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Corticotropin-Releasing Activity in the Rat Gastric Antrum (42393)

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Abstract. Rat gastric antrum, duodenum, pancreas, and spleen were extracted in acetic acid, treated with acetone, and purified on a C-18 cartridge. These extracts, in a dose equivalent to one respective organ, were examined for CRF bioactivity *in vitro* using rat half pituitaries, with gastric antrum extract showing a significant CRF activity. The antrum extract showed a dose-related CRF activity *in vitro* using rat pituitary cell culture, and the dose-response curve appeared to be parallel with that of synthetic rat hypothalamic CRF. Subsequent ion-exchange chromatography on a SP–Sephadex column showed that antrum CRF coeluted with basic materials (SP-III fraction), while rat hypothalamic CRF coeluted with weakly basic materials (SP-II fraction). The SP-III fraction was further purified by gel filtration on Sephadex G-50. CRF activity was eluted in two areas: large mol wt fraction (10,000–15,000) and small mol wt fraction (1500–2000). Hypothalamic CRF was eluted between them. The CRF activities of the two fractions were completely abolished by trypsin digestion, suggesting a peptide nature. The large molecular weight fraction exhibited a steeper dose-response curve than the hypothalamic CRF *in vitro* using cell culture, and the response to a dose equivalent to two antra exceeded the maximum response exhibited by the hypothalamic CRF. However, the fraction failed to increase serum corticosterone when injected in pharmacologically blocked rats. On the other hand, the small molecular weight fraction showed a lesser CRF activity and a similar dose-response curve to that of the hypothalamic CRF as tested *in vitro.* This fraction significantly stimulated corticosterone secretion *in vivo* as well. The small molecular weight activity did not appear to be due to other peptides or amines which have been reported as causing ACTH release. Although the physiological roles of the small molecular weight antrum CRF are unknown, it is possible that this CRF plays a role during stress as a tissue CRF.

Corticotropin-releasing factor (CRF) was first isolated from ovine hypothalami and characterized as having 41 amino acids by Vale *et al.* (1). The same group also characterized CRF from rat hypothalami (2) which was found to be identical to human hypothalamic CRF (3). Subsequently, antisera generated against ovine and rat CRF made it possible to conduct immunocytochemical studies as well as radioimmunoassay (RIA). The CRF activity determined by RIA or immunocytochemistry was not confined to the hypothalamus, but was also demonstrated in other neural tissues (4, 5), and several other organs including the gut and pancreas (5). In light of other gut–brain peptides, the presence of CRF-like immunoreactivity in the gut and pancreas was not surprising. Its physiological interpretations, however, have been complicated by the fact that not all CRF antisera are capable of recognizing CRF in the tissue (5). In fact, we have also failed to detect CRF-like immunoreactivity in the rat gastrointestinal tract using immunocytochemistry or homologous RIA for rat CRF (unpublished data).

On the other hand, the presence of bioactive CRF in tissues other than the hypothalamus has been known for nearly three decades, the so-called tissue CRF proposed by Brodish (6). Yet the source of this tissue CRF has remained obscure, nor has it been determined whether tissue CRF is related to hypothalamic CRF. Tissue CRF seems to play an important role in the regulation of ACTH secretion, as does the hypothalamic CRF, particularly during severe and sustained stress conditions (6).

In order to search for the source of tissue CRF, we screened various rat abdominal organs for CRF bioactivity and found that the gastric antrum showed a significant CRF activity when tested *in vitro.* Its physicochemical characteristics were then investigated and compared with those of synthetic rat hypothalamic CRF.

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Materials and Methods. *Tissue extraction and characterization.* Gastric antra were isolated from 150 adult CD strain rats of both sexes (Charles River Breeding Laboratories, Wilmington, Mass.) after decapitation and washed with clean water. After weighing, tissues were frozen and stored at −80°C until used. Frozen tissues were added directly to 20 vol of boiling 2 *M* acetic acid (vol/wt) and maintained at 95°C for 10 min. After cooling on ice, the tissues were homogenized by Polytron (Brinkmann Instruments, Inc., Westbury, N.Y.) at 4°C for 20 min and then centrifuged at 15,000*g* for 30 min. Ice-cold acetone, containing 0.02% 2-mercaptoethanol (2-ME), was added to the supernatant at a final concentration of 66%. The precipitates were removed by centrifugation and the supernatant was then evaporated *in vacuo* to dryness. The residual concentrates were dissolved in 500 ml of 1 *M* acetic acid and insoluble materials were removed by centrifugation and filtered through a GF/B filter (Whatman, LabSales, Inc., Hillsboro, Oreg.). Materials in the soluble fraction were then adsorbed on a column (2.5 × 23 cm) of LRP-1 resin (C-18, Whatman), washed with 1 *M* acetic acid, and eluted with a solution of H_2O:CH_3CN:10% trifluoroacetic acid = 40:60:1 (vol/vol) containing 0.02% 2-ME. The eluate was evaporated *in vacuo* and dissolved in 1 *M* acetic acid. One-fifth of this fraction was stored and used to measure CRF activity using a cell culture assay system. In the preliminary study, several organs including the gastric antrum, duodenum, pancreas, and spleen were isolated from 20 rats and then treated with procedures equivalent to those described above. They were examined for CRF activity using a half-pituitary *in vitro* assay. The remaining portion of the gastric antrum extract eluted from the C-18 column was further purified on a SP-Sephadex C-25 (H^+-form, Pharmacia Inc., Piscataway, N.J.) column (5 × 13 cm) at room temperature, preequilibrated with 1 *M* acetic acid. Successive elutions with 1 *M* acetic acid, 2 *M* pyridine, and finally 2 *M* pyridine acetate (pH 5.0) yielded three respective fractions: SP-I, SP-II, and SP-III. Each fraction was evaporated, and then lyophilized repeatedly to completely remove pyridine. One-seventh of each fraction was reserved for the cell culture assay. Six-sevenths of the SP-III fraction, which showed CRF activity and represented 102 fragments of the rat gastric antrum, were subjected to gel filtration on a Sephadex G-50 Fine column (2.5 × 100 cm; Pharmacia, Inc.) and eluted with 2 *M* acetic acid containing 0.01% 2-ME at room temperature. Column effluents were monitored by measuring absorbance at 280 nm and collected in 10-ml fractions. An aliquot of each fraction was lyophilized and examined for CRF activity using the cell culture assay. Synthetic hypothalamic CRF (generously provided by Dr. J. Rivier) was used as a reference preparation in each step of the purification. The recoveries of the hypothalamic CRF and arginine vasopressin (AVP; purchased from Peninsula Laboratories, Inc., Belmont, Calif.) through the Sephadex G-50 Fine column were 90 and 95%, respectively. The column was calibrated with ribonuclease A (Sigma Chemical Co., St. Louis, Mo.), bovine pancreatic trypsin inhibitor (Sigma), and bacitracin (Sigma) as well as the hypothalamic CRF and AVP.

Preparation of primary monolayer rat anterior pituitary cell culture. Corticotropin-releasing activity was primarily estimated by the anterior pituitary cell culture system as described elsewhere (7). Briefly, anterior pituitaries obtained from male rats of approximately 250 g were enzymatically dispersed with Collagenase Type II (Worthington Biochemical, Freehold, N.J.), DNAase II (Sigma), and pancreatin (GIBCO, Grand Island, N.Y.). The cells were collected by centrifugation and washed three times with Dulbecco's modified Eagle's medium (DMEM) containing 10% horse serum, 2.5% heat-inactivated fetal calf serum, and 1% antibiotic–antimycotic solution (GIBCO). The cells were resuspended in an appropriate volume of the same medium, and 1.0-ml aliquots (containing 1.25 × 10^5 cells) were added to each well of 24 multiwell tissue culture plates (Falcon, Oxnard, Calif.). The plates were incubated in a water-jacketed incubator (Queue System, Parkersburg, W.Va.) at 37°C in a water-saturated atmosphere of 95% air/5% CO_2 for 4 days. The cells were then washed with Hepes-buffered DMEM, and further incubated for 3 hr in 0.5 ml of the same medium containing test samples. Ascorbic acid (2.5 × 10^{-4} *M*) and bovine

serum albumin (BSA; 0.25%) were routinely added to the medium during the final incubation in order to prevent possible oxidation and adsorption of the test materials. ACTH level in the incubation medium was determined by RIA.

In vitro assay for CRF activity using a rat half-pituitary method. An *in vitro* assay for CRF activity was also performed using the rat half-pituitary method as previously described (8). In short, after two 1.5-hr incubations in Krebs–Ringer bicarbonate buffer containing 0.2% glucose and 0.25% bovine serum albumin (KRBG–BSA), the pituitaries were incubated for 1 hr in 1 ml KRBG–BSA and then in KRBG–BSA (controls), or KRBG-BSA-containing test materials, for another hour. The amount of ACTH released during the second 1 hr incubation expressed as a ratio of the amount released during the first 1 hr incubation was used as the index of CRF activity. The absolute amounts of ACTH released during the second 1 hr incubation were also used as the response parameter.

In vivo assay for CRF activity. An *in vivo* assay for CRF activity using rats pretreated with chlorpromazine, morphine, and Nembutal (CPZ-M-N) as described previously (9) was also used to evaluate the CRF bioactivity of test materials. Twenty minutes after intrajugular injection of test materials, blood was collected from the same vein. Serum was separated by centrifugation and frozen at −20°C until assayed for corticosterone.

Trypsin digestion. One-tenth of the pooled fractions 26–29 and 46–48 of the Sephadex G-50 gel filtration which showed CRF activity were lyophilized and dissolved in 1 ml of 1% NH_4HCO_3 (pH 8.2). Each solution was incubated with or without 100 μg of trypsin (Worthington) at 37°C for 5 hr. Following incubation, the mixture was heated at 100°C for 10 min to inactivate trypsin, lyophilized repeatedly to completely remove ammonium bicarbonate, and then assayed for CRF activity.

Radioimmunoassay of corticosterone, ACTH, CRF, and AVP. Serum corticosterone level was measured by RIA using a RIA kit for cortisol (Cambridge Medical Diagnostics, Inc., Billeria, Mass.). The primary antiserum against cortisol-3-BSA cross-reacted with corticosterone by 30% as compared to cortisol. Corticosterone was used as the reference standard. ACTH in the incubation media was measured by RIA using ^{125}I-labeled $ACTH_{1-24}$ and a rabbit antiserum generated against porcine ACTH (generously provided from NIAMDD, National Pituitary Agency). $ACTH_{1-24}$ was also used as the reference standard. A homologous RIA for rat hypothalamic CRF was performed using a rabbit antiserum generated against unconjugated synthetic rat CRF (r569), ^{125}I-labeled rat CRF as the tracer, and synthetic rat CRF as the standard. Samples were incubated with diluted antiserum to make a final dilution of 1:20,000, in a volume of 600 μl at 4°C for 48 hr, and 100 μl of tracer was then added and further incubated for 16 hr. The bound and free peptide was separated by charcoal–dextran. At 1:20,000 dilution, r569 bound 31% of the added tracer. A linear standard curve was demonstrated in a logit/log scale in a range between 8 and 128 pg synthetic rat CRF per tube, with a 50% intercept at 30 pg per tube. This RIA was specific for rat CRF, as indicated by a failure to cross-react with other peptide hormones known to be present in the rat tissue. AVP was measured by RIA using ^{125}I-labeled AVP, and a rabbit antiserum generated against AVP (AVP TG 84/74; kindly provided by Dr. T. Görcs). AVP was used as the reference standard. Samples were incubated with tracer and diluted antiserum at a final dilution of 1:350,000 in a volume of 700 μl at 4°C for 16 hr. The bound and free peptides were separated by charcoal–dextran solution. At 1:350,000 dilution, the antibody bound 52% of the added tracer. The sensitivity of this RIA was 2 pg per tube with a 50% intercept at 20 pg per tube.

Statistical analysis. Statistical analysis was performed by analysis of variance and subsequent Duncan's New Multiple Range tests.

Results. *CRF activity in the gastric antrum extract after the treatment with C-18 reverse-phase column.* Tissue extracts of rat gastric antrum, duodenum, pancreas, and spleen were preliminarily assayed for CRF activity using the half-pituitary *in vitro* assay. Among those organs, the gastric antrum extract exhibited a significant CRF activity (Table I). Subsequently, the CRF activity of the gastric antrum was investigated. As shown in Fig. 1, the gastric

TABLE I. *In Vitro* Half-Pituitary CRF Assay for the Extracts of Various Rat Organs

Organ	S/B	S (ACTH, ng/ml)
Control	0.96 ± 0.12	0.49 ± 0.02
Gastric antrum	4.83 ± 0.97*	2.73 ± 0.48*
Duodenum	2.65 ± 0.53	1.21 ± 0.18*
Pancreas	0.89 ± 0.04	0.64 ± 0.31
Spleen	1.41 ± 0.25	0.53 ± 0.22
Hypothalamic CRF	6.69 ± 1.67*	2.92 ± 0.32*

Note. One equivalent of each rat organ was added per incubation beaker. The average wet tissue weight of each rat extract is as follows: gastric antrum, 612 mg; duodenum, 479 mg; spleen, 601 mg; pancreas, 425 mg. The concentration of the hypothalamic CRF added to a beaker was 4 ng/ml. Results are expressed as a ratio of changes in ACTH concentration between the first and second incubation (S/B) and as ACTH concentration in the medium of the second incubation (S). Each value represents mean ± SEM of four replicate incubations.

* Significantly different from control, $P < 0.01$.

antrum extract increased ACTH release from anterior pituitary cultured cells in a dose-related manner. The highest concentration of the antrum extract added to a culture well was equivalent to one rat antrum fragment.

SP–Sephadex ion-exchange chromatography of the gastric antrum extract. The C-18-treated antrum extract was dissolved in 1 *M* acetic acid and then placed on a SP–Sephadex (H^+-form) column. The extract was eluted stepwise with 1 *M* acetic acid, 2 *M* pyridine, and 2 *M* pyridine acetate (pH 5.0). Acidic or neutral materials were eluted in the 1 *M* acetic acid fraction (SP-I), weakly basic materials in the 2 *M* pyridine fraction (SP-II), and basic materials in the 2 *M* pyridine acetate fraction (SP-III). Each fraction was assayed for CRF activity using the cell culture method. Most of the hypothalamic CRF appeared in the SP-II fraction, while gastric CRF activity was eluted exclusively in the SP-III fraction (Table II). These results suggest that the substance with CRF activity in the gastric antrum is different from the hypothalamic CRF.

Sephadex G-50 gel filtration of the SP-III fraction of the gastric antrum extract. The SP-III fraction was then loaded on a Sephadex G-50 Fine column and eluted with 2 *M* acetic acid containing 0.01% 2-ME. Aliquots of fractions were assayed for CRF activity using the cell culture method. Two peaks of CRF activity, designated as fractions A and B, were observed on the gel filtration, as shown in Fig. 2; fraction A was eluted near the void volume and fraction B was eluted just before bacitracin. Both fraction A (R_f = 1.17) and B (R_f = 1.96) appeared quite different from the hypothalamic CRF (R_f = 1.42) (Fig. 2). The molecular weights of the two fractions were estimated to be 10,000–15,000 and 1500–2000, respectively. Fraction A exhibited a considerably high CRF activity, while fraction B showed a moderate activity. Dilution curves of the two fractions in terms of CRF activity were shown in Fig. 3. Fraction A demonstrated a steeper dose-response curve than the hypothalamic CRF, and the response to the dose equivalent to two antra exceeded the maximum response to the hypothalamic CRF. In

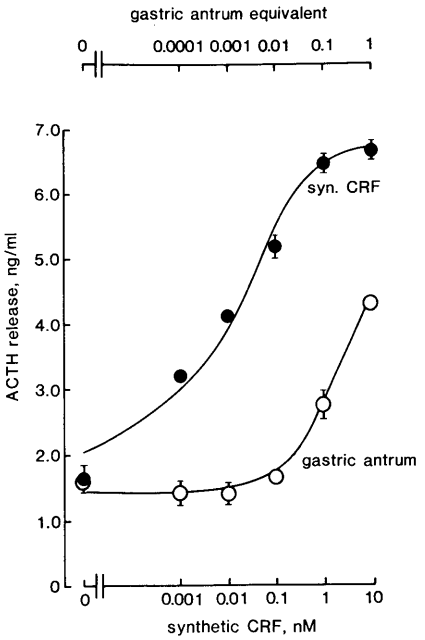

FIG. 1. Dose-response effects of synthetic rat hypothalamic CRF and gastric antrum extract on ACTH release in monolayer rat pituitary cell culture during a 3-hr incubation period. Each point represents mean ± SEM of four replicate cultures. Points without brackets indicate that SEM is within area of point. The highest concentration of the antrum extract added to a culture well was equivalent to one rat antrum fragment (612 mg wet tissue weight).

TABLE II. CRF ACTIVITY ON SP-SEPHADEX ION-EXCHANGE CHROMATOGRAPHY OF THE GASTRIC ANTRUM EXTRACT

	ACTH (ng/ml)	
	Hypothalamic CRF	Gastric antrum extract
Control	1.06 ± 0.15	1.06 ± 0.15
SP-I fraction	1.31 ± 0.06	1.16 ± 0.27
SP-II fraction	7.75 ± 0.40*	1.12 ± 0.12
SP-III fraction	1.75 ± 0.12	5.30 ± 0.10*

Note. The rat gastric antrum extract, corresponding to 120 fragments, was loaded on a SP-Sephadex ion-exchange column, and each eluted fraction was assayed for CRF activity using a cell culture system. One equivalent of rat gastric antrum was added to each culture well. Hypothalamic CRF (20 pmole) was treated in the same manner, and one-hundredth of each fraction per culture well was used for CRF activity. Each value represents mean ± SEM of duplicate cultures.

* Significantly different from control, $P < 0.01$.

contrast, the small molecular weight fraction showed a parallel dose-response curve to the hypothalamic CRF.

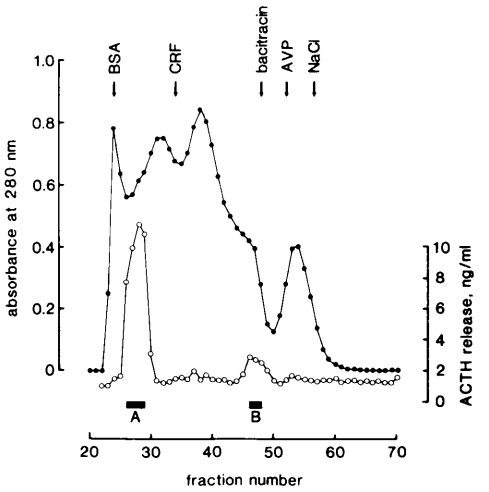

FIG. 2. Sephadex G-50 gel filtration of the basic peptides fraction (SP-III fraction) of gastric antrum extract obtained from SP-Sephadex ion-exchange chromatography. Each fraction was assayed for ACTH-releasing activity using a pituitary cell culture system. One-tenth of each fraction was added to a culture well. Each value of ACTH concentration represents mean of duplicate cultures. The Sephadex G-50 gel filtration was run at room temperature in the following conditions: column, Sephadex G-50 Fine, 2.5 × 100 cm; solvent, 2 *M* acetic acid containing 0.01% 2-ME; fraction size, 10 ml/tube; flow rate, 15 ml/hr.

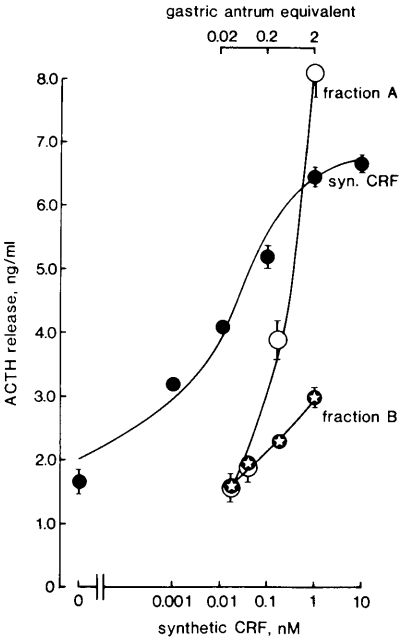

FIG. 3. Dose-response effects of fractions A and B obtained from Sephadex G-50 gel filtration (see Fig. 2) on ACTH release in pituitary cell cultures during a 3-hr incubation period, in comparison with that of rat hypothalamic CRF. Each value represents mean ± SEM of duplicate cultures. Points without brackets indicate that SEM is within area of point.

In vivo CRF activity in the gastric antrum extract. In order to confirm an *in vivo* stimulation of ACTH release, fractions A, B, and the synthetic hypothalamic CRF (0.01 nmole/100 g body wt) were injected into CPZ-M-N-treated rats. As shown in Table III, injection

TABLE III. CRF ACTIVITY OF FRACTIONS A AND B IN SEPHADEX G-50 GEL FILTRATION AS TESTED *IN VIVO*

	Serum corticosterone (ng/ml)
Saline control	20.40 ± 1.91
Fraction A	27.18 ± 3.30
Fraction B	38.38 ± 5.47*
Hypothalamic CRF	90.38 ± 8.61**

Note. Fraction A or B equivalent to two rat gastric antra was injected into a CPZ-M-N treated rat. The injected amount of hypothalamic CRF was 1×10^{-11} mole per rat. Each value represents mean ± SEM of four rats.

* Significantly different from control, $P < 0.05$.

** Significantly different from control, $P < 0.01$.

of the hypothalamic CRF significantly increased serum corticosterone levels, as expected. Administration of fraction B also caused a significant elevation in serum corticosterone levels. On the other hand, fraction A which showed an extremely high CRF activity *in vitro* (Fig. 3) failed to increase serum corticosterone levels. These results cast doubt upon the physiological significance of fraction A.

Trypsin treatment of fractions A and B in Sephadex G-50 gel filtration. As shown in Table IV, the CRF activities of both fractions A and B were completely abolished by trypsin digestion, while the activities remained unchanged after incubation without the enzyme under the same experimental conditions used in the enzymatic degradation experiment. These results suggest a peptide nature for fractions A and B.

Discussion. The acid extract of the rat gastric antrum treated with acetone and a C-18 reverse-phase column showed a considerable amount of CRF activity in two *in vitro* assays: the half-pituitary system (Table I) and the anterior pituitary cell culture system (Fig. 1). Moreover, the ACTH release from anterior pituitary cultured cells was dose-related (Fig. 1).

On SP-Sephadex ion-exchange chromatography, the portion of the gastric antrum extract with the most CRF activity was eluted in the SP-III fraction which contained basic materials, while the hypothalamic CRF was eluted in the SP-II fraction which contained weakly basic substances (Table II). That the substance(s) with CRF activity in the gastric antrum behaved differently from the hypothalamic CRF may indicate that these two materials are not identical. Absence of immunoreactive CRF in the rat stomach extract as determined by a homologous RIA for rat CRF also supports this view (unpublished data).

Our results show that the gastric antrum contains two peaks of CRF *in vitro* activity on Sephadex G-50 gel filtration; one (large molecular weight fraction) was eluted relatively near the void volume after BSA, and the other (small molecular weight fraction) was eluted just before bacitracin which has a molecular weight of 1450 (Fig. 2). It is noteworthy that neither of them was coeluted with the hypothalamic CRF on the Sephadex G-50 gel filtration.

The large molecular weight fraction exhibited a considerably high CRF activity in an *in vitro* assay. The stimulation of ACTH release by this fraction, however, far exceeded the maximum ACTH release by the hypothalamic CRF (Fig. 3). This finding led us to question its physiological significance. Since the gastric antrum extract had been treated with a C-18 reverse-phase column, the CRF activity was not due to salts and amines in the extract which are not retained on the column. Loss of the activity after trypsin digestion (Table IV) indicated that this substance is a peptide. On the other hand, failure to increase serum corticosterone level in CPZ-M-N rats after injection of the large molecular weight fraction (Table III) suggests that this substance may affect only cultured cells. It was of interest to note that when culture plates were stained by toluidine blue after assay incubation, considerable amounts of fine precipitates of toluidine blue were found exclusively in the culture wells in which the large molecular weight fraction was added, but no visible damage of the cultured cells was observed under microscopic examination (data not shown). Therefore, it is possible that some protein with a mol wt of 10,000–15,000 as estimated on gel filtration

TABLE IV. THE EFFECT OF TRYPSIN DIGESTION ON FRACTIONS A AND B IN SEPHADEX G-50 GEL FILTRATION

	ACTH (ng/ml)	
	Treatment without trypsin	Treatment with trypsin
Fraction A	6.30 ± 0.32*	1.04 ± 0.09
Fraction B	2.08 ± 0.16*	1.22 ± 0.08
Trypsin	—	1.06 ± 0.09
Control	0.98 ± 0.12	

Note. Fraction A or B was incubated with or without trypsin at 37°C for 5 hr. Following incubation, each mixture was heated at 100°C for 10 min to inactivate trypsin, lyophilized, and then assayed for CRF activity using a cell culture. 1.5 Antrum equivalents of each treated fraction were added to a culture well. Each value represents mean ± SEM of four replicate cultures.

* Significantly different from control, $P < 0.01$.

chromatography caused ACTH release from cultured cells, but not from the pituitary gland *in vivo*. Thus, its physiological significance as a CRF could be questionable. Extreme caution must be made in interpretation of results when an anterior pituitary cell culture method is used to screen physiological hypophysiotropic factors of tissue extracts.

On the other hand, the small molecular weight fraction showed a significant CRF activity in both *in vitro* and *in vivo* assay systems, though its CRF activity was not striking (Fig. 3, Table III). The CRF activity of this fraction contained in one rat antrum fragment may be estimated to be equal to about 1–10 pg of the hypothalamic CRF. The CRF activity of the fraction was completely abolished by trypsin digestion, suggesting its peptide nature (Table IV). It has been known that arginine vasopressin (AVP), oxytocin, angiotensin II, vasoactive intestinal polypeptide (VIP), peptide histidine isoleucine (PHI), epinephrine and norepinephrine, as well as CRF induce secretion of ACTH from corticotropic cells *in vitro* (10). As mentioned above, the effects of catecholamines could be ruled out. The mass of the substance(s) in the small molecular weight CRF fraction was estimated to be 1500–2000. Since the molecular weights of VIP and PHI (3326 and 2996, respectively) are considerably larger than that of the small molecular weight CRF, it is unlikely that the small CRF is VIP and/or PHI. Although it is well known that AVP possesses an intrinsic ACTH-releasing activity as well as a synergistic action with CRF, an anterior pituitary cell culture system is relatively refractory to AVP (10). In fact, AVP was ineffective in stimulating ACTH release in our cell culture system in concentrations ranging from 1×10^{-10} to 1×10^{-6} *M* (data not shown). Furthermore, the elution position of AVP was apparently different from that of the small molecular weight fraction (Fig. 2). Oxytocin and angiotensin II were also reported to slightly increase ACTH release *in vitro* (11, 12), and the molecular weights of both peptides are approximately 1000. Oxytocin is known to be eluted in the SP-II fraction with 2 *M* pyridine on a SP–Sephadex ion-exchange column because of its weakly basic property (13). Nevertheless, a possibility that the small molecular weight fraction contains angiotensin II remains, although neither immunoreactivity nor bioactivity of the peptide has been reported in the gastric antrum.

The presence of tissue CRF has been suggested by Brodish (6), but the source of tissue CRF has not been clarified. CRF-like immunoreactivity has been reported to occur in endocrine cells of the pancreas and gastrointestinal tract, the liver, pituitary, adrenal, lung, placenta, and several endocrine tumors (5). However, the chemical identity of this tissue CRF has not been determined. Hashimoto *et al.* (14) suggested that the adrenal gland might secrete tissue CRF, but whether it was active *in vivo* was not reported. In the present study, CRF bioactivity was demonstrated in the rat gastric antrum. The activity of stomach CRF in the small molecular weight fraction does not seem to be strong enough to represent tissue CRF. However, it is possible that the synthesis and release of this CRF-like substance(s) in the gastric antrum could be enhanced under certain conditions such as severe and sustained stress, as described by Brodish (6).

It has recently been reported that intravenous or intracerebral injection of CRF resulted in a dose-related inhibition of gastric acid secretion (15–17). Considering its possible preventive roles in the genesis of stress-induced gastrointestinal ulcers, it may also be of interest to investigate the effect of gastric CRF-like substance(s) on gastric acid secretion.

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