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The Mechanism of Activity-Dependent Sodium Channel Inhibition by the Antidepressants Fluoxetine and Desipramine

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ABSTRACT

The effect of monoamine uptake inhibitor-type antidepressants on sodium channels of hippocampal neurons was investigated. Members of the tricyclic group of antidepressants are known to modify multiple targets, including sodium channels, whereas selective serotonin-reuptake inhibitors (SSRIs) are regarded as highly selective compounds, and their effect on sodium channels was not investigated in detail. In this study, a representative member of each group was chosen: the tricyclic antidepressant desipramine and the SSRI fluoxetine. The drugs were roughly equipotent use-dependent inhibitors of sodium channels, with IC_{50} values ~100 μM at −150 mV holding potential, and ~1 μM at −60 mV. We suggest that therapeutic concentrations of antidepressants affect neuronal information processing partly by direct, activity-dependent inhibition of sodium channels. As for the mechanism of inhibition, use-dependent inhibition by antidepressants was believed to be due to a preferential affinity to the fast-inactivated state. Using a voltage and perfusion protocol by which relative affinities to fast- versus slow-inactivated states could be assessed, we challenged this view and found that the affinity of both drugs to slow-inactivated state(s) was higher. We propose a different mechanism of action for these antidepressants, in which slow rather than fast inactivation plays the dominant role. This mechanism is similar but not equivalent with the novel mechanism of use-dependent sodium channel inhibition previously described by our group (*Neuroscience* **125:**1019–1028, 2004; *Neuroreport* **14:**1945–1949, 2003). Our results suggest that different drugs can produce use-dependent sodium channel inhibition by different mechanisms.

It has become increasingly evident that the monoamine hypothesis cannot fully explain the pathophysiological mechanism of depression and the action of antidepressants (Shytle et al., 2002; Castren, 2005). Although the uptake blocker-type antidepressants inhibit monoamine transporters in the low nanomolar range (Torres et al., 2003), their therapeutic effect appears only at a much higher plasma and brain concentration (Muscettola et al., 1978; Bolo et al., 2000). In therapeutic (i.e., low micromolar) concentrations, however, these drugs affect other protein targets as well; most importantly, they inhibit several types of ion channels (Sernagor et al., 1989; Hennings et al., 1999; Deak et al., 2000; Pacher et al., 2000; Yang and Kuo, 2002; Eisensamer et al., 2003; Gumilar et al., 2003; Choi et al., 2004). Thus, the activity of neurons is modified by antidepressants in a complex way, by elevating monoamine levels and by directly modulating ion channels.

Neuronal sodium channels are ubiquitous and are crucial in dendritic integration, action potential initiation, and conduction. The potency of tricyclic antidepressants as sodium channel inhibitors was shown by the low micromolar IC_{50} values in vitro (e.g., Bou-Abboud and Nattel, 1998; Nicholson et al., 2002; Pancrazio et al., 1998), and by their in vivo efficiency against neuropathic pain (Namaka et al., 2004).

Binding of antidepressants to sodium channels has been demonstrated (McNeal et al., 1985; Nicholson et al., 2002). Binding of [^{3}H]batrachotoxin was inhibited by imipramine and amitriptyline, whereas [^{3}H]saxitoxin binding was not altered (Nicholson et al., 2002). The exact location of the binding site remains unresolved; it may be different from the local anesthetic binding site (Barber et al., 1991) or may overlap with it (Wang et al., 2004).

The main properties of sodium channel inhibition by antidepressants (use dependence, voltage dependence, and a hyperpolarizing shift of the inactivation curve) are shared by a variety of drugs (Deffois et al., 1996; Kuo et al., 2000), most notably local anesthetics and certain anticonvulsants. Be-

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ABBREVIATIONS: SSRI, selective serotonin-reuptake inhibitor; RSI, resting state inhibition; SDI, state-dependent inhibition; GBR 12909, vanoxerine.

cause of the shared properties of inhibition, it has been assumed that the underlying mechanism must be the same (Ogata and Narahashi, 1989; Kuo and Bean, 1994; Nau et al., 2000; Yang and Kuo, 2002; Wang et al., 2004). However, as we have shown (Mike et al., 2003, 2004), use-dependent inhibition can also be explained by an alternative mechanism, in which the slow-inactivated state has the highest affinity to the drug, and the onset rate of inhibition is limited not only by slow association to inactivated channels (a binding reaction) but also by slow inactivation (a gating transition). Our aim, therefore, was to clarify the mechanism of sodium channel inhibition by the tricyclic antidepressant agent desipramine and to extend the investigation to a chemically unrelated antidepressant, the SSRI fluoxetine, because the interaction of this latter compound with sodium channels has not been studied in detail previously.

Materials and Methods

All experimental procedures were approved by the local ethics committee and were in accordance with National Institutes of Health guidelines. Pregnant rats (17–18-day gestation) were anesthetized with a mixture of ketamine (50 mg/ml) and xylazine (10 mg/ml). The uterus was dissected out, placed in a laminar airflow box, and kept sterile. Individual fetuses were isolated; their whole brains were put into ice-cold minimal essential medium, and kept there during further dissection. Hippocampi of four to six fetuses were dissected out, incubated in 0.25% trypsin for 10 min, mechanically dissociated in minimal essential medium containing 10% fetal bovine serum, and plated at a density of 150 to 300 $\times$ 10^3 per 35-mm Petri dish (precoated with poly-L-lysine, 2 μg/ml). At 24 h after plating, the medium was replaced with B27-supplemented Neurobasal medium (Invitrogen, Carlsbad, CA), containing 25 μM 2-mercaptoethanol, 0.5 mM glutamine, and 25 μM glutamate. Half of the medium was changed twice a week thereafter to the same medium (i.e., Neurobasal + B27) without glutamate. Electrophysiological experiments were performed on neurons cultured for 7 to 24 days. Chemicals used for culture, and experiments were obtained from Sigma, unless otherwise mentioned.

Transmembrane currents were recorded by whole-cell or outside-out patch configurations of the standard patch-clamp technique (Hamill et al., 1981) using an Axopatch 200B amplifier and the pClamp software (Molecular Devices, Sunnyvale, CA). Borosilicate glass patch pipettes (1.4–3.8 MΩ) were coated with Sylgard (Dow-Corning, Midland, MI) to minimize capacitance. Series resistance was in the range of 3.5–9 MΩ (recordings with series resistance values exceeding 9 MΩ were excluded from analysis), and was compensated to 60–80%. The same relatively large-diameter pipettes were used for outside-out macropatches. Experiments were performed at room temperature (22°C). Pipettes were filled with an intracellular solution of the following composition: 70 mM CsCl, 70 mM CsF, 10 mM NaCl, 10 mM HEPES, and 10 mM Cs-EGTA; the pH was adjusted to 7.3 with CsOH. The composition of the external solution was 150 mM NaCl, 5 mM KCl, 1.4 mM $CaCl_2$, 10 mM glucose, and 5 mM HEPES; pH was adjusted to 7.3 with NaOH. Because decreased external sodium concentration was shown to modify the rates and equilibria of both fast and slow inactivation, we avoided partial substitution of external sodium ions. Instead, all major results found in whole-cell experiments were repeated in outside-out patches at only one of the drug concentrations (30 μM) to confirm that drug-induced changes are not affected by errors of inadequate voltage control. Currents were low-pass–filtered at 10 kHz and sampled at a rate of 100 kHz.

Drug application was performed using an improved version (Mike et al., 2004) of the dual U-tube method (Mike et al., 2000). Solution exchange time constants were in the 1–20-ms range and were not dependent on the duration of drug application.

Subtraction of leak and capacitive artifacts was performed off line, using a standard P/n protocol. Stability of passive properties was monitored by comparing successive responses with test voltage pulses applied at the end of each voltage protocol. Data in which significant shift of passive properties was recorded were not used for analysis.

Curve fitting was performed by the Solver function of Microsoft Excel. Time constants τ, or τ_1 and τ_2 were extracted from monoexponential or biexponential equations: $I(t) = (I_{max} - I_{min}) \times \exp(-t/\tau) + I_{min}$ and $I(t) = (I_{max} - I_{min}) \times [A_1 \times \exp(-t/\tau_1) + A_2 \times \exp(-t/\tau_2)] + I_{min}$, where A_1 and A_2 are the contribution of components to the amplitude. Concentration-inhibition curves were fit to the Hill equation: $I = I_{control}/[1 + ([D]/IC_{50})^{n_H}]$, where [D] is the drug concentration, IC_{50} is the concentration that causes 50% inhibition, and n_H is the Hill coefficient.

Statistical significance was determined using unpaired Student's t test or analysis of variance followed by Tukey-Kramer multiple comparisons test; $p < 0.05$ was considered significant. Results are presented as mean ± S.E.M. (unless otherwise noted) and the number of cells tested (n).

Results

Concentration-Inhibition Relationship at Different Holding Potentials. Both fluoxetine and desipramine inhibited sodium currents in a concentration-dependent manner. The potency of antidepressants was dependent on the holding potential but also influenced by other parameters of the stimulation protocol, such as the frequency of depolarizations or pulse duration (due to the use-dependent nature of inhibition).

Onset of drug action was monitored by 10-Hz trains, each consisting of 10 depolarizations. The holding potential was varied during the experiment according to the pattern illustrated in Fig. 1A, bottom. It was of the following values: −150, −120, −90, or −60 mV. For all holding potentials, sodium currents were evoked by 10-ms pulses to −20 mV. Interpulse interval was thus 90 ms, whereas intertrain interval was 9 s. Increasing the intertrain interval to 19 s had no effect on the extent or the time course of inhibition by the drugs (data not shown), which argues against the possibility that use-dependence is caused by state-dependent access to the binding site.

From −150 to −90 mV, the amplitude and kinetics of single depolarization-evoked currents were similar. Amplitudes of currents evoked from holding potentials −120, −90, and −60 mV were 100 ± 0.9, 91 ± 1.5, and 35 ± 3.1% of the amplitudes evoled from −150 mV, respectively (calculated from the first depolarization of the last trains at each holding potential). Currents evoked from −120 and −150 mV were of the same amplitude; the small (~9%) decrease observed with currents evoked from −90 mV was already significant ($p < 0.01$), whereas at −60 mV, already substantial inactivation was present. The relative amplitude of the currents evoked by the second, third, etc., depolarization differed more, depending on the holding potential, because of the voltage dependence of the recovery rate from inactivation. For holding potentials −150, −120, −90, and −60 mV, the tenth/first amplitude ratio of last trains was 0.98 ± 0.002, 0.94 ± 0.007, 0.88 ± 0.011, and 0.82 ± 0.017, respectively (n = 41; all differences between pairs of groups were significant, except −90 versus −60 mV). Because at this high number of cells (n) the S.E.M. value may be misleading; the 90% confidence interval values (supposing normal distribution) are more in-

formative: 0.96–1.01, 0.87–1.02, 0.76–1.00, and 0.64–1.01, for −150, −120, −90, and −60 mV holding potentials, respectively. The protocol used for studying the holding potential dependence can be seen in Fig. 1A. For illustration, a cell with a larger-than-average decay within trains was chosen so that alteration of peak amplitudes within trains (Fig. 1B) could be better seen. Drug application was started at −120 mV holding potential; before and after that, the membrane was kept at different potentials for 40 s (or 50 s in the case of −60 mV). Washout was monitored using the same protocol (Peak amplitudes from a whole experiment, including both onset and washout, are shown in Fig. 3B).

At strongly hyperpolarized membrane potential values (−150 and −120 mV), the inhibition had a tonic and a phasic component. Development of the tonic component is best seen at the amplitudes of currents evoked by the first pulses of each train at −120 mV before and during drug application (Fig. 1A, see the highest points of each group from f to g).

The tonic component at strongly hyperpolarized membrane potential values (−150 and −120 mV) probably reflects association to resting state (note that a significant inhibition is already present at the very first evoked current after start of fluoxetine perfusion); therefore, we will call this component "resting state inhibition" (RSI). Because RSI was not use-dependent (not dependent on intertrain interval, as we have discussed), it was most likely due not to stabilization of an inactivated conformation but possibly to steric occlusion of the conduction pathway. Phasic inhibition at strongly hyperpolarized potentials (Fig. 1A, compare f and g or b and h; the inhibition within a train was augmented in the presence of the drugs), on the other hand, was probably due to the increased affinity of antidepressants to inactivated states (it was caused either by an increased association of the drug during the 10-ms depolarized periods or by stabilization of the drug-bound inactivated state, which hindered recovery during the interpulse periods). This component, which developed because of the preferential affinity toward inactivated states, will be called "state-dependent inhibition" (SDI). At −90 mV, the tonic component of drug-induced inhibition was not entirely attributable to RSI; rather, it is a mixture of RSI

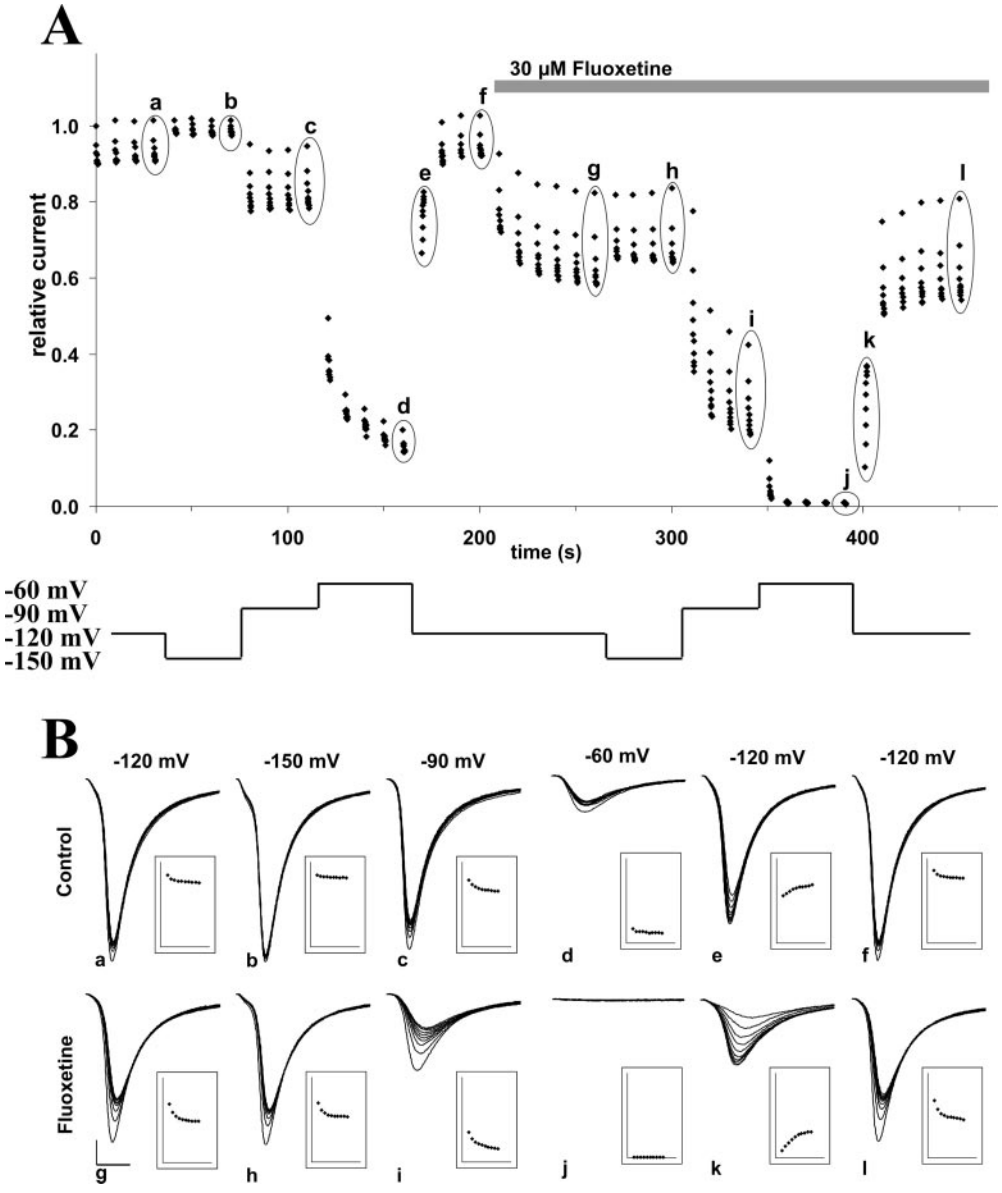

Fig. 1. Example for the holding potential-dependent inhibition of sodium channels caused by the antidepressants. The effect of 30 μM fluoxetine. A, peak amplitudes of currents evoked by 10-Hz trains of depolarizations delivered every 10 s during the experiment, whereas holding potentials were varied as shown at the bottom of the figure. Letters (from a to l) mark amplitudes for which the corresponding currents are shown in B. Amplitudes are expressed as relative to the amplitude of the first evoked current. B, individual currents evoked by trains of depolarizations. Letters (from a to l) indicate their position during the experiment as seen in A. Insets show peak amplitudes of individual trains on an expanded time scale. Top, control; Bottom, in the presence of 30 μM fluoxetine. Scale bars, 1 nA, 1 ms.

and SDI. Whenever the holding potential allowed a fraction of the ion channel population to inactivate, the component of SDI developed, and it became more dominant with both increased depolarization and increased concentration of the drugs. In Fig. 1, SDI is reflected both by the enlarged phasic inhibition within a train (f versus g, b versus h, or c versus i) and by the enlarged tonic component at −90 and −60 mV holding potentials (e.g., see the first amplitudes at −90 mV in the presence of fluoxetine). Under physiological conditions (~−60 mV), SDI was the major component of antidepressant-induced inhibition. The exact mechanism of this component is the main subject of this study.

SDI can be due either to state-dependent affinity or to state-dependent accessibility of the binding site. The former hypothesis, called the "modulated receptor model" (Hille, 1977), supposes that association is possible to all conformational states of the channel, but with a different affinity. The latter hypothesis, the "guarded receptor model" (Starmer et al., 1984), assumes that only certain states are accessible for drug binding.

Because the onset of SDI did not depend on previous activation, and the extent of SDI was dependent on the frequency and not the number of pulses (data not shown), we found that the modulated receptor model was more adequate in explaining our experimental findings than the guarded receptor model. Therefore, throughout the description of our results, we will propose hypotheses within this conceptual framework.

Repeated trains of depolarizations from holding potentials −150, −120, and −90 mV evoked similar successive groups of currents, indicating that at these holding potentials, the equilibrium was reached within 9 s (i.e., during the time between two trains). At −60 mV holding potential, however, the amplitude was progressively decreasing, and equilibrium was not reached within 50 s (in 98 of 98 cells). The time constants of both fast inactivation and recovery from fast inactivation were in the range of 0.2 to 20 ms (see below), so equilibrium between resting and fast-inactivated states should have been reached within a few hundred milliseconds. This indeed was indicated by the fact that the exponential fit of the decrease of amplitude within the train (Fig. 1B, insets) gave time constants in the range of 136–204 ms. The fact that the equilibrium was not reached as fast as expected (based on the time constants of fast inactivation and recovery from fast inactivation) revealed that under control conditions at −60 mV, slow inactivation did take part in determining the steady-state conformational equilibrium, and thus the availability of ion channels for activation.

In the presence of both antidepressants, prolonged development of equilibrium was observed already at more negative membrane potentials: at −90 mV at a 3 μM concentration of either drug, it was observable in roughly half of the experiments (five of nine cells), and, at higher concentrations, in all (34 of 34) cells. At −120 mV, it was apparent in all cells at 100 μM concentration, and in four of nine cells at 30 μM, which may indicate that antidepressants promoted the appearance of slow inactivation.

Summarizing the points illustrated by Fig. 1, the antidepressants caused a low potency inhibition (RSI) at resting conformation of the ion channels, most probably by steric occlusion of the pore. Upon depolarization, a high-potency SDI developed. Properties of state dependence in the case of these drugs suggest that it can be explained by the modulated receptor model rather than the guarded receptor model. Under control conditions, occurrence of slow inactivation is observed only at −60 mV holding potential, whereas in the presence of the antidepressants, slow inactivation seems to be promoted, being present at more negative holding potentials as well.

Figure 1 suggests that slow inactivation seems to occur at more negative potentials in the presence of the drugs (e.g., at −120 and −90 mV in the presence of 30 μM fluoxetine in Fig. 1). Therefore, we have to ask whether this indicates accelerated entry into and slower recovery from this state. The traditional view explains this phenomenon solely by slow and membrane potential-dependent association of the drugs. In subsequent sections of this article, we challenge this oversimplified explanation and investigate whether preferential affinity to the slow-inactivated state was an essential element of sodium channel inhibition by antidepressants.

Concentration-inhibition curves are shown in Fig. 2. The extent of drug-induced inhibition was calculated from the amplitude of the current evoked by the first depolarization of the last train at each holding potential (in the example shown in Fig. 1, these relative amplitudes are shown by the points right below letters b, c, d, and f in control and h, i, j and l in the presence of fluoxetine). Note that the curves constructed this way mostly reflect RSI at holding potentials −150 and −120 mV but predominantly reflect SDI at −90 and especially −60 mV. The potency of both drugs was thus profoundly dependent on the holding potential. The IC_{50} values for fluoxetine were 107.9 μM (−150 mV holding potential, n_H = 1.19), 74.1 (−120 mV, n_H = 1.39), 23.9 (−90 mV, n_H = 1.45), and 1.11 μM (−60 mV, n_H = 0.90). For desipramine the following IC_{50} values were found: 83.4 (−150 mV, n_H = 1.11), 56.7 (−120 mV, n_H = 1.40), 33.3 (−90 mV, n_H = 1.21), and

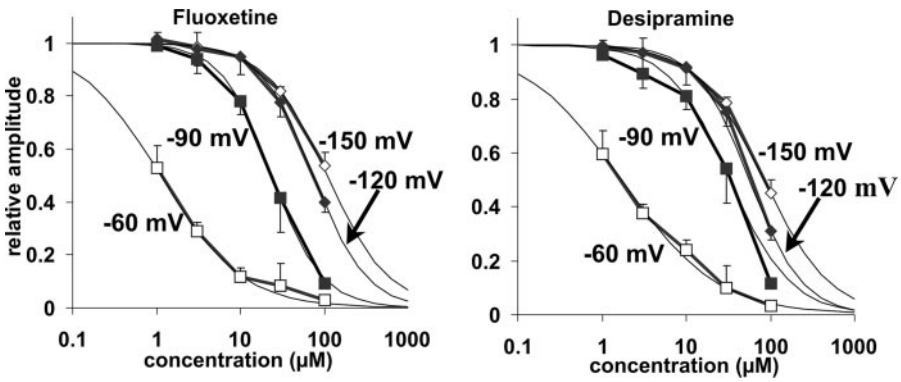

Fig. 2. Concentration − inhibition curves for fluoxetine (left) and desipramine (right) at holding potentials −150, −120, −90, and −60 mV. Each data point is an average of four to six individual measurements. Thin lines show curves obtained by fitting the Hill equation (see *Materials and Methods*) to data.

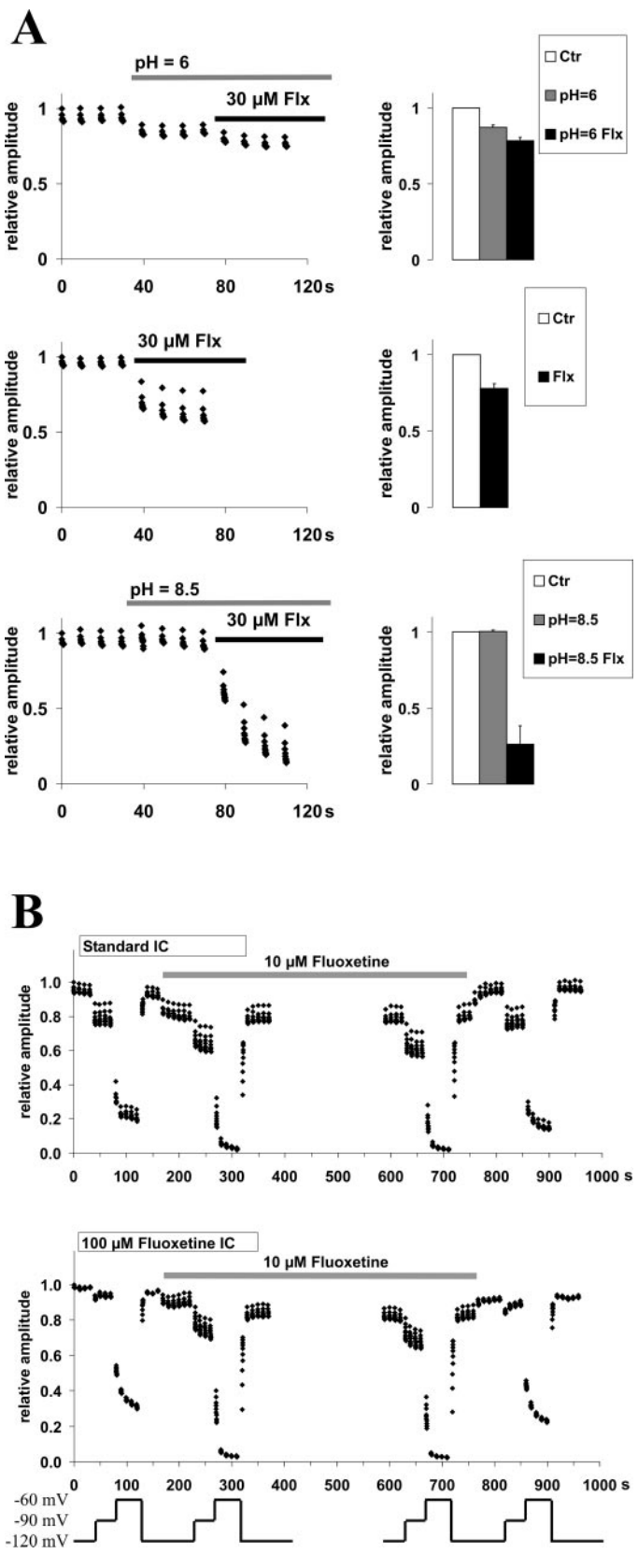

Fig. 3. Experiments on the accessibility of the binding site. A, the effect of changing the pH of the extracellular fluid on sodium channel inhibition by fluoxetine. Left, examples for plots of peak amplitude values as a function of time, obtained using the 10-Hz protocol (the holding potential was −120 mV). Right, mean ± S.E.M. values for $n = 4$ to 6 individual cells. Top, pH was lowered to 6.0 before fluoxetine perfusion. Middle, control (pH = 7.3). Bottom, pH was increased to 8.5 before fluoxetine perfusion. B, example for the lack of inhibition by intracellularly applied 100 μM fluoxetine. Top and bottom, plots of peak amplitudes obtained by the 10 Hz protocol as described at Fig. 1 (only this time both onset and offset are shown; furthermore, −150-mV holding potential was not investigated—see bars below figures indicating the holding potential). Top, ("Standard IC"), the intracellular solution contained no fluoxetine. Bottom, ("100 μM Fluoxetine IC"), 100 μM fluoxetine was dissolved in the intracellular solution. No significant difference in the extent of inhibition was observed.

1.68 μM (−60 mV, $n_H = 0.76$). The inhibitory effect of antidepressants was substantially influenced by the membrane potential, especially between −90 and −60 mV, which means, on the one hand, that the drugs were very potent around the resting membrane potential and, on the other hand, that the inhibition was most voltage-sensitive in this membrane potential range.

Accessibility of the Binding Site. Inhibition was more potent at depolarized membrane potentials. However, because drugs were applied from the extracellular side, this seems to be in contradiction with the fact that the majority of both drugs are in their positively charged forms at pH 7.3 and would only be consistent with the idea that the drugs entered a binding site from the intracellular side (after permeating the membrane). To investigate this possibility, we studied the accessibility of the antidepressant binding site from the extracellular and intracellular sides.

One possible approach to study the accessibility is manipulation of the extracellular pH. Fluoxetine and desipramine both contain a tertiary amine nitrogen that is positively charged in neutral pH (p*K*a values for fluoxetine and desipramine are 10.3 and 9.9, respectively). Alkalinization of the extracellular fluid increases the ratio of the neutral form of fluoxetine (from 0.1% at pH 7.3 to 1.55% at pH 8.5), thus helping to overcome a supposed hydrophobic barrier. Acidification, on the other hand, practically eliminates the neutral form (0.005% at pH 6.0). We compared the inhibition caused by 30 μM fluoxetine at three different pH values: 6.0, 7.3, and 8.5 (Fig. 3A). Acidification of the external fluid in itself caused a small inhibition of sodium currents that was probably due to channel blocking properties of hydrogen ions (Woodhull, 1973). Control amplitude of currents (relative to the amplitude measured at pH 7.3) was 0.87 ± 0.02 at pH 6.0 but 1.00 ± 0.01 at pH 8.3. The tenth/first amplitude ratio was not changed significantly. These results indicate that the gating of channels in itself was not affected noticeably. (A significant effect on the recovery rate from either the fast- or slow-inactivated states, as well as on the onset rate of slow inactivation, would have been obviously visible when the 10 Hz protocol was used; a change in the inactivation—recovery from inactivation equilibrium would have altered the ratio of amplitudes.) In the presence of 30 μM fluoxetine, the amplitude of the first current of the train (which reflects RSI) was 78.0 ± 3.0% of the control amplitude at pH 7.3; the inhibition significantly increased at pH 8.5 (26.10 ± 12.4% of the control) and decreased at pH 6.0 (90.2 ± 0.6% of the control) ($n = 3$ to 6; $p < 0.01$ and $p < 0.05$ for pH 8.5 and 6.0, respectively). Ratios of tenth/first amplitudes changed similarly, indicating that SDI became larger at pH 8.5 and smaller at pH 6.0; tenth/first amplitude ratios at pH 7.3, 8.5, and 6.0 were 0.70 ± 0.02, 0.28 ± 0.08, and 0.94 ± 0.01, respectively. These results suggest either that the active species is neutrally charged and thus may have significant hydrophobic interac-

tions with its binding site or that fluoxetine molecules have to overcome a hydrophobic barrier on their path to the binding site.

For potassium channels, it has been proposed that fluoxetine acts as an open channel blocker from the inside of the cell (Choi et al., 2004). To test this possibility, we studied the effect of high concentration of fluoxetine applied intracellularly. When 100 μM drug was dissolved in the pipette solution and recording of sodium currents was started within 5 s after break-in (i.e., reaching whole-cell configuration), no significant decrease of sodium currents was observed during diffusion of the pipette solution into the cell; peak amplitude at 4 min was 100.6 $\pm$ 0.8% of the peak amplitude at $<$5 s (n = 8). Under control conditions (no fluoxetine in the pipette), the same percentage was 100.5 $\pm$ 0.1% (n = 8). In contrast, when 1 mM QX314 (the lidocaine derivative sodium channel inhibitor, which is known to have an intracellular binding site) was included in the pipette, sodium currents decreased to 35.4 $\pm$ 6.7% of the amplitude evoked by the first depolarization within a few minutes after break-in [the time constant of decay was 36.0 $\pm$ 6.4 s (n = 5)]. Furthermore, when (during the continuous intracellular presence of 100 μM fluoxetine) 10 μM fluoxetine was applied extracellularly, the extent of inhibition (92.7 $\pm$ 1.5% of control at $-$120 mV, n = 6) was not significantly different from the results obtained with standard pipette solution (94.2 $\pm$ 2.9% of control, n = 4) and the onset kinetics was apparently identical. Figure 3B shows an example for the effect of 10 μM fluoxetine using the protocol described above, with either control or 100 μM fluoxetine-containing pipette solution.

The fact that intracellular application of fluoxetine was ineffective shows first of all that voltage-dependent association of the positively charged form is not a significant source of voltage-dependent inhibition. Our experiments suggest that 1) the majority of drug molecules are either neutral at their binding sites or have to overcome a hydrophobic barrier to reach it, and 2) the binding site seems to be more easily accessed from the extracellular side. As for the reason for the slow onset of inhibition, slow accumulation within the cell (implying intracellular accessibility) can be excluded.

Time-Dependent Shift of Inactivation Curves. A characteristically slow development of equilibrium was also observed while we measured inactivation curves. Three separate protocols with prepulse durations 0.4, 2, and 8 s were used (Fig. 4A). In control, inactivation curves obtained with the three protocols differed only slightly: half-inactivation voltages ($V_{1/2}$) were $-$64.02 $\pm$ 1.22, $-$64.31 $\pm$ 1.16, and $-$64.82 $\pm$ 1.14 mV (n = 20), respectively. A time-dependent leftward shift of the inactivation curve during recording was observed even without drug application (less than 5 mV). Because of this, and because of the cell-to-cell variability of $V_{1/2}$ in control, drug induced changes were expressed by the shift of $V_{1/2}$ ($\Delta V_{1/2}$), calculated from control values, both before and after drug application, as $\Delta V_{1/2} = (V_{1/2\ \mathrm{control}} + V_{1/2\ \mathrm{washout}})/2 - V_{1/2\ \mathrm{drug}}$. Using 8-s prepulses, the shifts caused by 3, 10, 30, and 100 μM fluoxetine were $-$2.82 $\pm$ 0.79, $-$8.44 $\pm$ 0.76, $-$21.50 $\pm$ 1.68, and $-$43.24 $\pm$ 1.78, respectively. In the case of desipramine, the corresponding values were: $-$3.57 $\pm$ 1.34, $-$7.66 $\pm$ 0.53, $-$14.49 $\pm$ 3.57, and $-$38.67 $\pm$ 1.46 for 3, 10, 30, and 100 μM, respectively (n = 3 to 5; $\Delta V_{1/2}$ values significantly different from zero are marked by asterisks in Fig. 4C) In contrast to control, in the presence of antidepressants, the hyperpolarizing shift markedly depended on prepulse duration. Figure 4B shows an example for the prepulse duration-dependent shift of inactivation curves in the presence of 30 μM fluoxetine and desipramine. Averaged concentration- and prepulse duration-dependence is illustrated in Fig. 4C. The difference between the shift at 0.4 s versus 8 s was significant at $\geq$3 μM fluoxetine and $\geq$30 μM desipramine (paired t test; significant differences are indicated by signs in Fig. 4C). Data presented in Fig. 4 were obtained by whole-cell measurements. Excision of the membrane patch in itself caused a substantial (12.6 $\pm$ 0.43 mV, n = 8) leftward shift in the steady-state inactivation curve (the reasons and mechanism of which were not addressed in this study). Nevertheless, the degree of drug-induced shift was similar in outside-out patches. In the pres-

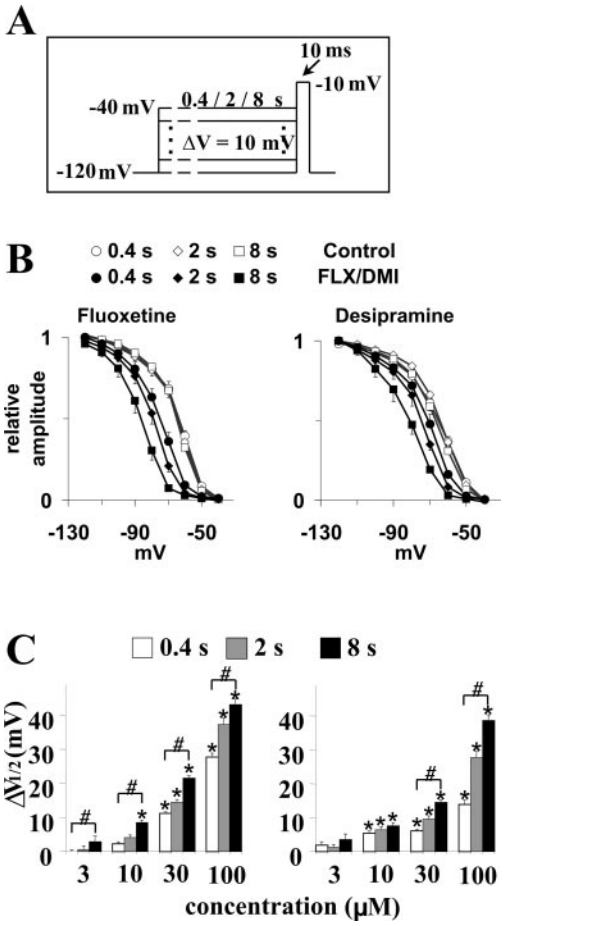

Fig. 4. Effect of fluoxetine and desipramine on steady-state sodium channel inactivation. A, voltage protocol used for the study. Prepulse duration was 0.4, 2, or 8 s. B, example for the prepulse duration-dependent shift of inactivation curves in the presence of 30 μM fluoxetine (left) and 30 μM desipramine (right). Current amplitudes are expressed as relative to the amplitude evoked from a $-$120-mV holding potential. Whereas in the control (open symbols) no significant difference was observed depending on the time provided for equilibration, in the presence of antidepressants (filled symbols), the shift was larger with longer time for equilibration (n = 4 for each data point). C, summary of results regarding the concentration-dependent and prepulse duration-dependent shift of inactivation curves. $\Delta V_{1/2}$ values were calculated for each individual cell. Each column is the average of measurements from n = 4 to 6 cells. Significant differences (compared with control obtained using the same prepulse duration) are marked by asterisks. The level of significance is not indicated; it ranged from $p < 0.05$ to 0.001. Significant difference between results with prepulse durations 0.4 and 8 s was found at all concentrations, as indicated by signs.

ence of 30 μM fluoxetine ($n = 10$), it was 6.18 ± 1.63, 8.27 ± 2.19, and 10.67 ± 3.23 mV; 30 μM desipramine caused 5.00 ± 2.16, 4.50 ± 2.99, and 8.25 ± 4.48 mV ($n = 4$) shift of $V_{1/2}$, for 0.4-, 2-, and 8-s prepulse durations, respectively.

Shift of the inactivation curve can be caused by stabilizing either the fast- or the slow-inactivated state. The slow onset of the shift could be explained in both cases: by slow gating (if the slow-inactivated state is stabilized) or by slow association (if the fast-inactivated state is stabilized).

Gating Transition Rates in the Presence of Antidepressants. Both fast and slow inactivated states can be stabilized by either accelerating transition into the relevant inactivated state or reducing the rate of recovery from it. We studied, therefore, the effect of antidepressants on the relevant gating transition rates.

During the discussion of drug effects on gating transition rates, however, we need to bear in mind that affinity is assumed to be state-dependent. Therefore, if we do experiments in the continuous presence of the drugs, we will see not only the effect of voltage on gating but also the combined effects of voltage on gating and binding. The interpretation of the results, therefore, is not straightforward, as we will discuss.

Fast-Inactivated State. In previous studies with the tricyclic antidepressant imipramine, high concentrations of the drug (30–300 μM) were shown to accelerate the decay phase of sodium currents (Yang and Kuo, 2002), whereas low (but already effective) concentrations (2–5 μM) have no effect on it (Ogata and Narahashi, 1989). Possible mechanisms for acceleration of the decay phase are rapid association during activation, which results in either steric occlusion of the conduction pathway (channel block), or acceleration of inactivation. (Detectable association must happen within the time window in which a significant fraction of the channels is open, and it must either block open channels, or promote inactivation. This mechanism requires a high association rate: $\sim 1 \times 10^7$ $M^{-1}s^{-1}$. Alternatively, acceleration of the decay phase may happen with a mechanism that does not require this high association rate, if we assume that drug-bound channels can conduct. Because the drug associates even to resting channels (although with a lower affinity), in the presence of the drug, a fraction of the channels will be drug-bound even at hyperpolarized membrane potential. When the process of activation and inactivation is started by depolarization, drug-bound channels already inactivate with the increased rate inflicted upon them by the drug molecule.

We studied the decay phase of evoked sodium currents in both whole-cell and outside-out patch measurements. Activation time and decay kinetics were significantly faster in outside-out patches, whereas the voltage-dependence of activation did not differ significantly. In neither of the two configurations did any of the antidepressants significantly alter the decay time constant of sodium currents at any concentration (1–100 μM). Because outside-out patch data reflect channel kinetics more faithfully, we illustrate antidepressant effect using results obtained in this configuration (Fig. 5A). Depolarization-evoked currents are illustrated on the left in Fig. 5A. The first 1.5 ms of decay was sufficiently well fit by a monoexponential equation; time constants varied between 0.28 and 0.67 ms (0.42 ± 0.048 ms) in control and were not significantly changed in the presence of up to 30 μM fluoxetine ($\tau_{Flx}/\tau_{Ctr} = 0.976 \pm 0.045$ paired t test, $p = 0.934$, $n = 6$) or 30 μM desipramine ($\tau_{DMI}/\tau_{Ctr} = 1.003 \pm 0.098$ paired t test, $p = 0.979$, $n = 4$) (Fig. 5A).

We also measured the recovery from fast inactivation. A standard double-pulse protocol was used. Activation and inactivation were evoked by the first 10-ms depolarization to −20 mV (this duration is enough to produce >99% inactivation). After an interpulse interval, the extent of recovery was tested by a second identical depolarization. The length of the interpulse interval was varied between 0.25 and 512 ms; the cells were kept at −150-mV holding potential during this time. The ratio of the second (test pulse-evoked) and the first (control pulse-evoked) current amplitudes was calculated and plotted as a function of interpulse interval duration. The average of recovery curves ($n = 13$) were fit by a biexponential function. The fast time constant, which contributed 89.4% of the total amplitude, was 1.16 ms; the slow time constant was 24.2 ms. There was no significant change in the fast time constant of recovery at any of the concentrations from 1 to 30 μM (Fig. 5B). Values were in the range of 1.00 to 1.22 ms, contributing 85.7 to 92.0% of the total amplitude. Slow time constants showed a concentration-dependent tendency to increase (up to ~100 ms), but this did not cause significant change in the overall shape of recovery curves, due to this component's small contribution to the amplitude. (Current amplitudes shown in Fig. 5, B–D were normalized to the current evoked by the first pulse in the presence of the drug. Therefore, the figure does not show the reduction of amplitude, which was significant in the presence of 10 and 30 μM concentrations of either of the drugs.) In summary, neither the onset of fast inactivation nor the recovery from fast inactivation was significantly affected by antidepressants.

Slow-Inactivated State. To study slow inactivation, a similar double-pulse protocol was used, consisting of two depolarizations (to −20 mV) from a hyperpolarized (−150 mV) holding potential (Fig. 5C, inset). The current evoked by the first depolarization served as a control; the duration of this depolarization—which was intended to evoke slow inactivation—was varied between 0.25 and 4096 ms. Before the second depolarization, the membrane was held for 10 ms at hyperpolarized holding potential (−150 mV); this duration was found to be enough for nearly full (85–95%) recovery from fast inactivation. The extent of slow inactivation was estimated by the ratio of the currents evoked by the second and first depolarizations. Data points were fit by biexponential equations. The fast time constant was 9.83 ms in control; it was responsible for only 8.0% of the decrease in amplitude. This component probably included residual fast inactivation (the fraction of fast-inactivated channels that could not recover within 10 ms) and intermediate forms of inactivation. [A form of inactivation with an intermediate onset and offset kinetics has been shown to be responsible for a small fraction of inactivation in skeletal muscle sodium channels (Kambouris et al., 1998). In addition, a rapid onset-slow offset form of inactivation has been observed in pyramidal cells (Mickus et al., 1999).] The slow time constant, which probably corresponded to slow inactivation, was 2347 ms in control; considerable (>25%) slow inactivation took place only during depolarizations longer than 1 s (Fig. 5C). In the presence of ≥3 μM fluoxetine and ≥10 μM desipramine, the time-dependent decrease in availability was significantly accelerated, as shown by a marked and concentration-dependent decrease in the slow time constant (paired t test, based on fits to individ-

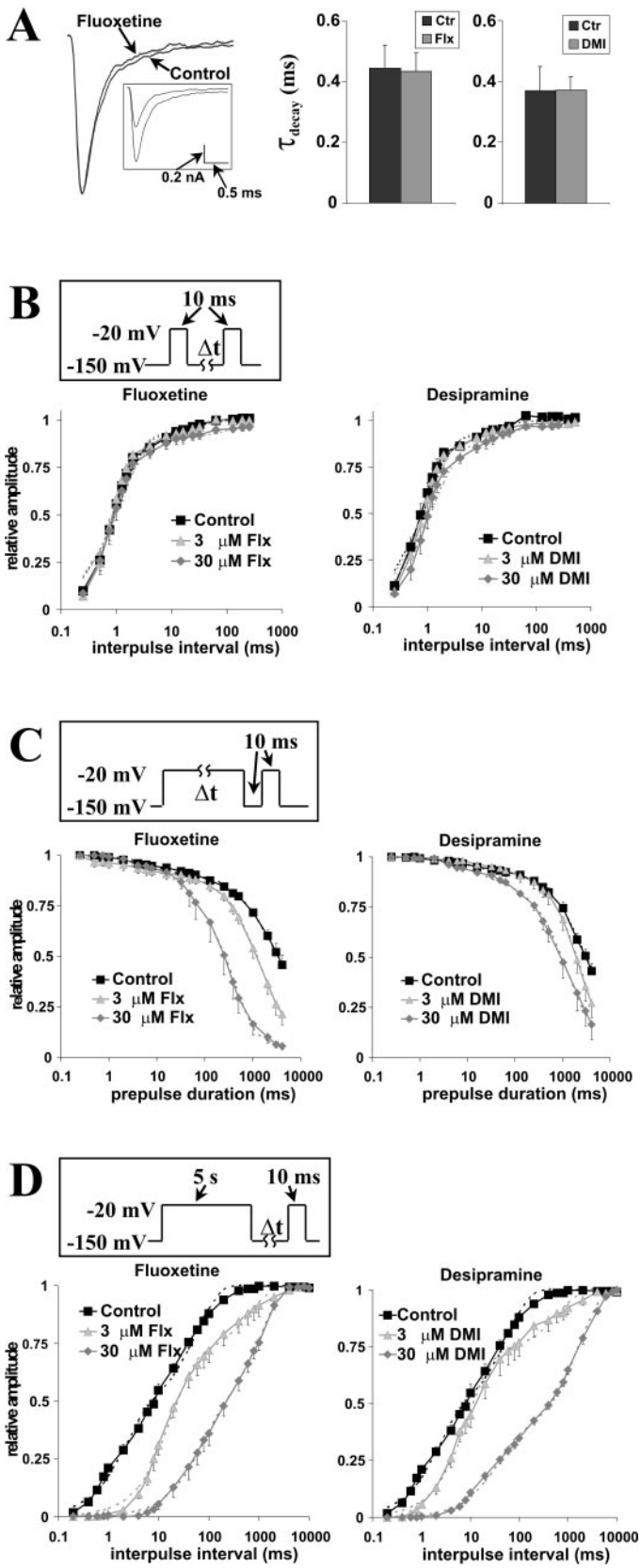

Fig. 5. Modification of gating kinetics by antidepressants. A, decay rate (which reflects the onset of fast inactivation) is not significantly affected by antidepressants. Left, example for a depolarization-evoked sodium current in the absence and presence of 30 μM fluoxetine. The current evoked in the presence of fluoxetine was scaled up to match the amplitude of control current. Inset shows currents with the original amplitudes. Right, average of decay time constants in the absence (Ctr) and presence (Flx and DMI) of the drugs. Single exponentials were fit to the decay phase of currents. B to D, different aspects of gating kinetics was studied using double-pulse protocols. Control data, as well as results obtained in the presence of 3 and 30 μM concentrations of either fluoxetine (left) or desipramine (right) are shown. Data points are mean of three to seven individual measurements; error bars show S.E.M. Dashed lines show biexponential curves fit to data. The double-pulse voltage protocol used in that particular series of experiments is illustrated in boxes. Amplitudes were normalized to the amplitude of current evoked by the first pulse. B, recovery from fast inactivation is not affected significantly by the drugs. Interpulse intervals were varied between 0.25 and 512 ms. C, onset of slow inactivation is accelerated in the presence of fluoxetine and desipramine. First pulse duration was varied between 0.25 and 4096 ms. D, rate of recovery from slow inactivation is reduced in the presence of fluoxetine and desipramine. Interpulse intervals were varied between 0.2 ms and 10 s.

ual measurements). Slow time constants of biexponential equations fit to mean data were 2347.3, 1493.7, 647.4, and 418.2 ms in the presence of 1, 3, 10, and 30 μM fluoxetine, respectively, and 2817.5, 2941.6, 2226.2, and 1234.2 in the presence of 1, 3, 10, and 30 μM desipramine, respectively. Neither the fast time constants nor the proportion of decrease for which they were responsible changed significantly. Fast time constants ranged from 3.4 to 14.1 ms and the corresponding relative amplitude from 4.6 to 12.5%; neither showed any concentration-dependent tendency.

Recovery from slow inactivation was investigated using a protocol similar to that used for recovery from fast inactivation, except that the duration of the first depolarization was 5 s (instead of 10 ms) (Fig. 5D, inset). The extent of recovery was measured after interpulse intervals ranging from 0.25 ms to 10 s. Results for control and for 3 and 30 μM concentrations of both drugs are shown in Fig. 5D. Biexponential equations were fit to recovery curves. Time constants (with relative contribution of the component to the amplitude) were 2.21 ms (45%) and 58.25 ms (55%) in control. The fast time constant probably reflects recovery from fast inactivation, because 5 s in the absence of drugs is only enough for ~50% slow inactivation. Both drugs markedly slowed recovery. At 3 μM, they changed both time constants by a factor of approximately 4 to 10; time constants for fluoxetine were 19.43 ms (68%) and 670.8 ms (32%) and for desipramine, 9.75 ms (72%) and 621.6 ms (28%). Either drug at 30 μM caused a roughly 15- to 30-fold reduction of time constants; for fluoxetine, they were 65.03 ms (40%) and 1059.1 ms (60%), and for desipramine, 36.07 ms (35%) and 1738.7 ms (65%). Particular time constants cannot be ascribed to specific transitions, because the process of recovery is too complex. At the beginning of recovery, the ion channel population is not homogenous but is distributed among bound and unbound fast-inactivated and slow-inactivated subpopulations. The distribution is dependent on the concentration of the drug. (We also need to be aware of the existence of multiple slow inactivated states with different recovery time constants, as we have mentioned.) Furthermore, recovery of fast-inactivated and slow-inactivated bound channels can be started either by dissociation or by recovery from inactivation, and the fraction of the subpopulation that starts with the former or the latter remains unclear.

Drug-induced changes in the time-dependent availability plots (Fig. 5, C and D) can be explained by two basic mechanisms. The conventional explanation assumes that altered rates reflect association and dissociation. However, it follows

from the modulated receptor hypothesis that state-dependent affinity and alteration of relevant transition rates mutually presume one another; i.e., we can suppose that there is no preferential affinity without altered gating rates. If this is so, a presumed preferential affinity to slow inactivated state must be reflected by altered gating transition rates. What is the source of alterations in the time-dependent availability plots? Is it only increased affinity of the drugs to fast-inactivated channels or is slow inactivation also involved? If the slow-inactivated state was also preferred by the drugs, the rates of progression and regression of inhibition would be determined not only by the increased affinity of the drugs to slow inactivated channels but also by the altered gating rates. Resolving this problem is only possible by assessing the relative affinities of the drugs to the fast- and slow-inactivated states.

Relative Affinity to Fast- and Slow-Inactivated States. The protocol we designed for studying the relative affinity to fast- and slow-inactivated states consisted of four trials. Trial 1 served as a control, trial 2 investigated the effect of slow inactivation, trial 3 examined the effect of drugs on predominantly fast-inactivated channels, and trial 4 tested the effects of drugs on predominantly slow-inactivated channels.

For assessing the association to fast-inactivated state (trials 1 and 3), we designed a protocol that provides enough time for fluoxetine to bind to predominantly fast inactivated channels but does not allow significant slow inactivation. The fraction of ion channels in the fast-inactivated state was maximized by applying three consecutive 100-ms depolarizations (this duration—at least in the absence of drugs—is not enough for significant slow inactivation), with 5-ms gaps between them (enough to reach roughly 80% recovery from fast inactivation) (Fig. 6A). The protocol obviously cannot provide a homogenous population of fast-inactivated channels: a minor fraction of channels may undergo "intermediate" (Kambouris et al., 1998) or "prolonged" (Mickus et al., 1999) inactivation, depending on the type of channels. The former was described as a separate kinetic component, both its onset and recovery rates being between characteristic rates of fast and slow inactivation, and the latter as a fast onset-slow recovery inactivation process. Nevertheless, at any type of sodium channel, the 3 × 100-ms pulse depolarization protocol results in a distribution of ion channels, where the majority (> 85%) is in the fast-inactivated state. Drug application (trial 3) was provided throughout this short train. The extent of inhibition was tested 50 ms after the end of the third pulse. This interval, spent at −150 mV, was enough for almost full (96.4 ± 1.1%) recovery in the absence of drugs (trial 1). This was meant to be so, because trial 1 served as a control for trials 2, 3, and 4. To compare association to fast-inactivated state with association to slow-inactivated state, an identical protocol was applied, except that it was preceded by a long (20-s) depolarization (Fig. 6A, "S"). This protocol was performed both without (trial 2) and with drug application (trial 4). To summarize the protocol, the four trials were the following: 1) control (no drug application) with only the 3 × 100-ms pulse train of depolarization; 2) control with the 20-s depolarization right before the short train; (3) the 3 × 100-ms pulse train with concurrent drug application; and 4) the 20-s depolarization followed by the short train and concurrent drug application. Note that drug application in both trial 3 and 4 was only during the 310 ms of the short train. Peak amplitude values of the four trials are marked as F_{Ctr}, S_{Ctr}, F_{drug}, and S_{drug}, respectively. The depolarizing prepulse was chosen to be this long to make a significant fraction of ion channels reach slow inactivated state. The relatively long recovery interval (50 ms) was needed, because

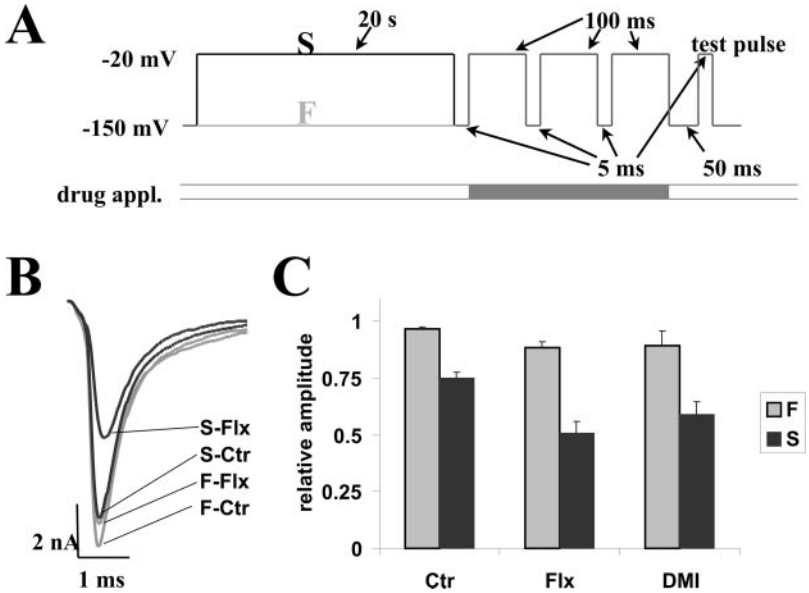

Fig. 6. Test of association to fast- versus slow inactivated channels. A, voltage and perfusion protocol. Four consecutive trials were performed. In trials 1 (gray line) and 2 (black line), the effect of voltage protocols was tested in the absence of the drug. In trials 3 and 4, the same voltage protocols were repeated while drug was perfused during the train of three consecutive 100-ms depolarizations (for a total time of 310 ms; indicated by the shaded area). The inhibition caused by drug perfusion was significantly larger after the 20 s depolarization (trial 4 versus 2) than without depolarization (trial 3 versus 1). B, example for the effect of 30 μM fluoxetine. F-Ctr, trial 1; S-Ctr, trial 2; F-Flx, trial 3; S-Flx, trial 4. C, averaged amplitudes of test-pulse-evoked currents measured without drug application (Ctr) and after brief application of fluoxetine (Flx) or desipramine (DMI). Current amplitudes were tested after the protocol which favors either the fast-inactivated (F, gray columns) or the slow-inactivated (S, black columns) state. Current amplitudes were normalized to the current evoked in trial 1 by the first depolarization of the 3 × 100-ms train.

we wanted to study the ratio of drug-bound, slow-inactivated channels; during this interval, nearly 95% of fast-inactivated channels recovered, even in the presence of 30 μM drug (Fig. 5B), whereas from drug-bound, slow-inactivated channels, only ~25% recovered (Fig. 5D). This interval was enough even for the majority of unbound slow-inactivated channels to recover (Fig. 6 and see below). Differences in the extent of recovery using this protocol, therefore, most sensitively reflected differences in the occupancy of the drug-bound, slow-inactivated state. (After the 20-s depolarization, the 3 × 100-ms depolarization did not cause further slow inactivation; in fact, a slight recovery from slow inactivation occurred, because during the 5-ms spent at −150 mV, more channels recovered from slow inactivation than the number of channels that slow inactivated during the 100-ms depolarization.)

The effect of the protocol on sodium currents is illustrated in Fig. 6B; relative amplitudes of currents for control (no drug applied), fluoxetine, and desipramine are shown in Fig. 6C. All amplitude values are expressed as relative to the current evoked by the first 100-ms depolarization in trial 1.

For fluoxetine and desipramine, drug-induced inhibition was 32.5 and 21.5%, respectively, when the 20-s prepulse was included (S_{drug}/S_{Ctr}; ratio of black columns in Fig. 6C) but only 8.4 and 7.4% when the prepulse was excluded (F_{drug}/F_{Ctr}; ratio of gray columns in Fig. 6C), suggesting that the slow-inactivated state was preferred by both drugs. The difference was significant at $p < 0.001$ in the case of 30 μM fluoxetine ($n = 8$) and at $p < 0.01$ in the case of 30 μM desipramine ($n = 5$) (paired t test). This indicates that the affinity of both drugs to the slow-inactivated state was higher than to the fast-inactivated state, and the difference was obvious even with this very brief (310 ms) pulse of antidepressants. (The drug application pulse could not be made longer, because we wanted to give the drug pulse to mostly depolarized channels, and longer depolarizations would have induced too much slow inactivation.) As expected, the inhibition caused by the 310-ms pulse of antidepressants caused a smaller inhibition than when drugs were continuously perfused (Fig. 5C), but a relatively small effect of the drugs without the long prepulse (trial 3) and a relatively small effect of the long prepulse without drugs (trial 2) allowed us to detect the superadditivity of the two effects when applied together (trial 4).

Discussion

Study of the mechanism of action of antidepressants on sodium channels has thus far focused on tricyclic antidepressants. A detailed analysis of the mechanism has been performed using imipramine (Ogata and Narahashi, 1989; Yang and Kuo, 2002) and amitriptyline (Nau et al., 2000; Wang et al., 2004). All these studies suppose that fast-inactivated conformation is preferred by antidepressants. In this study, we provide evidence that the principal mechanism of use-dependent sodium channel inhibition by the tricyclic desipramine was a preferred binding to (and stabilization of) the slow-inactivated state. In addition, we investigated the SSRI fluoxetine, which is believed to be more selective than tricyclic compounds, and proved that it inhibited sodium channels by a similar mechanism and with the same potency as desipramine.

In the first four sections of *Results* (shown in Figs. 1 to 5), we introduced the hypothesis of stabilization of the slow-inactivated state as a possible alternative explanation. Already in this section, certain hints suggested that this hypothesis is more plausible than the conventional explanation.

In the last section (see Fig. 6), we directly compared affinities to fast- versus slow inactivated states and found that both antidepressants preferred slow inactivated conformation.

Let us first discuss the hints from the first four sections, which argue for stabilization of the slow-inactivated state as a likely mechanism:

1. Slow inactivation was clearly detectable at a −60-mV holding potential, even in the absence of the drugs (Fig. 1). Slow inactivated channels are sure to be present during drug perfusion too; the only question is whether this conformation is made more or less energetically stable by drug binding. Because the delayed development of equilibrium was not reduced but was seen even at more negative potentials, it seemed likely that antidepressants promoted, rather than hindered transition into the slow-inactivated state.
2. Slow inactivation upon prolonged depolarization was detected even in control (Fig. 5C).
3. Recovery curves after prolonged depolarization in this article (Fig. 5D), and in other studies, are consistently biphasic even in the absence of drugs, indicating the presence of slow inactivation.

The presence of the drug, again, is not likely to remove slow inactivation. Therefore, in the context of the modulated receptor hypothesis, it is sensible to ask how rates of slow inactivation and recovery from slow inactivation are affected by the presence of the drugs. We suggest that altered time-dependent availability plots (Fig. 5, C and D) reflect not only binding rates but also are due in part to altered gating. This concept—however logical it may be—is a novel one. Similar studies on time-dependent availability were performed using the tricyclic antidepressants amitriptyline (Nau et al., 2000; Wang et al., 2004) and imipramine (Ogata and Narahashi, 1989; Yang and Kuo, 2002); similar results were obtained, but similar conclusions have not been drawn. Assuming that the fast-inactivated state was the only one preferred by antidepressants, the authors supposed that altered rates reflect association to and dissociation from ion channels. However, if—as we propose—the slow-inactivated state is equally or even more affected by drug binding, the effects on gating transition rates also have to be taken into account.

Slow-inactivation plots (Fig. 5C) with drugs present, therefore, reflect rates of association to both fast- and slow-inactivated states, as well as the rate of transition from fast- to slow-inactivated state. These together form the overall rate of decrease in availability. It is therefore incorrect to discuss the overall effect as a rate of association.

Plots of recovery from slow inactivation (Fig. 5D) with drugs present reflect a combination of dissociation and gating (recovery from inactivation) rates. If recovery from one of the inactivated states is slow enough to be comparable with dissociation rates (which is more likely in the case of the slow-inactivated state), then it will significantly affect the overall rate of recovery. To discuss the overall rate of recovery as "rate of dissociation," therefore, is incorrect.

To summarize the latter two points, results of the double-pulse protocols can be explained by binding reactions only or by the complex interaction of binding and gating rates. Gating rates are especially likely to affect overall rates if the slow-inactivated state is preferred by the drug.

To assess the relative affinity of the two antidepressants to fast- versus slow-inactivated states, we developed a special protocol. Although the test did not allow quantitative estimation of relative affinities or association rates to different conformational states, it did demonstrate in a qualitative manner that fluoxetine and desipramine have a preference toward the slow-inactivated state.

Although stabilization of fast inactivated state used to be the established mechanism for the action of local anesthetics and sodium channel inhibitor anticonvulsants, scattered evidence has repeatedly been found for the similar importance of the slow-inactivated state (Khodorov et al., 1976; Quandt, 1988; Chen et al., 2000; Fozzard et al., 2005). This suggests that the hypothesis of preferential affinity to the fast-inactivated state as a general explanation of use-dependent inhibition may be worth re-examination in the case of other sodium channel inhibitors. The novelty in our approach is that the preference toward fast- versus slow-inactivated states can be determined by applying a relatively simple voltage- and drug-application protocol, so the question of relative affinities can be addressed without mutagenesis experiments.

We have previously proposed stabilization of slow inactivated state as a mechanism of use-dependent inhibition in the case of the dopamine reuptake inhibitor GBR 12909 (Mike et al., 2004). The mechanism of inhibition by antidepressants differs from this previously proposed mechanism in two important aspects: a very slow rate of dissociation (with a time constant of several minutes) was found in the case of GBR 12909, and conduction of drug-bound channels was not impaired. Inhibition seemed to be principally caused by stabilization of nonconducting states, not by steric occlusion of the pore.

In the case of antidepressants, the dissociation was much faster, and we had no reason to suppose that drug-bound channels could conduct. Open channel block has been previously demonstrated in the case of both imipramine and amitriptyline (Yang and Kuo, 2002; Wang et al., 2004) using inactivation deficient mutants. In these studies, a concentration-dependent acceleration of the current decay was observed. This evidence, by the way, is not fully convincing; because slow inactivation of these mutant channels was not disabled, the phenomenon could be explained in part by an acceleration of the rate of slow inactivation. The extent to which the acceleration of current decay is due to open channel block versus altered rate of slow inactivation is currently unknown.

Our results suggest that either fluoxetine binds into a hydrophobic environment or it has to cross a hydrophobic barrier to reach its binding site. Based on our results, access of the charged form of antidepressants from the intracellular side can be excluded—unlike the case of the well described local anesthetic site. This suggests that either the accessibility or the location of the binding site is different (Barber et al., 1991).

Thus far, the significance of sodium channel inhibition by antidepressants has been thought to be the cardiac side effects of tricyclic drugs (Marshall and Forker, 1982) and their analgesic potency (Namaka et al., 2004). The fact that these drugs obviously affect neuronal sodium channels at similar (supposedly therapeutic) concentrations raises the question of whether their sodium channel inhibition effect plays a role in their antidepressant action. The fact that the SSRI fluoxetine—which has a different monoamine transporter selectivity and less G protein-coupled receptor-mediated side effects than tricyclic compounds—was found to act on sodium channels with a similar mechanism and at a similar concentration range as desipramine also suggests that this effect might be of therapeutic significance. The inhibition was found to be highly sensitive to experimental parameters (holding potential, frequency, pulse duration, etc.), as well as to extracellular pH, which suggests that the inhibition in vivo is highly selective depending on local activity patterns. The identification of the mechanism of action has special importance because this makes it possible to identify the neurons and subneuronal structures likely to be most affected, the kinds of activity patterns that are more sensitive, and the ways in which neuronal information processing is altered during antidepressant treatment. In this respect, it is important to note that the susceptibility of neuronal sodium channels to slow inactivation is an important determinant of dendritic integration (Mickus et al., 1999) and is specifically targeted by G protein-coupled receptor-mediated phosphorylation (e.g., Carr et al., 2003).

The message of this study is that for a more thorough understanding of the short-term effects of antidepressants on the brain, direct effects (such as the currently studied modulation of sodium channels) can be as important as indirect (monoamine-mediated) modulation.

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