Inhibition of K^+ currents of outer hair cells in guinea pig cochlea by fluoxetine

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Abstract

The effects of fluoxetine (Prozac[®]), a widely used antidepressant drug, on K^+ channel in outer hair cells isolated from guinea pig cochlea were studied using the whole-cell patch clamp technique. Fluoxetine potently inhibited leak K^+ currents with an IC_{50} of 0.78 μ M. The inhibition was reversible and voltage-independent. At 45- to 103-fold higher concentrations than the plasma levels, fluoxetine reversibly blocked voltage-activated K^+ currents. Kinetics of the current in the presence of fluoxetine resembled the control current, and the inhibition was not use-dependent. Neither the activation curve nor the reversal potential was affected by fluoxetine. This inhibition was voltage-dependent with an electric distance (δ value) of the binding site of at least 26% of the membrane field from the cytoplasmic side. Use-independent inhibition suggests that fluoxetine blocks the channel before its opening or instantly blocks the open channel. This is the first study of the action of this compound on K^+ channel of outer hair cells of the mammalian inner ear. We conclude that the block of the leak K^+ currents can occur at therapeutic levels of fluoxetine. Since the voltage-activated K^+ currents are not potently blocked by fluoxetine, this action might not be related to its antidepressant action or adverse effects.

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1. Introduction

Fluoxetine (Prozac[®]) is widely used to treat depression and at higher concentrations to treat obsessive–compulsive behavior (Fontaine and Chouinard, 1989; Gram, 1994; Wong et al., 1995). The antidepressant activity of fluoxetine is thought to be primarily due to its ability to inhibit the reuptake of serotonin in brain neuronal cells (Wong et al., 1974). In addition to this inhibitory action, fluoxetine has been reported to modulate the activity of ion channels, especially that of the voltage-activated K^+ channels. Fluoxetine inhibits delayed rectifier K^+ currents in rabbit corneal epithelial cells and in cultured human lens epithelium (Rae et al., 1995), in isolated canine and human jejunal circular smooth muscle cells (Farrugia, 1996), and in cerebellar granule neurons (Yeung et al., 1999). This compound also

inhibits voltage-gated $K_v1.1$ channels expressed in *Xenopus* oocytes (Tytgat et al., 1997), $K_v1.1$ channels (Yeung et al., 1999) and $K_v1.3$ channels (Choi et al., 1999) expressed in the Chinese hamster ovary cell line, and voltage-activated K^+ currents in PC12 cells (Hahn et al., 1999), as well as HERG K^+ currents, a rapid component of the delayed rectifier K^+ currents of cardiac ventricular myocytes (Thomas et al., 2002). However, the effect of fluoxetine on K^+ channel of outer hair cells is unclear.

In the mammalian organ of Corti, the sensory hair cells including inner hair cells and outer hair cells transduce sound vibrations into electrical signals. The outer hair cells increase the sensitivity of hearing by selectively amplifying sound reception (Beisel et al., 2000; Jentsch, 2000). The activities of K^+ channels of outer hair cells are thought to be indirectly responsible for this function (Jentsch, 2000). In this study, we examined the effects of fluoxetine on K^+ currents of outer hair cells isolated from the guinea pig cochlea and its cellular mechanisms underlying K^+ channel inhibition.

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2. Materials and methods

Adult albino guinea pigs (230–420 g) were sacrificed under methoxyflurane (Metofane) anesthesia, and the temporal bones were rapidly removed. One bulla was opened and the organ of Corti was exposed to minimum essential medium (MEM). The other bulla was kept at 4 °C for later dissection. The outer coils of the sensory epithelium of the organ of Corti were dissected out with fine forceps and transferred to MEM supplemented with collagenase (type V, 0.25 mg/ml, Sigma–Aldrich, St. Louis, MO). After 15 min of collagenase treatment at a room temperature of 22–25 °C, the cochlea coils were rinsed twice with MEM in order to stop the enzyme digestion. The coils were gently transferred with a 70 μ l volume of MEM to a perfusion chamber. This procedure usually yielded a large number of healthy isolated outer hair cells (Zajic and Schacht, 1987). The outer hair cells retained their normal morphology for an average time of 4 h. Stable currents could be usually recorded from such outer hair cells for more than 1 h when perfused with control solutions, drug-containing solutions, followed by control solutions for washout.

The care and use of the experimental animals reported in this study were approved by the Northwestern University Center for Comparative Medicine and the Northwestern University Institutional Animal Care and Use Committee.

Experiments were performed at room temperature (22–25 °C) using the whole-cell patch clamp technique. The patch pipettes had resistance of approximately 5 M Ω when filled with an internal solution and measured in an external solution. The internal pipette solution contained (mM): KCl 135, MgCl₂·6H₂O 2, BAPTA (1,2-bis (2-aminophenoxy) ethane-*N,N,N',N'*-tetraacetic acid) 10, HEPES-Na 10, ATP-Na₂ 1, GTP-Na 0.1, with pH adjusted to 7.3 with 1 N KOH, and osmolarity adjusted to 300 $\pm$ 5 mosM with D-glucose. The external solution contained (mM): NaCl 140, KCl 5, MgCl₂·6H₂O 2, HEPES-Na 5, HEPES-acid 5, EGTA 1, with pH adjusted to 7.4 with 1 N NaOH, and osmolarity adjusted to 300 $\pm$ 10 mosM with D-glucose (Lin et al., 1995). Glucose of 0.9–1.8 g was usually added to 1 l of external solution to reach the proper osmolarity. Fluoxetine hydrochloride was purchased from Sigma–Aldrich. Stock solutions of fluoxetine were made up in distilled water, and stored at –20 °C until used. Perfusion solutions of fluoxetine were prepared using the external solution immediately before the experiment. During the recordings, the cells were continuously perfused at a rate of 1.5–2.0 ml/min with control or drug-containing solutions. The solution volume of the bath chamber was kept at approximately 0.8 ml.

Currents were recorded with an Axopatch 200B amplifier using a CV-203 BU headstage (Axon Instruments, Union City, CA). Seal resistance of at least 1.0 G Ω was achieved before establishing the whole-cell configuration. Series resistance compensation of 70–80% was typically employed. The steady-state current was measured at the end of a 200-ms pulse. Currents recorded were low-pass filtered at 5 kHz,

digitized with a Digidata 1200 interface (Axon Instruments), and acquired by an IBM-compatible PC and pClamp software (version 6.0.3). Data were analyzed using Clampfit, Microsoft Excel 97, Origin version 6.0 (Microcal Software), or SigmaPlot version 5.0 (SPSS, Chicago, IL).

All data were expressed as mean $\pm$ S.E.M. and *n* as the number of cells. Statistical significance was determined at a level of *P* $\leq$ 0.05 using Student's *t*-test with a two-tailed distribution.

3. Results

Fig. 1A shows that whole-cell currents of outer hair cells exhibited both inward and outward components, when elicited with 200-ms voltage steps to the levels ranging from –70 to +30 mV from a holding potential of –30 mV in 10-mV increments. The inward leak current was reversibly reduced by 5 mM Cs⁺, suggesting that this component was partially carried by K⁺ ions (data not shown; Housley and Ashmore, 1992). The outward current increased in amplitude at increasing depolarizing pulses between –20 and +30 mV. The outward component inactivated slowly and was blocked by 30 mM tetraethylammonium (data not shown), indicating voltage-activated K⁺ current as previously reported (Lin et al., 1995; Nenov et al., 1997).

Since therapeutic doses of fluoxetine result in a plasma concentration of approximately 1 μ M (Altamura et al., 1994), we examined the effects of 1 μ M fluoxetine on the

Fig. 1. Effects of 1 μ M fluoxetine on both leak K⁺ current- and voltage-activated K⁺ current of an outer hair cell. Currents were elicited by 200-ms test pulses to potentials ranging from –70 to +30 mV in 10-mV increments at a holding potential of –30 mV, in control (A), in 1 μ M fluoxetine (B), and washout (C). The corresponding current–voltage relationships are shown in (D). ● = control, ■ = 1 μ M fluoxetine, and ○ = washout. Fluoxetine suppressed the inward leak current, while it had very little effect on the outward-going current.

Fig. 2. Concentration-dependent inhibition of leak K⁺ current of outer hair cells by fluoxetine. (A) Currents were evoked by 200-ms test pulses to potentials from -70 to -30 mV in 10-mV increments at a holding potential of -30 mV, and recorded before and during cumulative application of increasing concentrations of fluoxetine (3, 10, 30, and 100 μM) with 3-min intervals between the drug applications. The currents recovered after washing for 5 min. (B) Percentage inhibition values of $25 \pm 3\%$, $35 \pm 3\%$, $37 \pm 4\%$, $42 \pm 3\%$, and $46 \pm 7\%$ at 1, 3, 10, 30, and 100 μM of fluoxetine concentrations, respectively, at a test potential of -70 mV were fitted to a Hill equation, $y = A[C]^{n_H} / \{[IC_{50}]^{n_H} + [C]^{n_H}\}$, where y is percentage of inhibition, A is the maximal block, C is the drug concentration, IC_{50} is the drug concentration for 50% of the maximal block and n_H is the Hill coefficient, to yield a maximal block of 43%, a Hill coefficient of 1.00 and an IC_{50} of 0.78 μM. Each point is the mean $\pm$ S.E.M. for five to 12 cells.

K⁺ currents. Fig. 1B shows that fluoxetine inhibited leak K⁺ current, while exerting no effect on voltage-activated K⁺ current, as compared to control of Fig. 1A. Recovery of leak K⁺ current occurred after washout (Fig. 1C). The current–voltage relationship is plotted in Fig. 1D, which shows a 23% inhibition of the inward leak K⁺ current by 1 μM fluoxetine measured at a test potential of -70 mV.

Fig. 2A reveals concentration-dependent inhibition of fluoxetine on leak K⁺ current, while the drug concentration was increased cumulatively from 3 to 10, 30, and 100 μM, in an outer hair cell. The current recovered to 85% of the initial current after washout. It should be noted that a substantial block of leak K⁺ current was observed at a low drug concentration (3 μM), while there were block ceilings at higher concentrations (30–100 μM). For instance, 33%

inhibition of leak K⁺ current measured at the test potential of -70 mV was found in the presence of 3 μM fluoxetine. However, fluoxetine at 100 μM only blocked 52% of the control current.

Fig. 2B shows that at a test potential of -70 mV, fluoxetine at concentrations of 1, 3, 10, 30, and 100 μM inhibited the leak K⁺ current by $25 \pm 3\%$ ($n=5$), $35 \pm 3\%$ ($n=10$), $37 \pm 4\%$ ($n=12$), $42 \pm 3\%$ ($n=12$), and $46 \pm 7\%$ ($n=5$), respectively. These data were fitted to a Hill equation to yield a maximal block of 43%, a Hill coefficient of 1.00, and an IC_{50} of 0.78 μM. The inhibition of leak K⁺ current was also evaluated at other voltages. For instance, fluoxetine at 100 μM ($n=5$) suppressed leak K⁺ current by $45 \pm 5\%$ at -60 mV, $44 \pm 4\%$ at -50 mV, and $48 \pm 7\%$ at -40 mV. Thus, the inhibition of the leak current was not voltage-dependent.

To study the effect of fluoxetine on voltage-activated K⁺ current, a protocol that eliminated the inward component of K⁺ currents of outer hair cells was designed with depolarizing steps ranging from -30 to $+70$ mV in 10-mV increments (Fig. 3). The voltage-activated K⁺ current was inhibited by fluoxetine in a concentration-dependent man-

Fig. 3. Effects of fluoxetine on the voltage-activated K⁺ current of an outer hair cell. Currents were elicited by 200-ms test depolarizing pulses to potentials between -30 and $+70$ mV in 10-mV increments from a holding potential of -30 mV. (A) to (E), membrane currents were recorded before and during cumulative applications of increasing concentrations of fluoxetine (3, 10, 30, and 100 μM), with a 3-min interval between each drug application. (F) The currents recovered to 90% of control after washout for 5 min.

Fig. 4. Current–voltage relationships and time course of fluoxetine block of voltage-activated K^+ current of outer hair cells. (A) Current–voltage curves for the traces shown in Fig. 3A–F. Current amplitude measured at the end of a 200-ms pulse for each test potential is plotted as I – V relationships. ●=control, ▼=3 μ M, ▲=10 μ M, ◆=30 μ M, ■=100 μ M fluoxetine, and ○=washout. (B) Time course of current block in response to the cumulative applications of increasing concentrations of fluoxetine (3, 10, 30, and 100 μ M), with a 3-min interval between each drug application. The currents were elicited from a holding potential of -30 mV to a test potential of $+70$ mV. The inhibition of the currents by fluoxetine reached a steady-state level within 3 min after drug application.

ner. Fig. 3A–E shows the currents recorded before and during cumulative applications of increasing concentrations of fluoxetine (3, 10, 30, and 100 μ M) at 3-min intervals. In the control, voltage-activated K^+ currents increased in amplitude with increasing depolarization (Fig. 3A). In the presence of fluoxetine, the increase in amplitude of the current became smaller at higher depolarizations, especially in the presence of 30 or 100 μ M fluoxetine (Fig. 3D and E). Washout for 5 min reversed the inhibition to 90% of the control current (Fig. 3F). Current–voltage relationships are shown in Fig. 4A as derived from current records of Fig. 3A–F. The control plot (solid circles of Fig. 4A) shows a near linear increase in the current at potentials more positive than 0 mV. Fluoxetine reduced voltage-activated K^+ current amplitude for the entire voltage ranges over which this current was activated, and in a concentration- and voltage-dependent manner. For instance, the I – V plot in the presence of 100 μ M fluoxetine became almost a flat line at potentials between $+20$ and $+70$ mV (solid squares of Fig. 4A), indicating a voltage-dependent inhibition by the drug. After washout, the current recovered to the level close to the control current (open circles of Fig. 4A). The time course of

inhibition of voltage-activated K^+ current is illustrated in Fig. 4B. The inhibition quickly reached a steady state within 3 min at each concentration of fluoxetine upon application of the first test pulse.

The concentration–response relationship measured at a test potential of $+70$ mV for inhibition of voltage-activated K^+ current is illustrated by the solid line of Fig. 5A. Fluoxetine at 1, 3, 10, 30, 100, and 300 μ M inhibited the current by $5 \pm 1\%$ ($n=5$), $11 \pm 4\%$ ($n=6$), $23 \pm 5\%$ ($n=5$),

Fig. 5. Concentration-dependent and voltage-dependent block by fluoxetine of voltage-activated K^+ current of outer hair cells. (A) Concentration–response curves for inhibition of K^+ currents by fluoxetine. The inhibition calculated as percentage block at the end of 200-ms depolarizing steps from a holding potential of -30 mV to a test potential of 0 mV (dotted line), $+40$ mV (dashed line) or $+70$ mV (solid line), is plotted against fluoxetine concentration. Each point is the mean $\pm$ S.E.M. for five to six cells. The data were fitted to a Hill equation to give the following IC_{50} and n_H : 103 μ M, 0.77 at 0 mV; 69 μ M, 0.82 at $+40$ mV; 45 μ M, 0.85 at $+70$ mV, respectively. (B) Voltage dependence of current inhibition by fluoxetine. The smoothed line represents the activation curve of voltage-activated K^+ current, corresponding to G/G_{max} of right axis. Percentage of current block (solid straight line) is plotted as a function of membrane potential in the presence of 100 μ M fluoxetine ($n=5$), corresponding to percentage block of left axis. In the voltage ranges ($+20$ to $+70$ mV), where activation had reached saturation, the voltage dependence of inhibition was calculated according to the Woodhull model (Woodhull, 1973), yielding $\delta=0.26$ and $K_{d(0)}=103$ μ M. In this calculation, the cationic form of fluoxetine is the active form to block the channel. If one assumes that both the neutral form and the cationic form are active to block the channel by binding to the same site, and that the block by the neutral form is not voltage-dependent, while the block by the cationic form is voltage-dependent, then the data can be fitted satisfactorily with $K_n=3$ μ M, $K_{C(0)}=140$ μ M, and $\delta=0.32$, as shown by the dotted line. The total block is dissected out into the block by the neutral form (medium dashed line) and the block by the cationic form (short dashed line).

$39 \pm 7\%$ ($n=6$), $67 \pm 5\%$ ($n=5$), and $85 \pm 1\%$ ($n=2$), respectively. These data were fitted with the logistic equation: $Y = 100 - \{1/[1 + (IC_{50}/C)^{n_H}]\} \times 100$, where IC_{50} is the drug concentration for 50% inhibition of currents, C is the drug concentration, and n_H is the Hill coefficient. The fitted curve yielded an IC_{50} for fluoxetine of $45 \mu\text{M}$, with a Hill coefficient of 0.85. IC_{50} for fluoxetine inhibition were different at different test potentials: at test potentials of 0 mV (dotted line) and $+40$ mV (dashed line), IC_{50} values were 103 and $69 \mu\text{M}$, respectively, with Hill coefficients near unity (Fig. 5A).

The voltage dependence of the steady-state activation curve in the presence of fluoxetine was evaluated to determine whether the inhibition was a result of a positive shift of the activation curve. Activation curves were fitted with the Boltzmann equation: $G/G_{\text{max}} = 1/\{1 + \exp[(V_{0.5} - V_m)/k]\}$, where G and G_{max} are conductance and maximal conductance, respectively, and $V_{0.5}$ is the voltage at which the conductance is half-maximal, V_m is the test potential, and k is the slope factor for the activation curve. In the control, $V_{0.5}$ was -7.4 ± 1.0 mV and k was 9.0 ± 0.9 ($n=10$). In the presence of $100 \mu\text{M}$ fluoxetine, $V_{0.5}$ was -7.0 ± 1.7 mV and k was 7.5 ± 1.5 ($n=5$). These results demonstrate that fluoxetine does not significantly affect the activation curve of voltage-activated K^+ channel. The reversal potential was not affected by fluoxetine: -57 ± 3 mV in control ($n=10$) and -55 ± 6 mV in the presence of the drug ($n=4$), suggesting that fluoxetine does not alter the ion selectivity of the channel.

In order to quantitate the voltage dependence of inhibition of the K^+ current, percentage of current block in the presence of $100 \mu\text{M}$ fluoxetine is plotted as a function of membrane potential, together with the activation–voltage curve (Fig. 5B). The current was activated at -20 mV, and the activation reached saturation at $+20$ mV (smooth line of Fig. 5B). In the presence of $100 \mu\text{M}$ fluoxetine ($n=5$), at potentials where conductance was saturated between $+20$ and $+70$ mV, the degree of inhibition increased in a voltage-dependent manner (solid straight line of Fig. 5B): $56 \pm 5\%$ at $+20$ mV ($P < 0.01$ vs. values at $+70$ mV), $56 \pm 3\%$ at $+30$ mV ($P < 0.01$), $60 \pm 4\%$ at $+40$ mV ($P < 0.05$), and $67 \pm 5\%$ at $+70$ mV. According to the Woodhull model (Woodhull, 1973), the voltage dependence of block was determined by the equation: $f = [C]/\{[C] + K_d^* \times \exp(-z\delta EF/RT)\}$, where $f = \{1 - (I_{\text{fluoxetine}}/I_{\text{control}})\} \times 100$; z , F , R , and T are the charge valence of fluoxetine, the Faraday constant, the gas constant, and the absolute temperature, respectively; δ is the fractional electrical distance with reference to the inner membrane, i.e., the fraction of the transmembrane field sensed by a single charge ($z = 1$) at the receptor site; and K_d^* is the affinity at the reference voltage (0 mV). In this study, K_d^* and δ were calculated as $103 \mu\text{M}$ and 0.26, respectively.

In order to determine whether the fluoxetine inhibition of voltage-activated K^+ current depended on the open state of the channel, a pulse protocol was used in which fluoxetine block was compared with and without test pulses during the

first 3 min of fluoxetine perfusion. Fig. 6A shows that the current elicited by a 200-ms step depolarization to $+70$ mV from a holding potential of -30 mV were inhibited by $100 \mu\text{M}$ fluoxetine. The inhibited current could be scaled up to match the control current, indicating that there was no kinetic change in the current (the dotted line of Fig. 6A). The time course of current inhibition and recovery measured in another cell is shown in Fig. 6B. A 3-min fluoxetine ($100 \mu\text{M}$) application was sufficient for the block to reach the steady-state level ($66 \pm 4\%$, $n=3$). Fig. 6C shows data in a

Fig. 6. Non use-dependence of inhibition of voltage-activated K^+ current of outer hair cells by fluoxetine. (A) Currents were activated by a 200-ms depolarizing pulse to $+70$ mV from a holding potential of -30 mV. This cell produced a 73% inhibition in the presence of $100 \mu\text{M}$ fluoxetine. The current in the presence of $100 \mu\text{M}$ fluoxetine was scaled up to match the control current (dotted trace), indicating that there is no kinetic change except for a scaled-down current in the presence of the drug. (B) The pulse protocol of (A) was used at an interval of 10 s between pulse stimulations. Fluoxetine ($100 \mu\text{M}$) was applied to the bath for 5 min. The currents were inhibited by 63%. There was a full recovery after washing for 7 min. (C) The same protocol as that in B was used, except for the first 3 min of fluoxetine application during which no test pulses were applied. After resuming depolarizing pulses, the currents were already suppressed by 66%. A full recovery of the block could be seen after washing for 10 min.

third cell in which depolarizing test pulses were not applied during the first 3 min of perfusion of 100 μ M fluoxetine. Test pulses that followed revealed block of the current. After washout, the current recovered to the control level. The percentage of current inhibition by 100 μ M fluoxetine without the first 3-min depolarization was measured as $65 \pm 6\%$ ($n=4$). Thus, the inhibition without pulsing (Fig. 6C) was comparable to that during repeated pulsing (Fig. 6B), suggesting that fluoxetine block is not use-dependent.

4. Discussion

Fluoxetine has been found to be a potent inhibitor of leak K^+ current of outer hair cells of the guinea pig cochlea. Fluoxetine also blocked the voltage-activated K^+ current of outer hair cells at higher concentrations. These inhibitions were concentration-dependent and reversible. The block of leak K^+ current was voltage-independent. The inhibition of voltage-activated K^+ current was voltage-dependent, but not use-dependent. Neither the activation curve nor the reversal potential was affected by fluoxetine. This is the first report of the action of this compound on K^+ currents of outer hair cells of the mammalian inner ear.

The leak component of K^+ currents of outer hair cells suggests that the K^+ channel is open even at the resting potential (Dallos, 1996). Although the leak conductance is mainly K^+ selective, the leak current is likely to be due to nonselective cation currents (Housley and Ashmore, 1992; Kros, 1996). Our results are consistent with these observations. For instance, the inhibition of leak K^+ current amounted to 46% in the presence of 100 μ M fluoxetine, representing a maximal block. Inhibition of 25% at 1 μ M is more than the half-maximal block of fluoxetine (Fig. 2B). Thus, the IC_{50} of 0.78 μ M was obtained for fluoxetine inhibition by extrapolation. Since the therapeutic plasma concentration of fluoxetine is about 1 μ M (Altamura et al., 1994), the IC_{50} of 0.78 μ M for fluoxetine inhibition suggests that the block of leak K^+ current in outer hair cells can occur at the therapeutic level of fluoxetine although fluoxetine concentration in inner ear fluids (endolymph and perilymph) is unknown. This finding is similar to the previous observations that fluoxetine inhibits K^+ currents in cerebellar granule neurons (Yeung et al., 1999) and blocks $K_{V1.3}$ channels expressed in the Chinese hamster ovary cell line (Choi et al., 1999).

From more recent reports, the KCNQ4 gene is thought to encode the voltage-activated component of the K^+ channel in outer hair cells of the cochlea (Robbins, 2001; Trussell, 2000). KCNQ4, expressed in the basal membrane of outer hair cells, may be responsible for K^+ efflux across the basal membrane (Kharkovets et al., 2000). Loss or inhibition of KCNQ4 might result in a chronic K^+ overload of the outer hair cells, causing a slow degeneration of outer hair cells and leading to a hearing sensitivity reduction (Beisel et al., 2000; Jentsch, 2000; Kubisch et al., 1999). In our study, the

IC_{50} for fluoxetine inhibition of voltage-activated K^+ current was different at different test potentials, as shown in Fig. 5A. These levels from the test potentials of +70–0 mV are 45- to 103-fold higher than the therapeutic plasma concentration of fluoxetine. A single clinical report (Cunningham et al., 1990) described a patient with a paroxysmal diminution of hearing in her left ear during fluoxetine therapy at therapeutic doses. The results of our work make it unlikely that a reduction of hearing sensitivity during fluoxetine administration at therapeutic doses for treatment of depression is due to the inhibition of the voltage-activated K^+ current. In patients with obsessive–compulsive disorder who take a daily dose as high as several hundred milligrams of fluoxetine (Fontaine and Chouinard, 1989), however, the influence of the accumulation of fluoxetine and its active metabolite, norfluoxetine, on the hearing system such as the organ of Corti should be considered (Renshaw et al., 1992), as the plasma and brain level of fluoxetine would potentially rise to a level at which voltage-activated K^+ currents of outer hair cells are blocked.

Fluoxetine is known to block a variety of voltage-dependent K^+ channels in other preparations (Choi et al., 1999; Farrugia, 1996; Hahn et al., 1999; Rae et al., 1995; Thomas et al., 2002; Tytgat et al., 1997; Yeung et al., 1999). Fluoxetine block has been reported variably to be use-independent (Thomas et al., 2002; Yeung et al., 1999; the present study), use-dependent (Hahn et al., 1999; Tytgat et al., 1997), voltage-independent (Hahn et al., 1999; Thomas et al., 2002; Tytgat et al., 1997; Yeung et al., 1999; the present study for the leak current inhibition), or voltage-dependent (Choi et al., 1999; Tytgat et al., 1997; the present study for the voltage-activated current inhibition). Depending on the types of K^+ channels, the IC_{50} values of fluoxetine vary from a few to several hundred micromolars. In the $K_{V1.3}$ K^+ channels expressed in Chinese hamster ovary cells, fluoxetine at low concentrations produced a time-dependent block without altering the rising phase of the K^+ current. In addition, the block was enhanced by depolarization. These results are interpreted as an open channel block (Choi et al., 1999). At higher concentrations, fluoxetine produced a tonic block, which might be due to a fast open channel block or the block at the resting state (Choi et al., 1999).

Fluoxetine block of $K_{V1.1}$ channel expressed in *Xenopus* oocytes was use- and voltage-dependent (Tytgat et al., 1997). They postulated that fluoxetine exerted two types of block: voltage-independent block and voltage-dependent block. The voltage-independent block was due to binding of this drug to a high-affinity site in the last closed state before channel opening. The voltage-dependent block was due to the binding of fluoxetine to a low-affinity site in the open state. Repetitive depolarization rendered the last closed state and open state available for fluoxetine binding, resulting in use-dependent block. However, the lack of use-dependent block might result from an ultrafast open channel block, as reported in the fluoxetine block of the HERG K^+ channels

(Thomas et al., 2002). In our study, the use-independent block and the scaling down of the control current by fluoxetine, as shown in Fig. 6C and A, respectively, are consistent with the notion that fluoxetine can block the channel before its opening or block the channel instantly when it opens.

Another question is whether the block is due to the cationic form or the neutral form, since fluoxetine being a weak base with pK_a of 9.4 can exist as the neutral and cationic forms in varying proportion depending on the ambient pH. It has been postulated that, like local anesthetics, the neutral form of fluoxetine is able to penetrate the membrane; once entering the cell, it is the cationic form that blocks the channel from inside of the membrane (Maertens et al., 1999). A similar conclusion has been drawn from fluoxetine block of $K_v1.3$ channels (Choi et al., 1999). However, the block by fluoxetine of volume-regulated anion channel was attributed to the neutral form (Maertens et al., 1999).

In an attempt to integrate all of these observations of fluoxetine block of various channels, we speculate that while both the neutral form and the cationic form are active in blocking the same site in the K_v channel, they differ in their mechanism of blocking action. The neutral form takes the hydrophobic pathway to reach the blocking site and binds with a high affinity without voltage dependence (Hille, 1977). The cationic form takes the hydrophilic pathway from inside to bind to the low-affinity site in the open channel with voltage dependence (Tytgat et al., 1997). Fig. 5B illustrates the fit of the data by this dual block model, $y = [n/K_n + C/K_C] / [1 + n/K_n + C/K_{C(0)}]$, where n is the concentration of the neutral form; C is the concentration of the cationic form; K_n and K_C are the equilibrium dissociation constants of binding of the neutral and cationic forms, respectively. While the block caused by the neutral form is not voltage-dependent, the block caused by the cationic form is voltage-dependent with the form of $K_C = K_{C(0)} \exp(-z\delta EF/RT)$. With $pK_a = 9.4$ and at $pH = 7.4$, fluoxetine at $100 \mu M$ would yield $1 \mu M$ of the neutral form and $99 \mu M$ of the cationic form. The data for $100 \mu M$ fluoxetine were satisfactorily fitted with the following parameters: $K_n = 3 \mu M$, $K_{C(0)} = 140 \mu M$, and $\delta = 0.32$ (the dotted line of Fig. 5B). The dotted line represents the total block, which can be dissected out into the block by the neutral form of fluoxetine and the block by the cationic form (Fig. 5B). As expected, the cationic form exhibits a voltage-dependent increase in the blocking action. The neutral form exhibits a voltage dependence of block opposite to the cationic form despite the assumption that the neutral form binds to the blocking site in a voltage-independent manner. This apparent voltage dependence is due to an increase in competition of the cationic form with the neutral form to bind to the same site, as the membrane becomes more depolarized. In this dual block model, the neutral form of fluoxetine is more potent than the cationic form in blocking the K^+ channel.

In conclusion, fluoxetine inhibits both leak K^+ current- and voltage-activated K^+ current of outer hair cells of the

guinea pig cochlea. These inhibitions are concentration-dependent and reversible. Inhibition of leak K^+ current- and voltage-activated K^+ current may have different physiological and pathological implications. The leak component of K^+ currents of outer hair cells may be a potent target of fluoxetine with a submicromolar affinity. Since fluoxetine only weakly blocks voltage-activated component of the K^+ currents, this action is probably not related to its therapeutic action or adverse effects.

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