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EFFECTS OF SECRETIN AND GASTRIC INHIBITORY POLYPEPTIDE ON HUMAN PANCREATIC GROWTH HORMONE-RELEASING FACTOR(1-40)-STIMULATED GROWTH HORMONE LEVELS IN THE RAT

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Synthetic human pancreatic growth hormone-releasing factor containing 40 amino acids ([hpGRF (1-40)]-OH) significantly stimulated plasma growth hormone (GH) levels in both sodium pentobarbital and urethane anesthetized rats. Synthetic secretin, gastric inhibitory polypeptide (GIP), and glucagon significantly decreased plasma GH levels while synthetic vasoactive intestinal peptide (VIP) had no effect. Secretin and GIP also altered the in vivo plasma GH response to [hpGRF(1-40)]-OH. Whether this effect is the result of an interaction at the pituitary level or is due to an extra-pituitary effect of secretin and GIP awaits further study.

Two similar growth hormone-releasing factors ([hpGRF]) isolated from human pancreatic tumors and bearing striking structural similarities to the secretin-glucagon family of peptides have recently been reported (1,2). Both of these peptides share the identical first 40 residues with one ending at this point as a free acid ([hpGRF(1-40)]-OH (1). The other is C-terminally extended by 4 amino acids and bears a terminal amide group ([hpGRF(1-44)]-NH_2) (2). Both are capable of producing statistically identical maximal stimulation of GH levels (2). The considerable sequence overlap existing between these two peptides and the secretin-glucagon series creates renewed interest in the pituitary effects of this latter group of peptides as well as in their potential interactions with hpGRF. The present study utilizes peptides produced synthetically by solid-phase methodology to begin physiological studies investigating these possibilities.

ABBREVIATIONS: [hpGRF (1-40)]-OH and [hpGRF (1-44)]NH_2, human pancreatic growth hormone-releasing factors consisting of 40 and 44 amino acids, respectively; GH, growth hormone; GIP, gastic inhibitory polypeptide; VIP, vasoactive intestinal peptide.

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METHODS

Peptide synthesis: [hpGRF (1-40)]-OH protected peptide was assembled on an Ala-Merrifield resin according to a previously described solid-phase approach (3). It was deprotected and cleaved from the support by the improved HF-dimethylsulfide-cresol (25:65:10) reagent recently described (4). The crude peptide was purified by gel filtration on Sephadex G-50, ion-exchange chromtography on CM-cellulose using an NH_4Ac gradient, and preparative hplc on Whatman LRP-1 ODS silica (3) by elution with an acetonitrile-15% acetic acid system. Homogeneity was confirmed by analytical hplc on 300 Å ODS silica (Synchrom Synchropak), tlc, and amino acid analysis of acid and enzyme hydrolysates.

Animals: Male, Charles Rivers CD strain rats weighing 200-240 g were used in bioassays. The animals were maintained under controlled temperature (24±2°C) and light (0500-1900h) for at least one week prior to an assay. Diet consisted of Purina rodent laboratory chow and tap water ad libitum.

GH bioassays: Rats were fasted for 23-25h before an experiment except as otherwise indicated. All assays were conducted from 1000-1200h. The rats were anesthetized with sodium pentobarbital (Nembutal, 6 mg per 100 g body weight) or with urethane (150 mg per 100 g body weight). Exactly 30 min following anesthetic injection, 0.5 ml of saline or saline containing test peptide was rapidly injected subcutaneously. Solutions of the test peptides were prepared in gelatin-coated test tubes and injected using gelatin-coated sytinges to help prevent adsorption of peptide to the containers. Exactly 15 min after peptide injection, a 1 ml blood sample was quickly drawn from the jugular vein.

Plasma GH determination: Immediately after withdrawal, blood samples were transferred to chilled test tubes containing EDTA (1.5 mg per ml blood). Plasma was separated by centrifugation. Aliquots of the plasma were distributed into clean test tubes, frozen in ethanol-dry ice, and stored at -20°C until assayed for hormone concentration. Plasma GH levels were estimated using NIADDKD rat GH RIA components and protocol with the following modifications. The first and second antibody incubations were performed at room temperature for 24h and 2h respectively [^{125}]iodo-GH was added 2h after addition of first antibody. The between-assay and within-assay coefficients of variation were less than 10% using pooled plasma samples corresponding to 0.7, 1.4, and 2.8 ng rat GH per tube.

Significance of results was estimated using Duncan's new multiple range test.

RESULTS AND DISCUSSION

Synthetic [hpGRF (1-40)]-OH significantly increases plasma GH levels in both sodium pentobarbital and urethane anesthetized rats 15 minutes after subcutaneous injection of the peptide (Table 1). The magnitude of stimulation as compared to the corresponding saline control is greater in the urethane anesthetized animals. This is probably related to the differences in the known effects of these two anesthetics on GH levles. Urethane is known to significantly increase somatostatin levels in the hypothalamo-hypophseal portal blood as compared to sodium pentobarbital (5). Somatostatin can non-competitively block [hpGRF (1-44)]-NH_2-stimulated GH release in vitro but, even at high somatostatin concentrations, this blockage is not total (1). Our data on

Table 1. Stimulation of plasma GH levels by synthetic [hpGRF(1-40)]-OH in sodium pentobarbital and urethane anesthetized rats.

Dose [hpGRF(1-40)]-OH (μg/100g BW)	Plasma GH (ng/ml) Sodium Pentobarbital*		Urethane	
-	309±45	(10)	31±7	(7)
0.1	286±66	(7)	43±4	(4)
0.5	313±72	(7)	49±8	(6)
1.0	320±84	(7)	51±9	(5)
5.0	593±82	(7)	91±22	(6)
10	1269±263§	(7)	467±190§	(5)

Values are the mean±SEM (n); §$p<0.01$ vs saline; *fed rats.

[hpGRF (1-40)]-OH stimulation of GH during urethane anesthesia support this latter finding. Both of the above findings are in contrast to a recent report in which it was shown that [hpGRF (1-44)]-NH_2 was unable to stimulate GH release in conscious rats with fasting-stimulated circulating somatostatin levels (6). Whether this lack of stimulation is due to the [hpGRF (1-44)]-NH_2 dose or is due to some other factor cannot be determined from the data. However, when fasted rats were first pretreated with antibodies to somatostatin, stimulation of GH release was obtained (6).

Intravenous injection of [hpGRF (1-44)]-NH_2 has been shown to rapidly increase plasma GH levels in sodium pentobarbital anesthetized rats with the effect being most pronounced 5 minutes after injection and then rapidly disappearing (2). The present study indicates that [hpGRF (1-40)]-OH retains biological activity when given subcutaneously and, as might be expected, exerts its effect over a longer period of time as compared to the intravenous route of administration. However, given a potency estimate of 30% for the 40 residue peptide as compared to the larger 44 residue molecule (2), the degree of stimulation seen in this study using subcutaneous administration is somewhat less than expected based on the previous intravenous study (2). This might reflect lower

Table 2. Effects of secretin family peptides on plasma GH levels in sodium pentobarbital anesthetized rats.

Dose (µg/100g BW)	Plasma GH (ng/ml) Glucagon	GIP	Secretin	VIP
-	411±49 (9)	294±26 (7)	341±34 (5)	332±40 (9)
0.01	-	-	360±29 (5)	-
0.05	-	258±39 (6)	386±57 (6)	-
0.10	-	205±34*(7)	333±59 (6)	315±34 (6)
0.25	-	202±38* (7)	-	-
0.50	-	193±14* (6)	141±23§(6)	321±54 (6)
1.0	399±52 (6)	169±22§(6)	212±22*(6)	278±33 (6)
5.0	316±47 (6)	-	-	304±40 (6)
10	272±16*(6)	113±13§(7)	-	261±26 (6)

Values are the mean±SEM (n); *$p<0.05$ vs saline; §$p<0.01$ vs saline.

circulating plasma levels of the peptide due to slow absorption from the subcutaneous depot.

The stimulatory effect of glucagon on plasma GH is well-documented in humans (7). However, a reduction in plasma GH following administration of glucagon has been reported for rat (8) and duck (9). In the present study a significant decrease in plasma GH was obtained with synthetic glucagon (Table 2) confirming the earlier reported finding in rat (8). Synthetic secretin and GIP produced dramatic decreases in plasma GH while synthetic VIP had negligible effect at the doses studied (Table 2). However, at a very high dose (30µg/100 g BW) (Table 3), the inhibitory effect of both GIP and secretin on plasma GH levels was diminished as compared to the lower effective doses (Table 2). This result is not unlike that observed for antagonist analogs of substance P (10), and somatostatin (11) wherein both agonist and antagonist activity can be observed depending on dose. This suggests to us that secretin and GIP could conceivably be competitive inhibitors of hpGRF. In contrast to GIP given by itself, combinations of the high dose of GIP with [hpGRF (1-40)]-OH significantly augmented the release of GH as compared to [hpGRF (1-40)]-OH alone

Table 3. Alteration of the response to [hpGRF(1-40)]-OH by secretin and GIP.

Dose (µg/100g BW)			Plasma GH
[hpGRF(1-40)]	Secretin	GIP	(ng/ml)
-	-	-	275±46
5	-	-	1131±132§
-	-	30	230±20
-	30	-	211±37
5	-	5	1291±196§
5	-	30	1706±138§Θ
5	5	-	656±77*Θ
5	30	-	1064±174§

Values are the mean±SEM; n=6 for all. *$p<0.05$ vs saline; §$p<0.01$ vs saline; $^{\Theta}p<0.01$ vs GRF (5µg).

(Table 3). No effect was observed with secretin at 30µg/100 g BW in combination with [hpGRF (1-40)]-OH but, interestingly, at a lower dose (5µg/100 g BW) secretin significantly decreased the release of GH in response to [hpGRF (1-40)]-OH (Table 3).

Whether these results are indicative of secretin and GIP interacting with the pituitary receptor or are due to an extra-pituitary mechanism cannot be ascertained from the present study. Future studies investigating these two possibilities will include *in vitro* pituitary studies, different combinations of *in vivo* peptide doses, and be extended to the other recently characterized member of the secretin-glucagon family, porcine intestinal peptide having N-terminal histidine and C-terminal isoleucine amide (12).

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