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Ryuto Hirasawa* Kozo Hashimoto†

Zensuke Ota‡

*Okayama University,

†Okayama University,

‡Okayama University,

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Ryuto Hirasawa, Kozo Hashimoto, and Zensuke Ota

Abstract

The role of alpha-1 adrenergic mechanism in the shaking stress-induced adrenocorticotropic hormone (ACTH), and plasma noradrenaline secretion and pressor response were investigated using conscious rats. We also studied whether or not central corticotropin releasing hormone (CRH) is involved in the shaking stress-induced ACTH secretion. The shaking stress caused significant elevations of plasma ACTH, noradrenaline, and systolic blood pressure. Intra-third ventricular administration of alpha-1 adrenergic blocker, bunazosin, inhibited the shaking stress-induced ACTH secretion, but did not alter stress-induced noradrenaline secretion and pressor response. Furthermore, intra-third ventricular administration of CRH antagonist, alpha-helical CRH, significantly attenuated stress-induced ACTH secretion. These results indicate that alpha-1 adrenergic pathway and CRH at least partly mediate the shaking stress-induced ACTH secretion.

KEYWORDS: shaking stress, adrenocorticotropic hormone, noradrenaline, alpha-adrenergic mechanism, blood pressure

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A Role of Central Alpha-1 Adrenergic Mechanism in Shaking Stress-Induced ACTH and Noradrenaline Secretion

Ryuto Hirasawa*, Kozo Hashimoto and Zensuke Ota

Third Department of Internal Medicine, Okayama University Medical School, Okayama 700, Japan

The role of alpha-1 adrenergic mechanism in the shaking stress-induced adrenocorticotropic hormone (ACTH), and plasma noradrenaline secretion and pressor response were investgated using conscious rats. We also studied whether or not central corticotropin releasing hormone (CRH) is involved in the shaking stress-induced ACTH secretion. The shaking stress caused significant elevations of plasma ACTH, noradrenaline, and systolic blood pressure. Intra-third ventricular administration of alpha-1 adrenergic blocker, bunazosin, inhibited the shaking stress-induced ACTH secretion, but did not alter stress-induced noradrenaline secretion and pressor response. Furthermore, intra-third ventricular administration of CRH antagonist, alpha-helical CRH, significantly attenuated stress-induced ACTH secretion. These results indicate that alpha-1 adrenergic pathway and CRH at least partly mediate the shaking stress-induced ACTH secretion.

Key words : **shaking stress, adrenocorticotropic hormone, noradrenaline, alpha-adrenergic mechanism, blood pressure**

Certain kinds of stress are known to evoke the release of adrenocorticotropin (ACTH) and peripheral sympathetic nervous system (SNS) activation. Some of neurotransmitters in the hypothalamus which stimulate ACTH secretion can also evoke sympathoadrenomedullary changes, and it is possible that they mediate stress-induced ACTH and cardiovascular changes (1). It has been suggested that the alpha-adrenergic mechanism is involved in ACTH secretion at the pituitary level (2). However, at the hypothalamic level the action of catecholamines in the control of ACTH secretion remains controversial (3).

In this study, we examined the role of central alpha-1 adrenergic mechanism in the shaking stress induced-ACTH and noradrenaline secretion, and systolic blood pressure elevation in awake rats.

Materials and Methods

Cannula implantation. Male Wistar rats (weighing 250-300 g) were used *in vivo* experiments. The rats anesthetized with intraperitoneal sodium pentobarbital (45 mg/kg b.w., Somnopentyl, Pitman-Moore Inc, NJ, USA) and a stainless steel guide cannula (C313G) was implanted stereotaxically in the third venticle. This allowed us to inject alpha-1 adrenergic antagonist, bunazosin, directly into the cerebrospinal fluid. The rats were caged individu-

* To whom correspondence should be addressed.

ally and received standard rat biscuits and water *ad libitum*. Five or six days later, femoral artery was cannulated using PE50 polyethylene tubing (Intamedic, Clay Adams, USA) under pentobarbital anesthesia. The catheters were tunneled under the skin to exit at the nape of the neck. The catheters were filled with 0.9 % saline containing heparin sodium (500 U/ml) to prevent blood coagulation.

Two days later, the experiment was carried out in a quiet room. Ninety minutes prior to the experiment, a PE50 polyethylene tube was connected to the cannula for collecting blood samples and monitoring arterial blood pressure. The connecting tubing and cannula were filled with 0.9 % saline, containing hepalin sodium (500 U/ml), and the end of the tubing was suspended outside of the cage. Thus the rats were able to move freely.

Experiment 1. Sixty minutes prior to giving the stress, 1 ml of blood was withdrawn from the cannula by heparinized syringe, followed by 1 ml saline replacement. Plasma was separated for noradrenaline measurement and blood cells were resuspended in saline and mixed for later replacement.

Thirty min later, 0.3 ml of blood was collected for baseline ACTH assay, followed by 0.3 ml of saline replacement. Three μl of vehicle (0.9 % saline) or bunazosin (10 μg/3 μl) (provided by Esai Co, Tokyo, Japan) was injected intra-third ventricularly. Then, the cannula was connected through a pressure monitoring kit (SCK-590, Spectramed Medical Products, Ltd, Tokyo, Japan) to a Nihon Koden recorder (connection broad: RMP-6004, blood pressure amplifier: AP641, heart rate counter: AT601G, recticorder: WT-625G, Tokyo, Japan) for continuous measurements of arteral pressure and heart rate.

Thirty min later, shaking stress at 300 cycles/min (rotated horizontally) was given to the rats for 3 min using a Daiichi shaker (Daiichi radioisotope Labo., Ltd, Tokyo, Japan). Between 2.5 and 3 min after the onset of the stress, 1.3 ml blood was collected for noradrenaline and ACTH measurements, followed by an equivalent amount of blood replacement. Blood sample (0.3 ml) was collected 10 min after the onset of the stress for ACTH measurement.

Experiment 2. Thirty-five min before giving the stress, 1 ml of blood was withdrawn by heparinized syringe through the femoral artery cannula for measuring noradrenaline levels, followed by the replacement with 1 ml saline.

Thirty min later, 0.3 ml of blood was collected for ACTH assay. The femoral artery cannula was collected through a pressure monitoring kit. Then, 3 μl of vehicle (0.9 % saline) or corticotropin releasing hormone (CRH) antagonist, alpha-helical CRH (9–41) (Peninsula Lab., Inc., USA) (30 μg/3 μl) was injected intra-third ventricularly.

Five min later, shaking stress was given to the rats for 3 min. Between 2.5 and 3 min and at 10 min after the onset of the stress, blood was collected for ACTH and/or noradrenaline measurements, followed by an equivalent amount of blood replacement.

Hormone assay. Blood sample was collected in a chilled plastic tube and centrifuged (1,200 *g*) at 4 °C, and the plasma was stored at −20 °C pending assay. The plasma ACTH concentration was measured with a commercially available radioimmunoassay kit (DPC ACTH kit, Nippon DPC Co., Tokyo, Japan). The plasma noradrenaline extraction was carried out as soon as possible. The plasma noradrenaline concentration was determined by ion-pairing reversed phase high performance liquid chromatography (HPLC) with amperometric detection as described previously (5).

Statistical analysis. Values are presented by analysis of variance, followed by Duncan's new multiple range test or Student's *t*-test.

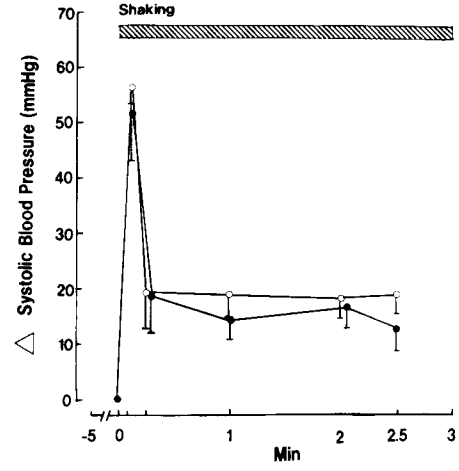

Fig. 1 The effect of intra-third ventricle administration of alpha-1 adrenergic antagonist (bunazosin, 10 μg) on shaking stress-induced pressor responce in awake rats (○ vehicle group (n = 7), ● bunazosin treated group (n = 8)).
All points and bars represent mean ± SEM.

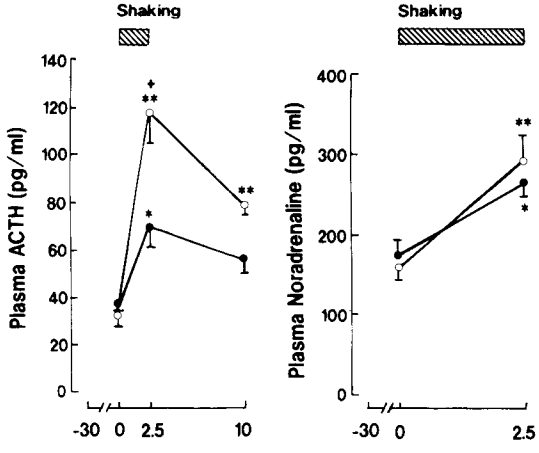

Fig. 2 The effect of intra-third ventricle administration of alpha-1 antagonist (bunazosin, 10μg) on shaking stress-induced plasma ACTH (○ vehicle group (n = 8), ● bunazosin treated group (n = 8)) and noradrenaline (○ vehicle group (n = 6), ● bunazosin treated group (n = 10)) elevations in awake rats. All points and bars represent mean ± SEM.
(The ACTH levels at "0" min indicate the values at 60 min before giving the stress. The noradrenaline levels at "0" min indicate the values at 30 min before giving the stress.)
* $p < 0.05$ *vs* baseline levels, ** $p < 0.01$ *vs* baseline levels, + $p < 0.01$ *vs* vehicle group.

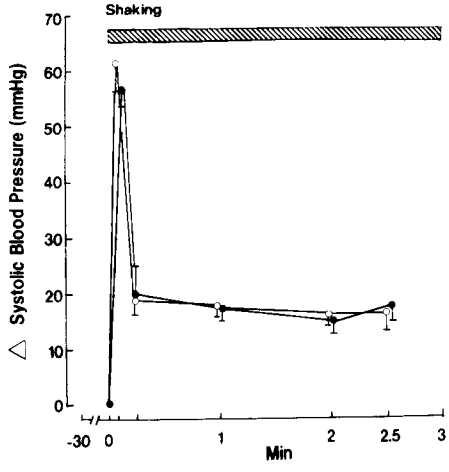

Fig. 3 The effect of intra-third ventricle administration of CRH antagonist (alpha-helical CRH, 30μg) on shaking stress-induced pressor responce in awake rats (○ vehicle group (n = 7), ● alpha-helical CRH treated group (n = 7)).
All points and bars represent mean ± SEM.

Result

Experiment 1. Systolic blood pressure increased shortly after the onset of shaking stress and peaked at 5 sec (61 ± 12 mmHg, mean ± SD), and it continued to be elevated by 15–20 mmHg above baseline levels during stress (Fig. 1, vehicle group). When the stress was removed, systolic blood pressure gradually went down to baseline levels. An intra-third ventricular administration of busazosin (10μg) did not attenuate stress-induced systolic blood pressure elevation. The shaking stress caused significant elevation of plasma ACTH at 2.5 and 10 min after the onset of stress, but plasma ACTH levels at 2.5 min were greater than those at 10 min. Plasma noradrenaline levels were also elevated at 2.5 min (Fig. 2, vehicle group). An intra-third ventricular administration of bunazosin significantly attenuat-

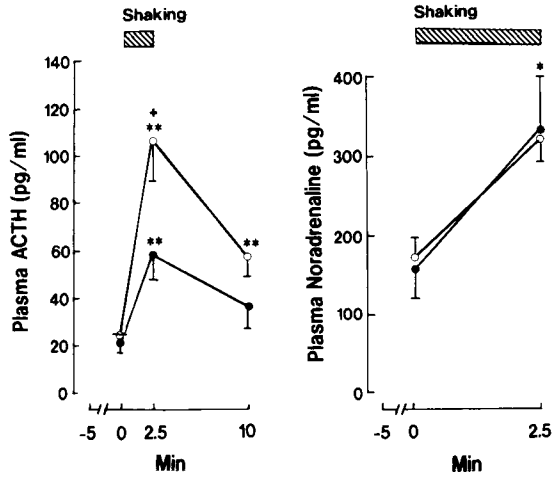

Fig. 4 The effect of intra-third ventricle administration of CRH antagonist (alpha-helical CRH, 30μg) on shaking stress-induced plasma ACTH (○ vehicle group, (n = 6); ● alpha-helical CRH treated group, (n = 4)) and noradrenaline (○ vehicle group, (n = 6); ● alpha-helical CRH treated group, (n = 5)) elevations in awake rats. All points and bars represent mean ± SEM.
The ACTH levels at "0" min indicate the values at 35 min before giving the stress. The noradrenaline levels at "0" min indicate the values at 5 min before giving the stress.
* $p < 0.05$ *vs* baseline levels, ** $p < 0.01$ *vs* baseline levels, + $p < 0.01$ *vs* vehicle group.

ed plasma ACTH elevation, while it did not affect the stress-induced elevation of plasma noradrenaline levels (Fig. 2).

Experiment 2. Intra-third ventricularly administered alpha-helical CRH (30 μg/3 μl) did not affect the shaking stress-induced pressor response (Fig. 3) and elevation of noradrenaline levels (Fig. 4). However, the shaking stress-induced ACTH secretion was significantly attenuated in alpha-helical CRH injected group (Fig. 4).

Discussion

The shaking stress induces acutely both mental and physical stress in the rats, and it evokes the release of ACTH and peripheral SNS activation (4). In the present study, both bunazosin and alpha-helical CRH treatment significantly reduced the stress-induced plasma ACTH elevation, but not pressor response and plasma noradrenaline elevation. It has been reported that the paraventricular nucleus of the hypothalamus, which contains the cell bodies of CRH neurons, receives direct noradrenergic projetions from the brainstem (6, 7, 8). The action of catecholamines on the regulation of CRH at the hypothalamic levels remains controversial. Central catecholamines have been reported to cause stimulatory (9–19), inhibitory (20–26) or no definite effect (27) on ACTH secretion. The differences in the species, administered doses, and experimental design might be responsible for the discrepancy (3). It has been demonstrated that some stressful stimuli increase hypothalamic noradrenaline turnover and adrenocortical activity in a parallel manner in the rats (11, 28, 29) and that noradrenaline might be an essential component of the ACTH renponse to the stress (18). Gibson *et al.* (30) reported that pretreatment with prazosin, alpha-1 adrenergic blocker, attenuated restraint stress-induced pituitary adrenocortical responses. Futhermore, Takao *et al.* (19) reported that central noradrenaline stimulated ACTH secretion mainly *via* the central alpha-adrenergic mechanism, and endogenous CRH might be at least partly involved in the noradrenaline-induced ACTH secretion in awake rats.

The present study demonstrated that intra-third ventricular administration of bunazosin and alpha-helical CRH attenuated shaking stress-induced ACTH secretion. Our results suggest that central alpha-1 adrenergic mechanism and endogenous CRH are at least partly involved in the shaking stress-induced elevation of plasma ACTH. It is possible that shaking stress stimulates alpha-1 adrenergic mechanism to evoke CRH secretion.

We previously reported that central angiotensinergic pathway partially mediated the shaking stress-induced activation of the SNS and systolic blood pressure elevation, but was not involved in shaking stress-induced ACTH secretion (4). Jones (31) suggested that alpha-1 adrenergic receptors in the hypothalamus are involved in the contorol of pressor responses elicited by intracerebroventricularly administered angiotensin II, since microinjection of prazosin in this site reduced pressor responses (31). Plasma catecholamine elevation could be one evidence of the peripheral SNS activation (32, 33). Our data suggest that the shaking stress evokes peripheral SNS activation, but central alpha-1 adrenergic mechanism is not involved in these activation and pressor responses.

Thus, our results lead us to speculate that central alpha-1 adrenergic mechanism at least partially mediates the shaking stress-induced ACTH secretion, but is not involved in activation of the peripheral SNS and blood pressure elevation evoked by shaking stress.

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