Functional Modulation of Na\textsubscript{1.2} Voltage-Gated Sodium Channels Induced by Escitalopram

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Escitalopram, a selective serotonin reuptake inhibitor (SSRI), may induce seizures, particularly in epileptic patients. In this study, we investigated the effect of escitalopram in Na\textsubscript{1.2} voltage-gated sodium channels (VGSCs) transfected HEK293 cells. Na\textsubscript{1.2} VGSCs current decreased by approximately 50.7±8.3% under treatment with 100 µM escitalopram. The IC\textsubscript{50} of escitalopram against Na\textsubscript{1.2} VGSCs was 114.17 µM. Moreover, the treatment with 100 µM escitalopram changed the voltage-dependence of inactivation and the voltage at half-maximal inactivation shifted significantly from −50.3±3.7 to −56.7±6.0 mV toward negative potential under treatment with 100 µM escitalopram. Surprisingly, the treatment with 100 µM escitalopram also changed the voltage-dependence of activation and the voltage at half-maximal activation shifted significantly from −13.8±4.6 to −21.5±3.9 mV toward negative potential under treatment with 100 µM escitalopram. These findings suggested that escitalopram might be able to inhibit Na\textsubscript{1.2} VGSCs current and affects both activation and inactivation states of Na\textsubscript{1.2} VGSCs.

Key words escitalopram; Na\textsubscript{1.2} voltage-gated sodium channels (VGSCs); HEK293

MATERIALS AND METHODS

Generating HEK293 Cells Expressing Na\textsubscript{1.2} VGSCs and Cell Culture  
The cDNA of Na\textsubscript{1.2} VGSCs was gifted from Dr. Masaharu Noda. In brief, the cDNA of Na\textsubscript{1.2} VGSCs inserted into pCI-neo Mammalian Expression Vector (Promega, Fitchburg, WI, U.S.A.) was transfected with FuGENE® 6 Transfection Reagent (Promega) to HEK293 cells (JCRB Cell Bank, Osaka, Japan) as following the manufacturer’s instruction. Cells were cultured as described previously with a slight modification. To select HEK293 cells expressing Na\textsubscript{1.2} VGSCs, cells were cultured with media containing 500 µg/mL Geneticin (Nacalai Tesque, Inc., Kyoto, Japan).

Electrophysiology  
Whole-cell VGSCs current were recorded as described previously. All experiments were performed at 22–25°C in a bath solution containing (mM) 100 NaCl, 40 tetraethylammonium (TEA)-Cl, 0.03 CaCl\textsubscript{2}, 10 2-[4-(2-hydroxyethyl)-1-piperazinyl]ethanesulfonic acid (HEPES), 10 MgCl\textsubscript{2}, 6H\textsubscript{2}O and 10 d-glucose, and the pH was adjusted to 7.4 with NaOH. The microelectrode solution consisted of (mM) 115 CsCl, 25 NaCl, 2 MgCl\textsubscript{2}, 6H\textsubscript{2}O, 1 CaCl\textsubscript{2}, 11 ethylene glycol bis(2-aminoethyl ether)-N,N,N',N'-tetraacetic acid (EGTA) and 10 HEPES, and the pH was adjusted to 7.4 with CsOH. The patch microelectrodes used for recording were made from borosilicate capillary glass (Narishige, Tokyo, Japan) and had resistances of 3–5 MΩ. Whole-cell current were recorded using an EPC-7 plus amplifier (HEKA Elektronik, Lambrecht, Germany) with low-pass filtering at 3 kHz and digitized with a Digidata1440A (Molecular Devices, Sunnyvale, CA, U.S.A.). Leak current were subtracted by a P/4 pulse protocol. These recorded data were sampled and analyzed using pCLAMP10.6 software (Molecular Devices). Escitalopram oxalate was obtained from H. Lundbeck A/S (Batch: V 4013, Copenhagen, Denmark) and dissolved in dimethyl sulfoxide (DMSO) as a stock solution. The final concentration of DMSO in the bath solution was set at 0.1%. To
explore the effect of escitalopram on Na\textsubscript{1.2} VGSCs current, the bath was perfused with bath solution in which escitalopram was dissolved at the indicated concentration.

**Data Analysis** Data were analyzed using a combination of pCLAMP 10.6 software, Origin 6.1, Microsoft Excel, and GraphPad InStat 3 software. The results are presented as the mean±standard deviation (S.D.) As statistical analysis, One-way ANOVA was performed followed by Dunnett multiple comparisons test to compare the effect of various concentration of escitalopram with vehicle treatment. In addition, Student’s paired $t$-test was performed for statistical analysis to compare various parameters of activation or inactivation curves fitted by Boltzmann function between before and after treatment of escitalopram.

**RESULTS**

First, we investigated the effect of escitalopram on Na\textsubscript{1.2} VGSCs current. Figure 1A shows the effect of 100 µM escitalopram on the Na\textsubscript{1.2} VGSCs current evoked by depolarization from a holding potential of −80 mV to a testing potential from −100 mV to 40 mV in steps of 10 mV in HEK293 cells. Under treatment with 100 µM escitalopram, the Na\textsubscript{1.2} VGSCs current decreased by around 50% compared to those before treatment with escitalopram (Fig. 1A). Figure 1B shows the current-voltage relationship before and after treatment with 100 µM
escitalopram and after washing out. In cells with a peak current at −10 mV, the inhibitory effect of 100 µM escitalopram started at around −20 mV and reached a maximum at −10 mV, after which inhibition persisted with further depolarization potentials (Fig. 1B). After washing out of escitalopram, the inhibitory effect of escitalopram on Na\(_{v}\)1.2 VGSCs current was almost fully restored (Figs. 1A, 1B). Treatment with 10, 30, 100, 300 and 500 µM escitalopram decreased Na\(_{v}\)1.2 VGSCs peak current by 19.2±7.6, 28.3±7.5, 50.7±8.3, 76.5±0.5 and 82.5±5.6% compared to that before treatment with escitalopram, respectively. Compared to treatment with vehicle alone, treatment with more than 30 µM of escitalopram significantly inhibited Na\(_{v}\)1.2 VGSCs peak current (Fig. 1C). When the inhibitory effect toward Na\(_{v}\)1.2 VGSCs peak current obtained under treatment with 500 µM escitalopram was taken to represent a maximal inhibitory response and those data sets were fitted with a logistic function: \( L/I = \exp(-k(S-\text{IC}_{50})) \), the IC\(_{50}\) was determined to be 114.2 µM. When lamotrigine, which is known as an antiepileptic drug and inhibitor of VGSCs including Na\(_{v}\)1.2, was treated in the same experimental condition, Na\(_{v}\)1.2 VGSCs current was also inhibited in a dose-dependent manner (supplemental data). Figure 2 shows the effect of escitalopram on the activation and inactivation of Na\(_{v}\)1.2 VGSCs. The voltage at half-maximal activation changed significantly from −13.8±4.6 to −21.5±3.9 mV under treatment with 100 µM escitalopram. In addition, the slope factor (k) of the activation curve also changed significantly from −7.7±2.3 to −5.7±1.3 (Fig. 2A). The voltage at half-maximal inactivation also changed significantly from −50.3±3.7 to −56.7±6.0 mV under treatment with 100 µM escitalopram. In contrast, there was no significant difference in the slope factor of the inactivation curve between before (5.7±1.9) and after treatment with 100 µM escitalopram (6.1±1.4) (Fig. 2B).

**DISCUSSION**

The present study was designed to update our understanding of SSRIs which might have other target molecule except for serotonin transporter. Thus, we investigated whether or not escitalopram affected Na\(_{v}\)1.2 VGSCs current. Although escitalopram is an SSRI and has been considered to interact with other biological molecules including VGSCs, we demonstrated that escitalopram inhibited the Na\(_{v}\)1.2 VGSCs current amplitude in a dose-dependent manner. Moreover, escitalopram also caused a significant hyperpolarizing shift in both the activation and inactivation curves of Na\(_{v}\)1.2 VGSCs. It has been reported that citalopram, which is a racemic mixture of \((R)\)-(−)citalopram and \((S)-(+)\)-citalopram, inhibited both Na\(_{v}\)1.7 and Na\(_{v}\)1.8 VGSCs current, with IC\(_{50}\) values of 174 and 100 µM, respectively.\(^{15}\) In addition, it has been shown that both citalopram and escitalopram may cause QRS prolongation via sodium channels.\(^{14}\) These findings suggest that it is reasonable for escitalopram to have an inhibitory effect on Na\(_{v}\)1.2 VGSCs current. However, it is also fact that IC\(_{50}\) of escitalopram against Na\(_{v}\)1.2 VGSCs was pretty high when the plasma concentration of escitalopram was considered for the clinical treatment. It has been reported that postmortem serum, which was obtained from a woman presented to the emergency department after having witnessed seizures, contained 7300 ng/mL citalopram.\(^{15}\) Therefore, the side effects of escitalopram via the modulation of Na\(_{v}\)1.2 VGSCs current might be caused when escitalopram is given as an overdose or is used in patients with a genetic modification in VGSCs, such as epileptic patients.\(^{1,2}\) Moreover, it has been considered that the genetic modification of Na\(_{v}\)1.2 related to generate the generalized epilepsy with febrile seizures.\(^{3,4}\) This result also may support that the side effect induced by escitalopram, such as epilepsy, might be caused via the functional modification of Na\(_{v}\)1.2. In contrast, it also has been known three types of VGSCs, such as Na\(_{v}\)1.1, Na\(_{v}\)1.3 and Na\(_{v}\)1.6 other than Na\(_{v}\)1.2, that are predominantly expressed in the central nervous system. Considering the effect of antiepileptic drugs in various VGSCs expressed in central nervous system,\(^{5-8}\) escitalopram also may inhibit not only Na\(_{v}\)1.2 but also other subtype of VGSCs in central nervous system in similar manner. In this study, it has also been demonstrated that escitalopram demonstrated the hyperpolarized shift in the voltage-dependence of both activation and inactivation. Several antiepileptic drugs or local anesthesia have demonstrated the hyperpolarized shift in the voltage-dependence of inactivation.\(^{9,10}\) In this respect, the inhibition mode of escitalopram may be similar to such...
drugs in the inactivation. Recently, it has been reported that a certain genetic mutation of Na\textsubscript{1.2} which demonstrated the hyperpolarized shift in the voltage-dependence of both activation and inactivation was identified in a patient with sporadic neonatal epileptic encephalopathy.\textsuperscript{16} Considering these studies, the inhibition mode of escitalopram that affected both of activation and inactivation states on Na\textsubscript{1.2} may be unique and induce a similar condition like a patient with epileptic encephalopathy mentioned above by the hyperpolarized shift in the voltage-dependence of both activation and inactivation. Though it is puzzling to explain how those phenomena affect the function of Na\textsubscript{1.2}, the shift of the voltage-dependence of both activation and inactivation toward negative potential may lead the imbalance of sodium influx followed by the disturbance of normal excitation of neurons at a certain range of membrane potential.

In summary, escitalopram inhibited Na\textsubscript{1.2} VGSCs current in a dose-dependent manner. Moreover, both voltage-dependence of the activation and inactivation were shifted toward hyperpolarization by treatment with escitalopram. Further studies will be needed to clarify the correlation between the electrophysiological mechanisms of the inhibitory effect of escitalopram on Na\textsubscript{1.2} VGSCs and its pharmacological action.

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Conflict of Interest Yoshihiko Nakatani received a research Grant from GlaxoSmithKline K.K. (GSK Japan Research Grant 2017). Taku Amano declares no conflict of interest.

Supplementary Materials The online version of this article contains supplementary materials.

REFERENCES


4) Citrome L. Vortioxetine for major depressive disorder: An indirect comparison with duloxetine, escitalopram, levomilnacipran, sertraline, venlafaxine, and vilazodone, using number needed to treat, number needed to harm, and likelihood to be helped or harmed. *J. Affect. Disord.*, 196, 225–233 (2016).


