Effect of the entorhinal cortex on diurnal ACTH and corticosterone release in rats

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Abstract

OBJECTIVE: In our previous study, a lesion in the entorhinal cortex was found to significantly attenuate the elevation of adrenocorticotropic hormone (ACTH) in plasma during immobilization stress. The aim of the present study was to investigate whether the entorhinal cortex exerts a modulatory effect on circadian ACTH and corticosterone release.

MATERIALS AND METHODS: Ibotenic acid (15 μg/μl) was stereotaxically bilaterally injected into the entorhinal cortex of rats. Two weeks after the injections, ACTH and corticosterone levels in plasma were measured at 0800 h, 1300 h and 1800 h.

RESULTS: Compared with sham-operated control rats, rats with entorhinal cortex lesions produced by ibotenic acid showed either significantly elevated plasma ACTH or plasma corticosterone levels at 0800 h, but no difference at 1300 h or 1800 h.

CONCLUSION: The results of the present study indicate that the entorhinal cortex plays a certain role in the regulation by the central nervous system of the circadian rhythm of the hypothalamic-pituitary-adrenal (HPA) axis.

INTRODUCTION

The simultaneous evaluation of the circadian rhythm of plasma adrenocorticotropic hormone (ACTH) and serum cortisol is a clinically reliable tool that make it possible to appreciate the neuroendocrine changes that occur in Alzheimer's disease (AD), and a selective impairment of hypothalamic-pituitary-adrenal (HPA) axis function in patients with AD has been observed (Magri *et al.*,2006; Umegaki *et al.*,2000a). However, the mechanism underlying the dysfunction of the HPA axis in AD remains unclear.

A wealth of evidence suggests that the entorhinal cortex plays an important role in AD (Artacho-Pérula and Insausti, 2007; Dickerson, 2007; Di Paola *et al.*, 2007). The entorhinal cortex is a gateway to the hippocampus, and many types of sensory input and other information reach the hippocampus via this cortex. It receives input from the neocortex, including the temporal and frontal lobes, amygdala and olfactory bulbs (Chrobak *et al.,* 2000). Information enters the hippocampal formation via the entorhinal cortex and exits via the fornix. Additionally, the entorhinal cortex is the primary supplier of converging neocortical

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sensory input to the ipsilateral dentate gyrus of the hippocampal formation (Ueki *et al.,* 1997).

Previously, we demonstrated that lesions in the entorhinal cortex produced by ibotenic acid, significantly attenuate the elevation of ACTH in plasma during immobilization stress (Umegaki *et al.,* 2003). This suggests that the entorhinal cortex could be involved in the processing of stress responses. However, the effect of the entorhinal cortex on circadian HPA axis function remains to be elucidated. In the present study, we examined the effects of the entorhinal cortex on diurnal ACTH and corticosterone release in rats.

MATERIALS AND METHODS

Subjects

All experiments were conducted on adult male Sprague-Dawley rats (Shanghai SLAC Laboratory Animal Co, LTD, Shanghai, China). Rats initially weighing between 280 and 330 g were individually housed in opaque microisolation cages to eliminate confounding environmental cues, in temperature (22–23°C) and humidity-controlled rooms. The animals were fed rat chow and water *ad libitum* and were allowed to acclimatize to a 12-h light cycle (lights on between 0800 h and 2000 h) for a period of 1 wk before experimental manipulation. The experiments described below were performed according to the protocols approved by the Animal Care Committee of the University of Zhejiang, in accordance with the guidelines established by the China Council for Animal Care.

Surgery

Three groups of rats were used: 1) normal controls (Normal, n=5); 2) sham-operated rats (ES, n=5); and 3) ibotenic acid-lesioned rats (EI, n=5). The rats were anaesthetized with sodium pentobarbital (50 mg/kg, i.p.) and mounted in a stereotaxic frame (Narishige Scientific Instrument Laboratory, Tokyo, Japan). To insert the stainless steel injection needle, the skull was exposed and a burr hole was drilled overlying the injection coordinates. Ibotenic acid was injected through the steel needle (outside tip diameter, 28 μm), which was connected to a 1.0-μl syringe via 30-cm tubing filled with injection solution. The coordinates for the entorhinal cortex were calculated relative to the bregma with the incisor bar set at –3.30 mm. The coordinates used were anterior-posterior –6.04 mm, medial-lateral ± 6.50 mm, and dorsal-ventral 7.00 mm from the skull surface in accordance with the Paxinos and Watson atlas (Paxinos and Watson, 1986). Entorhinal cortex lesions were produced by pressure-injecting 0.1 μl of ibotenic acid (15 μg/μl in 0.9% NaCl with 0.2% Trypan Blue; Sigma Chemical Co., St. Louis, MO, USA) bilaterally during a 5-min period. The tip of the needle was allowed to remain in the brain for 5 min after injection in order to minimize the dorsal diffusion of the drug along the needle tract. Sham-operated rats were treated in an identical manner to the ibotenic acid-lesioned rats but were injected with the same volume of 0.9% NaCl with 0.2% Trypan Blue without ibotenic acid.

A recovery period of 12 days was allowed to the operated rats, then all rats were anesthetized with diethyl ether, and a catheter was inserted into the jugular vein for repeated blood sampling. A 2-cm longitudinal incision was made in the neck directly over the trachea. The underlying muscles were separated using blunt dissection, and the right jugular vein was catheterized with Silastic tubing (Shiniest Polymer, Nagoya, Japan). A heparin-saline solution (10 U/ml) was used to prime the catheters to prevent blood clot formation. The catheter was threaded through the vein over a distance of 2.5 cm, which allowed the tip of the cannula to rest in or near the atrium. The free end of the catheter was plugged with a knot and the catheter was exteriorized and secured at the back of the neck with a special cap. The rats were kept in individual cages with free access to water and food. The catheters were aspirated and reprimed daily with 1% heparinized saline to maintain patency.

Procedures

During the 3 days following catheterization, venous blood samples were taken to examine plasma ACTH and corticosterone concentrations at 0800 h, 1300 h and 1800 h. At each sampling, the contents of the venous catheter were aspirated, 0.6 ml of blood was withdrawn and the catheter was flushed with 1% heparinized saline. To measure ACTH and corticosterone, blood was collected from the jugular vein catheter into chilled tubes containing ethylene diamine tetraacetic acid (EDTA; Sarstedt, Nümbrecht, Germany) and spun at 10,000 r.p.m. for 10 min. Plasma was removed, aliquoted into storage tubes, and stored at –20°C until assayed. Plasma ACTH and corticosterone concentrations were determined using commercially available enzyme-linked immunosorbent assay (ELISA) kits (R&D Systems, MN, USA) following the manufacturer's protocol. Fifteen microliters of plasma were assayed in duplicate for each sample.

Statistical analysis

Hormone data are presented as mean ± SEM. Statistical analysis was performed using StatView 5.0 (SAS Institute, Cary, NC, USA). Statistical significance was determined by one-way analysis of variance (ANOVA) followed by Fisher's least significant difference (LSD) protected *t*-test. Statistical significance was accepted if $p<0.05$.

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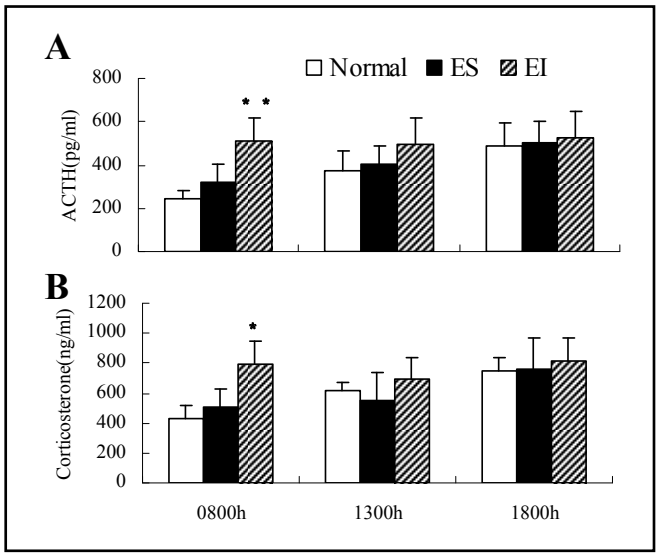

FIG. 1. Plasma ACTH (A) and corticosterone (B) concentrations in control (Normal, open bars), sham-operated (ES, closed bars) and ibotenic acid-lesioned (EI, hatched bars) rats under basal conditions at 0800 h, 1300 h and 1800 h. Values are expressed as mean ± SEM. *, *P* 0.05 and **, *P* 0.01 vs. normal and sham-operated rats.

TABLE 1. Body weight (g) of control (Normal), sham-operated (ES) and ibotenic acid-lesioned (EI) rats at the beginning and end of the study.

	Normal	ES	EI
Beginning	261.40 ± 7.33	263.46 ± 8.40	262.24 ± 11.46
End	387.38 ± 23.31	383.20 ± 30.51	367.60 ± 13.24

RESULTS

Body weight

Body weights were not significantly different between the three groups (Table 1).

Alternations in diurnal ACTH and corticosterone levels

During the nadir (0800 h) of the circadian rhythm, plasma ACTH and corticosterone concentrations were significantly ($p<0.05$) elevated in ibotenic acid-lesioned rats (Fig. 1). No significant differences in plasma ACTH or corticosterone levels were observed between the 3 treatment groups at either 1300 h or 1800 h.

DISCUSSION

The present results demonstrate that diurnal levels of both ACTH and corticosterone were altered by lesions in the entorhinal cortex. Ibotenic acid-lesioned rats showed a significant elevation in plasma ACTH and corticosterone levels at the nadir (0800 h) of the circadian rhythm, compared with controls. In contrast, there were no significant alterations in plasma ACTH or corticosterone levels between the 3 groups at either 1300 h or 1800 h.

It has long been known that the HPA axis has a characteristic that is fundamental to homeostatic regulation in mammals: a circadian rhythm in basal activity (Munck et al., 1984). In the rat, a daily peak in ACTH and corticosterone release occurs near the onset of darkness, corresponding to the end of inactivity in this nocturnal animal. This peak may serve to prepare the organism for the upcoming period of increased activity (Rusak and Zucker, 1979). In the present study, basal pituitary-adrenal activity appeared to be elevated during the trough of the circadian rhythm in ibotenic acid-lesioned rats compared with controls. High plasma ACTH and corticosterone levels were maintained throughout the course of the day in lesioned rats, while in normal and sham-operated animals, the levels tended to increase later in the day. Throughout the study, plasma ACTH and corticosterone levels tended to increase around 1300 h, probably due to the fact that, although rats consume the majority of their food during the dark cycle, they were consistently observed to be feeding at this time. It has previously been reported that plasma ACTH and corticosterone concentrations tend to rise just before feeding bouts (Dallman *et al.,* 1995).

This HPA axis diurnal cycle of activity is thought to be driven by daily fluctuations in the activity of corticotropin-releasing hormone (CRH) neurons within the paraventricular nucleus of the hypothalamus, which are controlled by input from the suprachiasmatic nucleus inputs and hippocampal feedback regulation (Jacobson and Sapolsky, 1991; Sage *et al.,* 2001; Ritter *et al.,* 2003). The present results suggest that the entorhinal cortex is also involved in the regulation of the circadian rhythms of the HPA axis.

In AD, a major neurodegenerative dementia disease, the entorhinal cortex as well as the hippocampus are severely damaged, and the nadir of plasma ACTH and corticosteron levels is reportedly elevated (Braak and Braak, 1992; Ferrari *et al*., 2001; Hartmann *et al*., 1997; Umegaki *et al.,* 2000b); this is consistent with the present results. This suggests that the hippocampus and entorhinal cortex play important roles in the regulation of the HPA axis.

The hippocampus has rich expression of glucocorticoid receptors and it is hypothesized that it is involved in the negative feedback mechanisms of the HPA axis (Jacobson and Sapolsky, 1991). O'Mara (2005) suggests in his review that the ventral subiculum directly projects to the paraventricular nucleus to inhibit the HPA axis. The present study, however, suggests that the entorhinal cortex may also be involved in the negative feedback mechanism of the HPA axis. Because the entorhinal cortex has close reciprocal connections with the hippocampus, it may transmit the inhibitory signals of the HPA axis.

Our series of studies raises the possibility that both the hippocampus and the entorhinal cortex are involved in the activation of the HPA axis (Umegaki *et al.,* 2003; Umegaki *et al.,* 2006; Zhu *et al*., 2001a; Zhu *et al*., 2001b). In conjunction with our previous finding that the entorhinal cortex regulates the stress response induced by immobilization, the present results suggest that the entorhinal cortex may be closely involved in both the HPA response capacity and basal secretion.

In conclusion, the present study strongly suggests that the entorhinal cortex plays a role in regulating the diurnal variations of the HPA axis.

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