Differential effects of fluoxetine enantiomers in mammalian neural and cardiac tissues

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Abstract. Racemic fluoxetine is a widely used SSRI antidepressant compound having also anticonvulsant effect. In addition, it was shown that it blocked several types of voltage gated ion channels including neural and cardiac calcium channels. In the present study the effects of enantiomers of fluoxetine (R(-)-fluoxetine and S(+)-fluoxetine) were compared on neuronal and cardiac voltage-gated Ca$^{2+}$ channels using the whole cell configuration of patch clamp techniques, and the anticonvulsant action of these enantiomers was also evaluated in a mouse epilepsy model. In isolated pyramidal neurons of the dorsal cochlear nucleus of the rat the effect of fluoxetine (S(+), R(-) and racemic) was studied on the Ca$^{2+}$ channels by measuring peak Ba$^{2+}$ current during ramp depolarizations. All forms of fluoxetine reduced the Ba$^{2+}$ current of the pyramidal cells in a concentration-dependent manner, with a K$_d$ value of 22.3±3.6 µM for racemic fluoxetine. This value of K$_d$ was higher by one order of magnitude than found in cardiac myocytes with fluoxetine enantiomers (2.4±0.1 and 2.8±0.2 µM). Difference between the effects of the two enantiomers on neuronal Ca$^{2+}$ current was observed only at 5 µM concentration: R(-)-fluoxetine inhibited 28±3% of the peak current, while S(+)-fluoxetine reduced the current by 18±2% (n=13, P<0.05). In voltage clamped canine ventricular cardiomyocytes both enantiomers of fluoxetine caused a reversible concentration-dependent block of the peak Ca$^{2+}$ current measured at 0 mV. Significant differences between the two enantiomers in this blocking effect was observed at low concentrations only: S(+)-fluoxetine caused a higher degree of block than R(-)-fluoxetine (56.3±2.2% versus 49.1±2.2% and 95.5±0.9% versus 84.5±3.1% block with 3 and 10 µM S(+) and R(-)-fluoxetine, respectively, P<0.05, n=5). Studied in current clamp mode, micromolar concentrations of fluoxetine shortened action potential duration of isolated ventricular cells, while higher concentrations also suppressed maximum velocity of depolarization and action potential amplitude. This shortening effect was significantly greater in the case of S(+) than R(-)-fluoxetine at 1 and 3 µM concentrations, whereas no differences in their effects on depolarization were observed. In pentylenetetrazole-induced mouse epilepsy model fluoxetine pretreatment significantly increased the 60 min survival rate, survival duration and seizure latency. These effects were more pronounced with the R(-) than the S(+) enantiomer. The results indicate that fluoxetine exerts much stronger suppressive effect on cardiac than neuronal calcium channels. At micromolar concentrations (between 1 and 10 µM) R(-)-fluoxetine is more effective than the S(+) enantiomer on neuronal, while less effective on cardiac calcium channels. The stronger anticonvulsant effect of the R(-) enantiomer may, at least partially, be explained by these differences. Used as an antidepressant or anticonvulsant drug, less severe cardiac side-effects are anticipated with the R(-) enantiomer.

Introduction

Fluoxetine is a widely used antidepressant compound, its action is primarily attributed to inhibition of the reuptake of serotonin (SSRI) in the central nervous system. Recent studies indicated, however, that fluoxetine had several additional effects, many of them involving inhibition of various types of ion channels, such as muscular and neuronal nicotinic receptors (1,2), volume-regulated anion channels (3), delayed rectifier K$^+$ channels in smooth muscle cells (4), voltage-gated Na$^+$ and K$^+$ channels in neurons (5,6) and epithelial cells (7), voltage-gated Ca$^{2+}$ channels in cardiac myocytes (8), nerve terminals (9) and hippocampal pyramidal cells (10).
Several reports indicated that some conventional antiepileptic drugs were found to inhibit Ca\(^{2+}\) channels (11-14), and vice versa, dihydropyridine type Ca\(^{2+}\) channel blockers were claimed to suppress epileptiform activity (15-18). Therefore, it is not surprising that fluoxetine was found to enhance the anticonvulsant potency of traditionally used antiepileptic drugs (19,20), and moreover, the drug exerted frank anticonvulsant action in animal as well as human studies (21-25).

Beyond the antidepressant and anticonvulsant actions of fluoxetine discussed above, cardiovascular side-effects, like dysrhythmias, atrial fibrillation, bradycardia (26-33) and syncope (34-38) were reported in association with fluoxetine treatment and overdose in man. These cases appear to be due to the inhibitory action of fluoxetine on cardiac Ca\(^{2+}\) and Na\(^{+}\) channels (8).

In all of the cited studies and case reports racemic fluoxetine was applied. However, pharmacological properties of enantiomers of fluoxetine were recently published (39-41). The two enantiomers of fluoxetine were found to be nearly equipotent inhibitors of serotonin reuptake but S(+)-fluoxetine was more slowly eliminated than the R(-) enantiomer (39,40). Based on their different pharmacokinetic properties and an absence of central stimulant effect of S(+)-fluoxetine, the S(+)- enantiomer is currently developed as an antimigraine drug, while R(-)-fluoxetine was suggested for treatment of syncope (34-38) were reported in association with fluoxetine treatment and overdose in man. These cases appear to be due to the inhibitory action of fluoxetine on cardiac Ca\(^{2+}\) and Na\(^{+}\) channels (8).

Materials and methods

Ion current measurements in cochlear neurons isolated from rat brain. The neuron isolation procedure was similar to that described earlier (42). Briefly, after the decapitation of the 5 to 11-day-old rat (n=15) the brain was removed into ice-cold artificial cerebrospinal fluid (aCSF), where Na\(^{+}\) was replaced by equimolar sucrose (‘low sodium’ aCSF). The normal aCSF contained: 125 mM NaCl; 2.5 mM KCl; 10 mM glucose; 1.25 mM NaH\(_2\)PO\(_4\); 26 mM NaHCO\(_3\); 2 mM CaCl\(_2\); 1 mM MgCl\(_2\); 3 mM myo-inositol; 0.5 mM ascorbic acid; 2 mM Na-pyruvate. Osmolarity of the aCSF solution was 335 mOsm/l, and the pH was set to 7.2 by NaOH. The dorsal cochlear nuclei were then removed and put into an incubation chamber, the bath solution was changed to HEPES buffered aCSF containing 1 mg/ml trypsin inhibitor (type I-S, Sigma). The pH of this solution was adjusted to 7.3 by application of HCl. After the cells settled down and adhered to the bottom of the experimental chamber, the bath solution was changed to HEPES buffered aCSF supplemented with the following channel blockers to minimize the interfering currents: 1 µM tetrodotoxin; 1 mM CsCl; 2 mM 4-aminopyridine and 5 mM TEA. In addition, the Ca\(^{2+}\) content of this solution was replaced by 5 mM Ba\(^{2+}\) in order to increase the amplitude of the ionic current flowing through the high-voltage activated Ca\(^{2+}\) channels. Thus, the charge carrier was Ba\(^{2+}\) when measuring currents through Ca\(^{2+}\) channels. When the effect of fluoxetine was investigated, fluoxetine was dissolved in the Ba\(^{2+}\) based extracellular solution described above, and this solution was applied with a gravity-driven perfusion system, similarly to the application of the control extracellular solution. The currents were recorded in the whole cell configuration of the patch clamp technique by using an Axopatch 200A amplifier connected to a TL-1 interface (Axon Instruments, CA). Digitization rate was 5 kHz and the current signals were filtered at 2 kHz with a 4-pole Bessel filter. The capacitive transients were electronically compensated, while leak correction was performed by measuring the leak current evoked by small voltage steps from a holding potential of -75 mV, and subtracting the extrapolated leak current from the total current. Series resistance varied between 2.5 and 17 MΩ, and it was compensated by at least 40%. No correction was made for the junction potential typically of 2-3 mV.

Electrophysiological measurements in isolated canine ventricular myocytes. Single canine ventricular myocytes were obtained from hearts of adult mongrel dogs using the segment perfusion technique as described earlier (43). 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, product no. M-0518), supplemented with taurine (2.5 g/l), pyruvic acid (175 mg/l), ribose (750 mg/l), allopurinol (13.5 mg/l) and NaHPO\(_4\) (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. 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 calcium was restored. Before use, the cells were stored overnight at 14°C in modified JMM solution (pH 7.4).
Action potentials were recorded from Ca
2+ -tolerant canine ventricular cells superfused with modified Krebs solution containing: 120 mM NaCl, 5.4 mM KCl, 2.7 mM CaCl
2, 1.1 mM MgCl
2, 1.1 mM NaH
2PO
4, 24 mM NaHCO
3 and 6 mM glucose. The solution was equilibrated with carbogen at a temperature of 37°C and the pH was adjusted to 7.4. Transmembrane potentials were recorded using glass microelectrodes filled with 3 M KCl and having tip resistance between 20 and 40 MΩ. These electrodes were connected to the input of an Axoclamp-2B amplifier (Axon Instruments). The cells were continuously paced through the recording electrode at a steady cycle length of 1000 ms using 1 ms wide rectangular current pulses with 120% threshold amplitude. Action potentials were digitized at 100 kHz using Digidata 1200 A/D card (Axon Instruments) and stored for later analysis.

Ca
2+ current was recorded from cells superfused with oxygenated Tyrode solution containing 140 mM NaCl, 5.4 mM KCl, 2.5 mM CaCl
2, 1.2 mM MgCl
2, 0.35 mM Na
2HPO
4, 5 mM HEPES, 10 mM glucose and 3 mM 4-aminopyridine, at pH 7.4. Suction pipettes, fabricated from borosilicate glass, had tip resistance of 2 MΩ after filling with pipette solution composed of 110 mM KCl, 40 mM KOH, 10 mM HEPES, 10 mM EGTA, 20 mM TEACl, 3 mM K-ATP and 0.25 mM GTP. The pH of this solution was adjusted to 7.2 with KOH. Membrane currents were recorded with an Axopatch-1D amplifier (Axon Instruments) using the whole cell configuration of the patch clamp technique. 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. 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. Outputs from the clamp amplifier were digitized at 20 kHz using an A/D converter (Digidata 1200, Axon Instruments) under software control (pClamp 6.0, Axon Instruments). Ca
2+ current was measured at a rate of 0.2 Hz using depolarizing voltage pulses of 400 ms duration clamped to 0 mV from the holding potential of -40 mV. In cardiac cells fluoxetine 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.

Study of anticonvulsant effect of fluoxetine in mouse epilepsy model. Mice of either sex, weighing 30±1.2 g, were injected with pentylenetetrazole (100 mg/kg, s.c.) in order to evoke epileptic activity. Single doses of 10 mg/kg fluoxetine (racemic, S(+), or R(-) enantiomer) were administered subcutaneously either 30 or 60 min before the application of pentylenetetrazole. Seizure activity was evaluated continuously during the period of 60 min following the injection of pentylenetetrazole according to the following parameters. Survival rate (expressed as percentage) was defined as the number of animals surviving the 60-min post-pentylenetetrazole period divided by the total number animals exposed to pentylenetetrazole in any particular group. Seizure latency represents time elapsed from the pentylenetetrazole-injection to the first appearance of seizures. Survival duration was calculated only for those animals which failed to survive the critical 60-min period of evaluation.

Statistics. All values presented are arithmetic means ± SEM. Statistical significance was determined by using Student’s t-test performed following ANOVA. Differences were considered significant when the P-value was <0.05.

The entire investigation conforms with 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 with the principles outlined in the Declaration of Helsinki.

Results

Effect of fluoxetine on Ba
2+ current in rat cochlear neurons. To measure Ba
2+ current flowing through Ca
2+ channels, voltage ramps rising from -100 mV to 40 mV during 200 ms were applied. These ramps were repeated at a rate of 0.33 Hz, the holding potential between the ramps was -75 mV. The activation of Ca
2+ channels during the ramps was seen as an inward current mediated by Ba ions. The effect of fluoxetine was measured as a reduction of the peak inward current. Fluoxetine was applied in a non-cumulative manner. The cell was exposed to one concentration of the drug (either S(+), R(-), or the racemic form) for 20-30 sec using a fast flow system. This time was sufficient to achieve steady-state effect. Exposures were separated with 5-min periods of washout. The next exposure (to another form of the same concentration) was applied only after full reversion of the drug-effect. All three forms of fluoxetine suppressed peak Ba
2+ current in a concentration-dependent manner studied between concentrations of 0.1 and 100 µM, the concentration-response curve obtained for racemic fluoxetine is shown in Fig. 1A. Fitting these results to the Hill equation yielded a Kd value of 22.3±3.5 µM and Hill coefficient of 0.87±0.1 (n=5). Significant differences between the two enantiomers were observed only at a concentration of 5 µM, where R(-)-fluoxetine caused 28±3% decrease in the current in contrast to the 18±2% reduction observed with 5 µM S(-)-fluoxetine (P<0.05, n=13) (Fig. 1B). The effect of fluoxetine was fully reversible even at the highest applied concentration of 100 µM (Fig. 1C), where the current was largely suppressed by the drug. One hundred µM of S(+), R(-) and racemic fluoxetine caused comparable, 81±3%, 76±2% and 80±4% block, respectively. Fluoxetine appeared to cause a certain degree of desensitization, as its blocking effect decreased with time. This desensitization was extremely rapid, it was over within a few seconds after exposure to the drug (Fig. 1D). This desensitization was prominent at low concentrations (0.1-1 µM), but it was never observed with 100 µM fluoxetine.

Effect of fluoxetine enantiomers on action potential configuration and L-type Ca
2+ current in isolated canine ventricular myocytes. Results obtained with racemic fluoxetine were reported earlier (8), therefore, the effects of the S(+) and R(-) enantiomers were studied and compared in these experiments. Fluoxetine (0.1-10 µM) shortened action potential duration (both APD
50 and APD
90) in a concentration-dependent manner (Table I). This effect was associated with depression of the plateau potential at 10 µM concentration. Concentrations higher than 10 µM caused progressive depolarization and loss of excitability, therefore, the effects of these concentrations
Figure 1. Effect of fluoxetine on the calcium channels of pyramidal neurons isolated from the dorsal cochlear nucleus of the rat. (A), Concentration-dependent effect of fluoxetine on peak Ba$^2+$ current. Effects were evaluated after reaching steady-state level of inhibition (within 20-30 sec). Symbols and bars represent mean ± SEM values, n=5. The blocking effect was statistically significant at each concentration comparing to control (not indicated). (B), Differential effects of 5 µM S(+)-fluoxetine and R(-)-fluoxetine on the Ba$^2+$ current. After taking the control trace, 5 µM S(+)-fluoxetine was applied first, and after reaching steady-state effect (20 sec) the cell was superfused with 5 µM R(-)-fluoxetine for further 20 sec. (C), Reversibility of the effect of fluoxetine. The cell was treated with 100 µM R(-)-fluoxetine for 25 sec, then washed with fluoxetine-free medium for 5 min. (D), Desensitization observed with 1 µM fluoxetine. After taking control record, 1 µM S(+)-fluoxetine was applied. The first subsequent trace (dashed line) was taken immediately (3 sec) after application of fluoxetine, dotted line indicates the trace taken after reaching the steady-state effect at 18 sec.

Table I. Effects of R(-)-fluoxetine and S(+)-fluoxetine on action potential characteristics in isolated canine ventricular myocytes.

<table>
<thead>
<tr>
<th></th>
<th>APD$_{50}$ (ms)</th>
<th>APD$_{90}$ (ms)</th>
<th>APA (mV)</th>
<th>$V_{max}$ (V/s)</th>
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<tr>
<td><strong>R(-)-fluoxetine (n=5)</strong></td>
<td></td>
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<tr>
<td>Control</td>
<td>157±5</td>
<td>216±7</td>
<td>117.9±1.5</td>
<td>313±5</td>
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<td>Fluoxetine 0.1 µM</td>
<td>150±4</td>
<td>209±4</td>
<td>118.1±1.4</td>
<td>314±6</td>
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<tr>
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<td>115.1±1.6</td>
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<td>154±10</td>
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<td>Washout</td>
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<td>224±10</td>
<td>115.8±2.0</td>
<td>290±31</td>
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<td><strong>S(+)-fluoxetine (n=5)</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
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<tr>
<td>Control</td>
<td>170±12</td>
<td>236±11</td>
<td>117.9±2.2</td>
<td>316±7</td>
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<tr>
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<td>223±10</td>
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<td>314±11</td>
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<td>Fluoxetine 10 µM</td>
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<tr>
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<td>117.9±2.4</td>
<td>305±16</td>
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</table>

APD$_{50}$ and APD$_{90}$ action potential duration measured at 50 and 90% repolarization, respectively. APA, action potential amplitude; $V_{max}$, maximum velocity of depolarization. Mean ± SEM values are given. *Significant changes from control. †Significant differences between effects of R(-)-fluoxetine and S(+)-fluoxetine (P<0.05).
were not analyzed. Significant differences in the APD-shortening effect was observed between the S(+) and R(-) enantiomer of fluoxetine: S(+) fluoxetine shortened APD₅₀ significantly from 0.1 µM, while R(-)-fluoxetine only from 1 µM. Furthermore, the magnitude of shortening was significantly greater with 1 and 3 µM of S(+) fluoxetine than with the R(-) enantiomer. At 10 µM concentration, however, no significant differences were observed between the effects of the two enantiomers on action potential duration. At this concentration both enantiomers also depressed action potential upstroke: reduction of maximum velocity of depolarization (Vₘₐₓ) and action potential amplitude (APA) was observed. In this respect no differences were seen between the enantiomers. All these effects of fluoxetine developed rapidly (within 2 min) and were fully reversible within the 5-min period of washout (except the APD₅₀ value following 10 µM of S(+) fluoxetine).

In voltage-clamped canine ventricular myocytes both enantiomers of fluoxetine (n=5 for each) caused concentration-dependent block of the peak Ca²⁺ current measured at 0 mV (Fig. 2). Reduction in the amplitude of the peak current was not accompanied with changes in the time course of inactivation (Fig. 2A). Similarly to the effects on action potentials, the suppressive effect of fluoxetine on Iₖᵩ developed rapidly and was largely reversible (Fig. 2B). The concentration-response curve, presented in Fig. 2C, shows that both enantiomers inhibited Iₖᵩ significantly from the lowest (0.1 µM) concentration studied, having moderate differences in the Kᵦ values (2.4±0.1 and 2.8±0.2 µM) and Hill coefficients (1.41±0.05 and 1.24±0.13) obtained for the S(+) and R(-) enantiomer, respectively. Significant differences in the blocking effect was observed only at 3 and 10 µM concentrations: S(+) fluoxetine caused a higher degree of block than R(-)-fluoxetine (56.3±2.2% versus 49.1±2.2% and 95.5±0.9% versus 84.5±3.1% block with 3 and 10 µM S(+) and R(-)-fluoxetine, respectively, P<0.05, n=5). The current was fully abolished by 100 µM fluoxetine. In summary, cardiac preparations appear to be more sensitive to the S(+) than the R(-) enantiomer, as indicated by the stronger effect of the former at 1-3 µM concentrations on action potential duration and at 3-10 µM concentrations on Iₖᵩ. In both cases the effect of the R(-) enantiomer was more readily reversible than that of the S(+) enantiomer (Table I and Fig. 2A and B).

**Figure 2.** Effect of the R(-) and S(+) enantiomer of fluoxetine on the Ca²⁺ current in canine ventricular cardiomyocytes. (A), Superimposed Iₖᵩ records obtained before, during (2 min), and after superfusion with 3 µM fluoxetine. Washout lasted for 5 min. Currents were measured during step depolarizations to 0 mV, each lasting for 400 ms, applied from the holding potential of -40 mV at a rate of 0.2 Hz. (B), Representative record showing the time scale of development and reversion of the fluoxetine-induced changes in Iₖᵩ. The drug was applied in a cumulative manner (a, control; b, 0.1; c, 1; d, 3; e, 10; f, 30 µM fluoxetine, each concentration for 2 min, g: 5 min washout). (C), Cumulative concentration-response curve obtained with the S(+) and R(-) enantiomers of fluoxetine in canine ventricular myocytes (n=5 for each). The solid line was generated by fitting data to the Hill equation. Symbols and bars represent mean values ± SEM, asterisks indicate significant differences (P<0.05) between the effects of the two enantiomers.

**Anticonvulsant effect of fluoxetine in pentyleneetetrazole-treated mice.** From the 32 control animals (exposed to 100 mg/kg pentyleneetetrazole alone) 31 died within the subsequent 60 min of evaluation (3.1% survival rate). Pretreatment with 10 mg/kg fluoxetine 30 min before application of pentyleneetetrazole increased the rate of survival, however, this increase was highly variable according to the fluoxetine enantiomer applied. Pretreatment with 10 mg/kg racemic or S(+) fluoxetine produced an increase of 8.9% for both groups, in contrast to the 33% increase observed with the R(-) enantiomer. These differences were much less pronounced when fluoxetine was applied 60 min prior to the injection of pentyleneetetrazole (Fig. 3A). The protective effect of 5 mg/kg fluoxetine (applied 60 min before pentyleneetetrazole) on the rate of survival was negligible: only 1 animal survived in the R(-) and none in the S(+) fluoxetine group (out of 12 and 13, respectively, not shown in Fig. 3).

Survival duration, monitored within the period of 60 min following the injection of pentylentetrazole, was increased significantly by pretreatment with either 10 mg/kg racemic or the same concentration of R(-)-fluoxetine, whereas the protective effect was not significant in the case of the S(+) enantiomer (Fig. 3B). Interestingly, this parameter appeared to be insensitive to the time of fluoxetine-treatment, since the values were almost identical in the cases of 30 and 60 min pretreatment. Survival duration was also longer with 5 mg/kg R(-) than S(+) fluoxetine (15.3±4 versus 8.8±1.8 min survivals were observed, n=12 and n=13, respectively, not shown). Qualitatively similar increase in seizure latency was obtained with: 10 mg/kg racemic or R(-)-fluoxetine, but not the S(+) enantiomer (Fig. 3C).
Anticonvulsant action of fluoxetine. Our present results indicate that fluoxetine exerts an anticonvulsant action in the pentylentetrazole-induced seizure model. These data are in accordance with those of previous studies demonstrating an anticonvulsant effect of fluoxetine in other models of epilepsy. Fluoxetine was found to be effective against maximal electroshock-induced tonic extension in rats (44), audiogenic seizures in mice and rats (21,25), and focally evoked limbic motor seizures in rats (22). Several clinical observations and animal studies showed that fluoxetine enhanced the anti-convulsant potency of various antiepileptic drugs (19,20,45-47). Regarding the effective anticonvulsant dose of the drug, fluoxetine exerted 50% protective effect at 5 mg/kg in the limbic motor seizure model, whereas, in the genetically epilepsy-prone rats (GEPR-9) the ED50 was ~16 mg/kg. In our present seizure model fluoxetine showed a significant anticonvulsant action at the dose of 10 mg/kg. We found a marked difference in the anticonvulsant activity of the two enantiomers, since R(-)-fluoxetine was more potent to increase the survival rate than the S(+) enantiomer. The time-course of anticonvulsant action of racemic and R(-)-fluoxetine was also different. Racemic fluoxetine exhibited equal anticonvulsant activity applied either 30 or 60 min before the pentylentetrazole-injection, while R(-)-fluoxetine had stronger anticonvulsant action when applied 30 min before the convulsant drug. The opposite was observed with the S(+) enantiomer. These observations can be well explained by recent results indicating considerable differences in the pharmacokinetic properties of the two enantiomers of fluoxetine (39-41). S(+)-fluoxetine was found to be more slowly eliminated than the R(-) enantiomer, and it was suggested that its N-demethyl metabolite, S(+)norfluoxetine is more potent than R(-)-norfluoxetine. Both metabolites may accumulate on chronic treatment with racemic fluoxetine (40).

In spite of previous data reporting that serotonin is involved in regulation of seizure susceptibility (44,48-50), it seems unlikely that inhibition of serotonin reuptake is the only mechanism by which fluoxetine exerts its anticonvulsant effect. Fluoxetine was shown to suppress the high K+ induced burst firing in rat hippocampal neurons (10) considered as a model for epilepsy not involving serotonergic transmission (51). Thus the other possible explanation for the anticonvulsant action of fluoxetine may be related to its inhibitory effect on ionic channels. Several reports indicated that altered activity of voltage-gated Na+ and Ca2+ channels was involved in the enhancement of neuronal discharges during epilepsy (51-53), and conventional anticonvulsant drugs were found to block voltage-dependent Na+ and Ca2+ channels (11,13,14,53).

Our previous results demonstrating that fluoxetine was a considerably more potent inhibitor of Ca2+ than Na+ channels support the possibility that inhibition of Ca2+ channels by fluoxetine may contribute to its anticonvulsant action (10).

Effects on calcium channels. In the present study we have compared the actions of racemic, R(-) and S(+)-fluoxetine on calcium channels in isolated rat cochlear neurons and canine ventricular myocytes. We have shown that fluoxetine decreased the high-voltage activated neuronal Ca2+ current (mainly L-type) in a concentration-dependent manner, and at a concentration of 5 µM this effect of R(-)-fluoxetine was significantly greater than that of the S(+) enantiomer. In our experiments, performed in coheular neurons, the IC50 for suppression of Ica was 22.3 µM suggesting that the sensitivity of these neurons to fluoxetine is lower than that was seen in hippocampal cells (10). On the other hand, blocking potency on Icai, comparable to our results, were obtained in PC12 cells (IC50 = 13 µM) (54) and in synaptosomes (for inhibition of calcium uptake, IC50 = 27 µM) (55). The discrepancy between the IC50 values may likely be attributed to differences in the dominant Ca2+ channel type in the neurons examined.

The lower range of fluoxetine concentrations (0.1-5 µM) blocking neuronal Ca2+ current in this study overlaps the therapeutic plasma concentrations (0.15-1.5 µM) of the drug. Under certain conditions (e.g. in case of drug interactions or reduced metabolism in elderly) the plasma concentration of fluoxetine can reach even higher levels. Moreover, during chronic treatment fluoxetine was shown to accumulate in human brain twenty times higher than its plasma level (56,57).
Thus, a significant inhibition of Ca\(^{2+}\) channels by fluoxetine may occur in a patient chronically treated with fluoxetine, exerting its anticonvulsant or antimigraine activity.

In isolated canine cardiomyocytes both enantiomers shortened action potential duration, but the effectivity of the two enantiomers was different. S(+)–fluoxetine shortened APD\(_{50}\) significantly from the concentration of 0.1 \(\mu\) M, while R(−)–fluoxetine from 1 \(\mu\) M only. In addition, the magnitude of shortening was also significantly greater in the case of 1 and 3 \(\mu\) M of S(+)–fluoxetine. At the higher concentration of 10 \(\mu\) M both enantiomers decreased also action potential amplitude and the maximum rate of depolarization. No difference was observed between the two enantiomers from this point of view indicating an equal potency of blocking cardiac Na\(^{+}\) channels. The shortening effect of fluoxetine on cardiac action potentials may be best explained with inhibition of the L-type Ca\(^{2+}\) channel. Indeed, both enantiomers blocked peak Ca\(^{2+}\) current significantly, however, this effect was more pronounced at 3 and 10 \(\mu\) M concentrations of the S(+) than the R(−) enantiomer. The 2.4 and 2.8 \(\mu\) M \(K_d\) values, obtained for S(+) and R(−)–fluoxetine in this study, is very close to the value of 3 \(\mu\) M, reported previously by Pacher et al (8) with racemic fluoxetine in canine ventricular cells. These cardiac electrophysiological actions of fluoxetine enantiomers may be proarrhythmic due to impairment of atrioventricular or intraventricular conduction and shortening of repolarization. Moreover, the negative inotropic action observed in rat papillary muscle (8) and the vasodilator effect of racemic fluoxetine (58) may also be due to inhibition of L-type Ca\(^{2+}\) channels and may explain the cardiovascular side-effects (dysrhythmias, syncope) observed occasionally in patients treated with fluoxetine (26,27,29,30,32-34,36,38).

In conclusion, our present data indicate that the two enantiomers of fluoxetine have different anticonvulsant effects and inhibitory activities on neuronal and cardiac Ca\(^{2+}\) channels at micromolar concentrations. While R(−)–fluoxetine exerts stronger anticonvulsant action associated with inhibition of neuronal Ca\(^{2+}\) channels, the S(+) form appears to be more potent in shortening of cardiac action potential due to inhibition of cardiac Ca\(^{2+}\) channels. It is important to emphasize that these differences in the action of the two enantiomers on Ca\(^{2+}\) current were evident only within a narrow range of concentrations (3-10 \(\mu\) M) and were not great in magnitude. It is tempting to speculate, however, that the S(+) isomer may be more responsible for the undesired cardiovascular side-effects that sometimes develop during chronic fluoxetine treatment. Used as an antidepressant or anticonvulsant drug, less severe cardiac side-effects are anticipated with R(−)–fluoxetine.

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