The dopamine reuptake inhibitor bupropion and dopamine D2/3 receptor agonist pramipexole have been clinically proven to improve both depression and treatment-resistant depression. We examined its influence on the duration of immobility during the forced swim test in adrenocorticotropic hormone (ACTH)-treated rats and further analyzed the possible role of the dopamine nerve system in this effect. Bupropion and pramipexole significantly decreased the duration of immobility in normal and ACTH-treated rats. We previously demonstrated that the chronic administration of ACTH caused a significant decrease in hippocampal cell proliferation and neurogenesis. In this study, we used the mitotic marker 5-bromo-2'-deoxyuridine to investigate the effects of bupropion and pramipexole on cell proliferation in the subgranular zone of the hippocampal dentate gyrus following chronic treatment with ACTH. The ACTH treatment for 14 d decreased adult hippocampal cell proliferation. The chronic administration of bupropion for 14 d blocked the loss of cell proliferation resulting from the chronic treatment with ACTH, whereas pramipexole did not. The administration of bupropion may have treatment-resistant antidepressive properties, which may be partly attributed to the normalization of hippocampal cell proliferation.

**Key words** adrenocorticotropic hormone; bupropion; pramipexole; cell proliferation; 5-bromo-2'-deoxyuridine

Major depressive disorder is a serious mental health problem and achieving remission from treatment-resistant depression remains an important clinical issue. A number of similarities have been reported between the features of depression and chronic glucocorticoid administration in laboratory animals. We previously showed that the chronic administration of adrenocorticotropic hormone (ACTH) to rats counteracted the decrease in immobility time induced by the tricyclic antidepressant, imipramine or desipramine, in the forced swim test, which is widely used as a predictor of antidepressant activity.1) The chronic co-administration of lithium, an agent that potentiates the actions of antidepressants in patients with depression including those with treatment-resistant depression, significantly decreased the duration of immobility, even when given concurrently with ACTH.3) Electroconvulsive therapy is considered to be the most effective biological treatment for depression, particularly severe intractable depression. We previously demonstrated that repeated electroconvulsive stimuli decreased the duration of immobility of rats repeatedly treated with ACTH during the forced swim test.5) As described above, we reported that the repeated treatment of rats with ACTH serves as a valuable animal model of tricyclic antidepressant-resistant depressive conditions.

Chronic treatment with antidepressants may increase cell proliferation reverse stress-induced decreases in hippocampal cell proliferation and neurogenesis.3,4) The ability to promote hippocampal neurogenesis is a feature of both classical antidepressants, such as tricyclic drugs and selective serotonin re-uptake inhibitors.5,6) Moreover, hippocampal cell proliferation and neurogenesis may be key factors in the actions of antidepressant drugs. We previously reported that the number of 5-bromo-2'-deoxyuridine (BrdU)-positive cells in the subgranular zone (SGZ) of the hippocampal dentate gyrus was significantly lower with the chronic administration of ACTH than the control value.3,6) This effect was not influenced by the chronic administration of imipramine or lithium, but was reversed by the co-administration of imipramine and lithium for 14 d in ACTH-treated rats.7) Furthermore, electroconvulsive stimuli increased cell proliferation in both saline-treated and ACTH-treated rats. These findings suggest that the treatment-resistant antidepressant effects of electroconvulsive stimuli may be attributed, at least in part, to an enhancement in hippocampal cell proliferation.8)

Dopamine agonists were previously shown to be effective against treatment-resistant depression in clinical studies9–11). We previously reported that the dopamine uptake inhibitor bupropion (10 mg/kg, intraperitoneally (i.p.)) and dopamine D2/D3 receptor agonist pramipexole (1 mg/kg, subcutaneously (s.c.)) decreased the duration of immobility of rats repeatedly treated with ACTH during the forced swim test.12,13) The present study was undertaken to determine the effect of chronic bupropion and pramipexole treatment on adult hippocampal cell proliferation quantified using BrdU immunohistochemistry in the SGZ of ACTH-treated rats.

**MATERIALS AND METHODS**

**Animals** Male Wistar rats (Charles River, Yokohama, Japan) with an initial weight of 220–230 g were utilized in the present study. Rats were group-housed, 4 per cage, under a constant light-dark cycle (lights on, 07:00–19:00) and fed 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 following drugs were used: bupropion hydrochloride (Sigma-Aldrich Co., St. Louis, MO, U.S.A.), pramipexole hydrochloride (Boehringer Ingelheim, Germany), and ACTH (1–24)-zinc (Cortrosyn-Z; Daichi-Sankyo, Tokyo, Japan). Bupropion and pramipexole were dissolved in saline. Rats were administered bupropion (intraperitoneal injection) and pramipexole (subcutaneous injection) for 14 d. ACTH (Cortrosyn-Z) was injected subcutaneously once daily (09:00 to 10:00 h) at 100 µg/rat (at an injection volume of 0.2 mL/rat) for 14 d. Control rats received an equivalent volume of vehicle (saline 0.2 mL/rat) subcutaneously for the same period of time. The thymidine-analog 5-bromo-2′-deoxyuridine (BrdU) (Sigma-Aldrich Co.) is known to be incorporated into dividing cells during the S-Phase and thus serves as a marker for cell proliferation. BrdU (75 mg/kg) was administered intraperitoneally 4 times per day at 6-h intervals (10.00 a.m., 4.00 p.m., 10.00 p.m., and 4.00 a.m.).

Immunohistochemistry Rats were transcardially perfused with ice-cold saline six hours after the last BrdU injection, followed by a fixative containing 4% paraformaldehyde and 0.35% glutaraldehyde in 0.1 M phosphate buffer (PB, pH 7.4) under deep anesthesia with pentobarbital sodium (80 mg/kg, i.p.). The brain was then rapidly removed en bloc from the skull, post-fixed for 24 h in a fixative containing 4% paraformaldehyde in 0.1 M PB (pH 7.4), and cryoprotected in 20% sucrose in 0.1 M PB with sodium azide. Brains snap-frozen with powdered dry ice were cut coronally on a cryostat into 20-µm thick sections containing the dentate gyrus of the hippocampus. The sections were collected in 10 mM phosphate-buffered saline (PBS) with 0.1% sodium azide for staining. Standard free-floating immunohistochemistry was used to detect BrdU-immunopositive signals in the dentate gyrus. Free-floating sections were subjected to deoxyribonucleic acid denaturation by incubating sections for 2 h in 50% formamide/2×standard saline citrate (SSC) at 65°C, followed by a 2×SSC rinse. Sections were incubated for 20 min in 2× HCl, PBS, and for 15 min in 0.1 M boric acid, pH 8.5. Following incubation in 1% normal goat serum in PBST (10 mM PBS containing 0.2% Triton X-100) for 30 min, sections were exposed to a mouse anti-BrdU monoclonal antibody (diluted 1:1000 in PBST; Abcam, Cambridge, MA, U.S.A.) for 18 h at 4°C. Sections were then washed in PBST (5×5 min) and incubated with a goat anti-mouse IgG Alexa Fluor 594 antibody (diluted 1:1000 in PBST; Invitrogen, Carlsbad, CA, U.S.A.) for 2 h at room temperature. All slides were analyzed under a fluorescence microscope (Olympus BX50-FLA; Olympus, Tokyo, Japan) using a mercury lamp through a 530–550 nm band-pass filter to excite Alexa Fluor 594. The light emitted from Alexa Fluor 594 was collected through a 590 nm long-pass filter. The stained cells were photographed at a magnification of ×200. Serial sections were collected for each brain to generate five sets representative of the entire hippocampus (−2.3 mm to +4.3 mm from the bregma). Each set contained sections at least 20-µm thick (400 µm apart) covering the entire anteroposterior extent of the hippocampus. The mean count obtained was then multiplied by 5 (the number of series) to obtain an estimate for the total number of BrdU-positive cells throughout the dentate gyrus. BrdU-positive cells of the dentate gyrus were counted by an investigator blinded to the experiments.

Statistical Analysis All values are expressed as the group mean±S.E.M. All data were assessed using a one-way ANOVA and 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 the Chronic Treatment with Bupropion and Pramipexole for 14 d on Cell Proliferation in the SGZ of the Hippocampal Dentate Gyrus in Saline- and ACTH-Treated Rats The administration of neither bupropion (10 mg/kg, i.p.) nor chronic pramipexole (1 mg/kg, s.c.) for 14 d affected the number of BrdU-positive cells in the SGZ of the hippocampal dentate gyrus (Fig. 1). Treatment with ACTH (100 µg/rat, s.c.) for 14 d significantly decreased this number. This effect was not influenced by the chronic administration of pramipexole, but was significantly reversed by the administration of bupropion for 14 d. Treatment with pramipexole for 14 d also significantly decreased the number of BrdU-positive cells in the SGZ.

DISCUSSION

In this study, we examined the effect of bupropion and pramipexole on cell proliferation in the hippocampus of ACTH-treated rats. The chronic administration of bupropion reversed the cell proliferation compared with treatment of ACTH. Previous studies have shown that antidepressants, monoamine reuptake inhibitors, affect serotonin (5-HT) receptors. Several lines of evidence suggest a functional interaction between 5-HT2 receptors and the monoaminergic system. Chronic ACTH treatment was shown to increase the expression of 5-HT2A receptor mRNA in the frontal cortex, while bupropion inhibited this effect in rats chronically treated with ACTH. Therefore, bupropion may be a promising way to improve the efficacy of the treatment of resistant depression by inhibiting 5-HT2A receptor function. Furthermore, 5-HT is well-known as a potent regulator of adult hippocampal neurogenesis. Treatment with the 5-HT2 receptor agonist α-methyl-5-HT was shown to decrease the number of BrdU-positive cells in cell proliferation and differentiation of the adult dentate gyrus. Furthermore, treatment with the 5-HT3 receptor antagonist cinanserin increased the proportion of BrdU-positive cells in newborn cells. The chronic ACTH treatment may possibly be involved in decreasing cell proliferation with the activation of 5-HT2A receptors. Therefore, it is likely that enhancing the proliferation of bupropion involves the inhibitory effect of 5-HT2A receptors in ACTH-treated rats.

The dopamine D2/D3 receptor agonist pramipexole has been introduced for the treatment of both early and advanced Parkinson’s disease. Furthermore, the efficacy of pramipexole was reported in patients with treatment-resistant depression. We demonstrated that pramipexole decreased the duration of immobility in chronic ACTH-treated rats. The adult mammalian brain retains neurogenic areas in the subventricular zone (SVZ) of the anterior lateral ventricle, except for the SGZ in the hippocampus dentate gyrus. The activation of D3 receptors has been shown to promote SVZ proliferation in rats. Furthermore, pramipexole promoted adult neurogenesis in the SVZ in Parkinson’s disease model rats. The 14-d
The intracerebroventricular infusion of the D3 agonist 7-hydroxy-di-propyl-amino-tetraline increased BrdU labeling of neural precursors in the SVZ in rats, but failed to affect neurogenesis in mice. However, the effect of the treatment with pramipexole has not been examined on neurogenesis in the SGZ of rats. In the present study, pramipexole significantly decreased the number of BrdU-positive cells in normal rats. Why pramipexole decreased the effect on neurogenesis in the SGZ in rats remains unknown. We previously confirmed that the number of Ki-67 (an endogenous marker of cell proliferation)-positive cells in the SGZ was lower in rats treated with pramipexole than in control rats (data not shown). On the other hand, we showed that the administration of pramipexole into the intranucleus accumbens rather than the medial prefrontal cortex decreased immobility in normal and ACTH-treated rats during the forced swim test. Therefore, the nucleus accumbens, not the hippocampus may play a critical role in the antidepressant effect of pramipexole.

In the present study, bupropion, but not pramipexole enhanced cell proliferation in the SGZ of the hippocampus dentate gyrus. When combined with the findings of previous studies, our results support the efficacy of the dopamine reuptake inhibitor, bupropion in improving treatment-resistant depression by triggering cell proliferation.

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