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Biochemistry

Isolation, primary structure, and synthesis of human hypothalamic somatocrinin: Growth hormone-releasing factor

(hypothalamus/pituitary/stalk median eminence/immunoaffinity chromatography/microsequencing)

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ABSTRACT **The hypophysiotropic peptide, growth hormone-releasing factor (GRF), was isolated from human hypothalamic–hypophysial tissues by means of acid extraction, immunoaffinity chromatography, gel filtration, and two steps of reverse-phase high-performance liquid chromatography. Amino acid sequence determination using a gas-phase sequencer and reverse-phase liquid chromatography of the native peptide and its synthetic replicates showed its primary structure to be as follows: H-Tyr-Ala-Asp-Ala-Ile-Phe-Thr-Asn-Ser-Tyr-Arg-Lys-Val-Leu-Gly-Gln-Leu-Ser-Ala-Arg-Lys-Leu-Leu-Gln-Asp-Ile-Met-Ser-Arg-Gln-Gln-Gly-Glu-Ser-Asn-Gln-Glu-Arg-Gly-Ala-Arg-Ala-Arg-Leu-NH_2, which is identical to that of the GRF recently isolated and characterized from a human pancreatic tumor that had caused acromegaly. Human hypothalamic GRF shows major homologies (93%, 89%, and 86%, respectively) when its primary structure is compared to that of the hypothalamic GRF from the porcine, bovine, caprine, and ovine species.**

From a tumor of the pancreas that had caused acromegaly, we recently isolated and characterized three structurally related peptides with high intrinsic activity specifically to stimulate the secretion of hypophysial growth hormone (1). The most potent of these peptides, hpGRF-44 (human pancreas growth hormone-releasing factor) was shown to be composed of 44 amino acids with an amidated COOH terminus, the other two peptides, hpGRF-37 and hpGRF-40, being shown to correspond to the sequences 1–37 and 1–40 of hpGRF-44. The latter two peptides possess a free COOH terminus. Because of its higher potency in the *in vitro* assay and because it has an amidated COOH terminus, we proposed that hpGRF-44 was the mature form of the peptide, hpGRF-37 and hpGRF-40 being postsecretory degradation products still endowed with biological activity (1). Subsequent cloning of the precursor molecule for GRF from the tumor confirmed the proposal that hpGRF-44 was the mature peptide (2, 3) because it was shown to be preceded in the precursor by a dibasic cleavage sequence (Arg-Arg) and terminated by a cleavage and amidation signal (Gly-Arg).

In the original paper reporting the primary structure of hpGRF-44 (1), we had concluded that the material characterized from the tumor had all the biological characteristics of the long-postulated but still not characterized hypothalamic human GRF (hGRF). We also had raised the question whether the tumor-derived material ectopically produced was indeed identical to the physiological hGRF of hypothalamic origin. Subsequently, we reported (4) that immunoreactive and bioactive hGRF purified from the hypothalami of four human brains had the same elution characteristics and immunoreactivity as hpGRF-44, with a minor fraction corresponding to hpGRF-40. Because only minute amounts of the hypothalamic material were purified, the possibility of determining even the amino acid composition was out of the question.

Ascertaining the identity of hypothalamic hGRF and comparing it with that of the tumor-derived hpGRF still remained to be done both because of the fundamental questions involved and because regulatory agencies both in this country and overseas have been asking whether hpGRF-44 is truly a replicate of the hypothalamic material when given to patients in one clinical context or another or whether it should be considered as a drug endowed with the biological activities of the as yet uncharacterized hypothalamic hGRF. Therefore, we organized the collection of several thousand stalk median eminence fragments of human pituitary and hypothalamus origin and report here the isolation of hypothalamic hGRF and its complete structural characterization and synthesis.

MATERIALS AND METHODS

Source of Human Tissues. Three batches of human pituitary stalk–hypothalamic median eminence fragments were separately processed through the whole isolation procedure to isolate the hypothalamic hGRF. The first batch consisted of 600 acetone-preserved fragments provided by S. Reichlin (New England Medical Center), 737 fragments processed into acetone powder provided by A. Parlow (Harbor-UCLA Medical Center), 109 frozen fragments provided by T. Shibasaki and K. Shizume (Tokyo Women's Medical College), and 100 frozen fragments provided by R. Gaillard (Hôpital Cantonal, Geneva). The second batch consisted of 610 frozen fragments provided by H. Friesen (University of Manitoba Faculty of Medicine), 379 frozen fragments obtained from P. Chatelain, J. C. Job, and F. Dray (Association France Hypophyse, Paris), and 43 frozen fragments provided by T. Shibasaki and K. Shizume (Tokyo Women's Medical College). The third batch of 2050 acetone-preserved frozen fragments was provided by A. Parlow (Harbor-UCLA Medical Center).

Extraction of Tissues. Each batch of tissue was either lyophilized or vacuum-dried and extracted by the following procedure. Tissues were boiled in water (12 ml/g) for 2 min, immediately cooled in an ice-bath, and then homogenized with an equal volume of 0.6 M HCl containing phenylmethylsulfonyl fluoride and peptstatin A (10 μg/ml each) and 0.5% 2-mercaptoethanol by using a Polytron (Brinkmann) for 5

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Abbreviations: GRF, growth hormone-releasing factor; hGRF, human GRF; hGRF-44-NH_2 and hGRF-44-OH, the 44-amino-acid COOH-terminal amide and COOH-terminal acid forms of hGRF, respectively; hpGRF, pancreatic hGRF; irGRF, immunoreactive GRF; MBHA, *p*-methylbenzhydrylamine.

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min. The homogenate was centrifuged at 20,000 rpm for 30 min in a JA-20 rotor (Beckman model J2-21 centrifuge), and the supernatant was decanted. The pellet was reextracted with one-half volume of HCl but at 0.3 M concentration and containing the same amount of all the additives. The supernatants were combined and extracted three times with twice their total volume of petroleum ether/diethyl ether, 2:1 (vol/vol). The aqueous phase from the last extraction was centrifuged at 10,000 rpm for 30 min in a JA-10 rotor to break up the emulsion. The supernatant was removed by suction, and an equal volume of 40 mM Na_2HPO_4 was added to the aqueous phase. The pH was adjusted to 7.4 with 5 M NaOH, and the extract was degassed with a vacuum aspirator for 30 min. Any remaining precipitate was removed by centrifugation.

Construction of the GRF Immunoaffinity Column. A pool of antiserum (98 ml) from four rabbits immunized against hpGRF-40 by using the methylated bovine serum albumin technique (5) was precipitated once with saturated $(NH_4)_2SO_4$ to concentrate the IgG (6). The precipitate was redissolved in half its serum volume of borate-buffered saline (pH 8.3) (0.100 M H_3BO_3/0.025 M $Na_2B_4O_7 \cdot 10H_2O$/0.075 M NaCl) and dialyzed three times against 2 liters of the same buffer in a Spectrapor no. 1 membrane tubing (M_r cutoff, 6000–8000; cylinder diameter, 14.6 mm; Spectrum Medical Industries, Los Angeles) at 4°C for 24 hr. The volume of the retentate was adjusted to its original serum volume with the same buffer, and the remaining insoluble particles were removed by centrifugation to yield a purified IgG solution. Inhibition of the binding of the hpGRF-40 tracer to the purified IgG by synthetic hpGRF fragments revealed that the pooled antibodies recognized the midportion of the peptide.

Seventy milliliters of Affi-Gel 10 (Bio-Rad) were transferred to a 3.5 × 12 cm glass column containing a coarse-porosity fritted disc sealed above the stopcock at the bottom and a 14/20 standard ground joint at the top. The gel was washed in the cold room four times with 35 ml of ice-cold 2-propanol and four times with 35 ml of ice-cold water. The total washing should be accomplished within 30 min to minimize hydrolysis of the active ester. Thirty-five milliliters of the purified IgG solution was added, and the mixture was rocked in the cold-room for 24 hr. The remaining activated gel was blocked by rocking with 7 ml of 1 M glycine amide (pH 8.00) for another 24 hr. The solvent was drained, and 700 ml of phosphate-buffered saline (pH 7.4) (0.020 M Na_2HPO_4/0.150 M NaCl/0.01% NaN_3) followed by 280 ml of 1 M CH_3COOH and another 700 ml of the phosphate-buffered saline were pumped successively through the column with a Varioperplex II pump (LKB) at 35 ml/hr to prewash the gel. The irreversible binding sites on the immunoaffinity column were blocked by rocking with 340 μg of synthetic hpGRF(25–44)NH_2 in 35 ml of the phosphate-buffered saline for 4 hr in the cold room, and the excess blocking peptide was removed by washing the column once more through the complete prewash cycle.

Isolation of hGRF. The tissue extract was pumped through the GRF-immunoaffinity column twice at the same flow rate as above. After washing off the unbound material with 700 ml of phosphate-buffered saline, the adsorbed immunoreactive GRF (irGRF) was eluted with 1 M CH_3COOH, and frac-

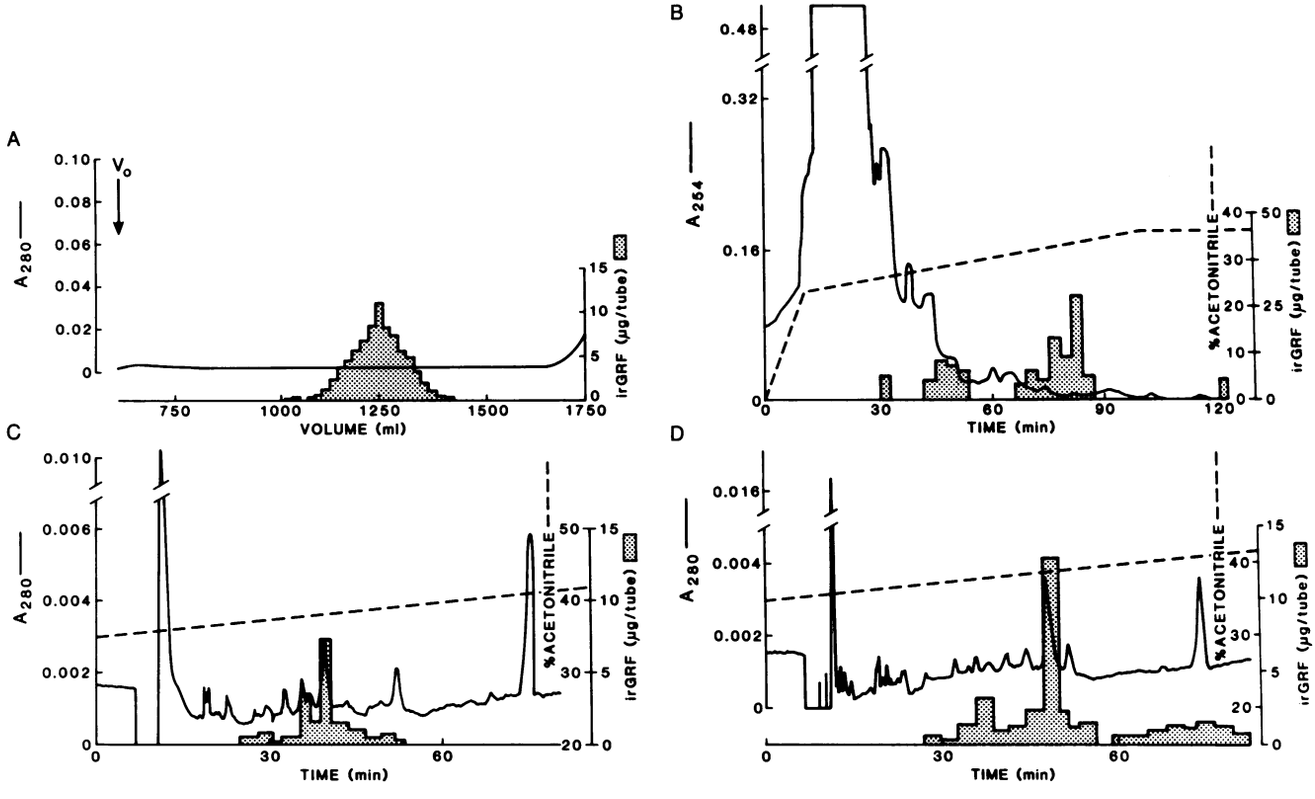

FIG. 1. Isolation of hGRF from a batch of 1032 human pituitary stalk–hypothalamic median eminence fragments. (*A*) The immunoaffinity-purified irGRF was chromatographed on a Sephadex G-75 column (4.5 × 117 cm, V_{bed} = 1900 ml) and developed in 1 M CH_3COOH/0.2% 2-mercaptoethanol at 1 ml/min. (*B*) The gel-filtered irGRF fractions eluted between 1098 and 1368 ml were pooled and pumped onto a semipreparative 1 × 25 cm Ultrasphere ODS column (5-μm particle size; Altex, Berkeley, CA) with a 0.25 M triethylammonium phosphate, pH 3.0/acetonitrile mobile phase. Fractions of 2.5 ml were collected at 1 ml/min. (*C*) The irGRF species eluted between 78 and 87 min in *B* were pooled and purified on a 0.46 × 25 cm Aquapore RP-300 column (7-μm particle size; Brownlee Labs) with a 0.2% heptafluorobutyric acid/acetonitrile solvent system. Fractions of 2.5 ml were collected at 1 ml/min. (*D*) The irGRF species eluted between 72 and 77 min in *B* were pooled and purified in the same manner as in *C*. At all chromatography steps, 1% aliquots of the column fractions were subjected to RIA after drying in a vacuum centrifuge (Savant) in the presence of 100 μg of serum albumin.

tions of 7 ml were collected. Location of the irGRF in the column effluents was performed with a RIA using the same pool of hpGRF-40 antisera as that used to construct the affinity column. The fractions containing irGRF were pooled and further purified by gel filtration on Sephadex G-75 and two steps of reverse-phase high-performance liquid chromatography (HPLC), all done at room temperature. Details of the chromatographic conditions are given in the figure legends.

Structural Characterization. Amino acid analyses (7) and the sequence determination (8) were performed as described, except for a new program (designated *MHNVAC*, supplied by M. Hunkapiller of Applied Biosystems, Foster City, CA) obviating the use of the vacuum system in the gas-phase sequencer. Also, the resultant phenylthiohydantoin–amino acid residues were identified by using an improved HPLC system suggested by W. Touchstone (Baylor College of Medicine, Houston, TX) and M. Hunkapiller.

Synthesis. The GRF peptides were synthesized by solid-phase methodology (9) on a peptide synthesizer (Beckman model 990). Peptides with an amidated COOH terminus were prepared with a *p*-methylbenzhydrylamine resin (MBHA resin) (10); for peptides with a free COOH terminus, a chloromethyl-substituted polystyrene resin (substituted with 0.9 mmol of chlorine per g) (9) was used. Derivatized amino acids (Bachem Fine Chemicals, Torrance, CA) used in the synthesis were of the L-configuration, and the N^{α}-amino function was protected exclusively with the *t*-butyloxycarbonyl group. Side-chain functional groups were protected as follows: benzyl for serine, threonine, glutamic acid, and aspartic acid; tosyl for arginine; 2-chlorobenzyloxycarbonyl for lysine; and 2,6-dichlorobenzyl for tyrosine. Coupling of the COOH-terminal amino acid to the MBHA resin was carried out with dicychohexylcarbodiimide and to the chloromethyl resin by using a previously published procedure (11). The subsequent amino acids were coupled according to the following schedule: (*a*) wash two times (i.e., twice for 0.5 min per wash; see below) with CH_2Cl_2; (*b*) wash with 50% CF_3COOH/5% 1,2-ethanedithiol in CH_2Cl_2; (*c*) deprotect with 50% CF_3COOH/5% 1,2-ethanedithiol in CH_2Cl_2; (*d*) wash three times with CH_2Cl_2; (*e*) wash two times with CH_3OH; (*f*) wash two times with 10% triethylamine in CH_2Cl_2; (*g*) wash two times with CH_3OH; (*h*) repeat steps *f* and *g*; (*i*) wash two times with CH_2Cl_2; (*j*) couple protected amino acid at a concentration of 1 mmol/g of resin with an equivalent amount of dicyclohexylcarbodiimide in CH_2Cl_2, except for leucine, arginine, asparagine, and glutamine, which were coupled in 30% dimethylformamide in CH_2Cl_2; (*k*) wash with CH_2Cl_2; (*l*) wash two times with 50% dimethylformamide in CH_2Cl_2; (*m*) wash with 10% triethylamine in CH_2Cl_2; (*n*) wash two times with CH_3OH; (*o*) wash two times with CH_2Cl_2; (*p*) acetylate unreacted N^{α}-amino group with 25% acetic anhydride in CH_2Cl_2; (*q*) wash two times with CH_2Cl_2; and (*r*) wash two times with CH_3OH. The duration of each washing step was 0.5 min. Deprotection and acetylation were carried out in 20 min. Coupling of the protected amino acid was performed for 2 hr and, in the cases of asparagine and glutamine, a 1.2 equivalent of 1-hydroxybenzotriazol was included. After the last amino acid had been incorporated, the *t*-butyloxycarbonyl protecting group was removed (12) before 1 g of the peptide–resin conjugate was treated with a mixture of 14 ml of HF, 1.5 ml of anisole, and 0.25 ml of methylethyl sulfide at −20°C for 0.5 hr and at 0°C for 0.5 hr. The HF was removed in vacuo at 0°C, and the resulting peptide and resin mixture was washed twice with $(CH_3CH_2)_2O$ and twice with $CHCl_3$ and $(CH_3CH_2)_2O$ alternately. The peptide was extracted five times with 2 M CH_3COOH, and the extract was lyophilized. The lyophilized product was first purified on a column of Sephadex G-50 fine (Pharmacia) developed in 30% CH_3COOH to remove the truncated fragments and salt. The next step of purification was by CM-32 carboxymethylcellulose cation-exchange chromatography (Whatman) developed with a gradient generated by adding 2.5 volumes of 0.4 M NH_4OAc at pH 6.5 to 1 volume of 0.01 M NH_4OAc at pH 4.5. Final purification was achieved by partition chromatography on Sephadex G-50 fine using the solvent system 1-butanol/ethanol/pyridine/0.2 M CH_3COOH, 4:1:1:7 (vol/vol). The chromatographic fractions were monitored by UV absorption at 280 nm (ISCO model UA5 absorbance monitor) and TLC on 0.25-mm-thick precoated silica gel 60 plates (EM Laboratories, Elmsford, NY) with the solvent system 1-butanol/pyridine/CH_3COOH/water, 6:6:1.2:4.8 (vol/vol), and the spots were detected with ninhydrin spray.

Starting with 6.00 g of MBHA resin substituted by 0.6 mmol of amine per g, 7623 mg of crude peptide was obtained from the HF treatment. After purification, 426 mg of hGRF-44-NH_2 was obtained (2.3% yield based on substitution of the amino group on the MBHA resin).

The corresponding hGRF-44-OH was synthesized on 2.70 g of chloromethyl-substituted polystyrene resin esterified to the extent of 0.42 mmol of leucine per gram. The crude peptide (2818 mg) was purified by the same procedure used for the hGRF-44-NH_2, and 200 mg of product was obtained (3.5% yield based on substitution of the leucine on the chloromethyl resin).

RESULTS

The isolation of hypothalamic hGRF was achieved by using the five-step purification procedure described here involving

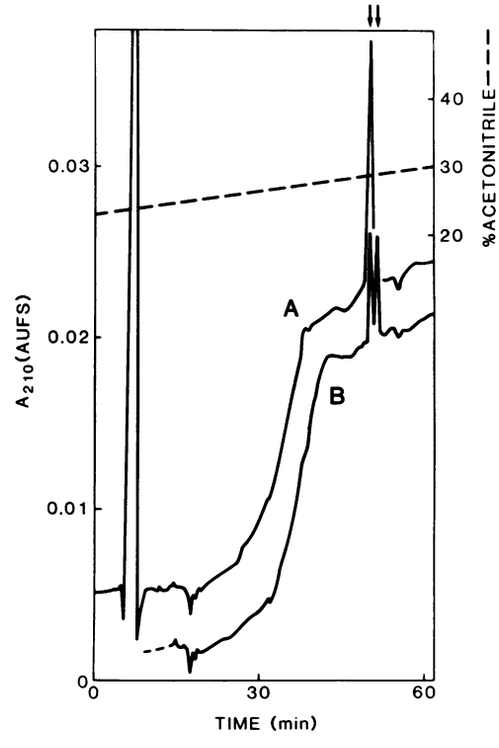

FIG. 2. Reverse-phase liquid chromatography of native hGRF-44 and synthetic replicates containing either a free acid or an amidated COOH terminus. Samples were analyzed on a 0.46 × 25 cm Aquapore RP-300 column (7-μm particle size; Brownlee Labs) with a 0.25 M triethylammonium phosphate, pH 3.0/acetonitrile mobile phase as eluant at 1 ml/min. Traces: A, the sample injected was a mixture of 50 pmol of synthetic hGRF-44-NH_2 and an equal amount of native hGRF-44; B, the sample injected was a mixture of 50 pmol of synthetic hGRF-44-OH and an equal amount of native hGRF-44. The right arrow corresponds to the retention time of synthetic hGRF-44-OH and the left arrow to that of the hGRF-44-NH_2. AUFS, absorbance units full-scale.

acid extraction, immunoaffinity chromatography, gel filtration, and semipreparative and analytical HPLC. Four hundred picomoles of hGRF-40 and 500 pmol of hGRF-44 were isolated from the first batch of human tissues. From the second batch, 900 pmol of hGRF-40 and 1300 pmol of hGRF-44 were isolated, whereas from the third batch we obtained 1100 pmol of hGRF-40 and 500 pmol of hGRF-44. Only materials isolated from the second batch of tissues were used for the final characterization, as shown in Figs. 1 and 2.

Gel filtration of the irGRF recovered from the immunoaffinity column yielded one major zone of GRF activity (Fig. 1*A*) which, upon semipreparative HPLC, was separated into two main irGRF fractions (Fig. 1*B*). Each of the two irGRF fractions was further purified on an analytical HPLC system, which yielded one major peak of irGRF with distinct 280-nm UV adsorption (Fig. 1 *C* and *D*). The peak with GRF immunoreactivity in Fig. 1 *C* and *D* was subjected to amino acid analysis, and results showed that the former was in agreement with the amino acid composition of hpGRF-40 and the latter was identical to hpGRF-44 (Table 1).

Direct Edman degradation of two 500-pmol samples of the 44-amino-acid hypothalamic hGRF peptide from Fig. 1*D* in a gas-phase protein sequencer (Applied Biosystems model 470A) yielded its primary structure up to 43 amino acids from the NH_2 terminus on the second analysis (Table 2). Identification of the COOH-terminal leucine was deduced by comparing the established sequence of hGRF-44(1–43) with the amino acid composition of hGRF-44. The only uncertainty lay in the nature of the COOH terminus, whether it was an amide or a free acid. To answer this question, synthetic replicates of hGRF-44 possessing either an amidated or a free COOH terminus were synthesized by solid-phase methodology and cochromatographed with native hGRF-44 on an analytical HPLC system. Results in Fig. 2 clearly show coelution of native hGRF-44 with synthetic hGRF-44-NH_2 and separation from synthetic hGRF-44-OH. Thus, the complete structural characterization of hGRF required less than 1.2 nmol of peptide isolated from 1032 fragments of human pituitary stalk median eminence.

Table 1. Amino acid composition of the purified irGRF from Fig. 1 *C* and *D*

Amino acid	Residues in eluted irGRF, mean ± SD	
	Elution at 40 min from Fig. 1*C**	Elution at 49 min from Fig. 1*D*†
Asx	3.59 ± 0.05 (4)	3.72 ± 0.11 (4)
Thr	0.99 ± 0.06 (1)	0.97 ± 0.02 (1)
Ser	3.76 ± 0.10 (4)	3.84 ± 0.14 (4)
Glx	6.74 ± 0.06 (7)	6.63 ± 0.11 (7)
Gly	3.93 ± 0.11 (3)	3.71 ± 0.11 (3)
Ala	4.07 ± 0.16 (4)	4.84 ± 0.05 (5)
Val	0.86 ± 0.01 (1)	1.03 ± 0.01 (1)
Met	0.89 ± 0.21 (1)	0.81 ± 0.10 (1)
Ile	1.71 ± 0.02 (2)	1.94 ± 0.22 (2)
Leu	3.59 ± 0.20 (4)	4.84 ± 0.33 (5)
Tyr	2.18 ± 0.12 (2)	2.12 ± 0.09 (2)
Phe	0.90 ± 0.04 (1)	1.08 ± 0.04 (1)
His	0.00	0.00
Trp	0.00	0.00
Lys	2.27 ± 0.15 (2)	2.28 ± 0.08 (2)
Arg	4.50 ± 0.04 (4)	6.19 ± 0.01 (6)
Cys	0.00	0.00
Pro	0.00	0.00

Ten to 15 pmol of peptide were hydrolyzed at 110°C for 20 hr in 50 μl of constant-boiling HCl containing 2% (vol/vol) thioglycollic acid and analyzed in a Liquimat III amino acid analyzer (Kontron) as described (7). Each value is the mean ± SD of two determinations and is not corrected for hydrolysis losses.

*Values in parentheses correspond to the integer amino acid composition of hpGRF-40.

†Values in parentheses correspond to the integer amino acid composition of hpGRF-44.

DISCUSSION

The efficient isolation of hGRF from the limited amount of tissues available was due to high cross-reactivity between the hpGRF-40 antibody and hGRF. We took advantage of this finding to concentrate the peptide from a large volume of crude extract on an immunoaffinity column constructed with this antibody before any subsequent purification. Final yield of the hGRF from the three batches of human tissues was not uniform. Only the second batch yielded the highest amount of hGRF-44. Interestingly, none of the tissues from this batch had ever been treated with acetone, whereas most of the tissues in the first batch and all of those in the third batch

Table 2. Gas-phase sequence analysis of hGRF-44

Cycle no. N	Residue no.	>PhNCS–AA	Yield, pmol
1	1	Tyr	149
2	2	Ala	107
3	3	Asp	43.8
4	4	Ala	136
5	5	Ile	142
6	6	Phe	92.5
7	7	Thr	54.0
8	8	Asn	98.2
9	9	Ser	62.2
10	10	Tyr	88.2
11	11	Arg	69.5
12	12	Lys	77.0
13	13	Val	86.1
14	14	Leu	108
15	15	Gly	62.3
16	16	Gln	22.8
17	17	Leu	53.8
18	18	Ser	31.0
19	19	Ala	50.6
20	20	Arg	33.2
21	21	Lys	20.8
22	22	Leu	37.1
23	23	Leu	7.87
24	24	Gln	21.4
25	25	Asp	8.26
26	26	Ile	22.6
27	27	Met	19.5
28	28	Ser	15.5
29	29	Arg	9.27
30	30	Gln	7.63
31	31	Gln	7.18
32	32	Gly	12.9
33	33	Glu	7.65
34	34	Ser	2.59
35	35	Asn	6.16
36	36	Gln	0.95
37	37	Glu	8.36
38	38	Arg	5.68
39	39	Gly	3.62
40	40	Ala	5.40
41	41	Arg	3.86
42	42	Ala	3.72
43	43	Arg	0.97
44*	44	X	—

The amount applied was 500 pmol, the average repetitive yield was 90.9%, and the initial yield was 40.0%. >PhNCS, phenylthiohydantoin; AA, amino acid.

*The carboxyl-terminal leucine was identified by comparison of the above sequence for hGRF-44(1–43) with the amino acid composition for hGRF-44.

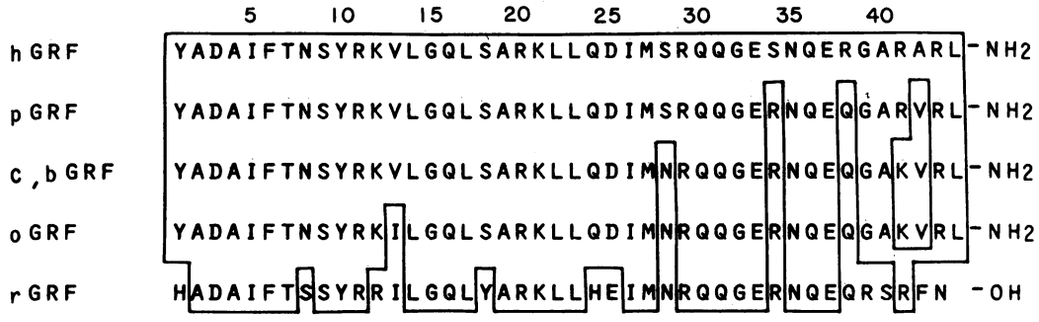

FIG. 3. Amino acid sequences of mammalian hypothalamic GRFs. The letters h, p, c, b, o, and r designate the human, porcine, caprine, bovine, ovine, and rat species, respectively.

were preserved in acetone. Unless further studies are performed, it is premature to speculate whether acetone promotes degradation of the peptide.

The structure of hGRF determined in this study was found to be identical to that of hpGRF-44, the growth hormone-releasing peptide isolated from a human pancreatic tumor and endowed with the highest potency of the various hpGRF peptides obtained in that tumor (1). This finding is in keeping with earlier observations indicating that biologically active peptides produced ectopically by tumors have usually been found to be identical to the products of the physiological source of such peptides (13).

The methodology described here has been used by us to isolate and characterize porcine (14), bovine (15), ovine, and caprine hypothalamic GRFs (unpublished data). With these tissues, obtained fresh from the slaughterhouse, only one major GRF peptide form was isolated. All of these GRF peptides of hypothalamic origin contain 44 amino acids and are amidated at their COOH termini (Fig. 3). Only in the case of a second pancreatic tumor did we (16) and Rivier *et al.* (17) isolate exclusively hpGRF-40, a peptide with a free COOH terminus. In contrast, rat hypothalamic GRF characterized by Spiess *et al.* (18) contains only 43 amino acids and the COOH terminus is not amidated (Fig. 3). In addition, rGRF-43 is structurally rather different from the other GRF peptides (14 amino acid substitutions as compared to hGRF-44). It is possible that rodents may have a quite different gene structure coding for GRF from that of the higher mammals.

Characterization of hypothalamic hGRF having now been completed, with the conclusion that the material of hypothalamic origin is identical to the previously sequenced tumor-derived GRF (hpGRF), there is no further need to keep the nomenclature "human pancreas GRF" or the abbreviation "hpGRF." Because of the significant species variations in the primary structures of the various GRFs already characterized, it is advisable, however, to refer to the material by prefixing with the lower-case first letter of the adjective describing that species, such as hGRF, oGRF, bGRF, pGRF, etc.

With the characterization of hypothalamic hGRF, medical use of its synthetic replicate truly represents replacement or substitution therapy with the native molecule.

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