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PHYSIOLOGICAL ROLES OF SOMATOCRININ AND SOMATOSTATIN IN THE REGULATION OF GROWTH HORMONE SECRETION

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Summary. Somatocrinin, a 44 amino acid peptide with potent growth hormone (GH) releasing activity in anesthetized rats, was tested in conscious freely-moving rats. When high doses of 1 to 10 μg were administered (iv) at random times between spontaneous GH pulses, the responses were inconsistent. When similar doses were tested under identical conditions but in rats pretreated with antibodies against somatostatin, all animals demonstrated a marked and immediate increase in plasma GH of 5 to 10 fold. Similarly, a 1 μg dose of somatocrinin was also ineffective in increasing plasma GH when administered to rats subjected to a 72 h fast, a paradigm known to enhance endogenous somatostatin secretion. However, plasma GH increased over 20 fold if rats were pretreated with antibodies against somatostatin. These results demonstrate the dynamic and opposite roles exerted by somatocrinin and somatostatin in regulating GH secretion.

We have shown that somatocrinin (hpGRF-44), a 44 amino acid peptide isolated from a human pancreatic tumor that had caused acromegaly, is a potent and specific stimulus for the release of growth hormone (GH) in rats anesthetized with sodium pentobarbital (1,2). Similar results were also obtained with two fragments of hpGRF-44, both of which were also isolated from the tumor and consist of the first 40 (hpGRF-40) and 37 (hpGRF-37) amino acids of hpGRF-44. For obvious reasons, animals anesthetized with sodium pentobarbital are not the best model for studies designed to investigate mechanisms regulating GH secretion. The results reported here were obtained in conscious freely-moving rats outfitted with a chronic indwelling venous catheter and individually maintained in isolation chambers. In animals so prepared, the GH response to hpGRF was erratic and inconsistent in comparison to those obtained

ABBREVIATIONS:
hpGRF-44, hpGRF-40 and hpGRF-37: human pancreatic growth hormone releasing factors composed of 44, 40 and 37 amino acids, respectively.

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in anesthetized rats. This suggested that somatostatin, the hypothalamic inhibitor of GH secretion, has a dynamic role in the pituitary response to somatocrinin. To demonstrate such a role of endogenous somatostatin, the studies were repeated in rats pretreated with antibodies against somatostatin. The ability of the hpGRF to induce GH release was also tested in rats subjected to a 72 h fast, treatment known to stimulate secretion of hypothalamic somatostatin (3). In this animal model, elimination of endogenous somatostatin led to a consistant GH response to hpGRF.

MATERIALS & METHODS

Animals: Male Sprague-Dawley rats weighing 300-400 gm were used in these experiments. They were housed in a vivarium with controlled temperature (19-22°C) and humidity and a 14 h light:10 h dark lighting schedule (light on at 0600h) and were given food and water ad libitum except when subjected to a 72 h fast.

One to four weeks prior to performing the experiments, rats were prepared with chronic indwelling venous catheters. The catheter consisted of a 45 cm piece of PE 50 tubing (Clay Adams, Bocton, Dickson & Co., Parsippany, N.J.) to which a 3 cm Silastic extension (0.025 x 0.047'', Dow-Corning, Midland, MI) was attached. The Silastic tubing was inserted into the external jugular vein and advanced to the superior vena cava. The free end of the catheter was passed subcutaneously to the nape of the animal and exteriorized. A rodent harness (Spalding Medical Products, Arroyo Grande, CA) with a spring extension to the outside of the cage, through which the catheter was passed, was attached to the animal. The catheter, thus exteriorized, was then connected to a single-channel, fluid swivel to allow free movement of the rat. The infusion (0.1 ml/h) of a 2% solution of heparinized saline was then initiated to keep the catheters patent. Animals were subsequently housed individually in isolation chambers in the vivarium.

Experimental procedures: On the days of experiment, animals were left completely undisturbed in their isolation chambers. Blood samples (0.15 ml) were withdrawn via the indwelling catheter as indicated at the time points in the figures and centrifuged immediately. Plasma was removed for radioimmunoassay and the red blood cells were resuspended in saline and returned to the animals. Peptides and somatostatin antibodies were administered by the same intravenous catheter.

Radioimmunoassay procedures: Radioimmunoassays of GH were performed by the double-antibody method using reagents provided by the National Pituitary Agency of the National Institutes of Health, with the exception that the antiserum used was provided by Dr. K. Sinha (4). Samples were first diluted 1/5 and then assayed in triplicate using 20-100 µl.

Antibody preparations: Antibodies against somatostatin were prepared as described (5).

Peptides: The peptides were synthesized by solid phase techniques using a Beckman 990 peptide synthesizer (6). The peptides were dissolved in water and diluted with saline to attain working concentrations such that injection volume was always 0.5 ml.

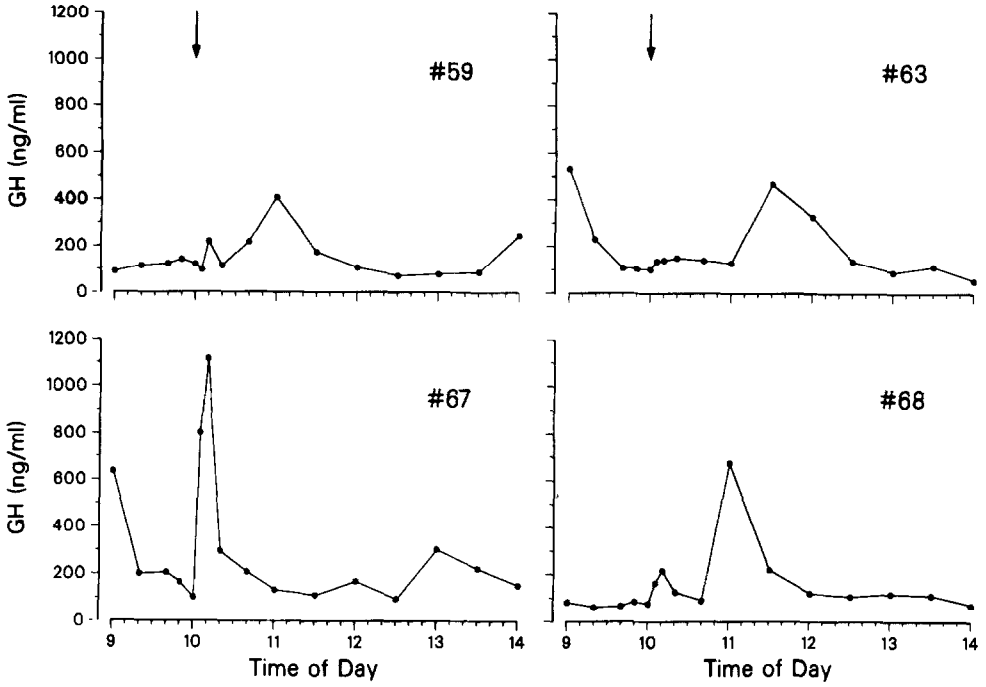
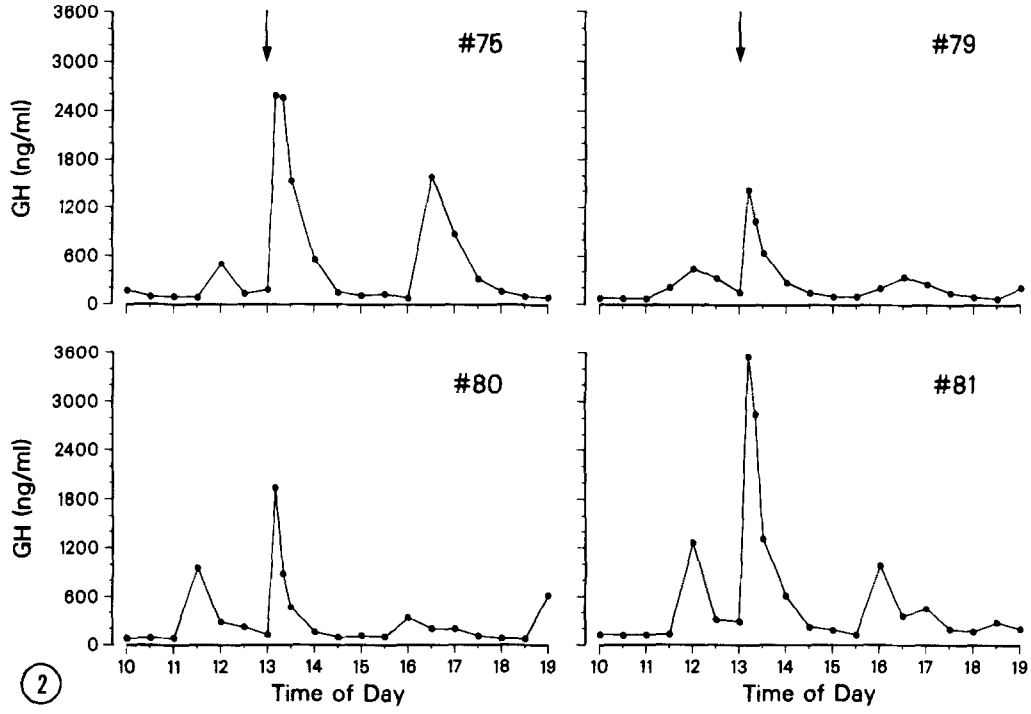


Fig. 1 The effect of 10 μg hpGRF-44 or -40 (iv) on GH secretion in 4 individual, conscious, freely-moving male rats. Injections (indicated by arrows) were made at a time known to be between spontaneous GH pulses. Note the absence of response in rat #63 and the partial response in rats #59 and #68 as compared to the response in rat #67.

RESULTS

The administration of hpGRF-44 and -40 to conscious, freely-moving, male rats during the interval between spontaneous GH pulse failed to elicit a consistent increase in plasma GH concentrations. Of 15 rats treated with 500 ng to 10 μg hpGRF per animal, only 5 demonstrated an increase in GH comparable to what we had observed in rats anesthetized with sodium pentobarbital (2). Fig. 1 illustrates 4 individual examples of the inconsistency observed in the response. In contrast, all rats (n=9) pretreated with 5.8 mg protein of antibodies against somatostatin 4 h before injection of 1 μg hpGRF-44 or -40 demonstrated an immediate and dramatic increase in plasma GH (Fig. 2, note the change scale on the Y axis when compared to Fig. 1).

In an additional experiment 5 rats were subjected to a 72 h fast and then tested for their GH response to 1 μg of hpGRF-44 (iv). As illustrated (Fig. 3) hpGRF was unable to stimulate GH secretion in fasted rats. However, if these rats were pretreated with 5.8 mg protein of antibodies against



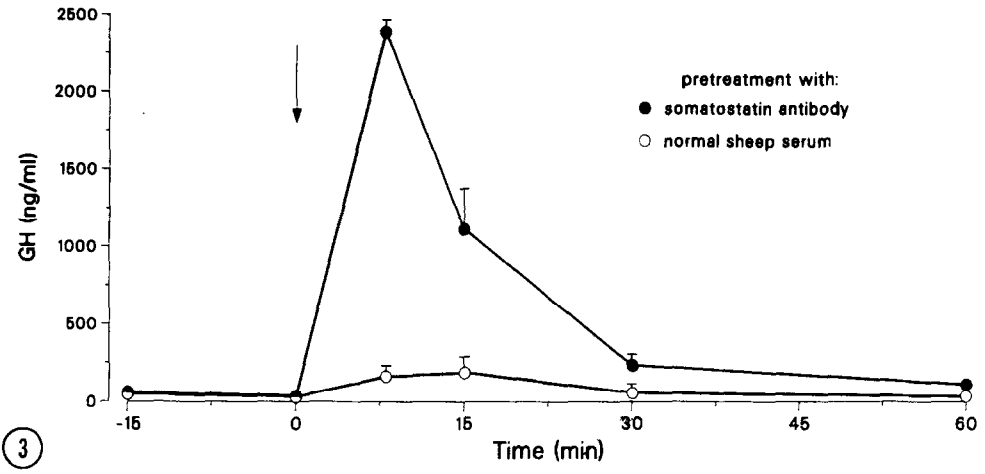


Fig. 2 The effect of 1 μg hpGRF-44 or -40 (iv) on GH secretion in 4 individual, conscious, freely-moving male rats pretreated with 5.8 mg protein of antibodies against somatostatin. Injections (indicated by arrows) were made at a time known to be between spontaneous GH pulses. Note the change in dose of hpGRF administered and scale of GH concentrations as compared to Fig. 1.

Fig. 3 The effect of 1 μg hpGRF-44 (iv, indicated by arrow) on GH secretion in conscious freely-moving male rats fasted for 72 hrs and pretreated with 5.8 mg protein of normal sheep serum or antibodies against somatostatin 2 hrs prior to injection of hpGRF. Data expressed as mean ± SEM, n = 5.

somatostatin 2 h before injection of hpGRF-44, we consistantly observed a marked increase in plasma GH concentrations.

The interval between spontaneous GH pulses in rats which were refractory to hpGRF was 2.8 ± 0.1 h. Similarly, the time interval between the GH pulse induced by hpGRF and the next spontaneous GH pulse was 3.2 ± 0.1 h in rats responding to somatocrinin. However, in these rats, the interval between spontaneous GH pulses was significantly extended ($p<0.01$) to 4.4 ± 0.2 h by the administration of hpGRF during the interval between spontaneous pulses.

DISCUSSION

These studies establish the rapid and potent bioactivity of somatocrinin in conscious, freely-moving rats. However, the inconsistency of hpGRF to stimulate GH release in normal animals suggests that some mechanism antagonistic to somatocrinin is also involved in regulating GH secretion. That this role is, in part, due to somatostatin is demonstrated by the observations that hpGRF will consistently stimulate GH secretion in rats pretreated with antibodies to somatostatin. We have also observed this dynamic role of somatostatin in rats subjected to a 72 h fast. Fasting is known to interrupt normal GH pulses, presumably via endogenous somatostatin (3). Using such animals we failed to observe any increase in plasma GH concentrations following iv injection of 1 µg hpGRF-44; a response could regularly be elicited with somatocrinin in these rats if they were first pretreated with antibodies against somatostatin.

Spontaneous GH pulses in rats occur at 3 to 3.5 h intervals and are entrained to by the light/dark cycle (7). As noted, the time interval between spontaneous GH pulses in rats responding to hpGRF was 4.4 h which was significantly longer than the interval observed in animals refractory to somatocrinin. This increased time interval was undoubtedly due to the GH surge induced by hpGRF since the interval between the induced and spontaneous peak of GH was the expected 3 to 3.5 h. This observation suggests that although the spontaneous ultradian rhythm of GH pulses is entrained to the light/dark cycle, it can also be influenced by the appearance of an experimentally induced GH pulses.

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REFERENCES

1. Guillemin R, Brazeau P, Böhlen P, Ling N, Esch F, Wehrenberg WB (1982) Science (in press).
2. Wehrenberg WB, Ling N, Brazeau P, Esch F, Böhlen P, Baird A, Ying S and Guillemin R (1982) Biochem Biophys Res Commun (in press).
3. Tannenbaum GS, Epelbaum J, Colle E, Brazeau P and Martin J (1978) Endocrinology 102:1909-1913.
4. Sinha YN, Selby FW, Lewis UJ, Vanderlaan WP (1972) Endocrinology 91:784-792.
5. Brazeau P, Epelbaum J, Tannenbaum GS, Rorstad O, Martin JB (1978) Metabolism 27:1133-1137.
6. Ling N, Esch F, Davis D, Mercado M, Regno M, Böhlen P, Brazeau P and Guillemin R (1980) Biochem Biophys Res Commun. 95:945-950.
7. Tannenbaum GS and Martin JB (1976) Endocrinology 98:562-569.