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Effects of imipramine on extracellular serotonin and noradrenaline concentrations in ACTH-treated rats

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Abstract

We investigated the effect of imipramine on extracellular serotonin (5-HT) and noradrenaline concentrations in the medial prefrontal cortex of rats treated with adrenocorticotropic hormone (ACTH) for 14 days using in vivo microdialysis. Chronic ACTH treatment did not affect basal extracellular 5-HT and noradrenaline concentrations compared with chronic saline treatment. Acute imipramine treatment plus chronic ACTH treatment significantly increased extracellular 5-HT concentrations, compared with imipramine treatment alone. 8-hydroxy-2-di-*n*-propylamino tetralin (8-OH-DPAT), a 5-HT_{1A} receptors full agonist, caused a significant decrease in extracellular 5-HT concentrations. However, its inhibitory effect was attenuated by the treatment with ACTH for 14 days. These findings suggest that chronic treatment with ACTH enhances the increasing effect release of 5-HT by imipramine through the desensitization of somatodendritic 5-HT_{1A} autoreceptors.

Keywords: Imipramine; 8-hydroxy-2-di-*n*-propylamino tetralin; 5-HT_{1A} receptor; Microdialysis; Adrenocorticotropic hormone

1. Introduction

Imipramine, a traditional tricyclic antidepressant, is useful for depression. The pharmacological mechanism of imipramine's effect is inhibition of the serotonin (5-HT) and noradrenaline uptake carriers. In vivo microdialysis studies have shown that imipramine increases the output from 5-HT and noradrenaline synapses in the medial prefrontal cortex (Mochizuki et al., 2002a). Increased concentrations of 5-HT and noradrenaline in the synaptic cleft can lead to an increase in the transmission of serotonergic and noradrenergic signals. This ability of imipramine to facilitate serotonergic and noradrenergic neurotransmission may contribute to its antidepressant properties. Animals treated with imipramine show antidepressant-like effects in the forced swim test, widely used as a predictor of antidepressant activity. We have already found that the effect on immobility time of imipramine (10 mg/kg, i.p.) was blocked by chronic treatment with adrenocorticotropic hormone (ACTH: 100 μg/rat, s.c., 14 days) in the rat forced swim test (Kitamura et al., 2002). It is conceivable that the inhibition by chronic ACTH treatment of the immobility-decreasing effect of imipramine is associated with the change in extracellular 5-HT and noradrenaline concentrations in the central nervous system. However, the effects of imipramine on extracellular 5-HT and noradrenaline levels under conditions where the hypothalamic–pituitary–adrenal axis is functioning abnormally are not clearly understood.

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Particular attention has focused on the somatodendritic 5-HT_{1A} autoreceptor as a possible site for the control of the activity of ascending serotonergic neurons (Sprouse and Aghajanian, 1987; Jolas et al., 1993). The activation of somatodendritic 5-HT_{1A} autoreceptors inhibits the firing-dependent release of 5-HT in projection areas (Hamon et al., 1988; Sharp et al., 1989). Direct and indirect agonism of the somatodendritic 5-HT_{1A} autoreceptors appears to induce desensitization after chronic treatment with antidepressants, as measured using electrophysiological and neurochemical indexes. This mechanism is thought to be involved in the enhancement by antidepressant drugs of extracellular 5-HT concentrations in some forebrain areas (Bel and Artigas, 1993; Mochizuki et al., 2002b). Namely, these findings suggest that the functions of somatodendritic 5-HT_{1A} autoreceptors play a key role in determining extracellular 5-HT concentrations and the antidepressive effect. However, the sensitivity of these autoreceptors when the hypothalamic–pituitary–adrenal axis is affected remains unclear.

In the present study, we investigate the effect of imipramine on extracellular 5-HT and noradrenaline concentrations in the medial prefrontal cortex of ACTH-treated rats using an in vivo microdialysis method. In addition, we studied whether chronic treatment with ACTH modifies the function of somatodendritic 5-HT_{1A} autoreceptors, by examining the effect of 8-OH-DPAT, a 5-HT_{1A} receptor full agonist, on extracellular 5-HT concentrations in the medial prefrontal cortex.

2. Materials and methods

2.1. Animals

Male Wistar rats (Charles River, Japan) with an initial weight of 180–230 g were utilized in this study. The rats were kept under a constant light–dark cycle (lights on, 07:00–19:00 h), with access to 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.

2.2. Drugs

The following drugs were used in this study: imipramine hydrochloride (Wako, Osaka, Japan), (±)-8-hydroxy-2-di-*n*-propylamino tetralin hydrobromide (8-OH-DPAT: Research Biochemicals Inc. South Natick, MA), and ACTH-(1-24)-zinc (Cortrosyn-Z: Daiichi Seiyaku, Tokyo, Japan). Imipramine and 8-OH-DPAT were dissolved in saline. Rats were injected with imipramine (10 mg/kg, i.p.), 8-OH-DPAT (0.03 mg/kg, s.c.), and saline at 2 ml/kg body weight. Rats were administered ACTH (100 μg/rat, s.c. (injection volume was 0.2 ml/rat): Cortrosyn-Z) or saline (0.2 ml/rat, s.c.) once daily (9:00 to 10:00) for 14 days.

2.3. Microdialysis procedures

Rats were implanted stereotaxically under anesthesia with pentobarbital (30 mg/kg, i.p.) with an AG-4 guide cannula (Eicom Co., Kyoto, Japan) leading to the surface of the medial prefrontal cortex at the following coordinates relative to the bregma: A+3.2,ML+0.8, DV+1.0 mm. Dialysis probes were implanted at the same time as the guide cannula so that 3.0 mm of probe was exposed to the tissue of the medial prefrontal cortex. Rats were housed individually after these operations. The separate groups of rats were used for each experiment once only. Experiments were performed in freely moving rats. On the following day, 20 h after surgery, perfusion was started using artificial cerebrospinal fluid (145 mM NaCl, 3.0 mM KCl, 1.3 mM $CaCl_2$, 1.0 mM $MgCl_2$) at a flow rate of 2 μl/min. Dialysate samples were collected in sample vials containing 60 μl of 0.05 M acetic acid. Fifty microliters of dialysate was injected into the HPLC system to determine the extracellular levels of 5-HT and noradrenaline. The average of the first three baseline samples was taken as 100%.

2.4. Analytical procedures

The high-performance liquid chromatography (HPLC) system consisted of an EP-300 liquid chromatograph pump (EP-300: Eicom, Kyoto, Japan), a DGU-4A degasser (DG-300: Eicom, Kyoto, Japan), a reversed phase ODS column, Eicompak SC-5ODS ö2.1 mm×150 mm (Eicom, Kyoto, Japan), and an ECD-300 electrochemical detector (+750 mV against Ag/AgCl reference electrode: Eicom, Kyoto, Japan). The mobile phase was an acetic-citrate buffer (pH 3.5), 190 mg/l sodium 1-octanesulfonate, 5 mg/l EDTA, and 16% methanol, degassed and pumped at a flow rate of 0.25 ml/min. The peaks were recorded using a Powerchrom integrator (Eicom, Kyoto, Japan).

A standard solution containing authentic 5-HT and noradrenaline was injected every working day, and the amounts of 5-HT and noradrenaline were determined by comparison with the peak height of the standard. The detection limit was 0.1–0.2 pg. At the end of the experiment, the brains were examined histologically for correct probe placement. Only data from rats in which the microdialysis probes were correctly located were used in the calculation of the results.

2.5. Measurement of plasma corticosterone

Twenty four hours after administration of the last dose of ACTH or saline, the plasma corticosterone levels ware measured. Plasma corticosterone levels were measured using a commercially available radio-immunoassay kit (Rat Corticosterone [^{125}I] RIA System, Amersham Biosciences Corp. USA).

2.6. Data analysis

All the data are given as the mean±S.E.M. of individual values of the rats from each group. The 5-HT and noradrenaline concentrations of dialysate samples were expressed as absolute values (pg/30 min dialysate sample). The average of the absolute values in three consecutive samples before the administration of a drug or saline was determined as the basal concentration (100%). Mean percentages were calculated from each basal value for each 30-min sample across the rats in all

groups. Statistical comparisons between the two groups at each time point were performed using one-way analysis of variance (ANOVA) followed by an unpaired *t*-test (two-tailed). Probability values of less than 0.05 were considered to show a significant difference.

3. Results

3.1. Effects of acute administration of imipramine on extracellular 5-HT and noradrenaline concentrations in the medial prefrontal cortex after ACTH treatment for 14 days.

Fig. 1 illustrates the time-course of the effect of the acute administration of imipramine (10 mg/kg, i.p.) on extracellular 5-HT and noradrenaline concentrations in the medial prefrontal cortex of saline-treated rats and ACTH-treated rats. The mean baseline levels of 5-HT and noradrenaline were not affected by chronic treatment with ACTH at 100 μg/rat for 14 days (5-HT: saline group 1.12±0.30 pg/60 μl, ACTH group 0.93±0.39 pg/60 μl; noradrenaline: saline group 0.73±0.10 pg/60 μl, ACTH group 0.75±0.08 pg/60 μl). With regard to 5-HT concentrations, the response to imipramine was significantly greater in the ACTH-treated rats than saline-treated controls at 30 min after imipramine's administration [$F(1,18)=5.77$, $P<0.05$]. Imipramine also raised extracellular noradrenaline levels, however the magnitude of the increase was not greater in the ACTH-treated rats.

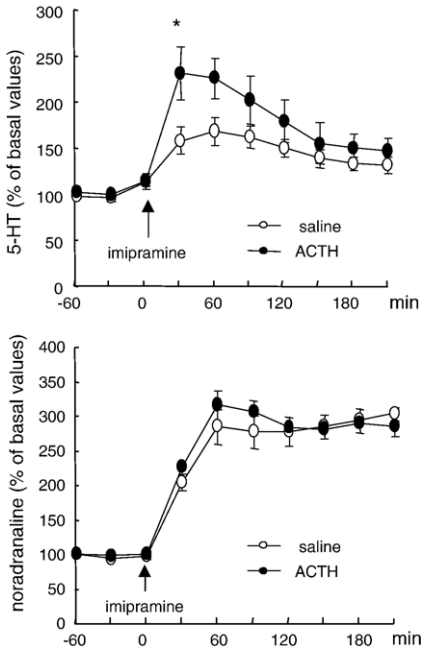

Fig. 1. Effects of chronic ACTH treatment on the imipramine-induced increases in extracellular 5-HT and noradrenaline concentrations in the medial prefrontal cortex. The animals were given saline (open circles) or ACTH (100 μg/rat, s.c., black circles) for 14 days. They were given imipramine (10 mg/kg, i.p.) at 0 min. The results are expressed as percentages of the mean of three measurements taken before drug administration. Each point represents the mean±S.E.M for ten rats. Data were analyzed with the unpaired *t*-test. *$P<0.05$, significantly different from the saline group.

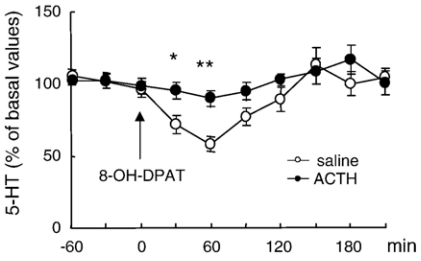

Fig. 2. Effects of chronic ACTH treatment on the 8-OH-DPAT-induced decreases in extracellular 5-HT concentrations in the medial prefrontal cortex. The animals were given saline (open circles) or ACTH (100 μg/rat, s.c., black circles) for 14 days. They were given 8-OH-DPAT (0.03 mg/kg, s.c.) at 0 min. The results are expressed as percentages of the mean of three measurements taken before drug administration. Each point represents the mean±S.E.M. for ten rats. Data were analyzed with the unpaired *t*-test. *$P<0.05$, **$P<0.01$, significantly different from the saline group.

3.2. Effects of acute administration of 8-OH-DPAT on extracellular 5-HT concentrations in the medial prefrontal cortex after ACTH treatment for 14 days.

Fig. 2 illustrates the time-course of the effect of acute administration of 8-OH-DPAT at 0.03 mg/kg (s.c.) on extracellular 5-HT concentrations in the medial prefrontal cortex of saline-treated rats and ACTH-treated rats. 8-OH-DPAT produced a decrease in extracellular 5-HT concentrations in the medial prefrontal cortex. In the rats given ACTH for 14 days, the effect of the treatment with 8-OH-DPAT was attenuated at 30 and 60 min (30 min: $F(1, 18)=7.91$, $P<0.05$; 60 min: $F(1, 18)=16.53$, $P<0.01$).

3.3. Effects of chronic treatment of ACTH for 14 days on plasma corticosterone levels in rats.

Chronic ACTH treatment significantly increased the plasma corticosterone levels (saline group: 1.1±0.2 μg/dl, ACTH group: 13.9±0.2 μg/dl, $P<0.01$).

4. Discussion

We previously reported that the chronic administration of ACTH (100 μg/rat, s.c.) counteracts the decrease in immobility time induced by acute treatment with imipramine (10 mg/kg, i.p.) in rats (Kitamura et al., 2002). The precise mechanism of this counteraction is unclear. One would expect ACTH or ACTH-stimulated corticosterone to influence the immobility-reducing effect of imipramine. It is well established that corticosterone acts on neurotransmission. In support of this, we observed that chronic ACTH (100 μg/rat, s.c., 14 days) treatment in rats produced a significant rise in corticosterone levels, a condition termed hypercorticism in this study. Imipramine acts upon 5-HT uptake carriers, influencing 5-HT neuronal activity. The treatment of rats with corticosterone led to a significant decrease in the binding of cortical and hippocampal 5-HT uptake carriers (Arora and Meltzer, 1986). Accordingly, it is possible to inhibit the release of 5-HT and noradrenaline in chronic ACTH-treated rats.

We observed the influence of a single administration of imipramine (10 mg/kg, i.p.) on extracellular 5-HT and noradrenaline concentrations in the medial prefrontal cortex of rats after 14 days of chronic ACTH treatment (100 μg/rat, s.c.). Acute administration of imipramine only at 10 mg/kg (i.p.) increased extracellular 5-HT and noradrenaline concentrations in the medial prefrontal cortex. This is consistent with previous reports (Mochizuki et al., 2002a; Ferraro et al., 2005). However, imipramine further elevated extracellular levels of 5-HT in the rats treated with ACTH at 100 μg/rat (s.c.) for 14 days in the present study. On the other hand, the increase in noradrenaline concentrations was not affected by imipramine in the rats chronically treated with ACTH. Accordingly, the increase in 5-HT and noradrenaline concentrations induced by imipramine was not blocked by chronic ACTH treatment. In addition, only one dose (10 mg/kg) of imipramine was tested in this study. It is possible to cause a leftward shift in the dose–response curve or increase in efficiency for imipramine to promote the release of 5-HT by its action at the 5-HT transporters. Further work is in progress to study the dose–response relationship for imipramine to increase 5-HT and noradrenaline concentrations in the medial prefrontal cortex in chronic ACTH-treated rats.

Somatodendritic 5-HT_{1A} autoreceptors, which are located on the cell bodies and dendrites of 5-HT neurons in the raphe nucleus, exert negative feedback control of cell firing (Sprouse and Aghajanian, 1987; Jolas et al., 1993). This results in a decrease in the turnover (Hamon et al., 1988) and release (Sharp et al., 1989) of 5-HT in all areas of the brain to which these neurons project. The excess 5-HT in the extracellular space of the raphe nucleus activates somatodendritic 5-HT_{1A} autoreceptors of serotonergic neurons, and reduces the neuronal activity and release of 5-HT by nerve terminals in the forebrain (for a review, see Artigas et al., 1996). Accordingly, it is important to recognize that the release of endogenous 5-HT in the nerve terminals of the forebrain is a function of somatodendritic 5-HT_{1A} autoreceptors in the raphe nucleus. In the present study, we investigated the influence of 8-OH-DPAT, a 5-HT_{1A} receptors full agonist, on extracellular 5-HT concentrations in the medial prefrontal cortex of ACTH-treated rats. The effect of 8-OH-DPAT was significantly attenuated by the chronic treatment with ACTH for 14 days. It was reported that corticosterone induced a functional desensitization of somatodendritic 5-HT_{1A} autoreceptors in the dorsal raphe nucleus (Laaris et al., 1995). Namely, it seems that chronic treatment with ACTH produced a desensitization of somatodendritic 5-HT_{1A} autoreceptors after 14 days. In the present study, it is reasonable to assume that the effect of imipramine on extracellular 5-HT concentrations in chronic ACTH-treated rats is due to the desensitization of somatodendritic 5-HT_{1A} autoreceptors. It is worth noting that the ability of 5-HT to inhibit the firing of serotonergic neurons was reduced by the chronic administration of antidepressants (Mochizuki et al., 2002b). Namely, the functional desensitization of somatodendritic 5-HT_{1A} autoreceptors has been observed following chronic treatment with ACTH as well as antidepressants. Further study of the relationship between the desensitization of somatodendritic 5-HT_{1A} autoreceptors and antidepressive effects should be made using chronic ACTH-treated rats.

In summary, the acute administration of imipramine clearly increased extracellular 5-HT concentrations in the medial prefrontal cortex of ACTH-treated rats. This effect seemed to involve the desensitization of somatodendritic 5-HT_{1A} autoreceptors caused by chronic treatment with ACTH.

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