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Corticotropin-Releasing Factor Activates the Noradrenergic Neuron System in the Rat Brain

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EMOTO, H., M. TANAKA, C. KOGA, H. YOKOO, A. TSUDA AND M. YOSHIDA. *Corticotropin-releasing factor activates the noradrenergic neuron system in the rat brain.* PHARMACOL BIOCHEM BEHAV 45(2) 419–422, 1993.— The effect of corticotropin-releasing factor (CRF) on central noradrenaline (NA) metabolism was examined by measuring levels of the major metabolite of NA, 3-methoxy-4-hydroxy-phenylethyleneglycol sulfate (MHPG-SO_4) in several rat brain regions. Various doses of CRF ranging from 0.5 -10 μg injected ICV significantly increased MHPG-SO_4 levels in several brain regions including the hypothalamus, amygdala, midbrain, locus coeruleus (LC) region, and pons + medulla oblongata excluding the LC region. Plasma corticosterone levels were also significantly increased after ICV CRF administration up to 0.5 μg. The present results that CRF not only elevates plasma corticosterone levels but also increases NA metabolism in many brain regions suggest its neurotransmitter and/or neuromodulator role exerting the excitatory action on central NA neurons.

Corticotropin-releasing factor Noradrenaline MHPG-SO_4 Corticosterone Brain

WE reported that a variety of stressful stimuli cause marked increases in noradrenaline (NA) metabolism and NA release in rat brain regions by measuring levels of NA and its major metabolite, 3-methoxy-4-hydroxyphenylethyleneglycol sulfate (MHPG-SO_4), using our fluorometric method (25,28) as well as by measuring extracellular NA levels with in vivo microdialysis (27,32). These increases show characteristics related to the particular brain region studied and/or to the nature of the stressor (25,26,28). The mechanism by which the central NA neuron system is activated due to multiple forms of stress is one of the major problems to investigate in the research on stress. Neurochemically, corticotropin-releasing factor (CRF) might be one of the substrates inducing negative emotions or an integrator of the organism's reactions to stress as suggested in behavioral (2,7,16) and clinical studies (18,19). CRF was isolated and characterized as a 41-residue peptide from ovine hypothalami (29) that characteristically exerts its action on the hypothalamic-pituitary-adrenal cortical (HPA) axis (21) and autonomic nervous function (10). There are many supportive findings for its role as a putative neurotransmitter and/or neuromodulator in the CNS (4,8,22–24). The present study was undertaken to evaluate the neurochemical effect of CRF on brain noradrenergic neuronal activity by measuring MHPG-SO_4 as a marker of NA metabolism. This is the first study to assess noradrenergic neuronal activity in eight brain regions including the LC region, simultaneously, following ICV CRF injection.

METHOD

Animals

Male Wistar rats (230–270 g) were housed four per standard cage containing wood shavings and maintained at constant temperature (24 ± 1°C) and humidity (50 ± 10%) in a room illuminated for 12 h/day (light 0700 h). They were allowed free access to food and water.

Drugs

CRF (Sigma Chemical Co., St. Louis, MO) was dissolved in physiological saline.

Experimental Procedure

Rats were anesthetized with sodium pentobarbital (50 mg/kg, IP) and implanted with a polyethylene cannula into the right lateral ventricle. Four days after surgery, we confirmed the exact position of the cannula by the fact that the cerebrospinal fluid (CSF) flowed out from the cannula. Saline (5 μl)

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or one of four doses of CRF (0.1, 0.5, 1.0, and 10.0 μg, dissolved in 5 μl saline) per conscious rat was injected ICV using a microsyringe attached with a microtubing (5 μl/min) in the home cage according to the method of de Wied (5). Each group consisted of eight rats. One hour after the respective injection, rats were sacrificed by decapitation. The brain was removed and dissected into the discrete eight regions, including the hypothalamus, amygdala, hippocampus, midbrain, cerebral cortex, and pons + medulla oblongata excluding the LC region and thalamus according to the method of Gispen et al. (11), and frozen on solid CO_2. The LC region was dissected by the method of Reis and Ross (20). Blood was collected from the cervical wound into heparinized tubes. Dissected tissues and separated plasma were stored at −80°C until assayed MHPG-SO_4 levels in brain regions and plasma corticosterone levels were determined fluorometrically by the method developed by us (14) and by the method of van der Vies (31), respectively.

Statistical Analysis

Data were analysed by analysis of variance (ANOVA) and subsequent Newman-Keuls test for the significant main effects.

RESULTS

Figure 1 depicts levels of MHPG-SO_4 in eight brain regions of animals from the five treatment groups. ANOVA of hypothalamus levels revealed a significant group effect, $F(4, 35) = 6.9$, $p < 0.05$. Newman-Keuls tests indicated that the group given 1.0 μg CRF showed significantly higher levels of MHPG-SO_4 as compared with the saline group. ANOVA of LC region levels revealed a significant group effect, $F(4, 35) = 13.9$, $p < 0.05$. Newman-Keuls tests indicated that the groups given 0.5, 1.0, and 10.0 μg CRF showed significantly higher levels of MHPG-SO_4 as compared with the saline group. ANOVA of amygdala levels revealed a significant group effect, $F(4, 35) = 3.91$, $p < 0.05$. Newman-Keuls tests indicated that the 1.0-μg CRF group showed significantly higher levels of MHPG-SO_4 as compared with the saline group. ANOVA of residual pons + medulla oblongata levels revealed a significant group effect, $F(4,35) = 4.82$, $p < 0.05$. Newman-Keuls tests indicated that the 1.0-μg CRF group showed significantly higher levels of MHPG-SO_4 as compared with the saline group. ANOVA of midbrain levels revealed a significant group effect, $F(4, 35) = 5.12$, $p < 0.05$. Newman-Keuls tests indicated that the 1.0- and 10.0-μg CRF groups showed significantly higher levels of MHPG-SO_4 as

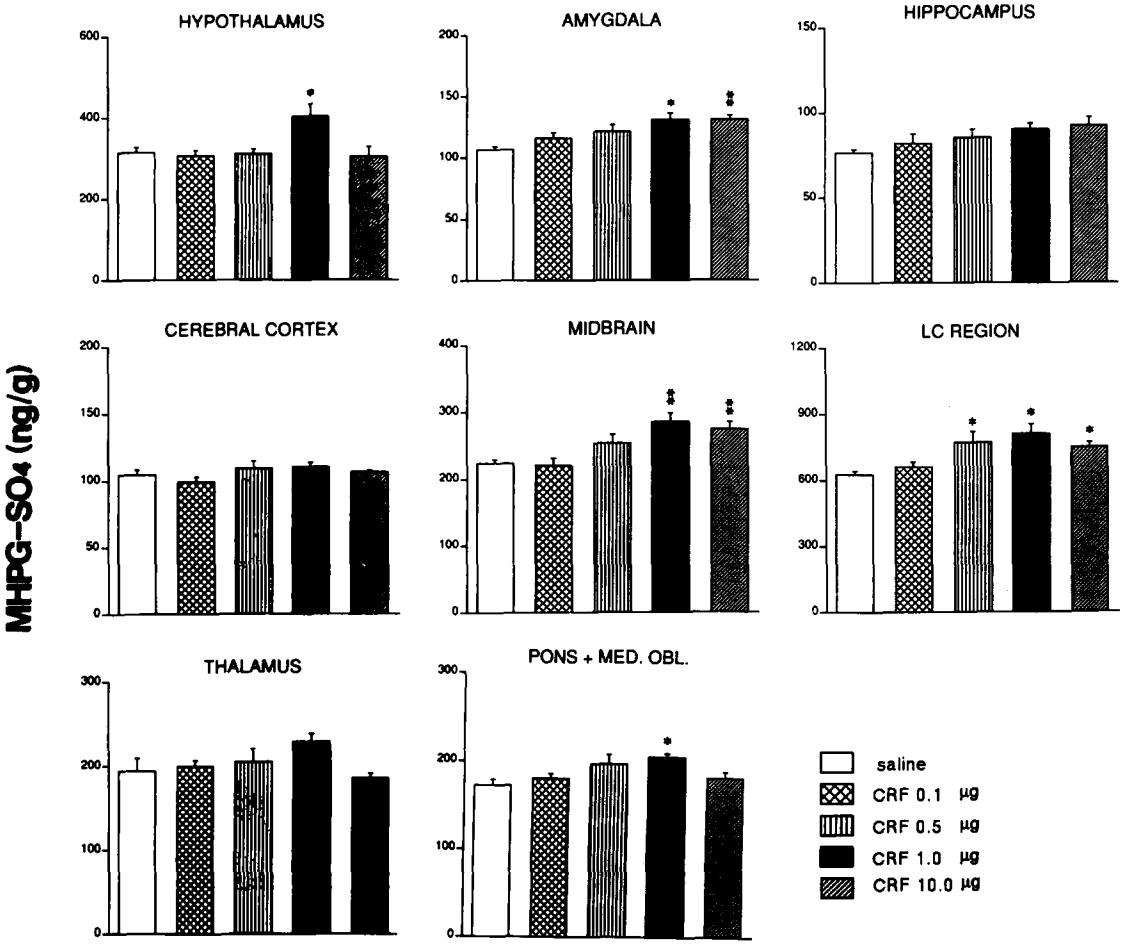

FIG. 1. Effects of ICV corticotropin-releasing factor (CRF) administration on increases in levels of MHPG-SO_4 in eight brain regions. Each value indicates the mean ± SEM of eight rats. Statistical significance between each dose of CRF vs. saline control was compared by the Newman-Keuls test (*$p < 0.05$, **$p < 0.01$).

compared with the saline group. Figure 2 depicts levels of plasma corticosterone in the experimental groups. ANOVA of corticosterone levels revealed a significant group effect, $F(4, 35) = 10.07$, $p < 0.05$, and Newman-Keuls tests indicated that the 0.5-, 1.0-, and 10.0-μg CRF groups showed significantly higher levels of corticosterone as compared with the saline group.

DISCUSSION

In the present study, the results that ICV administration of CRF significantly increased plasma corticosterone levels, consistent with previous investigations, support the well-established finding that CRF activates the anterior pituitary gland, with consequent release of corticosterone from the adrenal cortex into the circulation (21). Further, this could be a suggestive finding that the action to the HPA system by CRF is via a central mechanism.

CRF caused significant increases in MHPG-SO_4 levels in five brain regions, including the hypothalamus, amygdala, LC region, midbrain, and residual pons + medulla oblongata. These results suggest that CRF causes an increase in NA release as well as NA metabolism in these areas because MHPG-SO_4 was reliably identified as the main metabolite of NA, especially in the rat brain (14,17). The present findings are in agreement with previous studies showing that CRF increases MHPG levels in the prefrontal cortex, hypothalamus, and brain stem in mice (6) and the MHPG : NA ratio in the frontal cortex and hippocampus (16). Further, bilateral infusion of CRF at 1 μg into the LC produced significant increases in the concentration of DHPG, one of the NA metabolites, in the amygdala and posterior hypothalamus (2).

Although the mechanism by which CRF activates the NA neuron system is unclear, it is likely that exogenous CRF activates it via CRF receptors, which may exist on the noradrenergic cell body and/or on the nerve terminal sites. This is supported by the findings that a direct application of CRF into the LC increases the firing rates of LC neurons, demonstrated with the electrophysiological study (30), and that extensive distribution of CRF binding sites were shown throughout the CNS (4). This receptor-mediated CRF action on the NA neurons is further supported by our preliminary study, in which the CRF antagonist α-helical CRF partially blocked the increase in MHPG-SO_4 levels induced by immobilization stress (unpublished data).

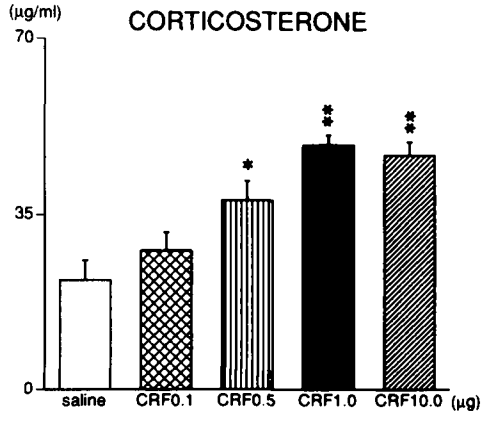

FIG. 2. Effects of ICV corticotropin-releasing factor (CRF) administration on increases in levels of plasma corticosterone in eight brain regions. Each value indicates the mean ± SEM of eight rats. Statistical significance between each dose of CRF vs. saline control was compared by the Newman-Keuls test (*$p < 0.05$, **$p < 0.01$).

Unexpectedly, there were a few inconsistencies concerning the effect of the high-dose range and the regional effect in contrast to the previous reports. We failed to detect statistically significant effects in the hippocampus, thalamus, and cerebral cortex; nevertheless, CRF tended to increase MHPG-SO_4 levels in the present study. Although the reason for this discrepancy is unclear, in the cerebral cortex it may be derived from the difference of tissue sampling for this area. We collected the whole cortex in this investigation, compared to the frontal or prefrontal cortex in previous reports. One more possible reason we considered is that CRF may act locally on the NA neuronal system. In our previous studies, diazepam attenuated stress-induced increases in MHPG-SO_4 levels in the LC but not in the thalamus and midbrain, to which the LC projects their fibers, and psychological stress and conditioned fears, where the emotional factors are predominantly involved, increase MHPG-SO_4 levels preferentially in the LC, hypothalamus, and amygdala but not in the cerebral cortex, hippocampus, and thalamus, where they are projected from the LC. These findings suggest there might be a possibility that certain alterations of noradrenergic activity in the LC are not always reflected to the changes in its terminal sites, as mentioned by Tanaka et al. (27). Further, because it is demonstrated that the spatial distribution within the rat LC of neurons projecting to particular brain regions (15), the activating effect of CRF to brain NA metabolism via heterogeneous CRF receptors that may be distributing to NA neurons, could not be always exerted on all noradrenergic terminal and cell body sites. There was not shown a dose-dependent manner in several regions. This may be due to different technical methods and substrates for the measurement of NA metabolites in contrast to other investigations. By measuring extracellular NA release using in vivo microdialysis, we provided the direct evidence that ICV injection of CRF at a dose of 3 μg increases NA levels in the anterior hypothalamus (9), but we shall need further study to clarify these discrepancies.

Increases in NA metabolism, particularly in the hypothalamus, amygdala, and LC region, caused in the regions examined may be related to provoking negative emotional responses in animals because similar regional selectivity was found by our above-mentioned psychological stress paradigms on NA neuronal activity. While numerous reports are shown in review by Dunn and Berridge (7) and by many investigators, centrally administered CRF produces stress-like anxiety-related behavioral changes (1) that were blocked by inhibiting agents of CRF (3). Also, stress is likely to provoke CRF activity in several brain regions (12,13). Moreover, clinical studies showed that CRF levels in human CSF were elevated in depressed patients (19) and that there was also a marked reduction in the number of CRF receptors in the frontal cortex of suicide victims (18). These findings would surely support that the brain CRF is closely related with provoking the emotions of anxiety and/or fear. Together, activated NA metabolism in the hypothalamus, amygdala, and LC region induced by CRF might be involved as one of several possible neural mechanisms for eliciting such hyperemotional responses as anxiety and/or fear.

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