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T-Type Calcium Channels Are Inhibited by Fluoxetine and Its Metabolite Norfluoxetine

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ABSTRACT

Fluoxetine, a widely used antidepressant that primarily acts as a selective serotonin reuptake inhibitor, also inhibits various neuronal ion channels. Using the whole-cell patch-clamp technique, we have examined the effects of fluoxetine and norfluoxetine, its major active metabolite, on cloned low-voltage-activated T-type calcium channels (T channels) expressed in tsA 201 cells. Fluoxetine inhibited the three T channels $Ca_V3.1$, $Ca_V3.2$, and $Ca_V3.3$ in a concentration-dependent manner (IC_{50} = 14, 16, and 30 μM, respectively). Norfluoxetine was a more potent inhibitor than fluoxetine, especially on the $Ca_V3.3$ T current (IC_{50} = 5 μM). The fluoxetine block of T channels was voltage-dependent because it was significantly enhanced for T channels in the inactivated state. Fluoxetine caused a hyperpolarizing shift in steady-state inactivation, with a slower rate of recovery from the inactivated state. These results indicated a tighter binding of fluoxetine to the inactivated state than to the resting state of T channels, suggesting a more potent inhibition of T channels at physiological resting membrane potential. Indeed, fluoxetine and norfluoxetine at 1 μM strongly inhibited cloned T currents (~50 and ~75%, respectively) in action potential clamp experiments performed with firing activities of thalamocortical relay neurons. Altogether, these data demonstrate that clinically relevant concentrations of fluoxetine exert a voltage-dependent block of T channels that may contribute to this antidepressant's pharmacological effects.

Fluoxetine (Prozac; Eli Lilly & Co., Indianapolis, IN) is a psychoactive drug widely prescribed in many psychiatric disorders, including depression, obsessive-compulsive disorder and bulimia nervosa. Like most antidepressants, fluoxetine causes side effects including nausea, gastrointestinal complaints, headaches, anxiety, insomnia, drowsiness, and loss of appetite (Cookson and Duffett, 1998). Fluoxetine is metabolized via the cytochrome P450 enzyme system (Stark et al., 1985; Wong et al., 1995) into multiple metabolites, including norfluoxetine, the major active metabolite, with pharmacological properties that are similar to or more potent than those of the parent drug (Hiemke and Hartter, 2000). The therapeutic action of fluoxetine primarily results from the inhibition of serotonin reuptake (Stark et al., 1985; Wong et al., 1995), thus enhancing serotoninergic neurotransmission. Besides this mechanism, fluoxetine has several other modulatory effects, such as inhibition of G protein-coupled receptors (Stanton et al., 1993; Palvimaki et al., 1996), blockade of monoamine oxidases (Mukherjee and Yang, 1999), and modulation of several ionic channels. Indeed, it has been reported that fluoxetine is a potent blocker of K^+ channels (Thomas et al., 2002; Choi et al., 2003; Kobayashi et al., 2003; Kennard et al., 2005), Na^+ channels (Pancrazio et al., 1998), and Ca^{2+} channels (Deak et al., 2000).

T-type Ca^{2+} channels, a subgroup of voltage-gated Ca^{2+} channels, are the target of many antipsychotic drugs, including penfluridol, clozapine, fluspiriline, haloperidol, and pimozide (Enyeart et al., 1992; Santi et al., 2002; Yunker, 2003). After the study by Deak et al. (2000) reporting that fluoxetine inhibits T-type Ca^{2+} currents in rat hippocampal pyramidal cells, it is of importance to characterize fluoxetine's action on the three T-type Ca^{2+} channel subtypes ($Ca_V3.1$ or α_{1G}, $Ca_V3.2$ or α_{1H}, and $Ca_V3.3$ or α_{1I}) that have been characterized recently by molecular cloning and patch-clamp techniques (Cribbs et al., 1998; Perez-Reyes et al., 1998; Klugbauer et al., 1999; Lee et al., 1999; Monteil et al., 2000a,b). In this article, we report the first electrophysiological study of the inhibitory effects of fluoxetine and norfluoxetine on the recombinant T-type Ca^{2+} channels. Because T-type Ca^{2+}

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ABBREVIATIONS: HP, holding potential.

channels are broadly involved in physiology, including cardiac pacemaker, hormone secretion, fertilization, neuronal firing, epilepsy, cardiac hypertrophy (Huguenard, 1996), sleep (Anderson et al., 2005), and pain (Todorovic et al., 2001; Bourinet et al., 2005), their inhibition by fluoxetine should be investigated for a better understanding of this antidepressant's therapeutic action and side effects.

Materials and Methods

Cell Culture and Transfection Protocols. The tsA 201 cell line was cultivated in Dulbecco's modified Eagle's medium supplemented with glutamax and 10% fetal bovine serum (Invitrogen, Cergy-Pontoise, France). Transfection was performed using jet-polyethyleneimine (Qbiogene, Illkirch, France) with a DNA mix containing 10% of a green fluorescent protein plasmid and 90% of either one of the pCDNA3 plasmid constructs that codes for human $Ca_V3.1$ (Cribbs et al., 1998; Perez-Reyes et al., 1998; Klugbauer et al., 1999; Lee et al., 1999; Monteil et al., 2000a,b), $Ca_V3.2$ (Cribbs et al., 1998) and $Ca_V3.3$ (Gomora et al., 2002). Electrophysiological recordings were performed 2 to 4 days after transfection. A Chinese hamster ovary cell line stably expressing Cav3.1, isoform "bc" (Cribbs et al., 1998; Perez-Reyes et al., 1998; Klugbauer et al., 1999; Lee et al., 1999; Monteil et al., 2000a,b; Chemin et al., 2001), was used in some experiments, and no difference was observed in fluoxetine sensitivity between the two recipient cell models. The mouse neuroblastoma/rat glioma hybrid cell line NG 108-15 was cultured in Dulbecco's modified Eagle's medium supplemented with glutamax, 10% fetal bovine serum, and 2% hypoxanthine/aminopterine/thymidine (Invitrogen), as reported elsewhere (Chemin et al., 2002b).

Electrophysiology and Calcium Current Analysis. Whole-cell Ca^{2+} currents were recorded at room temperature. Extracellular solution contained 2 mM $CaCl_2$, 160 mM tetraethylammonium chloride, and 10 mM HEPES, pH to 7.4 with tetraethylammonium-OH. Pipettes had a resistance of 2 to 3 MΩ when filled with a solution containing 110 mM CsCl, 10 mM EGTA, 10 mM HEPES, 3 mM Mg-ATP, and 0.6 mM GTP, pH to 7.2 with CsOH. The sampling frequency for acquisition was 10 kHz, and data were filtered at 2 kHz. For action potential clamp studies (Fig. 5), a thalamocortical relay cell firing activity was generated using NEURON (Hines and Carnevale, 1997). This simulation environment (available at http://www.neuron.yale.edu/neuron/about/what.html) allows simulation of neuronal activities that can be used as waveforms in voltage-clamp experiments on Ca_V-transfected cells (Chemin et al., 2002a). The Ca^{2+} current data were analyzed as described previously (Chemin et al., 2001). Current-voltage relationships were fitted using a combined Boltzmann and linear ohmic relationships, where $I = G_{max} \times (V_m - V_{rev})/(1 + e^{(V_m - V_{1/2})/slope})$. To minimize the consequence of current rectification near reversal potential on the determination of conductance, the current values greater than +30 mV were not considered for the fit. The normalized conductance-voltage curves were fitted with the following Boltzmann equation: $G/G_{max} = 1/(1 + e^{(V_{1/2} - V_m)/slope})$. The steady-state inactivation curves were fitted using $I/I_{max} = 1 - 1/(1 + e^{(V_{1/2} - V_m)/slope})$. Kinetics of the recovery from inactivation were calculated with a double-exponential expression: $\%I = A_1(1 - e^{-V_m/t_1}) + A_2(1 - e^{-V_m/t_2})$, where A_1 and A_2 are the relative amplitudes of each exponential and t_1 and t_2 their respective time t constants. To better evaluate the role of fluoxetine on the recovery process, we defined the global recovery (t_g) as $A_1t_1 + A_2t_2$. The dose-response curves were fitted using a sigmoidal Hill function, $I = 1/(1 + e^{(EC_{50} - V_m)/Hill\ slope})$. Student's t tests were used to compare the different values and were considered significant at $P < 0.05$. Results are presented as the mean ± S.E.M., and n is the number of cells used.

Pharmacological Agents. Fluoxetine and norfluoxetine were purchased from Sigma-Aldrich (Lyon, France). The drugs were dissolved in water at 10 mM as a stock solution and keep at −20°C. Drugs were applied to cells by a gravity-driven perfusion device controlled by solenoid valves.

Results

Fluoxetine Inhibition of Recombinant T-Type Ca^{2+} Channels. Figure 1 shows typical trace recordings of Ca_V3 currents expressed in the tsA 201 cells. Application of fluoxetine (10–20 μM) inhibits the three cloned T-type Ca^{2+} channels (Fig. 1, A–C, left). A similar inhibition was obtained at every potential, as observed on the corresponding current-voltage curves for $Ca_V3.1$, $Ca_V3.2$, and $Ca_V3.3$ currents (Fig. 1, A–C, right). The fluoxetine block reached its maximum after ~40 s and was totally reversible upon washout, as illustrated for $Ca_V3.3$ currents (Fig. 1D). The average current inhibition induced by 10 μM fluoxetine was 42% (I/I_{Ctrl} = 0.58 ± 0.02, n = 12) for $Ca_V3.1$ currents, 43% (I/I_{Ctrl} = 0.57 ± 0.01, n = 7) for $Ca_V3.2$ currents, and 27% (I/I_{Ctrl} = 0.73 ± 0.02, n = 9) for $Ca_V3.3$ currents (Fig. 1E). Likewise, in proliferative NG 180-15 cells that display native T-type Ca^{2+} channels related to $Ca_V3.2$ channels (Chemin et al., 2002b),

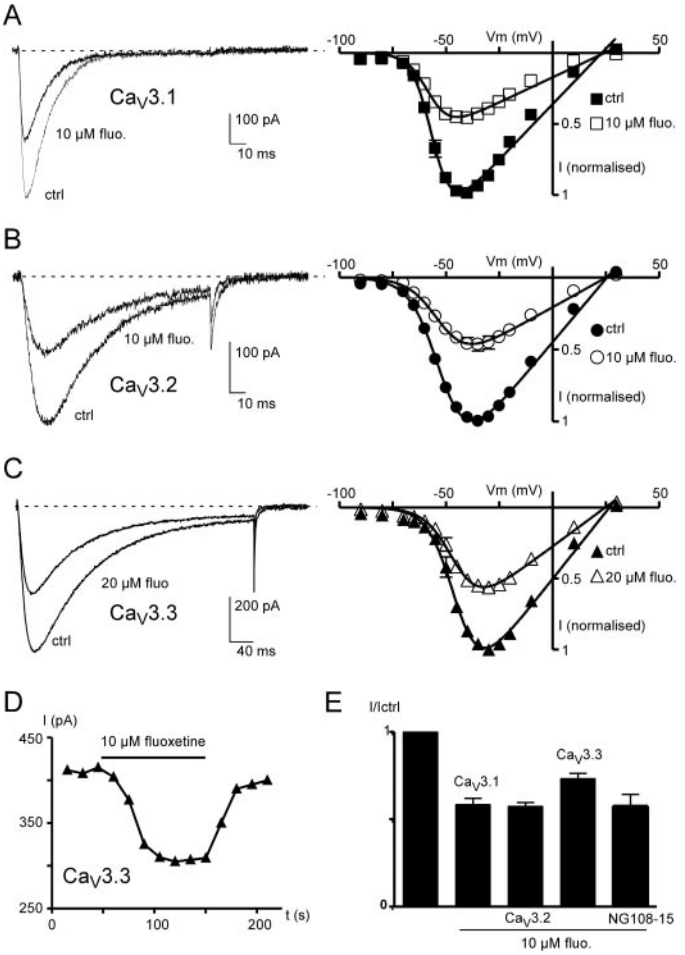

Fig. 1. Fluoxetine inhibits cloned T-type/Ca_V3 Ca^{2+} channels. A to C, effect of 10 μM fluoxetine (fluo., open symbols) on Ca^{2+} currents (2 mM external Ca^{2+}) elicited by a −30 mV test pulse (left) and on current-voltage curves (right) obtained for $Ca_V3.1$ currents (A, squares; n = 7) and $Ca_V3.2$ currents (B, circles; n = 11) and 20 μM fluoxetine on $Ca_V3.3$ currents (C, triangles; n = 5). The holding potential during the interpulse (10 s) was −100 mV. D, time course of fluoxetine inhibition on $Ca_V3.3$ currents. E, comparison of the effect of 10 μM fluoxetine on the $Ca_V3.1$, $Ca_V3.2$, and $Ca_V3.3$ currents elicited by a −30-mV test pulse and on T currents from proliferative NG 108-15 cells.

10 μM fluoxetine inhibited T currents by 43% (I/I_{Ctrl} = 0.57 ± 0.06, n = 5; Fig. 1E).

Ca_V3 Current Inhibition by Fluoxetine and Norfluoxetine Is Concentration-Dependent. Increasing concentrations of fluoxetine and norfluoxetine were then applied to tsA201 cells expressing the various T-type Ca^{2+} channels (Fig. 2). The T-current inhibition was concentration-dependent for $Ca_V3.1$, $Ca_V3.2$, and $Ca_V3.3$ channels (Fig. 2, A and B). Analysis of the dose-response curves of fluoxetine revealed IC_{50} values of 14.5 ± 1.1 μM (n = 6) for $Ca_V3.1$ currents, 15.9 ± 1.3 μM (n = 7) for $Ca_V3.2$ currents, and 31.1 ± 3.5 μM (n = 7) for $Ca_V3.3$ currents (Fig. 2B). Hill slope factors were 0.9 ± 0.1, 0.8 ± 0.1, and 0.6 ± 0.1, for $Ca_V3.1$, $Ca_V3.2$, and $Ca_V3.3$ currents, respectively (Fig. 2B). Norfluoxetine, the active metabolite of fluoxetine, was more potent than the parent molecule in blocking Ca_V3 currents, as illustrated for $Ca_V3.3$ currents (Fig. 2, C and D). As for fluoxetine, norfluoxetine produced a concentration-dependent block of the recombinant T-type Ca^{2+} channels (Fig. 2, E and F). The IC_{50} values obtained with norfluoxetine were 13.8 ± 0.1 μM (n = 5), 7.01 ± 0.03 μM (n = 3), and 5.5 ± 0.2 μM (n = 3) for $Ca_V3.1$, $Ca_V3.2$, and $Ca_V3.3$ currents, respectively, whereas the Hill slope factors were 1.35 ± 0.03, 1.58 ± 0.03, and 2.93 ± 0.02 for $Ca_V3.1$, $Ca_V3.2$, and $Ca_V3.3$ currents, respectively (Fig. 2F).

Effects of Fluoxetine on the Steady-State Inactivation of Ca_V3 Currents. Next, we investigated whether the effects of fluoxetine on T-type Ca^{2+} currents would be state-dependent. A double-pulse protocol was used to assess whether fluoxetine affects the voltage dependence of the steady-sate inactivation of Ca_V3 channels. A family of $Ca_V3.1$ currents evoked by this protocol is depicted in Fig. 3A before (top traces) and during application of 10 μM fluoxetine (bottom traces). Fluoxetine significantly shifted the steady-state inactivation curves of $Ca_V3.1$ currents toward hyperpolarized potentials from −75.7 ± 0.6 mV in control condition to −86.7 ± 1 mV (10 μM fluoxetine, n = 9; Fig. 3B). A slight shift (2 mV), but not statistically significant, was observed in the steady-state activation curve (Fig. 3B). Neither activation nor inactivation kinetics were changed after fluoxetine treatment for $Ca_V3.1$ currents or for $Ca_V3.2$ and $Ca_V3.3$ currents (data not shown). As a consequence of the steady-state inactivation shift, the window current (or steady-state current) generated by the $Ca_V3.1$ subunit was markedly decreased (Fig. 3B). Similar results were obtained with the $Ca_V3.2$ and $Ca_V3.3$ subunits (Fig. 3C). In the presence of 10

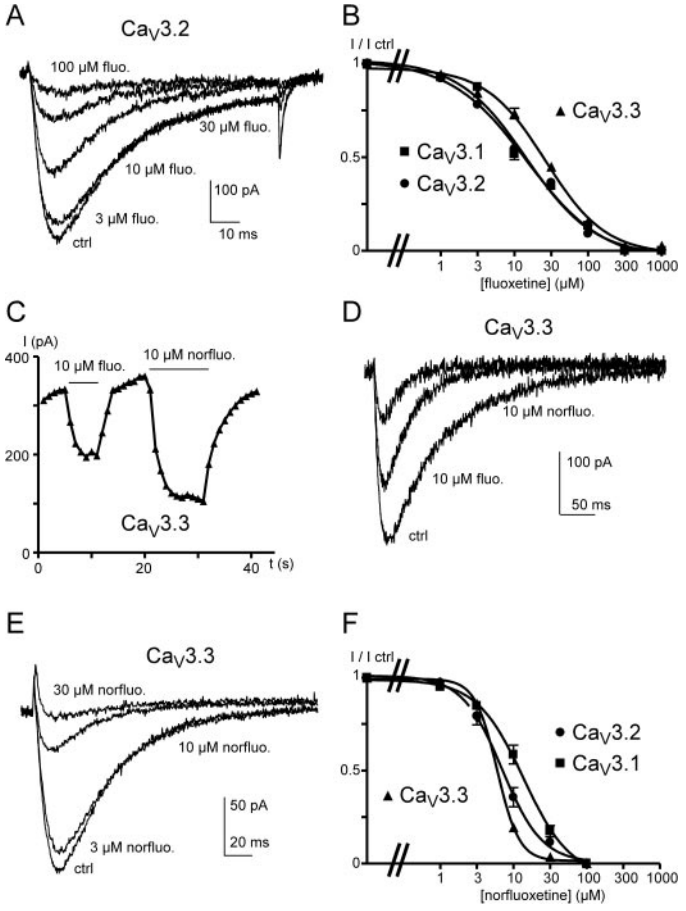

Fig. 2. Concentration-dependent inhibition of Ca_V3 currents by fluoxetine and norfluoxetine. A, effect of increasing concentrations of fluoxetine (fluo.) on Ca^{2+} currents generated by the $Ca_V3.2$ subunit. B, dose-response curves for fluoxetine inhibition of $Ca_V3.1$ (■), $Ca_V3.2$ (●), and $Ca_V3.3$ currents (▲). Dose-response curves were established by fitting the normalized currents with a sigmoidal Hill equation (see *Materials and Methods*). C, time course and inhibitory effects of 10 μM fluoxetine and 10 μM norfluoxetine (norfluo.) on $Ca_V3.3$ currents. D, examples of $Ca_V3.3$ current traces obtained after the consecutive application of 10 μM fluoxetine and 10 μM norfluoxetine. E, effect of increasing concentrations of norfluoxetine on $Ca_V3.3$ currents. F, dose-response curves for norfluoxetine inhibition of $Ca_V3.1$ (■), $Ca_V3.2$ (●), and $Ca_V3.3$ currents (▲).

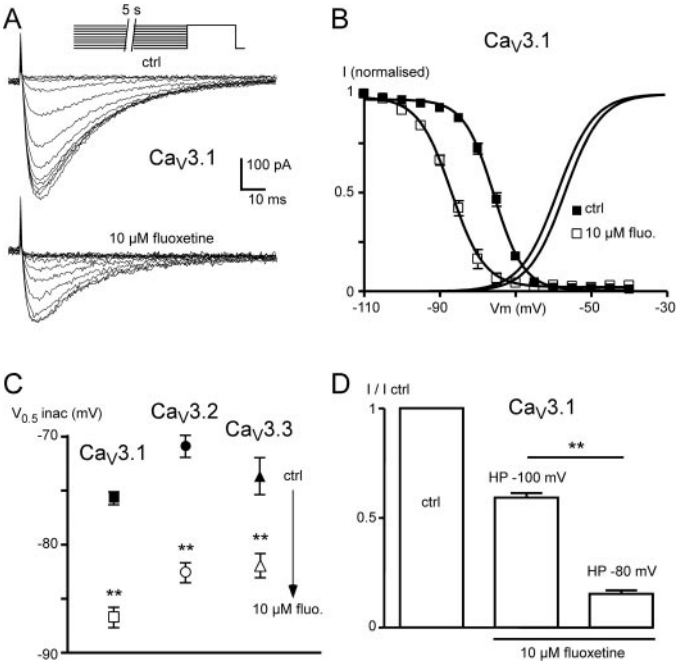

Fig. 3. Effects of fluoxetine on the steady-state inactivation of Ca_V3 currents. A, typical traces showing the effect of 10 μM fluoxetine on the steady-state inactivation of the $Ca_V3.1$ subunit. The steady-state inactivation was estimated from the variation of the current amplitude at −30 mV after a 5-s predepolarization of increasing amplitude (−110 to −40 mV with 5-mV increments). B, normalized steady-state activation and inactivation curves for the $Ca_V3.1$ subunit in the presence (□) and in the absence (■) of 10 μM fluoxetine (fluo.). Steady-state activation curves were obtained from the current-voltage curve presented in Fig. 1 [$V_{0.5}$ = −56.4 ± 1.1 mV for control and $V_{0.5}$ = −58.4 ± 0.9 mV (n = 7) in the presence of 10 μM fluoxetine, P = 0.18], whereas steady-state inactivation curves were obtained from the protocol described in A. C, effects of 10 μM fluoxetine on the $V_{0.5}$ value of the steady-state inactivation for the three Ca_V3 subunits (n = 10, 15, and 11). D, block of $Ca_V3.3$ currents by fluoxetine was dependent on the HP.

μM fluoxetine, the half-potential ($V_{0.5}$) of steady-state inactivation was shifted approximately 10 mV toward the hyperpolarized potential for the three T-type Ca^{2+} channel subunits (Fig. 3C), whereas no significant change was observed for the corresponding slope factors for $Ca_V3.1$, $Ca_V3.2$, and $Ca_V3.3$ subunits. Such an effect on the voltage dependence of the steady-state inactivation suggests that fluoxetine interacts with the inactivated state of the channels. A direct method to evaluate fluoxetine binding onto inactivated T channels was to measure current inhibition at various holding potentials (HPs). The inhibition of $Ca_V3.1$ currents by 10 μM fluoxetine was examined for HPs of -100 and -80 mV (Fig. 3D). As expected, 10 μM fluoxetine strongly blocked $Ca_V3.1$ currents at HP -80 mV (by 85 $\pm$ 1%, $n = 5$), whereas at HP -100 mV, fluoxetine reduced $Ca_V3.1$ currents by 41 $\pm$ 2% ($n = 5$).

Fluoxetine Slows the Recovery from Inactivation of Ca_V3 Currents. To determine whether the channel is maintained as blocked in the inactivated state by fluoxetine, we investigated the recovery from short inactivation using a two paired-pulse protocol (Fig. 4). Figure 4A shows typical examples of current traces recorded for different values of interpulse intervals for $Ca_V3.1$ currents in the absence (top traces) and presence of 10 μM fluoxetine (bottom traces). The time course of the recovery process was then plotted (Fig. 4B), showing that fluoxetine significantly slowed t_g, the time constant, the global recovery from short inactivation, by a factor of approximately 2 for $Ca_V3.1$ (188 $\pm$ 21 and 391 $\pm$ 20 ms before and after fluoxetine application, respectively; $n = 7$) and $Ca_V3.2$ (381 $\pm$ 28 and 706 $\pm$ 45 ms before and after fluoxetine application, respectively; $n = 5$) currents, whereas these effects were not significant for $Ca_V3.3$ currents at this holding potential (250 $\pm$ 49 and 390 $\pm$ 68 ms, before and after fluoxetine application, respectively; $n = 10$; Fig. 4, B and C).

Enhanced Fluoxetine Inhibition of Ca_V3 Currents in Action Potential Clamp Experiments. Both the slowing in the recovery kinetics and the decrease in the window current component in the presence of fluoxetine strongly suggested that this drug would be a potent inhibitor of Ca_V3 currents involved in neuronal firing activities. To appreciate better the physiological impact of the fluoxetine block of T currents, we performed voltage-clamp experiments on transfected cells using a thalamocortical relay cell-firing activity generated with the NEURON model as waveform (Fig. 5A, see *Materials and Methods*). As observed previously (Kozlov et al., 1999; Chemin et al., 2002a), the $Ca_V3.3$ currents strongly participate in the Ca^{2+} entry during sustained neuronal activities (Fig. 5B). Fluoxetine application (1 and 10 μM) strongly decreased the amplitude of $Ca_V3.3$ currents and accelerated the decay of Ca^{2+} entry through $Ca_V3.3$ channels (Fig. 5, C and D). To quantify these effects, we measured the integral of the inward Ca^{2+} currents before (Ctrl) and after application of fluoxetine (1 and 10 μM) and norfluoxetine (1 μM). Application of 1 μM fluoxetine and norfluoxetine on $Ca_V3.3$ currents resulted in 47% of inhibition ($I/I_{Ctrl} = 0.53 \pm 0.11$, $n = 6$) and 74% of inhibition ($I/I_{Ctrl} = 0.26 \pm 0.09$, $n = 5$), respectively (Fig. 5, C and E). The average current inhibition induced by 10 μM fluoxetine was 82% for $Ca_V3.3$ currents ($I/I_{Ctrl} = 0.18 \pm 0.02$, $n = 8$), 93% for $Ca_V3.1$ currents ($I/I_{Ctrl} = 0.07 \pm 0.01$, $n = 7$), and 86% for $Ca_V3.2$ currents ($I/I_{Ctrl} = 0.14 \pm 0.02$, $n = 9$) (Fig. 5E). Considering the significant block of micromolar concentrations of both fluoxetine and norfluoxetine in action potential clamp experiments, the data strongly suggest that fluoxetine inhibition of

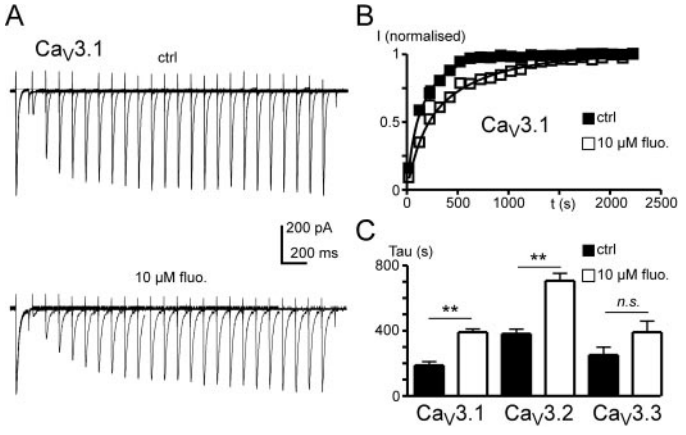

Fig. 4. Fluoxetine slows the recovery from inactivation of Ca_V3 currents. A and B, effects of 10 μM fluoxetine (fluo.) on the time course of recovery from short inactivation for $Ca_V3.1$ currents at a HP of -100 mV. Recovery from short inactivation was measured using a double pulse at -30 mV (lasting 100 ms) with an interpulse at -100 mV of increasing duration. C, effects of 10 μM fluoxetine on the τ of recovery for the three Ca_V3 subunits. The τ of recovery was deduced from the curves presented in B (see *Materials and Methods*).

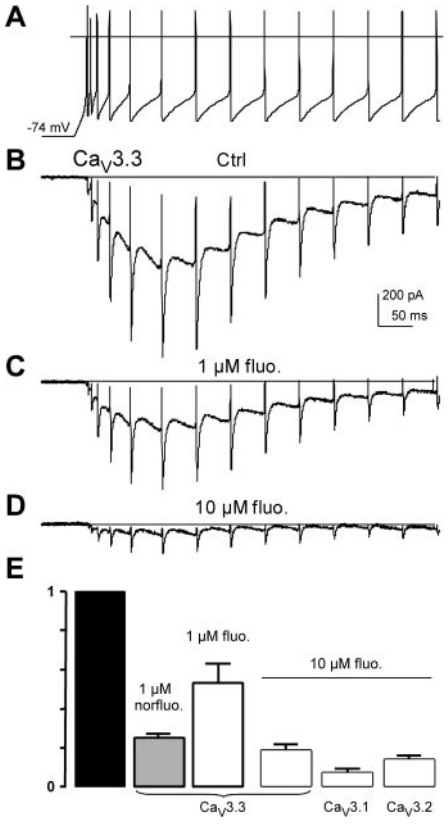

Fig. 5. Fluoxetine strongly blocked $Ca_V3.3$ currents during thalamic relay cell-like activities. A, firing activity typical of those recorded on thalamic relay neurons was used as waveform (action potential clamp) to induce Ca^{2+} currents in a cell expressing $Ca_V3.3$ channels (B). C and D, fluoxetine strongly blocked $Ca_V3.3$ current during thalamic relay cell-like activities at 1 μM (C) and 10 μM (D). Traces presented in B, C, and D were obtained on the same cell. E, normalized integral of the total current generated by the three Ca_V3 subunits during thalamic relay cell-like activities in the presence of 1 μM fluoxetine ($n = 6$), 1 μM norfluoxetine ($n = 5$), and 10 μM fluoxetine ($n = 7$ for $Ca_V3.1$, $n = 9$ for $Ca_V3.2$, and $n = 8$ for $Ca_V3.3$).

neuronal T-type Ca^{2+} currents could significantly affect neuronal activities.

Discussion

The main finding of the present study is that fluoxetine inhibits the three Ca_V3 T-type Ca^{2+} channel isotypes as expressed in human embryonic kidney 293/tsA 201 cells. We describe that fluoxetine preferentially binds to inactivated T-type Ca^{2+} channels and stabilizes them in their inactivated state. We also provide evidence that fluoxetine can strongly inhibit T-type Ca^{2+} channels during neuronal firing activities. Altogether, our data significantly extend a previous electrophysiological study, the first to our knowledge, describing that fluoxetine inhibits voltage-gated calcium channels, including T-type Ca^{2+} channels, in rat hippocampal pyramidal cells (Deak et al., 2000). Moreover, we report that norfluoxetine, the major active metabolite of fluoxetine, is even more potent that the parent molecule in blocking T-type Ca^{2+} channels.

Recombinant Ca_V3 channels are efficiently inhibited by micromolar concentrations of fluoxetine and norfluoxetine. It is noteworthy that the use of neuronal activities clearly shows that a 1 μM concentration of both fluoxetine and norfluoxetine significantly inhibited neuronal T-type Ca^{2+} channels (47 and 74% inhibition, respectively, for $Ca_V3.3$ current; Fig. 5). The therapeutic plasma concentration of fluoxetine is estimated between 0.15 and 1.5 μM (Orsulak et al., 1988; Altamura et al., 1994). Under steady-state conditions, the plasma concentrations of norfluoxetine are higher than those of fluoxetine (Altamura et al., 1994), and for both compounds, it was shown that brain concentration can reach much higher levels during long-term fluoxetine treatment (Karson et al., 1993). In addition, in elderly patients, decreased elimination increases the plasma concentration of fluoxetine (Pato et al., 1991; Borys et al., 1992). For all of these reasons, the fluoxetine concentrations that block T-type Ca^{2+} channels are within the range of those observed clinically after oral administration of the drug, and a significant reduction of these channels may occur in patients who receive long-term treatment with fluoxetine.

The IC_{50} values obtained with fluoxetine for blocking T-type Ca^{2+} channels are similar to those obtained for the inhibition of other ionic channels in different preparations. For example, the IC_{50} values of fluoxetine block of cloned shaker potassium channel (Kv1.3 and Kv1.4) and voltage-activated potassium channel (hsK1-3) range between 3 and 33 μM (Choi et al., 1999, 2003; Terstappen et al., 2003). We show that norfluoxetine is more potent than fluoxetine in inhibiting Ca_V3 channels, especially $Ca_V3.3$ currents. Likewise, it has been reported that norfluoxetine is a more potent and more selective 5-hydroxytryptamine reuptake inhibitor than fluoxetine (Hiemke and Hartter, 2000) and produced a stronger inhibitory effect than fluoxetine on Kv1.3 currents (Choi et al., 1999).

Inhibition of recombinant T-type Ca^{2+} channels is highly dependent on their inactivation state. Indeed, fluoxetine induces an approximately -10 mV shift of the steady-state inactivation curves for each T-channel isotype without having a statistically significant effect on the activation curves. Therefore, fluoxetine block is more potent at a physiological holding potential for which T-channels are inactivated (Huguenard, 1996). These results and the fact that fluoxetine slows the recovery kinetics from inactivation indicate that this drug interacts with inactivated T channels and maintains the channels in the inactivated state. These findings contrast with mechanistic studies on other ionic conductances showing that fluoxetine binds to the open state of cloned nicotinic acetylcholine receptor and Kv1.4 channels (Garcia-Colunga et al., 1997). Binding to the inactivated T-type Ca^{2+} channels is an important feature of the fluoxetine block, because this property could also contribute to tissue selectivity of the fluoxetine effects. For example, dihydropyridines that bind preferentially to the inactivated state of L-type Ca^{2+} channels are useful as antihypertensive drugs by acting on vascular smooth muscle while having little effect on the heart (Triggle, 1992).

By stimulating tsA 201 cells overexpressing Ca_V3 channels with firing activities typical of thalamocortical relay neurons, we demonstrate that fluoxetine block of T currents is more potent during physiological stimulations. This suggests that fluoxetine could be an important regulator of firing activities dependent on T-type Ca^{2+} channels (Destexhe et al., 1998). For example, the mechanism of T-type Ca^{2+} channels inhibition by fluoxetine (binding to and stabilization of the inactivated T-type Ca^{2+} channels) suggests that this drug could impair low-threshold spikes related to T channels in the thalamus (Huguenard and Prince, 1992; Huguenard, 1996; Destexhe et al., 1998). Thalamic T channels are involved in slow-wave sleep (Anderson et al., 2005), and fluoxetine block of T-type Ca^{2+} channels may explain insomnia, a side effects of this drug (Gram, 1994). T-type Ca^{2+} channels of the thalamus are also involved in the pathogenesis of epilepsy (Tsakiridou et al., 1995). In animal models of epilepsy, fluoxetine decreases the probability of audiogenic convulsions in genetically seizure-prone rodents and hippocampal seizures in rat (Prendiville and Gale, 1993; Wada et al., 1995). The anticonvulsant action of fluoxetine has been observed both in animal and human studies. Again, anticonvulsive properties of fluoxetine (Leander, 1992; Prendiville and Gale, 1993; Wada et al., 1995) could be related to the block of thalamic T-type Ca^{2+} channels. In addition, fluoxetine enhances the effects of various conventional anticonvulsants in mice, such as phenytoin (Leander, 1992), which also inhibits T currents (Todorovic et al., 2000). Fluoxetine may also affect non-neuronal T-type Ca^{2+} channels. Indeed, cardiac T-type Ca^{2+} channels participate in the control of the pacemaker activity (Lei and Kohl, 1998), and their inhibition by fluoxetine may explain the bradycardia induced by this drug (Pacher et al., 2000). In addition, we observed a net decrease in the window current component of cloned T-type Ca^{2+} channels. This effect on window current may explain the relaxation induced by fluoxetine on vascular smooth muscles (Farrugia, 1996) by decreasing intracellular calcium levels.

In summary, the data presented here show that fluoxetine (Prozac) potently inhibits the three T-type Ca^{2+} channel isotypes. Fluoxetine preferentially blocks inactivated T-type Ca^{2+} channels, which corresponds to their status in physiological conditions. Indeed, inhibition of T-type Ca^{2+} channels represents a novel mechanism by which fluoxetine is pharmacologically active and could account for some of the clinical effects in treated patients. Fluoxetine inhibition of T-type Ca^{2+} channels should therefore be taken into account in

further studies investigating the pharmacological properties of this widely prescribed antidepressant.

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