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Research Report

Effects of continuous administration of paroxetine on ligand binding site and expression of serotonin transporter protein in mouse brain

Kazufumi Hirano[a], Takahiro Seki[b], Norio Sakai[b], Yasuhiro Kato[c], Hisakuni Hashimoto[c], Shinya Uchida[a], Shizuo Yamada[a,*]

[a]*Department of Biopharmaceutical Sciences and Center of Excellence (COE) Program in the 21st Century, School of Pharmaceutical Sciences, University of Shizuoka, 52-1 Yada, Shizuoka 422-8526, Japan*

[b]*Department of Molecular and Pharmacological Neuroscience, Graduate School of Biomedical Sciences, Hiroshima University, 1-2-3 Kasumi, Minami-ku, Hiroshima 734-8551, Japan*

[c]*Department of Hospital Pharmacy, Hamamatsu University of School of Medicine, 1-20-1 Handayama, Hamamatsu 431-3192, Japan*

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Abstract

Selective serotonin reuptake inhibitors (SSRIs), such as paroxetine, are utilized in the treatment of depression and anxiety disorders. Although SSRIs potently interfere with the activity of brain serotonin transporter (SERT) after acute treatment, clinical improvement of psychiatric diseases is observed only after the repeated administration for several weeks (2–6 weeks). The present study was undertaken to investigate the effects of continuous administration of paroxetine on specific [^{3}H]paroxetine binding sites and expression of SERT protein in mouse brain. Mice continuously and subcutaneously received paroxetine at doses of 2.67 or 13.3 μmol/kg/day for 21 days by using osmotic minipumps, and the steady-state plasma drug levels were within the range of reported concentrations in the clinical therapy. Continuous administration of paroxetine at theses doses produced significant (25–46%) reduction of [^{3}H]paroxetine binding in each brain region (cerebral cortex, striatum, hippocampus, thalamus, midbrain) of mice. In Western blot analysis, expression levels of SERT protein in the thalamus and midbrain of mice were significantly (51% and 61%, respectively) decreased on day 21 after the implantation of minipumps at the higher dose. In conclusion, this study has firstly shown that continuous administration of paroxetine induces significant reduction of not only ligand binding sites of SERT but the protein expression level in mouse brain. Such down-regulation of SERT may partly underlie the therapeutic effect of long-term treatment with SSRIs in human.

Theme: Neurotransmitters, modulators, transporters and receptors
Topic: Uptake and transporters

Keywords: Selective serotonin reuptake inhibitor; Serotonin transporter; Chronic treatment

1. Introduction

Selective serotonin reuptake inhibitors (SSRIs), such as paroxetine, are one of the most frequently prescribed therapeutic agents in all medicines, and they show diverse therapeutic actions on various psychiatric disorders including depression, obsessive–compulsive disorder, panic disorder and other conditions as well [15,29]. Serotonin (5-hydroxytryptamine, 5-HT) has long been known to have a multitude of different physiological actions (e.g., mood, anxiety, sleep, temperature, appetite, sexual behavior and eating behavior) due to the wide variety of 5-HT receptors [2]; and serotonin transporter (SERT) predominantly regulates the synaptic concentration of released 5-HT [28]. It is considered that depression and other psychiatric disorders are caused by chronically low levels of seroto-

* Corresponding author. Fax: +81 54 264 5635.
E-mail address: yamada@ys7.u-shizuoka-ken.ac.jp (S. Yamada).

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nergic neurotransmission, and SSRIs potently and selectively interfere with the activity of brain SERT under in vitro [23] and in vivo [12,13] conditions, resulting in the enhancement of serotonergic neurotransmission. Although SSRIs inhibit brain SERT activity after acute treatment, it is known that clinical improvement of psychiatric diseases is observed only after the repeated treatment for several weeks (2–6 weeks) [5,6]. This delayed therapeutic response is a common property of all antidepressants, suggesting that acute uptake inhibition initiates the cascade of events that eventually bring about clinical alleviation. Therefore, it is predicted that adaptive processes in brain serotonergic system would underlie the antidepressive effect of SSRIs.

It is well known that serotonergic neurotransmission is regulated by not only SERT but also its autoreceptors. Namely, two types of serotonergic autoreceptors with different neuronal locations have been identified. One is somatodendritic 5-HT_{1A} autoreceptors in the raphe nuclei, which play an important role in the regulation of serotonergic cell firing and neurotransmitter release into the synaptic clefts [11,34], and the other is 5-HT_{1B} autoreceptors that are located on axon terminals in the serotonergic projection areas such as hippocampus, hypothalamus and frontal cortex [20,34]. To date, several studies have been undertaken to elucidate the mechanism of antidepressant actions. It has been reported that the 5-HT_{1A} agonist-stimulated [^{35}S]GTPγS binding in the dorsal raphe was attenuated after chronic treatment of SSRIs without changes in receptor density [24,27], suggesting that somatodendritic 5-HT_{1A} receptors are desensitized through the changes in G-protein expression level and/or regulatory process of the receptor (e.g., phosphorylation) [11]. Similarly, the sustained administration of SSRI also induced subsensitivity of 5-HT_{1B} autoreceptors in the serotonergic projection areas [22]. These findings were also supported by the clinical study that therapeutic efficacy of SSRIs was augmented by coadministration of 5-HT_{1A} receptor antagonists such as pindolol [1]. Thus, the lag time between onset of medication and therapeutic efficacy is hypothesized to involve the progressive desensitization of 5-HT autoreceptors.

In case of brain SERT, chronic antidepressant treatment has been reported to induce significant reduction of radioligand binding sites and SERT function in rat brain [3,4,25,32], while no change [10] and increase [14] were also reported. Thus, the results in the alteration of brain SERT are inconsistent, and it is probable that such discrepancy stems from the distinction of treatment regimen. Because the half-lives of antidepressants in human are considerably longer than those in rodents [7,13], daily oral administration of SSRIs makes it possible to produce sustained pharmacological effects throughout the day under the clinical condition but not in animals. Thus, it is important to make efforts in rodent studies to simulate serum levels after treatment with SSRIs reported in clinical studies. Previously, less well studied is the effect of long-term SSRI treatment on the ligand binding sites and protein expression of SERT. Therefore, we have simultaneously measured specific [^{3}H]paroxetine binding and expression levels of SERT protein in the brain of mice maintained at the clinically relevant steady-state plasma concentration of paroxetine. In fact, such steady-state paroxetine level was achieved by subcutaneous implantation of the drug-containing osmotic minipumps.

2. Materials and methods

2.1. Materials

[^{3}H]Paroxetine (706.7 GBq/mmol) was purchased from Dupont-NEN Co. Ltd. (Boston, MA). Paroxetine hydrochloride was kindly donated by GlaxoSmithKline Pharmaceuticals (West Sussex, UK). Rabbit polyclonal anti-SERT antibody (against *carboxyl*-terminal region of SERT) was made as described previously [33], and all other drugs and materials were obtained from commercial sources.

2.2. Drug treatment

Male ICR strain mice at 5 weeks of age (SLC, Shizuoka, Japan) were housed five per cage in the laboratory with free access to food and water and maintained on a 12-h dark/light cycle in a room with controlled temperature (24 ± 1 °C) and humidity (55 ± 5%). All animal procedures were in strict accordance with the guidelines approved by the Experimental Animal Ethical Committee of University of Shizuoka. Mice were administered paroxetine (2.67 or 13.3 μmol/kg/day) for 5, 10, 15 or 21 days subcutaneously by means of osmotic minipumps (model 2004, Durect, Cupertino, CA), and control animals received vehicle (50% ethanol in distilled water). Subcutaneous implantation of osmotic minipumps was performed under aseptic condition. Briefly, a small incision was made in the skin between the scapulae under light anesthesia with diethyl ether, and a small pocket was formed by spreading the subcutaneous connective tissues apart with a hemostat. The pump was inserted into the pocket, and the skin incision was closed with sutures on day 0. After the procedure mice were housed separately.

2.3. Measurement of [^{3}H]paroxetine binding in mouse brain

Osmotic minipumps were removed from mice on day 21, and specific [^{3}H]paroxetine binding in mouse brain was measured at 72 and 120 h (washout period) after cessation of drug treatment. Mice were exsanguinated from descending aorta under light anesthesia with diethyl ether, and brain was perfused with 0.9% NaCl from the aorta. Then, the whole brain tissue was removed and divided into five brain regions (cerebral cortex, hippocampus, striatum, thalamus