Effects of Imipramine and Bupropion on the Duration of Immobility of ACTH-Treated Rats in the Forced Swim Test: Involvement of the Expression of 5-HT2A Receptor mRNA

Yoshiihsa Kitamura,*a,b Yoshihisa Fujitani,b Kouhei Kitagawa,a,b Toshiaki Miyazaki,b Hidenori Sagara,a Hiromu Kawasaki,c Kazuhiro Shibata,b Toshiaki Sendo,b and Yutaka Gomita,b

a Department of Pharmaceutical Care and Health Sciences, Graduate School of Medicine, Dentistry and Pharmaceutical Sciences, Okayama University; b Department of Pharmaceutical Sciences, Graduate School of Medicine, Dentistry and Pharmaceutical Sciences, Okayama University; and c Department of Hospital Pharmacy, Okayama University Medical School.

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We examined the effect of chronic administration of imipramine and bupropion, monoamine reuptake inhibitors, on the duration of immobility in the forced swim test and serotonin (5-HT)2A receptor function in the form of 5-HT2A receptor mRNA levels in rats chronically treated with adrenocorticotropic hormone (ACTH). The immobility-decreasing effect of bupropion without imipramine did not influence the chronic ACTH treatment. The effect on the expression of 5-HT2A receptor mRNA of chronic ACTH treatment was decreased by bupropion, but not imipramine. These results suggest that bupropion has the effect of reducing immobility time in the forced swim test in the tricyclic antidepressant-resistant depressive model induced by chronic ACTH treatment in rats, and that decreased 5-HT2A receptor mRNA levels may be involved in this phenomenon.

Key words: adrenocorticotropic hormone; imipramine; bupropion; forced swim test; 5-HT2A receptor

It is well documented in neurochemical and behavioral studies that antidepressants, monoamine reuptake inhibitors, affect serotonin (5-HT)2A Receptors. The repeated administration of monoamine reuptake inhibitors induced a decrease in the density of 5-HT2A receptors in naïve rats.1–3) Several lines of evidence suggest functional interaction between 5-HT2A receptors and the monoaminergic system.

Psychoendocrinological studies have focused on the regulation of the hypothalamic-pituitary-adrenal (HPA) axis in patients with depression.4) The HPA axis has been postulated to play an important role in the functions of 5-HT2A receptors. The psychoneuroendocrine mechanism whereby steroid hormones regulate 5-HT2A receptor function will impact our understanding of hypercortisolism, a condition typical of affective disorders and treatment-resistant depression,5) and the effect of tricyclic antidepressants.6) Kuroda et al. have reported that, in rats, chronic treatment with adrenocorticotropic hormone (ACTH) increases the binding of [3H]-Ketanserin to 5-HT2A receptors in the forebrain neocortex.7) We reported that the chronic administration of corticosterone increases the density of 5-HT2A receptors in the frontal cortex.8) In addition, we confirmed that chronic treatment with ACTH increased the binding of [3H]-Ketanserin to 5-HT2A receptors. Namely, activating the HPA axis enhanced the function of the 5-HT2A receptors. Some clinical studies have demonstrated increases in the number of 5-HT2A receptor-binding sites in the postmortem brains of both suicides and depressed subjects.9–12) Animal models of ACTH treatment may shed light on the mechanism governing the 5-HT2A receptor’s up-regulation, a condition associated with the pathophysiology of depression.

We previously reported that the chronic administration of corticosterone and ACTH potentiated (±)-1-(2,5-dimethoxy-4-iodophenyl)-2-aminopropane (DOI)-induced wet-dog shakes, via the activation of 5-HT2A receptors.13) Furthermore, this effect of ACTH was not inhibited by the chronic administration of imipramine.13) The reason why the chronic administration of imipramine did not inhibit the increase in DOI-induced wet-dog shakes in the ACTH-treated rats is unknown. Also, the mechanism by which the hyperfunction of 5-HT2A receptors is inhibited by monoamine reuptake inhibitors remains to be elucidated. To the best of our knowledge, there is no published data on the effect of monoamine reuptake inhibitors on the expression of 5-HT2A receptor mRNA in chronic ACTH-treated rats.

In rodents, the forced swim test is widely used as a predictor of antidepressant activity.14) Many antidepressants, including the noradrenaline/5-HT reuptake inhibitor imipramine and the dopamine reuptake inhibitor bupropion, reduce the immobility of rodents in the forced swim test.15–17) Although these drugs affect the duration of immobility in the forced swim test in native rats, few attempts have been made to examine their effects in a model where the HPA axis is abnormally activated. In the present study, with regard to the monoamine reuptake inhibitors imipramine and bupropion, we examined the effect on the duration of immobility in the forced swim test in ACTH-treated rats, and also examined the 5-HT2A Receptor mRNA levels in the frontal cortex.

MATERIALS AND METHODS

Animals Male Wistar rats (Charles River, Japan), initially weighing 210—230 g, were kept on a constant light–dark cycle (lights on: 07:00—19:00 h), with standard laboratory food and tap water in an air-conditioned room (23 ± 1°C with approximately 60% humidity). All experiments were conducted according to the guidelines for Animal Experimentation at Okayama University Medical School. Every effort was made to minimize the number and suffering of animals used.

Drugs The drugs used were imipramine hydrochloride (Wako, Osaka, Japan), bupropion hydrochloride (Sigma, St.

* To whom correspondence should be addressed. e-mail: ykita@pharm.okayama-u.ac.jp © 2008 Pharmaceutical Society of Japan
Louis, MO, U.S.A.) and ACTH-(1-24)-zinc (Cortrosyn-Z: Daiichi Seiyaku, Tokyo, Japan). Imipramine and bupropion were dissolved in saline. The rats were injected with imipramine (10 mg/kg, i.p.) and bupropion (10 mg/kg, i.p.) at 2 ml/kg body weight. ACTH (Cortrosyn-Z) was injected subcutaneously once daily (9:00 to 10:00) at a dose of 100 μg/rat (injection volume was 0.2 ml/rat, s.c.) for 1—14 d. Control rats received an equivalent volume of saline, 0.2 ml/rat (s.c.), for the same treatment period.

**Measurement of Immobility** To measure immobility, rats were placed individually in plastic cylinders (height 37 cm, diameter 15.5 cm) containing 20 cm of water at 25 °C, as described by Porsolt et al.[14] Two swim sessions were conducted in the initial 13-min pretest; a 6-min test followed 24 h later. The total period of immobility during the 6-min test period was recorded using the TARGET series/7M analysis program (Neuroscience, Tokyo, Japan).

**Measurement of Cortex 5-HT2A Receptor mRNA by Real-Time Quantitative Polymerase Chain Reaction (PCR).** Total RNA Extraction  At 1 d after the last drug administration, rats were sacrificed by decapitation. The brain was quickly removed and dissected on ice. Samples were frozen at −80 °C before homogenization. Total RNA was isolated from rat frontal cortex with TRIzol Reagent (GIBCO) according to the manufacturer’s directions. The RNA samples were dissolved in RNase-free water and quantified with UV-spectrophotometry at 260 nm.

**Primers** Primers for rat 5-HT2A receptors and glyceraldehyde-3-phosphate dehydrogenase (GAPDH) were designed for real-time PCR. Specific primers designed based on gene bank data were as follows: 5-HT2A receptor primers, Forward: 5′-AGCCCGTTCAACTCCAGAA-3′, Reverse: 5′-TTTTGCTCATTGCTGATGGA-3′; Reverse: 5′-TCCACCACCCCTGTTGCTGTA-3′.

**Reverse Transcription** Extracted RNA was reverse transcribed with reverse transcriptase using the procedure of the supplier (Gene AmpRNA PCR kit, Applied Biosystems). The reverse transcription reaction mixture contained 0.02 μg/μl of RNA, 2.5 μM of the oligo-d(T)16 primers, 1 mM of each dNTP, 2.5 mM of reverse transcriptase, and 1×RT buffer, 1.0 U/μl of RNase inhibitor, and 5 mM MgCl2. The reverse transcription reaction was performed at 42 °C for 60 min, followed by heating at 99 °C for 5 min.

**Real-Time PCR:** Real-time PCR was performed with the SYBR® Green PCR Core Reagents (Applied Biosystems). For detection and quantification, a GeneAmp 5700 (Applied Biosystems) was used. Reactions were performed in a reaction mixture (25 μl) consisting of 1×SYBR PCR buffer, 200 μM of each dNTP, 0.025 U/μl of AmpliTaq Gold, 2.5 mM MgCl2, and 4 pmol of each primer. PCR was performed with a 10 min preincubation at 95 °C followed by 40 cycles of 30 s at 95 °C, 1 min at 60 °C, and 2 min at 72 °C. The products were verified by melting curve analysis, agarose gel electrophoresis, and DNA sequencing. The Real-time PCR method was validated by using serially diluted cDNA to establish a standard curve. To quantify the gene expression profile in each sample, the efficiency of each standard curve was determined from its slope and comparative threshold according to the manufacturer’s instructions. For each sample, the amount of targeted mRNA (arbitrary units) was normal-
ized to the amount of mRNA of a housekeeping gene, GAPDH. Also, PCRs without the RT reaction were performed for each sample in order to exclude contamination of the genomic DNA. The data analysis was performed with GeneAmp 5700 software.

**Statistics** Data are given as the mean±S.E.M. All data were analyzed by the one-way analysis of variance (ANOVA); the group means were compared using Tukey’s test for multiple comparisons. Probability values of less than 0.05 were considered to show a significant difference.

**RESULTS**

**Effects of Imipramine and Bupropion on the Duration of Immobility in ACTH-Treated Rats** Following a 14-d chronic administration of imipramine and bupropion to normal rats, we examined the effect on the duration of immobility in the forced swim test. Imipramine (10 mg/kg, i.p.) and bupropion (10 mg/kg, i.p.) significantly decreased the duration of immobility in normal rats. Furthermore, we tested the effect of imipramine and bupropion on the duration of immobility in the forced swim test in rats treated with ACTH for 14 d. The immobility-decreasing effect of imipramine was blocked by chronic treatment with ACTH (imipramine: F3,28=9.68, p<0.01), however, the immobility-decreasing effect of bupropion was not (F3,28=6.53, p<0.01) (Fig. 1).

**Effects of Imipramine and Bupropion on the Expression of 5-HT2A Receptor mRNA in ACTH-Treated Rats** Figure 2 shows the expression of 5-HT2A receptor mRNA. The administration of imipramine (10 mg/kg, i.p.) and bupropion (10 mg/kg, i.p) for 14 d significantly decreased the level of 5-HT2A receptor mRNA. Treatment with ACTH (100 μg/rat s.c.) for 14 d significantly increased the expression. This effect of ACTH was not decreased by the chronic administration of imipramine (10 mg/kg, i.p.) (F3,12=20.45,
We previously reported that the effect of the chronic administration of imipramine (10 mg/kg, i.p.) on immobility time in the forced swim test is inhibited by chronic ACTH treatment in rats. The inhibition of the immobility-decreasing effect of imipramine is reversed by coadministration of lithium and imipramine. Namely, chronic treatment of rats with ACTH may prove to be an effective model of tricyclic antidepressant-treatment-resistant depression. The current results confirmed the effect of chronic imipramine administration in ACTH-treated rats. Furthermore, the administration of bupropion reduced the immobility time of naive and ACTH-treated rats in the forced swim test in this study. Several studies have reported an increased 5-HT_{2A} receptor density or 5-HT_{2A} receptor-mediated response in depressed patients normalized by antidepressant treatment. Using drugs with significant antagonistic properties toward the 5-HT_{2A} receptor (mianserin and mirtazapine), in addition to selective serotonin reuptake inhibitor (SSRI)s enhanced therapeutic responses in patients with major depression. In an animal study, a reduction in the number of 5-HT_{2A} receptors caused by the administration of an antisense oligonucleotide decreased the duration of immobility in the forced swim test in mice. Altogether, these findings indicate the sensitization of 5-HT_{2A} receptor function to be an essential part of antidepressant drug action. In this study, chronic ACTH treatment increased the expression of 5-HT_{2A} receptor mRNA in the frontal cortex. This result suggested glucocorticoids to be an important regulatory factor for the 5-HT_{2A} receptor gene. Therefore, the activation of the HPA axis may regulate 5-HT_{2A} receptor mRNA levels via the activation of glucocorticoid receptors. Furthermore, we reported the effect of ACTH on the immobilization of rats in the forced swim test after administration of the 5-HT_{1A} receptor agonist 8-hydroxy-2-di-n-propylamino tetralin (8-OHDPAT). The immobility-decreasing effect of 8-OH-DPAT, unlike that of imipramine, was not blocked by the administration of ACTH for 14 d. 8-OH-DPAT inhibited the wet-dog shakes induced by DOI in chronic ACTH-treated rats.

Namely, we suggested that the decreased 5-HT_{2A} receptor function may be involved in the antidepressant-like effect in ACTH-treated rats. A number of studies have shown that other antidepressants are related to 5-HT_{2A} receptor function. Fluvoxamine, a SSRI, has been reported to have no effect on 5-HT_{2A} receptor density. Paroxetine, a SSRI, and milnacipran, a serotonin noradrenaline reuptake inhibitor (SNRI), have been reported to decrease 5-HT_{2A} receptor density or have no effect. Furthermore, other SSRIs, including fluoxetine, citalopram and sertraline, have also been reported to have varying effects. Therefore, further studies are needed to determine whether or not 5-HT_{2A} receptors are involved in the antidepressant-like actions of SSRIs and SNRIs in ACTH-treated rats.

Bupropion is used clinically as an atypical antidepressant. Furthermore, bupropion is used against treatment-resistant depression. We previously observed that bupropion reduced the duration of immobility in a dose-dependent manner (saline: 267.7±12.4 s; 2.5 mg/kg: 227.1±18.0 s; 5 mg/kg: 231.9±15.1 s; 10 mg/kg: 180.2±27.9 s). Notably, bupropion at 10 mg/kg (i.p.) significantly shortened the duration of immobility. In this study, we therefore adopted a dose of 10 mg/kg. The duration of immobility following chronic administration of bupropion was decreased by chronic treatment with ACTH for 14 d. Bupropion inhibited the enhancing effect on the expression of 5-HT_{2A} receptor mRNA in the chronic ACTH-treated rats. It is difficult to explain why bupropion inhibited the effect on the expression of 5-HT_{2A} receptor mRNA in the chronic ACTH-treated rats. It was reported that the level of 5-HT_{2A} receptor mRNA and the number of 5-HT_{2A} receptor-binding sites were increased in rats exposed to the neurotoxin 6-OHDA. These increases were abolished after the chronic administration of the dopamine receptor agonist apomorphine or SKF-38393. Namely, bupropion may inhibit the expression of 5-HT_{2A} receptors by stimulating the dopaminergic nerve system. On the other hand, in our preliminary study, an agent for Parkinson’s disease, selegiline, a monoamine oxidase B inhibitor, decreased the duration of immobility in ACTH-treated rats, like bupropion. Activation of the dopaminergic system may prove to be a promising way to improve the treatment of depression or treatment-resistant depression. Studies are in progress to clarify the relationship between the duration of immobility and activity of the dopaminergic nerve system.

Imipramine and bupropion have affinity for noradrenaline uptake sites and appear to act as inhibitors. It has been well established that chronic administration of desipramine results in a down-regulation of 5-HT_{2A} receptors activity in rats. We reported that desipramine inhibited DOI-induced wet-dog shaking behavior in naive and ACTH-treated rats. This result suggested that a noradrenaline reuptake inhibitor may improve the hyperfunction of 5-HT_{2A} receptors induced by...
chronic ACTH treatment. However, imipramine did not have any effect on the 5-HT$_{2A}$ receptor mRNA levels in ACTH-treated rats. Bupropion is a weak reuptake inhibitor that shows ca. 2.5-fold selectivity for dopamine versus noradrenaline.\(^{41,42}\) Thus, the contribution of the noradrenaline system to the inhibition of 5-HT$_{2A}$ receptor mRNA expression by imipramine and bupropion is comparatively small in ACTH-treated rats.

In the present study, bupropion decreased the duration of immobility in the forced swim test, and decreased 5-HT$_{2A}$ receptor mRNA levels in the frontal cortex in a tricyclic antidepressant-resistant depressive model induced by chronic ACTH treatment in rats. Stimulation of the central dopaminergic nerve system may prove to be a promising way to improve the efficacy of the treatment of resistant depression by the inhibition of 5-HT$_{2A}$ receptor function.

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### REFERENCES