Acta Med. Okayama, 2007
Vol. 61, No. 6, pp. 311-317

Acta Medica Okayama
http://www.lib.okayama-u.ac.jp/www/acta/

Original Article

The Influence of Hyperactivity of the Hypothalamic-pituitary-adrenal Axis and Hyperglycemia on the 5-HT$_{2A}$ Receptor-mediated Wet-dog Shake Responses in Rats

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Hyperactivity of the hypothalamic-pituitary-adrenal (HPA) axis induces hyperglycemia and serotonin (5-HT)$_{2A}$ receptor supersensitivity. In the present study, to investigate the effect of hyperglycemia on the function of 5-HT$_{2A}$ receptors, we compared the 5-HT$_{2A}$ receptor-mediated wet-dog shake responses in rats treated with adrenocorticotropic hormone (ACTH), dexamethasone and streptozotocin. ACTH (100 μg/rat per day, s.c.), dexamethasone (1 mg/kg per day, s.c.) and streptozotocin (60 mg/kg, i.p.) produced significant hyperglycemia at 14 days after the start of these treatments, and the hyperglycemia was most pronounced in the streptozotocin-treated rats. The wet-dog shake responses induced by (±)-1-(2,5-dimethoxy-4-iodophenyl)-2-aminopropane (DOI), a 5-HT$_{2A}$ receptor agonist, were significantly enhanced at 14 days after repeated treatment with ACTH and dexamethasone. However, streptozotocin-induced diabetes had no effect on the wet-dog shake responses. The results of the present study suggest that hyperglycemia is not strongly associated with the enhanced susceptibility of 5-HT$_{2A}$ receptors under the condition of hyperactivity of the HPA axis.

Key words: hyperglycemia, ACTH, dexamethasone, streptozotocin, 5-HT$_{2A}$ receptor

Several epidemiological and clinical studies have indicated that patients with either type-1 or type-2 diabetes mellitus have a higher prevalence of psychiatric disorders than the general population [1], and hyperactivation of the hypothalamic-pituitary-adrenal (HPA) axis has been reported in patients with diabetes mellitus, especially in those with poor glycemic control and ketoacidosis [2, 3]. It is well known that repeated administration of the adrenocorticotropic hormone (ACTH) or corticosteroid can readily produce not only psychological side effects, such as depression accompanied by anxiety, excitement and sleeplessness, but also hyperglycemia [4, 5]. Therefore, these reports led us to speculate that both HPA axis hyperactivity and hyperglycemia may be responsible for the psychiatric disorders that tend to occur in patients receiving chronic high dose steroid treatment or those who suffer from diabetes mellitus.

Received March 13, 2007; accepted June 15, 2007.
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Serotonin (5-HT) and dopamine receptors play important roles in the pathophysiology of psychiatric disorders, including depression. For example, elevated numbers of 5-HT$_{2A}$ receptors and reduced dopamine turnover have been reported in the post-mortem brains of suicide victims and depressed subjects [6, 7]. In our previous studies using experimental animals, we found that repeated ACTH treatment increases the wet-dog shake response induced by (±)-1-(2,5-dimethoxy-4-iodophenyl)-2-aminopropane (DOI), a 5-HT$_{2A}$ receptor agonist in rats [8-10]. Moreover, it has reported that chronic dexamethasone administration induces 5-HT$_{2A}$ receptor supersensitivity in the rat brain [10]. However, the relationship between hyperglycemia and 5-HT$_{2A}$ receptor supersensitivity is not clear.

The primary aim of this study was to clarify whether or not hyperglycemia is related to the enhanced 5-HT$_{2A}$ receptor function under the condition of hyperactivity of the HPA axis induced by either ACTH or corticosteroid. In the present study, we investigated the effects of repeated treatment with ACTH and dexamethasone on 5-HT$_{2A}$ receptor-mediated wet-dog shake responses in the rat, and then compared them to the responses in streptozotocin-induced diabetic rats. Furthermore, to clarify whether the function of central dopaminergic receptors is altered by hyperactivity of the HPA axis and hyperglycemia, we also examined the effects on dopamine receptor-mediated stereotyped behavior in the rat.

Materials and Methods

Animals. Male Wistar strain rats (at 5-6 weeks of age) were obtained from Charles River (Yokohama, Japan). All animals were housed at 2 rats / cage (42 × 26 × 15 cm). The animal room was maintained at 22 ± 1 °C under a 12 h/12 h light/dark cycle with lights on from 7:00 AM. Food and water were available *ad libitum*. All animal experiments were performed in compliance with the Guidelines for Animal Experimentation and with the approval of the Committee of Animal Experimentation, Ehime University School of Medicine.

Drugs. The following drugs were used: Cortrosyn-Z (ACTH, zinc hydroxide suspension of tetracosactide acetate;); Daiichi-Sankyo Pharmaceutical Co. Ltd., Tokyo, Japan), dexamethasone sodium phosphate (Banyu Pharmaceutical Co. Ltd., Tokyo, Japan), streptozotocin (Sigma-Aldrich Co., St. Louis, MO, USA), (±)-1-(2,5-dimethoxy-4-iodophenyl)-2-aminopropane (DOI; Sigma-Aldrich) and apomorphine hydrochloride (Sigma-Aldrich). Streptozotocin (STZ) was dissolved in 0.05 M citrate buffer at pH 4.5 immediately before administration. Apomorphine hydrochloride was dissolved in saline containing 0.1% ascorbic acid and the solution was kept on ice in the dark to protect against oxidative degradation. DOI and dexamethasone were dissolved in saline. ACTH was injected subcutaneously to a volume of 0.2 ml/rat. All other drugs were injected at a volume of 0.1 ml per 100 g body weight.

DOI-induced wet-dog shake responses. All observations of DOI-induced wet-dog shake responses were performed between 10:00 AM and 2:00 PM. DOI-induced wet-dog shakes were observed in individual clear polycarbonate cages (42 × 26 × 30 cm). Immediately after the subcutaneous administration of DOI (1 mg/kg), the number of wet-dog shakes was recorded over a 30-min period [9].

Apomorphine-induced stereotyped behavior. It is well established that stereotypy is a dopamine-dependent behavior, and apomorphine, a mixed D_1/D_2 dopamine receptor agonist, causes stereotyped behavior in animals [10]. The stereotyped behavior induced by apomorphine was observed in the individual wire mesh cages (20 × 15 × 15 cm) and scored. The degree of stereotyped behavior was as follows: 0, no stereotyped behavior; 1, discontinuous sniffing; 2, continuous sniffing; 3, continuous sniffing and discontinuous licking or biting; 4 continuous sniffing and continuous licking or biting.

Measurement of plasma corticosterone. Blood samples for measurement of the plasma corticosterone levels were collected from the postcaval vein under ether anesthesia, and trunk blood was then collected in heparinized tubes. The blood was centrifuged at 10,000 g for 15 min, and then the plasma was removed and stored at −20 °C until analysis. The blood was collected between 11:00 AM and 12:00 PM. The plasma corticosterone concentrations were determined using a commercially available enzyme immunoassay kit (Assay Designs, Inc., Ann Arbor, MI, USA) following the manufacturer's instructions.

Experimental procedures. The animals were subcutaneously administered ACTH (100 μg/rat), dexamethasone (1 mg/kg) and saline once daily for 14 days. The behavioral test (DOI-induced wet-dog shake responses and apomorphine-induced stereotyped behavior) was performed 24 h after the last treatments. Streptozotocin (60 mg/kg, i.p.) was intraperitoneally administered 14 days before the experiments, and the control rats were administered the vehicle alone. The blood glucose levels were determined using a glucose analyzer (Arkray glucocard Diameter-alpha GT-1661; Arkray, Inc., Kyoto, Japan).

Statistical analysis. The values were expressed as the means and S.E.M. of each group. The data were analyzed using Student's *t*-test. *P* values of less than 0.05 were considered to be significant. We used the Statcel QC (OMS Publishing Inc., Tokyo, Japan) statistical analysis software package.

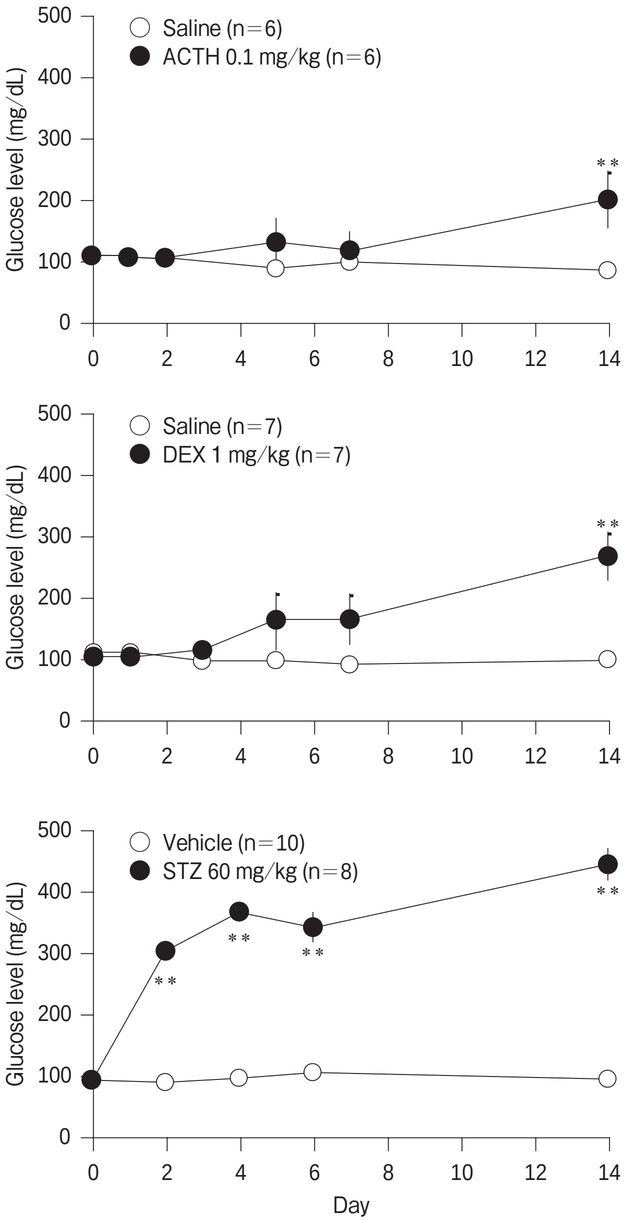

Fig. 1 Chronological changes in the blood glucose levels in the rats treated with ACTH, dexamethasone and streptozotocin. ACTH (100 μg/rat, s.c.) and dexamethasone (DEX, 1 mg/kg, s.c.) were administered once daily for 14 days, and streptozotocin (STZ, 60 mg/kg, i.p.) was administered on day 0. Each point represents the mean ± S.E.M. (n = 6–10). $^{**}p < 0.01$ (Student's *t*-test).

Results

Fig. 1 shows the chronological changes in the blood glucose levels with the daily administration of ACTH and dexamethasone or after a single injection of streptozotocin. The plasma glucose levels gradually increased with the daily administrations of ACTH (100 μg/rat, s.c.) and dexamethasone (1 mg/kg, s.c.), and the plasma glucose levels significantly ($p < 0.01$, respectively) increased at 14 days in comparison to the saline-treated control rats. On the other hand, a single injection of streptozotocin (60 mg/kg, i.p.) increased the plasma glucose levels significantly ($p < 0.01$) and markedly 2 days after the injection, and thereafter hyperglycemia persisted. The plasma corticosterone levels were not significantly different between the streptozotocin- and vehicle-treated control rats (Fig. 2).

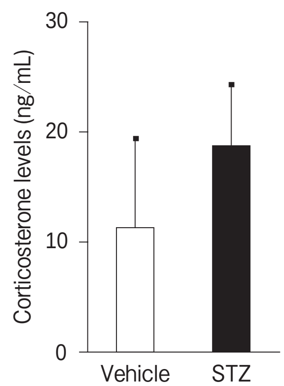

Fig. 2 Plasma corticosterone levels in the rats treated with streptozotocin (STZ) and the vehicle. Each column represents the mean ± S.E.M. (n = 8).

The administration of apomorphine (1 mg/kg, s.c.) caused a marked stereotyped behavior, and its maximum effect was observed at from 10–30 min after the apomorphine administration (Fig. 3). ACTH, dexamethasone and streptozotocin had no effect on the time course of the stereotyped behavior score or the total score.

Fig. 4 shows the number of DOI-induced wet-dog shakes in the rats treated with ACTH, dexamethasone and streptozotocin. The number of wet-dog shakes significantly increased in the groups with ACTH and dexamethasone in comparison to the saline-treated control groups. However, no significant difference was observed in the number of wet-dog shakes between the streptozotocin-induced diabetic rats and nondiabetic rats.

Discussion

Glucocorticoid excess results in insulin resistance

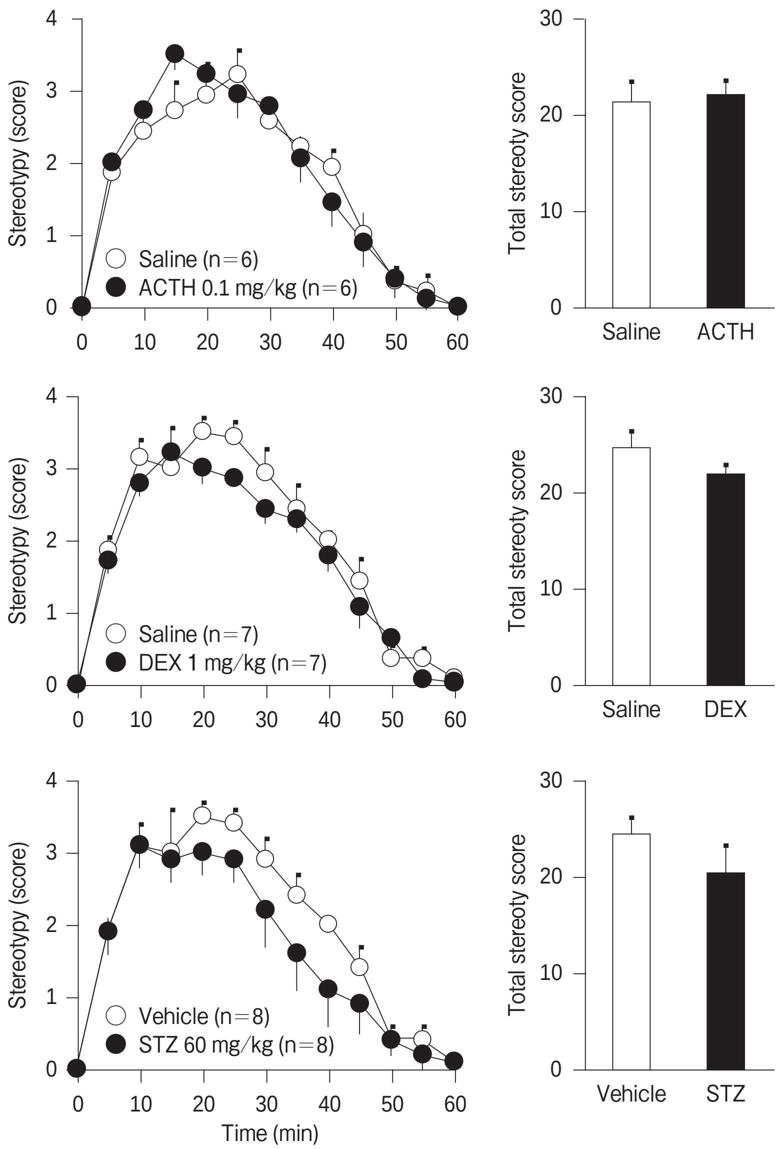

Fig. 3 Stereotyped behavior induced by apomorphine in the rats treated with ACTH, dexamethasone (DEX) and streptozotocin (STZ). Each point represents the mean score or total score for 1 h with S.E.M. (n = 6–8).

[12] by blunting insulin's ability to suppress the hepatic glucose production and stimulate peripheral glucose utilization [13]. Glucocorticoids also have a direct inhibitory effect on glucose-induced insulin release in β-cells [14]. In a preliminary study, we examined the dose-response effect of ACTH (50–100 μg/rat) and dexamethasone (0.5–1 mg/kg) on blood glucose levels in rats. The results showed that 14 days of treatment with ACTH (50 μg/rat) or dexamethasone (0.5 mg/kg) did not change the blood glucose levels; however, treatment with ACTH (100 μg/rat) or dexamethasone (1 mg/kg) gradually increased the blood glucose levels. On the other hand, streptozotocin (60 mg/kg, i.p.) produced significant hyperglycemia 2 days after the injection, and the hyperglycemia at 14 days after the treatment with the streptozotocin was markedly higher than that at 14 days after treatment with ACTH (100 μg/rat, s.c.) or dexamethasone (1 mg/kg, s.c.).

Diabetic patients have been shown to have disrupted circadian patterns of cortisol secretion, with elevated cortisol levels [2]. Chan *et al.* [15] reported that plasma ACTH and corticosterone levels in streptozotocin-induced diabetic rats were significantly higher (approximately 2-fold) at 8:00 AM, but they were not different at 1:00 PM or 6:00 PM. In the present study, the diabetic and control rats showed the same corticosterone levels at 11:00 AM to 12:00 PM. Therefore, the disparity of results may be related to the differences in the time of experiment. However, we previously observed that the plasma corticosterone levels in rats following a 14-day chronic ACTH treatment (100 μg/day, s.c.) were approximately 12-fold higher than those in the saline-treated rats [8]. Therefore, ACTH and dexamethasone produced a more marked HPA axis hyperactivity than streptozotocin.

Studies showing the presence of glucocorticoids and their binding sites in the central nervous system indicate that these hormones may affect the central neurotransmission [16]. Both the dopaminergic brain systems and glucocorticoids are considered to be involved in certain psychopathological conditions in humans, including depression [17]. Moreover, psychiatric abnormalities, such as depression, euphoria or manic psychoses have also been observed in psychiatrically healthy patients receiving chronic high dose steroid treatment for such medical disorders such as Addison's disease, rheumatoid arthritis, asthma, dermatological or hematological diseases [18, 19]. Studies using experimental animals have shown that glucocorticoids modulate such behavior in animals as the locomotor activity [20] and stereotyped behavior [21]. Danilczuk *et al.* [21] reported that single and large doses of prednisolone (4–20 mg/kg) or dexamethasone (4–8 mg/kg) intensified and prolonged the stereotypy induced by apomorphine or amphetamine in rats. However, in this study, 14-day administration of ACTH (100 μg/rat) and low doses of dexamethasone (1 mg/kg) had no effect on apomorphine-induced

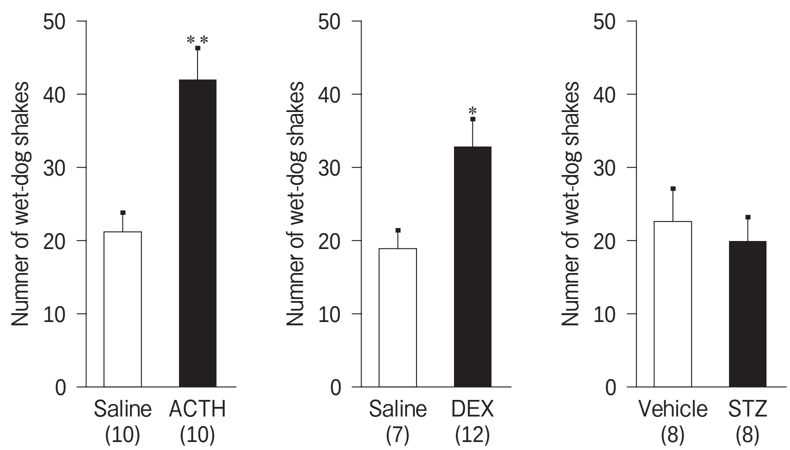

Fig. 4 DOI-induced wet-dog shake responses in the rats treated with ACTH, dexamethasone (DEX) and streptozotocin (STZ). Each column represents the mean $\pm$ S.E.M. (n = 6–12). $*p < 0.01$, $**p < 0.001$ (Student's *t*-test).

stereotyped behavior. Therefore, the discrepancy in our results may be related to the differences in the dose of ACTH or dexamethasone.

The disparity of results may also be related to the differences in the treatment period. Lim *et al.* [22] observed a decrease in the turnover of dopamine and an increase in the maximum binding number of dopamine D_2 receptors 4 weeks after a single administration of streptozotocin in the striatum of diabetic rats, thus suggesting that the upregulation of dopamine receptors might be due to decreased dopamine metabolism. An *in vivo* brain microdialysis study has also shown that the basal release of dopamine and dihydroxyphenylacetic acid (DOPAC) in the rat ventral striatum was lower in diabetic than in normal rats [23]. However, Sumiyoshi *et al.* [24] reported that 4 weeks after a single administration of streptozotocin, there was no change in the dopamine D_2 receptor density in the rat striatum. In this study, apomorphine-induced stereotyped behavior was not affected 2 weeks after the treatment with streptozotocin.

The systemic administration, as well as the microinjection of DOI, a $5\text{-HT}_{2A/2C}$ receptor agonist, into the medial prefrontal cortex elicits dose-dependent wet-dog shake or head twitch responses [25, 26]. These responses can be blocked by selective 5-HT_{2A} receptor antagonists [25], indicating that these responses are mediated by central 5-HT_{2A} receptors. We have previously reported that the chronic administration of ACTH (100 μg/rat) potentiates DOI-induced wet-dog shaking behavior in rats [9, 27]. Similarly, the glucocorticoid analog dexamethasone (1 mg/kg) has been reported to enhance DOI-induced wet-dog shaking behavior in rats [28]. These treatments with ACTH and dexamethasone have been reported to increase the binding of [^{3}H] ketanserin to 5-HT_{2A} receptors in the forebrain neocortex [29, 30]. In this study, we confirmed these phenomena using behavioral pharmacology. On the other hand, streptozotocin-induced diabetes has been reported to cause an increase in the density of 5-HT_{2A} receptors 4 weeks after streptozotocin treatment without affecting dopamine D_2 receptors in the rat striatum [24]. In the present study, the DOI-induced wet-dog shake responses in diabetic rats were not significantly affected at 2 weeks after streptozotocin treatment. These findings indicated that the enhancement of 5-HT_{2A} receptor function in rats treated with ACTH or dexamethasone is more closely related to activation of the HPA axis than to hyperglycemia.

In conclusion, our results indicated that repeated treatment with ACTH or dexamethasone enhanced the susceptibility of 5-HT_{2A} receptors, and this enhancement was more associated with the HPA axis hyperactivity than hyperglycemia. It has been reported that glucocorticoids inhibit the glucose uptake in adipocytes and fibroblasts, decrease local cerebral glucose utilization, and inhibit glucose uptake in hippocampal neurons *in vitro* [31]. Moreover, it has been suggested that the prolonged exposure of hippocampal neurons to elevated glucocorticoid levels can lead to neurodegeneration or suppressed neurogenesis [31]. Therefore, further studies will be necessary to clarify the effects of long-term hyperglycemia.

Acknowledgments. This work was supported by the Japanese Health Science Foundation and a Grant-in-Aid for Scientific Research (No. 17590127) from the Japanese Ministry of Education, Science, Sports and Culture in Japan.

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