PROCEEDINGS OF THE SOCIETY FOR EXPERIMENTAL BIOLOGY AND MEDICINE **183**, 363–367 (1986)

Interaction of Human Pancreatic Growth Hormone-Releasing Factor, Thyrotropin-Releasing Hormone, and Somatostatin on Growth Hormone Release in Chickens (42432)

J. E. TAYLOR, C. A. BALL, AND F. C. LEUNG[1]

Merck Sharp & Dohme Research Laboratories, Department of Animal Physiology, P.O. Box 2000, Rahway, New Jersey 07065

Abstract. The effects of synthetic somatostatin (SRIF) on serum growth hormone (GH) concentrations stimulated by exogenous administration of synthetic thyrotropin-releasing hormone (TRH) and/or human pancreatic GH-releasing factor (hpGRF) were investigated in 4-week-old cockerels. In addition, the additive effects of TRH and hpGRF on serum GH were examined. TRH and hpGRF, when given in combination intravenously, produced an additive effect on serum GH concentration that peaked 10 min after the injection. The somatostatin did not significantly affect basal GH concentrations when given alone, but did significantly decrease the magnitude of the GH response to hpGRF. In contrast, SRIF did not significantly decrease the stimulatory effects of TRH on GH release. These results suggest that TRH and hpGRF are potent GH releasers *in vivo* and that their stimulating effects on GH release are additive, suggesting different mechanisms for their stimulation. The results obtained from the combination studies suggest that the main site of the stimulatory action of hpGRF is at the pituitary, and that SRIF significantly inhibited the rise in serum GH induced by a synthetic hpGRF, but not that induced by TRH.

Thyrotropin-releasing hormone (TRH) was shown to be a potent growth hormone (GH) releaser in the domestic fowl, both *in vivo* and *in vitro* (1). Numerous reports have since suggested that TRH may influence GH secretion in birds (2–7). Klandorf *et al.* (8) recently showed that administration of TRH antiserum indeed lowered basal concentrations of GH. That observation suggests that TRH is involved in regulating GH release in the chicken. Even though the chicken GH (cGH)-releasing factor has not been isolated and characterized, studies using synthetic human pancreatic GH-releasing factor (hpGRF) have reported it to be a potent GH releaser both *in vivo* and *in vitro* in birds (9–11). Somatostatin (SRIF), a peptide that inhibits GH release but has no effect on basal GH concentration, significantly inhibits the stimulating effects of hpGRF *in vitro* and of TRH *in vivo* (1, 9). Thus, it appears that there are two stimulating factors and one inhibiting factor controlling GH release in birds. Careful examination of published data revealed that TRH is a much more potent GH releaser than hpGRF *in vivo* (9–11), but hpGRF is a more potent GH releaser than TRH *in vitro* (9). Recently, we reported that the stimulatory effects of TRH on serum GH release *in vivo* in chickens could be suppressed by α-methyl-*para*-tyrosine, phenoxybenzamine, and chloridine (12). These findings indicated that in chickens, the hypothalamic noradrenergic system mediated the stimulatory effect of TRH in serum GH release. There is no published information with regard to the effect of SRIF on the stimulatory effects of hpGRF *in vivo*. Therefore, we further examined the interactions of SRIF, TRH, and hpGRF on GH release *in vivo*, and our findings are detailed in this report.

Materials and Methods. Four-week-old Hubbard × Hubbard and Arbor Acres cockerels (approximately 1 kg) were used in all experiments. Synthetic SRIF was obtained from Sigma Chemical Company (St. Louis, Mo.), synthetic $hpGRF_{44}$ was obtained from Bachem, Inc. (Torrance, Calif.), and synthetic TRH was purchased from Beckman (Palo Alto, Calif.).

Four experiments were conducted with 36 birds, which were randomly divided into four treatment groups of 9 birds each. Chickens

[1] Present address: Pacific Northwest Laboratory, Biology and Chemistry Department, P.O. Box 999, Richland, Wash. 99352.

0037-9727/86 $1.50

were individually housed in a temperature (25°C)- and light (14 hr light/10 hr dark)-controlled environment. Food and water were available *ad libitum*. Experiment 1 examined the effect of various doses of SRIF on serum GH concentration. Experiment 2 examined the effect of a maximum dose of TRH and/or $hpGRF_{44}$ on serum GH concentration. These maximum doses of TRH and $hpGRF_{44}$ were established from previous experiments (9, 13). Experiment 3 examined the effect of SRIF (100 μg) in combination with $hpGRF_{44}$ (10 μg) on serum GH concentration, and Experiment 4 examined the effect of SRIF (100 μg) in combination with TRF on serum GH concentration. In all four experiments, chickens were fasted 2 hr before iv injection via the brachial vein, and control chickens received saline vehicle injections. Approximately 1 ml of blood was collected at 0, 10, 30, and 60 min after injection via heart puncture. Serum samples were stored at −20°C until assayed. Serum GH concentration was determined by a homologous radioimmunoassay for cGH, using a double-antibody procedure developed in our laboratory (13).

Serum GH concentrations were expressed as nanograms per milliliter ± SE, using the purified pituitary cGH (FLcGH-2) as a standard. For statistical analysis, all experimental data were subjected to one-way analysis of variance and Student–Newman–Keuls multiple-range test, with $P < 0.05$ considered significant.

Results. The effect of SRIF on serum GH release in 4-week-old cockerels is shown in Fig. 1. Serum concentration of GH was significantly elevated in birds that received 100 μg of SRIF 30 min after injection. At doses of 1, 10, and 100 μg/bird, serum GH concentration was not affected.

The effects of TRH and hpGRF, alone and in combination, on serum GH release are shown in Fig. 2. Birds treated with TRH (1 μg/bird), $hpGRF_{44}$ (10 μg/bird), and the two hormones in combination showed significant increases in serum GH concentrations as compared to control birds. The peak response of GH was observed at 10 min after injection and a return to basal levels was seen after 60 min. The stimulatory effects of TRH on GH were approximately three- to fourfold greater than those produced by hpGRF. However,

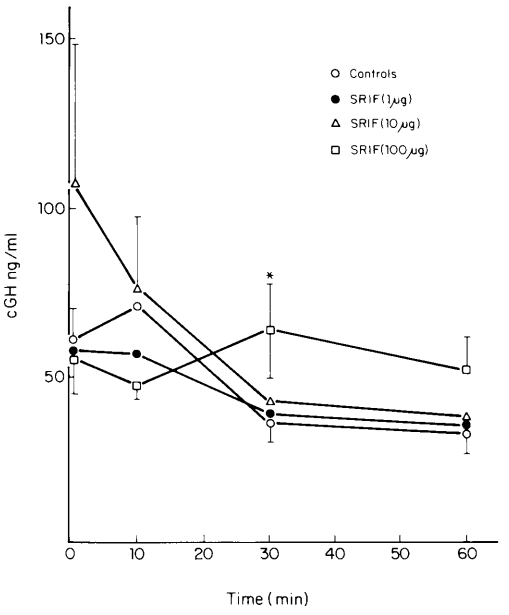

FIG. 1. Effects of somatostatin (SRIF) on serum chicken growth hormone (cGH) concentrations when injected into 4-week-old cockerels. Values represent means ± SE; $N = 9$ birds/group. $^*P < 0.05$, as compared to controls.

TRH and hpGRF in combination produced an elevation of GH release that was significantly higher than the rises seen when either was administered separately.

The effects of SRIF and/or hpGRF on serum release are shown in Fig. 3. A significant stimulation of GH release was seen 10 min after the hpGRF injection, and serum GH concentrations returned to control values 60 min after the injection. However, when SRIF was given in combination with hpGRF, the stimulatory effects of hpGRF on GH release were significantly inhibited. SRIF alone did not produce any significant effects on serum GH concentrations in any experiments.

The effects of SRIF and/or TRH on GH release are shown in Fig. 4. Ten minutes after the injection of TRH, serum GH concentration was significantly higher than that of the control birds. However, when TRH and SRIF were given in combination, the magnitude of the GH response was reduced, although it was not statistically significant in the birds that were given TRH alone.

Discussion. The studies reported here clearly demonstrate that SRIF blocked hpGRF-induced GH release, but did not affect

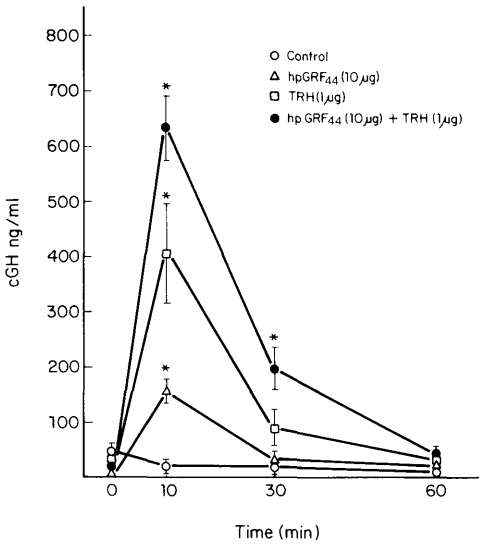

FIG. 2. Mean serum chicken growth hormone (cGH) concentrations (ng/ml) from 4-week-old cockerels injected with human pancreatic growth hormone-releasing factor$_{44}$ (hpGRF$_{44}$; 10 μg/bird, △); thyrotropin-releasing hormone (TRH; 1.0 μg/bird, □); and hpGRF$_{44}$ and TRH (10 and 1.0 μg/bird, ●). Control birds received a saline vehicle injection, O. Vertical bars represent SE; $N = 9$ birds/group. *$P < 0.05$, as compared to controls.

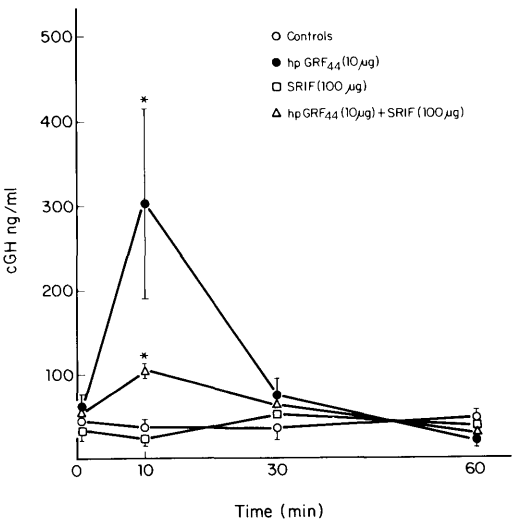

FIG. 3. Mean serum chicken growth hormone (cGH) concentrations (ng/ml) from 4-week-old cockerels injected with growth hormone-releasing factor$_{44}$ (GRF$_{44}$; 10 μg/bird, ●); somatostatin (SRIF; 100 μg/bird, □); and GRF$_{44}$ and SRIF (10 and 100 μg/bird, △). Control birds received a saline vehicle injection, O. Vertical bars represent SE; $N = 9$ birds/group. *$P < 0.05$, as compared to controls.

basal GH release or that evoked by TRH. Our results also show that the exogenous administration of synthetic TRH and hpGRF, alone or in combination, caused a release of GH in immature chickens. When TRH and hpGRF were given in combination, they produced an additive effect on GH release. These results are in agreement with work previously published (9, 14). The synergistic/additive effects of TRH and hpGRF on GH release suggest that they act through different mechanisms in stimulating GH release in chickens. We previously reported (9) that TRH and hpGRF in combination also produced synergistic and additive effects on GH release *in vitro,* suggesting that the TRH may act via a different receptor from that of hpGRF at the pituitary in stimulating GH release. The other possibility is that TRH may act on the hypothalamus in addition to the pituitary in stimulating GH release in chickens.

Since catecholamines do not directly affect GH release *in vitro* (15, 16), they must be involved in the control of GH secretion in chickens by way of the hypothalamus. We re-

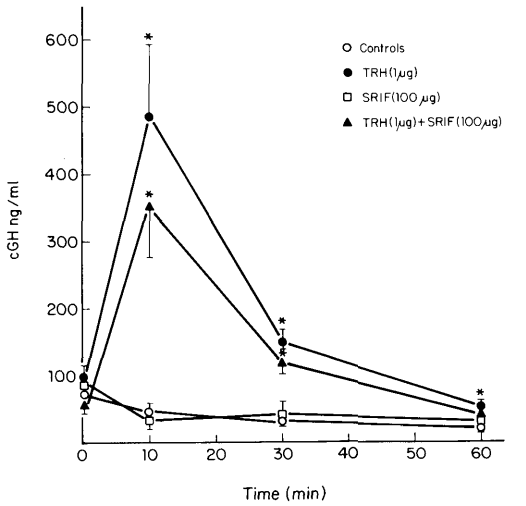

FIG. 4. Mean serum chicken growth hormone (cGH) concentrations (ng/ml) from 4-week-old cockerels injected with thyrotropin-releasing hormone (TRH; 1 μg/bird, ●); somatostatin (SRIF; 100 μg/bird, □); and TRH and SRIF (1 and 100 μg/bird, ▲). Control birds received a saline vehicle injection, O. Vertical bars represent SE; $N = 9$ birds/group. *$P < 0.05$, as compared to controls.

cently reported (12, 17) that noradrenergic blockers significantly inhibited the stimulatory effects of TRH but not the stimulatory effects of hpGRF in 4-week-old cockerels *in vivo*. These results support the idea of hypothalamic involvement in TRH stimulatory effects on GH release in addition to the direct action on the pituitary.

The reduction in basal concentrations of GH after the administration of TRH antiserum indicates that TRH is a factor in the control of GH secretion in birds (8). The roles that TRH and hpGRF play in controlling GH release may be different. TRH and hpGRF are both potent GH releasers *in vivo* in immature birds (9), but only hpGRF stimulates GH release in adult birds (11, 18). The TRH had a slight stimulatory effect on GH release in autosomal dwarfs, but had great GH-releasing activity in the sex-linked dwarfs (10). The hpGRF has been reported active in both autosomal and sex-linked dwarfs (10). This information suggests that there is diversity in the mechanisms by which TRH and hpGRF stimulate GH release in chickens.

A recent report concerning the purification of chicken SRIF from the hypothalamus further supports a physiological role for SRIF in regulating GH release in the avian species (19). We observed no significant effect in GH concentration when SRIF and TRH were given in combination, whereas Harvey *et al.* (1) reported a significant reduction. The discrepancy may be due to differences in the strains and/or ages of the birds used. Harvey *et al.* (1) used an 8- to 10-week-old layer strain of chickens, and we used a 4-week-old broiler strain.

In conclusion, our data suggest that TRH, GRF, and SRIF are all involved in the regulation of GH release in chickens. The stimulatory effects of TRH and hpGRF *in vivo* are additive in nature, and SRIF significantly inhibits the stimulatory effects of hpGRF but not those caused by TRH. This suggests that the stimulatory effects of TRH and hpGRF are mediated through different pathways; it is possible that the TRH stimulating effect is not only at the pituitary but at another site as well.

---

1. Harvey S, Scanes CG, Chadwick A, Bolton NJ. The effect of thyrotropin-releasing hormone (TRH) and somatostatin (GHRIH) on growth hormone and prolactin secretion *in vitro* and *in vivo* in the domestic fowl (*Gallus domesticus*). Neuroendocrinology **26**: 249–260, 1978.

2. Hall TR, Chadwick A, Bolton NJ, Scanes CG. Prolactin release *in vitro* and *in vivo* in the pigeon and domestic fowl following administration of synthetic thyrotropin-releasing factor (TRF). Gen Comp Endocrinol **26**:298–306, 1975.
3. Hall TR, Chadwick A. Effect of growth hormone inhibiting factor (somatostatin) on the release of growth hormone and prolactin from pituitaries of the domestic fowl *in vitro*. J Endocrinol **168**:163–164, 1976.
4. Harvey S. Thyroid hormones inhibit growth hormone secretion in domestic fowl (*Gallus domesticus*). J Endocrinol **96**:329–334, 1983.
5. Harvey S, Sterling RJ, Klandorf H. Concentrations of triiodothyronine, growth hormone and luteinizing hormone in the plasma of thryoidectomized fowl (*Gallus domesticus*). Gen Comp Endocrinol **50**:275–281, 1983.
6. Harvey S, Sterling RJ, Phillips JG. Diminution of thyrotropin releasing hormone-induced growth hormone secretion in adult domestic fowl (*Gallus domesticus*). J Endocrinol **89**:405–410, 1981.
7. Proudman JA, Opel H. Effect of feed or water restriction on basal and TRH stimulated growth hormone secretion in the growing turkey poult. Poult Sci **60**: 659–667, 1981.
8. Klandorf H, Harvey S, Fraser HM. Physiological control of growth hormone secretion by thyrotropin-releasing hormone in the domestic fowl. J Endocrinol **105**:351–355, 1985.
9. Leung FC, Taylor JE. *In vivo* and *in vitro* stimulation of growth hormone release in chickens by synthetic human pancreatic growth hormone releasing factors (hpGRFs). Endocrinology (Baltimore) **113**:1913–1915, 1983.
10. Scanes CG, Carsia RV, Lauterio TJ, Huybrechts L, River J, Vale W. Synthetic human pancreatic growth hormone release factor (GRF) stimulates growth hormone secretion in the domestic fowl (*Gallus domesticus*). Life Sci **34**:1127–1134, 1984.
11. Harvey S, Scanes CG. Comparative stimulation of growth hormone secretion in anesthetized chickens by human pancreatic growth hormone releasing factor (hpGRF) and thyrotropin releasing hormone (TRH). Neuroendocrinology **39**:314–320, 1984.
12. Leung FC, Taylor JE, Ball CA. Potent interaction between thyrotropin releasing hormone (TRH) and human pancreatic growth hormone-releasing factor (hpGRF) in stimulating chicken growth hormone (cGH) *in vivo*. In: Proceedings of the 7th International Congress of Endocrinology. Amsterdam, Excerpta Medica Foundation, p1013, 1984.
13. Leung FC, Taylor JE, Steelman SL, Bennett CD, Rodkey JA, Long RA, Serio R, Weppelman RM, Olson G. Purification and properties of chicken growth

hormone and the development of a homologous radioimmunoassay. Gen Comp Endocrinol **86**:389–400, 1984.

14. Harvey S, Scanes CG. Interaction between human pancreatic growth hormone releasing factor (hpGHF) and thyrotropin releasing hormone (TRH) on growth hormone secretion in domestic fowl. Horm Metab Res **17**:113, 114, 1985.
15. Buonomo FC, Lauterio TJ, Scanes CG. Episodic growth hormone secretion in the domestic fowl (*Gallus domesticus*): Alpha adrenergic regulation. Comp Biochem Physiol **78C**:409–413, 1984.
16. Hall TR, Chadwick A. Hypothalamic control of prolactin and growth hormone secretion in the pituitary gland of the pigeon and the chicken and *in vitro* studies. Gen Comp Endocrinol **49**:135–143, 1983.
17. Leung FC, Taylor JE, Ball CA. Potent interaction between thyrotropin releasing hormone (TRH) and human pancreatic growth hormone releasing factor (hpGHF) in stimulating chicken growth hormone (cGH) *in vivo:* Hypothalamic noradrenergic mediation in TRH stimulation of cGH release. Domest Anim Endocrinol **2**:183–190, 1985.
18. Harvey S, Scanes CG, Marsh JA. Stimulation of growth hormone secretion in dwarf chickens by thyrotropin releasing hormone (TRH) or human pancreatic growth hormone releasing factor (hpGHF). Gen Comp Endocrinol **55**:493–497, 1984.
19. Hasegawa Y, Miyamoto K, Nomura M, Igarashi M, Kangawa K, Matsuo H. Isolation and amino acid compositions of four somatostatin-like substances in chicken hypothalamic extract. Endocrinology (Baltimore) **115**:433–435, 1984.

Received May 6, 1986. P.S.E.B.M. 1986, Vol. 183.
Accepted September 3, 1986.