). No such shift in the steady-state inactivation curve was observed in the case of I_{to}. Similarly, norfluoxetine caused no change in the steady-state current–voltage relationship of the membrane or in the density of the inward rectifier K^+ current, I_{K1}. All these effects of norfluoxetine developed rapidly and were fully reversible. Comparing present results with those obtained previously with fluoxetine, it can be concluded that norfluoxetine displays stronger suppression of cardiac ion channels than fluoxetine. Consequently, the majority of the cardiac side effects observed during fluoxetine treatment are likely to be attributed to its metabolite norfluoxetine.

Keywords Fluoxetine · Norfluoxetine · Cardiac cells · Electrophysiology · Action potentials · Calcium current · Potassium current · Antidepressant drugs

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Introduction

Fluoxetine (Prozac) is a widely used antidepressant compound, its primary action is based on inhibition of serotonin reuptake in the central nervous system. Recent studies indicate, however, that fluoxetine has several additional effects on neuronal (Stauderman et al. 1992; Tytgat et al. 1997; Pancrazio et al. 1998), cardiac (Pacher et al. 2000; Magyar et al. 2003), smooth muscle (Farrugia 1996), and epithelial (Rae et al. 1995) cells. These effects appear to involve direct inhibition of the ion channels in the cell membrane. It is generally believed that a significant part of the therapeutic activity of fluoxetine is attributable to its most important active metabolite norfluoxetine (Fuller and Snoddy 1991), which is produced via demethylation of fluoxetine by cytochrome P450-2C9 enzyme (von Moltke et al. 1997; Ingelman-Sundberg 2004). Norfluoxetine was shown to inhibit neuronal K^+ channels (Choi et al. 1999, 2001), serotonin-mediated currents (Choi et al. 2003) and nicotinic acetylcholine receptors (López-Valdés and García-Colunga 2001), however, in contrast to the well-characterized cellular electrophysiological actions of fluoxetine, little is known about such effects of norfluoxetine in cardiac membranes. The goal of the present study was, therefore, to characterize the cellular electrophysiological actions of norfluoxetine in isolated canine ventricular cardiomyocytes, comparing these effects of norfluoxetine to our previous results obtained with fluoxetine. Since norfluoxetine caused much stronger suppression on cardiac ion channels than fluoxetine, it was concluded that the majority of the cardiac side effects observed during

fluoxetine treatment may likely be attributed to its metabolite norfluoxetine.

Materials and methods

Cell isolation Single canine ventricular cells were obtained from hearts of adult mongrel dogs using the segment perfusion technique as described previously (Magyar et al. 2000). Briefly, the animals (10–20 kg) were anesthetized with i.v. injection of 10 mg/kg ketamine hydrochloride (Calypsolvet) plus 1 mg/kg xylazine hydrochloride (Rometar). After opening the chest the heart was rapidly removed and the left anterior descending coronary artery was perfused using a Langendorff apparatus. Ca^{2+}-free JMM solution (Minimum Essential Medium Eagle, Joklik modification; Sigma Chemicals, product no. —0518, St Louis, MO, USA), supplemented with taurine (2.5 g/l), pyruvic acid (175 mg/l), ribose (750 mg/l), allopurinol (13.5 mg/l) and NaH_2PO_4 (200 mg/l), was used during the initial 5 min of perfusion to remove Ca^{2+} and blood from the tissue. After addition of $NaHCO_3$ (1.3 g/l), the pH of this perfusate was 7.0 when gassed with carbogen (95% O_2 + 5% CO_2). Cell dispersion was performed for 30 min in the same solution containing also collagenase (660 mg/l, Worthington Cls-1), bovine albumin (2 g/l) and $CaCl_2$ (50 μM). During the isolation procedure the solutions were gassed with carbogen and the temperature was maintained at 37°C. The cells were rod shaped and showed clear striation when the external Ca^{2+} was restored. Before use, the cells were stored overnight at 14°C in modified JMM solution (pH 7.4).

Action potential recording To record action potentials from the myocytes, the viable cells were sedimented in a plexiglass chamber allowing for continuous superfusion with modified Krebs solution (containing in mM: NaCl 120, KCl 5.4, $CaCl_2$ 2.7, $MgCl_2$ 1.1, NaH_2PO_4 1.1, $NaHCO_3$, glucose 6) having pH adjusted to 7.4±0.05 when gassed with carbogen. Transmembrane potentials were recorded at 37°C using glass microelectrodes filled with 3 M KCl and having tip resistance between 20 MΩ and 40 MΩ. These electrodes were connected to the input of an Axoclamp-2B amplifier (Axon Instruments, Union City, CA, USA). The cells were continuously paced through the recording electrode at steady cycle length of 1,000 ms using 1 ms wide rectangular current pulses with 120% threshold amplitude. Outputs from the clamp amplifier were digitized at 100 kHz using a Digidata 1200 A/D card (Axon Instruments) and stored for later analysis, which was performed under the control of pClamp 6.0 software (Axon Instruments).

Voltage clamp Transmembrane ion currents were recorded in oxygenated Tyrode solution (containing in mM: NaCl 140, KCl 5.4, $CaCl_2$ 2.5, $MgCl_2$ 1.2, Na_2HPO_4 0.35, HEPES 5, glucose 10, pH 7.4) at 37°C. Suction pipettes, fabricated from borosilicate glass, had tip resistance of 2 MΩ after filling with pipette solution (composed of in mM: KCl 110, KOH 40, HEPES 5, EGTA 10, TEACl 20, K-ATP 3 and GTP 0.25 mM, or alternatively, K-aspartate 100, KCl 45, $MgCl_2$ 1, EGTA 10, HEPES 5, K-ATP 3, when measuring Ca^{2+} or K^+ currents, respectively). The pH of these pipette solutions was adjusted to 7.2 with KOH. I_{Ca} was blocked by 5 μM nifedipine, and 3 mM 4-aminopyridine was used to suppress I_{to} (both drugs were applied externally). Currents were recorded with an Axopatch-200B amplifier (Axon Instruments) using the whole cell configuration of the patch clamp technique (Hamill et al. 1981). After establishing high (1–10 GΩ) resistance seal by gentle suction, the cell membrane beneath the tip of the electrode was disrupted by further suction or by applying 1.5 V electrical pulses for 1–5 ms. After this step, the intracellular solution was allowed to equilibrate with the pipette solution for a period of 5–10 min before starting the measurement. Ionic currents were normalized to cell capacitance, determined in each cell using short (25 ms) hyperpolarizing pulses from 0 mV to −10 mV. The series resistance was typically 4–8 MΩ before compensation (usually 50–80%). Experiments were discarded when the series resistance was high or substantially increasing during the measurement. The applied experimental protocols are described in the Results section where appropriate.

Drug application Norfluoxetine (Sigma) was dissolved in distilled water and was added to the bath in a cumulative manner applying each concentration for 2 min. This period of time was sufficient to achieve steady-state effects in both action potential and ion current measurements.

Statistics All values presented are arithmetic means ± SEM. Statistical significance was determined using Student's *t*-test. Differences were considered significant when the *P* value was less than 0.05.

The entire investigation conforms the *Guide for the Care and Use of Laboratory Animals* published by the US National Institutes of Health (NIH publication no. 85-23, revised 1996) and the principles outlined in the Declaration of Helsinki.

Results

Effect of norfluoxetine on action potential configuration

Cumulative concentration-dependent effects of norfluoxetine on the configuration and parameters of the action potential are shown in Fig. 1 and Table 1. Norfluoxetine (1–10 μM) markedly accelerated repolarization, i.e., shortened the duration of action potentials measured at both 50% and 90% levels of repolarization. The drug also attenuated early (phase-1) repolarization, and depressed the level of the late plateau (Fig. 1A, B). In addition, norfluoxetine decreased action potential amplitude and the maximum rate of depolarization (V_{max}) in a concentration-dependent manner, having a 2.42±0.36 μM EC_{50} value for

the V_{max}-block (Fig. 1C). The effects of the same fluoxetine concentrations on V_{max} are also depicted in this figure for comparison (data taken from Pacher et al. 2000). As shown in Fig. 1C, the V_{max}-depressant effect of norfluoxetine was much stronger than that of fluoxetine. These effects of norfluoxetine on action potential characteristics developed rapidly and were fully reversible. Norfluoxetine had no effect on the resting potential at the concentrations studied.

Effect of norfluoxetine on the calcium current

Peak I_{Ca} was measured at a rate of 0.2 Hz using depolarizing voltage pulses of 400 ms duration clamped from the holding potential of −40 mV to the test potential of +5 mV. K^+ currents were blocked by the externally applied 4-aminopyridine and internally applied TEACl. Stability of I_{Ca} was monitored at least for 5 min before cumulative application of norfluoxetine (from 0.1 μM to 10 μM, each concentration for 2 min). Norfluoxetine caused marked suppression of peak I_{Ca} without changing the time course of inactivation (Fig. 2A). The decay of I_{Ca} was fitted as a sum of two exponential components at +5 mV, having time constants of 14.2±1 ms and 83±8 ms in control, and 13.7±0.9 ms and 77±9 ms, respectively, in the presence of 3 μM norfluoxetine (not significant [NS], n=7). The effect of norfluoxetine on I_{Ca} developed rapidly (within 2 min) and was fully reversible upon washout (Fig. 2B). The norfluoxetine-induced block of I_{Ca} was concentration-dependent (Fig. 2C). Inhibition of the current was statistically significant from the concentration of 0.3 μM (inhibition of 20.3±2.5%, P<0.05, n=7) and above. Fitting results to the Hill equation yielded an EC_{50} of 1.13±0.08 μM and a Hill coefficient of 1.19±0.12. For comparison, concentration-dependent effects of 1–100 μM fluoxetine on I_{Ca} is also included in this panel (data taken from Pacher et al. 2000). The 5.4±0.94 μM EC_{50} value obtained for fluoxetine indicates that cardiac I_{Ca} is more sensitive to norfluoxetine than to fluoxetine.

Current–voltage relations for I_{Ca} were obtained by applying a series of test pulses increasing up to +40 mV in 5-mV steps in the absence and presence of norfluoxetine, and peak values of I_{Ca} were plotted against their respective test potentials. No shift in the current–voltage relationship was observed in the seven myocytes after application of 3 μM norfluoxetine (Fig. 2D). Ca^{2+} conductance (G_{Ca}) was calculated at each membrane potential by dividing the peak current by its driving force (the difference between the applied test potential and the reversal potential for I_{Ca}, estimated to be +55 mV). Ca^{2+} conductance was significantly reduced by 3 μM norfluoxetine at each membrane potential studied, however, when G_{Ca} values were normalized to the respective G_{Ca} obtained at +30 mV, the G_{Ca}–V_m relationships were fully identical (Fig. 2E). These results indicate that voltage dependence of activation of I_{Ca} is not affected by 3 μM norfluoxetine.

In contrast to the unchanged voltage dependence of activation, the voltage dependence of inactivation was altered by norfluoxetine in a reversible manner. In order to study the voltage dependence of steady-state inactivation of I_{Ca}, test depolarizations to +5 mV were preceded by a set of prepulses clamped to various voltages between −55 mV and +5 mV for 500 ms. Peak currents measured after these prepulses were normalized to the peak current measured after the −55 mV prepulse and plotted against the respective prepulse potential. The data were fitted to the two-state Boltzmann function (Fig. 2F). Superfusion of the cells with 3 μM norfluoxetine shifted the midpoint potential by almost 7 mV to the left (from the control value of −20.9±0.8 mV to −27.7±1.4 mV, P<0.05, n=7), however, no significant difference was observed between the respective slope factors (3.9±0.3 mV and 4.4±0.3 mV, NS, n=7).

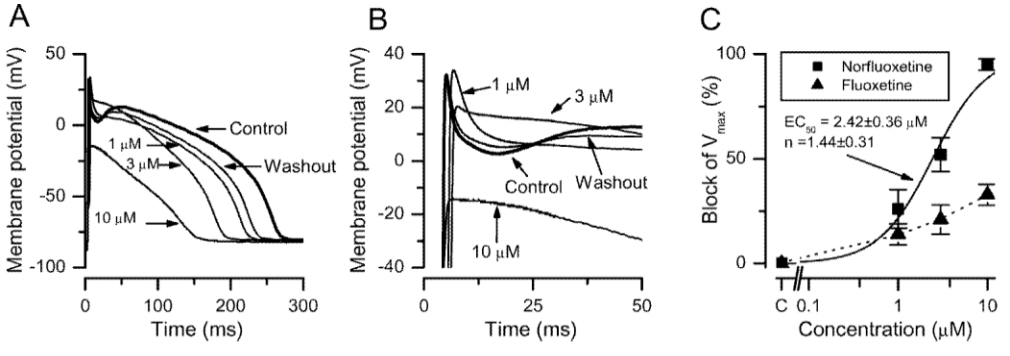

Fig. 1A–C Concentration-dependent effect of norfluoxetine on action potential configuration in single canine ventricular myocytes. **A** Superimposed action potentials recorded at 1 Hz before and after superfusion with 1, 3, and 10 μM norfluoxetine, each for 2 min. Finally, the cell was washed in drug-free solution for a further 3 min. **B** The same action potentials are shown on an extended scale to demonstrate the drug-induced changes in the notch. **C** Suppressive effect of norfluoxetine on the maximum velocity of depolarization, V_{max} (n=12). Data were fitted to the Hill equation (*solid line*). *Symbols* and *bars* represent mean ± SEM. Our earlier results obtained with similar concentrations of fluoxetine in five myocytes (*dotted line*) are also included for comparison (data taken from Pacher et al. 2000)

Table 1 Cumulative concentration-dependent effects of norfluoxetine on action potential parameters in canine ventricular myocytes (n=12). *RP* resting membrane potential, *APA* action potential amplitude, V_{max} maximal velocity of depolarization, APD_{50} and APD_{90} action potential duration measured at 50% or 90% level of repolarization respectively

	RP (mV)	APA (mV)	V_{max} (V/s)	APD_{50} (ms)	APD_{90} (ms)
Control	−81.8±1.0	112.5±1.5	201±26	149±12	221±12
1 μM	−82.3±0.7	111.8±1.9	140±18*	119±11***	196±13**
3 μM	−82.2±0.9	102.9±2.3***	116±17**	90±7***	157±8***
10 μM	−82.2±1.3	69.4±4.2***	12±4***	76±4***	124±5***
Washout	−82.4±1.9	110.6±1.8	189±26	146±12	225±14

Mean ± SEM values are given
Asterisks denote significant changes from control (*P<0.05, **P<0.01, ***P<0.001) determined using Student's t-test for paired data

Effect of norfluoxetine on the transient outward current

The transient outward current, I_{to}, was studied at +50 mV using voltage pulses of 200 ms duration arising from the holding potential of −80 mV. Each of these test pulses was preceded by a 5 ms long prepulse to −40 mV in order to inactivate the Na current.

Similarly to I_{Ca}, I_{to} was also depressed by norfluoxetine without visible changes in current kinetics (Fig. 3A). The decaying branch of I_{to} was fitted as a sum of two exponentials, yielding time constants of 1.3±0.1 ms and 6.4±0.4 ms in control, and 1±0.8 ms and 6.6±1.2 ms, respectively, in the presence of 3 μM norfluoxetine (NS, n=5), indicating that time course of inactivation was unaltered in the presence of norfluoxetine. The suppressive effect of norfluoxetine on I_{to} completely developed within 2 min and was fully reversible (Fig. 3B). I_{to} was decreased by norfluoxetine in a concentration-dependent manner (Fig. 3C). This effect developed at relatively low

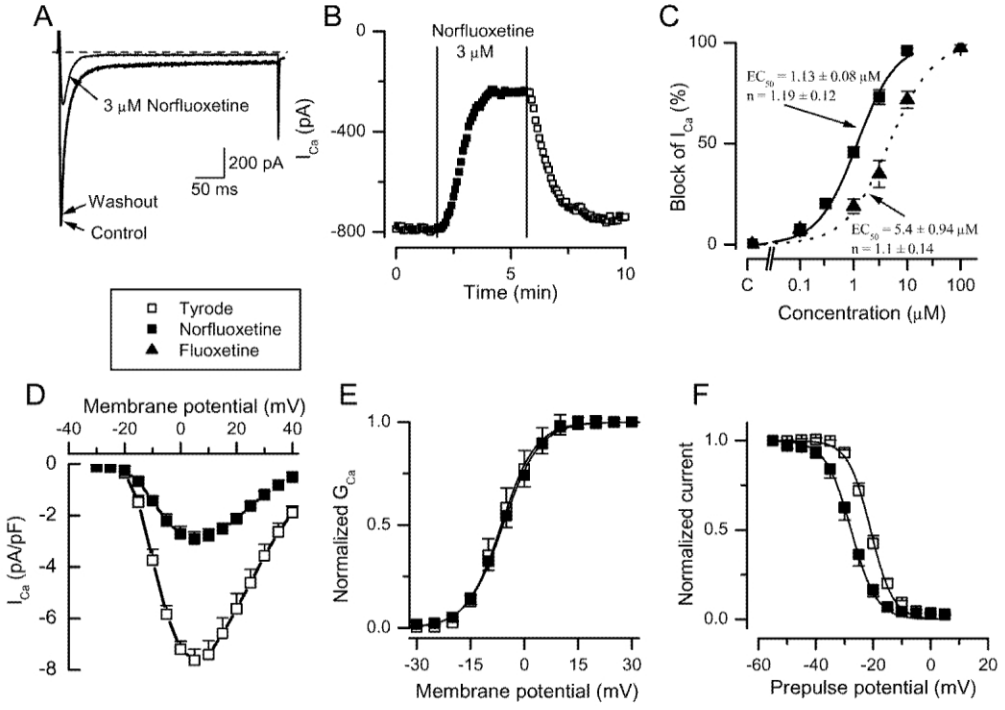

Fig. 2A–F Effect of norfluoxetine on the calcium current studied in seven myocytes. **A** Superimposed I_{Ca} records, obtained before, during, and after 2 min superfusion with 3 μM norfluoxetine. The *dashed line* denotes zero current. **B** Representative experiment showing the time scale of development and reversion of the norfluoxetine-induced changes in I_{Ca}. **C** Cumulative concentration-dependent effect of norfluoxetine on peak I_{Ca} measured at +5 mV. *Solid line* was obtained by fitting data to the Hill equation. Our earlier results obtained with 1–100 μM fluoxetine in six myocytes (*dotted line*) are also included for comparison (data taken from Pacher et al. 2000). **D** Current–voltage relationship of peak I_{Ca} in the absence and presence of 3 μM norfluoxetine. **E** Voltage-dependent activation of Ca^{2+} conductance (G_{Ca}) in control and after application of 3 μM norfluoxetine as calculated from the previously shown current–voltage curves. At each membrane potential G_{Ca} was normalized to that obtained at +30 mV, and the results were fitted to a two-state Boltzmann model (*solid curves*). **F** Voltage dependence of steady-state inactivation of I_{Ca} determined using paired-pulse protocol in the presence and absence of 3 μM norfluoxetine. *Solid curves* were obtained by fitting data to a two-state Boltzmann model. Estimated midpoint potentials and slope factors are given in the text. *Symbols* and *bars* are mean ± SEM values

concentrations (suppression of 28.5±3.9% was observed in the presence of 0.3 μM norfluoxetine, $P<0.05$, $n=5$). The Hill equation, used to describe the concentration-dependency of the norfluoxetine effect, yielded an EC_{50} of 1.19 ±0.17 μM, a value very close to that obtained for I_{Ca} (1.13 ±0.08 μM). The Hill coefficient, again, was close to unity (0.82±0.06). For comparison, 3 μM fluoxetine had no significant effect on I_{to} (Pacher et al. 2000).

Current–voltage relations for I_{to} were obtained in the absence and presence of norfluoxetine by applying a series of test pulses increasing from −40 mV to +65 mV in 5 mV-steps, and peak values of I_{to} were plotted as a function of the test potential (Fig. 3D). When the conductance of the I_{to} channels (G_{to}) was calculated in a way similar to that applied for I_{Ca} the voltage dependence of activation of I_{to} was generated (Fig. 3E). Although the norfluoxetine-induced block of I_{to} showed little voltage dependence, the drug caused a small, but statistically significant leftward shift on the steady-state activation curve. The midpoint potentials, obtained by fitting the data to a two-state Boltzmann function, were 10.9±0.7 mV and 2.9±3.5 mV in the absence and presence of 3 μM norfluoxetine, respectively ($P<0.05$, $n=6$), while the estimated slope factors were practically identical (14.8±1.8 mV and 12.7 ±0.7 mV, NS, $n=6$). Indicating the reversible nature of the norfluoxetine effect on activation, the midpoint potential obtained after washing out norfluoxetine was 8.7±3.5 mV, statistically not different from the control value (not shown).

In contrast to results obtained for I_{Ca}, norfluoxetine failed to alter the voltage dependence of inactivation of I_{to} (Fig. 3F). The estimated midpoint potentials (−35.7 ±0.7 mV vs. −36.1±1.9 mV) and slope factors (3.3 ±0.4 mV vs. 4±0.6 mV) determined in the absence and presence of 3 μM norfluoxetine, respectively, were not different statistically (NS, $n=6$). In these experiments the 400 ms prepulses were varied from −60 mV to +10 mV in 10-mV steps, while the test potential was set to +50 mV.

Effect of norfluoxetine on the inward rectifier K^+ current

The steady-state current–voltage relationship of the membrane of canine ventricular cells was determined using 400 ms long voltage commands clamped to potentials ranging from −135 mV to +45 mV increasing in 10-mV steps. Currents measured at the end of these pulses were plotted against the respective test potentials. As shown in Fig. 4, norfluoxetine (3 μM) failed to modify the steady-state current–voltage relationship in canine cardiomyocytes. The negative branch of the I–V curve depends on the amplitude of I_{K1}. The current densities measured at −125 mV were −35.9±2.3 pA/pF and −32.7±2.7 pA/pF, respectively, before and after application of 3 μM norfluoxetine (NS, $n=5$) indicating that norfluoxetine—at least at this concentration—had no suppressive effect on I_{K1}.

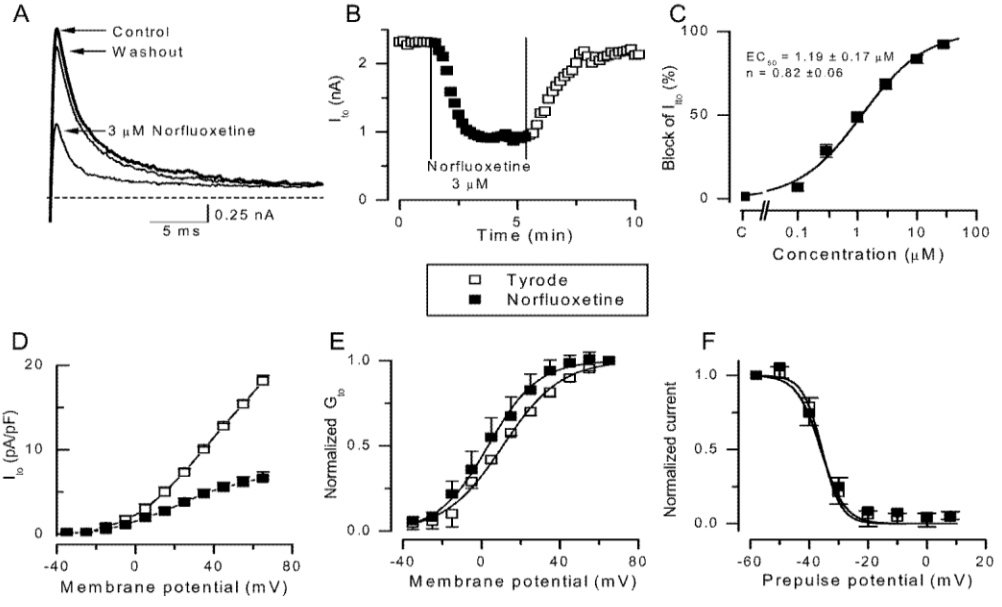

Fig. 3A–F Effect of norfluoxetine on the transient outward current. **A** Superimposed I_{to} records, obtained before, during, and after 2 min superfusion with 3 μM norfluoxetine. **B** Representative experiment showing the time scale of development and reversion of the norfluoxetine-induced changes in the current. **C** Cumulative concentration-dependent effects of norfluoxetine on peak I_{to} measured at +50 mV. The *solid line* was obtained by fitting data to the Hill equation ($n=5$). **D** Current–voltage relationship obtained in six cells for I_{to} in the absence and presence of 3 μM norfluoxetine. **E** Voltage-dependent activation of the I_{to} channels, defined as conductance (G_{to}), calculated from the current–voltage curves in control and in the presence of 3 μM norfluoxetine. At each membrane potential G_{to} was normalized to its value obtained at +65 mV, and the results were fitted to a two-state Boltzmann model (*solid curves*). Estimated midpoint potentials and slope factors are given in the text. **F** Voltage dependence of steady-state inactivation of I_{to} determined using paired-pulse protocol in the presence and absence of 3 μM norfluoxetine ($n=6$). *Solid curves* were obtained by fitting data to a two-state Boltzmann model. *Symbols* and *bars* represent mean ± SEM values

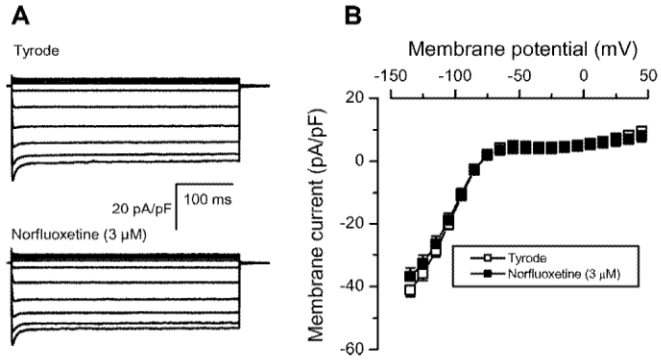

Fig. 4A, B Effect of norfluoxetine on the inward rectifier K^+ current. **A** Current families, obtained in Tyrode solution and in the present of 3 μM norfluoxetine, were elicited with test pulses of 400 ms duration clamped to voltages ranging from −135 mV to −5 mV and increasing in 10-mV steps. **B** Steady-state current–voltage relations obtained in five cells in the absence and presence of 3 μM norfluoxetine. In these experiments the range of test pulses was extended to +45 mV. The current measured at the end of each test pulse was plotted as a function of the respective test potential. *Symbols* and *bars* denote mean ± SEM

Discussion

Effects of norfluoxetine on cardiac ion currents

Micromolar concentrations of norfluoxetine evoked multiple effects on action potential configuration in canine ventricular cells: the drug decreased the maximum rate of rise and amplitude of the action potential, attenuated early repolarization, depressed the plateau, and accelerated terminal repolarization, while the resting membrane potential was unaltered. These actions can fully be explained by the norfluoxetine-induced changes in ion currents, since suppression of I_{Ca} (leading to plateau depression and action potential shortening) and I_{to} (resulting in reduction of early repolarization) was demonstrated, while I_{K1} (responsible for the highly negative resting potential) was not affected by norfluoxetine. Although I_{Na} was not measured directly in this study, reduction of V_{max} is generally accepted as a measure of Na^+ channel blockade (Hondeghem 1978).

Interestingly, the EC_{50} values estimated for I_{Ca} and I_{to} were practically equal (1.1 μM and 1.2 μM, respectively), furthermore, EC_{50} of the V_{max}-block (2.4 μM) was also close to these values. Taking into account that the Hill coefficients obtained for I_{Ca}, I_{to}, and the V_{max}-block (1.2, 0.8, and 1.4, respectively) were all close to unity, it seems possible that norfluoxetine binds to a single binding site which may be a common structure of many 6-TM channels, but is absent from the members of the Kir superfamily, mediating the I_{K1} current. The minor differences observed between effects of norfluoxetine on the kinetic properties of I_{Ca} and I_{to} (negative shifts in voltage dependence of inactivation and activation, respectively) may probably be related to different amino acid environments of the binding site. Similar differences can be seen when comparing the channel-blocking effect of norfluoxetine in various preparations. For instance, the suppressive effect of the drug was clearly voltage dependent on the cloned neuronal potassium channel Kv 3.1 (Choi et al. 2001), whereas little voltage dependence was observed in our cardiac I_{to} current.

Comparison with effects of fluoxetine

In our previous work, performed with fluoxetine in various mammalian cardiac tissues including canine myocytes, fluoxetine was shown to evoke changes in action potential morphology similar to those described in the present study with norfluoxetine (Pacher et al. 2000). The fluoxetine-induced depression of plateau and shortening of action potentials in canine ventricular myocytes were attributed to inhibition of I_{Ca}. Present results indicate that I_{Ca} is more sensitive to norfluoxetine than fluoxetine, since the 1.1 μM EC_{50} value obtained for norfluoxetine was five times lower than the EC_{50} of 5.4 μM found with fluoxetine by Pacher et al. (2000). Similar conclusion can be drawn regarding the suppression of I_{Na}, although the EC_{50} value for the fluoxetine-induced V_{max}-block was not determined. However, according to Fig. 1C, the EC_{50} must be well above 10 μM in the case of fluoxetine, a value being again at least five times higher than the respective EC_{50} obtained with norfluoxetine. Surprisingly, I_{to} was not inhibited by 10 μM fluoxetine (Pacher et al. 2000), in contrast with the present results obtained with norfluoxetine (EC_{50}=1.2 μM). The exact reason for this difference remains to be elucidated, however, it can be speculated that I_{to} channel protein can distinguish between the two structures. The most important conclusion of this study—based on the comparison above—is that not only fluoxetine, but also its active metabolite norfluoxetine, may exhibit cardiovascular depressant effects in clinically relevant concentrations. Since the inhibitory effects of norfluoxetine on I_{Ca}, I_{Na} and I_{to} are much stronger than those of fluoxetine, the majority of cardiac side effects, being attributed previously to fluoxetine, may likely be ascribed to the presence of norfluoxetine. More detailed evaluation in a functional assay using isolated hearts might be helpful to give further support in this respect.

Clinical implications

The norfluoxetine-induced shortening of action potential duration is potentially proarrhythmic due to reduction of the effective refractory period, and the concomitant facilitation of development of re-entry type arrhythmias. In addition, inhibition of I_{Ca} may lengthen atrioventricular conduction, resulting in atrioventricular block, while depression of V_{max} may result in compromised intraventricular conduction due to inhibition of I_{Na}. At the same time, these actions of norfluoxetine (i.e., suppression of I_{Na} and I_{Ca}) may also be considered antiarrhythmic (class I + IV type, respectively). Furthermore, the reduction of the Ca^{2+} window current, due to the leftward shift of the

steady-state inactivation curve (shown in Fig. 2F) may decrease the incidence of early after depolarizations.

Clinically, serotonin-reuptake inhibitors, including fluoxetine and its metabolite norfluoxetine, are believed to cause less cardiovascular side effects than tricyclic antidepressants. However, there is an increasing number of case reports on dysrhythmias, like atrial fibrillation or bradycardia (Buff et al. 1991; Friedman 1991; Masquelier et al. 1993; Drake and Gordon 1994; Hussein and Kaufman 1994; Roberge and Martin 1994; Graudins et al. 1997; Anderson and Compton 1997) and syncope (Ellison et al. 1990; McAnally et al. 1992; Cherin et al. 1997; Livshits and Danenberg 1997; Rich et al. 1998) associated with fluoxetine treatment and overdose. The upper range of therapeutic plasma concentrations of fluoxetine was reported to vary between 0.15 μM and 1.5 μM in humans. In addition, similar concentrations of its active metabolite, norfluoxetine is also present in the plasma of fluoxetine-treated patients (Orsulak et al. 1988; Kelly et al. 1989; Keck and McElroy 1992; Januzzi et al. 2002). Under extreme conditions (e.g., decreased metabolism in the elderly, acute overdose or drug interactions), these plasma concentrations of fluoxetine and norfluoxetine can reach higher levels (Pato et al. 1991; Borys et al. 1992; Hale 1993; Eap et al. 2001). Furthermore, recent data indicate that fluoxetine (and probably norfluoxetine as well) can be accumulated in the tissues: 20 times accumulation of fluoxetine has been detected in human brain during chronic fluoxetine treatment (Karson et al. 1993; Komorski et al. 1994). Considering the micromolar EC_{50} values obtained with norfluoxetine for I_{Ca} and the V_{max}, depressed atrioventricular and intraventricular conduction can well be anticipated in patients treated with fluoxetine. It must be emphasized, however, that the norfluoxetine-induced electrophysiological alterations are not necessarily always harmful. They are, of course, in patients having deficient impulse conduction, but they may be beneficial in cases with long QT syndrome. Therefore, in depressed patients having also cardiac disorders, ECG control is strongly recommended during the fluoxetine therapy.

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