Proc. Natl. Acad. Sci. USA
Vol. 80, pp. 4851–4855, August 1983
Medical Sciences

Characterization of rat hypothalamic corticotropin-releasing factor

(peptide isolation/sequence microanalysis/peptide mapping/peptide synthesis)

JEAN RIVIER, JOACHIM SPIESS, AND WYLIE VALE

Peptide Biology Laboratory, The Salk Institute, P.O. Box 85800, San Diego, California 92138

Communicated by W. Maxwell Cowan, April 14, 1983

ABSTRACT **A polypeptide was purified from rat hypothalamic extracts on the basis of its high intrinsic activity to release corticotropin (ACTH) from cultured rat anterior pituitary cells and its immunoactivity in a radioimmunoassay directed against the NH_2 terminus (residues 4–20) of ovine hypothalamic corticotropin-releasing factor (CRF). Based on Edman degradation, peptide mapping, and amino acid analysis, the primary structure of this rat CRF was established to be: H-Ser-Glu-Glu-Pro-Pro-Ile-Ser-Leu-Asp-Leu-Thr-Phe-His-Leu-Leu-Arg-Glu-Val-Leu-Glu-Met-Ala-Arg-Ala-Glu-Gln-Leu-Ala-Gln-Gln-Ala-His-Ser-Asn-Arg-Lys-Leu-Met-Glu-Ile-Ile-NH_2. The hypophysiotropic potency of synthetic rat CRF did not deviate significantly from the potencies of the isolated native peptide or of synthetic ovine CRF. The close structural relationship between rat and ovine hypothalamic CRF is indicated by an 83% sequence homology.**

Harris proposed that the neuroregulation of adrenocorticotropic hormone (ACTH; corticotropin) may be mediated by a substance later termed corticotropin-releasing factor (CRF), which reaches the adenohypophysis by the hypothalamic–hypophysial portal system (1). Early experimental observations by Guillemin and Rosenberg (2), and Saffran and Schally (3), supported the presence of such factors in the hypothalamus that would increase the rate of ACTH secretion by the pituitary gland incubated *in vitro* or maintained in organ culture. More than 25 years later, ovine CRF (oCRF) was isolated (4, 5), characterized (5, 6), and synthesized (7). Subsequently the amino acid sequence of the protein precursor of oCRF was deduced from the cDNA (8). With the availability of synthetic oCRF and antibodies raised against it, various aspects of oCRF's distribution and of its hypophyseal, vascular, and neural actions have been investigated (9–18), and immunoneutralization experiments in the rat with anti-oCRF antisera have demonstrated that this or related peptides are indeed involved in the physiologic regulation of ACTH secretion (19). As oCRF was purified from a side fraction (5) containing only a small percentage of the total ACTH-releasing activity in ovine hypothalamic extracts (20), it was necessary to establish the structure of the predominant CRF in fresh tissue extracts. Because of its importance as an experimental animal and because its CRF differed from oCRF with respect to immunologic and chromatographic behavior, the rat was chosen as a source of CRF. We report here the isolation, characterization, and total synthesis of rat CRF (rCRF), a 41-residue peptide belonging to the family that includes oCRF, sauvagine (21), and urotensin I (22). In view of the fact that the latter three peptides have a spectrum of biological activities on the cardiovascular and central nervous systems of the mammal with differences in relative potencies (23–25), it was furthermore important to characterize CRF in the species in which its physiological functions have been most thoroughly investigated.

The publication costs of this article were defrayed in part by page charge payment. This article must therefore be hereby marked "*advertisement*" in accordance with 18 U.S.C. §1734 solely to indicate this fact.

EXPERIMENTAL PROCEDURES

rCRF was purified from lyophilized rat hypothalamic fragments provided by A. Parlow under the aegis of National Institute of Arthritis, Diabetes, and Digestive and Kidney Diseases. We used two separate biological tests to follow CRF activities throughout our purification schemes: (*i*) an *in vitro* method for assaying the ability of CRF to stimulate the secretion of ACTH and β-endorphin by primary cultures of rat pituitary cells (5, 26) and (*ii*) a radioimmunoassay (RIA) with the C-24 antibody directed toward the NH_2 terminus (residues 4–20) of oCRF and with HPLC-purified ^{125}I-labeled CRF, [Tyr^{13},Nle^{21}]CRF, or [Nle^{21},Tyr^{32}]CRF as the radioligand (22). C-24 CRF antibody was generated in a rabbit immunized with [Tyr^{22},Gly^{23}]oCRF-(1–23) coupled to human α globulin by bisdiazotized benzidine. Sensitivity of the assay is <1 fmol.

Peptides were made by the solid-phase approach. *t*-Butyloxycarbonyl N$^{\alpha}$ protection (Boc) was used throughout. Side chains were protected as follows: benzyl esters (OBzl) for both aspartic and glutamic acids, benzyl ethers (OBzl) for both serine and threonine hydroxyl groups, xanthydryl (Xan) for both asparagine and glutamine, tosyl (Tos) for both histidine and arginine, and chlorobenzyloxycarbonyl (2ClZ) for lysine. Couplings (1–2 hr) were mediated by dicyclohexylcarbodiimide in CH_2Cl_2 or dimethylformamide for asparagine, glutamine, and arginine derivatives. A 3-fold excess of amino acid based on the original substitution of the resin was used. Double couplings were automatically performed at residues 14, 18, and 40. Syntheses were started with as many as 12 g of resin per run in a modified Beckman 990 B synthesizer. In that case, the peptide resin weighed 25 g and was cleaved in HF (150 ml) batchwise (8 g) in the presence of anisole (20 ml) and methyl ethyl sulfide (2 ml). Two trifluoroacetic acid (60% in CH_2Cl_2, 5% ethanedithiol) deblockings were performed at each step (10 and 15 min, respectively). An isopropyl alcohol (1% ethanedithiol) wash followed CF_3COOH treatment prior to the two triethylamine (12% in CH_2Cl_2) neutralization steps (which included an intermediate MeOH wash). Each coupling step was followed by acetylation [10% $(CH_3CO)_2O$ in CH_2Cl_2 for 15 min].

For purification of the synthetic peptides, cartridges fitting Waters Associates preparative liquid chromatograph 500 (LC-500) were packed with 15- to 20-μm C_{18}, C_4, or phenyl silicas (300 Å, Vydac). A gradient of CH_3CN in TEAP buffer (27) was generated by a low-pressure Eldex gradient maker. Crude peptides (directly after HF cleavage and lyophilization) were loaded (3 g per run) through the pump; cuts were made, analyzed, and often rerun under different conditions until pure product was obtained. Desalting of the purified fractions, independently checked for purity, was achieved by using a gradient of CH_3CN in 0.1% CF_3COOH (28). The center cut was lyophilized to yield

Abbreviations: ACTH, adrenocorticotropic hormone or corticotropin; CRF, corticotropin-releasing factor; rCRF, rat CRF; oCRF, ovine CRF; RIA, radioimmunoassay; GH-RF, growth hormone (somatotropin)-releasing factor.

(10–15%) the desired product ($[\alpha]_D^{22} = -93.7°$, concentration = 1 g/100 ml in 1% AcOH after correction of peptide content by amino acid composition).

RESULTS AND DISCUSSION

Acetone defatting of the rat hypothalamic tissues and acid extraction followed by gel filtration on Sephadex G-50 has been described earlier in a scheme used for the initial purification of rat hypothalamic growth hormone (somatotropin)-releasing factor (GH-RF) (29). Several zones exhibiting CRF-like immunoactivity were found. A high molecular weight zone appearing in the void volume of the gel permeation step (representing <10% CRF-like immunoreactivity) and a zone (representing ≈90% CRF-like immunoreactivity) also containing GH-RF-like activity with a partition coefficient K_{av} of 0.20–0.31 were both biologically active as well as immunoreactive. Multiple low molecular weight peaks (K_{av} = 0.5–1.1) exhibited lower intrinsic activities (maximal secretory rate at maximum concentration of added product) than did oCRF in the *in vitro* bioassays and were not active in our CRF assay. We suspect that the smaller molecular weight bioactive substances with lower intrinsic activity than CRF may be related to neurohypophysial peptides or catecholamines. Although these weak ACTH secretagogs may have limited independent effects, they may be important as potentiators of the action of CRF (9, 16–18, 29). The nature of the high molecular weight fraction (K_{av} = 0.0–0.15) has not been further investigated and may represent a precursor molecule (30). An estimate of the relative content in the rat hypothalamus of the high molecular weight versus the 4- to 5-kilodalton species may depend upon several factors, including detection methods, tissue collection, and extraction procedures. The zone representing 90% of the total CRF-like immunoreactivity detected after gel filtration was further purified batchwise by preparative HPLC with the Waters Associates preparative LC-500 system (step 1 in Table 1). Under these conditions rCRF and GH-RF activities could be clearly separated. Retention volume for rat GH-RF was 1,600–1,800 ml. A total of 100,000 rat hypothalami were processed in three batches (which we will refer to as batch numbers 1, 2, and 3, respectively) of 60,000, 20,000, and 20,000 hypothalami, which had a total dry weight of 500 g. Yields were 5.9, 1.6, and ≈1.8 nanomol of rCRF, which had respective purities of 50–55%, 80–90%, and >90% as shown by sequence and amino acid analyses (31). Overall recovery was >50% as assessed by RIA of both starting materials and final product. This corresponds to an approximate 10^7-fold purification. Whereas we used batch 1 to optimize semipreparative and analytical chromatographic conditions that led to a preparation pure enough for sequence analysis and peptide mapping, batch 2 was used for confirmatory sequence analysis; batch 3 was purified, and an aliquot was further oxidized to [Met(O)^{21,38}]-rCRF in order to obtain an amino acid analysis that reflected the composition derived from sequence analysis and for chromatographic comparison with the synthetic replicate (Fig. 1). Details of the purification of batch 2 are outlined in Table 1.

A semipreparative step (1) on Vydac C_4 was followed by five analytical steps on different packing materials (Vydac C_4-, diphenyl- or C_{18}-derivatized 300-Å silicas) with two solvent systems (TEAP buffer or 0.1% CF_3COOH and CH_3CN). Step 7 was used to concentrate the earlier fractions from step 6 and free them from the 2-mercaptoethanol that had been added at each step to minimize oxidation of the methionine residue(s) prior to sequence analysis. Batch 3, which was purified following a strategy similar to that shown in Table 1, yielded before and after H_2O_2 treatment both rCRF and [Met(O)^{21,38}]rCRF as illustrated in Fig. 1. In contradistinction with oCRF, which was isolated in its oxidized methionine form from a minor side fraction of extracts originally used for the characterization of luteinizing hormone-releasing factor (20), rCRF was isolated in its native form and found to be the major ACTH-releasing substance with high intrinsic activity in fresh rat hypothalamic extracts.

Problems associated with the purification of hypophysi-

Table 1. Rat CRF purification from 20,000 rat hypothalami: Batch 2

HPLC step	Column Size, cm	Column Packing*	Flow rate, ml/min	Solvent A composition†	Loading volume, ml‡	Gradient shape†	UV detection	Retention volume,¶§ ml	Hypothalami equivalents¶
1	5 × 30	Vydac C_{18}	75	TEAP	4,000	7% B to 60% in 40 min	1.0 AUFS at 280 nm	1,900–2,200	20,000
2	1 × 25	Vydac C_4	3	0.1% CF_3COOH	14	40% B to 100% in 45 min	1.0 AUFS at 280 nm	76.5–81.5	3,000
3	0.45 × 25	Vydac diphenyl	1.2	TEAP	6	40% B to 80% in 30 min	2.0 AUFS at 235 nm	20.4–22.8	5,000
4	0.45 × 25	Vydac C_{18}	1.2	0.1% CF_3COOH	4	40% B to 80% in 30 min	2.0 AUFS at 210 nm	34.2–35.4	4,000
5	0.45 × 25	Vydac C_{18}	1.2	0.1% CF_3COOH	3	50% B to 80% in 30 min	0.05 AUFS at 280 nm	31.8–32.4	10,000
6	0.45 × 25	Vydac diphenyl	1.2	0.1% CF_3COOH	3	40% B to 50% in 20 min	0.35 AUFS at 210 nm	30.0–30.6	5,000
7	0.45 × 25	Vydac diphenyl	1.2	0.1% CF_3COOH	3	40% B to 50% in 20 min	0.20 AUFS at 210 nm	30.0–30.7	20,000

* Particle size was 5 μm except for step 1 which used 20 μ silica. All silicas were end-capped.

† TEAP buffer (pH 2.25) was made as described in ref. 27. B was 60% CH_3CN diluted in solvent A.

‡ Except for step 1, active fractions were loaded 1 ml at a time at regular intervals into a 2-ml loop after being diluted with H_2O (1:1, vol/vol).

§ Retention volume of the active zone was measured in ml from the time of start of the gradient (does not include loading volume). 2-Mercaptoethanol was added to all fractions (collected in polypropylene tubes) as they were eluted from the column (final concentration, 0.2–0.5%). Fractions were stored at −20°C. Aliquots for assays (0.1–1% of total fraction volume; never less than 5 μl) were measured with micropipettes and plastic tips and were transferred into polypropylene tubes containing bovine serum albumin (10 μl; 10 mg/ml) and dried in a Savant rotary evaporator. Losses prior to assays and inconsistent results could be minimized in that manner.

¶ In order to determine recoveries of biological activity throughout the purification scheme, one has to keep record of the original number of hypothalami (hypothalami equivalents or fragments) that are applied per chromatographic run, so that a specific activity can be deduced. When 3,000 hypothalami equivalents were loaded (step 2), seven identical runs were performed to process the total 20,000 hypothalami.

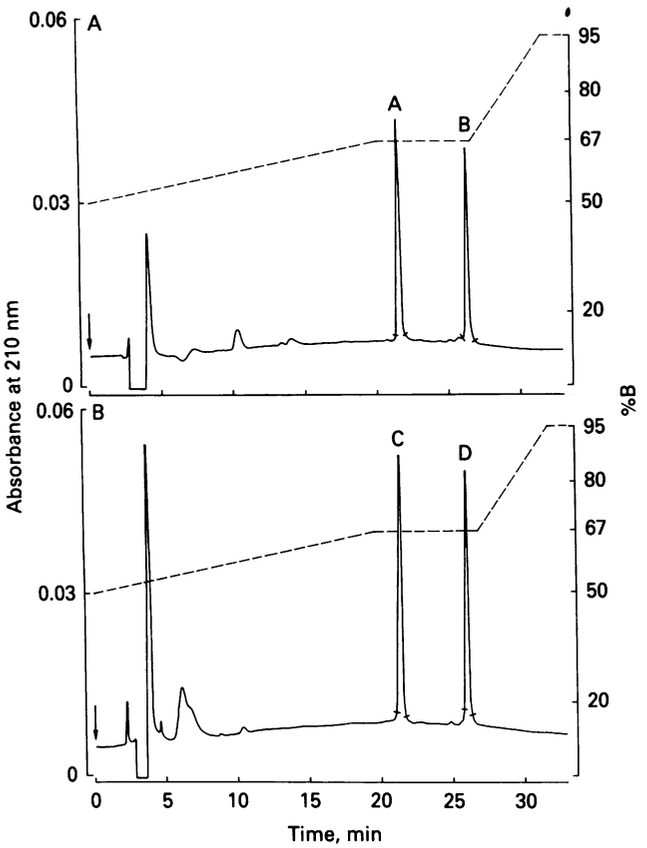

FIG. 1. (*A*) To an aliquot of rCRF (1,200 hypothalami equivalent in 0.25 ml of 0.1% CF_3COOH in 35% CH_3CN/65% H_2O) from step 7 in Table 1 diluted with water (0.25 ml) was added H_2O_2 (30%, 50 μl) at 0°C. The solution was kept at that temperature for 0.5 hr and was chromatographed under the conditions described below. The major peak (>90%) [Met(O)^{21,38}]rCRF was collected for rechromatography in the presence of an equivalent amount of nonoxidized material to generate chromatogram *A*. (*B*) Synthetic [Met(O)^{21,38}]rCRF was preparatively generated by treatment at 0°C of the synthetic rCRF (5 mg) in 1% AcOH (1 ml) with an excess of H_2O_2 (100 μl) for 0.5 hr. Aliquots (0.7 μg) of both synthetic peptides were rechromatographed to generate chromatogram *B*. Kinetic studies have shown that prior to generating [Met(O)^{21,38}]rCRF, both intermediates [Met(O)^{21}]rCRF and [Met(O)^{38}]-rCRF are formed and can be easily separated from both reduced and fully oxidized materials. The samples of native and synthetic peptides were applied in two successive runs to a Vydac C_4 (no. 2115-6) end-capped (5-μm 300-Å pore size; 4.5 × 250 nm) column, which was eluted under gradient conditions shown by the dotted line. The solvent in *A* was 0.1% CF_3COOH; the solvent in B was 0.1% CF_3COOH in 60% CH_3CN/40% H_2O. Flow rate was 1.2 ml/min. UV detection at 210 nm is expressed in absorbance units full scale. Peaks A and C are native and synthetic [Met(O)^{21,38}]rCRF; B and D are native and synthetic rCRF.

otropic peptides from ovine or rat hypothalami have been well recognized (4, 32). We had observed that large-pore C_{18}-derivatized silica (Vydac, 5-μm particle size, 300-Å pore size) gave significantly better resolution of CRF and, in general, of peptides larger than 25 residues than similarly derivatized silicas with pore sizes ranging from 80 to 120 Å (33). Usage of similar silicas with different selectivities obtained by derivatization and end-capping (C_4, diphenyl, and C_{18}) and of two solvents (TEAP and 0.1% CF_3COOH) with different ion-pairing capabilities has resulted in good resolution and excellent recovery of rCRF biological and immunological activities.

The primary structure of rCRF was determined by Edman degradation using the *o*-phthaldialdehyde strategy and peptide mapping on reverse-phase HPLC after clostripain digestion (31). The *o*-phthaldialdehyde strategy consists of selectively blocking one or several primary amine termini during Edman degradation at a cycle where a nonreactive proline residue is present, thus allowing for further degradation of the proline-containing peptide.

A homology (83%) with oCRF is present (Fig. 2): substitutions are found at positions 2, 22, 23, 25, 38, 39, and 41. With the exception of the position 2 substitution, oCRF and rCRF share a common 1–21 sequence, thus explaining the ability of the RIA with the C-24 antibody directed against the NH_2-terminal 4–20 residues to read both CRFs well, whereas most RIAs developed against the whole oCRF sequence had been shown in several laboratories (23, 30) to only poorly detect rCRF. RIAs with the C-24 antisera have been used to detect CRF-like immunoactivity in the hypothalamus/median eminence/pituitary of sheep, dog, rat, and human (9, 13, 14).

rCRF was synthesized in a stepwise manner on a paramethylbenzhydrylamine resin. The following sequence was assembled by using experimental procedures similar to those described earlier (32): Boc-Ser(OBzl)-Glu(OBzl)-Glu(OBzl)-Pro-Pro-Ile-Ser(OBzl)-Leu-Asp(OBzl)-Leu-Thr(OBzl)-Phe-His-(Tos)-Leu-Leu-Arg(Tos)-Glu(OBzl)-Val-Leu-Glu(OBzl)-Met-Ala-Arg(Tos)-Ala-Glu(OBzl)-Gln(Xan)-Leu-Ala-Gln(Xan)-Gln(Xan)-Ala-His(Tos)-Ser(OBzl)-Asn(Xan)-Arg(Tos)-Lys(2ClZ)-Leu-Met-Glu(OBzl)-Ile-Ile-paramethylbenzhydrylamine resin. After complete deprotection and cleavage by hydrofluoric acid, the crude preparation of rCRF was purified by using preparative HPLC techniques developed in our laboratory (35). The synthetic peptide was shown to be greater than 98% pure by HPLC with several solvent systems and columns; it had the expected amino acid composition, and a peptide map after incubation with clostripain was generated by reverse-phase HPLC (31). No evidence for contaminating peptides could be shown. Native [Met(O)^{21,38}]rCRF could be generated by H_2O_2 oxidation in 0.1% CF_3COOH in the presence of 10–20% CH_3CN. Synthetic [Met(O)^{21,38}]rCRF was generated from synthetic rCRF after H_2O_2 oxidation in 1% AcOH as reported earlier for oCRF[5]. As can be seen (Fig. 1), both natural and synthetic peptides cannot be distinguished from their chromatographic behavior in that system. Similarly, all other tests, including sequence analysis and peptide mapping of natural and synthetic rCRF, cannot show any significant difference.

The abilities of native rCRF, synthetic rCRF, and oCRF to stimulate ACTH secretion by cultured rat pituitary cells are compared in Fig. 3. In two assays, one of which is shown in Fig. 3, both synthetic rCRF and oCRF and native rCRF exhibit similar potencies and intrinsic activities. In the same assay, [Met(O)^{21,38}]rCRF was found to have only 1% of CRF potency, hence justifying our efforts to keep our extracts in the presence of reducing agents (2-mercaptoethanol) throughout the purification scheme. In the rat, rCRF has been shown to be equipotent to oCRF and to exhibit similar activities (unpublished data).

We had shown that acetylated des-Ser-Gln-Glu-oCRF and des-Ser-Gln-Glu-Pro-Pro-oCRF had full intrinsic activity and 100% and 28% of oCRF's potency, respectively, whereas oCRF-(10–41) was almost devoid of biological activity, indicating that the NH_2-terminal five or six residues were not indispensable for intrinsic activity (7). Thus, substitution of Gln-2 in oCRF by Glu-2 in rCRF would not have been expected to modify either the spectrum of activities or potency of rCRF. Substitutions at positions 22, 23, and 25 (and 38, 39, and 41), which are within a sequence stretch that was predicted [by using Chou and Fasman statistical analysis (36)] or proposed [by using circular dichroism spectroscopy and other physicochemical methods (T. Kaiser, M. Goodman, and P. Pallai, personal communication; unpublished data)] to have a highly amphiphilic α-helical sec-

```
                    5         10        15        20        25        30        35        40
RAT CRF             S E E P P I S L D L T F H L L R E V L E M A R A E Q L A Q Q A H S N R K L M E I I■
OVINE CRF           S Q E P P I S L D L T F H L L R E V L E M T K A D Q L A Q Q A H S N R K L L D I A■
SUCKER U-I          N D D P P I S I D L T F H L L R N M I E M A R I E N E R E Q A G L N R K Y L D E V■
CARP U-I            N D D P P I S I D L T F H L L R N M I E M A R N E N Q R E Q A G L N R K Y L D E V■
                         5         10        15        20        25        30        35        40
SAUVAGINE           Z G P P I S I D L S L E L L R K M I E I E K Q E K E K Q Q A A N N R L L L D T I■
```

FIG. 2. The CRF family homologous amino acids are italicized. A, alanine; D, aspartic acid; E, glutamic acid; F, phenylalanine; G, glycine; H, histidine; I, isoleucine; K, lysine; L, leucine; M, methionine; N, asparagine; Q, glutamine; R, arginine; S, serine; T, threonine; V, valine; Y, tyrosine; Z, pyrrolidonecarboxylic acid; ■, amidated COOH terminus.

ondary structure encompassing residues 8–31 and possibly 7–41, could have had an important effect on biological activity. By using Chou and Fasman P_α values assigned to Thr-22 (0.83), Lys-23 (1.16), Asp-25,39 (1.01), Leu-38 (1.21), and Ala-41 (1.42) for oCRF and Ala-22 (1.42), Arg-23 (0.98), Glu-25,39 (1.51), Met-38 (1.45), and Ile-41 (1.02) for rCRF, we show a significant increase in α helix-forming potential. Furthermore, the character of the side chains has been conserved (Ala-22/Thr-22, Met-38/Leu-38, and Ile-41/Ala-41, neutral; Arg-23/Lys-23, basic; Glu-25,39/Asp-25,39, acidic). The strengthening of the potential α-helical secondary structure of rCRF versus that of oCRF may further suggest that this structure is indeed the biologically active form that we proposed would interact with the receptor. Finally, all substitutions, with the exception of Ile-41 for Ala-41, may have resulted from single base changes.

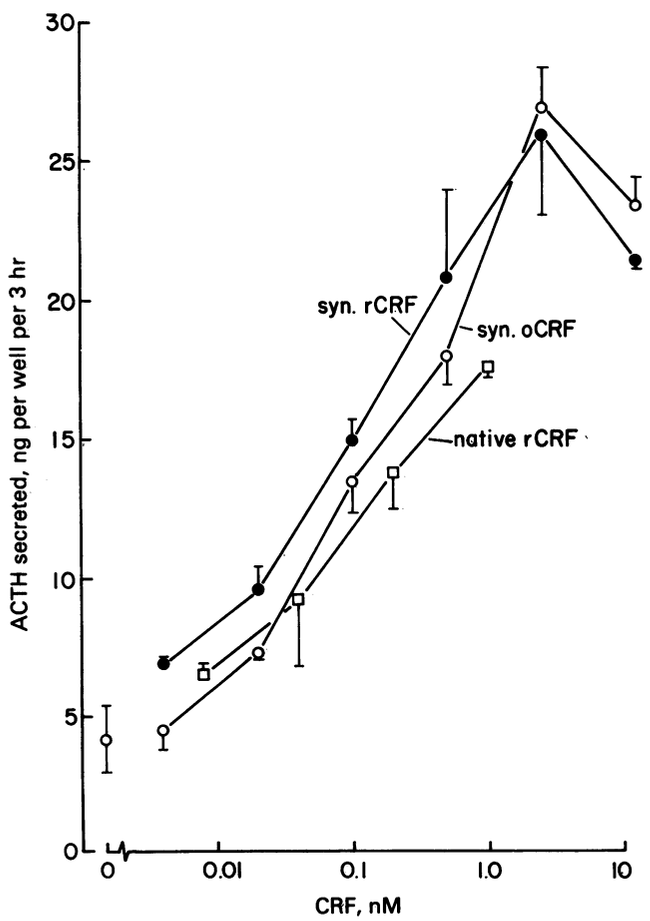

FIG. 3. Effects of native rCRF, synthetic oCRF, and synthetic rCRF on ACTH secretion over a 4-hr period by rat pituitary cells in monolayer culture for 3–5 days. Cells were dissociated and cultured as described (29). ACTH levels are estimated by double antibody RIA. Concentration of purified native rCRF was determined by amino acid analysis.

Ovine CRF and human pancreatic tumor GRF (35) differ from the corresponding rat hypothalamic peptides by 17% and 33%, respectively. This marked nonalignment between mammalian regulatory peptides has not been seen for the smaller hypothalamic hypophysiotropic peptides.

We thank Dr. C. Rivier for supervising the ACTH RIAs in our attempts to isolate rCRF by bioassay and for RIA data reported in Fig. 2. We are grateful to Dr. Al Parlow who, through the National Hormone and Pituitary Program, provided the rat hypothalamic fragments. We acknowledge the technical assistance of J. Vaughan, R. Galyean, R. McClintock, R. Kaiser, G. Yamamoto, C. Douglas, C. Jenkins, A. Wells, D. Dalton, J. Heil, R. Lee, and L. Keadle and manuscript preparation by L. Wheatley, D. Weigand, and S. McCall. This research was supported by National Institutes of Health Grants AM26741, AM20917, HD13527, and AA03504 and by grants from The Rockefeller Foundation and the March of Dimes Birth Defects Foundation. Research was conducted in part by The Clayton Foundation for Research, California Division. W.V. and J.S. are Clayton Foundation Investigators.

1. Harris, G. W. (1948) *Physiol. Rev.* **28**, 139–179.
2. Guillemin, R. & Rosenberg, B. (1955) *Endocrinology* **57**, 599–607.
3. Saffran, M. & Schally, A. V. (1955) *Can. J. Biochem. Physiol.* **33**, 408–415.
4. Rivier, J., Rivier, C., Spiess, J. & Vale, W. (1983) *Anal. Biochem.* **127**, 258–266.
5. Vale, W., Spiess, J., Rivier, C. & Rivier, J. (1981) *Science* **213**, 1394–1397.
6. Spiess, J., Rivier, J., Rivier, C. & Vale, W. (1981) *Proc. Natl. Acad. Sci. USA* **78**, 6517–6521.
7. Rivier, J., Spiess, J., Rivier, C., Lederis, K. & Vale, W. (1983) in *17th European Peptide Symposium*, Prague, Czechoslovakia, Aug. 29–Sept. 3, 1982, eds. Blaha, K. & Malon, P. (de Gruyter, Berlin), in press.
8. Furutani, Y., Morimoto, Y., Shibahara, S., Noda, M., Takahashi, H., Hirose, T., Asai, M., Inayama, S., Hayashida, H., Miyata, T. & Numa, S. (1983) *Nature (London)* **301**, 537–540.
9. Vale, W. W., Rivier, C., Spiess, J., Brown, M. & Rivier, J. (1983) in *Brain Peptides*, eds. Krieger, D., Brownstein, M. & Martin, J. (Wiley, New York), in press.
10. Brown, M. R., Fisher, L. A., Spiess, J., Rivier, C., Rivier, J. & Vale, W. (1982) *Endocrinology* **111**, 928–931.
11. Sutton, R. E., Koob, G. F., LeMoal, M., Rivier, J. & Vale, W. (1982) *Nature (London)* **297**, 331–333.
12. Fisher, L. A., Rivier, J., Rivier, C., Spiess, J., Vale, W. & Brown, M. R. (1982) *Endocrinology* **110**, 2222–2224.

13. Swanson, L. W., Sawchenko, P. E., Rivier, J. & Vale, W. W. (1983) *Neuroendocrinology* **36,** 165–186.
14. Bloom, F. E., Battenberg, E. L. F., Rivier, J. & Vale, W. (1982) *Regul. Peptides* **4,** 43–48.
15. Gibbs, D. M. & Vale, W. (1982) *Endocrinology* **111,** 1418.
16. Gillies, G. E., Linton, E. A. & Lowry, P. J. (1982) *Nature (London)* **299,** 355–357.
17. Turkelson, C. M., Thomas, C. R., Arimura, A., Chang, J., Chang, J.-H. & Shimiza, M. (1982) *Peptides* **1,** 111.
18. Beny, J. L. & Baertschi, A. J. (1982) *Experientia* **38,** 270.
19. Rivier, C., Rivier, J. & Vale, W. (1982) *Science* **218,** 377–379.
20. Burgus, R., Amoss, M., Brazeau, P., Brown, M., Ling, N., Rivier, C., Rivier, J., Vale, W. & Villarreal, J. (1976) in *Hypothalamus and Endocrine Functions,* eds. Labrie, F., Meites, J. & Pelletier, G. (Plenum, New York), pp. 355–372.
21. Montecucchi, P. C., Anastasi, A., deCastiglione, R. & Erspamer, V. (1980) *V. Int. J. Peptide Protein Res.* **16,** 191–196.
22. Lederis, K., Leter, A., McMaster, D., Moore, G. & Schlesinger, D. (1982) *Science* **218,** 162–164.
23. Vale, W., Rivier, C., Brown, M. R., Spiess, J., Koob, G., Swanson, L., Bilezikjian, L., Bloom, F. & Rivier, J. (1983) in *Recent Progress in Hormone Research,* ed. Greep, R. (Academic, San Diego, CA), Vol. 39, in press.
24. Brown, M. R., Fisher, L. A., Spiess, J., Rivier, J., Rivier, C. & Vale, W. (1982) *Regul. Peptides* **4,** 107–114.
25. MacCannell, K. L., Lederis, K., Hamilton, P. L. & Rivier, J. (1982) *Pharmacology* **25,** 116–120.
26. Vale, W., Grant, G., Amoss, M., Blackwell, R. & Guillemin, R. (1972) *Endocrinology* **91,** 562–572.
27. Rivier, J. (1978) *J. Liq. Chromatogr.* **1,** 343–367.
28. Bennett, H. P. J., Hudson, A. M., McMartin, C. & Purdon, G. (1977) *Biochem. J.* **168,** 9–15.
29. Vale, W., Vaughan, J., Yamamoto, G., Spiess, J. & Rivier, J. (1983) *Endocrinology* **112,** 1553–1555.
30. Linton, E. A. & Lowry, P. J. (1982) *Neuropeptides* **3,** 45–52.
31. Spiess, J., Rivier, J. & Vale, W. (1983) *Biochemistry,* in press.
32. Guillemin, R., Brazeau, P., Bohlen, P., Esch, F., Ling, N. & Wehrenberg, W. B. (1982) *Science* **218,** 585–587.
33. Rivier, J., McClintock, R., Eksteen, R. & Karger, B. L. (1983) in *Hormone Drugs,* eds. Gueriguian, J. L., Bransome, E. D., Jr., & Outschoorn, A. S. (U.S. Pharmacopeial Convention, Inc., Rockville, MD), pp. 554–564.
34. Marki, W., Spiess, J., Tache, Y., Brown, M. & Rivier, J. E. (1981) *J. Am. Chem. Soc.* **103,** 3178–3185.
35. Rivier, J., Spiess, J., Thorner, M. & Vale, W. (1982) *Nature (London)* **300,** 276–278.
36. Chou, P. Y. & Fasman, G. D. (1978) *Annu. Rev. Biochem.* **47,** 251–276.