The 5-HT$_{1A}$ Receptor Full Agonist, 8-OH-DPAT Inhibits ACTH-Induced 5-HT$_{2A}$ Receptor Hyperfunction in Rats: Involvement of 5-HT$_{1A}$ Receptors in the DOI-Induced Wet-Dog Shakes in ACTH-Treated Rats

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Received August 29, 2006; accepted October 25, 2006; published online October 31, 2006

We examined the influence of 8-hydroxy-2-di-*n*-propylamino tetralin (8-OH-DPAT), a serotonin 1A (5-HT$_{1A}$) receptor full agonist, on the wet-dog shake response induced by the (±)-1-(2,5-dimethoxy-4-iodophenyl)-2-aminopropane (DOI), a 5-HT$_{2A}$ receptor agonist, in adrenocorticotropic hormone (ACTH)-treated rats. Chronic ACTH (100 μg/rat, s.c.) treatment for 14 d increased the wet-dog shake response induced DOI. The 8-OH-DPAT inhibited the wet-dog shake response induced by DOI in rats with ACTH for 14 d. On the other hand, the 8-OH-DPAT-induced hypothermia and flat body posture were inhibited when ACTH was administered for 14 d. These findings suggest that chronic treatment with ACTH decreased the sensitivity of the 5-HT$_{1A}$ receptor system; however, the inhibitory effects from the 5-HT$_{1A}$ receptors to the 5-HT$_{2A}$ receptor system is not inhibited in ACTH-treated rats.

Key words 5-HT$_{2A}$ receptor; wet-dog shake; 5-HT$_{1A}$ receptor; (±)-1-(2,5-dimethoxy-4-iodophenyl)-2-aminopropane (DOI); 8-hydroxy-2-di-*n*-propylamino tetralin (8-OH-DPAT)

Adrenal steroids regulate diverse processes and systems in the brain. Several studies have indicated that the activation of the hypothalamic-pituitary-adrenal axis changes the serotonin (5-hydroxytryptamine, 5-HT) function in the brain. The 5-HT receptor subtypes, particularly 5-HT$_{2A}$ and 5-HT$_{1A}$ receptors, have been postulated to play an important role in the pathogenesis of depression.[1,2] Some clinical studies have demonstrated increases in the number of 5-HT$_{2A}$ receptor-binding sites in the postmortem brains of both suicides and depressed subjects.[3—5] Our previous studies have already reported that chronic adrenocorticotropic hormone (ACTH) treatment increases the wet-dog shake response induced by (±)-1-(2,5-dimethoxy-4-iodophenyl)-2-aminopropane (DOI), 5-HT$_{2A}$ receptor agonist.[6] Thus, we suggested that chronic ACTH-treated rats could induce the hyperactivity of the 5-HT$_{2A}$ receptor *via* the activation of the hypothalamic-pituitary-adrenal axis.

On the other hand, numerous investigations have demonstrated interactions between the brain 5-HT$_{1A}$ receptor function and the hypothalamic-pituitary-adrenal axis. It was reported that chronic treatment with corticosterone decreased 5-HT$_{1A}$ receptor binding and the levels of 5-HT$_{1A}$ receptor mRNA in the hippocampus.[7,8] We demonstrated that hypercorticism, which raises circulating corticosterone levels, decreased the 5-HT$_{1A}$ receptor binding in hippocampal membranes.[9] Thus, the enhancement of the hypothalamic-pituitary-adrenal axis produced dysfunction of the 5-HT$_{1A}$ receptor mechanism.

Furthermore, several studies have suggested that there are functional interactions from 5-HT$_{2A}$ receptor function to 5-HT$_{1A}$ receptor function. It has been reported that 8-hydroxy-2-di-*n*-propylamino tetralin (8-OH-DPAT), 5-HT$_{1A}$ receptor full agonist, inhibited DOI-induced head twitch behavior in naive rats.[10—13] These results supported the hypothesis that 5-HT$_{1A}$ receptors exert an inhibitory control over the activation of 5-HT$_{2A}$ receptors. Such interactions may play an important role in the mechanism of action of antidepressant drugs.[14—16] As previous studies have been used for normal rats, the model of the hypothalamic-pituitary-adrenal axis activation is interesting from the viewpoint of the mechanism of interaction between 5-HT$_{2A}$ and 5-HT$_{1A}$ receptor function, or of the action of the 5-HT$_{1A}$ receptor agonist.

In the present study, we examined the influence of 8-OH-DPAT on the effect of chronic ACTH treatment increasing the DOI-induced wet-dog shakes, and also examined 8-OH-DPAT-induced hypothermia and flat body posture, as an index reflective of 5-HT$_{1A}$ receptor function.

MATERIALS AND METHODS

Animals Male Wistar rats (Charles River, Japan) with an initial weight of 180—230 g were utilized in this study. Rats were kept on a constant light–dark cycle (light 07:00—19:00 h), with standard laboratory food and tap water in an air-conditioned room (23±1 °C with approximately 60% humidity).

Drugs The following drugs were used in this study: (±)-1-(2,5-dimethoxy-4-iodophenyl)-2-aminopropane (DOI: Research Biochemicals Inc., South Natick, MA, U.S.A.), (±)-8-hydroxy-2-di-*n*-propylamino tetralin hydrobromide (8-OH-DPAT: Research Biochemicals Inc., South Natick, MA, U.S.A.), ketanserin tartrate (Sigma-Aldrich Co., St. Louis, MO, U.S.A.) and ACTH-(1-24)-zinc (Cortrosyn-Z: Daiichi Seiyaku, Tokyo, Japan). 8-OH-DPAT, DOI and ketanserin were dissolved in saline. Rats were injected with 8-OH-DPAT, DOI and ketanserin 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

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0.2 ml/rat, s.c.) for 1—14 d. Control rats received an equivalent vehicle volume saline 0.2 ml/rat (s.c.) for the same treatment duration.

Experimental Procedures Measurement of 5-HT_{2A} Receptor-Mediated Behavioral Response: The DOI-induced wet-dog shake response was induced 24 h after the administration of the last dose of ACTH. Two animals were put into clear plastic cages (22×38×18 cm) at an ambient temperature of 23±1 °C for at least 2 h before drug administration. The animals were treated with DOI (1 mg/kg, s.c.) and returned to their cages. Immediately after injection, the number of wet-dog shakes was recorded over a 30-min period, as reported previously.[17]

Measurement of 5-HT_{1A} Receptor-Mediated Behavioral Response: 8-OH-DPAT-induced hypothermia and flat body posture were observed in the same rats 24 h after the administration of the last dose of ACTH. Adaptation to the environment was likewise the measurement of the 5-HT_{2A} receptor-mediated behavioral response. Their body temperature was measured with a thermision probe (connected to an electronic thermometer) inserted 2 cm into the rectum. Their temperature was measured immediately before drug administration. The animals were then treated with 8-OH-DPAT and returned to their cages. The hypothermic response to 8-OH-DPAT was calculated from the decrease in body temperature. Flat body posture observation periods of 1 min were initiated 5 min after the drug injection, and this observation was repeated every 5 min over a period of 30 min. Flat body posture was scored, using the ranked intensity scale (0=absent, 1=equivocal, 2=present, 3=marked) described by Tricklebank *et al.*[18] Scores were summed for 6 observation periods. Their body temperature was measured again 30 min following 8-OH-DPAT administration.

Experiments Experiment 1. The Effect of Ketanserin on the DOI-Induced Wet-Dog Shake Response in Rats: DOI at doses of 0.3—3 mg/kg (s.c.) and saline was administered to rats. Ketanserin, the 5-HT_{2A} receptor antagonist (0.03—0.3 mg/kg, s.c.) was administered 15 min before DOI (1 mg/kg, s.c.) treatment.

Experiment 2. The Effects of ACTH on the DOI-Induced Wet-Dog Shake Response in Rats: Rats were administered ACTH (100 μg/rat, s.c.) once daily for a period of 1—14 d. The DOI (1 mg/kg, s.c.)-induced wet-dog shake responses were measured 24 h after the final administration of ACTH.

Experiment 3. The Effect of ACTH on the Inhibitory Effect of 8-OH-DPAT on the DOI-Induced Wet-Dog Shake Response in Rats: Rats were administered ACTH (100 μg/rat, s.c.) once daily for a period of 14 d. We measured the DOI-induced wet-dog shake response 24 h after the final administration of ACTH. 8-OH-DPAT (0.03—0.3 mg/kg, s.c.) was administered 15 min before the administration of DOI (1 mg/kg, s.c.).

Experiment 4. The Effects of ACTH on 8-OH-DPAT-Induced Hypothermia and Flat Body Posture in Rats: Rats were administered ACTH (100 μg/rat, s.c.) once daily for a period of 1—14 d. The 8-OH-DPAT (0.3 mg/kg, s.c.)-induced hypothermia and flat body posture were measured 24 h after the final administration of ACTH.

Statistics Values are expressed as the means±S.E.M. The data were analyzed by one-way analysis of variance (ANOVA), and the group means were compared using Student's *t*-test or Dunnett's test for multiple comparisons. Flat body posture was analyzed with Wilcoxon rank sum test. Probability values less than 0.05 were considered to show a significant difference.

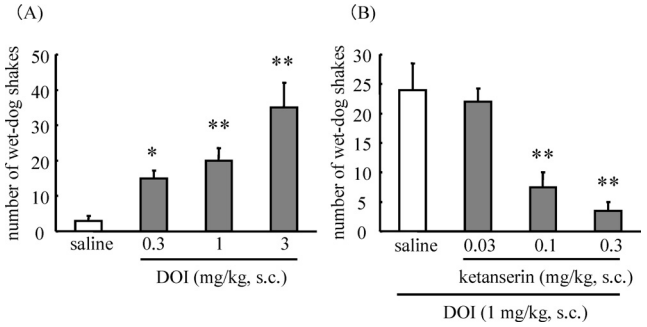

Fig. 1. Wet-Dog Shake Response Induced by DOI in Rats

(A) Dose-response for DOI on the wet-dog shake response in rats. (B) The effect of ketanserin on the DOI-induced wet-dog shake response in rats. Ketanserin was administered 15 min before DOI administration (1 mg/kg, s.c.). Values are expressed as the means±S.E.M. of 6 animals. Data were analyzed by one-way ANOVA, followed by Dunnett's test. * $p<0.05$, ** $p<0.01$, significantly different from saline.

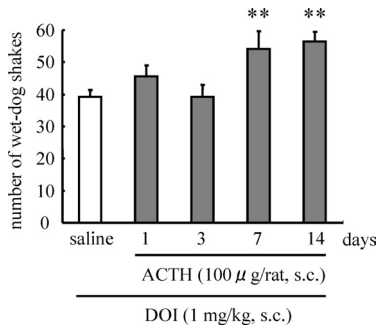

Fig. 2. Effects of ACTH Treatment Period on DOI-Induced Wet-Dog Shake Response in Rats

ACTH (100 μg/rat, s.c.) was administered to rats once daily for 14 d. DOI-induced wet-dog shakes were measured the day following the final treatment with ACTH. Values are expressed as the means±S.E.M. of 8 animals. Data were analyzed by one-way ANOVA, followed by Dunnett's test. ** $p<0.01$, significantly different from saline.

RESULTS

Experiment 1. The Effect of Ketanserin on the DOI-Induced Wet-Dog Shake Response in Rats: DOI (0.3—3 mg/kg, s.c.) produced a dose-dependent increase in wet-dog shakes ($F_{(3,20)}=8.27$, $p<0.01$) (Fig. 1A). Ketanserin (0.03—0.3 mg/kg, s.c.) decreased the wet-dog shakes induced by DOI (1 mg/kg, s.c.) in a dose-dependent manner ($F_{(3,20)}=8.57$, $p<0.01$) (Fig. 1B).

Experiment 2. The Effects of ACTH on the DOI-Induced Wet-Dog Shake Response in Rats: Chronic treatment with ACTH (100 μg/rat, s.c.) for 1—14 d chronologically increased the DOI (1 mg/kg, s.c.)-induced wet-dog shake response ($F_{(4,35)}=4.66$, $p<0.01$) (Fig. 2).

Experiment 3. The Effect of ACTH on the Inhibitory Effect of 8-OH-DPAT on the DOI-Induced Wet-Dog Shake Response in Rats: 8-OH-DPAT significantly inhibited the DOI (1 mg/kg, s.c.)-induced wet-dog shake response in naive rats ($F_{(3,28)}=12.80$, $p<0.01$) (Fig. 3). On the other hand, the inhibitory effect of 8-OH-DPAT on the DOI-induced wet-dog shake response was likewise observed in rats treated with

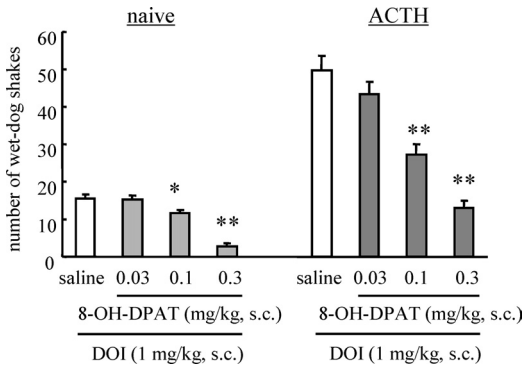

Fig. 3. 8-OH-DPAT Prevention of DOI-Induced Wet-Dog Shakes in Naive or ACTH-Treated Rats

ACTH (100 μg/rat, s.c.) was administered to rats once daily for 14 d. DOI-induced wet-dog shakes were measured the day following the final treatment with ACTH. 8-OH-DPAT (0.03—0.3 mg/kg, s.c.) was administered 15 min before DOI (1 mg/kg, s.c.) administration. Values are expressed as the means±S.E.M. of 6—8 animals. Data were analyzed by one-way ANOVA, followed by Dunnett's test. * $p<0.05$, ** $p<0.01$, significantly different from saline.

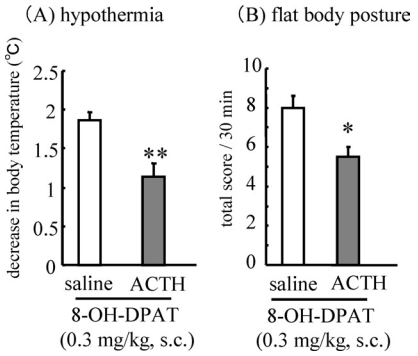

Fig. 4. Effects of ACTH on 8-OH-DPAT-Induced Hypothermia and Flat Body Posture in Rats

ACTH was administered to rats once daily for 14 d. 8-OH-DPAT (0.3 mg/kg, s.c.)-induced hypothermia was measured the day following the final ACTH treatment (100 μg/rat, s.c.). Values are expressed as the means±S.E.M. of 6 animals. Data were analyzed by Student's *t*-test (hypothermia) and Wilcoxon rank sum test (flat body posture). * $p<0.05$, ** $p<0.01$, significantly different from saline.

ACTH (100 μg/rat, s.c.) for 14 d ($F_{(3,20)}=12.20$, $p<0.01$) (Fig. 3).

Experiment 4. The Effects of ACTH on 8-OH-DPAT-Induced Hypothermia and Flat Body Posture in Rats: The chronic treatment of ACTH (100 μg/rat, s.c.) for 14 d significantly decreased the 8-OH-DPAT (0.3 mg/kg, s.c.)-induced hypothermia and flat body posture (hypothermia : $p<0.01$; flat body posture : $p<0.05$) (Fig. 4).

Discussion In this study, we demonstrated that chronic treatment with ACTH produced changes in brain serotonergic neural function, affecting the 5-HT_{2A} and 5-HT_{1A} receptor function, and the behavioral interaction of 5-HT_{2A} and 5-HT_{1A} receptor function. The one major finding was that the inhibitory effect of 8-OH-DPAT on the DOI-induced wet-dog shake response was observed by the chronic administration of ACTH for 14 d. Numerous investigations have demonstrated a possible interaction between 5-HT_{2A} and 5-HT_{1A} receptor function in naive rats. It had been reported that the DOI-induced wet-dog shake response and head twitch response were inhibited by the administration of 8-OH-DPAT.[10—13] It is reasonable to suggest that activation of the 5-HT_{1A} receptor function exerts an inhibitory effect on the 5-HT_{2A} receptor function *via* the 5-HT_{1A} receptor function.

We observed that plasma corticosterone levels in rats following a 14-d chronic ACTH treatment (100 μg/d, s.c.) were significantly higher than those in saline treatment rats (saline 1.1±0.2 μg/dl; ACTH 13.9±3.0 μg/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. Furthermore, chronic ACTH treatment, a treatment that up-regulates the hypothalamic-pituitary-adrenal axis, inhibited the 8-OH-DPAT-induced hypothermia and flat body posture in the present study. Numerous investigations have demonstrated a possible interaction between the 5-HT_{1A} receptor and the hypothalamic-pituitary-adrenal axis. We previously reported that binding of [^{3}H]-8-OH-DPAT to the 5-HT_{1A} receptor in the hippocampus decreased 24 h after both the acute and chronic (14 d) administration of corticosterone (50 mg/kg, s.c.).[9] An autoradiographic study showed that chronic exposure to high levels of corticosterone decreased the binding at 5-HT_{1A} receptors in the dentate gyrus and CA4 hippocampus region in rats.[7] Additionally, chronic but not acute treatment with corticosterone decreased 5-HT_{1A} receptor binding in rat CA1 hippocampus region (in the ventral part only) and the dentate gyrus.[8] Regarding 5-HT_{1A} receptor binding, the general consensus is that the chronically elevated corticosterone down-regulates 5-HT_{1A} receptor binding in the hippocampus. In the present study, the inhibitory effect of 8-OH-DPAT on the DOI-induced wet-dog shake response was not inhibited by the chronic administration of ACTH. With respect to the inhibitory effect of 5-HT_{1A} receptors on 5-HT_{2A} receptors, we assumed that the 5-HT_{1A} receptor-mediated action was not inhibited by the enhancement of the hypothalamic-pituitary-adrenal axis. Namely, these studies thus suggest that 5-HT_{1A} receptor function is not always suppressed on the activation of the hypothalamic-pituitary-adrenal axis.

In this study, the chronic treatment of ACTH inhibited the 5-HT_{1A} receptor function. However, the inhibitory effect of the 5-HT_{2A} receptor function due to the 5-HT_{1A} receptor stimulation was not inhibited by ACTH. These findings suggest that the inhibitory effect of the 5-HT_{2A} receptor due to the 5-HT_{1A} receptor may be related to some aspect of the antidepressive effect of the 5-HT_{1A} receptor agonist.

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