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Original Article

Effects of Imipramine and Lithium on the Suppression of Cell Proliferation in the Dentate Gyrus of the Hippocampus in Adrenocorticotropic Hormone-treated Rats

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We examined the influence of chronic adrenocorticotropic hormone (ACTH) treatment on the number of Ki-67-positive cells in the dentate gyrus of the hippocampus in rats. ACTH treatment for 14 days decreased the number of such cells. The administration of imipramine or lithium alone for 14 days had no effect in saline-treated rats. The effect of ACTH was blocked by the administration of imipramine. Furthermore, the coadministration of imipramine and lithium for 14 days significantly increased the number of Ki-67-positive cells in both the saline and ACTH-treated rats. The coadministration of imipramine and lithium normalized the cell proliferation in the dentate gyrus of the hippocampus in rats treated with ACTH.

Key words: ACTH, imipramine, lithium, proliferation, Ki-67

Psychoendocrinological studies have focused on the regulation of the hypothalamic-pituitary-adrenal (HPA) axis in patients with depression [1]. Cortisol hypersecretion in depression is believed to result from abnormalities in the HPA axis [1, 2]. Multiple neuroendocrinological abnormalities appear in subjects with depressive disorders, including increases in cortisol secretion and the attenuation of cortisol suppression in response to dexamethasone [3]. Cortisol hypersecretion in depression is believed to result from abnormalities in the HPA axis. Patients with Cushing's disease, a hyperadrenocorticism following a pituitary tumor, an adrenal tumor, or a tumor producing ectopic adrenocorticotropic hormone (ACTH), often exhibit mental changes including depression [4, 5]. Tricyclic antidepressants are not effective for the treatment of depression in patients with Cushing's disease, but steroid-suppressive agents such as metyrapone and aminogluethimide are effective for depression in such cases [6], suggesting an etiological link between depressive illness and the disinhibition of the HPA axis observed in subjects with Cushing's disease. In addition, steroid-suppressive agents, such as metyrapone and ketoconazole, and a glucocorticoid receptor antagonist, RU486, are effective treatments for antidepressant treatment-resistant

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depression [5, 7, 8]. These results suggest that abnormal activation of the HPA axis correlates with treatment-resistant depression. We previously reported that the chronic administration of ACTH to rats counteracted the decrease in immobility time induced by a tricyclic antidepressant, imipramine or desipramine, in the forced swim test, which is widely used as a predictor of antidepressant activity [9]. Furthermore, chronic coadministration of lithium, an agent that potentiates the actions of antidepressants in patients with depression, including those with treatment-resistant depression [10], significantly decreased the duration of immobility, even when given concurrently with ACTH [9]. Chronic treatment of rats with ACTH may therefore be an effective model of tricyclic antidepressant treatment-resistant conditions.

Chronic treatment with antidepressants may increase cell proliferation and granule cell survival and reverse stress-induced decreases in hippocampal cell proliferation and neurogenesis [11, 12]. The ability to promote hippocampal neurogenesis is a feature of both classical antidepressants, such as selective serotonin re-uptake inhibitors, and tricyclic drugs [13-15]. Moreover, hippocampal cell proliferation and neurogenesis might be key factors in the actions of antidepressant drugs.

The nuclear protein Ki-67 is an endogenous marker of cell proliferation, being expressed in all phases of the cell cycle except the resting phase [16, 17]. We hypothesized that chronic ACTH treatment would lead to a decrease in cell proliferation within the dentate gyrus that could be reversed by the coadministration of imipramine and lithium in rats. This study examined the immunolocalization of Ki-67 in the rat dentate gyrus to evaluate the effect of imipramine and lithium on adult hippocampal cell proliferation.

Materials and Methods

Animals. Male Wistar rats (Charles River, Yokohama, Japan) with an initial weight of 220-230 g were utilized. The rats were group-housed, 4 per cage, under a constant light-dark cycle (lights on, 07:00-19:00 hours) and fed standard laboratory food and tap water in an air-conditioned room (23 ± 1℃ 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 the animals used. Four to 6 animals were used for each group.

Drugs. The following drugs were used: imipramine hydrochloride (Sigma), lithium carbonate (Taisho Pharmaceutical, Tokyo, Japan) and ACTH-(1-24)-zinc (Cortrosyn-Z: Daiichi-Sankyo, Tokyo, Japan). Imipramine was dissolved in saline. Lithium was suspended in a 0.5% methylcellulose solution. The rats were administered imipramine and lithium at a dosage of 2 ml/kg of body weight for 14 days. ACTH (Cortrosyn-Z) was injected subcutaneously once daily (09:00 to 10:00 hours) at 100 μg/rat (the injection volume was 0.2 ml/rat) for 14 days. The control rats received an equivalent volume of vehicle (saline 0.2 ml/rat) subcutaneously for the same period of time.

Immunohistochemistry. One day after the last drug administration, rats were transcardially perfused with ice-cold saline followed by a fixative containing 4% paraformaldehyde and 0.35% glutaraldehyde in 0.1 M phosphate buffer (PB, pH 7.4) under deep pentobarbital sodium anesthesia (80 mg/kg, i.p.). After the perfusion, the brain was 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 15% sucrose in 0.1 M PB with sodium azide for ~48 h. 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 Ki-67-immunopositive signals in the dentate gyrus of the hippocampus. For this purpose, an adjacent set of equally spaced brain sections containing the hippocampus was selected from all the animals examined. The sections were soaked in 10 mM PBS containing 0.2% Triton X-100 (PBST) for 30 min at room temperature. After incubation in 1% normal goat serum for Ki-67 in PBST for 30 min, the sections were exposed to a rabbit anti-Ki-67 monoclonal antibody (diluted 1:500 in PBST; Lab Vision, Fremont, CA, USA) for 18 h at 4℃. The sections were then washed in PBST (5 × 5 min) and incubated with a goat anti-rabbit IgG Alexa Fluor 488 antibody (diluted 1:500 in PBST; Invitrogen, Carlsbad, CA, USA) for 2 h at

room temperature. Following washes in PBS (3× 10 min), the sections were stained with 10 μg/ml of Hoechst33258 for 30 sec to visualize cell nuclei. All slides were analyzed under a fluorescence microscope (Olympus BX50-FLA; Olympus, Tokyo, Japan) using a mercury lamp through a 470–490 nm or 360–370 nm band-pass filter to excite the Alexa Fluor 488 or Hoechst dye, respectively. The light emitted from Alexa Fluor 488 or Hoechst was collected through a 515–550 nm band-pass filter or 420 nm long-pass filter, respectively. The stained cells were photographed at a magnification of ×200. The Ki-67-positive cells of the dentate gyrus of the hippocampus were counted manually on two slices per animal using a microscope by an investigator blinded to the experiments.

Statistical analysis. All values are expressed as the group mean ± S.E.M. The group means were assessed using Student's *t*-test, and compared using Tukey's test for multiple comparisons.

Results

Effects of chronic ACTH treatment on the number of Ki-67-positive cells in the dentate gyrus of the hippocampus. Fig. 1 and 2 show the number of Ki-67-positive cells in the dentate gyrus of the hippocampus. At 1 day after the final administration of ACTH (100 μg/rat, s.c., 14 days), the number was significantly decreased compared with the control value ($p < 0.01$).

Effects of chronic treatment with imipramine and lithium for 14 days on the number of Ki-67-positive cells in the dentate gyrus of the hippocampus in saline and ACTH-treated rats. Neither chronic administration of imipramine (10 mg/kg, i.p.) nor chronic administration of lithium (100 mg/kg, p.o.) for 14 days affected the number of Ki-67-positive cells in the dentate gyrus of the hippocampus. However, the coadministration of imipramine (10 mg/kg, i.p.) and lithium (100 mg/kg, p.o.) for 14 days significantly increased the number ($F(3, 39) = 7.821$, $p < 0.01$) (Fig. 3). Treatment with ACTH (100 μg/rat, s.c.) for 14 days significantly decreased the number (Fig. 4). This effect was not influenced by the chronic administration of lithium, but was enhanced by the administration of imipramine, or coadministration of imipramine and lithium for 14 days ($F(4, 43) = 9.702$, $p < 0.01$) (Fig. 4).

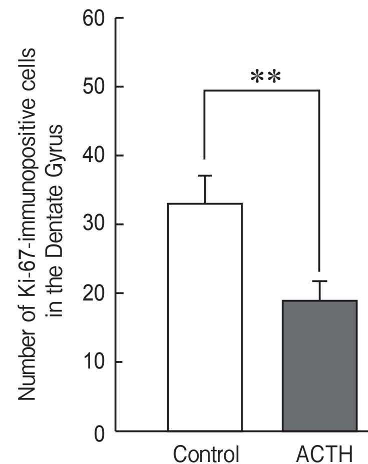

Fig. 1 Effects of chronic ACTH treatment on the number of Ki-67-positive cells in the dentate gyrus of the hippocampus. Rats were administered ACTH (100 μg/rat, s.c.) once daily for a period of 14 days. The number of Ki-67-positive cells in the dentate gyrus of the hippocampus was measured the day after the final ACTH treatment. The rats were killed by decapitation and their brains were removed 1 day after the final administration of ACTH. Values are expressed as the mean ± S.E.M. for 5 animals. Data were analyzed with Student's *t*-test. $^{**}p<0.01$, significantly different from the control value.

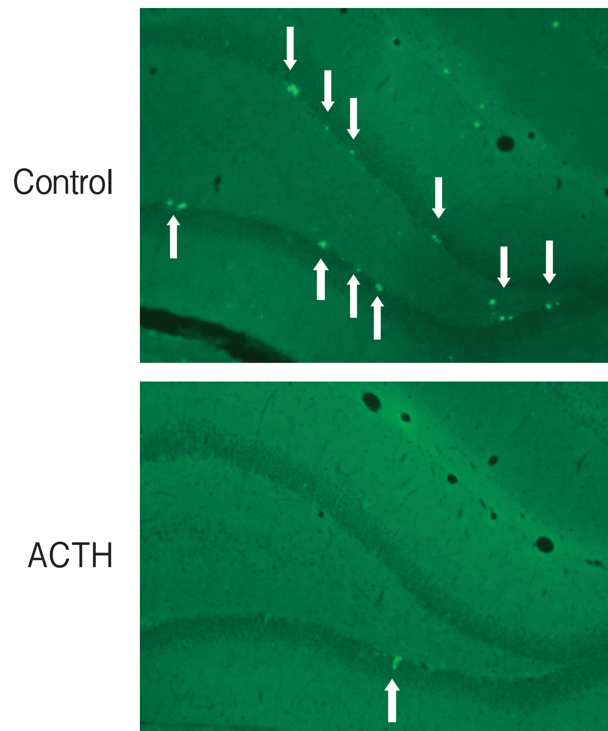

Fig. 2 Representative fluorescence photomicrographs of the effect of chronic ACTH treatment on the number of Ki-67-positive cells in the dentate gyrus of the hippocampus.

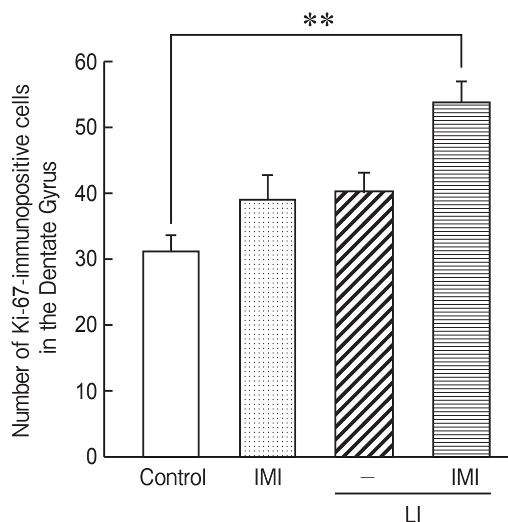

Fig. 3 Effects of chronic treatment with imipramine and lithium for 14 days on the number of Ki-67-positive cells in the dentate gyrus of the hippocampus of saline-treated rats. Rats were administered imipramine (IMI: 10 mg/kg, i.p.) and lithium (LI: 100 mg/kg, p.o.) once daily for a period of 14 days. Values are expressed as the means ± S.E.M. for 5–6 animals. Data were analyzed with one-way ANOVA, followed by Tukey's test. $^{**}p<0.01$, significantly different from the control value.

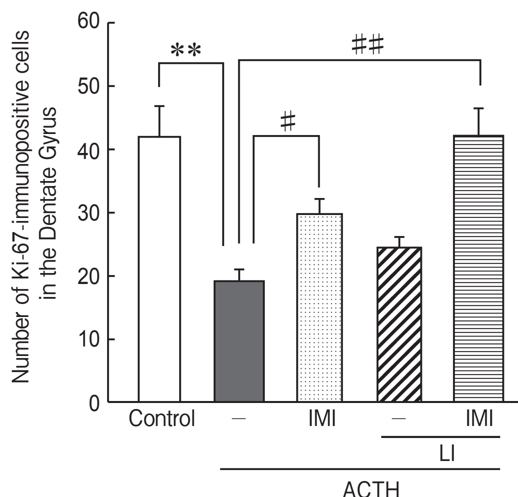

Fig. 4 Effects of chronic treatment with imipramine and lithium for 14 days on the number of Ki-67-positive cells in the dentate gyrus of the hippocampus of ACTH-treated rats. Rats were administered imipramine (IMI: 10 mg/kg, i.p.), lithium (LI: 100 mg/kg, p.o.) and ACTH (100 μg/rat, s.c.) once daily for a period of 14 days. Values are expressed as the means ± S.E.M. for 4–6 animals. Data were analyzed with one-way ANOVA, followed by Tukey's test. $^{**}p<0.01$, significantly different from the control value. $^{\#}p<0.05$, $^{\#\#}p<0.01$, significantly different from the ACTH value.

Discussion

We observed that chronic ACTH treatment reduced the number of Ki-67-positive cells in the dentate gyrus of the hippocampus. We previously found that plasma corticosterone levels in rats following a 14-day chronic ACTH treatment (100 μg/day, s.c.) were significantly higher than those in saline-treated rats (saline: 1.1 ± 0.2 mg/dl; 14 days: 13.9 ± 3.0 mg/dl). We thereby demonstrated that chronic ACTH treatment in rats produced a significant elevation in corticosterone levels compared with nontreated controls, a condition termed hypercorticism. Given the repetitive activation of the HPA axis, it seems probable that glucocorticoids play an important role in the development of aberrant brain function after chronic ACTH treatment, *e.g.*, in the suppression of cell proliferation and neurogenesis. It has been reported that a glucocorticoid receptor antagonist normalized the depressive effects of chronic stress on adult neurogenesis [18]. In the present study, chronic ACTH treatment led to strong activation of the HPA axis, suggesting reductions in adult hippocampal cell proliferation and neurogenesis.

Furthermore, neither imipramine nor lithium alone affected the number of Ki-67-positive cells in the dentate gyrus in saline-treated rats. Several studies have shown an increase in hippocampal cell proliferation and neurogenesis in rodents following chronic treatment with antidepressants such as fluoxetine (a selective serotonin re-uptake inhibitor), imipramine, and desipramine (tricyclic antidepressants). A decrease in cell proliferation and neurogenesis in the dentate gyrus of the hippocampus has been demonstrated in different animal models of depression [11, 12]. It has also been shown that stress-induced changes in the levels of hippocampal cell proliferation and neurogenesis can be reversed by the chronic administration of antidepressants [13–15]. It is difficult to explain why imipramine and lithium did not increase the number of Ki-67-positive cells in the dentate gyrus in saline-treated rats. It has been reported that chronic, but not subacute, treatment with antidepressants increases cellular proliferation in the hippocampus [13]. Furthermore, there are reports, in mice, of strain differences in neurogenesis [19]. Thus, differences in the number of injections, the animal species (mouse or rat), the animal

strain, or the experimental design could be reflected in the experimental results.

The coadministration of imipramine and lithium for 14 days significantly increased the number of Ki-67-positive cells in ACTH-treated rats. In the central nervous system, lithium alters the dynamics of neurotransmission within serotonergic pathways. In biochemical studies, lithium increased 5-HT synthesis in the brain, 5-HT turnover in various brain regions in rats [20], and 5-HT release from nerve endings [21]. These findings suggest that the mechanism of cell proliferation involves a facilitation of central 5-HT neurotransmission by the coadministration of lithium and imipramine. However, the possibility that the noradrenergic system is involved in the effects observed in the present study can not be excluded. Studies are underway to clarify the serotonergic and noradrenergic functions on the coadministration of imipramine and lithium in chronic ACTH-treated rats.

We previously reported that the decrease in immobility induced by single and chronic administration of imipramine was blocked by the chronic administration of ACTH for 14 days [9]. Thus ACTH-treated rats may serve as an animal model of tricyclic antidepressant treatment-resistant depression. In the present study, we confirmed by means of Ki-67 labeling that chronic ACTH treatment suppresses adult cell proliferation, suggesting that the depressed hippocampal cell proliferation is closely related to the pathophysiology of depression or treatment-resistant depression. In addition, we confirmed that hippocampal cell proliferation is required for lithium to augment the effect of imipramine in this rodent model of treatment-resistant depression.

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References

1. Carroll BJ, Curtis GC and Mendels J: Neuroendocrine regulation in depression Ⅱ. Discrimination of depressed from nondepressed patients. Arch Gen Psychiatry (1976) 33: 1051-1058.
2. Carpenter WT and Bunney WE Jr: Adrenal cortical activity in depressive illness. Am J Psychiatry (1971) 128: 31-40.
3. Carroll BJ, Feinberg M, Greden JF, Tarika J, Albala AA, Haskett RF, James NM, Kronfol Z, Lohr N, Steiner M, de Vigne JP and Young E: A specific laboratory test for the diagnosis of melancholia, standardization, validation, and clinical utility. Arch Gen Psychiatry (1981) 38: 15-22.
4. Kelly WF, Checkley SA and Bender DA: Cushing's syndrome, tryptophan and depression. Br J Psychiatry (1980) 136: 125-132.
5. Murphy BEP, Dhar V, Ghadirian AM, Chouinard G and Keller R: Response to steroid suppression in major depression on resistant to antidepressant therapy. J Clin Psychopharmacol (1991) 11: 121-126.
6. Sonino N, Boscaro M, Ambroso G, Merola G and Mantero F: Prolonged treatment of Cushing's disease with metyrapone and aminoglutethimide. IRCS J Med Sci (1986) 14: 485-486.
7. Murphy BEP, Filipini D and Ghadirian AM: Possible use of glucocorticoid receptor antagonists in the treatment of major depression: preliminary results using RU486. J Psychiatr Neurosci (1993) 18: 209-213.
8. Wolkowitz OM, Reus VI, Manfred F, Ingbar J, Brizendine L and Weingartner H: Ketoconazole administration in hypercortisolemic depression. Am J Psychiatry (1993) 150: 810-812.
9. Kitamura Y, Araki H and Gomita Y: Influcence of ACTH on the effects of imipramine, desipramine and lithium on duration of immobility of rats in the forced swim test. Pharmacol Biochem Behav (2002) 71: 63-69.
10. De Montigny C, Gruberg F and Deschenes JD: Lithium induces rapid relief of depression in tricyclic antidepressant drug non-responders. Br J Psychiatry (1981) 138: 252-256.
11. Fuchs E, Czeh B and Flugge G: Examining novel concepts of the pathophysiology of depression in the chronic psychosocial stress paradigm in tree shrews. Behav Pharmacol (2004) 15: 315-325.
12. Malberg JE and Duman RS: Cell proliferation in adult hippocampus is decreased by inescapable stress: reversal by fluoxetine treatment. Neuropsychopharmacology (2003) 28: 1562-1571.
13. Santarelli L, Saxe M, Gross C, Surget A, Battaglia F, Dulawa S, Weisstaub N, Lee J, Duman R, Arancio O, Belzung C and Hen R: Requirement of hippocampal neurogenesis for the behavioral effects of antidepressants. Science (2003) 301: 805-809.
14. Kodama M, Fujioka T and Duman RS: Chronic olanzapine or fluoxetine administration increases cell proliferation in hippocampus and prefrontal cortex of adult rat. Biol Psychiatry (2004) 56: 570-580.
15. Malberg JE, Eisch AJ, Nestler EJ and Duman RS: Chronic antidepressant treatment increases neurogenesis in adult rat hippocampus. J Neurosci (2000) 20: 9104-9110.
16. Kee N, Sivalingam S, Boonstra R and Wojtowicz JM: The utility of Ki-67 and BrdU as proliferative markers of adult neurogenesis. J Meurosci Methods (2002) 115: 97-105.
17. Taupin P: BrdU immunohistochemistry for studying adult neurogenesis: paradigms, pitfalls, limitations, and validation. Brain Res Rev (2007) 53: 198-214.
18. Oomen CA, Mayer JL, de Kloet ER, Joëls M and Lucassen PJ: Brief treatment with the glucocorticoid receptor antagonist mifepristone normalizes the reduction in neurogenesis after chronic stress. Eur J Neurosci (2007) 26: 3395-3401.
19. Schauwecker PE: Genetic influence on neurogenesis in the dentate gyrus of two strains of adult mice. Brain Res (2006) 1120: 83-92.
20. Ghoshdastidar D and Poddar MK: Long term effect of lithium on brain regional catecholamine metabolism. Indian J Exp Biol (1990) 28: 444-450.
21. Hotta I and Yamawaki S: Possible involvement of presynaptic 5-HT autoreceptors in effect of lithium on 5-HT release in hippocampus of rat. Neuropharmacology (1988) 27: 987-992.