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Possible role of adrenoceptors in the hypothalamic paraventricular nucleus in corticotropin-releasing factor-induced sympatho-adrenomedullary outflow in rats

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ABSTRACT

Aims: A functional interaction between the corticotropin-releasing factor (CRF) system and noradrenergic neurons in the brain has been suggested. In the present study, we investigated the interrelationship between the central CRF-induced elevation of plasma catecholamines and adrenoceptor activation in the paraventricular nucleus of the hypothalamus (PVN) using urethane-anesthetized rats.

Main methods: In rats under urethane anesthesia, a femoral venous line was inserted for infusion of saline, and a femoral arterial line was inserted for collecting blood samples. Next, animals were placed in a stereotaxic apparatus for the application of test agents. Catecholamines in the plasma were extracted by alumina absorption and were assayed with high-performance liquid chromatography with electrochemical detection. Quantification of noradrenaline in rat PVN microdialysates was performed with high-performance liquid chromatography with electrochemical detection.

Key findings: We showed that centrally administered CRF elevated noradrenaline release in the PVN. Furthermore, we demonstrated that microinjection of phenylephrine into the PVN induced elevation of plasma levels of adrenaline, but not of noradrenaline, whereas microinjection of isoproterenol into the PVN induced elevation of plasma levels of noradrenaline, but not of adrenaline. Bilateral blockade of adrenoceptors in the PVN revealed that phentolamine significantly suppressed the CRF-induced elevation of plasma adrenaline level, while propranolol significantly CRF-induced elevation of plasma noradrenaline level.

Significance: Our results suggest that centrally administered CRF-induced elevation of plasma levels of adrenaline and noradrenaline can be mediated via activation of α-adrenoceptors and β-adrenoceptors, respectively, in the rat PVN.

1. Introduction

Corticotropin-releasing factor (CRF), a 41-amino acid peptide, is a key stress-related neuropeptide and was originally established as the primary physiological regulator of adrenocorticotropic hormone (ACTH) secretion (Rivier and Vale, 1985). However, it has been well documented that the peptide exhibits a broad distribution in extrahypothalamic brain regions, acting as a neurotransmitter/neuromodulator, and forms circuits in the brain (Sawchenko and Swanson, 1983; Cummings and Seybold, 1988). Increasing evidence indicates that within the central nervous system, the peptide also acts as a regulator of the autonomic nervous system and of cardiovascular function (Brown and Fisher, 1985; Brown et al., 1985; Fisher, 1988; Yokotani et al., 2001; Okada et al., 2003). Specifically, Brown et al. (1982) reported that intracerebroventricularly administered CRF produces an elevation of the plasma concentrations of noradrenaline and adrenaline in conscious rats. They revealed that CRF acts within the brain to stimulate sympathetic outflow by experiments with hypophysectomy, adrenalectomy and ganglion blocker, indicating that the central effects of CRF is thought to be entirely independent of ACTH release.

By using selective receptor antagonists, we previously reported that the centrally administered CRF-induced elevation of plasma noradrenaline was mediated by the activation of α_1 and β adrenoceptors in the brain, and that of plasma adrenaline is mediated by the activation of α_1 adrenoceptors in the brain (Yorimitsu et al., 2008). More recently, we reported that intracerebroventricularly administered isoproterenol, a β adrenoceptor agonist, elevated plasma noradrenaline, but not adrenaline (Ando et al., 2015). Collectively, these observations suggest that centrally administered CRF might elevate plasma catecholamines via activation of brain adrenoceptors.

Noradrenaline is one of the major neurotransmitters involved in brain function, and it acts as a warning signal under stress conditions.

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It activates α or β adrenoceptors in the brain to elicit several kinds of biological responses, such as increased blood pressure and heart rate and secretion of ACTH (Moore and Bloom, 1979; Woodruff et al., 1986; McCall, 1988; Schreihofer and Guyenet, 2000). The paraventricular nucleus of the hypothalamus (PVN) is a complex integrative center important for neurohumoral regulation and the maintenance of cardiovascular and body fluid homeostasis (Kenney et al., 2003; Benarroch, 2005). The PVN receives dense noradrenergic projections from the brainstem with an additional smaller contribution arising from the locus coeruleus (Sawchenko and Swanson, 1983). An ultrastructural study demonstrated that noradrenergic axon terminals contact gastric pre-autonomic neurons in the PVN (Balcita-Pedicino and Rinaman, 2007). Furthermore, functional analyses suggest that adrenoceptors in the PVN play an important role in regulating sympathetic outflow (Scheurink et al., 1990; Williams and Morilak, 1997; Chen et al., 2006; Zhang and Felder, 2008). Collectively, these observations suggest that noradrenergic neurons projecting to the PVN can activate adrenoceptors located in the PVN and can mediate the excitatory effects of noradrenaline to induce sympathetic outflow.

In the present study, to identify the brain sites for CRF-induced sympatho-adrenomedullary outflow, we used a brain microdialysis technique to examine pharmacologically the possibility that centrally administered CRF can release noradrenaline in the PVN. Furthermore, the effects of microinjection into the PVN of phenylephrine, an α_1-adrenoceptor agonist, or isoproterenol, a β adrenoceptor agonist on the plasma levels of noradrenaline and adrenaline were examined.

2. Materials and methods

2.1. Animals

Male Wistar rats weighing approximately 350 g were maintained in an air-conditioned room at 22–24 °C under a constant day-night rhythm for >2 weeks and given food (laboratory chow, CE-2; CLEA Japan, Hamamatsu, Japan) and water ad libitum. All experiments were conducted in compliance with the guiding principles for the care and use of laboratory animals approved by Aichi Medical University.

2.2. Microdialysis

Microdialysis experiments were performed according to methods published in a previous report (Okada et al., 2000). Briefly, a stainless steel guide cannula held on the tip of an L-shaped stainless steel apparatus was implanted stereotaxically just above the right PVN. For intracerebroventricular administration of CRF, another stainless steel cannula (o.d. 0.35 mm) was inserted into the left lateral cerebral ventricle, and the peptide was applied in a volume of 10 μl using a 50-μl Hamilton syringe at the following coordinates (in mm): AP +1.59, L 1.5, V −4.62 (AP, anterior from bregma; L, lateral from the midline; V, below the surface of the brain). The PVN was perfused with Ringer's solution (147 mM NaCl, 4 mM KCl and 2.3 mM $CaCl_2$) at a flow rate of 2 μl/min using a microinfusion pump (EP-60, Eicom, Kyoto, Japan). Three consecutive dialysates were used to measure the baseline release of noradrenaline. Noradrenaline was measured directly with high-performance liquid chromatography (HPLC) with electrochemical detection (Okada et al., 2002).

2.3. Microinjection

With rats under urethane anesthesia (1.2 g/kg, intraperitoneally), a femoral venous line was inserted for infusion of saline (1.2 ml/h), and an arterial line was inserted for collection of blood samples, as described previously (Ando et al., 2015). Next, the animal was placed in a stereotaxic apparatus, as described previously (Ando et al., 2015). Holes were drilled in the skull for administration of test substances. The stereotaxic coordinates of the tip of the cannula were as follows (in mm): PVN: AP, −1.8 mm; L, 0.3 mm; V, 7.6 mm: lateral ventricle: AP, −0.8 mm; L, 1.5 mm; V, 4.0 mm, according to the rat brain atlas of Paxinos and Watson (2005). Four hours were allowed to elapse before the application of test substances.

In the experimental group, a stainless steel injection cannula (o.d. 0.3 mm) was attached to PE-20 tubing, which was then connected to a Hamilton microsyringe (0.5-μl syringe for PVN injection; 10-μl syringe for intracerebroventricular injection). To examine effects of adrenoceptor agonists on plasma catecholamine levels, phenylephrine (5 or 10 nmol), isoproterenol (5 or 10 nmol) or sterile saline as a vehicle was injected unilaterally into the PVN in a volume of 50 nl over 30 s. Next, to examine effects of bilateral blockade of adrenoceptors in the PVN on CRF-induced elevation of plasma catecholamine levels, adrenoceptor antagonists in combination with CRF were administered. Phentolamine (6 nmol/side), propranolol (6 nmol/side) or dimethyl sulfoxide (DMSO) as a vehicle was injected bilaterally into the PVN in a volume of 100 nl over 60 s. After 30 min, CRF (1.5 nmol) or sterile saline was injected intracerebroventricularly in a volume of 10 μl over 30 s. At the end of the experiments, brains were removed, post-fixed in 4% paraformaldehyde solution and cut on a cryostat, and then brain sections were stained in hematoxylin solution for histological verification of injection sites.

2.4. Measurement of plasma catecholamines

Arterial blood samples (250 μl) were collected in a heparinized tube through an arterial catheter and were preserved on ice during the experiments. Immediately after the final sampling, plasma was prepared by centrifugation (3000 ×*g* for 10 min at 4 °C). According our previous reports, catecholamines in the plasma were extracted by alumina and were assayed with HPLC with electrochemical detection (Ando et al., 2015).

2.5. Treatment of data and statistics

Results are expressed as the mean ± S.E.M. of the net change above the respective basal value. The data were analyzed by Student's *t*-test (Fig. 1) or by one-way ANOVA with SPSS v22.0, followed by a post-hoc analysis with the Bonferroni method (Figs. 2, 3, 5). *P* values < 0.05 were taken to indicate statistical significance.

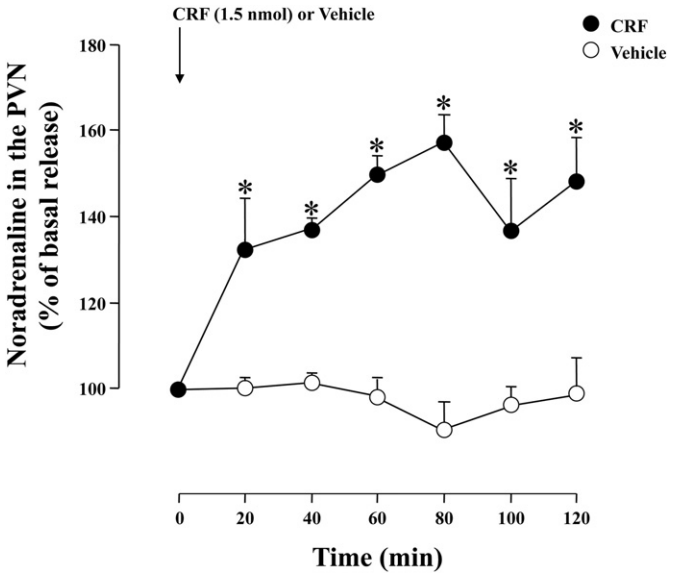

Fig. 1. Effects of intracerebroventricularly (i.c.v.) administered CRF on the release of noradrenaline in the PVN. ●, CRF (n = 6); ○, vehicle (10 μl saline/animal, i.c.v.) (n = 4). *Significantly different (p<0.05) from vehicle.