PROCEEDINGS OF THE SOCIETY FOR EXPERIMENTAL BIOLOGY AND MEDICINE 175, 79–83 (1984)

Growth Hormone and Prolactin Response to Thyrotropin Releasing Hormone and Growth Hormone Releasing Factor in the Immature Turkey (41770)

J. A. PROUDMAN

Avian Physiology Laboratory, ARS, U.S. Department of Agriculture, Beltsville, Maryland 20705

Abstract. Synthetic thyrotropin releasing hormone (TRH) and human pancreatic growth hormone releasing factor (hpGRF) stimulated growth hormone (GH) secretion in 6- to 9-week-old turkeys in a dose-related manner. TRH and hpGRF (1 and 10 μg/kg, respectively) each produced a sixfold increase in circulating GH levels 10 min after iv injection. Neither TRH nor hpGRF caused a substantial change in prolactin (PRL) secretion in unrestrained turkeys sampled through intraatrial cannulas. However, some significant increases in PRL levels, possibly related to stress, were noted.

Thyrotropin releasing hormone (TRH) is a potent stimulus of thyrotropin and prolactin (PRL) secretion in mammals and, in some species, also increases circulating growth hormone (GH) levels. In birds, TRH produces a sharp increase in GH secretion in immature chickens (1, 2) and ducks (3), but has little or no effect on GH levels in adult chickens (2, 4). The GH response to TRH in immature chickens is reportedly not dose-related (1, 2), suggesting that this peptide may not be the physiological releasing hormone for GH. The effect of TRH on PRL secretion in birds is uncertain. TRH stimulates crop sac growth in the pigeon (5), but it does not increase circulating PRL levels in immature chickens (1, 5). TRH increases PRL release from pigeon (5) and chicken (1, 5) pituitaries cultured *in vitro,* but not from turkey pituitary cells (6). Human pancreatic growth hormone-releasing factor (hpGRF) is an extremely potent releaser of GH in rats, and in physiological studies is nearly indistinguishable from rat hypothalamic GRF (7–10). The effect of synthetic hpGRF on GH secretion in birds has not been reported. The present study investigates the effects of TRH and hpGRF on GH and PRL secretion in the immature turkey.

Materials and Methods. *Experiment I.* Nicholas Large White male turkeys were raised in wire batteries under constant light with free access to feed and water. At 6 weeks of age, birds were selected at random to receive either vehicle (saline) or TRH (Beckman Instruments, Palo Alto, Calif.) at a dose of either 0.1, 0.25, 0.5, 1.0, 5.0, or 10.0 μg/kg of body weight. The doses selected were based on studies using the young chicken (1, 2) and ranged from doses found ineffective (0.1 and 0.25 μg/kg) to one which consistently produced a maximal response (10 μg/kg). A pretreatment blood sample was drawn from each of three birds and TRH or vehicle was injected into the brachial vein. Additional 1.5-ml blood samples were collected from the contralateral vein at 5, 10, and 20 min following injection. Treatments were rotated successively until each was administered to five birds. No bird received more than one injection. Sera were stored at −70°C until analysis.

Experiment II. Nicholas Large White male turkeys were raised in floor pens under a 14L:10D photoperiod (lights on at 0600 hr) with free access to feed and water. Experiments were conducted while the birds were between 7 and 9 weeks of age. Birds were selected at random to receive either vehicle, TRH (0.1 or 1.0 μg/kg) (Boehringer Mannheim Biochemicals, Indianapolis, Ind.), or hpGRF(1-44) (0.1, 1.0, or 10.0 μg/kg) (Peninsula Laboratories, Belmont, Calif.). Treatments were administered and blood samples collected using a cannula placed via the jugular vein into the atrium (11). Six birds were cannulated 2 days prior to sampling and placed in individual wire cages. On the day of sampling, cannulas were passed through the tops of the cages at least 2 hr prior to treatment and connected to three-way stopcocks. A pretreatment blood sample was drawn, the treatment was administered in a volume of <1 ml, and the cannula was flushed with 3 ml of saline. Additional 2.5-ml blood samples were collected in heparinized syringes at 10, 20, 40, 60, and 120

0037-9727/84 $1.50

min following treatment. This procedure was repeated on groups of four to six birds (some cannulas failed to work) until each treatment had been administered to five birds. Treatments were always administered at about 1300 hr, and were represented as equally as possible across the 2-week period. No bird was used more than once. Plasmas were stored at −70°C until analysis.

Radioimmunoassays. The concentrations of GH and PRL present in serum and plasma samples were measured by the homologous radioimmunoassay procedures of Proudman and Wentworth (12) and Proudman and Opel (13), respectively. All samples from a complete experiment were analyzed for GH or PRL in a single assay so as to avoid interassay variability. Potency estimates were calculated using the spline-function program of the LKB Model 1270 gamma counter.

Statistical analysis. Analysis of variance was done to establish differences in treatment and differences over time. Data were $\log_{10}$-transformed to correct for heterogeneity of variances. Where treatment by time interactions were present, a Dunnett's procedure (14) was applied to compare means for a given treatment to the zero time (pretreatment) mean for that treatment. The Dunnett's procedure was a two-sided comparison performed at the 0.05 level of significance to establish a significant rise or decline in hormone levels following treatment.

Results. *Experiment I.* TRH produced a significant increase in GH secretion in turkey poults within 5 min of administration at all dose levels studied. The data (Table I) suggest that increasing doses of TRH (up to 1 μg/kg) were increasingly effective in stimulating GH secretion, but did so in a nonlinear manner. Examination of individual bird responses (not shown) revealed that even the lowest doses of TRH (0.1 and 0.25 μg/kg) produced a dramatic GH peak (>500 ng/ml) in some birds, and that increasing the dose of TRH administered increased the number of birds showing such a response. A small rise in GH levels in the saline-treated controls was noted, but this increase was not significant. PRL levels increased significantly ($P < .001$ by paired t test) in the control birds (from 7.4 ± 0.7 ng/ml pretreatment to 14.4 ± 1.1 ng/ml at 5 min) so that the effect of TRH on PRL secretion could not be determined in this experiment. The stress of handling for short-interval blood sampling has previously been shown to increase PRL levels in turkeys (15).

Experiment II. Cannulated birds were used in this experiment to avoid possible effects of handling stress on hormone levels. Table II shows that neither GH nor PRL levels increased in cannulated control birds sampled for 2 hr; in fact, significant declines in both hormones were observed at some time periods. GH levels increased about sixfold in birds receiving either 1 μg/kg TRH or 10 μg/kg hpGRF. The GH levels also increased significantly in birds receiving 0.1 μg/kg TRH or 1 μg/kg hpGRF, but to a lesser extent (about threefold) and for a shorter duration than was

TABLE I. SERUM GH RESPONSE TO TRH

Dose (μg/kg)	Time after injection (min)			
	0	5	10	20
0.00	60.7 ± 6.7	95.0 ± 10.7	96.0 ± 11.0	95.0 ± 19.9
0.10	58.5 ± 8.6	218.5 ± 83.0*	183.0 ± 60.0*	113.1 ± 26.5*
0.25	48.5 ± 13.0	236.3 ± 92.6*	357.1 ± 101.0*	352.5 ± 78.0*
0.50	48.4 ± 4.4	394.5 ± 76.8*	477.0 ± 70.1*	246.5 ± 25.6*
1.00	69.1 ± 11.1	414.4 ± 91.5*	493.9 ± 46.0*	370.0 ± 87.4*
5.00	42.7 ± 7.0	267.4 ± 62.0*	363.2 ± 81.4*	313.6 ± 69.6*
10.00	38.4 ± 12.7	365.5 ± 96.0*	301.0 ± 71.1*	463.8 ± 67.0*

Note. Values are means ± SE of five turkeys per dose in units of ng/ml.

* Post-treatment means significantly different from pretreatment (0 min) means as determined by Dunnett's procedure done at $\alpha = 0.05$.

TABLE II. PLASMA GH AND PROLACTIN RESPONSE TO TRH AND hpGRF

Treatment	Dose (μg/kg)	Time after injection (min) 0	10	20	40	60	120
GH (ng/ml)							
Control	0.0	46.3 ± 10.4	43.6 ± 11.1	39.5 ± 11.8	18.2 ± 7.1*	10.7 ± 2.9*	51.3 ± 12.7
TRH	0.1	30.9 ± 7.0	106.8 ± 49.5*	93.5 ± 48.8	33.8 ± 11.2	86.4 ± 20.8*	24.5 ± 6.2
	1.0	25.0 ± 7.5	151.5 ± 24.3*	127.0 ± 28.0*	88.5 ± 28.0*	48.4 ± 13.0*	72.9 ± 24.5*
GRF	0.1	33.1 ± 7.7	33.1 ± 11.4	26.3 ± 6.5	51.8 ± 15.3	50.0 ± 17.8	20.0 ± 6.8
	1.0	31.7 ± 11.6	94.9 ± 31.5*	57.8 ± 20.0	35.2 ± 14.4	41.9 ± 22.1	31.0 ± 7.7
	10.0	40.4 ± 8.6	223.8 ± 29.6*	191.8 ± 23.3*	132.1 ± 25.3*	124.2 ± 27.8*	71.0 ± 16.0
Prolactin (ng/ml)							
Control	0.0	12.1 ± 0.6	11.9 ± 1.0	11.3 ± 1.3	13.2 ± 1.4	10.8 ± 1.2	9.4 ± 0.6*
TRH	0.1	11.5 ± 3.5	12.5 ± 3.4	13.8 ± 5.6	12.5 ± 4.4	12.2 ± 3.7	12.6 ± 5.1
	1.0	11.3 ± 1.6	15.0 ± 1.8*	11.7 ± 1.4	12.0 ± 1.9	9.2 ± 1.4	8.9 ± 0.9
GRF	0.1	10.2 ± 2.1	9.9 ± 1.8	10.4 ± 1.8	9.0 ± 1.8	10.1 ± 2.1	9.7 ± 2.3
	1.0	9.4 ± 0.9	10.5 ± 2.1	10.6 ± 1.2	10.9 ± 2.1	8.8 ± 1.1	8.0 ± 1.1
	10.0	13.1 ± 3.3	21.4 ± 3.9*	18.9 ± 4.0*	16.9 ± 2.3*	15.0 ± 2.6*	10.3 ± 2.0

Note. Values are means ± SE of five turkeys per dose.

* Post-treatment means significantly different from pretreatment (0 min) means as determined by Dunnett's procedure dose at $\alpha = 0.05$.

observed with the higher dose levels. The lowest dose of hpGRF (0.1 μg/kg) produced no significant change in circulating GH levels.

PRL levels increased significantly in response to two treatments—1 μg/kg TRH and 10 μg/kg hpGRF. The increase produced by TRH was small and transitory; hpGRF increased PRL slightly more than did TRH, and this increase was sustained until 60 min after treatment.

Discussion. TRH and hpGRF markedly stimulated GH secretion in male turkey poults. The GH response to TRH was clearly dose-dependent, but individual differences in sensitivity to TRH masked the exact nature of the dose–response relationship. Experiment II suggests that hpGRF also increased GH levels in a dose-dependent manner. GH secretion was stimulated more effectively by TRH than by hpGRF on a *weight* basis, but since hpGRF is a much larger peptide than TRH (5040 vs 362 mol wt), its effectiveness on a *molar* basis was equal to or greater than that of TRH. The hpGRF doses found effective in the turkey were similar to those effective in mammalian species. Wehrenberg *et al.* (9) found more than a 10-fold increase in the GH levels of anesthetized rats given 1 μg of hpGRF (about 3.5 μg/kg), although further work (10) has shown that only 1 in 3 conscious unrestrained rats shows such a response unless somatostatin is simultaneously suppressed. Administration of hpGRF (1 μg/kg) to adult men stimulated GH secretion in four of six individuals (16), a response rate similar to that observed with the same dose in the turkey. The mean increase in responding men (10-fold) was substantially greater than the maximum individual increase (5- to 6-fold) observed at this dose in the turkey, but the turkey response may have been underestimated since the time-course of the response in the bird is unknown. The maximum response occurs 3–5 min following hpGRF injection in the anesthetized rat (9), but 20–30 min after injection in humans (16). Additional studies are required to define both the maximum effective dose and the time-course of the GH response in birds.

TRH is obviously not the potent stimulus for PRL secretion in the turkey that it is in mammals. When studied in unrestrained turkeys, TRH produced only a small increase in PRL secretion at a dose level shown to maximally stimulate GH secretion. Previous studies have shown TRH to have no consistent effect on *in vivo* PRL secretion in chickens

(1). Such results differ markedly from those in mammals, where TRH is a strong PRL stimulus. However, the control of PRL secretion in avian species is unlike that of mammalian species in that the avian hypothalamus exerts a stimulatory control over PRL while the mammalian hypothalamus has an inhibitory influence. The importance of TRH as a PRL releasing factor in mammals has recently been reviewed (17) and a comparative review (18) of the neuroendocrine control of PRL secretion in birds and mammals provides discussion of the differential effects of TRH and other brain chemicals on PRL secretion in these species. Less is known of the effects of hpGRF on PRL secretion, but studies in rats (9, 19) and man (15) have found no increase in circulating PRL levels following administration of hpGRF at dose levels which markedly stimulate GH secretion. The increase in turkey PRL in response to 10 μg/kg of hpGRF resulted primarily from a 4-fold increase in one individual. The possibility cannot be ruled out that this response may have resulted from some unknown stress, since previous work in this laboratory has shown extreme differences in individual sensitivity in the PRL response to stress (15). Additional studies with larger numbers of birds would be needed to establish unequivocally any PRL response to TRH or hpGRF. However, numerous studies in this laboratory have found that the physiological fluctuations in PRL in the mature turkey greatly exceed the changes shown here, suggesting that any small PRL response to relatively high levels of these peptides would probably not be physiologically meaningful.

This research has shown that TRH and hpGRF are both effective in stimulating GH secretion in a dose-related manner in the immature turkey, and that neither has a substantial effect on PRL secretion. Which, if either, of these very different peptides is the physiological GRF in birds is a question for further study.

The author thanks Gaynelle Campbell for technical assistance in collecting samples and performing radioimmunoassays, and Beverly Russell and Joyce McRae for surgically inserting cannulas. I also thank Dr. Estelle Russek, Department of Animal Science, University of Maryland, for performing the statistical analyses.

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. Scanes CG, Harvey S, Morgan BA, Hayes M. Effect of synthetic thyrotrophin-releasing hormone and its analogues on growth hormone secretion in the domestic fowl (*Gallus domesticus*). Acta Endocrinol **97**:448–453, 1981.
3. Pethes G, Scanes CG, Rudas P. Effect of synthetic thyrotropin releasing hormone on the circulating growth hormone concentration in cold and heat stressed ducks. Acta Vet Acad Sci Hung **27**:175–177, 1979.
4. 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.
5. Hall TR, Chadwick A, Bolton NJ, Scanes CG. Prolactin release *in vitro* and *in vivo* in the pigeon and the domestic fowl following administration of synthetic thyrotrophin-releasing factor (TRF). Gen Comp Endocrinol **25**:298–306, 1975.
6. Proudman JA, Opel H. Secretion of prolactin by superfused turkey anterior pituitary cells attached to cytodex beads. Poultry Sci **60**:1714, abstr, 1981.
7. Guillemin R, Brazeau P, Bohlen P, Esch F, Ling N, Wehrenberg WB. Growth hormone-releasing factor from a human pancreatic tumor that caused acromegaly. Science **218**:585–587, 1982.
8. Rivier J, Spies J, Thorner M, Vale W. Characterization of a growth hormone-releasing factor from a human pancreatic islet tumour. Nature (London) **300**:276–278, 1982.
9. Wehrenberg WB, Ling N, Brazeau P, Esch F, Bohlen P, Baird A, Ying S, Guillemin R. Somatocrinin, growth hormone releasing factor, stimulates secretion of growth hormone in anesthetized rats. Biochem Biophys Res Commun **95**:382–387, 1982.
10. Wehrenberg WB, Ling N, Bohlen P, Esch F, Brazeau P, Guillemin R. Physiological roles of somatocrinin and somatostatin in the regulation of growth hormone secretion. Biochem Biophys Res Commun **95**:562–567, 1982.
11. Opel H, Proudman JA. Two methods for serial blood sampling from unrestrained, undisturbed turkeys with notes on the effects of acute stressors on plasma levels of prolactin. Poultry Sci, submitted for publication.
12. Proudman JA, Wentworth BC. Radioimmunoassay of turkey growth hormone. Gen Comp Endocrinol **36**:194–200, 1978.
13. Proudman JA, Opel H. Turkey prolactin: Validation of a radioimmunoassay and measurement of changes

associated with broodiness. Biol Reprod **25**:573–580, 1981.

14. Steel RDG, Torrie JH. Principles and Procedures of Statistics. New York, McGraw–Hill, p111, 1960.
15. Opel H, Proudman JA. Effects of repeated handling and blood sampling on plasma prolactin levels in the turkey. Poultry Sci **61**:1390, abstr, 1982.
16. Thorner MO, Spiess J, Vance ML, Rogol AD, Kaiser DL, Webster JD, Rivier J, Borges JL, Bloom SR, Cronin MJ, Evans WS, MacLeon RM, Vale W. Human pancreatic growth-hormone-releasing factor selectively stimulates growth-hormone secretion in man. Lancet **1**:24–28, 1983.
17. Leong DA, Frawley LS, Neill JD. Neuroendocrine control of prolactin secretion. Amer Rev Physiol **45**:109–127, 1983.
18. Harvey S, Chadwick A, Border G, Scanes CG, Phillips JG. Neuroendocrine control of prolactin secretion. In: Scanes *et al,* eds. Aspects of Avian Endocrinology: Practical and Theoretical Implications. Grad Studies, Texas Tech Univ, Vol 26:pp41–64, 1982.
19. Cronin MJ, Rogol AD, MacLeod RM, Keefer DA, Login IS, Borges JLC, Thorner MO. Biological activity of a growth hormone-releasing factor secreted by a human tumor. Amer J Physiol **244**:E346–E353, 1983.

Received July 29, 1983. P.S.E.B.M. 1984, Vol. 175.
Accepted September 26, 1983.