RNA to Northern blot analysis using the ^{32}P-labeled pANF-1 insert as a hybridization probe (Fig. 3). With a 40-minute exposure, a single band was observed from atrial mRNA, suggesting that the ANF message is plentiful. No hybridizing mRNA's were detected in rat liver mRNA even after 3 days of exposure (results not shown). The length of ANF mRNA is 850 bases. Assuming that the poly(A)$^+$ tail is approximately 100 bases in length, the estimated size of ANF mRNA indicates that pANF-1 contains a nearly full-length cDNA insert.

To determine how many genes similar to ANF occur in the rat genome and whether these genes occur in other species, we subjected rat, mouse, and human DNA to Southern blot analysis and hybridization to ^{32}P-labeled pANF-1 insert DNA (Fig. 4). Under relatively stringent conditions, where sequences of less than 70 percent homology should not hybridize, only one intense band was detected by the probe in restriction enzyme digests of rat, mouse, and human DNA's. [Two very faint bands of hybridization were detected (not visible on the autoradiogram shown in Fig. 4). Whether these faint bands represent distantly related genes or artifacts of the hybridization procedure remains uncertain.] These observations allow two conclusions. First, there are no other closely related (more than 70 percent identical) preprohormone genes encoded in the rat genome. Second, the preproANF gene is highly conserved among human, rat, and mouse, which suggests an important role for this preproANF structure.

These results show that ANF is synthesized as a preprohormone in atrial tissue that has characteristics typical of processed secreted peptides, suggesting that ANF is a cardiac hormone. The conserved similarity between ANF genes from distant species supports the hypothesis that this peptide plays an important role in maintaining cardiovascular homeostasis and that its prepro form is important for its subsequent action. The availability of the plasmid pANF-1 should permit the identification of the various roles for preproANF. For example, this cDNA clone should allow the synthesis of large amounts of the protein, enabling the physiological role of preproANF to be tested. Furthermore, the physiological changes that lead to the synthesis of the protein can be studied at the transcriptional level. Finally, mutations in the transcriptional and regulatory portions of the gene for ANF may account for some pathophysiologic states. With the use of the cDNA clone and isolated genomic clones, we can now investigate the potential role of ANF in animal models of hypertension and congestive heart failure.

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20. We thank K. Klein for assistance with the Southern blot analysis, J. Buse for help in isolation of rat atria, J. B. West for technical help in the synthesis and isolation of the oligonucleotide probes, and N. Roosa for editorial assistance. Supported by NIH training grant HL-070208 (C.E.S.); a fellowship from the Medical Research Council of Canada and a Career Development Award from the Arthritis Society of Canada (A.D.D.); a Helen Hay Whitney Foundation grant (E.C.); American Heart Association grants AHA 80-148 and 82-240, NIH grants HL-19259 and NS-19583, and a grant from the R. J. Reynolds Company (C.H. and R.M.G.); a grant from Hoechst AG (W. Germany) (J.A.S.); and American Cancer Society grant JFR66 (J.G.S.).
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16 April 1984; accepted 22 May 1984

Growth Hormone–Releasing Factor Stimulates Pancreatic Enzyme Secretion

Abstract. *Growth hormone–releasing factors (GRF's) from two human pancreatic tumors (hpGRF's) that caused acromegaly and from the rat hypothalamus (rhGRF) were recently isolated and characterized. Although these peptides are potent growth hormone secretagogues, they have not until now been described to have actions outside the pituitary. These GRF's were shown to stimulate digestive enzyme secretion from an exocrine pancreatic preparation in vitro, rhGRF being more than 100 times as potent as hpGRF. Adenosine 3',5'-monophosphate mediates this action of the GRF's.*

The growth hormone–releasing factors (GRF's) isolated from the human pancreatic tumors (*1–3*) and the rat hypothalamus (*4*) belong structurally to the glucagon-secretin family of peptides that include vasoactive intestinal peptide (VIP), glucagon, secretin, gastric inhibitory peptide (GIP), and PHI, a peptide with histidine as its NH_2-terminus and isoleucine amide as its COOH-terminus. Because members of the glucagon-secretin family of peptides have various physiological effects in the gastrointestinal tract and because the tumor GRF's were of pancreatic origin, we pursued the possibility that GRF has an effect in the exocrine pancreas.

In our preparation of dispersed acini from the guinea pig pancreas, VIP, PHI, and secretin—but not glucagon or GIP—act to stimulate digestive enzyme secretion by increasing cellular adenosine 3',5'-monophosphate (cyclic AMP) (*5, 6*). In contrast to VIP, PHI, and secretin, secretagogues of enzyme secretion such as cholecystokinin, bombesin, and carbachol cause mobilization of cellular calcium and enzyme secretion with no change in cyclic AMP (*7*). Another characteristic of this preparation is that the enzyme secretion from acini incubated with a combination of an agent that acts by increasing cyclic AMP and an agent that causes mobilization of cellular calci-

um is greater than would be expected if the responses were additive (that is, a potentiated response) (*7*). Enzyme secretion with two agents having the same mechanism of action is no greater than the response with the agent causing greater enzyme secretion alone (*7*). We used the above characteristics to determine the mechanism of action of GRF-stimulated enzyme secretion.

Dispersed acini from guinea pig pancreas were prepared and incubated in standard solution (*8*). To measure enzyme secretion, we measured the release of amylase, one of the digestive enzymes, from the acini (*9, 10*). Cyclic AMP was measured with a radioimmunoassay kit (New England Nuclear) (*5, 11*). All GRF peptides and porcine PHI(27) were synthesized with the solid-phase approach (*2, 12*); porcine VIP was from Peninsula Laboratories (San Carlos, California); and the COOH-terminal octapeptide of cholecystokinin (CCK-OP) was a gift from Dr. Miguel Ondetti (Squibb Institute for Medical Research, Princeton, New Jersey).

Compared to control (no added peptide), 10 n*M* rat hypothalamic GRF [rhGRF(1-43)-OH] stimulated a twofold linear increase in amylase release from pancreatic acini incubated in standard solution at 37°C for 40 minutes (data not shown). The addition of theophylline (5 m*M*) had no effect on amylase release from control acini but stimulated a sevenfold linear increase, compared to control, in amylase release from acini incubated in the presence of 10 n*M* rhGRF(1-43)-OH for 40 minutes (data not shown). In all subsequent experiments, acini were incubated in standard solution with theophylline (5 m*M*).

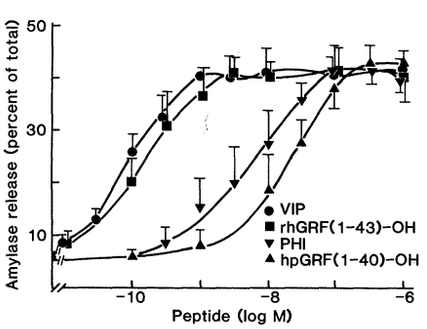

Fig. 1. Effect of various peptides on amylase release from dispersed pancreatic acini. Acini were suspended in standard incubation solution with theophylline (5 m*M*) and incubated with the peptide at 37°C for 30 minutes at the indicated concentrations. Values are expressed as the percentage of cellular amylase released into the extracellular medium during the incubation. Results are the means of at least five separate experiments and in each experiment each value was determined in duplicate. Vertical bars represent 1 S.D.

Table 1. Relative potencies of various GRF peptides for amylase release from guinea pig dispersed pancreatic acini and growth hormone release from rat adenohypophyseal cells. For amylase release, dispersed acini were suspended in standard incubation solution with theophylline (5 m*M*) and incubated at 37°C for 30 minutes with the peptides indicated, at various concentrations. Values for amylase release were determined in duplicate in at least five separate experiments. All of these peptides had similar efficacy for amylase release (data not shown). Growth hormone release was determined in 4-day-old monolayer cultures of rat adenohypophyseal cells (*2*). After a 4-hour incubation of cultured cells in a serum-free medium containing 0.1 percent bovine serum albumin and 0.5 m*M* ascorbic acid and various concentrations of GRF peptides at 37°C, growth hormone release was measured (*2*) with a radioimmunoassay kit distributed by the National Hormone and Pituitary Program. Values for growth hormone release were determined in triplicate in at least two experiments. All of these peptides had similar efficacy for growth hormone release. The EC_{50} for rhGRF(1-43)-OH–stimulated growth hormone release was 10 p*M*. Relative potencies were determined with the program BIOPROG (*13*). Values in parentheses represent 95 percent confidence limits.

Peptide	Relative potency for amylase release	Relative potency for growth hormone release
rhGRF(1-43)-OH	1.0	1.0
rhGRF(1-29)-NH_2	0.75 (0.49, 1.1)	1.96 (1.19, 3.23)
[Tyr^1]rhGRF(1-29)-NH_2	0.087 (0.052, 0.14)	0.96 (0.61, 1.40)
hpGRF(1-40)-OH	0.0094 (0.0060, 0.015)	0.38 (0.26, 0.60)
hpGRF(1-44)-NH_2	0.0063 (0.004, 0.009)	0.58 (0.39, 0.78)
rhGRF(3-40)-OH	<0.0002	<0.0002

In addition to rhGRF(1-43)-OH, VIP, PHI, and hpGRF(1-40)-OH, which is the most abundant form of hpGRF from the two pancreatic tumors (*1–3*), each increased amylase release from pancreatic acini (Fig. 1). The maximal increase in amylase release caused by these agents was similar (similar efficacy). However, these agents differed in the concentrations required for their effect on enzyme secretion (different potency). The concentration of peptide required to stimulate half-maximal amylase release (EC_{50}), a measure of potency, was 80 p*M* for VIP, 130 p*M* for rhGRF(1-43)-OH, 6 n*M* for PHI, and 21 n*M* for hpGRF(1-40)-OH. The relative potencies with 95 percent confidence limits (*13*) were 1.0 for VIP, 0.49 (0.34, 0.74) for rhGRF(1-43)-OH, 0.012 (0.007, 0.020) for PHI, and 0.0049 (0.003, 0.007) for hpGRF(1-40)-OH.

In a similar manner, we determined the relative potencies of various analogs of rhGRF and hpGRF to stimulate amylase release and compared these values to those for growth hormone release from rat pituitary cells (Table 1). The relative potencies of rhGRF(1-43)-OH and rhGRF(1-29)-NH_2 for amylase release were not significantly different from each other, indicating that the 14 amino acids at the COOH-terminal are not necessary for this action of rhGRF.

In contrast to rhGRF, the human pancreatic tumor GRF's, hpGRF(1-40)-OH and hpGRF(1-44)-NH_2, had potencies for amylase release that were less than 1 percent of that for rhGRF(1-43)-OH. Because rhGRF(1-29)-NH_2, like VIP, has a histidine in position 1 and is more than ten times as potent as [Tyr^1]rhGRF(1-29)-NH_2 and because the hpGRF's have a tyrosine in position 1, part, but not all, of the difference in potency between rat and human GRF may be attributable to a relative preference of the pancreatic GRF receptor for an NH_2-terminal histidine. In marked contrast to the findings in the pancreas, rhGRF and the hpGRF's were potent secretagogues of growth hormone release from cultured rat pituitary cells (Table 1), suggesting a striking

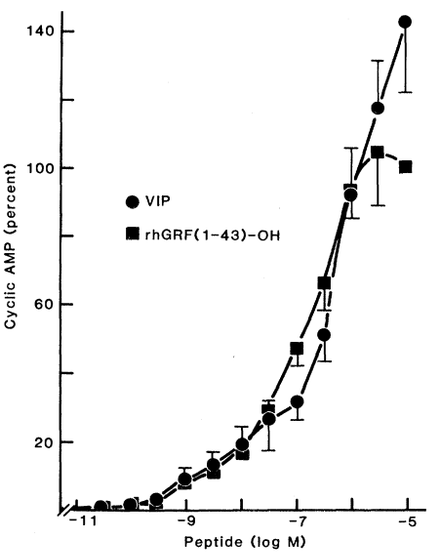

Fig. 2. Effect of VIP and rhGRF(1-43)-OH on cyclic AMP in dispersed pancreatic acini. Acini were suspended in standard incubation solution with theophylline (5 m*M*) and incubated with the peptide at 37°C for 30 minutes at the indicated concentrations. Results are expressed as a percentage of the value measured with $1 \times 10^{-5} M$ rhGRF(1-43)-OH. These results represent the means of five experiments and in each experiment each value was determined in duplicate. Vertical bars represent 1 S.D. At 10 μmol/liter rhGRF(1-43)-OH stimulated a 49 ± 16-fold increase in cyclic AMP.

Table 2. Amylase release stimulated by secretagogues in combination. Dispersed acini were suspended in standard incubation solution with theophylline (5 m*M*) and incubated with the indicated concentrations of peptides for 15 minutes at 37°C. Values are expressed as the percentage of cellular amylase released into the extracellular medium during the incubation. Results are mean ± 1 S.D. from five separate experiments, with each value determined in duplicate in each experiment. The numbers in parentheses represent the mean of the additive value calculated by adding the increase in amylase release caused by GRF alone to the value for amylase release obtained with CCK-OP alone.

Peptide	Amylase release (percentage of total)		
	Alone	+ VIP (10 n*M*)	+ CCK-OP (0.3 n*M*)
None	2.4 ± 1.3	13.2 ± 3.4	21.2 ± 5.2
rhGRF(1-43)-OH (10 n*M*)	13.6 ± 4.0	13.5 ± 3.6	34.7 ± 8.0* (32.4)
hpGRF(1-40)-OH (1 μ*M*)	13.8 ± 3.5	14.2 ± 3.2	35.0 ± 7.1* (32.6)

*Significantly greater ($P < 0.05$) than the additive value, by Student's paired *t*-test.

difference between receptors for GRF in the pituitary and the pancreas.

In order to elucidate the intracellular mechanism of action of GRF in the pancreas, we incubated pancreatic acini with combinations of rhGRF(1-43)-OH and hpGRF(1-40)-OH, plus either VIP or CCK-OP (Table 2). Amylase release from pancreatic acini incubated with VIP plus either rhGRF(1-43)-OH or hpGRF(1-40)-OH was not different from that observed with either agent alone. In contrast, amylase release from pancreatic acini incubated with CCK-OP plus either GRF was greater than the expected "additive value."

To further evaluate the mechanism of action of GRF's, we measured the ability of rhGRF(1-43)-OH and VIP to increase cellular cyclic AMP (Fig. 2). Both VIP and rhGRF(1-43)-OH caused increases in cyclic AMP. At concentrations of peptide that cause maximal amylase release [1 n*M* for VIP and 3 n*M* for rhGRF(1-43)-OH], these agents caused an approximately sixfold increase in cyclic AMP. At greater concentrations of peptide, these agents caused up to a 50-fold increase in cyclic AMP. Such "spare" cyclic AMP for enzyme secretion has been observed previously with VIP, PHI, and secretin in dispersed pancreatic acini from guinea pig (*5*). These findings together with the finding that theophylline augments rhGRF-stimulated amylase release and that GRF potentiates the amylase release caused by CCK-OP indicate that GRF acts to stimulate amylase release by increasing cellular cyclic AMP. Similarly, hpGRF has a direct stimulatory effect on cyclic AMP production in cultured anterior pituitary cells, suggesting that GRF acts in part to stimulate growth hormone release through increases in cyclic AMP (*14*).

Other members of the glucagon-secretin family of peptides that stimulate enzyme secretion in this preparation of pancreas do so by interacting with the VIP-preferring receptor (*5*). To determine if GRF also interacts with the VIP receptor, we measured its ability to inhibit binding of ^{125}I-labeled VIP to the acini. The finding that rhGRF(1-43)-OH was as effective and approximately one-half as potent as VIP in inhibiting the binding of ^{125}I-labeled VIP (Fig. 3) indicates that rhGRF interacts with the VIP receptor to cause enzyme secretion (compare Figs. 1 and 3).

Our results show that GRF has a VIP-like effect in the exocrine pancreas of the guinea pig—that is, GRF stimulates digestive enzyme secretion by interacting with the VIP-preferring receptor and increasing cellular cyclic AMP. Although GRF is a very potent secretagogue of enzyme secretion, the presence of GRF-like peptides in the normal gastrointestinal tract has not been reported. Thus, the physiological significance of these observations remains at issue. It will be important to determine whether GRF is present in the gastrointestinal tract of the rat and the guinea pig by using appropriate antibodies to rhGRF.

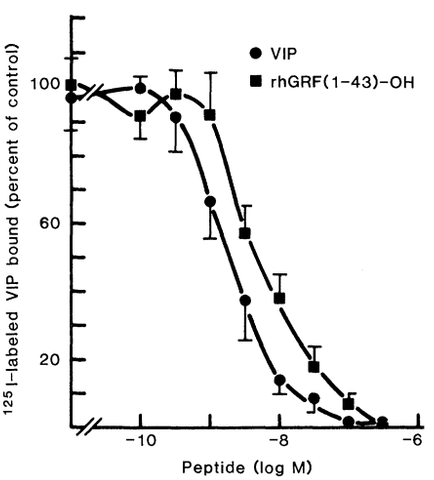

Fig. 3. Effect of rhGRF(1-43)-OH and VIP on binding of ^{125}I-labeled VIP to dispersed acini. ^{125}I-labeled VIP was prepared by modifications of the chloramine-T method (*5*) and was purified by high-performance liquid chromatography with a C_{18} column (Vydac) eluted with 0.1 percent trifluoroacetic acid and acetonitrile in an increasing linear gradient. Dispersed acini were incubated with ^{125}I-labeled VIP and the peptide at the indicated concentrations for 60 minutes at 37°C. Then total binding, saturable binding, and nonsaturable binding were measured (*5*). Nonsaturable binding for ^{125}I-labeled VIP (binding of ^{125}I-labeled VIP in the presence of 0.3 μ*M* unlabeled VIP) was less than 10 percent of total binding. Values are expressed as the percentage of saturable binding measured with no added peptide. Results are the means of three experiments and in each experiment each value was determined in duplicate. Vertical bars represent 1 S.D.

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15. Supported by funds from the Research Services of the Veterans Administration and by NIH grants AM 33010 (S.J.P.) and AM 26741 (P.B.L.). This research was conducted in part by the Clayton Foundation for Research, California Division. W.V. is a senior Clayton Foundation investigator. We thank M. Ondetti (Squibb Institute for Medical Research, Princeton, N.J.) for the COOH-terminal octapeptide cholecystokinin, R. Kaiser and R. Galyean for technical assistance, and G. Deming for help with preparation of the manuscript.

13 January 1984; accepted 26 April 1984