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Research Report

Inhibition of astroglial Kir4.1 channels by selective serotonin reuptake inhibitors

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ABSTRACT

The inwardly rectifying K⁺ (Kir) channel Kir4.1 is responsible for astroglial K⁺ buffering. We recently found that tricyclic antidepressants (TCAs) inhibit Kir4.1 channel currents, which suggests that astroglial Kir currents might be involved in the pharmacological action of antidepressants. We therefore further examined the effects of the currently most popular antidepressants, selective serotonin reuptake inhibitors (SSRIs), and other related agents on Kir4.1 channels heterologously expressed in HEK293T cells. The whole-cell patch clamp technique was used. Fluoxetine, the typical SSRI, inhibited Kir4.1 channel currents in a concentration-dependent manner with an IC₅₀ value of 15.2 μM. The inhibitory effect of fluoxetine was reversible and essentially voltage-independent. Fluoxetine had little or no effect upon Kir1.1 (ROMK1) or Kir2.1 (IRK1) channel currents. Other SSRIs, sertraline and fluvoxamine, also inhibited Kir4.1 channel currents whereas the tetracyclic (mianserin) or the 5-HT₁A receptor-related (buspirone) antidepressants did not. This study shows that SSRIs such as fluoxetine and sertraline preferentially block astroglial Kir4.1 rather than Kir1.1 or Kir2.1 channels in the brain, which may be implicated in their therapeutic and/or adverse actions.

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1. Introduction

There are more than fifteen inwardly rectifying potassium (Kır) channel subunits that can be functionally classified into four subfamilies (Isomoto et al., 1997; Kubo et al., 2005). They play crucial roles in determining the resting membrane potential of neurons (Kir2.x), in regulating neuronal excitability via G-protein-mediated pathways (Kir3.x) or intracellular ATP-dependent signaling (Kir6.x), and in transporting K⁺ in glial and epithelial cells (Kir1.x, Kir4.x and Kir5.x). Among the Kir subunits, Kir4.1 and Kir5.1 are expressed broadly, but not diffusely, in the brain with a high density in some regions such as the olfactory bulb, cerebral cortex, hippocampus and cerebellum (Takumi et al., 1995; Higashi et al., 2001; Hibino

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Abbreviations: Eₚ, K⁺ equilibrium potential; Kir, Inwardly rectifying potassium; GFP, Green fluorescent protein; SSRI, Selective serotonin reuptake inhibitor; TCA, Tricyclic antidepressant

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et al., 2004). Their expression appeared to occur only in astrocytes, specifically in the processes surrounding synapses or in the membrane domains facing the pia mater and blood vessels, where they are involved in buffering the excessive extracellular K+ elevated during neuronal activity (Poopalsundaram et al., 2000; Walz, 2000; Kofuji and Newman, 2004; Simard and Nedergaard, 2004).

The astroglial Kir channels are composed of two subtypes, the homo-tetramer of Kir4.1 (Kir4.1 channels) or the hetero-tetramer of Kir4.1 and Kir5.1 (Kir4.1/Kir5.1 channels) (Tanemoto et al., 2000; Higashi et al., 2001; Hibino et al., 2004). Although the assembly of Kir5.1 alone does not exhibit channel activity, both Kir4.1 and Kir4.1/Kir5.1 are functionally active as intermediate inwardly rectifying channels (Takumi

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**Fig. 1** – Effects of fluoxetine on Kir4.1 channel currents in HEK293T cells. (A) Representative response of Kir4.1 channels currents to fluoxetine (30 μM) and Ba2+ (3 mM). Transiently transfected HEK293T cells were voltage-clamped at −40 mV (\( E_K \)) in 30 mM [K+]o. A pair of voltage steps (500 ms in duration) to \( E_K ±70 \) mV (−110 and +30 mV) with a 200-ms interval were applied every 20 s. Upper panel: concatenated responses of Kir4.1 channel currents to the repeated voltage step protocol. Bars indicate the periods for which the cell was superfused with solutions which contained fluoxetine or Ba2+. Lower panel: macroscopic traces of Kir4.1 channel currents during (a) control, (b) application of fluoxetine, (c) washout of fluoxetine and (d) application of Ba2+. The arrowheads indicate the current level recorded at −40 mV. (B) Current−voltage relationships of Kir4.1 currents recorded from one HEK293T cell in the presence of different concentrations of fluoxetine (3, 10, 30 μM). The cell was voltage-clamped at −90 mV (\( E_K \)) in 5.4 mM [K+]o, and 500-ms steps to between −160 mV and −20 mV in 20 mV increments were delivered every 15 s. (C) Current inhibition (Iocular/Icontrol) by 10 and 30 μM fluoxetine at different membrane voltages. The Kir4.1 currents were recorded as in panel B and the inhibition ratio was determined as described in Experimental procedure. Symbols represent the mean±SEM of 3 or 4 separate experiments.
et al., 1995; Tanemoto et al., 2000). The channels conduct a large inward K\(^+\) current at membrane potentials negative to the K\(^+\) equilibrium potential (\(E_K\)) and a small, but significant, outward K\(^+\) current at those positive to the \(E_K\). Therefore, they can absorb the excessive extracellular K\(^+\), which is locally elevated near synaptic sites, and transport it to regions of low K\(^+\) such as blood vessels (Walz, 2000; Ishii et al., 2003; Kofuji and Newman, 2004; Simard and Nedergaard, 2004; Kucheryavyykh et al., 2007). Although spatial K\(^+\) buffering by astrocytes is critical in maintaining neuronal excitability in the brain, little is known about the functional control of Kir4.1 channels and their relationship to brain diseases.

We have recently found that TCAs such as nortriptyline can inhibit Kir4.1 channels in voltage- and time-dependent fashions (Su et al., 2007). Since astrocytes are now considered as one of the targets of antidepressant agents (Fuchs et al., 2004; Mercier et al., 2004; Czeh et al., 2006), our results suggest a novel possibility that antidepressants inhibition of Kir4.1-mediated astroglial K\(^+\) buffering may be implicated in their pharmacological actions. In the present study, therefore, we have examined the effects of the currently clinically most popular antidepressants, the selective serotonin reuptake inhibitors (SSRIs), and other related agents on astroglial Kir4.1 channel currents.

2. Results

2.1. Effects of fluoxetine on Kir4.1 channel currents

The effect of the typical SSRI fluoxetine was examined using the whole cell patch clamp technique in HEK293T cells transfected with Kir4.1 cDNA. The cell shown in Fig. 1A was held at \(-40\, \text{mV} (\sim E_K)\) in 30 mM [K\(^+\)]\(_o\). Symmetric paired-step pulses (±70 mV, 500 ms in duration, with a 200-ms interval) were given every 20 s. In control conditions, a large inward K\(^+\) current was evoked by hyperpolarizing voltage steps while a smaller but significant outward K\(^+\) current was seen during depolarizing steps. This is the intermediate inward rectifying current that is

![Fig. 2 - Comparison of the effects of fluoxetine upon Kir4.1, Kir2.1 and Kir1.1 channels. (A) Representative responses of Kir2.1 and Kir1.1 channels to 100 \(\mu\)M fluoxetine. The cells expressing Kir2.1 (left) and Kir1.1 (right) channels were voltage-clamped at \(-40\, \text{mV} (\sim E_K)\) in 30 mM [K\(^+\)]\(_o\) and stimulated as described in Fig. 1A. The arrowheads indicate the basal current level at \(-40\, \text{mV}\. (B) Concentration–response curves for fluoxetine inhibition of Kir4.1, Kir2.1 and Kir1.1 channels. The relationship between the current ratio at \(-110\, \text{mV}\) and the concentration of fluoxetine was fitted with Hill’s equation (lines). Symbols represent the mean ± SEM of 3 or 4 separate experiments.](image-url)
characteristic of Kir4.1 channels (Fig. 1Aa). When fluoxetine (30 μM) was applied to the bath, both outward and inward currents were markedly inhibited (Fig. 1Ab). The current gradually recovered during washout of the drug (Fig. 1Ac). When Ba²⁺ (3 mM) was added to the bath at the end of the experiment, the Kir4.1 channel current was blocked completely (Fig. 1Ad).

Fig. 1B shows current–voltage relationships of Kir4.1 currents recorded from the same HEK293T cell which was bathed in the solution with a physiological level (5.4 mM) of [K+]o and held at −90 mV (~E_k). Fluoxetine concentration-dependently inhibited Kir4.1 channel currents at a whole range of membrane potentials examined (Fig. 1B).

Fig. 1C shows the I_{Drug}/I_{Control} ratios at different membrane potentials in the presence of 10 and 30 μM fluoxetine. The magnitude of inhibition was only slightly greater at potentials between −60 and −20 mV. Thus, the inhibitory action of fluoxetine on Kir4.1 channels is practically voltage-independent.

2.2. Effects of fluoxetine upon other Kir channels

We next examined the effects of fluoxetine on constitutively active Kir2.1 and Kir1.1 channels to determine whether fluoxetine is selective for Kir4.1 channels. The HEK293T cells expressing either Kir2.1 or Kir1.1 channels were held at −40 mV in 30 mM [K+]o, and the symmetric paired-step pulses (±70 mV, 500 ms in duration, 200-ms interval) were applied in the same manner as described in Fig. 1A. In control conditions, the currents recorded from the cells expressing Kir2.1 channels showed strong

![Figure 3](image-url)  
**Fig. 3** – Effects of different antidepressant compounds on Kir4.1 channel currents. (A) Representative traces of Kir4.1 channel current responses to sertraline (30 μM), fluvoxamine (100 μM), mianserin (100 μM) and buspirone (100 μM). The HEK293T cells expressing Kir4.1 channels were held at −40 mV (~E_k) in 30 mM [K+]o and stimulated as described in Fig. 1A. Arrowheads indicate the basal current levels recorded at −40 mV. (B) Concentration–response effects of different antidepressant compounds upon Kir4.1 channel currents. The relationship between the current ratio at −110 mV and the drug concentration was fitted with Hill’s equation (lines). The data for fluoxetine (closed circle) are those shown in Fig. 2B and are repeated here for comparison with the other compounds. Symbols represent the mean±SEM of 3 to 5 separate experiments.
increased during a hyperpolarizing step to ±110 mV and practically no outward current at +30 mV (Fig. 2A left). In contrast, the currents from the Kir1.1-transfected cells showed weak inward rectification with relatively large outward currents at positive potentials (Fig. 2A right). These current properties of Kir2.1 and Kir1.1 channels were consistent with those observed in our previous studies (Morishige et al., 1993; Kondo et al., 1996).

The application of 100 μM fluoxetine had negligible effect upon either Kir2.1 or Kir1.1 channel currents (Fig. 2A).

Fig. 2B shows the concentration–response curves for fluoxetine upon Kir 4.1, Kir2.1 and Kir1.1 channel currents recorded in 30 mM [K+]o. The IC50 value for fluoxetine inhibition of Kir4.1 was 15.2 μM (h=2.87) whereas 100 μM fluoxetine reduced Kir2.1 or Kir 1.1 currents by only about 10 to 30%. Thus the action of fluoxetine was selective for astroglial Kir4.1 channels compared with Kir2.1 or Kir1.1 channels.

2.3. The comparison of different antidepressants upon Kir4.1 channels

We compared the effects of fluoxetine on Kir4.1 channels with those evoked by different antidepressants (Fig. 3). These drugs included the SSRIs sertraline and fluvoxamine, the tetracyclic antidepressant mianserin and the 5-HT1A receptor partial agonist buspirone. The cells expressing Kir4.1 channels were held at –40 mV in 30 mM of [K+]o, and given the symmetric paired-step pulse protocol (+70 mV, 500 ms in duration, 200-ms interval). Sertraline potently inhibited Kir4.1 channel currents with an IC50 value of 7.2 μM (h=2.63) (Fig. 3B). Fluvoxamine also diminished Kir4.1 channel currents, but its effects were much weaker than those of sertraline or fluoxetine. Interestingly, in the presence of 100 μM fluvoxamine, Kir4.1 currents gradually increased during a hyperpolarizing step to –110 mV, and the outward current decreased rapidly during a depolarizing step to +30 mV (Fig. 3A). This voltage-dependent effect of fluvoxamine was similar to that recorded previously for nortriptyline (Su et al., 1999). In contrast to the SSRIs, the tetracyclic antidepressant mianserin and the 5-HT1A receptor partial agonist buspirone had negligible effect upon Kir4.1 channels (Figs. 3A and B).

3. Discussion

This study shows that (1) SSRIs such as fluoxetine and sertraline potently inhibit Kir4.1 channels that underlie astroglial K+–spatial buffering, (2) the inhibitory effect of fluoxetine was voltage-independent and was selective for Kir4.1 among Kir channels examined, and (3) the tetracyclic antidepressant mianserin and the 5-HT1A receptor partial agonist buspirone had little effect upon Kir4.1 channel activity.

We have recently shown that classical TCAs can inhibit Kir4.1 channel currents which are responsible for astroglial K+–spatial buffering (Su et al., 2007). This suggested a possibility that alteration of astroglial K+ buffering may be involved in the pharmacological actions of various antidepressants. The result in this study that the SSRIs, the currently most popular antidepressants, effectively block the Kir4.1 channel activity is consistent with this notion.

Previous studies showed that fluoxetine inhibits the voltage-dependent potassium channels, Kv1.x and HERG (Choi et al., 1999; Yeung et al., 1999; Thomas et al., 2002). The inhibitory actions of sertraline and fluoxetine for Kir4.1 channels were similar to slightly less potent than the actions on Kv1.3 (e.g., IC50 value=6 μM for fluoxetine) or HERG (e.g., IC50 value=3 μM for fluoxetine) channels, but were more potent than those on Kv1.1 channels (e.g., IC50 value=55–600 μM for fluoxetine) (Yeung et al., 1999; Choi et al., 1999; Thomas et al., 2002). Although the IC50 value of fluoxetine for Kir4.1 inhibition is higher than the plasma levels (i.e., 1 μM) during the clinical treatments, it is generally known that the cerebral concentrations of antidepressants are much higher than those in the plasma due to their high brain/plasma distribution ratios (e.g., 10:1 to 30:1) (Glotzbach and Preskorn, 1982; Karson et al., 1993; Henry et al., 2005). In fact, a clinical study with magnetic resonance spectroscopy has revealed that the brain concentrations of fluoxetine are approximately 17 and 25 μM following the repeated treatments with therapeutic doses for 3 and 5 weeks, respectively (Henry et al., 2005). Therefore, the drugs in the brain would inhibit Kir4.1-mediated astroglial K+ buffering after several weeks (steady state) during the clinical treatment. In addition, our results suggest that 25 μM of fluoxetine barely affects Kir currents mediated by Kir2.1 or Kir1.1 channels that are expressed in neurons (Ohno-Shosaku and Yamamoto, 1992; Kenna et al., 1994; Horio et al., 1996; Kubo et al., 2005). Previous studies using Xenopus oocytes (Kobayashi et al., 2004) also showed that fluoxetine can reduce activities of the G-protein-activated Kir channels, Kir3.1/3.2, Kir3.1/3.4 and Kir3.2 channels, with IC50 values of 16.9, 18.4 and 89.5 μM, respectively. However, these channels were inhibited only by 60–70% of the control even at the maximum concentrations of fluoxetine and were negligibly affected by other SSRIs (e.g., citalopram and fluvoxamine) (Kobayashi et al., 2003, 2004). Accordingly, it seems likely that SSRIs at therapeutic doses would more preferentially block astroglial Kir4.1 channels than neuronal Kir2.1, Kir1.1, and Kir3.x channels in the brain.

In our previous studies, the TCA nortriptyline inhibited Kir4.1 channels in a voltage- and time-dependent manner with stronger inhibition at more positive membrane potentials (Su et al., 2007). Analysis of nortriptyline blockade indicated that the agent acts as a deep pore blocker where it would pass 62% of the electrical field across the plasma membrane from the inside of the cell to reach its binding site (Su et al., 2007). On the other hand, the voltage dependency of SSRI-induced Kir4.1 inhibition was negligible. In addition, the Hill coefficients for SSRIs such as fluoxetine and sertraline were more than 2, whereas the value for nortriptyline was nearly unity (Su et al., 2007). These results suggest that SSRIs, unlike TCAs, act at multiple sites, which may exhibit positive cooperativity while being insensitive to membrane potentials. Interestingly, one of the SSRIs examined, fluvoxamine, exhibited the nortriptyline-like voltage-dependent feature in inhibiting Kir4.1 channel currents, suggesting that the drug may partly share the action sites with TCAs in inhibiting Kir4.1 channels.

It is well documented that most antidepressants inhibit reuptake of monoamines, in particular serotonin and norepinephrine, into the nerve terminals and elevate extracellular levels of the monoamines in synaptic clefts (Sanchez and Hyttel, 1999; Frazer, 2001). This would normalize deficits of the activity of monoaminergic neurons, which has long been
thought to be the principle mechanism of actions of antidepressants. However, it is pointed out that this “monoaminergic mechanism” cannot solely account for clinical features of antidepressants such as the delayed onset of their actions (usually 2 to 6 weeks despite the acute blockade of monoamine reuptake) (Briner and Dodel, 1998) and the differences between the therapeutic concentrations of drugs in the brain and their binding affinities to the monoamine transporters (Karson et al., 1993; Sanchez and Hyttel, 1999; Henry et al., 2005). Furthermore, antidepressants including SSRIs are known to be effective in treating diverse symptoms other than the depressive disorders, including chronic pain and anxiety disorders (e.g., panic disorders, obsessive-compulsive disorders and social phobia) (Pollack and Marzol, 2000; Ninan, 2003; Rani et al., 1996). Thus it is suggested that other actions of antidepressants than the monoaminergic mechanisms may also be involved in their pharmacological effects (Fuchs et al., 2004; Mercier et al., 2004; Malberg and Blendy, 2005). The inhibition of the astroglial Kir4.1 channels by antidepressants may serve as one of such mechanisms. A recent study using RNAi revealed that knockdown of Kir4.1 channels in cultured cortical astrocytes reduces 85% of K+ buffering currents, which will lead to increased neuronal excitability by increasing [K+]o surrounding neurons (Kucheryavykh et al., 2007). In addition, the knockdown of Kir4.1 channels accompanied a reduction of the glutamate clearance (Kucheryavykh et al., 2007). Therefore, the blockade of the Kir4.1 channels by antidepressants may increase the neuronal activity by reducing astroglial K+ and/or glutamate buffering, which could be involved in their clinical effects for depression and other related disorders. This possibility was supported by the findings that both systemic and i.c.v. administration of various K+ channel blockers such as tetraethylammonium (for all K+ channels), glibenclamide and glyburide (for ATP-sensitive K+ channels) and apamine (for Ca2+-activated K+ channels), all of which commonly increase the excitability of neurons, produced significant antidepressant effects in the animal models of depression (e.g., forced swimming tests) (Guo et al., 1995, 1996; Kaster et al., 2005). Alternatively, neuronal facilitation by the Kir4.1 blockade may lead to a proconvulsive action with antidepressants at very high concentrations or under sustained treatments (Barbey and Roose, 1998; Pisani et al., 1999). It should be noted, however, that the present results were obtained from in vitro system using HEK293T cells heterologously expressing Kir4.1 channels, which may not be necessarily relevant to the in vivo situation. Further studies such as in vivo assessment of the neural activity and the [K+]o level are required to delineate detailed mechanisms and pharmacological relevance of the inhibitory actions of antidepressants on astroglial Kir channels.

4. Experimental procedure

4.1. Transfection and cell culture

Transfection of Kir constructs and cell culture were performed as described previously (Tanemoto et al., 2000; Su et al., 2007). Briefly, human Kir4.1 inserted into an IRES vector (pIRES2-DsRed2, Clontech, Mountain view, CA) were co-transfected with a green fluorescent protein (GFP) marker plasmid (pCA-GFP) into HEK293T cells using Fugene6 transfectant (Roche Diagnostics, Tokyo, Japan). The transfected cells were then dissociated with 0.05% trypsin/EDTA (Invitrogen), seeded onto poly-l-lysine-coated glass cover slips and kept in Dulbecco’s modified minimal Eagle medium (Sigma-Aldrich, St. Louis, MO), containing 10% (v/v) fetal bovine serum (Sigma-Aldrich) and 1% (v/v) antibiotic/antimycotic cocktail (Invitrogen), in a humidified 5% CO2/37 °C environment. For the expression of Kir1.1 and Kir2.1 channels, HEK 293T cells were transfected with rat Kir1.1/pcDNA3 or mouse Kir2.1/pcDNA3, respectively, in the manner described above. Electrophysiological experiments were conducted 24–72 h after transfection.

4.2. Electrophysiology

The transfected cells on coverslips were transferred into a bath which was placed on the stage of an inverted fluorescence microscope (Axiovert 135, Carl Zeiss, Tokyo, Japan) and continuously perfused with a bathing solution at a flow rate of 1–1.5 ml/min. The bathing solution contained (in mM) 112 NaCl, 30 KCl, 5 HEPES, 2 CaCl2, 0.53 MgCl2, and 5.5 glucose (pH7.4). In experiments studying the current–voltage relationship of the effects of fluoxetine, extracellular K+ concentration ([K+]o) was reduced to 5.4 mM by replacing K+ with Na+ in the bath solution.

The transfected HEK 293T cells used for the whole-cell patch clamp experiments were identified by GFP expression. The recording micropipettes had input resistances of 1.4–1.8 MΩ when filled with an internal solution containing (in mM) 140 KCl, 2 MgCl2, 5 EGTA, and 5.5 glucose (pH7.25 with KOH). The cells were voltage-clamped at EK (e.g., −40 mV in 30 mM [K+]o) and stepped to depolarized or hyperpolarized potentials (e.g., ±70 mV) from EK. The clamp voltage and associated macroscopic currents were recorded with an Axopatch 200B amplifier (Axon Instruments, Union City, CA), fed through a digitizer (Digidata 1322A, Axon Instruments) and stored in a computer with the data acquisition system Clampex 9.2 (Axon Instruments). The membrane capacitance was also measured by the amplifier immediately after attainment of whole-cell mode while keeping the whole-cell capacity compensation and series resistance compensations throughout the recording. The signals were monitored on a dual beam oscilloscope and recorded on a thermal array recorder (RTA-1100, Nihon Koden, Tokyo, Japan). All experiments were performed at room temperature (22–25 °C).

4.3. Drugs

Fluoxetine hydrochloride, fluvoxamine maleate, mianserin hydrochloride, and bupropion hydrochloride were purchased from Sigma-Aldrich (St. Louis, MO). Sertraline hydrochloride was obtained from Pfizer Inc. (Groton, CT). Stock solutions (10 mM) were prepared with distilled water, stored at 4 °C until the day of the experiment and diluted in the bathing solution before the experiments. All other reagents were obtained from commercial sources.
4.4. Data analysis and statistics

For analysis, the recorded signals were reproduced off-line and analyzed with Clampfit 9.2 (Axon Instruments). In each cell, an excessive concentration (1–3 mM) of BaCl2 which totally blocked Kir channels was applied at the end of each experiment. The Kir channel currents were measured by subtracting the Ba2+-resistant currents from the total current. The response to the test drug was estimated as the current ratio (\(I_{\text{drug}}/I_{\text{control}}\)) at −110 mV, which was obtained by dividing the current recorded in the presence of the drug at the end of each voltage step with the equivalent current recorded in the absence of the drug. The \(I_{50}\) of each drug at membrane potential of −110 mV was determined by fitting the concentration–response data to Hill’s equation as follows:

\[
f(D) = \frac{1}{1 + (D/IC_{50})^h}
\]

where \(f(D)\) is the current ratio in the presence of the drug at a given concentration (D) and \(h\) is the Hill coefficient. All data are expressed as the mean ± SEM.

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