Available online at www.sciencedirect.com
SCIENCE DIRECT®
Biochemical Pharmacology 66 (2003) 2125–2132
Biochemical Pharmacology
www.elsevier.com/locate/biochempharm

Mechanism of block by fluoxetine of 5-hydroxytryptamine$_3$ (5-HT$_3$)-mediated currents in NCB-20 neuroblastoma cells

Jin-Sung Choi[a], Bok Hee Choi[a], Hye Sook Ahn[a,b], Myung-Jun Kim[a,b], Duck-Joo Rhie[a], Shin Hee Yoon[a,b], Do Sik Min[a], Yang-Hyeok Jo[a], Myung-Suk Kim[a], Ki-Wug Sung[b,c], Sang June Hahn[a,b,*]

[a]*Department of Physiology, College of Medicine, The Catholic University of Korea, 505 Banpo-dong, Socho-gu, Seoul 137-701, South Korea*
[b]*Medical Research Center, College of Medicine, The Catholic University of Korea, 505 Banpo-dong, Socho-gu, Seoul 137-701, South Korea*
[c]*Department of Pharmacology, College of Medicine, The Catholic University of Korea, 505 Banpo-dong, Socho-gu, Seoul 137-701, South Korea*

Received 23 May 2003; accepted 8 August 2003

Abstract

The effect of fluoxetine (Prozac) on 5-hydroxytryptamine$_3$ (5-HT$_3$)-mediated currents in NCB-20 neuroblastoma cells was examined using the whole-cell patch-clamp technique. Fluoxetine produced a significant reduction of peak amplitude without altering the activation time course of 5-HT$_3$-mediated currents. These effects were concentration-dependent, with an IC_{50} value of 4.15 μM. No voltage dependence was evident in fluoxetine's block of 5-HT$_3$-mediated currents over the entire voltage range tested. The extent of block by pre-application of fluoxetine was significantly greater than that by co-application. Fluoxetine also increased the apparent rate of current desensitization to 5-HT application. Using a first-order kinetics analysis, the open-channel blocking rate constants were 0.06 $\mu M^{-1}\ s^{-1}$ (k_{+1}, association rate constant) and 0.05 s^{-1} (k_{-1}, dissociation rate constant), with an apparent K_d ($=k_{-1}/k_{+1}$) of 0.83 μM. This value is close to an IC_{50} of 1.11 μM obtained from the reduction in τ, the time constant of desensitization. Intracellular application of fluoxetine for long durations had no effect on the amplitude or kinetics of 5-HT$_3$-mediated currents. Similarly, norfluoxetine, the major metabolite of fluoxetine, reduced the peak current, and enhanced the rate of current desensitization in a concentration-dependent manner with an IC_{50} of 2.66 μM, indicating that norfluoxetine is more potent than fluoxetine in blocking 5-HT$_3$-mediated currents. These results indicate that, at clinically relevant concentrations, fluoxetine and its metabolite, norfluoxetine, block 5-HT$_3$-mediated currents in NCB-20 neuroblastoma cells.

Keywords: Fluoxetine; Norfluoxetine; 5-HT$_3$-mediated currents; NCB-20 neuroblastoma cell

1. Introduction

5-HT is one of the major neuromodulators that regulate synaptic transmission in the central and peripheral nervous systems, and it is implicated in complex neuronal functions [1,2]. Among the subfamilies of 5-HT receptors, 5-HT$_3$ receptors are the only ionotropic ligand-gated channels that mediate transient inward currents [3]. Indeed, this receptor is remarkably sensitive to a number of pharmacological agents and is probably an important therapeutic target of drugs used to treat anxiety, emesis, and inflammatory pain [2,4–6].

Fluoxetine, a widely used antidepressant, is reported to selectively inhibit the reuptake of 5-HT and enhance its neurotransmission by increasing its concentration at the central and peripheral synapses without affecting other catecholaminergic transmission [7]. However, several studies showed that fluoxetine, in addition to its selective serotonin reuptake inhibitor action, blocks a wide variety of ligand- and voltage-gated ion channels in different preparations. For example, fluoxetine has been shown to cause open-channel block of nicotinic acetylcholine receptors, which belong to the same superfamily of 5-HT$_3$ receptors [8]. In addition, at clinically relevant concentrations, fluoxetine also blocks native 5-HT$_3$-mediated currents in rat nodose ganglion neurons [9,10]. However, previous electrophysiological studies on the effects of

* Corresponding author. Tel.: +82-2-590-1168; fax: +82-2-532-9575.
E-mail address: sjhahn@catholic.ac.kr (S.J. Hahn).
Abbreviations: 5-HT, 5-hydroxytryptamine; 5-OHi, 5-hydroxyindole.

0006-2952/$ – see front matter © 2003 Elsevier Inc. All rights reserved.
doi:10.1016/j.bcp.2003.08.012

fluoxetine on the 5-HT_3-mediated currents have focused only on the concentration–response effects, leaving the mechanism of action underlying the specific effects of fluoxetine on 5-HT_3-mediated currents largely undefined. Thus, the effects of fluoxetine and its metabolite, norfluoxetine, on 5-HT_3-mediated currents in NCB-20 neuroblastoma cells were investigated to further establish the pharmacological properties of 5-HT_3 receptors.

2. Materials and methods

2.1. Cell preparation

NCB-20 neuroblastoma cells (kindly provided by Dr. Lovinger, NIH, USA) used for electrophysiological experiments were grown under the conditions described previously [11]. At present, two different subunit 5-HT_{3A} and 5-HT_{3B} have been cloned [1]. In NCB-20 cells, the 5-HT_{3A} receptor is expressed at high density [11].

Cells were seeded onto 35 mm dishes containing poly-D-lysine (Sigma) coated coverslips 1 day prior to electrophysiological recordings. The cells were then incubated at 37° in a humidified atmosphere of 95% O_2 and 5% CO_2.

2.2. Electrophysiological recordings

The whole-cell configuration of the patch-clamp technique was used for voltage-clamp recordings at room temperature (22–23°) with an Axopatch 200B amplifier (Axon Instruments). Micropipettes, fabricated from PG10165-4 glass capillary tubing (World Precision Instruments) using a vertical puller (Model PP-83, Narishige), had a tip resistance of 2–3 MΩ when filled with the internal pipette solution. Capacitive currents were compensated with analog compensation, but leak subtraction was not used in this study. Currents were low-pass filtered at 2.5 kHz (four-pole Bessel filter) and sampled at 5 kHz. Data acquisition and data analysis were performed with an IBM Pentium computer, using pClamp 8.1 software (Axon Instruments).

2.3. Solutions and drugs

For whole-cell recordings, the electrodes were filled with a solution containing 140 mM CsCl, 10 mM EGTA, and 10 mM HEPES (pH 7.3 with CsOH, osmolality adjusted to 295 mOsm/kg with sucrose). The bath solution for whole-cell recordings contained 140 mM NaCl, 5 mM KCl, 1.3 mM $CaCl_2$, 1 mM $MgCl_2$, 20 mM HEPES, and 10 mM glucose (pH 7.3 with NaOH, osmolality adjusted to 300 mOsm/kg with sucrose). During the recording, the chamber (RC-13, Warner Instrument Corporation) was continuously perfused with bath solution. All drugs were rapidly applied with a superfusion system using θ-tubing. After establishing a whole-cell recording, cells were placed in front of one side of the θ-tubing pipette. The bath solution, which contained the agonist, flowed through different sides of the θ-tubing which was pulled to an inner diameter of about 300 μm. Solutions were rapidly switched around the cell using a piezoelectric-driven micromanipulator (P-287.70, Physik Instrumente), which displaced the θ-tubing laterally so that the cell could be exposed for a defined period of time in drug-containing solution, and then rapidly returned to drug-free solution. The rate of solution change was measured according to the following protocol. The change of current was recorded using an open patch pipette, which was voltage clamped at 0 mV during a switch from bath solution to a diluted (1:10 with water) bath solution. The relaxation due to a change in junction potential was well fitted by a single exponential function with a time constant of 24.77 ± 3.31 ms (N = 6). This solution exchange time did not impede the ability to estimate the activation time course of the 5-HT_3-mediated current. Solution flow was driven by gravity from wells placed above the preparation and application of solution was controlled by a valve placed upstream of the drug-containing tubing. Drugs were diluted in bath solution from stock solutions. Fluoxetine (Tocris Cookson Ltd) and norfluoxetine (Research Biochemicals Inc.) were dissolved in DMSO to yield stock solutions of 10 mM. The concentration of DMSO in the final dilution was less than 0.1% and this concentration of DMSO had no effect on 5-HT_3-mediated currents. All other chemicals were purchased from Sigma Chemical Co.

2.4. Data analysis

Concentration–response data were best fitted with the Hill equation using Origin 7.0 software (OriginLab Corp.):

$$Y = \frac{1}{\{1 + (IC_{50}/[D])^n\}} \quad (1)$$

where IC_{50} is the concentration of drug resulting in 50% block (or EC_{50}), D is the drug concentration, and n is the Hill coefficient. Interaction kinetics between drug and channel are described on the basis of a first-order blocking scheme [12]. The apparent rate constants for association (k_{+1}) and dissociation (k_{-1}) were calculated from the following equations:

$$\tau_D = \frac{1}{(k_{+1}[D] + k_{-1})} \quad (2)$$

$$K_d = \frac{k_{-1}}{k_{+1}} \quad (3)$$

in which τ_D is the drug-induced time constant, calculated from single exponential fits to the desensitizing current traces.

The 20–80% rise time (the time to rise from 20% of the peak to 80%) was measured using pClamp 8.1 software (Axon Instruments) and is commonly used to determine current activation.

Results are expressed as means ± SEM. Statistical significance was determined at the level 0.05 using Student's *t* test or ANOVA.

3. Results

Initially the concentration–response relationship for 5-HT activation of 5-HT_3-mediated currents in NCB-20 cells under whole-cell recording conditions was determined. All experiments were carried out at a holding potential of −50 mV unless otherwise stated. Applications of 5-HT for 5 s at 1 min intervals to NCB-20 cells caused a concentration-dependent inward current at −50 mV; 30 μM 5-HT induced a nearly maximal response. A previous investigation revealed that 5-HT-induced inward current in NCB-20 cells are mediated by 5-HT_3 channels [11,13]. These currents were completely blocked by 1 μM Y-25130, the selective 5-HT_3 antagonist (data not shown). These properties of 5-HT_3-mediated currents agreed well with those previously reported [11,13]. The EC_{50} value for the 5-HT response was 2.09 ± 0.13 μM (N = 8), so 3 μM 5-HT was used as the standard concentration in the present study. Maximum 5-HT_3-mediated currents at −50 mV averaged 1213.37 ± 188.39 pA and whole-cell capacitance was 42.41 ± 3.78 pF (N = 26).

The concentration dependence of the block of 5-HT_3-mediated currents by fluoxetine is presented in Fig. 1A. Under control conditions, 5-HT (3 μM) elicited a rapidly rising inward current (20–80% rise time = 205.78 ± 16.88 ms, N = 6) that desensitized slowly during prolonged (5 s) applications. Pre-application of fluoxetine (3 μM) for 5 s before and 5 s during 5-HT (3 μM) application did not modify the activation time course of the current (20–80% rise time = 194.55 ± 19.12 ms, N = 6, Student's *t* test, $P > 0.05$), but reduced the peak current in a concentration-dependent manner. Fluoxetine alone (up to 100 μM) did not induce any inward currents in NCB-20 cells (N = 5). A plot of the normalized current as a function of the fluoxetine concentration yields an IC_{50} value for peak current of 4.15 ± 0.24 μM and a Hill coefficient of 0.99 (N = 6) (Fig. 1B). In addition to reducing the peak current, fluoxetine also altered the time course of the current desensitization (Fig. 1C). Under control conditions, 5-HT_3-mediated current desensitization was well fitted to a single exponential function with a time constant of 3245.26 ± 383.91 ms (N = 6). In the presence of fluoxetine, there was an acceleration in the apparent rate of the

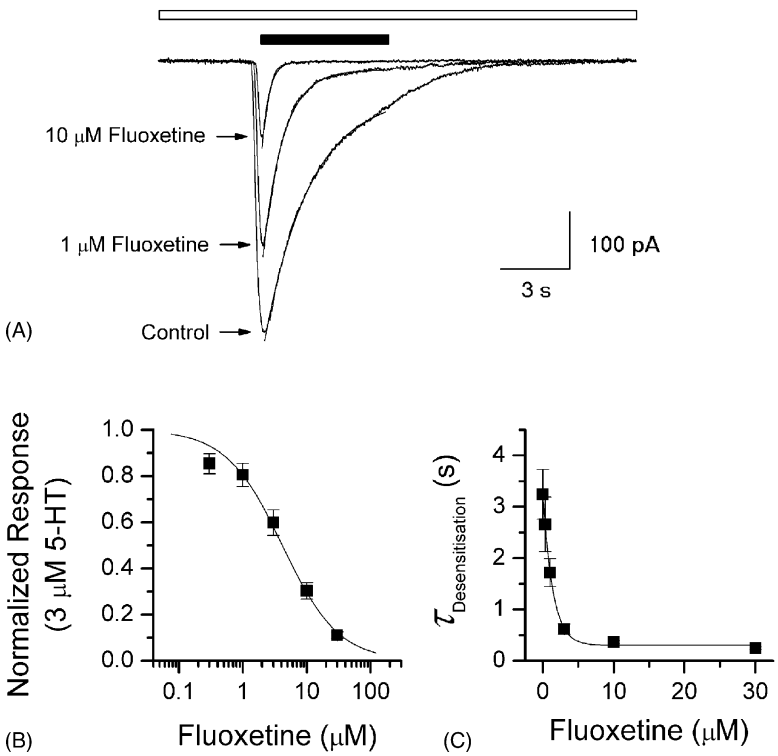

Fig. 1. Concentration dependence of fluoxetine block of 5-HT_3-mediated currents. (A) Superimposed whole-cell 5-HT_3-mediated current traces obtained by 5-s application (indicated by the closed horizontal bar) of 5-HT (3 μM) in the absence (control) and presence of 1 and 10 μM fluoxetine (indicated by the open horizontal bar) in NCB-20 neuroblastoma cells. Currents were well fitted to a single exponential decay. The holding potential was −50 mV. (B) Concentration–response curve for block of 5-HT_3-mediated currents. Currents were measured at the peak to generate the concentration–response curve. Nonlinear least-squares fit of the data yielded an IC_{50} value of 4.15 μM and a Hill coefficient of 0.99 (N = 6). (C) Plot of the time constant (τ) of current desensitization as a function of the fluoxetine concentration. The time constant was obtained from single exponential fits of the decay phase of the current traces (N = 6). Data are expressed as means ± SEM.

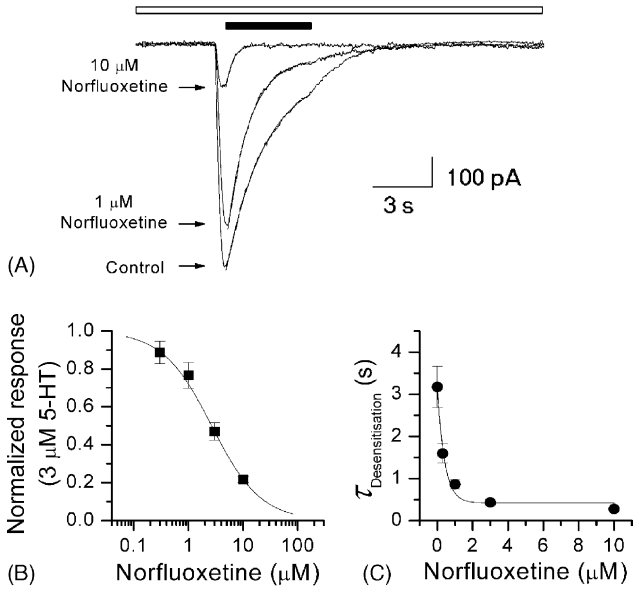

Fig. 2. Concentration dependence of norfluoxetine block of 5-HT_3-mediated currents. (A) Superimposed whole-cell 5-HT_3-mediated current traces obtained by 5-s application (indicated by the closed horizontal bar) of 5-HT (3 μM) in the absence (control) and presence of 1 and 10 μM norfluoxetine (indicated by the open horizontal bar) in NCB-20 neuroblastoma cells. Currents were well fitted to a single exponential decay. The holding potential was −50 mV. (B) Concentration–response curve for block of 5-HT_3-mediated currents. Currents were measured at the peak to generate the concentration–response curve. Nonlinear least-squares fit of the data yielded an IC_{50} value of 2.66 μM and a Hill coefficient of 0.97 (N = 5). (C) Plot of the time constant (τ) of current desensitization as a function of the fluoxetine concentration. The time constant was obtained from single exponential fits of the decay phase of the current traces (N = 5). Data are expressed as means ± SEM.

current desensitization in a concentration-dependent manner. The time constants of desensitization in the presence of 3 and 10 μM fluoxetine were 617.48 ± 52.08 ms and 366.56 ± 29.64 ms (N = 6), respectively. Figure 1C summarizes the concentration dependence of these time constants. Because fluoxetine modified the time course of the current desensitization, it was also useful to quantify the effect of fluoxetine on the rate of desensitization of 5-HT_3-mediated currents. Time constants (τ) at each concentration of fluoxetine were obtained from single exponential fits of the desensitization component of 5-HT_3-mediated currents. A nonlinear least-squares fit of the Hill equation to the normalized values of τ yielded an IC_{50} of 1.11 ± 0.35 μM (N = 6) which reduced τ to 50% of the control value.

Pre-application of norfluoxetine blocked 5-HT_3-mediated currents in a qualitatively similar fashion to fluoxetine (Fig. 2A); however, norfluoxetine displayed a higher potency to block 5-HT_3-mediated currents. For example, 10 μM fluoxetine and norfluoxetine reduced 5-HT_3-mediated currents by 69.7 and 78.3%, respectively. The concentration–response curve derived for the block of 5-HT_3-mediated currents by norfluoxetine is shown in Fig. 2B. Norfluoxetine was a more potent blocker of 5-HT_3-mediated currents than was fluoxetine with an IC_{50} of 2.66 ± 0.36 μM (N = 5). Norfluoxetine also induced a concentration-dependent increase in the rate of desensitization of the currents (Fig. 2C). Similarly, the concentration of norfluoxetine which reduced τ to 50% of the control value was 0.16 ± 0.02 μM (N = 5).

In order to judge the possible voltage dependence of the blocking effects of fluoxetine, 5-HT_3-mediated currents were recorded at different holding potentials from −50 to +50 mV. Representative current traces from a single cell are presented in Fig 3A. Fluoxetine reduced peak 5-HT_3-mediated currents over the entire voltage range tested. 5-HT_3-mediated currents (normalized to control at −50 mV) in the absence and presence of fluoxetine are plotted against holding potential (Fig. 3B). Current–voltage relationships for peak currents were almost linear with a reversal potential at about 0 mV. The reversal potentials of 5-HT_3-mediated currents were similar both in the absence and presence of fluoxetine. The mean fractional block was calculated at the different holding potentials tested. Fluoxetine blocked 5-HT_3-mediated currents both at −50 mV (49.64 ± 5.59%) and +50 mV (45.86 ± 3.54%, N = 4) to a similar extent (Fig. 3C). Thus, the actions of fluoxetine on 5-HT_3-mediated currents were independent of voltage.

To understand the mechanism of fluoxetine action, we compared the extent of channel block by co-application of 5 μM fluoxetine with pre-application of fluoxetine (Fig. 4A and B). Pre-application of fluoxetine blocked 52.36± 3.36% of the peak current (N = 4), while co-application of fluoxetine was much less effective, only blocking 23.69 ± 3.59% of 5-HT_3-mediated currents (N = 4).

The most notable feature of fluoxetine block of 5-HT_3-mediated currents was a concentration-dependent increase in the rate of channel desensitization. This action to accelerate the decay of 5-HT_3-mediated currents could be due to an open-channel blocking mechanism [14]. To clarify this hypothesis, 5-HT_3-mediated currents were recorded in the presence of 5-hydroxyindole (5-OHi, 10 mM), which is known to slow the desensitization of the 5-HT_3 channel to 5-HT [15] (Fig. 5A). Under control conditions, 5-HT_3-mediated currents induced by 3 μM 5-HT with 10 mM 5-OHi rapidly activated and reached their maximal peak and then declined slowly, with a time constant of 10.21 ± 2.40 s (N = 4). Fluoxetine induced a concentration-dependent increase in the rate of desensitization of 5-HT_3 channels during the application of 5-OHi and 5-HT. Although the current does not relax to the control amplitude in the absence of fluoxetine and remains depressed as shown in Fig. 5A, full recovery of current after washout period of 1 min was observed between successive current sweeps. In addition, the rate of current decay was fitted by a single exponential function, and thus, these time constants can be taken as an approximation of the drug–open-channel interaction kinetics. Because 5-HT_3-mediated currents undergo residual slow desensitization during the application of 5-OHi and 5-HT, we disregarded the time

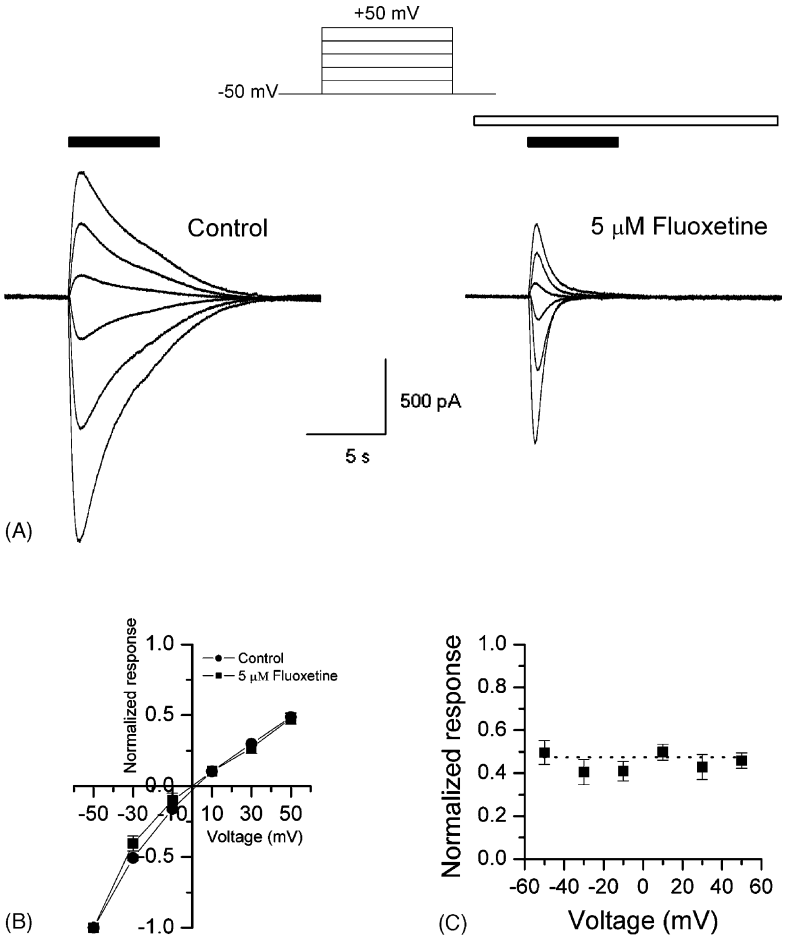

Fig. 3. Voltage-independent block of 5-HT_3-mediated currents by fluoxetine. (A) 5-HT_3-mediated currents evoked at various holding potentials in the absence and presence of 5 μM fluoxetine. (B) Current–voltage curves for 5-HT_3-mediated currents in the absence and presence of fluoxetine (5 μM). Currents were normalized to the control current at −50 mV. (C) Mean fractional block as a function of membrane potential (N = 5). Data are expressed as means ± SEM.

constant values at low concentrations (300 nM and 1 μM) of fluoxetine in calculating a good approximation of the time constant from the development of drug-induced block. The relationship between τ and drug concentration was well described by Eq. (2) (see Section 2; Fig. 5B). The slope of this function yielded an apparent association rate constant $k_{+1} = 0.06\ \mu M^{-1}\ s^{-1}$ and the intercept at the ordinate gave a dissociation rate constant $k_{-1} = 0.05\ s^{-1}$ (N = 4). The apparent K_d ($=k_{-1}/k_{+1}$) was 0.83 μM. This value is in good agreement with the IC_{50} value of 1.11 μM for fluoxetine that was obtained from the reduction in τ, the time constant of desensitization.

To determine on what side of the membrane 5-HT_3 channels are blocked by fluoxetine, 100 μM of fluoxetine was included in the pipette solution (Fig. 6). Intracellular application of fluoxetine for long durations (up to 20 min) had no effect on the amplitudes or the kinetics of 5-HT_3-mediated currents. When 5 μM of fluoxetine was subsequently added extracellularly, 5-HT_3-mediated currents were reduced to 45.80 ± 1.73% (N = 3) of control. The fact that fluoxetine was effective only when applied from the extracellular side of the cell membrane indicates that the binding site for fluoxetine is more accessible from the extracellular side.

4. Discussion

These results show that in NCB-20 cells, fluoxetine blocks 5-HT_3-mediated currents in a concentration-dependent and voltage-independent manner. One of the important observations of this study is that fluoxetine not only reduced the peak current but also enhanced the rate of current desensitization of 5-HT_3 channels, a result that is similar to the findings of previous studies of noncompetitive, voltage-independent block of 5-HT_3-mediated currents by ifenprodil in NG108 neuroblastoma cells [5]. In our study, however, these observations can be explained in one of three ways. First, fluoxetine blocks channels prior to the activation phase *via* an interaction with the closed channels (closed-channel block). Thus, ''blocked'' channels cannot contribute to the peak current, resulting in an apparent reduction in the peak current (see below). This conclusion is further supported by the observation that

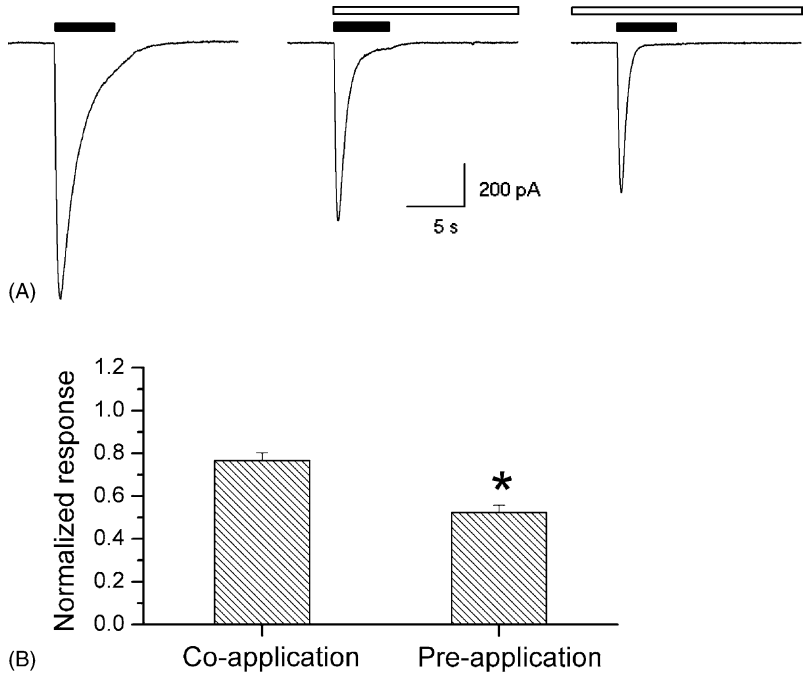

Fig. 4. Effects of fluoxetine (5 μM) on 5-HT_3-mediated currents following various modes of fluoxetine application. (A) Fluoxetine was applied 5 s before (pre-application) and during (co-application) 5-HT (3 μM) application. (B) Summary of normalized current. Greater block was observed for application (5 s) prior to 5-HT (3 μM) delivery than for co-application. Data are expressed as means ± SEM (N = 4). *Significantly different from co-application ($P < 0.05$).

fluoxetine is a noncompetitive inhibitor which interacts preferentially with the closed state of 5-HT_3 receptor in NIE-115 cells [16]. Second, the decrease in peak amplitudes of 5-HT_3-mediated currents may be due to a fast open-channel block by fluoxetine. If fluoxetine can reach its binding site when the 5-HT_3 receptor is activated (open state), and the drug association rate is rapid compared to the rate for channel opening, then the block of the currents develops when the channels begin to open. Thus, open-channel block can result in a reduction of the peak currents. However, if the association rate of fluoxetine is slow compared to the rate of channel opening, then channel desensitization to 5-HT will appear to be accelerated. Alternatively, fluoxetine may modulate the kinetics of the intrinsic desensitization mechanism to achieve this end. Thus, if a significant fraction of channels are rapidly desensitized by fluoxetine during the activation phase of the current, the desensitized channel would not carry current and diminished peak amplitude would be recorded. This hypothesis is consistent with the previous report that the time course of blocking effect of propofol is similar to the kinetics of 5-HT-induced desensitization [17]. Clearly, this phenomenon requires additional study with desensitization-deficient channels. These findings suggest that a reduction in peak current reflects the sum of the degree of block that occurs before the activation phase (closed-channel block) and that which occurs during the activation phase (fast open-channel block or desensitization). Similarly, the discrepancy in the IC_{50} values obtained for peak current (4.15 μM) and for desensitization τ (1.11 μM) suggest that the peak current measurement might reflect the sum of the degree of closed- and open-channel block, and measuring τ might reflect only the degree of open-channel block.

In the present study, fluoxetine induced a reduction in peak current in a voltage-independent manner. Fluoxetine predominates in its positively charged form at physiological pH, and charged forms of drugs have usually been associated with voltage dependence. In addition, the evidence for a mechanism of open-channel block includes voltage dependence. Because most of the blocking properties of fluoxetine on voltage-gated ion channels are linked to voltage dependence and open-channel block [18–21], the mechanism by which fluoxetine blocks 5-HT_3-mediated currents may differ from the way in which it interacts with voltage-gated ion channels. Thus, it is possible that the voltage-independence reflects the fact that fluoxetine interacted with the channels both in the closed and open state (see above). Additionally, it should be considered that if the major mechanism of action of fluoxetine on 5-HT_3-mediated currents is thought to involve the uncharged form of the drug, fluoxetine should block the current in a voltage-independent manner. This is consistent with results previously reported for fluoxetine block of volume-regulated anion channels, in which the blockade is mediated by the uncharged form of fluoxetine [22]. Furthermore, the open-channel blocker, diltiazem, reduces 5-HT_3-mediated currents more effectively as the membrane is depolarized, inhibiting 5-HT_3-mediated currents equally both on pre-application and on co-application [14]. This result suggests that there is no block of 5-HT_3-mediated currents before channel activation

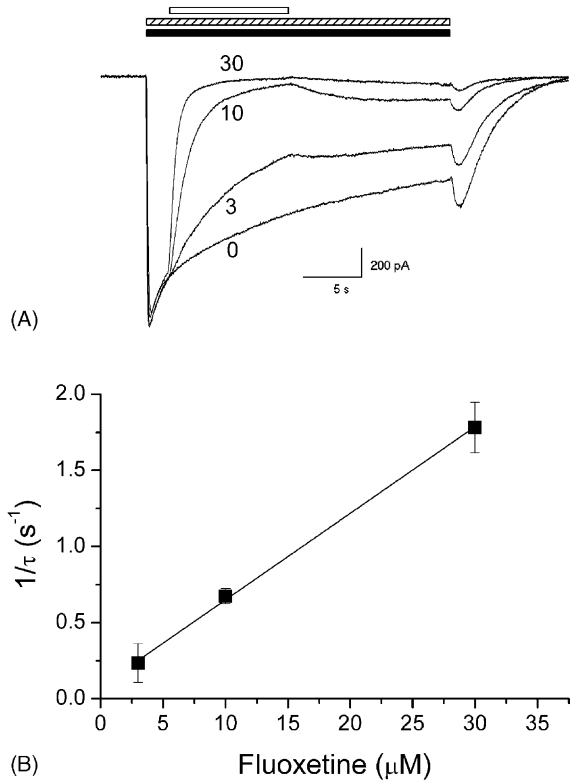

Fig. 5. Kinetics of fluoxetine-induced block. 5-HT_3-mediated currents were recorded in the presence of 5-OHi (10 mM), which is known to reduce the rate of desensitization of the 5-HT_3-mediated currents. (A) Fluoxetine (indicated by the open horizontal bar) induced a concentration-dependent increase in the rate of desensitization of the channels during the application of 5-OHi (indicated by the hatched horizontal bar) and 5-HT (indicated by the closed horizontal bar). Onset of block and recovery from block was fitted by a single exponential function. (B) The relationship between τ and drug concentration was well described by Eq. (2). $1/\tau$ for onset of block varied linearly with fluoxetine concentration. The slope of this function yielded an apparent association rate constant $k_{+1} = 0.06\ \mu M^{-1}\ s^{-1}$ and the intercept at the ordinate gave a dissociation rate constant $k_{-1} = 0.05\ s^{-1}$ (N = 4). The apparent K_d ($=k_{-1}/k_{+1}$) was 0.83 μM. This value is in good agreement with the IC_{50} value of 1.11 μM for fluoxetine that was obtained from the reduction in the time constant of desensitization, τ.

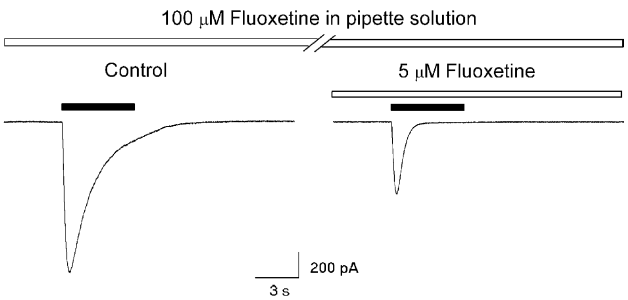

Fig. 6. Effects of intracellular fluoxetine on 5-HT_3-mediated currents. Internal application of 100 μM fluoxetine in the whole-cell configuration had no effect on the amplitude or the kinetics of 5-HT_3-mediated currents up to 20 min after application. Subsequent extracellular application of 5 μM fluoxetine (indicated by the open horizontal bar) blocked 5-HT_3-mediated currents effectively.

(i.e. open-channel block). In contrast to diltiazem, we found a greater depression of the 5-HT_3-mediated current with pre-application as opposed to co-application experiments. Whereas co-applied fluoxetine may act only after the channels are activated, pre-applied fluoxetine can block 5-HT_3-mediated currents by binding to both closed and open states of channels. Thus, the fact that greater block occurs with application prior to channel activation suggests that fluoxetine can bind to and inhibit the opening of the closed state of 5-HT_3 channels.

The present study shows that fluoxetine is a potent blocker of 5-HT_3-mediated currents in NCB-20 cells with an IC_{50} of 4.15 μM. Moreover, the active metabolite of fluoxetine, norfluoxetine, is more potent as a blocker of 5-HT_3-mediated currents than the parent compound ($IC_{50} = 0.97$ μM). These results agree with previous findings in native 5-HT_3 receptors of rat nodose ganglion neurons which were inhibited by fluoxetine with a similar potency ($IC_{50} = 1.3$ μM) [9,10]. This effect is also similar to the previously reported block by fluoxetine of nicotinic acetylcholine receptors, which belong to the same superfamily of 5-HT_3 receptors [8]. In addition, the IC_{50} value of fluoxetine for blocking 5-HT_3-mediated currents is similar to that obtained for the inhibition of voltage-gated ion channels in different preparations. For example, the IC_{50} values of fluoxetine block of cloned *shaker* potassium channel, Kv1.3, and voltage-activated calcium channels are reported to be 3.0 μM [19] and 1.1 μM [23], respectively. Because the mean therapeutic plasma concentrations of fluoxetine ranges from 1 to 2 μM and fluoxetine accumulates in brain at concentrations 20-fold higher than those observed in plasma [24,25], the fluoxetine-induced block of 5-HT_3-mediated currents is of clinical relevance.

Clinically, the 5-HT_3 receptor appears to play an important role in depression, emesis, and modulation of pain perception [4,26]. Despite the fact that fluoxetine is widely used as an antidepressant drug and its pharmacological action is primarily derived from inhibition of the reuptake of serotonin at the synaptic cleft in the central nervous system, fluoxetine has been reported to be effective for the relief of pain [26–28]. Although clinical relevance of 5-HT_3 block by fluoxetine is currently unknown, the inhibition of 5-HT_3 by fluoxetine may contribute to the therapeutic efficacy of fluoxetine in depression and also have pharmacological relevance to its analgesic effect.

In summary, at clinically relevant concentrations, fluoxetine blocks 5-HT_3-mediated currents in a concentration-dependent and voltage-independent manner and interacts with more than one state of 5-HT_3 channels.

Acknowledgments

This study supported by a grant from the Medical Research Center, Korea Science and Engineering Foundation, Republic of Korea (R13-2002-005-01002-0). We thank

Dr. Lovinger (NIH, USA) for providing us with NCB-20 cells and Kris Silver (Department of Entomology, Cornell University, USA) for reading the manuscript.

References

[1] Barnes NM, Sharp T. A review of central 5-HT receptors and their function. Neuropharmacology 1999;38:1083–152.
[2] Jones BJ, Blackburn TP. The medical benefit of 5-HT research. Pharmacol Biochem Behav 2002;71:555–68.
[3] Jackson MB, Yakel JL. The 5-HT_3 receptor channel. Annu Rev Physiol 1995;57:447–68.
[4] Greenshaw AJ. Behavioural pharmacology of 5-HT_3 receptor antagonists: a critical update on therapeutic potential. Trends Pharmacol Sci 1993;14:265–70.
[5] McCool BA, Lovinger DM. Ifenprodil inhibition of the 5-hydroxytryptamine$_3$ receptor. Neuropharmacology 1995;34:621–9.
[6] Jenkins A, Franks NP, Lieb WR. Actions of general anaesthetics on 5-HT_3 receptors in NIE-115 neuroblastoma cells. Br J Pharmacol 1996;117:1507–15.
[7] Wong DT, Horng JS, Bymaster FP, Hauser KL, Molloy BB. A selective inhibitor of serotonin uptake: Lilly 110140, 3-(*p*-trifluoromethylphenoxy)-*N*-methyl-3-phenylpropylamine. Life Sci 1974;15:471–9.
[8] García-Colunga J, Awad JN, Miledi R. Blockage of muscle and neuronal nicotinic acetylcholine receptors by fluoxetine (Prozac). Proc Natl Acad Sci USA 1997;94:2041–4.
[9] Fan P. Effects of antidepressants on the inward current mediated by 5-HT_3 receptors in rat nodose ganglion neurones. Br J Pharmacol 1994;112:741–4.
[10] Fan P. Inhibition of a 5-HT_3 receptor-mediated current by the selective serotonin uptake inhibitor, fluoxetine. Neurosci Lett 1994;173:210–2.
[11] Lovinger DM, Sung KW, Zhou Q. Ethanol and trichloroethanol alter gating of 5-HT_3 receptor-channels in NCB-20 neuroblastoma cells. Neuropharmacology 2000;39:561–70.
[12] Snyders DJ, Bennett PB, Hondeghem LM. Mechanisms of drug–channel interaction. In: Fozzard HA, Haber E, Jennings RB, Katz AM, Morgan HE, editors. The heart and cardiovascular system. 2nd ed. New York: Raven Press; 1992. p. 2165–93.
[13] Yang HS, Kim SY, Choi SJ, Kim KJ, Kim ON, Lee SB, Sung KW. Effect of 5-hydroxyindole on ethanol potentiation of 5-hydroxytryptamine (5-HT)$_3$ receptor-activated ion current in NCB-20 neuroblastoma cells. Neurosci Lett 2003;338:72–6.
[14] Gunthorpe MJ, Lummis SCR. Diltiazem causes open channel block of recombinant 5-HT_3 receptors. J Physiol 1999;519:713–22.
[15] Kooyman AR, van Hooft JA, Vijverberg HPM. 5-Hydroxyindole slows desensitization of 5-HT_3 receptor-mediated ion current in NIE-115 neuroblastoma cells. Br J Pharmacol 1993;108:287–9.
[16] Breitinger H-GA, Geetha N, Hess GP. Inhibition of the serotonin 5-HT_3 receptor by nicotine, cocaine, and fluoxetine investigated by rapid chemical kinetic techniques. Biochemistry 2001;40:8419–29.
[17] Barann M, Dilger JP, Bönisch H, Göthert M, Dybek A, Urban BW. Inhibition of 5-HT_3 receptors by propofol: equilibrium and kinetic measurements. Neuropharmacology 2000;39:1064–74.
[18] Tytgat J, Maertens Ch, Daenens P. Effect of fluoxetine on a neuronal, voltage-dependent potassium channel (Kv1.1). Br J Pharmacol 1997;122:1417–24.
[19] Choi JS, Hahn SJ, Rhie DJ, Yoon SH, Jo YH, Kim MS. Mechanism of fluoxetine block of cloned voltage-activated potassium channel Kv1.3. J Pharmacol Exp Ther 1999;291:1–6.
[20] Choi BH, Choi JS, Yoon SH, Rhie DJ, Min DS, Jo YH, Kim MS, Hahn SJ. Effects of norfluoxetine, the major metabolites of fluoxetine, on the cloned neuronal potassium channel Kv3.1. Neuropharmacology 2001;41:443–53.
[21] Perchenet L, Hilfiger L, Mizrahi J, Clément-Chomienne O. Effects of anorexinogen agents on cloned voltage-gated K^+ channel hKv1.5. J Pharmacol Exp Ther 2001;298:1108–19.
[22] Maertens C, Wei L, Voets T, Droogmans G, Nilius B. Block by fluoxetine of volume-regulated anion channels. Br J Pharmacol 1999;126:508–14.
[23] Deák F, Lasztóczi B, Pacher P, Petheö GL, Kecskeméti V, Spät A. Inhibition of voltage-gated calcium channels by fluoxetine in rat hippocampal pyramidal cells. Neuropharmacology 2000;39:1029–36.
[24] Karson CN, Newton JE, Livingston R, Jolly JB, Cooper TB, Sprigg J, Komoroski RA. Human brain fluoxetine concentrations. J Neuropsychiatry Clin Neurosci 1993;5:322–9.
[25] Komoroski RA, Newton JE, Cardwell D, Sprigg J, Pearce J, Karson CN. In vivo ^{19}F spin relaxation and localized spectroscopy of fluoxetine in human brain. Magn Reson Med 1994;31:204–11.
[26] Eschalier A, Kayser V, Guilbaud G. Influence of a specific 5-HT_3 antagonist on carrageenan-induced hyperalgesia in rats. Pain 1989;36:249–55.
[27] Wong DT, Bymaster FP, Engleman EA. Prozac (fluoxetine, Lilly 110140), the first selective serotonin uptake inhibitor and an antidepressant drug: twenty years since its first publication. Life Sci 1995;57:411–41.
[28] Rani PU, Naidu MUR, Prasad VBN, Rao TRK, Shobha JC. An evaluation of antidepressants in rheumatic pain conditions. Anesth Analg 1996;83:371–5.