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Block of Persistent Late Na^+ Currents by Antidepressant Sertraline and Paroxetine

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Abstract Antidepressants, such as traditional tricyclic antidepressants (TCAs), are the first-line treatment for various pain syndromes. Available evidence indicates that TCAs may target Na^+ channels for their analgesic action. In this report, we examined the effects of contemporary antidepressants sertraline and paroxetine on (1) neuronal Na^+ channels expressed in GH_3 cells and (2) muscle rNav1.4 Na^+ channels heterologously expressed in Hek293t cells. Our results showed that both antidepressants blocked Na^+ channels in a highly state-dependent manner. The 50% inhibitory concentrations (IC_{50}) for sertraline and paroxetine ranged ~18–28 μM for resting block and ~2–8 μM for inactivated block of neuronal and rNav1.4 Na^+ channels. Surprisingly, the IC_{50} values for both drugs were about 0.6–0.7 μM for the open channel block of persistent late Na^+ currents generated through inactivation-deficient rNav1.4 mutant Na^+ channels. For comparison, the open channel block in neuronal hNav1.7 counterparts yielded IC_{50} values around 0.3–0.4 μM for both drugs. Receptor mapping using fast inactivation-deficient rNav1.4-F1579A/K mutants with reduced affinities toward local anesthetics (LAs) and TCAs indicated that the F1579 residue is not involved in the binding of sertraline and paroxetine. Thus, sertraline and paroxetine are potent open channel blockers that target persistent late Na^+ currents preferentially, but their block is not mediated via the phenylalanine residue at the known LA/TCA receptor site.

Keywords Antidepressant · Sertraline · Paroxetine · Voltage-gated sodium channel · Open channel block · Use-dependent block

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Introduction

Antidepressants are drugs that treat depression and anxiety disorders (Baldessarini 2001). These drugs exert diverse pharmacological actions in four categories: norepinephrine-reuptake inhibitors (NRIs), selective serotonin-reuptake inhibitors (SSRIs), atypical antidepressants and monoamine oxidase inhibitors (MOIs). Most NRIs consist of a tertiary-amine or a secondary-amine tricyclic group. These tricyclic antidepressants (TCAs), such as amitriptyline, appear to have analgesic properties and are beneficial for patients with pain syndromes (Monks and Merskey 1999). Several targets for TCAs' analgesic action have been proposed, including (1) the antidepressive pathway, (2) the adenosine pathway (Sawynok 2003) and (3) the action potential pathway (Gerner and Wang 2006; Brau et al. 2001; Pancrazio et al. 1998).

Voltage-gated Na^+ channels are responsible for the generation and propagation of action potentials in excitable membranes. Mammalian Na^+ channel isoforms contain a large α-subunit (Nav1.1–Nav1.9) and one or two small β-subunits (β1–β4) (Catterall, 2000). The α-subunit consists of four homologous domains (D1–D4), each with six transmembrane segments (S1–S6). A TCA binding site has been identified in the Na^+ channel α-subunit (Wang et al. 2004a). This TCA receptor overlaps the local anesthetic (LA) receptor, including a well-conserved D4S6 residue,

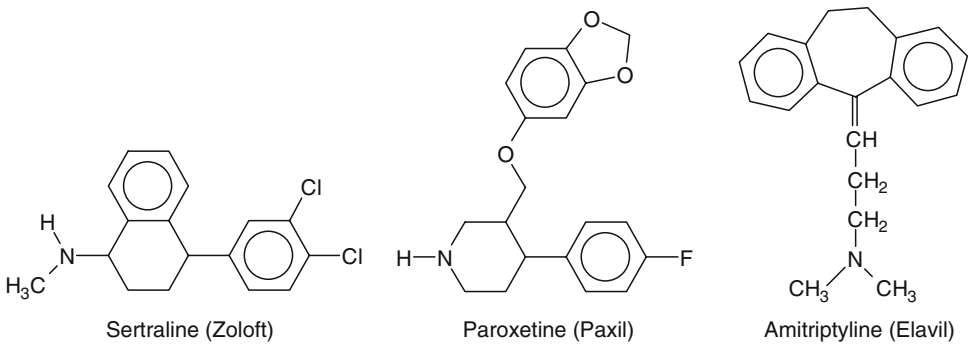

Fig. 1 Chemical structures of antidepressants: sertraline, paroxetine and amitriptyline. Both sertraline and paroxetine are secondary-amine drugs that belong to the SSRI group, whereas amitriptyline is a tertiary-amine TCA. These drugs are protonated in solution at physiological pH. U.S. trade names are in parentheses

phenylalanine (Catterall and Mackie 2001). The 50% inhibitory concentration (IC_{50}) values of TCAs for open and inactivated block of Na^+ channels are within their therapeutic plasma concentrations, suggesting that block of open and inactivated Na^+ channels in vivo may play a role in their analgesic action.

Contemporary SSRI drugs approved by the U.S Food and Drug Administration in the 1990s are also used for patients with chronic pain, but evidence for their analgesic action is scarce (Ansari 2000). Recently, using an electro-optical assay, Huang et al. (2006) found that 13 out of 18 antidepressants, including the SSRIs sertraline and paroxetine, inhibited Na^+ currents. Similarly, Dick et al. (2006) reported inhibitory action of the TCAs fluoxetine and paroxetine in resting and inactivated Nav1.7 Na^+ channels. These findings that SSRIs act as potent Na^+ channel blockers are somewhat surprising since the structures of SSRI drugs (Fig. 1) do not have a well-defined tricylic/phenyl hydrophobic moiety and an intermediate alkyl linker connecting it to the amine group like those of TCAs and LAs. Such results raise several important questions regarding the inhibitory action on Na^+ channels by SSRIs. First, do SSRIs block open Na^+ channels as potently as TCAs do? Second, are IC_{50} values of the open Na^+ channel block by SSRI drugs within their therapeutic plasma concentrations? Third, do SSRIs, TCAs and LAs share a common receptor in Na^+ channels? Fourth, is block of Na^+ channels by SSRIs isoform-specific? We therefore studied the effects of sertraline and paroxetine in neuronal GH_3 cells and in rNav1.4 wild-type, inactivation-deficient rNav1.4 Na^+ channels and their mutants with an altered LA receptor. For comparison, we also investigated the effects of these drugs in inactivation-deficient hNav1.7 Na^+ channels. The hNav1.7 isoform plays a major role in the development of acute and inflammatory pain (Nassar et al. 2004), and it has been implicated as an attractive target for novel analgesic drugs (Wood et al. 2004). Our results suggest that sertraline and paroxetine block Na^+ channels with the potency order of open > inactivated > resting channels. Unexpectedly, receptor mapping indicates that the critical phenylalanine residue at the LA/TCA receptor in Na^+ channels is not involved in binding with sertraline and paroxetine.

Materials and Methods

Cultures of GH_3 Cells and HEK293 Cells Stably Expressing Na^+ Channels

Rat pituitary GH_3 cells expressing neuronal Nav1.1, 1.2, 1.3 and 1.6 Na^+ channel isoforms endogenously (Vega et al. 2003) were purchased from American Type Culture Collection (Manassas, VA). Human embryonic kidney (HEK293) cells heterologously expressing rNav1.4 wild-type Na^+ channels and inactivation-deficient Na^+ channels (rNav1.4-L435W/L437C/A438W, i.e., $L_{435}L_{437}A_{438} \rightarrow W_{435}C_{437}W_{438}$ or WCW mutant) were reestablished from frozen vials as described (Wang et al. 2004b). Other inactivation-deficient Na^+ channels (e.g., IFM → QQQ mutant; Ramos and O'Leary 2004) have been used to study open channel blockers in *Xenopus* oocytes, but these mutant channels do not express well in mammalian cells (Grant et al. 2000). Cultured HEK293 cells and GH_3 cells were maintained at 37°C in a 5% CO_2 incubator in Dulbecco's modified Eagle medium (Life Technologies, Rockville, MD) containing 10% fetal bovine serum (HyClone, Logan, UT), 1% penicillin and streptomycin solution (Sigma, St. Louis, MO), 3 mM taurine and 25 mM HEPES (Life Technologies).

Transient Transfection of HEK293t Cells with S6 Mutants in the WCW cDNA Construct

We created additional S6 mutant channels in the rNav1.4-WCW cDNA background by site-directed mutagenesis as described (Wang et al. 2004b). Among these rNav1.4-WCW mutant channels are WCW-F1579A, WCW-F1579K and WCW-N784K. These specific residues in rNav1.4 channels (F1579 and N784) are located in the middle of S6 segments, and F1579, but not N784, appears to be critical for LA binding (Nau and Wang 2004). Human hNav1.7

clone (Klugbauer et al. 1995) was kindly provided by Dr. Norbert Klugbauer. An inactivation-deficient hNav1.7-WCW mutant was created by triple mutations at homologous positions (L396W/L398C/A399W mutant). For transient transfection, HEK293t cells were grown to ~50% confluence and transfected by calcium phosphate precipitation. Transfection of mutant channels (5–10 μg) along with rat β1-pcDNA1 (10–20 μg) and reporter CD8-pih3m (1 μg) was adequate for current recording. The rat β_1-subunit was included to increase the level of channel expression. Control experiments indicated that coexpression of the β_1-subunit did not affect binding affinities of sertraline or paroxetine, as shown previously for local anesthetics (Wright et al. 1999). Cells were replated 15 h after transfection in 35-mm dishes, maintained at 37°C in a 5% CO_2 incubator and used after 1–4 days. Transfection-positive cells were identified with CD8 immunobeads (Dynabeads, Lake Success, NY).

Solutions and Chemicals

Cells were perfused with an extracellular solution containing (in mM) 65 NaCl, 85 choline-Cl, 2 $CaCl_2$ and 10 HEPES (titrated with tetramethylammonium-OH to pH 7.4). The pipette (intracellular) solution consisted of (in mM) 100 NaF, 30 NaCl, 10 EGTA and 10 HEPES (titrated with cesium-OH to pH 7.2). Sertraline HCl and paroxetine HCl were purchased from Sigma. Drugs were dissolved in dimethylsulfoxide (DMSO) at 50 mM (sertraline) or 100 mM (paroxetine) as stock solutions, as suggested by the manufacturer, and stored at 4°C. Final drug concentrations up to 100 μM were made by serial dilution. The highest DMSO concentration in the bath solution (0.2%) had little effect on Na^+ currents.

Electrophysiology and Data Acquisition

The whole-cell configuration of a patch-clamp technique (Hamill et al. 1981) was used to record Na^+ currents in HEK293 cells at room temperature (22 ± 2°C). Electrode resistance ranged 0.5–1.0 MΩ. Command voltages were elicited with pCLAMP9 software and delivered by Axopatch 200B (Axon Instruments, Burlingame, CA) or by EPC-7 (List Electronics, Darmstadt, Germany[AU1]). Cells were held at −140 m*V* and dialyzed for 10–15 min before current recording. The capacitance and leak currents were canceled with the patch-clamp device and by P/-4 subtraction. Liquid junction potential was 5.9 m*V* at 20°C as estimated by pCLAMP9 and was not corrected. Peak currents at +30 m*V* were 2–20 nA for the majority of cells. Access resistance was 1–2 MΩ under the whole-cell configuration; series resistance compensation of >85% typically resulted in voltage errors of ≤3 m*V* at +30 m*V*. Dose–response studies were performed at +30 m*V* for the outward Na^+ currents. Such recordings allowed us to avoid the complication of series resistance artifacts and to minimize inward Na^+ ion loading (Cota and Armstrong 1989). Curve fitting was performed by Microcal Origin (Northampton, MA). An unpaired Student's *t*-test was used to evaluate estimated parameters (mean ± SEM or fitted value ± SE of the fit); $P < 0.05$ was considered statistically significant.

Results

Resting and Inactivated Block of Na^+ Channels by Sertraline and Paroxetine in GH_3 Cells and in Hek293t Cells Expressing rNav1.4 Isoform

Pituitary GH_3 cells express neuronal Nav1.1, 1.2, 1.3 and 1.6 Na^+ channel isoforms (Vega et al. 2003). Figure 2a shows that sertraline at 10 μM blocked about 25% of peak Na^+ currents at +30 m*V* when the cell was held at −140 m*V* (current traces on the left). If the cell was first held at a conditioning voltage of −70 m*V* for 10 s, however, the level of block was significantly enhanced and 90% of peak Na^+ currents were inhibited as measured at a brief test pulse (current traces on the right, Fig. 2a). An interpulse (−140 m*V* for 100 ms) was inserted between the conditioning pulse and test pulse (right inset). The reason that more block occurs with this pulse protocol is that most drug-bound inactivated Na^+ channels do not recover during the interpulse (−140 m*V*/100 ms), whereas the drug-free inactivated channels recover readily during this period. Similar state-dependent block of Na^+ currents by paroxetine was observed in GH_3 cells (Fig. 2b), although its potency was less than that of sertraline.

Under identical conditions, we found that both sertraline and paroxetine also blocked the resting and inactivated rNav1.4 Na^+ channel isoforms in a strong state-dependent manner (Fig. 3a, b). Dose–response curves for sertraline (Fig. 4a) and paroxetine (Fig. 4b) in GH_3 cells and in Hek293t cells expressing rNav1.4 Na^+ channels were constructed under various drug concentrations. The curves were fitted with a Hill equation, and the IC_{50} values and Hill coefficients were obtained (Table 1). The Hill coefficients varied from 1.11 to 2.27. No significant differences in IC_{50} values were detected in the resting block between neuronal and rNav1.4 Na^+ channel isoforms for sertraline or for paroxetine (Fig. 4, closed square vs. closed triangle; Table 1). The inactivated rNav1.4 channels, however, appeared more sensitive to sertraline and paroxetine than their neuronal counterparts expressed in GH_3 cells (Fig. 4, open square vs. open triangle; Table 1). The IC_{50} ratio of resting vs. inactivated rNav1.4 muscle Na^+ channels was 9.9 for sertraline and 5.0 for paroxetine. In comparison,

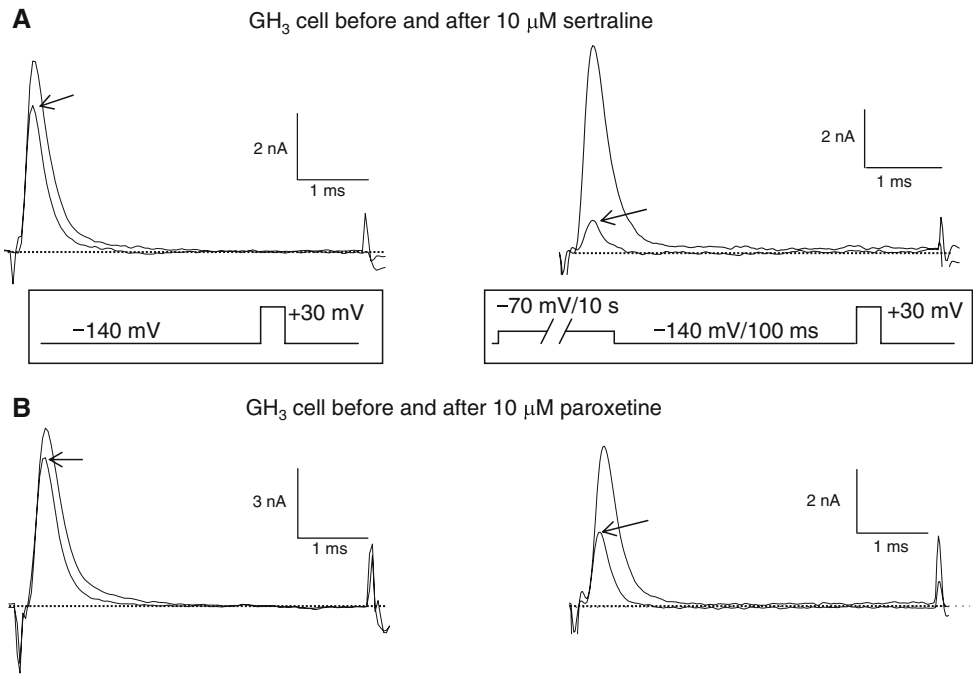

Fig. 2 Resting and inactivated block of Na^+ channels by sertraline and paroxetine in GH_3 cells expressing neuronal isoforms. (**A**) Traces shown on the *left* indicate resting block by 10 μM sertraline at −140 m*V* holding potential, whereas traces shown on the *right* indicate inactivated block by 10 μM sertraline at −70 m*V*. Pulse protocols are shown in the *inset*. Currents were evoked by a 5-ms test pulse at +30 m*V*. (**B**) Traces shown on the *left* indicate resting block by 10 μM paroxetine, whereas traces shown on the *right* indicate inactivated block by 10 μM paroxetine. Pulse protocols are the same as above. Current traces in the four panels were recorded from four different cells

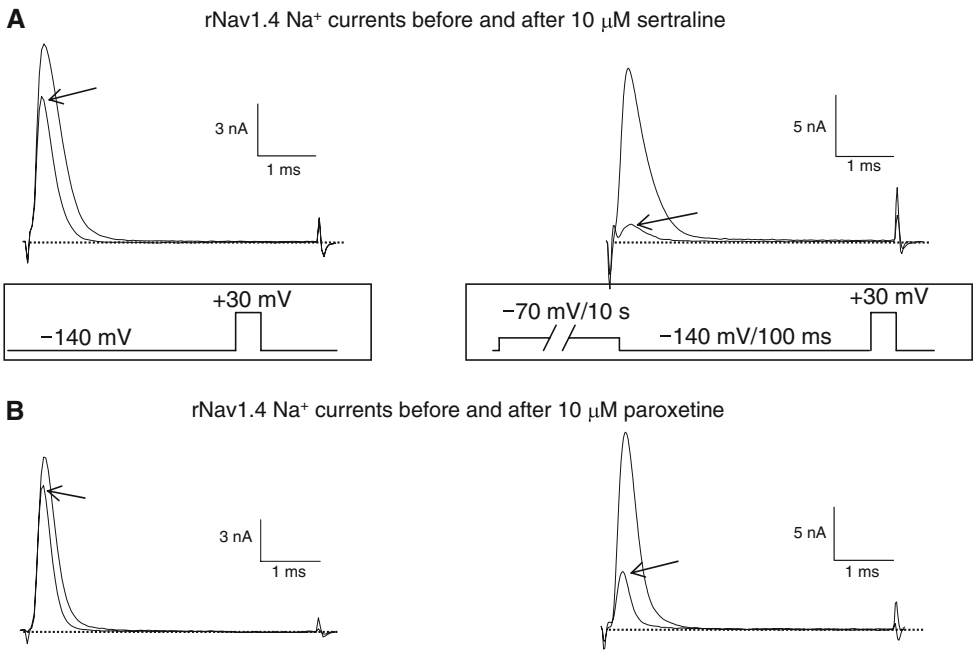

Fig. 3 Resting and inactivated block of rNav1.4 Na^+ channels by sertraline and paroxetine in Hek293t cells. (**A**) Traces shown on the *left* are resting block at 10 μM sertraline in Hek293t cells expressing rNav1.4 Na^+ channels, and traces shown on the *right* are inactivated block at 10 μM sertraline. Pulse protocols are shown in the *inset*. (**B**) Traces shown on the *left* are resting block at 10 μM paroxetine in Hek293t cells expressing rNav1.4 Na^+ channels, and traces shown on the *right* are inactivated block at 10 μM paroxetine. Pulse protocols are the same as shown in (**A**). Current traces in the four panels were recorded from four different cells

sertraline and paroxetine preferentially blocked inactivated over resting neuronal Na^+ channels in GH_3 cells by a factor of 3.3 and 4.9, respectively.

Open Channel Block of Persistent Late rNav1.4 Na^+ Currents by Sertraline and Paroxetine

Voltage-gated Na^+ channels have a very brief open time once they are activated due to fast inactivation (∼0.5 ms; Aldrich, Corey and Stevens, 1983). To study the open channel block by sertraline and paroxetine directly, we therefore used fast inactivation–deficient mutant Na^+ channels previously used to study the open channel block by TCAs (Wang et al. 2004a). Figure 5a shows the open channel blocking phenotype of inactivation-deficient Na^+ currents at various concentrations of sertraline (top traces) and paroxetine (bottom traces). Without drugs (trace 0), the current traces show that most Na^+ currents are maintained

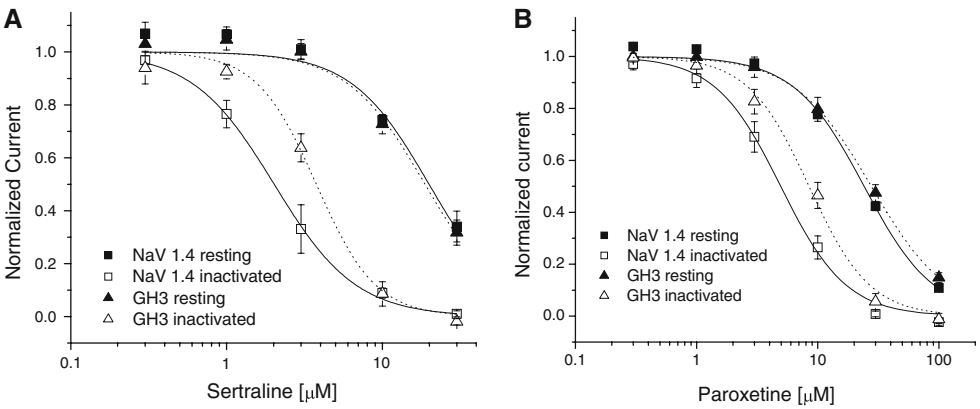

Fig. 4 Dose–response curve of resting and inactivated block by sertraline and paroxetine. Relative potency of sertraline (**A**) and paroxetine (**B**) of neuronal Na^+ channel isoforms (*dotted line*) and skeletal muscle Na^+ channel isoforms (*solid line*) was compared. Data were taken from experiments at various drug concentrations with pulse protocols identical to those described in Figs. 2 and 3

at the end of a 50-ms pulse. We chose the 50-ms duration because slow inactivation occurred significantly when the duration was further lengthened (Wang et al. 2003b). With drug present, the higher the drug concentrations, the greater the block of the maintained Na^+ currents. Dose–response curves were constructed for the block of the peak and maintained currents by sertraline and paroxetine as shown in Fig. 5b (top and bottom panels, respectively). Curve fitting was performed by the Hill equation; the IC_{50} values and Hill coefficients are listed in Table 1. The lists show that both sertraline and paroxetine have the highest affinities toward the open Na^+ channels, with IC_{50} values of $\sim$0.6–0.7 μM and Hill coefficients of $\sim$1.4. The IC_{50} values for resting channel block were 10.7- and 16.8-fold greater than those for open channel block of sertraline and paroxetine, respectively. It is not clear why the IC_{50} values for sertraline (19.90 μM) and paroxetine (24.16 μM) in wild-type rNav1.4 Na^+ channels are greater than those in inactivation-deficient rNav1.4 Na^+ channels (7.37 and 10.27 μM, respectively). One possibility is that we overestimate the resting block in inactivation-deficient rNav1.4 Na^+ channels if significant block in peak currents also occurs during channel opening when fast inactivation is hampered. Such interactions between local anesthetics and Na^+ channels along the activation pathway have been reported before (Wang et al. 2004b).

Use-Dependent Block of Neuronal Na^+ Channels, rNav1.4 Wild-Type and Fast Inactivation-Deficient rNav1.4 Na^+ Channels by Sertraline and Paroxetine

Most LAs and TCAs are capable of eliciting use-dependent block of Na^+ currents. We therefore determined whether sertraline and paroxetine display a similar use-dependent phenotype in GH_3 cells during repetitive pulses. We found that both SSRI drugs indeed elicited strong use-dependent block of neuronal Na^+ currents. Figure 6a shows that sertraline at 10 μM produced rapid use-dependent block of peak Na^+ currents in GH_3 cells when stimulated at 5 Hz for 25 pulses. Under identical conditions, a similar phenotype was found for paroxetine (Fig. 6b). Figure 6c shows the time courses of the use-dependent block of the peak Na^+ currents by sertraline (open squares) and paroxetine (closed squares) that reached a steady-state block of 64.4% and 70.5%, respectively. Without drugs, little or no use-dependent block of Na^+ currents was observed in GH_3 cells (open circles). Additional results showed that sertraline and paroxetine induced a comparable, although slightly greater, use-dependent block in wild-type muscle rNav1.4 Na^+ currents. Recovery from this use-dependent block was measured after 25 repetitive pulses at +50 m*V* for 20 ms. Figure 7a shows the time courses of recovery at various intervals after repetitive pulses. For sertraline, 68.8 ± 2.4% of currents recovered with a time constant of 1.19 ± 0.05 s ($n = 6$). For paroxetine, 88.9 ± 4.7% of currents recovered with a time constant of 13.6 ± 0.2 s ($n = 6$).

Since during repetitive pulses most Na^+ channels were rapidly inactivated within a few milliseconds (Fig. 6a, b), it was unclear whether transient open channels were also involved in this use-dependent phenotype. We therefore used fast inactivation–deficient rNav1.4-WCW mutant Na^+ channels to assess directly the role of open channel block by these drugs in their use-dependent phenotypes. Figure 8 shows that the use-dependent block of peak Na^+ currents by 10 μM sertraline (left traces) and paroxetine (right traces) is even greater than that in wild-type channels in GH_3 cells (Fig. 6a, b), both reaching a steady-state block of >95%. In addition, the peak current at pulse 1 decayed rapidly during depolarization, indicating rapid open channel block, as shown in Fig. 4. The time courses of block of peak inactivation-deficient Na^+ currents by sertraline (open squares) and paroxetine (closed squares) during repetitive pulses are shown in Fig. 8c. Without drugs, however, there was also significant reduction of peak rNav1.4-WCW Na^+ currents ($\sim$ 40%, open circles), a phenomenon probably due to enhanced slowed inactivation (Wang et al. 2003b). The recovery from open channel block by 10 μM sertraline as determined at −140 m*V* holding potential followed an

Table 1 Estimated IC_{50} values and Hill coefficients (in parentheses) in resting, open and inactivated Na^+ channels endogenously expressed in GH_3 cells or heterologously expressed in Hek293t cells

Na^+ channel isoforms	Drug	IC_{50} (Hill coefficient) in Na^+ channel (μM)			
		Resting state	Open state	Inactivated state	Ratio
Neuronal Na^+ channels in GH_3 cells	Sertraline	18.76 ± 1.46 (1.74 ± 0.23)	NA	3.76 ± 0.24 (2.28 ± 0.31)	5.0
	Paroxetine	27.71 ± 0.30 (1.36 ± 0.02)	NA	8.49 ± 0.55 (1.73 ± 0.17)	3.3
rNav1.4 wild-type Na^+ channels in Hek293t cells	Sertraline	19.90 ± 2.29 (1.75 ± 0.34)	NA	2.02 ± 0.06 (1.65 ± 0.07)	9.9
	Paroxetine	24.16 ± 1.34 (1.50 ± 0.12)	NA	4.96 ± 0.29 (1.61 ± 0.13)	4.9
rNav1.4-WCW channels in Hek293t cells	Sertraline	7.37 ± 0.43 (1.45 ± 0.13)	0.69 ± 0.03 (1.41 ± 0.04)	NA	10.7
	Paroxetine	10.27 ± 0.88 (1.14 ± 0.10)	0.61 ± 0.06 (1.43 ± 0.18)	NA	16.8
rNav1.4-WCW/784K channels in Hek293t cells	Sertraline	10.26 ± 0.38 (1.56 ± 0.11)	1.11 ± 0.15 (1.24 ± 0.19)	NA	9.2
	Paroxetine	13.73 ± 1.15 (1.12 ± 0.10)	0.70 ± 0.09 (1.11 ± 0.16)	NA	19.6
rNav1.4-WCW/1579A channels in Hek293t cells	Sertraline	5.60 ± 0.33 (1.47 ± 0.12)	0.78 ± 0.03 (1.87 ± 0.13)	NA	7.2
	Paroxetine	11.50 ± 0.86 (1.17 ± 0.09)	0.75 ± 0.04 (1.66 ± 0.15)	NA	15.3
rNav1.4-WCW/1579K channels in Hek293t cells	Sertraline	9.31 ± 0.24 (1.55 ± 0.07)	1.45 ± 0.07 (1.45 ± 0.09)	NA	6.4
	Paroxetine	23.58 ± 0.40 (1.32 ± 0.03)	3.16 ± 0.36 (1.27 + 0.16)	NA	7.5

In cells expressing neuronal and rNav1.4 wild-type Na^+ channels, currents were evoked by a 5-ms test pulse at +30 m*V*. In cells expressing Nav1.4-WCW mutants, currents were evoked by a 50-ms test pulse at +30 m*V*. Because of the limitation in the pulse duration of inactivation-deficient Na^+ currents, these IC_{50} values should be considered approximations for steady-state block. Values were derived as described in Figure 4, with $n = 5$–6. The ratios of IC_{50} values for the resting over the open or the inactivated state of corresponding Na^+ channels are listed. NA, not applicable

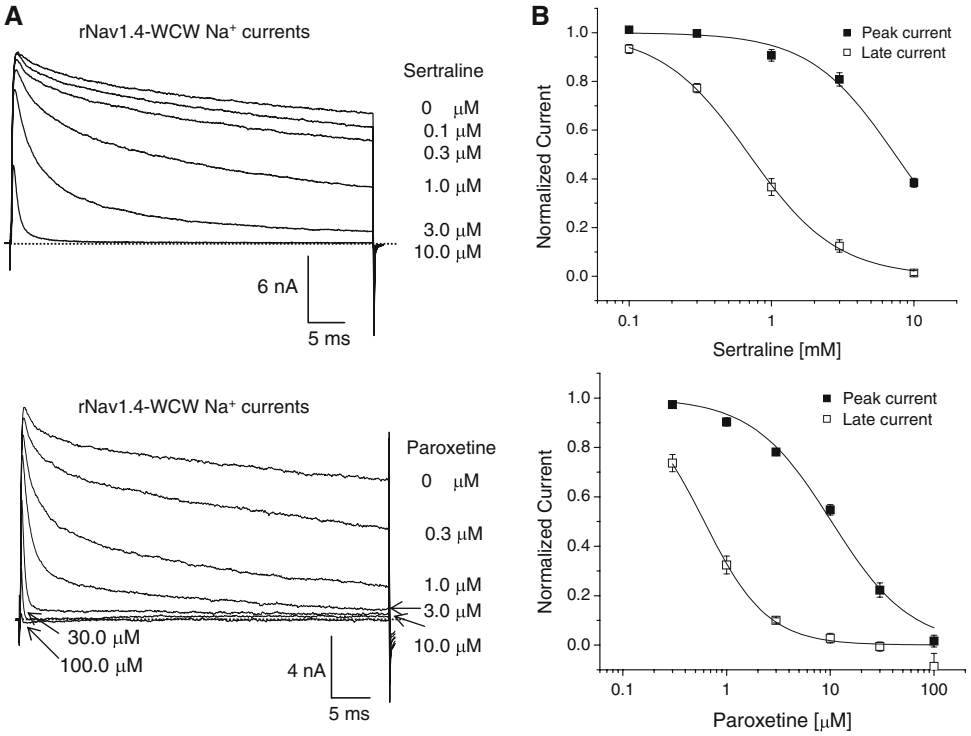

Fig. 5 Open channel block of rNav1.4-WCW mutant Na^+ currents by sertraline and paroxetine. (**A**) Currents were evoked by a 50-ms test pulse at +30 m*V*. Current traces were recorded before and after the application of sertraline (*top panel*) and paroxetine (*bottom panel*) at various concentrations. (**B**) Dose–response curve. Peak and late currents in the presence of various drug concentrations were normalized to the control saline response without drug and fitted with a Hill equation. At 100 μM, paroxetine might also alter leak currents, which could not be subtracted completely. For sertraline, the resting channel IC_{50} was 7.37 ± 0.43 (1.45 ± 0.13) and the open channel block IC_{50} was 0.69 ± 0.03 (1.41 ± 0.04) ($n = 6$). For paroxetine, the resting IC_{50} was 10.27 ± 0.88 (1.14 ± 0.10) and the open block IC_{50} was 0.61 ± 0.06 (1.43 ± 0.18) ($n = 5$)

exponential time course with a time constant of 2.60 ± 0.33 s ($n = 6$) and a majority component of 80.4 ± 12.4% (Fig. 7b). A similar slow recovery was found for the open channel block of paroxetine with a time constant of 4.98 ± 0.48 s ($n = 6$) and a component of 77.4 ± 10.1%. Such a slow recovery time course for the open channel block by sertraline and paroxetine therefore explains the rapid use-dependent phenotype found in Fig. 8 since once the peak current was blocked it could not recover within the 200-ms interpulse duration at the −140 m*V* holding potential. There were significant differences in the recovery time courses in wild-type and in rNav1.4-WCW mutant channels (Fig. 7a vs. b), indicating that fast inactivation also modulates the recovery time courses from block induced by sertraline and paroxetine. Such modulation has been reported for flecainide block of these channels before (Wang et al. 2003a).

The Phenylalanine Residue at the LA/TCA Receptor is not Critical for Sertraline and Paroxetine Binding

The blocking phenotypes of voltage-gated Na^+ channels by sertraline and paroxetine are comparable to those by LAs or TCAs. Both LAs and TCAs are known to target a specific receptor within the Na^+ channel α-subunit. The LA/TCA receptor in the Na^+ channel includes a most critical phenylanaline residue (Nau and Wang 2004), F1579, in the rNav1.4 isoform. We therefore asked whether residue F1579 is involved in binding of sertraline and paroxetine. Figure 9 shows the block of inactivation-deficient rNav1.4-F1579A Na^+ currents at various concentrations of sertraline and paroxetine. Dose–response curves were constructed using the data shown in Fig. 9, and the IC_{50} values and Hill coefficients are listed in Table 1. The data illustrate that both sertraline and paroxetine have high affinities toward the open rNav1.4-WCW/F1579A Na^+ channels, with IC_{50} values of ∼0.75–0.78 μM. These values were little changed from those (0.61–0.69 μM, $P > 0.05$) of rNav1.4-WCW inactivation-deficient Na^+ channels, suggesting that residue F1579 is not critical for binding of sertraline and paroxetine. Introduction of a positive charge at position F1579 by lysine substitution did reduce the binding affinities of sertraline and paroxetine somewhat (Table 1, $P < 0.05$), perhaps due to positive charge–charge repulsion between F1579K and the cationic ligand. However, this contention is not strongly supported by the results from a

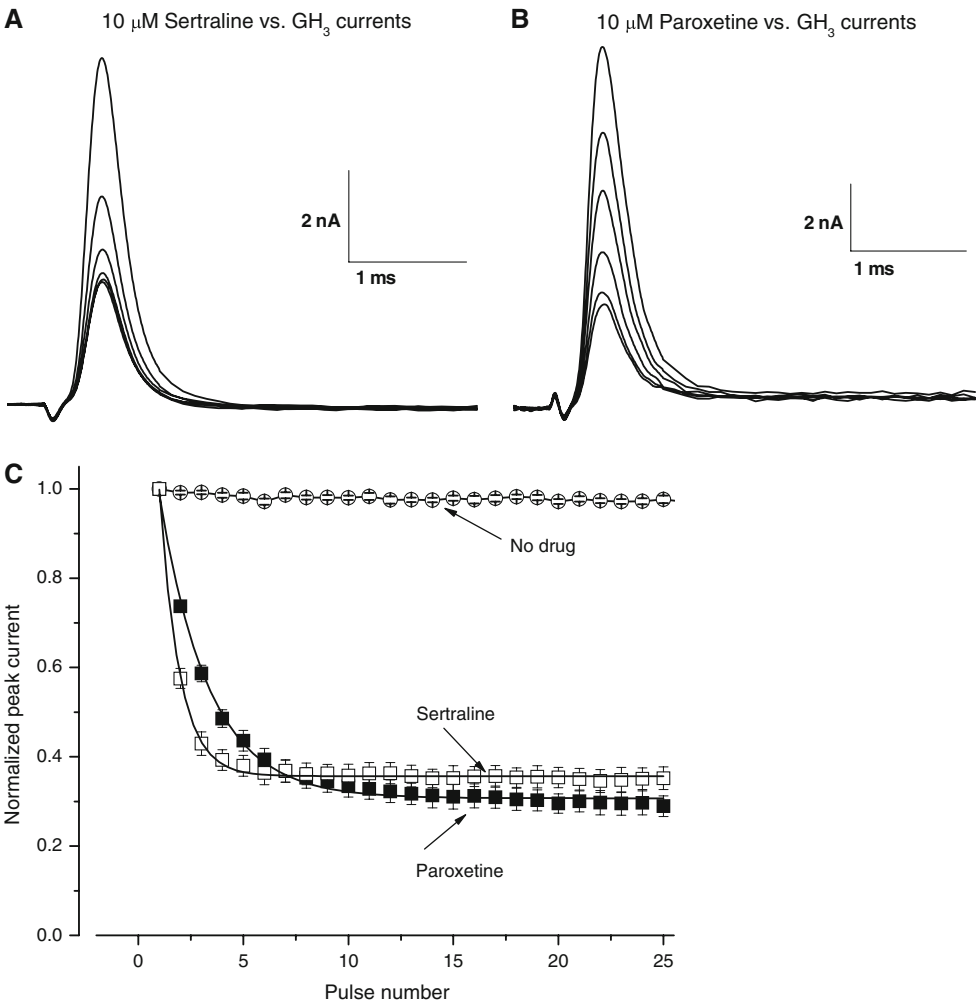

Fig. 6 Use-dependent block of Na^+ currents by sertraline and paroxetine. Use-dependent block in GH_3 cells by 10 μM sertraline (**A**) and 10 μM paroxetine (**B**). Traces correspond to pulses 1, 2, 3, 5, 10 and 25. The pulse (+50 m*V* for 20 ms) was applied repetitively at 5 Hz. (**C**) Rapid time course of use-dependent block during repetitive pulses. Peak currents as shown in (**A** and **B**) were measured, normalized with respect to the peak amplitude at pulse 1 and plotted against the corresponding pulse number. *Solid lines* are best fits of single exponential functions with a time constant (τ) of 0.94 ± 0.02 pulse for sertraline and 2.33 ± 0.06 pulse for paroxetine ($n = 6$)

homologous substitution at the nearby D2S6-N784K site (Nau and Wang 2004). Substitution of N784 with a lysine had a limited effect on sertraline binding and none at all on paroxetine binding (Table 1).

Open Channel Block of Fast Inactivation-Deficient hNav1.7-WCW Na^+ Channels by Sertraline and Paroxetine

Since muscle rNav1.4 channels are not involved in pain pathways, we investigated the open channel block of inactivation-deficient hNav1.7-WCW mutant Na^+ channels by sertraline and paroxetine. Figure 10a, top and bottom panels, shows the blocking phenotypes of inactivation-deficient hNav1.7-WCW currents by sertraline and paroxetine at various concentrations, respectively. The concentration- and time-dependent blocking phenotypes of open hNav1.7-$W_{396}C_{398}W_{399}$ mutant channels are similar to those of rNav1.4-$W_{435}C_{437}W_{438}$ shown in Fig. 5. The IC_{50} values were then determined by dose–response curves, which yielded 0.36 and 0.31 μM for sertraline (Fig. 10b, top) and paroxetine (Fig. 10b, bottom), respectively, for block of persistent late currents near the end of the pulse.

Discussion

We have examined the state-dependent block of neuronal and muscle Na^+ channels by sertraline and paroxetine. These two contemporary SSRI drugs are widely used to treat depression, with U.S. trademarks Zoloft (sertraline) and Paxil (paroxetine). Our results show, for the first time, that these two drugs block the open state of Na^+ channels preferentially over their resting and inactivated counterparts. Both drugs also elicit strong use-dependent block of Na^+ currents during repetitive pulses. Sertraline and paroxetine contain an *N*-methyl-L-naphthalenamine and a phenylpiperidine moiety, respectively (Fig. 1), and their binding site at Na^+ channels appears different from the known LA/TCA receptor. The implications of these findings are discussed.

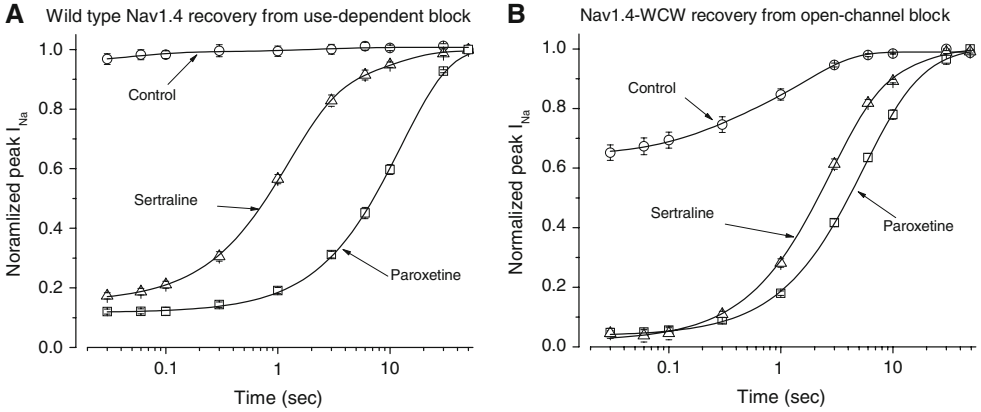

Fig. 7 Time courses for recovery from the use-dependent block of rNav1.4 wild-type and from the open channel block of rNav1.4-WCW mutant channels. (**A**) Recovery time courses for the use-dependent block of rNav1.4 wild-type were measured by a brief test pulse at various intervals after 25 repetitive pulses (+40 m*V* for 20 ms) at 5 Hz without and with 10 μM sertraline or paroxetine. Peak currents were determined, normalized with respect to the peak amplitude measured at 50 s after repetitive pulses and plotted against the time interval. Data ($n = 5$–6) were best fitted by two exponential functions; corresponding time constants for the major component are given in the text. Notice that without drug no use-dependent block was evident. This is consistent with $\tau = 1.7$ ms for recovery from rNav1.4 fast inactivation. (**B**) A 100-ms conditioning pulse at +30 m*V* was first applied to elicit the time-dependent block of 10 μM sertraline or paroxetine in rNav1.4-WCW currents. At various intervals, recovery from the time-dependent block was measured by a brief test pulse at +30 m*V*. Data were best fitted by two exponential functions; corresponding time constants for the major component are given in the text. Notice that without drug a fraction of peak currents (26.0 ± 2.8%) recovered slowly with a time constant of 1.7 ± 0.2 s ($n = 5$)

Sertraline and Paroxetine Block Na^+ Channels in a Highly State-Dependent Manner

Block of Na^+ channels by sertraline and paroxetine is highly state-dependent. The IC_{50} values follow the order of open (1x) < inactivated (~4–8x) < resting (~10–20x) states of rNav1.4 Na^+ channels (Table 1). There is no evidence to support that sertraline and paroxetine block of Na^+ currents is isoform-specific. Sertraline and paroxetine have the same resting affinities toward the muscle rNav1.4 and neuronal Na^+ channel isoforms in GH_3 cells. Moderate differences in the inactivated affinities for sertraline (3.76 vs. 2.02 μM) and for paroxetine (8.49 vs. 4.96 μM) were present between neuronal and muscle Na^+ channels, respectively, indicating that both inactivated Na^+ channels are sensitive to sertraline and paroxetine block. Using fluorescence detection of membrane potentials, Huang et al. (2006) found that, when stimulated at 10 Hz, sertraline and paroxetine block wild-type rNav1.3 Na^+ channels effectively in the 2–5 μM range. Our IC_{50} values for the inactivated channels (Table 1, 2–8 μM) are comparable to their data after electrical field stimulation, suggesting an important role of inactivated Na^+ channels in their fluorescence assay. Similarly, Dick et al. (2006) reported the IC_{50} values of 17 and 1.45 μM paroxetine for block of resting and inactivated hNav1.7 Na^+ channels, respectively. Neither of the above studies reported open Na^+ channel block by sertraline and paroxetine. This is perhaps because fast inactivation of Na^+ channels normally keeps the channel open for only a very brief period of time, ~0.5 ms. With such short opening, open channel block by sertraline and paroxetine is difficult to quantify directly. In this report, we used the fast inactivation–deficient rNav1.4-WCW and hNav1.7-WCW mutant Na^+ channels to measure the open channel block by sertraline and paroxetine, an approach used previously to study the TCA affinity in open Na^+ channels (Wang et al. 2004a). The pharmacological actions of sertraline and paroxetine on Na^+ channels are indeed very similar to those of TCAs, which elicit strong use-dependent block of Na^+ currents and have the highest affinities toward the open state of Na^+ channels. However, the IC_{50} ratio of inactivated vs. open states for amitriptyline is only about twofold, less than the four- to eightfold ratio found for sertraline and paroxetine.

Do Sertraline and Paroxetine Target the Phenylalanine Residue at the LA/TCA Receptor in Na^+ Channels?

Because sertraline, paroxetine, TCAs and LAs act so similarly, we thought that all these drugs might target the same receptor in Na^+ channels. This notion, however, was not supported by the results from receptor mapping. For example, rNav1.4-WCW/F1579A mutant channels are nearly as sensitive to sertraline and paroxetine block as rNav1.4-WCW inactivation-deficient Na^+ channels (Table 1, $P > 0.05$). Such a finding contrasts with the results found for LAs, in which the block was generally reduced by a factor of ≥10 when the residue F→A substitution at the LA receptor was

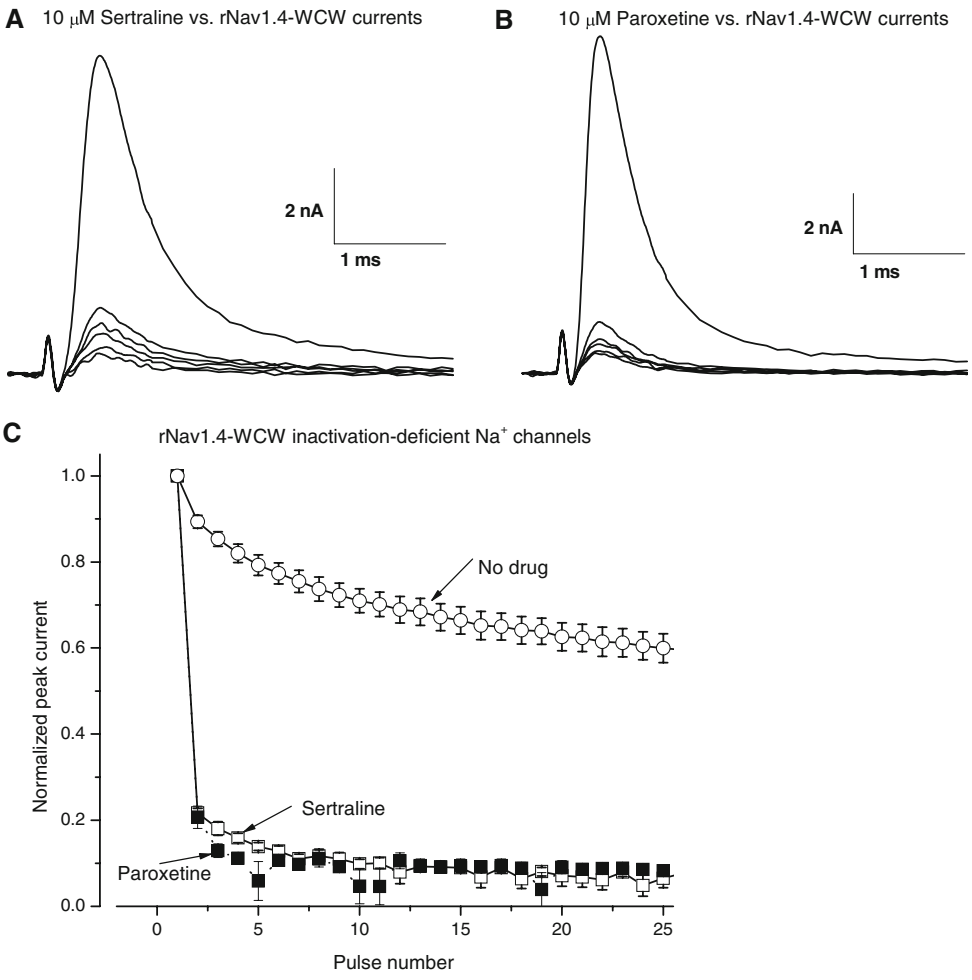

Fig. 8 Use-dependent block of inactivation-deficient Na^+ currents by sertraline and paroxetine. Use-dependent block of fast inactivation–deficient Na^+ channels by 10 μM sertraline (**A**) and 10 μM paroxetine (**B**). The pulse protocol was the same as that used in Fig. 6. (**C**) Rapid time course of use-dependent block during repetitive pulses. Peak currents as shown in (**A** and **B**) were measured, normalized with respect to the peak amplitude at pulse 1 and plotted against the corresponding pulse number. *Solid lines* are best fits by single exponential functions with a τ value of <1 pulse, too fast to be accurately estimated ($n = 6$)

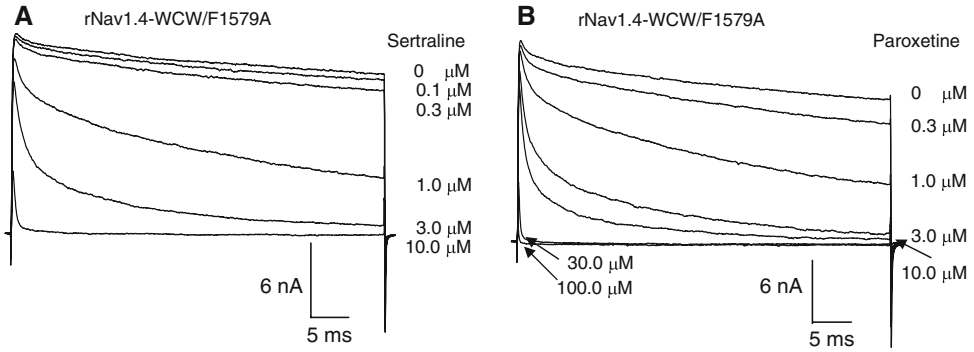

Fig. 9 Block of rNav1.4-WCW/F1579A Na^+ currents in the presence of various concentrations of sertraline (**A**) and paroxetine (**B**). Superimposed representative Na^+ current traces were recorded before and after the drug application at various concentrations. Currents were evoked by a 50-ms test pulse at +30 m*V*. The maintained Na^+ currents near the end of the 50-ms pulse were highly sensitive to drug block

made (Catterall and Mackie 2001; Nau and Wang 2004). For lysine substitution (rNav1.4-WCW/F1579K), however, the block by sertraline and paroxetine was reduced, though modestly (0.6–0.7 vs. 1.45–3.16 μM, Table 1; $P < 0.05$). The reason for this reduction is unclear. One possibility is the charge–charge repulsion introduced by the lysine side chain and cationic sertraline or paroxetine. If such charge–charge repulsion occurs, the binding site for sertraline and paroxetine may be near the cation binding site within the inner cavity commonly found in cation channels (Zhou and MacKinnon 2004). Unfortunately, the mutation at the equivalent position at D2S6 (N784K; i.e., opposite the

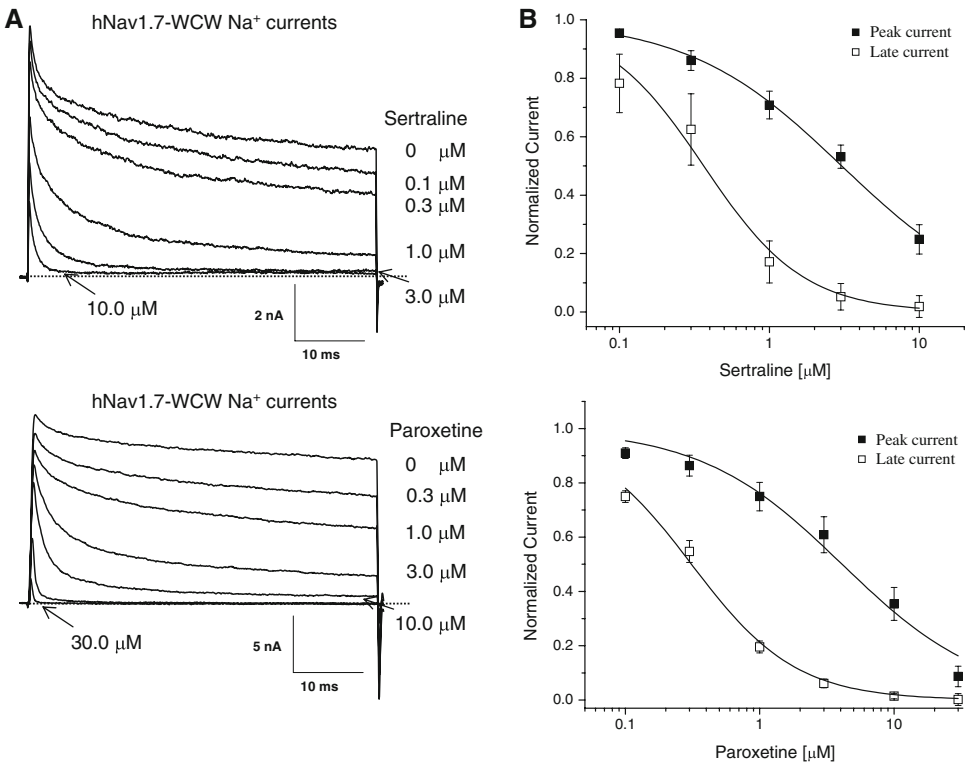

Fig. 10 Open channel block of hNav1.7-WCW mutant Na^+ currents by sertraline and paroxetine.(**A**) Currents were evoked by a 50-ms test pulse at +30 m*V*. Current traces were recorded before and after the application of sertraline (*top*) and paroxetine (*bottom*) at various concentrations. (**B**) Dose–response curve. Peak and late currents in the presence of various drug concentrations were normalized to the control saline response without drug and fitted with a Hill equation. For sertraline, the resting channel IC_{50} was 3.03 ± 0.19 (0.84 ± 0.05) and the open channel block IC_{50} was 0.36 ± 0.04 (1.30 ± 0.17) ($n = 8$, *top panel*). For paroxetine, the resting IC_{50} was 4.10 ± 0.59 (0.82 ± 0.09) and the open block IC_{50} was 0.31 ± 0.02 (1.12 ± 0.07) ($n = 7$, *bottom panel*)

F1579K position within the inner cavity) was even less effective at reducing the binding affinity of sertraline and paroxetine than the F1579K mutation. These results suggest that the cation binding site within the inner cavity is not involved in binding of cationic sertraline and paroxetine. Detailed receptor mapping for sertraline and paroxetine will be required to address the precise location of the sertraline and paroxetine receptor.

Our studies show that sertraline and paroxetine target the persistent late neuronal hNav1.7 Na^+ currents, with IC_{50} values of ~0.3–0.4 μM, which are near or above the therapeutic plasma concentrations of 0.1–0.3 μM (sertraline) and 0.08–0.15 μM (paroxetine) (Baldessarini 2001). However, sertraline and paroxetine may reach much higher concentrations in nervous tissues as they enrich in rodent brains 24- and 3.3-fold relative to plasma concentrations, respectively (Doran et al. 2005). With this consideration, both drugs may reduce or eliminate persistent late neuronal Na^+ currents in vivo. Such action by sertraline and paroxetine may in part explain their analgesic action in several clinical trials (Gonzalez-Viejo et al. 2005; Masand et al. 2006). In principle, selective block of persistent late neuronal Na^+ currents will be beneficial for patients with neuropathic and chronic pain originating from ectopic high-frequency discharges (Devor and Seltzer 1999). This blocking action of sertraline and paroxetine on the open neuronal Na^+ channel could work cooperatively with other pharmacological targets of these SSRI antidepressants on central and peripheral pain pathways.

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