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Corticotropin-Releasing Factor Administration Elicits a Stress-Like Activation of Cerebral Catecholaminergic Systems

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DUNN, A. J. AND C. W. BERRIDGE. *Corticotropin-releasing factor administration elicits a stress-like activation of cerebral catecholaminergic systems.* PHARMACOL BIOCHEM BEHAV 27(4) 685–691, 1987.—The cerebral content of the biogenic amines, dopamine (DA), norepinephrine (NE), and serotonin (5-HT) and their catabolites 30 min after CRF or saline injections was determined using HPLC with electrochemical detection. Injection of CRF (1.0 μg) into the lateral ventricles (ICV) of mice produced a behavioral activation in which their motor movements appeared as bursts of activity followed by periods of immobility. CRF administration (ICV or SC) did not alter the concentrations of DA, NE, tryptophan, 5-HT, or 5-hydroxyindoleacetic acid (5-HIAA) in any brain region measured. ICV CRF increased the concentrations of dihydroxyphenylacetic acid (DOPAC), the major catabolite of DA, and of 3-methoxy,4-hydroxyphenylethyleneglycol (MHPG), the major catabolite of NE, in several brain regions. DOPAC:DA ratios were consistently increased in prefrontal cortex, septum, hypothalamus, and brain stem relative to animals injected with saline. MHPG:NE ratios were also increased in the prefrontal cortex and hypothalamus, with a marginal effect ($p=0.06$) in brain stem. SC CRF significantly increased DOPAC:DA in prefrontal cortex, and MHPG:NE in prefrontal cortex, hypothalamus and brain stem. Pretreatment with naloxone did not prevent any of the neurochemical responses to ICV CRF, but naloxone alone increased DOPAC:DA in medial profrontal cortex, and decreased MHPG:NE in nucleus accumbens in CRF-injected mice. These results suggest that administration of CRF either centrally or peripherally induces an activation of both dopaminergic and noradrenergic systems in several regions of mouse brain. The pattern resembles that we observe in mice following stressful treatments such as footshock or restraint, but the effect of CRF on noradrenergic systems is less pronounced. Also, brain free tryptophan which is consistently increased in all brain regions by footshock or restraint was not altered by CRF. Thus CRF triggers a response in CNS catecholamine systems that resembles, but does not precisely mimic, that observed following commonly used stressors. This activation of CNS catecholamine metabolism may be related to some of the behavioral effects of CRF, but not all of them because naloxone, which prevents the effects of CRF on exploratory behavior, did not alter the catecholamine responses to CRF.

Corticotropin-releasing factor (CRF) Stress Dopamine Norepinephrine
Dihydroxyphenylacetic acid (DOPAC) 3-Methoxy,4-hydroxyphenylethyleneglycol (MHPG)
Serotonin (5-HT) 5-Hydroxyindoleacetic acid (5-HIAA) Tryptophan Corticosterone Naloxone

CORTICOTROPIN-releasing factor (CRF) has been characterized as a 41-amino acid polypeptide by Vale *et al.* [28]. Immunohistochemical studies indicate that the distribution of the peptide is not confined to hypothalamic neurons projecting to the median eminence region; CRF-containing cell bodies and fibers have also been identified in the cerebral cortex, septum, amygdala, brain stem, and spinal cord [19,24]. Autoradiographic studies of binding sites for CRF have shown localization in cerebral cortex, septum, amygdala and hippocampus [8,34]. Thus, like many other hypothalamic hormones, CRF probably has dual roles: a classical endocrine one involving release in the hypothalamic median eminence region and transport through the portal vascular system to stimulate the release of ACTH from the anterior pituitary; and an as yet undetermined role in other regions of the brain. A clinical significance for cerebral CRF may be inferred from the reported elevation of CRF in CSF from depressed patients [21], and decreased CRF in cortex and striatum of brains from Alzheimer's disease patients [2].

Intracerebroventricular (ICV) injection of CRF into rats in microgram quantities has been reported to cause a behavioral activation, similar to that which occurs in certain stress-

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ful situations [16,23]. It also, stimulates the peripheral sympathetic nervous system, increasing plasma concentrations of catecholamines, and indices of sympathetic function, such as blood pressure and heart rate [5]. Thus ICV CRF mimics some of the effects of stress.

To determine whether CRF produces a neurochemical response like that in stress, we have administered it ICV and SC to mice and measured the cerebral content of the catecholamines, indoleamines, and their major catabolites using HPLC with electrochemical detection (HPLC-EC).

METHOD

Synthetic human/rat CRF was obtained from Peninsula Laboratories. It was freshly dissolved in isotonic saline containing 0.001 M HCl shortly before use. Male CD-1 mice (25–35 g) were obtained from Charles River Laboratories (Wilmington, MA). They were group-housed until immediately before surgery when they were placed in individual Plexiglas cages with wood shavings as bedding and unlimited access to food and water. For ICV injections, plastic cannulae were inserted into both cerebral lateral ventricles under barbiturate anesthesia as previously described [14]. After surgery, mice were housed individually (4–7 days) and weighed about 30±4 g at the time of CRF injection. For SC injection mice were placed in individual cages 3 days before the experiment.

On the day of the experiment, each mouse received an injection of CRF or saline, and was immediately replaced in its home cage from which water and food had been removed. ICV injections were performed without anesthesia introducing 2 μl into each lateral ventricle. SC injections were in a total volume of 0.1 ml. In one experiment, mice were injected SC with naloxone (Endo Labs, 0.8 mg/kg) immediately before CRF. In some experiments, the subsequent behavior of each mouse was scored every 15 sec using a time-sampling procedure [7] by a trained observer unaware of the identity of the injectate. Behavior was scored as locomotion; grooming; rearing; movement (other than locomotion, rearing, or grooming); eating; drinking; and quiet (motionless). Each mouse was removed from the colony room, decapitated precisely 30 min later and the brain excised. Trunk blood was also collected. The brain was immediately chilled with ice-cold isotonic saline, and dissected into prefrontal cortex, nucleus accumbens septi, caudate-putamen, septum, hypothalamus, hippocampus, and brain stem as previously described [9,11]. In one experiment, only the medial part of prefrontal cortex [15] was examined. Each brain part was immediately weighed in a tared 1.5 ml Eppendorf tube and frozen on dry-ice. Samples were then homogenized within 24 hours using a Kontes Micro-Ultrasonic Cell Disrupter. The homogenizing medium was 0.4–1.0 ml of 0.1 M $HClO_4$ containing 0.1 mM EDTA and 10–100 ng of N-methyldopamine (depending on the region) as an internal standard. The homogenates were then frozen and subsequently centrifuged to remove the macromolecular precipitate. All samples were stored at −55° until shortly before analysis.

The HPLC apparatus consisted of an LDC Milton-Roy pump (Model 396), a Waters WISP 710B automatic samples injector, a Bioanalytical Systems TL-5A cell with a glassy carbon electrode, maintained at 0.80–0.85 V with respect to a Ag/AgCl reference electrode, a Bioanalytical Systems LC-3A detector, and a Hewlett-Packard 3390A integrator. The column was a 20 cm 5 micron ODS C18 reverse-phase column (HPLC Technology). The mobile phase was 0.05 M sodium phosphate-0.05 M sodium citrate (pH 3.0–3.2) containing 0.2 mM sodium octyl sulfonate (Eastman-Kodak), 0.1 mM EDTA, and 6–10% methanol (v/v) pumped at 1.0 ml/min. The column was maintained at 39° by a BAS column heater. The contents of norepinephrine (NE), dopamine (DA), dihydroxyphenylacetic acid (DOPAC), homovanillic acid (HVA), 3-methoxytyramine (3–MT), normetanephrine (NM), 3-methoxy,4-hydroxyphenylethyleneglycol (MHPG), tryptophan, serotonin (5-HT) and 5-hydroxyindoleacetic acid (5-HIAA), and NMDA were determined with reference to freshly diluted standards (Sigma Chemical Co., St. Louis, MO) using an internal standard correction procedure. Not all of these compounds were present in sufficient amounts in all brain regions to yield consistent and reliable determinations. Chromatographic conditions were adjusted by minor alterations of the pH, and methanol and octyl sulfonate concentrations so that all these compounds gave clear peaks that did not overlap with uric acid, tyrosine, dopa, and tyramine. In the mouse, the proportion of MHPG that is sulfated is very low [32] so that this important catabolite of NE can be measured in untreated extracts.

Plasma corticosterone was determined by radioimmunoassay following extraction of the total steroids with methylene chloride. The assay was performed using kits provided by Cambridge Medical Diagnostics, and also with the antibody and procedure described by Underwood and Williams [27].

Calculations including statistical analyses were performed on an Apple IIe computer using a commercial spreadsheet program, Visicalc™. Statistical analyses were performed by 2-factor ANOVA with post hoc Student's t-tests, and by Dunnett's test in those experiments with multiple comparisons to one control group.

TABLE 1

BEHAVIORAL RESPONSES TO ICV CRF

Treatment	Behavioral Scores: Grooming	Locomotor Activity	Other Movement
Saline	5.3 ± 1.9	18.6 ± 4.0	23.3 ± 4.7
0.2 μg CRF	3.2 ± 1.2	15.3 ± 4.2	28.7 ± 2.6
1.0 μg CRF	3.8 ± 3.6	16.7 ± 3.2	33.7 ± 3.7*

Scores are the mean ± SEM number of 15 sec behavioral scores in each category for the 30 min following ICV injection into mice (n=9) of saline (vehicle) or CRF. A Dunnett's test on the "other movement" scores fell just short of statistical significance. $t(22)=1.98$, for the difference between the 1 μg CRF and saline groups, but a Student t-test indicated statistical significance. $t(16)=1.86$, $*p<0.05$. Plasma corticosterone concentrations: Saline: 203 ± 53; 0.2 μg CRF: 172 ± 27; 1.0 μg CRF: 166 ± 23 ng/ml (n.s.).

RESULTS

The conditions for the assessment of the behavioral effects of CRF were not ideal because of the intermittent disturbances caused by the need to stagger the injections of CRF to enable the collection of the tissue for neurochemical analyses. Table 1 contains the results of one experiment in which CRF was injected ICV at doses of 0.2 and 1.0 μg. One

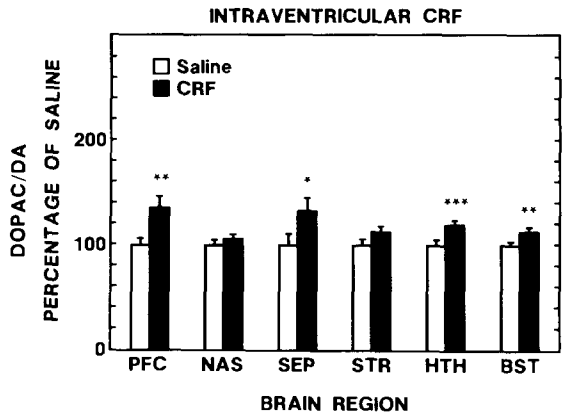

FIG. 1. The effect of ICV CRF on DOPAC:DA ratios in various brain regions. CRF (1 μg) or saline was injected into the lateral ventricles of mice and amine contents 30 min later measured by HPLC-EC. Data are the combined results of three separate experiments (two experiments each for prefrontal cortex and septum). The total numbers of animals were: 25 saline and 29 CRF, but there were occasional losses of samples and incomplete data because of inadequate HPLC resolution. PFC, prefrontal cortex; NAS, nucleus accumbens septi; SEP, septum; STR, striatum (rostral caudate-putamen); HTH, hypothalamus; BST, brain stem. DOPAC was immeasurable in the hippocampus (HPC). Mean DA and DOPAC concentrations were: PFC: 76, 48; NAS: 8160, 1090; SEP: 2180, 404; STR: 9270, 942; HTH: 650, 228; BST: 101, 68 pg/mg wet wt., respectively. Two-factor ANOVA (experiment, treatment) indicated that CRF treatment significantly increased DOPAC in PFC, $F(1,31)=8.3$, $p<0.01$, NAS, $F(1,48)=4.9$, $p<0.05$, SEP, $F(1,30)=10.4$, $p<0.005$, STR, $F(1,34)=5.4$, $p<0.05$, HTH, $F(1,46)=13.6$, $p<0.001$, and BST, $F(1,48)=32.5$, $p<0.001$. DA content was not altered in any region. DOPAC:DA ratios were increased in PFC, $F(1,30)=10.4$, $p=0.003$, SEP, $F(1,28)=4.2$, $p<0.05$, HTH, $F(1,46)=13.3$, $p<0.001$, and BST, $F(1,48)=8.3$, $p<0.005$. *Significantly different from saline ($p<0.05$); **$p<0.01$, ***$p<0.001$.

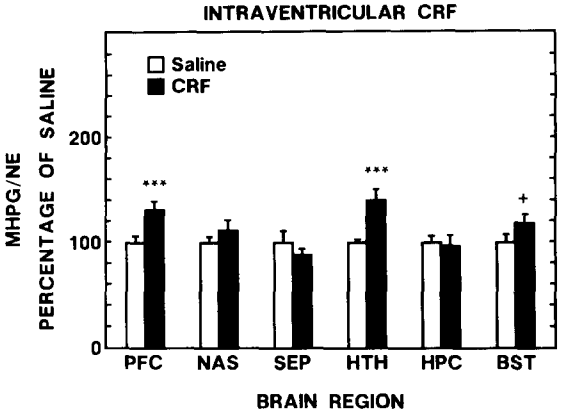

FIG. 2. The effect of 1 μg CRF ICV on MHPG:NE ratios in various brain regions. The same experiments as in Fig. 1. MHPG was undetected in STR. Mean NE and MHPG concentrations were: PFC: 535, 98; NAS: 747, 463; SEP: 743, 189; HTH: 1850, 186; HPC: 345, 95; BST: 894, 310 pg/mg wet wt., respectively. Two-factor ANOVA indicated significant effects of CRF on MHPG in PFC, $F(1,27)=10.9$, $p<0.005$, HTH, $F(1,42)=18.5$, $p<0.001$, and BST, $F(1,46)=4.3$, $p<0.05$. NE content was not altered in any region. MHPG:NE ratios were significantly increased in PFC, $F(1,27)=12.6$, $p<0.001$, and HTH, $F(1,42)=24.2$, $p<0.001$, and a marginal effect in BST, $F(1,47)=3.5$, $p=0.06$. **Significantly different from saline ($p<0.001$); $^{+}p=0.06$.

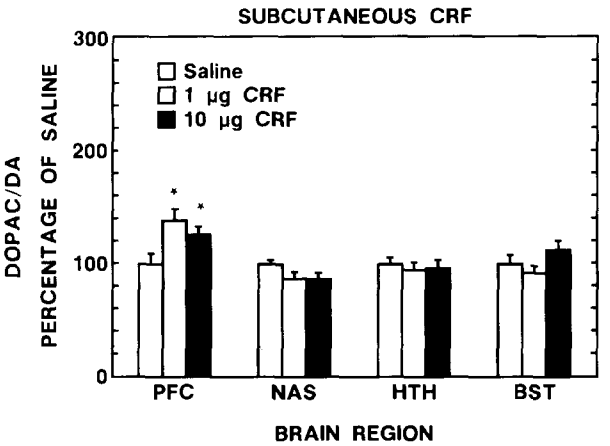

FIG. 3. The effect of SC CRF on DOPAC:DA ratios in various brain regions. CRF (1.0 or 10 μg) or saline was injected subcutaneously into mice (n=8) and amine contents 30 min later measured by HPLC-EC. *DOPAC:DA ratios were significantly increased by CRF in PFC, Dunnett's $t(19)=2.7$ (1 μg), $p<0.05$.

(but not 0.2) μg CRF resulted in behavioral changes in the mice obvious to an observer ignorant of the nature of the injections. Their motor movements appeared as bursts of activity followed by periods of immobility, a pattern not observed in saline-injected mice or those injected with 0.2 μg CRF. Perhaps because of its intermittent nature, this behavioral response was not reflected in increased locomotor activity scores, but movement scores other than locomotion, rearing, or grooming were elevated by the 1 μg dose of CRF. This effect fell just short of statistical significance on a Dunnett's test. We did not observe increases in grooming scores.

Figures 1 and 2 contain data combined from three separate experiments (including that of Table 1) in which cerebral catecholamines were measured following ICV CRF. Administration of 1 μg CRF ICV significantly increased the DOPAC contents of prefrontal cortex, nucleus accumbens, striatum, septum, hypothalamus, and brain stem, and the MHPG contents of prefrontal cortex, hypothalamus, and brain stem, without causing any statistically significant changes in the contents of the parent amines (DA and NE), or 5-HT, 5-HIAA or tryptophan. Because catabolite:amine ratios have been suggested to be a sensitive index of amine utilization, we have presented the data in this form in Figs. 1–6. DOPAC:DA ratios were significantly increased by ICV CRF in prefrontal cortex, septum, hypothalamus, and brain stem; small increases in nucleus accumbens, and the striatum were not statistically significant (Fig. 1). MHPG:NE ratios were significantly increased in the prefrontal cortex and hypothalamus (Fig. 2). An effect in brain stem was only marginally significant ($p=0.06$). In two of the three experiments, CRF injection significantly increased plasma corticosterone concentrations (e.g., Saline: 110±30; CRF 184±26 ng/ml; $p<0.02$). When 0.2 μg of CRF was injected ICV (the experiment of Table 1) the only statistically signficant changes observed were increases of DOPAC:DA in the striatum, and of MHPG:NE in the hypothalamus, suggesting that this dose was marginal for eliciting significant neurochemical changes.

Because it is possible that the actions of ICV CRF were

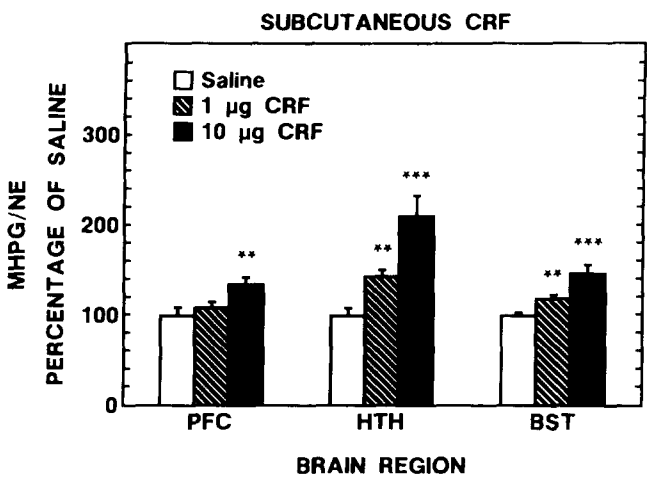

FIG. 4. The effect of SC CRF on MHPG:NE ratios in various brain regions. The same experiment as in Fig. 3. *MHPG:NE was significantly increased by 1 μg CRF in HTH, Dunnett's $t(20)=3.0, p<0.01$, and BST, $t(16)=2.4, p<0.05$, and by 10 μg CRF in PFC, $t(20)=3.8, p<0.01$, HTH, $t(20)=7.2, p<0.01$, and BST, $t(16)=5.6, p<0.01$.

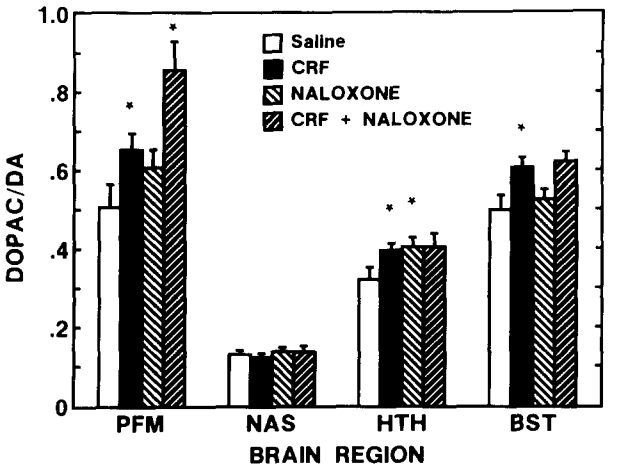

FIG. 5. The effect of ICV CRF and naloxone on DOPAC:DA ratios in various brain regions. Mice (n=8) were injected with naloxone (0.8 mg/kg) or saline immediately followed by 1 μg of CRF ICV. Brain tissue was sampled 30 min later. PFM: medial prefrontal cortex. ANOVA indicated significant effects of CRF in PFM, $F(1,26)=13.3, p=0.001$, and BST, $F(1,28)=21.6, p<0.001$, and of naloxone in PFM, $F(1,26)=7.9, p<0.01$. The interactions were not statistically significant. *CRF significantly different from the appropriate saline or naloxone control group ($p<0.05$).

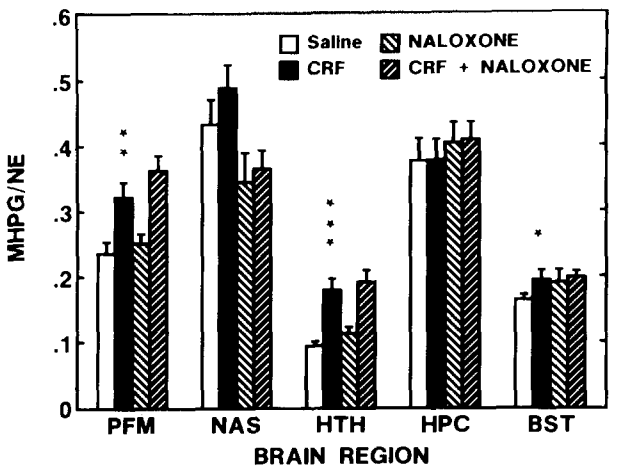

FIG. 6. The effect of ICV CRF and naloxone on MHPG:NE ratios in various brain regions. The same experiment as in Fig. 5. ANOVA indicated significant effects of CRF in PFM, $F(1,28)=29.7, p<0.001$, and HTH, $F(1,28)=43.9, p<0.001$, and of naloxone in NAS, $F(1,27)=9.1, p<0.01$. The interactions were not statistically significant. *Significantly different from CRF-saline group ($p<0.05$). **Significantly different from the saline-saline group ($p<0.01$, ***$p<0.001$).

due to leakage to the periphery and subsequent activation of the pituitary-adrenal axis, we also tested the effect of CRF injected subcutaneously at doses of 1.0 and 10 μg. DA, NE, 5-HT, 5-HIAA, and tryptophan were not significantly altered by either dose of CRF. However, DOPAC was signficantly elevated by 1 μg of CRF in prefrontal cortex and septum, and by 10 μg CRF in prefrontal cortex, hypothalamus, and brain stem. DOPAC:DA ratios were elevated significantly only in prefrontal cortex (Fig. 3). MHPG content was increased by 10 μg CRF in prefrontal cortex, and by both 1 and 10 μg CRF in hypothalamus and brain stem. MHPG:NE ratios were increased in precisely the same pattern (Fig. 4).

As expected, SC treatment with CRF significantly increased plasma corticosterone concentrations compared to saline (Saline: 76±13; 1 μg CRF: 282±21; 10 μg CRF: 310±31 ng/ml; $p<0.01$, Dunnett's test).

Naloxone has been reported to enhance the increases in catecholamine catabolites during stress [26]. On the other hand, naloxone prevents the CRF-induced increase in exploratory behavior in the multicompartment chamber in mice [1], and CRF-induced grooming in rats [10]. Therefore, we tested the effect of naloxone pretreatment on the responses to ICV CRF.

In this experiment, we examined only the medial part of prefrontal cortex (PFM), which in our studies has been the most responsive dopaminergic region in stress (unpublished observations, see also [15]). There were no statistically significant changes in the contents of DA, NE, tryptophan, or 5-HT in any of the regions tested. For DOPAC:DA ratios, ANOVA indicated increases in PFM and brain stem due to CRF, and in PFM due to naloxone (Fig. 5). Post-hoc tests revealed significant effects of CRF in PFM and brain stem, in both saline- and naloxone-treated mice. The effect of naloxone in PFM was only statistically significant in CRF-injected mice. The effects of CRF and of naloxone in hypothalamus were statistically significant when compared to saline by Dunnett's test ($p<0.05$). MHPG:NE ratios were significantly increased by CRF in PFM and hypothalamus, and decreased by naloxone in nucleus accumbens (Fig. 6). Post-hoc tests revealed significant effects of CRF in PFM and hypothalamus, in both saline- and naloxone-treated animals. The effect of naloxone in NAS was statistically significant only in CRF-injected mice. In this experiment the 5-HIAA:5-HT ratio was significantly decreased by CRF in hypothalamus (data not shown), but this effect was not observed in two other experiments. ANOVA did not indicate any significant interactions between the naloxone and CRF treatments in any metabolite or ratio in any region. Plasma corticosterone was significantly elevated by the CRF treat-

ment compared to saline-injected mice (Saline: 36±11; CRF 121±17; Naloxone 67±15; CRF-Naloxone 129±39 ng/ml; $F(1,28)=9.9$, $p<0.01$).

DISCUSSION

Because DOPAC is the major catabolite of DA, and MHPG is the major catabolite of NE in mouse brain, our neurochemical data suggest an activation by CRF of dopaminergic and noradrenergic systems in some brain areas. Although there is currently some debate as to whether production of DOPAC necessarily reflects increased release of DA [6,33], there can be little doubt that it reflects some metabolic activity of dopaminergic neurons. While some have also questioned the validity of measurements of MHPG production as an index of NE release [6], this issue is less controversial than that of DOPAC (see, for example [18]). We would like to conclude, at least tentatively, that the increased DOPAC:DA and MHPG:NE ratios reflect increases in the activity of cerebral dopaminergic and noradrenergic neurons, respectively.

In a previous study, van Loon *et al.* [30] failed to find any effect of intracisternal CRF (5 or 20 μg) on either DA or NE "turnover." These authors used inhibitors of catecholamine synthesis or degradation to determine rates of turnover, and it is possible that the use of these drugs may have masked the effect of the CRF. Moreover, they used rats rather than the mice used in the present study, and intracisternal rather than ICV injections, so that the access of the CRF to intracerebral sites was probably rather different.

Our intention in the experiments with ICV CRF was to activate cerebral CRF-receptors, but, leakage of the injected CRF to the periphery with subsequent activation of the pituitary-adrenal axis is possible, especially because of the observed increases in plasma corticosterone. Nevertheless, we consider that our effects with ICV CRF most likely result from direct action on the brain, because we have not observed changes in DOPAC:DA or MHPG:NE ratios following SC administration of ACTH or corticosterone (Dunn, unpublished observations, and submitted for publication). Moreover, the SC dose of CRF required to increase DOPAC and MHPG is substantially greater than that effective ICV. Also, we have not observed behavioral responses like those to CRF with either SC injections of ACTH or corticosterone, or ICV injections of ACTH (which consistently increase grooming in mice [7, 10, 14]). Finally, whereas ICV CRF caused behavioral activation, although we did not score behaviors following SC CRF, observations by an independent observer suggested that the 10 μg dose caused behavioral depression, indicated by a lack of activity. These differences in the behavioral responses to peripheral and central CRF mitigate against mediation by ACTH or corticosterone of the effects of ICV CRF.

An activation of cerebral noradrenergic systems by CRF would be predicted from the observed activation of locus coeruleus neurons following either ICV or iontophoretic application of CRF [29]. It may also be consistent with the general activation of electrophysiological activity observed in rats [13].

Extensive work by Brown *et al.* [5] has shown that ICV CRF potently activates the peripheral sympathetic nervous system and the adrenal medulla [4]. This is reflected not only in increases in circulating catecholamines and glucose, but also by increases in blood pressure and heart rate. Thus, an activation by CRF of central catecholaminergic systems complements the sympathetic activation, and would be consistent with the cerebral catecholaminergic systems representing the central arm of the sympathetic nervous system [22]. It is possible that the CNS catecholamine changes are secondary to the peripheral sympathetic ones, or *vice versa*. It is also possible that the neurochemical changes are secondary to changes in blood pressure, but it should be noted that whereas ICV CRF tends to produce increases in heart rate and blood pressure, peripheral administration decreases them [5]. Nevertheless, the hypotension elicited by SC CRF could account for both the behavioral depression and the CNS catecholamine changes.

The neurochemical pattern of results we observed following CRF administration resembles that we observe following stressful treatments such as footshock or restraint. Footshock treatment also increases DOPAC:DA ratios in prefrontal cortex, hypothalamus, and brain stem, and at higher footshock currents (0.5 mA) or with restraint, those in nucleus accumbens and caudate-putamen [9,11]. However, footshock or restraint treatment also substantially increases MHPG and MHPG:NE ratios in all regions [9], more markedly than observed here with ICV or SC CRF. Footshock and restraint treatments also consistently elevate brain tryptophan concentrations, an effect we have not observed following CRF administration. Thus the metabolite profile following injection of CRF resembles, but does not completely mimic those observed after footshock or restraint.

An important question is whether CRF injection itself might act as a stressor, initiating a stress response by a mechanism unrelated to the physiological actions of CRF. This response might include activation of the cerebral catecholaminergic systems. We cannot exclude this possibility, especially because both ICV and SC CRF elevated plasma corticosterone concentrations. However, the increases of corticosterone caused by ICV CRF were rather less than those observed following SC CRF, or footshock or restraint, and were absent in one experiment (Table 1). If ICV or SC CRF initiates a stress response, then the metabolic response to CRF lacks the normally observed effect of stressors on brain tryptophan. Nevertheless, given that ICV CRF can initiate stress-like behavior [1, 3, 16], it is tempting to suggest that it can also directly activate a CNS stress response.

Tanaka *et al.* [26] found that naloxone pretreatment enhanced the normal increase of brain MHPG observed in stressed rats, but we have not observed such an effect in mice (Dunn and Berridge, unpublished observations). Therefore, our failure to find significant alterations by naloxone of CRF-induced changes in DOPAC:DA or MHPG:NE ratios resembles our own data with footshock.

Sutton *et al.* [23] reported a locomotor activation in rats at doses of 1 or 10 μg of CRF ICV (confirmed by Veldhuis and de Wied [31]), and an increased sensitivity to "stressful aspects of the situation" in a novel open field. These effects were not mimicked by ACTH, and were apparently independent of the pituitary because they were present in hypophysectomized animals [12]. CRF has also been reported to enhance the behavioral effects of novelty in an open field approach test in rats, an effect that was opposite to that of benzodiazepines [3]. The pattern of behavior resembles that observed during mild stress, and this and the electrophysiological results referred to below led Koob and Bloom [16] to speculate that CRF may increase arousal or even mediate some behavioral responses in stress. The behavioral effects we observed in mice after ICV CRF are not inconsistent with these studies in rats. Despite the less than

optimal conditions for observation, we did observe some behavioral activation in the mice, although this was more evident to observers than is reflected in the behavioral scores. However, we did not observe increased locomotor activity or grooming scores in mice (see also [1,10]), in contrast to previous results in rats [3, 10, 20, 23, 31].

Whether or not the catecholaminergic activation we observed underlies the behavioral effects of ICV CRF remains to be determined. Koob *et al.* [17] presented evidence that dopaminergic blockade with alpha-flupenthixol prevented the CRF-induced locomotor activation in rats, but only at cataleptic doses and not at lower doses capable of reversing amphetamine-induced hyperactivity. Thus they concluded that dopaminergic systems were not involved in these effects of CRF. Likewise, Swerdlow and Koob [25] found that 6-hydroxydopamine-induced lesions of nucleus accumbens did not prevent the behavioral activation caused by ICV CRF. Certainly these results suggest that dopaminergic systems do not mediate the CRF-induced increase in activity, but they do not exclude a role for noradrenergic systems. In fact, when linear regression was performed on the results of the experiment presented in Table 1, there was a statistically significant correlation between the moving scores and the changes in hypothalamic MHPG:NE ratios, $r(19)=0.44$, $p<0.05$, but none of the other main neurochemical effects. However in our experience, naloxone reverses behavioral effects of ICV CRF such as decreased exploratory behavior [1], and grooming [10], but not the cerebral catecholamine response. Naloxone treatment did significantly decrease MHPG:NE ratios in NAS, but CRF did not significantly increase the ratio in this region (Fig. 5). This would tend to suggest that the catecholamine activation does not underlie the behavioral responses to CRF.

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