Effects of intravenous and intraventricular injection of antisera directed against corticotropin-releasing factor on the secretion of anterior pituitary hormones

(ether stress/immunoneutralization/pituitary hormone release)

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ABSTRACT To determine the physiological significance of corticotropin-releasing factor (CRF) in the control of pituitary hormone secretion, highly specific antibodies directed against the peptide were injected either intravenously or intraventricularly (third ventricle) and the effect on plasma levels of pituitary hormones was determined before and after application of ether stress for 1 min. The intravenous injection of CRF antiserum (0.5 ml) did not significantly alter basal corticotropin (ACTH) levels in freely moving ovariectomized rats but largely blocked the increase in plasma ACTH resulting from ether stress. These antibodies had no effect on the ether-induced decline in plasma growth hormone (GH), and they failed to modify plasma luteinizing hormone levels. In a second experiment, CRF antiserum (3 μl) or normal rabbit serum was injected into the third ventricle. A blood sample was drawn 24 hr later and immediately thereafter another injection of CRF antiserum or normal rabbit serum was made. There was no modification in the level of any of the hormones 24 hr after the first injections, and they were similar in CRF antiserum and normal rabbit serum-injected animals. After imposition of ether stress, the response of plasma ACTH was nearly completely blocked by the intraventricular CRF antiserum, but the degree of blockade was slightly less than that obtained by intravenous injection. The decline in plasma GH after ether stress was blocked by the intraventricular CRF antiserum. There was no effect of the intraventricular injection of the antiserum on the levels of the other pituitary hormones. The results with intravenous injection of the antiserum indicate that CRF plays an extremely important but probably not completely indispensable role in the release of ACTH after ether stress. The results of the intraventricular injection of the antiserum suggest strongly that endogenous CRF may also modify its own release in response to stress, augmenting it by a positive ultrashort loop feedback, and that the antiserum against the peptide blocked this action; however, an action at the pituitary of these intraventricularly injected antibodies cannot be completely ruled out. The blockade of the stress-induced suppression of GH release by the CRF antibodies suggests that CRF released intrahypothalamically during ether stress brings about an alteration in the hypothalamic control of GH secretion such that the stress-induced inhibition of GH release is blocked.

The control of corticotropin (ACTH) secretion is mediated by corticotropin-releasing factor (CRF) acting in concert with vasopressin and possibly other substances such as oxytocin, epinephrine, and angiotensin II (1–3). It has been shown recently that CRF has other actions as well, because intraventricular injection of the peptide resulted in decreased growth hormone (GH) and luteinizing hormone (LH) secretion (4–6). These are hypothalamic actions because there is no effect of this peptide directly on the pituitary to alter secretion of either hormone. The threshold dose of CRF to decrease plasma GH was lower than that to decrease plasma LH after injection of the peptide into the third ventricle (4). The effect on GH is probably mediated, at least in part, by a release of somatostatin because CRF can release somatostatin from median eminence tissue incubated in vitro (7). CRF appears to induce the stress pattern of hormone secretion by the rat pituitary gland via hypothalamic and direct actions on the pituitary. To assess the physiologic significance of these actions requires studies either with a CRF antagonist or with antisera directed against the peptide. In the present paper, we report the effects of intravenous and intraventricular injection of CRF antiserum on the secretion of pituitary hormones. The results indicate that, in addition to a very powerful effect to mediate stress-induced ACTH secretion by direct action on the pituitary, CRF also appears to be capable of modulating the secretion of both ACTH and GH by an intrahypothalamic action.

MATERIALS AND METHODS

Animals. Adult female Sprague–Dawley rats (Holtzmann, Madison, WI) weighing 180–200 g were housed in group cages under controlled conditions of light (on from 0500–1900 hr) and temperature (23°C–25°C) with ad libitum access to food and water. They were ovariectomized while anesthetized with ether. The animals were used for experiments 4–6 weeks after ovariectomy. Ovariectomized rats were used because their high levels of gonadotropins permitted easy evaluation of decreases in concentrations.

CRF Antibody (CRF-Ab) Preparation. A coupling procedure was used and is described elsewhere (8). Briefly, 200 μg of synthetic ovine CRF (a generous gift from W. Vale and C. Rivier, The Salk Institute) was conjugated to 1.0 mg of bovine serum albumin/0.04 M glutaraldehyde. The mixture was dissolved in 0.1 M phosphate buffer (pH 7.0) and Freund’s complete adjuvant, emulsified, and injected intradermally into male rabbits (New Zealand White, Hickory Hills Farms). The procedure was repeated monthly using incomplete Freund’s adjuvant for emulsion. This antiserum gives a parallel dose response with rat CRF and rat median eminence extracts. It is highly specific and there is no cross-reaction (<0.01%) with a wide variety of hypothalamic peptides, including somatostatin, vasopressin, oxytocin, growth hormone-releasing hormone, and thyrotropin-releasing hormone.

Abbreviations: ACTH, corticotropin; CRF, corticotropin-releasing factor; GH, growth hormone; LH, luteinizing hormone; CRF-Ab, CRF antibody.
EXPERIMENTAL PROCEDURES

Intravenous Injections. In-dwelling catheters were inserted into the right external jugular vein 24 hr prior to experimentation as described (9). The rats were returned to individual cages after cannulation. Undiluted CRF-Ab (0.5 ml) or an equal volume of normal rabbit serum was injected through the cannula 5 min prior to the application of ether stress for 1 min. Heparinized blood samples (0.9 ml) were withdrawn from the jugular cannula just before injection of either CRF-Ab or normal rabbit serum and at 2, 5, 15, and 30 min after initiation of either stress. The blood removed at each time was replaced by an equal volume of 0.9% NaCl. Ether stress was administered as described (10) by placing the rat in an ether jar containing cotton soaked with ether. The animal was left in the jar for 1 min and then removed.

Intraventricular Injections. A 23-gauge stainless steel cannula was implanted into the third ventricle 7–10 days before experimentation as described (11). After surgery, the animals were placed in individual cages. Three microliters of undiluted CRF-Ab was injected into the third ventricle 24 hr prior to and again 30 min before the application of ether stress. Heparinized blood samples were collected from the jugular vein cannula as described above.

RIA. Plasma ACTH was measured by RIA with anti-ACTH supplied by the National Institute of Arthritis, Diabetes, and Digestive and Kidney Diseases after an extraction procedure as described (4). Recovery of ACTH from plasma was 80% with an interassay coefficient of variation of 10.6% and intraassay coefficient of variation of 9.8%. Sensitivity of the plasma ACTH assay was 20 pg/ml.

Prolactin and GH were measured by the RIA kits supplied by the National Institute of Arthritis, Diabetes, and Digestive and Kidney Diseases, and the results were expressed in terms of the RP reference standards provided with the kits. LH was measured by the method of Niswender et al. (12). The RP-1 rat pituitary reference preparation was used and the LH levels were expressed in terms of the NIH-LH-S1 reference preparation to be comparable with previous results from this laboratory.

Statistics. Differences between groups across time were tested by the Student–Newman–Keuls multiple comparison test after analysis of variance or by Student's t test after calculating the area under the curve of plasma hormone concentrations from time zero to the time in question by planimetry.

RESULTS

The Effect of Intravenous Administration of CRF-Ab on the Response to Ether Stress. Injection of CRF-Ab failed to modify plasma ACTH during the 5 min prior to application of ether stress. Similarly, there was no effect of normal rabbit serum on the level of plasma ACTH. The intravenous injection of the antibodies against CRF dramatically inhibited the release of ACTH induced by ether. Plasma ACTH levels in the animals injected with antisera were significantly lower than those in the animals injected with normal rabbit serum at 2, 5, and 15 min after initiation of ether stress (P < 0.005, P < 0.005, and P < 0.05, respectively) (Fig. 1). There was a slight but significant residual response to ether stress in the animals injected with the antisera.

Effect of Intraventricular Injection of CRF-Ab. Three microliters of CRF antisera were injected into the third ventricle, 24 hr before, and again 30 min prior to application of ether stress. Plasma ACTH values were similar in normal rabbit serum- and in antisera-injected animals 24 hr after the first injection, and they were also not significantly altered by the second injection of either normal rabbit serum or CRF antisera (Fig. 2). The increase in plasma ACTH induced by ether was again highly significantly lowered by the intraventricular injection of CRF antisera on comparison to the results in the normal rabbit serum-treated animals. The lowering was significant at 2 and 5 min (P < 0.005) (see Fig. 2).

The Effect of Intraventricular Injection of CRF Antibodies on Plasma GH After Stress. Although the injection into the third ventricle of antibodies to CRF did not change the basal level of plasma GH after either the first or second injection on comparison to the values in the normal rabbit serum-treated animals, the response to ether was dramatically altered in the animals injected with CRF antisera (Fig. 3). There was a significant lowering of plasma GH in the normal rabbit serum-injected animals, which became significant within 5 min (P < 0.025) after ether stress. Values reached a minimum at 15 min and remained low for the 30-min duration of the experiment. In contrast, there was no significant lowering of plasma GH in the animals that had been preinjected with CRF antisera and the plasma GH was significantly higher than that in the normal rabbit serum-injected animals.

![FIG. 1. The effect of intravenous (IV) injection of antibodies directed against ovine CRF (CRF-Ab) or normal rabbit serum (NRS) on the response to ether stress as measured by plasma ACTH concentrations. Values are mean ± SEM. Arrow indicates the time of intravenous injection. Square indicates the time of application of ether stress for 1 min. **, P < 0.005 vs. normal rabbit serum-injected control.](image1)

![FIG. 2. Effect of intraventricular injection of CRF-Ab (CRF-AB) or normal rabbit serum (NRS) (3 μl into the third ventricle (3V)) on the ACTH response to ether stress. The first injection into the third ventricle occurred 24 hr before beginning of the experiment. At ~30 min, a blood sample was withdrawn and a second similar injection was given into the third ventricle. Arrows indicate the time of the injections. A second blood sample was drawn at time 0, and this was followed immediately by the application of ether stress for 1 min. Box indicates the time of the 1-min application of ether stress. **, P < 0.005.](image2)
injected animals at 15 and 30 min after initiation of the ether stress ($P < 0.025$ at both intervals when the area under the plasma GH curve from 0–15 or 0–30 min was compared by planimetry).

In contrast, intravenous injection of 0.5 ml of the CRF antisera did not alter the pattern or values of plasma GH, which was lowered after the application of ether stress in both the antibody and the normal rabbit serum-injected animals (Table 1).

Neither intravenous nor intraventricular injection of CRF antisera altered plasma levels of LH either in resting conditions or after ether stress (Table 1). Similarly, third-ventricular injection of CRF-Ab did not change the pattern and values of plasma prolactin either at rest or after application of ether stress (Table 1).

**DISCUSSION**

The present results indicate that CRF plays an important role in the induction of the stress-induced pattern of secretion of both ACTH and GH. Our results with intravenous injection of CRF antisera confirm the findings of Rivier and Vale (1), who similarly showed that antibodies directed against CRF could largely block the ACTH release in response to stress in the rat. In the present experiments, there was a small residual response, which could be due to either injection of insufficient antibodies to inactivate CRF action at the pituitary completely and/or a residual response possibly mediated via vasopressin, oxytocin, epinephrine, and/or other agents that activate the release of ACTH during stress (10, 13-15).

The results show that the intraventricular injection of minute quantities of CRF antisera can also largely block the ACTH release during stress. This effect may be due to a blockade of the recently shown ultrashort loop positive feedback of CRF to enhance its own release during stress (16). It is clear that antisera injected into the ventricle can be taken up via the adjacent hypothalamic tissue and act there (17). It is also possible, particularly since the first injection of the antiserum was given 24 hr prior to the application of stress, that the antisera could have been taken up by portal vessels and delivered to the pituitary. Consequently, the possibility that the action of the intraventricular CRF antibodies to block stress-induced ACTH release may have been in part via direct blockade of the action of CRF at the pituitary level cannot be completely ruled out.

Our results show that CRF antisera injected into the ventricle can block the lowering of plasma GH that follows stress in the rat. Since there is no action of CRF directly on the pituitary to affect GH release (3, 4), these effects are almost certainly due to an action of the antibodies to block the intrahypothalamic action of CRF to suppress GH secretion. This action may be mediated either by an inhibition of GH releasing factor release and/or by an augmentation of somatostatin release. Intraventricular injection of CRF has recently been shown to depress plasma GH levels (4, 6). The peptide stimulates somatostatin release from incubated median eminence fragments (7), so at least part of its action in vivo is presumably mediated by stimulation of somatostatin release.

In contrast, there was no effect of the CRF antibodies given intraventricularly on the stress-induced release of prolactin, and the pattern of plasma LH was similarly unaltered. Injection of CRF into the third ventricle failed to alter prolactin release; however, a much higher dose than that

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**Table 1. GH, LH, and prolactin release by ether stress with preinjection of CRF-Ab or normal rabbit serum into the third ventricle or intravenously**

<table>
<thead>
<tr>
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<th>Release at time (min) after onset of ether stress</th>
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<tbody>
<tr>
<td></td>
<td>−5</td>
</tr>
<tr>
<td>GH (i.v.)</td>
<td></td>
</tr>
<tr>
<td>CRF-Ab (n = 7)</td>
<td>73.4 ± 4.5</td>
</tr>
<tr>
<td>NRS (n = 5)</td>
<td>63.8 ± 5.8</td>
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<tr>
<td>LH (i.v.)</td>
<td></td>
</tr>
<tr>
<td>CRF-Ab (n = 7)</td>
<td>14.0 ± 3.3</td>
</tr>
<tr>
<td>NRS (n = 5)</td>
<td>17.2 ± 4.8</td>
</tr>
<tr>
<td>LH (3V)</td>
<td></td>
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<tr>
<td>CRF-Ab (n = 10)</td>
<td>14.3 ± 0.2</td>
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<tr>
<td>NRS (n = 6)</td>
<td>13.2 ± 3.5</td>
</tr>
<tr>
<td>PRL (3V)</td>
<td></td>
</tr>
<tr>
<td>CRF-Ab (n = 10)</td>
<td>29.0 ± 2.9</td>
</tr>
<tr>
<td>NRS (n = 6)</td>
<td>24.2 ± 4.1</td>
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</tbody>
</table>

Results are expressed as ng per ml of plasma (mean ± SEM). NRS, normal rabbit serum; 3V, third ventricle; PRL, prolactin.

*This sample was taken 24 hr after the first injection of 3 μl of antiserum or normal rabbit serum into the third ventricle, and immediately thereafter a second injection of 3 μl was made into the ventricle. This was followed 30 min later by collection of the time 0 sample.
necessary to lower plasma growth hormone did lower plasma LH (4). Consequently, we expected to find an alteration in LH secretion following the ether stress in the antisera-injected animals. Our failure to find this could mean that the dose of antisera was not sufficient to block the action of CRF on LHRH neurons and/or that under these conditions this action of CRF to inhibit LHRH neurons was not operative.

In summary, our results indicate the physiological significance of CRF to mediate the stress-induced pattern of both ACTH and GH secretion by hypothalamic as well as pituitary sites of action.

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