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Differential Effects of Corticotropin-Releasing Hormone on Central Dopaminergic and Noradrenergic Neurons

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Median eminence
Paraventricular nucleus
Corticotropin-releasing hormone

Abstract
Corticotropin-releasing hormone (CRH) has been shown to be a central mediator for most, if not all, stress-induced responses. Since stressful stimuli may decrease hypothalamic tuberoinfundibular and tuberohypophysial dopaminergic neuronal activities, we aimed to determine whether CRH is involved. Using central administration of various doses of ovine CRH (oCRH; 1, 3 and 10 μg/rat) into the lateral cerebroventricle of either male or female rats, the neurochemical changes in various parts of the central nervous system, including the hypothalamus, were determined by high-performance liquid chromatography at various times after the injection (30, 60, 120 and 240 min). The concentrations of 3,4-dihydroxyphenylacetic acid (DOPAC) and 3-methoxy-4-hydroxy-phenylethyleneglycol (MHPG), two major metabolites of dopamine and norepinephrine, respectively, in discrete brain regions were used as indices for catecholaminergic neuron activity. Plasma corticosterone levels increased significantly after all doses of oCRH and at all time points studied. oCRH also exerted significant stimulatory effects on noradrenergic neuron terminals in the frontal cortex, and on dopaminergic neuron terminals in the nucleus accumbens, hypothalamic paraventricular and periventricular nuclei, and intermediate pituitary lobe. Dopaminergic neuron terminals in the median eminence and the neural lobe of the pituitary, however, were not affected. There was no major difference in the responses between male and female rats. We conclude that CRH has a differential effect on central catecholaminergic neurons.

Introduction

Corticotropin-releasing hormone (CRH), a 41-amino acid peptide originally isolated from ovine hypothalami [45], is the major factor that regulates the synthesis and release of pituitary proopiomelanocortin-derived peptides, which include the adrenocorticotropin (ACTH) [37]. Besides its role in the control of pituitary hormones, CRH may also act as a neuromodulator or a neurotransmitter in the central nervous system. Several lines of evi-

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dence support this hypothesis: (1) CRH is widely distributed throughout the brain and spinal cord, as determined by both radioimmunoassay [35] and immunohistochemical [43] methods; (2) CRH alters neuronal discharge rate in a number of brain loci as measured either by extracellular recording in vivo [13, 46] or by intracellular recording in vitro [1]; (3) specific, high affinity ($K_d \approx 0.2$ n*M*) CRH receptor binding sites have a widespread and nonuniform distribution in the CNS [9]; (4) central administration of CRH elicits potent effects on a variety of animal behaviors [20].

Activation of the hypothalamopituitary-adrenal axis is the major endocrine response to a variety of stressful manipulations. It has also been shown that CRH administered intracerebroventricularly (i.c.v.) leads to anxiogenic behaviors [42] and large increases in plasma ACTH, heart rate, and mean arterial blood pressure [2], responses similar to those associated with stress. Thus, it has been postulated that CRH may be a primary central mediator that integrates endocrine and autonomic responses to stress [11, 14].

It is well documented that stress may affect brain aminergic neuronal systems [12, 49]. In animal models, acute stress activates norepinephrine (NE) neuronal systems; increased NE turnover and decreased NE concentration occur in regions receiving projection from the locus ceruleus [12, 49]. Studies from this laboratory have also shown that restraint stress decreases tuberoinfundibular (TI) [7] and tuberophypophysial (TH) [28] dopamingergic (DA) neuronal activities. We hypothesized that if CRH is a major central mediator for stress, we should observe stress-induced effects on central catecholaminergic neurons by administering CRH directly into the brain.

Although previous studies [3, 10, 12, 21, 31, 46] have shown that CRH can activate central noradrenergic and DA neurons, most have focused on the locus ceruleus noradrenergic and the mesolimbic DA systems. Few reports have focused on lateral tegmental noradrenergic and incertohypothalamic DA systems [41, 47], and none on the TIDA or THDA neurons.

The purpose of the present report was to determine further the effects of CRH on the activities of most central noradrenergic and DA terminals, with special focus on the hypothalamic regions. Since the basal activity of TIDA neurons differs between the sexes [8], both intact male and ovariectomized plus estrogen-treated (OVX+E_2) female rats were used for comparison. Brain NE and DA neuronal activities were estimated by measuring the concentrations of their major metabolites. The results show that while CRH activated DA and NE neuronal activities in several brain regions and raised plasma corticosterone levels, it had no effect on the activity of TIDA neurons.

Methods

Adult male and female Long-Evans rats weighing 200–225 g were purchased from Harlan Laboratories (Indianapolis, Ind.). All female rats were ovariectomized on day 0, and were implanted on day 4 with cannulae (23-gauge stainless tubing, 1 cm) in the lateral cerebral ventricle, and with Silastic capsules (20 mm, No. 602-285, Dow Corning, Midland, Mich., USA) containing estradiol-17β (E_2, Sigma Chemical Co., St. Louis, Mo., USA; 150 µg/ml corn oil) subcutaneously. The male rats were implanted with the same cannulae in the ventricle on day 3. On day 7, all the rats were injected i.c.v. through their cannulae with either artificial cerebrospinal fluid (ACSF, 3 µl) or ovine CRH (oCRH, 1, 3 or 10 µg/3 µl/rat, Peninsula, Belmont, Calif., USA). In the dose-response study, all the rats were decapitated 60 min after the injection. In the time-course study, the rats were decapitated 30, 60, 120 or 240 min after the injection of oCRH (10 µg/rat) and 60 min after ACSF.

Plasma samples were collected and stored at −20 °C until assayed for hormone levels. Brains and pituitaries were quickly removed and frozen on dry ice. Frontal brain sections (600 µm) were prepared by using a cryostat, beginning cutting at approximately A9.2 mm [19]. Sections were thaw-mounted onto glass slides. The median eminence (ME), frontal cortex (FCTX), nucleus accumbens (NA), periventricular nucleus (A14), and paraventricular nucleus (PVN) were dissected from these sections using a modification [27] of the punch technique of Palkovits [34]. The neural lobe (NL) and the intermediate lobe (IL) were dissected from frozen pituitary glands using a technique described previously [25]. Tissue samples were placed in 60 µl of 0.1 *M* phosphate-citrate buffer (pH 2.5) containing 15% methanol and stored at −20 °C.

The activities of dopaminergic neurons were assessed by measuring the concentration of 3,4-dihydroxyphenylacetic acid (DOPAC), the major metabolite of DA, in various brain regions innervated by DA neurons. This has been shown to be a reliable index for DA neuronal activity [26]. The activities of noradrenergic neurons were assessed by measuring 3-methoxy-4-hydroxy-phenylethyleneglycol (MHPG), the major metabolite of NE. In the rat brain, MHPG exists as a sulfonated conjugate which must be hydrolyzed before it can be detected electrochemically. Accordingly, supernatants were heated for 5 min at 94 °C in 0.15 *M* perchloric acid to liberate free MHPG [29]. The concentrations of DA, DOPAC, NE and MHPG in the brain tissue samples were determined by high-performance liquid chromatography with electrochemical detection as described previously [4]. The tissue pellets were dissolved in 1.0 *N* NaOH and assayed for protein content [30]. Plasma corticosterone levels were determined by a radioimmunoassay kit (ICN Biochemicals, Costa Mesa, Calif., USA).

Statistical analysis was conducted using one-way analysis of variance followed by Student-Newman-Keuls test.

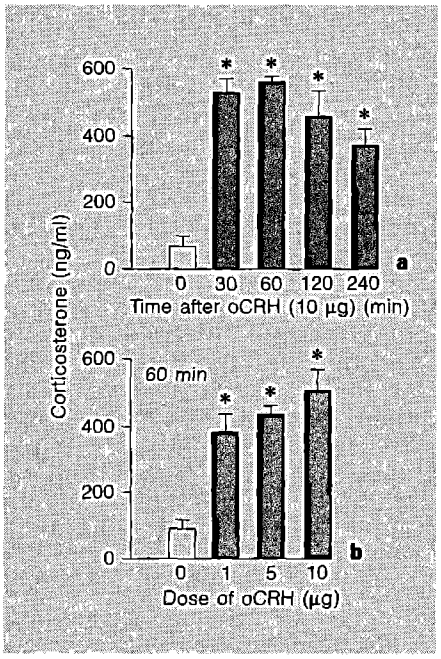


Fig. 1. Time- and dose-dependent effects of oCRH on plasma corticosterone levels in male rats. A single dose of oCRH (10 µg/rat) was injected i.c.v. and the animals were sacrificed 30, 60, 120 or 240 min later (**a**). Various doses of oCRH (1, 3 or 10 µg/rat) were injected i.c.v. and animals were sacrificed at 60 min (**b**). Animal numbers were 8–10/group. * $p < 0.05$, vs. ACSF-injected control.

Results

I.c.v. administration of oCRH, at all doses and at all sampling times, increased plasma corticosterone levels in the male rats (fig. 1). The levels peaked by 30 min, and declined somewhat after 60 min. The effects were dose-dependent (1–10 µg) and significant increases were observed after 1 µg oCRH.

In male rats, the 10 µg dose of oCRH significantly increased the DOPAC levels in the NA and PVN at all sampling periods, in the A14 at 60 min, and in the IL from 60 to 240 min after injection (fig. 2). When 60 min was used as the sampling time, either 3 or 10 µg of oCRH increased DOPAC levels in the NA, A14, PVN and IL (fig. 3), whereas the 1 µg dose was effective in the NA only. In contrast to the above regions, the DOPAC levels in the ME or NL were not affected at all [data not shown], indicating that central DA neurons were differentially affected by oCRH.

The MHPG levels in FCTX and A14 regions in the male rats were also significantly increased after 10 µg oCRH injection (fig. 4). The effects, however, were not strictly dose-dependent (1–10 µg) when sampled at 60 min (fig. 4). The increases in A14 did not reach statistical significance (fig. 4). Those in PVN were not affected at all [data not shown], indicating that central NE neurons were also differentially affected by oCRH.

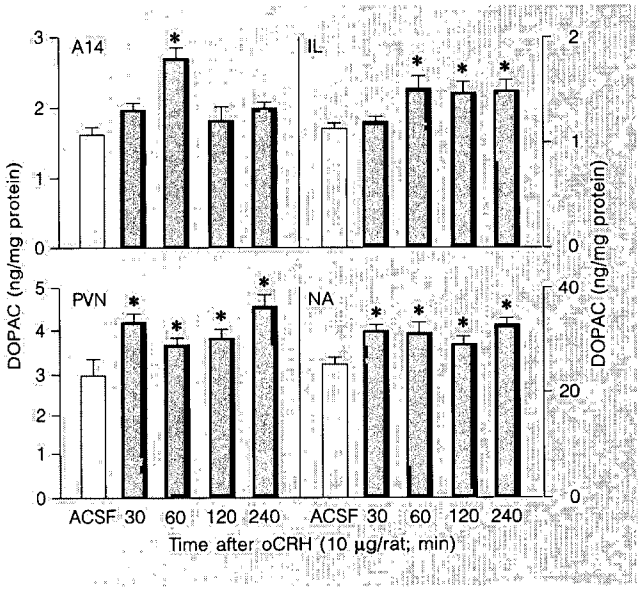
In OVX+E_2 rats, the effects of oCRH (10 µg) were essentially the same as those obtained in male rats except for the NL region (table 1). oCRH increased DOPAC levels in the NA, A14, PVN and IL, but not in the ME. It is noteworthy that the DOPAC level in the NL region was also increased in OVX+E_2, but not in male rats. oCRH also increased MHPG levels in the FCTX and the A14, but not in the PVN (table 1), similar to results for male rats.

The concentrations of DA and NE in all the regions measured did not change significantly after 10 µg oCRH injection. Since the storage pool of catecholaminergic neuron terminals is quite large, short-term activation of release usually does not change their content too much. We also calculated the DOPAC/DA or MHPG/NE ratios of each region as another index for neuronal activity, and the results were identical with those using DOPAC or MHPG alone.

Discussion

It has been shown repeatedly that central administration of CRH activates the hypothalamopituitary-adrenal axis [3, 15, 40]; our time- and dose-dependent studies confirm these earlier results. These results are important because they assured us of the effectiveness of our i.c.v. injection of CRH. It has been proposed that hypothalamic releasing hormones, e.g., gonadotropin-releasing hormone, thyrotropin-releasing hormone, may use the cerebral ventricles as a route for transport to the ME and eventually to the anterior pituitary gland [17, 18, 48]. Another study shows that there may be a positive ultrashort feedback loop for CRH such that intracerebral CRH stimulates its own release [32]. Both mechanisms can contribute to the activation of the hypothalamopituitary-adrenal axis.

There is also mounting evidence showing that central administration of CRH produces a variety of responses (hormonal, physiological, neurochemical, behavioral) that are similar to those produced by stress [11, 14]. For example, central noradrenergic systems are activated by stress as well as by CRH [10, 12, 31, 46]. There are two major central noradrenergic systems, one originating from the locus ceruleus and one from the lateral tegmental



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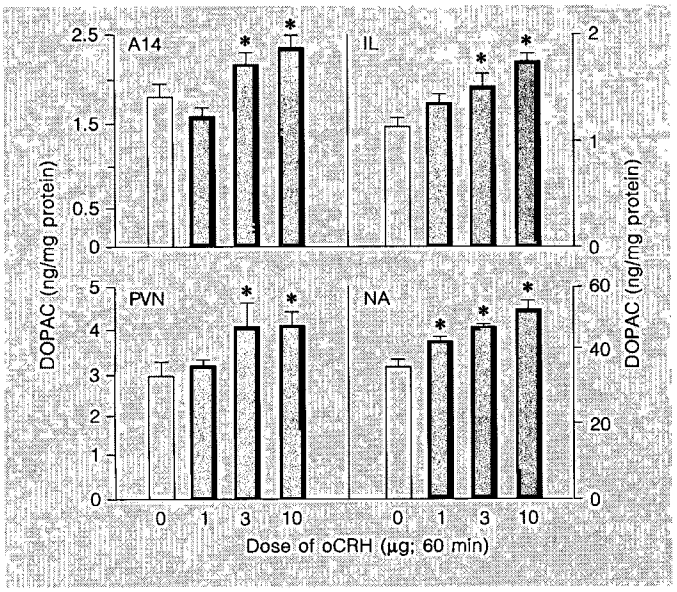

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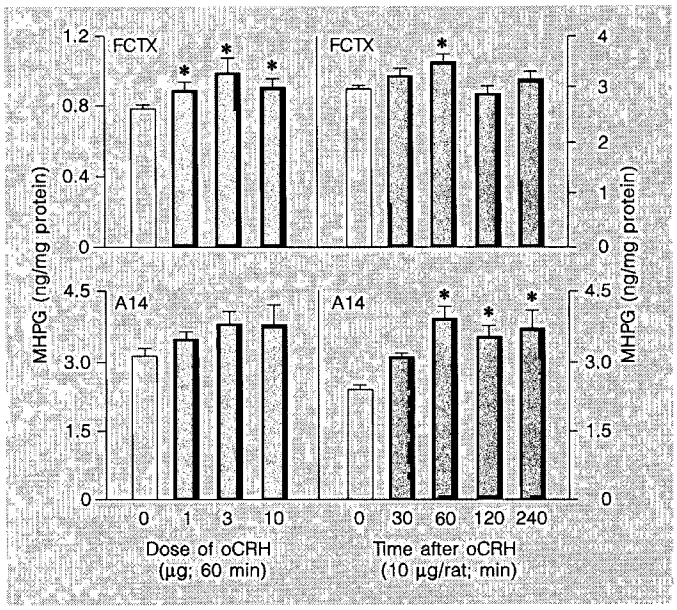

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Fig. 2. Time-dependent effects of i.c.v. administration of 10 µg oCRH in male rats on DOPAC concentrations in A14, PVN, IL and NA. * $p < 0.05$, vs. ACSF-injected control (n = 8–10).

Fig. 3. Dose-dependent effects of oCRH (1, 3 and 10 µg i.c.v.) in male rats on DOPAC concentrations in A14, PVN, IL and NA. * $p < 0.05$, vs. ACSF-injected controls (n = 8–10).

Fig. 4. Dose- and time-dependent effects of oCRH in male rats on MHPG concentrations in the FCTX and A14. * $p < 0.05$, vs. ACSF-injected controls (n = 8–10).

Table 1. Effects of oCRH on DOPAC and MHPG concentrations of selected brain regions of OVX+E_2 rats

	DOPAC, ng/mg protein						MHPG, ng/mg protein		
	ME	IL	NL	NA	A14	PVN	FCTX	A14	PVN
ACSF	11.62±1.12	1.61±0.13	0.88±0.08	27.5 ±1.47	1.47±0.12	1.79±0.28	1.95±0.14	8.54±0.27	12.03±0.96
30 min	12.83±0.96	2.42±0.16*	1.00±0.04	32.12±1.85	1.71±0.09	6.44±0.89*	2.10±0.11	7.95±0.28	12.24±1.67
60 min	11.90±0.59	2.36±0.18*	1.10±0.06*	34.05±1.40*	1.93±0.07*	2.69±0.47	2.53±0.08*	10.0±0.58*	13.78±1.25
120 min	12.21±0.54	2.76±0.30*	1.03±0.05	31.46±2.15	1.78±0.22	3.18±0.83	2.66±0.12*	8.54±0.51	13.78±1.48
240 min	11.19±0.81	2.90±0.19*	1.07±0.05	32.73±2.04	1.69±0.16	2.56±0.48	2.69±0.17*	8.19±0.34	12.27±0.75

Rats that had been ovariectomized 7 days previously and had received subcutaneous estrogen implants (OVX+E_2) and i.c.v. cannulation 3 days previously were used. Each rat received a single dose of oCRH (10 µg/rat i.c.v.) in the morning and was sacrificed at 30, 60, 120 or 240 min later. DOPAC and MHPG levels in various brain regions are expressed as mean ± SEM, n = 9–10 rats. * $p < 0.05$, vs. ACSF control.

region [6]. Most early studies focused on central areas that are innervated by the former system, e.g. the FCTX [10, 31, 46], and few reports focus on regions in the later system, e.g. the hypothalamus [3, 41]. Our data show that the noradrenergic neurons that project to the FCTX were consistently stimulated by oCRH, but those to the PVN and A14 were not, indicating that the two systems may be differentially activated by CRH.

As for the central DA systems, CRH also exerted mostly excitatory effects in various regions with the exception of ME and NL. That CRH can stimulate mesolimbic DA neurons and produces related behavioral responses has been reported [21, 31], and our results for the NA confirm these findings. The DA neurons in the hypothalamus, however, have not received as much attention. Part of the reason may be that hypothalamic nuclei are rather small and their specific functions are not recognized so well. An earlier study by Van Loon et al. [47] reported that CRH has no effect on dopamine synthesis/turnover rate in the whole hypothalamus region. The use of the micropunch technique in this study enabled us to dissect out different DA terminal regions in the hypothalamus, and the results revealed that CRH can have a differential effect on DA neurons in different regions of the hypothalamus. Using the whole hypothalamus may obscure the differences. There is morphological evidence showing that CRH neurons innervate DA neurons in the A14 region of macaques [44], and DA fibers are associated with CRH neurons in the PVN of rats [24]. Our results that oCRH induced time- and dose-dependent activations of dopaminergic neuron activity in the A14 and PVN regions provide direct evidence for the action of CRH.

As for the possible role of CRH in the PVN and A14, it has been shown that central administration of CRH also decreases plasma luteinizing hormone and growth hormone levels in gonadectomized rats [33, 38, 39], in addition to its corticotropin-releasing effect. Changes in the release of hypothalamic gonadotropin-releasing hormone and somatostatin may be involved [37–39], and the activation of DA neurons in the PVN and A14 by oCRH may be an underlying mechanism.

Earlier studies from this lab have shown that stress-induced prolactin secretion may be mediated by a decrease in TIDA neuron activity [7, 28]. The theory that CRH may be 'the' central mediator for stress [11, 14] has led us to the hypothesis that CRH may also be involved in the stress-induced reduction of TIDA neuron activity. Our results, however, do not support this. None of the three doses of oCRH induced any change in TIDA neuron activity at three time points in either sex.

A sexual difference in the activities of TIDA neurons and their responses to stress has been reported previously [8]. The basal TIDA neuron activity is higher in female than in male rats, and stress decreases the TIDA neuron activitiy in female rats only. If CRH is involved in this stress-induced decrease in TIDA activity, we should observe a significant effect of CRH on TIDA neurons in female, but not in male, rats. Our results, however, demonstrate that CRH had no effect on TIDA neuronal activity in either sex. These results indicate that the stress-induced decrease in TIDA neuronal activity may not be mediated by CRH alone.

The THDA neurons that project to the neurointermediate lobe of the pituitary gland have been subdivided into two separate systems: one terminates specifically in the NL and the other in the IL [25]. Using a special dissecting technique as reported previously [25], we can separate the NL and IL from frozen pituitary gland and measure their DOPAC and DA contents individually. This is also a firsthand finding that oCRH exhibits a differential effect on DA neuron activity in the IL and NL. Our finding that oCRH caused a slight but significant increase of DOPAC level in the NL region of OVX+E_2, but not male rats, indicates that some sexual difference may exist in that region, although no previous study has focused on this point before.

The synthesis and release of α-melanocyte-stimulating hormone (α-MSH) from the IL are influenced by both CRH [36] and THDA neurons [5, 16]. The DOPAC concentration in the IL has been shown to be a reliable index of THDA neuron activity [23]. Furthermore, plasma α-MSH levels also correlate well with THDA neuron activity [22]. The finding that oCRH had a consistent stimulatory effect on the IL's THDA neuronal activity provides another facet of CRH's regulation of the release of α-MSH. By itself, CRH has a stimulatory role on the release of α-MSH; by stimulating the THDA neuronal activity, it may also have an inhibitory effect. The physiological role of CRH on THDA neurons remains to be elucidated.

We conclude from these findings that although central administration of CRH produced many of the neurochemical effects similar to those induced by stress, it played no significant part in the control of TIDA neurons.

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