Voltage-dependent transient currents of human and rat 5-HT transporters (SERT) are blocked by HEPES and ion channel ligands

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Abstract The hyperpolarization-activated transient current of mammalian 5-hydroxytryptamine transporters (SERT) expressed in *Xenopus* oocytes was studied. Human (h) and rat (r) SERT transient currents are blocked by HEPES with changes in the waveform kinetics, and the blockade of hSERT has use-dependent properties. HEPES also changes the time course of the prepriming step, especially for hSERT. Transient currents at hSERT and rSERT are also blocked by spermine and spermidine in the mM range, and by fluoxetine, cocaine, QX-314, and QX-222 in the μM range. These pharmacological and kinetic properties of transient current blockade emphasize the similarities between the transient current and phenomena at ion channels. © 2002 Published by Elsevier Science B.V. on behalf of the Federation of European Biochemical Societies.

Key words: Ion channel; Voltage jump

1. Introduction

Mammalian 5-hydroxytryptamine (5-HT) transporters (SERT) expressed in heterologous cells display at least four uncoupled currents. These include a transport-associated current, a tonic leakage current, a hyperpolarization-activated transient current, and a proton current [1–4]. Of these, the voltage-dependent transient current is by far the largest, amounting to several μA. Therefore the transient current may reflect a channel-like state of the transporter [1,2]. Further studies of the transient current may reveal details of the transition between transporter and channel modes of SERT and perhaps, by analogy with structure–function studies on other channels, the residues that line the conducting pathway. Uncoupled channel-like currents also occur at other neurotransmitter transporters [2,5–8], increasing the relevance of uncoupled currents.

This study reports on several agents that block the voltage-dependent transient current. The study began when we discovered that HEPES, a component of the bathing solution in most of our previous studies, is itself a blocker of the transient current. We have studied some aspects of this blockade by HEPES and other agents. We also found instances of use-dependent blockade, which further strengthens the analogy with ion channels.

2. Materials and methods

2.1. Reagents and solutions

Cocaine, fluoxetine, HEPES, spermine and spermidine were from Sigma (St. Louis, MO, USA). QX-222 and QX-314 were from Tocris (Ballwin, MO, USA). The control recording solution (ND96) contained 96 mM NaCl, 2 mM KCl, 1 mM $MgCl_2$ and 5 mM HEPES, pH 7.4. The phosphate-buffered saline (PBS) solution was purchased from Irvine Scientific (Irvine, CA, USA).

2.2. Expressing hSERT and rSERT in Xenopus oocytes

The high-efficiency expression system for human and rat serotonin transporters in *Xenopus* oocytes [1,4] was used. In brief, 30 ng cRNA of rat or human SERT (rSERT, hSERT) was injected into stage VI oocytes, and cells were incubated at 18°C for 7–10 days. The incubation solution contained ND96 plus 2% horse serum.

2.3. Electrophysiology

Two-electrode voltage clamp procedures were used [9]. Signals were acquired by a Digidata interface and pCLAMP 8 software (Axon Instruments, Union City, CA, USA). The waveforms of the transient currents were fitted to single exponentials with non-linear routines in ORIGIN (Microcal Software, Northampton, MA, USA) and CLAMPFIT 8 (Axon Instruments).

3. Results and discussion

3.1. HEPES blocks the transient current at hSERT and rSERT

Most of our experiments were conducted in parallel with both hSERT and rSERT. The transient current is largest and therefore most conveniently measured at high negative potentials; we used −140 mV in this study. However, the transient current is also observable at more moderate potentials [1]. The transient current is also largest after a prepriming step to a positive potential [1]; +60 mV was employed in the present series.

Fig. 1 displays the basic phenomenon under observation. When the transient current is measured in a PBS solution, it is considerably larger than in our usual HEPES-buffered solution. The transient currents of both hSERT and rSERT were decreased by HEPES at concentrations greater than ∼1 mM. The hSERT transient current was decreased by only ∼10% at 1 mM HEPES; however, the blockade was increased by the use-dependent effect below. In the experiment of Fig. 1 and in several similar experiments, 1 mM HEPES blocked rSERT by ∼50%; in other experiments, 5 mM HEPES was required for 50% block of rSERT (for instance, Fig. 2). At present it can be stated that HEPES blockade of both rSERT and hSERT is characterized by an IC_{50} between 1 and 5 mM.

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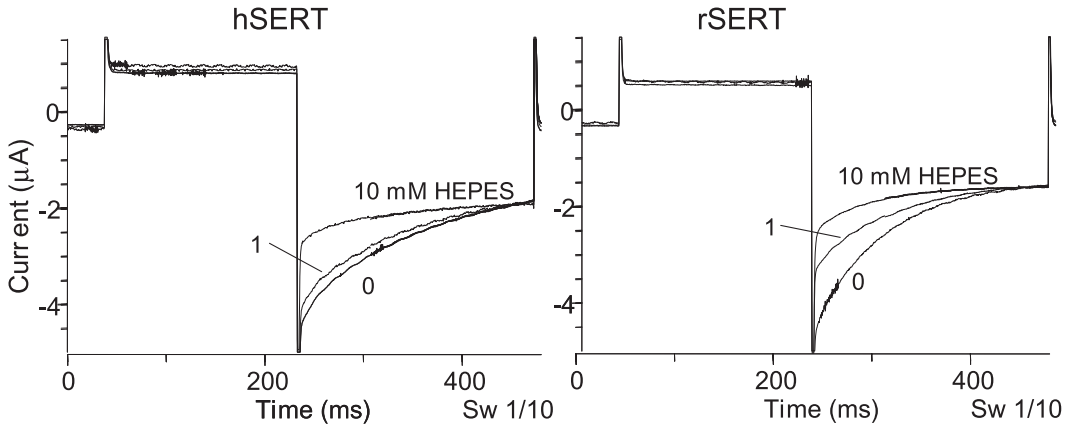

Fig. 1. Voltage-dependent transient currents recorded from oocytes expressing hSERT (left-hand panels) and rSERT (right-hand panels). Currents were recorded in PBS (zero HEPES) and in PBS containing HEPES (1 and 10 mM). The membrane potential was held at −40 mV, was jumped to a prepriming potential of +60 mV for 195 ms, and was then jumped to −140 mV for 240 ms to elicit the transient current. The traces are typical of at least three cells for each transporter, but other rSERT-expressing cells showed less inhibition by 1 mM HEPES.

3.2. *Effects on transient current waveform, and use-dependent blockade of hSERT by HEPES*

HEPES blockade changes the kinetics of the transient currents (Fig. 2). The effects on rSERT are simpler and will be described first. The decay of the transient current is described by the sum of two exponential processes [1]. One appropriate description assigns a time constant equal to the average of the individual time constants, weighted by their amplitudes (Fig. 2). This weighted average time constant was greater for the HEPES-blocked currents than for the control currents (Fig. 2), at all voltages in the range that gave transient currents large enough for systematic measurements. Thus the HEPES-blocked rSERT currents decay somewhat more slowly than unblocked currents.

The HEPES blockade of transient currents at hSERT displays an additional feature (Fig. 3). This blockade is use-dependent, increasing with repetitive stimulation at intervals of 1.2 s, whereas little or no use dependence is observed with rSERT (Fig. 3). Use-dependent blockade of hSERT recovers in 15–30 s; when elicited at such intervals, hSERT transient currents from multiple stimuli superimpose (data not shown). There is little or no use-dependent blockade of rSERT by HEPES (Fig. 3).

The hSERT transient current waveform changes during use-dependent blockade (Fig. 3B). In particular, as the peak current decreases with time, the faster exponential component increases in amplitude. In the example of Fig. 3, the contribution of the faster component was 22% at the first response, and approached a plateau of 40% after ~15 responses.

3.3. *HEPES affects prepriming time course*

Use-dependent blockade is common in the interactions of channel blockers with ion channels, especially at voltage-activated channels. In the usual interpretation, the blocker's access to its binding site differs with the state of the channel [10]. Many blockers access their binding sites more directly when

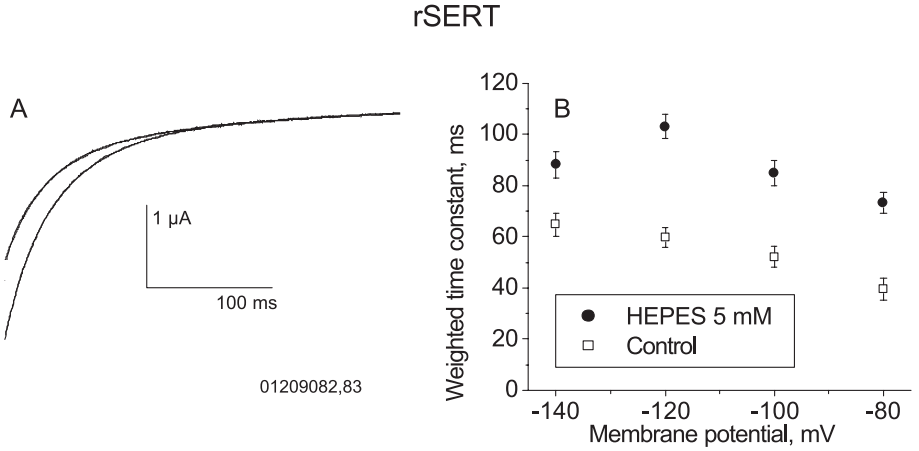

Fig. 2. Kinetic analysis of HEPES blockade of rSERT. A: Transient currents after a jump from a prepriming potential of +60 mV to a test potential of −140 mV, as in Fig. 1. The smaller transient is recorded in the presence of HEPES, 5 mM. A double exponential decay has been fitted to each waveform and is superimposed on the waveform. For the control trace, the time constants are 23 ms (41% of the total amplitude) and 67 ms, yielding a weighted average of 49 ms. For the HEPES trace, the time constants are 30 ms (65% of the total amplitude) and 177 ms, yielding a weighted average of 82 ms. B: Weighted time constants for the transient current decay at several voltages in the absence and presence of 5 mM HEPES. Data represent mean ± S.E.M. for five cells in each case.

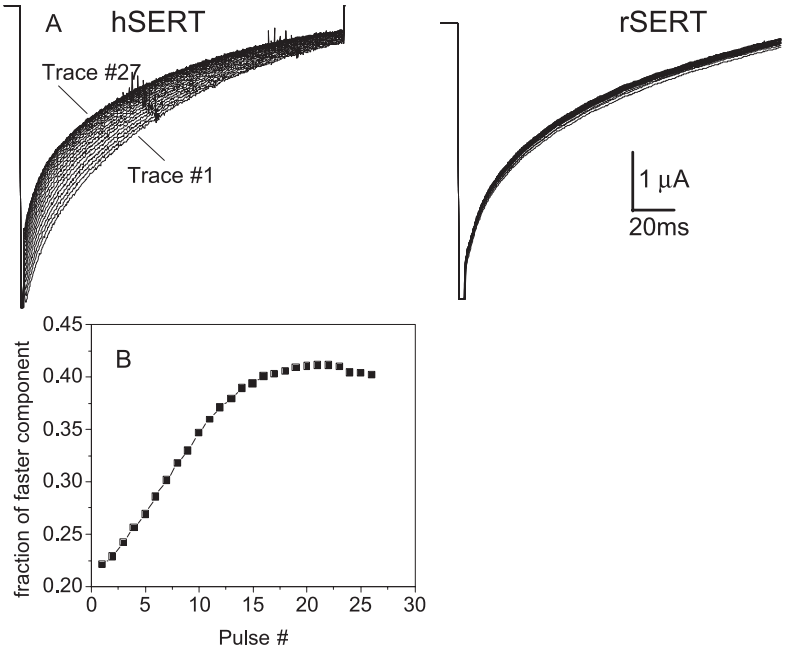

Fig. 3. A: Transient currents of hSERT and rSERT during repetitive hyperpolarizing stimuli at −140 mV at intervals of 1.2 s in ND solution (containing 5 mM HEPES). hSERT, but not rSERT, shows use-dependent block of the transient current. Membrane potential was jumped from the holding potential of −40 mV to a prepriming potential of +60 mV for 120 ms (not shown), and was then jumped to the test potential of −140 mV for 180 ms to elicit the transient current. Each plot is typical of data from at least 10 cells for hSERT or rSERT. B: Fractional contribution of the faster component as a function pulse number, for the experiment on hSERT in A.

the channel is open, then remain at their sites when the channel inactivates, so that additional block accumulates during the next episode when channels are open.

SERT displays a phenomenon analogous to inactivation: the hyperpolarization-activated current is transient during a maintained hyperpolarizing pulse. The interactions between HEPES and the transporter also change the time course of inactivation and recovery from inactivation. The phenomenon analogous to recovery from inactivation is best studied with prepriming pulses of various durations at +60 mV (Fig. 4). In the absence of HEPES, both hSERT and rSERT display a time constant of 95 ms for repriming at +60 mV. For rSERT, this time constant is increased slightly (37%) in the presence of HEPES. However, for hSERT the time constant for recovery is decreased by nearly two-fold, to 50 ms. A straightforward interpretation of this effect is that depolarizing potentials drive the positively charged HEPES molecule out of a binding site on hSERT located within the membrane field.

3.4. Spermine and spermidine block the transient current

The blockade by HEPES raises the possibility that the transient nature of the hyperpolarization-activated current could arise entirely from open-channel blockade in which the blocking molecule, like HEPES, would be an extracellular cation. Previous studies showed that polycationic channel blockers such as spermidine and spermine are released from oocytes [11]. We therefore tested for blockade of the transient current by extracellular spermine and spermidine, and we found that extracellular spermine and spermidine do block the transient current at millimolar concentrations (Fig. 5). Dose–response studies showed that at hSERT, the IC_{50} for spermidine is 10 mM. Spermine (5 mM) exerted a use-dependent blockade at hSERT (Fig. 5C) but not at rSERT. These data confirm the hypothesis that extracellular polyamines can block the transient currents; but the relatively high (mM) concentrations needed for block make it unlikely that released polyamines help to shape the waveform of the transient current. As an alternative explanation, the transient nature of the waveform would arise from an intracellular anionic blocking molecule which would be driven into the membrane field and bind to the transporter at negative intracellular potentials.

3.5. Other ligands also block the transient current

Elimination of blockade by removing HEPES from the buffers allowed us to study the blockade of the transient current by other drugs. The well-known SERT blockers fluoxetine and cocaine both blocked the transient current through rSERT (Fig. 6). The blockade by cocaine is half-maximal at ~1 μM, which is within a factor 5 of values for blockade of 5-HT uptake [12]. The blockade by fluoxetine was half-maximal at ~5 μM, which is similar to the value for blockade of 5-HT uptake in oocytes [1]. The cationic channel blockers QX-314 and QX-222 also blocked the transient current, the latter at concentrations below 10 μM, comparable to its effects on nicotinic acetylcholine receptors [13,14]. Fluoxetine, cocaine, QX-314, and QX-222 did not exert use-dependent block at rSERT and were not studied at hSERT.

Thus the transient currents of hSERT and rSERT can be blocked by several compounds that also interact with cation channels, and the blockade is associated with waveform changes. Furthermore hSERT displays use-dependent blockade by two molecules, HEPES and spermine. The data do not suggest a straightforward formal model for the interaction between the blockers and the transporter, and the residues that bind the blockers remain to be identified. Nonetheless the data provide additional evidence for similarities between (1) the

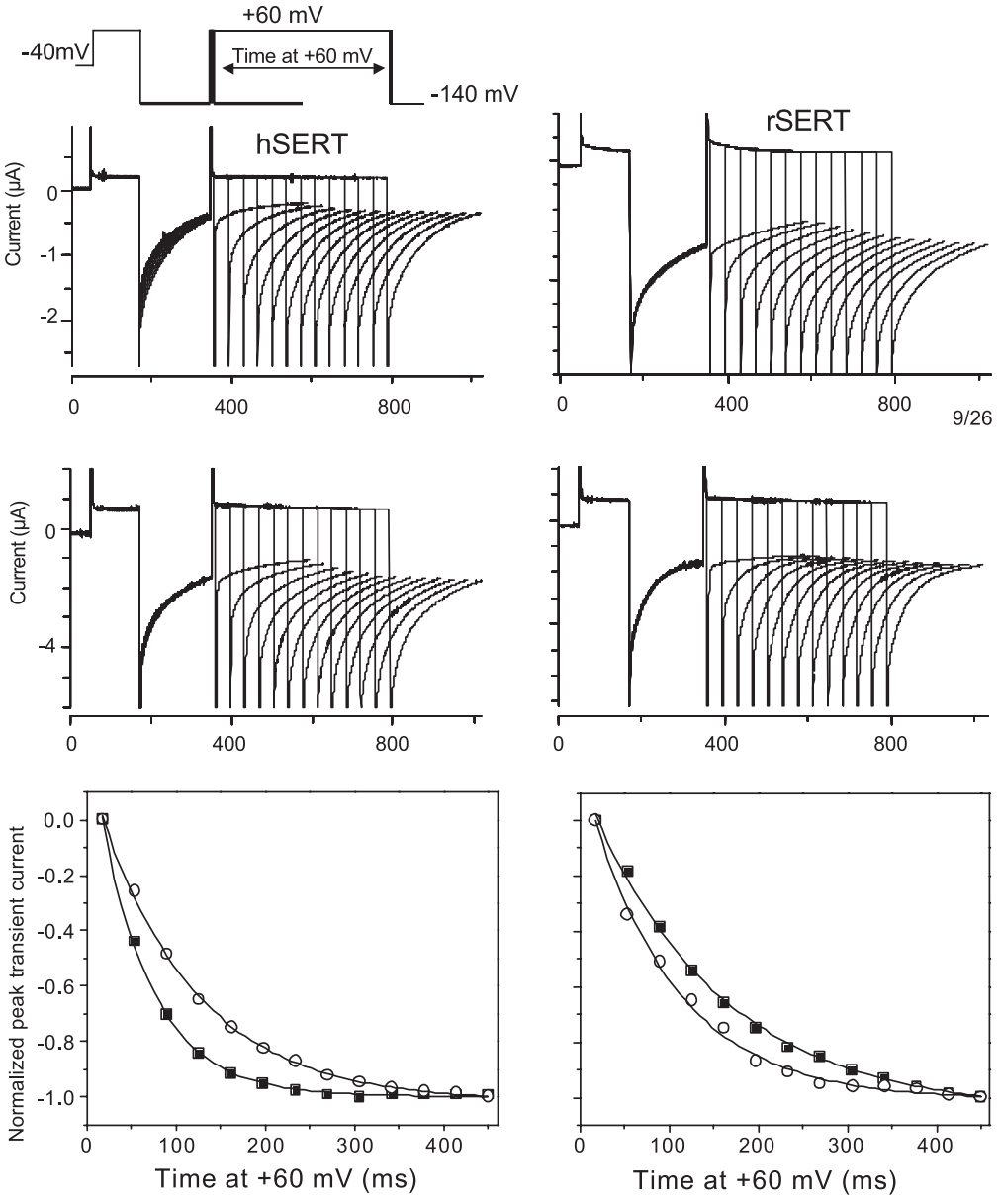

Fig. 4. Prepriming process of hSERT and rSERT in the presence (top panels) and absence (middle panels) of 5 mM HEPES. Membrane potential was held at −40 mV. Voltage was jumped to the prepriming potential of +60 mV for 120 ms, then to −140 mV for 180 ms, followed by another jump to +60 mV for various periods (9 ms for the first trace, with increments of 36 ms for the subsequent traces), then back to the test potential of −140 mV for 240 ms to elicit the transient current. Bottom panels are plots of the normalized peak transient current (the maximum current is set to 1 and the minimum current is set to 0) vs. the duration of the prepriming pulse at +60 mV. The lines are single exponentials fitted to the data. The time constant (mean ± S.E.M.) is 50 ± 5 ms ($n = 22$) for hSERT in the presence of 5 mM HEPES (closed symbols) and 94 ± 5 ms ($n = 6$) in the absence of HEPES (open symbols). For rSERT, the time constant is 130 ± 15 ms ($n = 10$) in the presence of 5 mM HEPES (closed symbols) and 95 ± 7 ms ($n = 8$) in the absence of HEPES (open symbols).

state of SERT responsible for the hyperpolarization-activated transient current and (2) the open state of ion channels.

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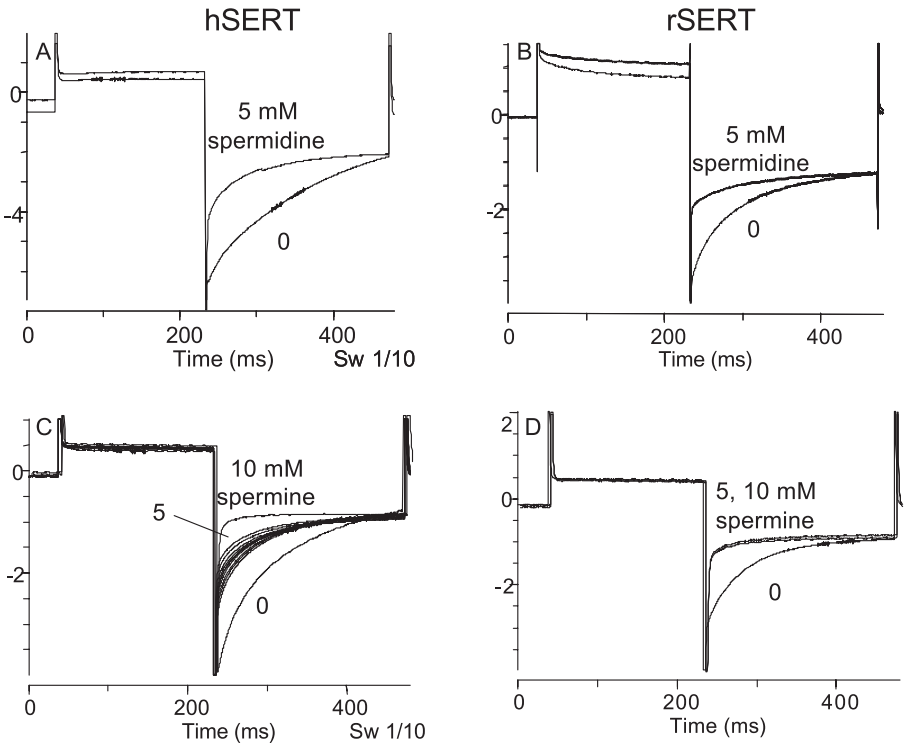

Fig. 5. Transient current of hSERT (A, C) and rSERT (B, D) in the absence and presence of spermidine (A, B) and spermine (C, D). Membrane potential was held at −40 mV, jumped to the priming potential of +60 mV for 195 ms, then to the test potential of −140 mV for 240 ms to elicit the transient current. For hSERT measured in 5 mM spermine, the traces are from multiple measurements at 1 s intervals to show the use-dependent block of the transient current by 5 mM spermine. As in the experiment of Fig. 3A, the larger current is the first trace. Each plot is typical of data from at least three cells.

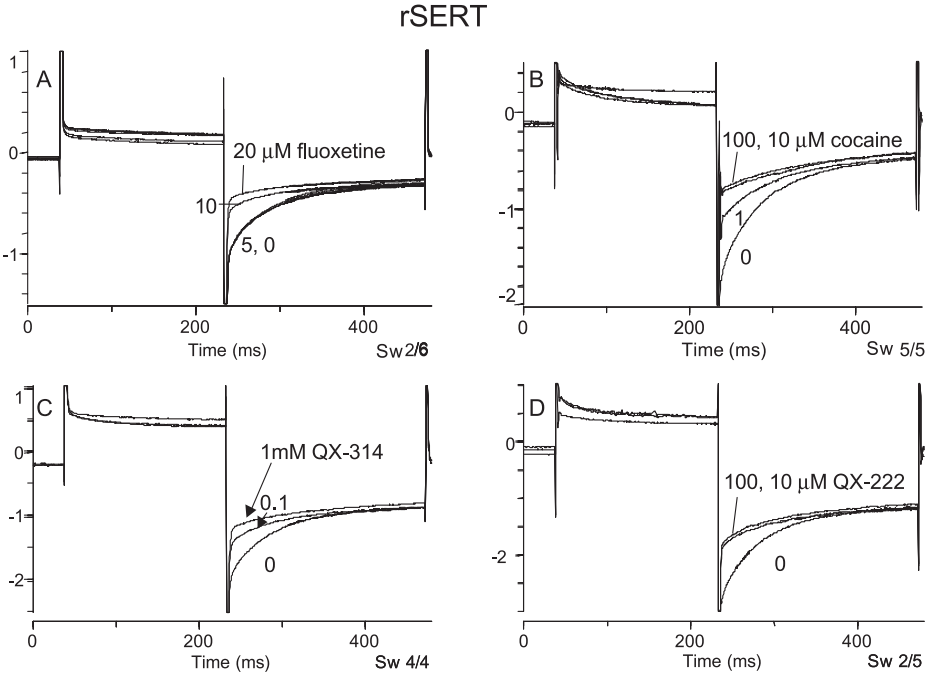

Fig. 6. Effects of fluoxetine (A), cocaine (B), QX-314 (C), and QX-222 (D) on the transient current and steady-state current of rSERT. For the transient current measurement, the membrane potential was held at −40 mV, was jumped to the priming potential of +60 mV for 195 ms, and was then jumped to the test potential of −140 mV for 240 ms. Each experiment is typical of data from at least three cells.

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