5-Hydroxytryptaminergic Receptor-Mediated Regulation of Growth Hormone Secretion in Holstein Steers Occurs *via* α_2-Adrenergic–Dependent and –Independent Mechanisms (44026)

P. J. GAYNOR,* C. R. SIMMONS,* K. J. LOOKINGLAND,† AND H. A. TUCKER*,[1]

Departments of Animal Science and Pharmacology and Toxicology,† Michigan State University, East Lansing, Michigan 48824-1225*

Abstract. ***In vitro*** **and** ***in vivo*** **experiments were used to determine the relationship between 5-hydroxytryptaminergic and α_2-adrenergic receptors in regulation of growth hormone secretion in cattle. Activation of 5-hydroxytryptaminergic receptors (10^{-8}, 10^{-6}, 10^{-4} *M* quipazine) or α_2-adrenergic receptors (10^{-8}, 10^{-6}, 10^{-4} *M* clonidine) had no effect on secretion of growth hormone from perifused anterior pituitary cells.** ***In vivo,*** **quipazine (0.2 mg/kg body wt, iv) and clonidine (8 μg/kg body wt, iv), when injected separately, each maximized secretion of growth hormone in Holstein steers. However, concurrent administration of quipazine and clonidine at these doses additively increased secretion of growth hormone (mean areas under curves = 439, 914, 1425, and 2359 ± a pooled SEM of 246 ng · ml^{-1} · min for vehicle, clonidine, quipazine, and quipazine plus clonidine treatments, respectively). Blockade of 5-hydroxytryptaminergic receptors with cyproheptadine (0.2 or 1.0 mg/kg body wt, sc, 0740 hr) decreased basal concentrations of growth hormone but had no effect on the ability of clonidine (8 μg/kg body wt, iv, 0840 hr) to increase secretion of growth hormone (mean areas under curves = 591, 1218, 363, 1087, and 1002 ± a pooled SEM of 177 ng · ml^{-1} · min for vehicle-vehicle, vehicle-clonidine, 0.2 mg cyproheptadine-vehicle, 0.2 mg cyproheptadine-clonidine and 1.0 mg cyproheptadine-clonidine treatments, respectively). Blockade of α_2-adrenergic receptors with either yohimbine (5 mg/kg body wt, sc, 0740 hr) or idazoxan (20 mg/kg body wt, sc, 0740 hr) suppressed both basal and 5-hydroxytryptaminergic receptor-stimulated (0.2 mg quipazine/kg body wt, iv, 0840 hr) secretion of growth hormone (mean areas under curves = 568, 1252, 410, and 558 ± a pooled SEM of 108 ng · ml^{-1} · min for vehicle-vehicle, vehicle-quipazine, yohimbine-vehicle, and yohimbine-quipazine treatments, respectively, and means of 553, 1468, 194, and 686 ± a pooled SEM of 221 ng · ml^{-1} · min for vehicle-vehicle, vehicle-quipazine, idazoxan-vehicle, and idazoxan-quipazine treatments, respectively). We conclude that two mechanisms in the central nervous system mediate 5-hydroxytryptaminergic receptor-stimulated secretion of growth hormone in cattle; one independent and another dependent on α_2-adrenergic receptors, possibly** ***via*** **regulation of basal growth hormone secretion. In contrast, α_2-adrenergic receptor-induced secretion of growth hormone occurs independently of 5-hydroxytryptaminergic receptors.**

[P.S.E.B.M. 1996, Vol 212]

[1] To whom requests for reprints should be addressed at 230 Anthony Hall, Department of Animal Science, Michigan State University, East Lansing, MI 48824-1225.

This research was supported by the United States Department of Agriculture Grants 91-37206-6718 and 93-37206-9347.

Received December 27, 1995. [P.S.E.B.M. 1996, Vol 212]
Accepted April 9, 1996.

0037-9727/96/2124-0355$10.50/0

Monoamine neurotransmitters within the hypothalamus regulate the release of two hypothalamic peptides, growth hormone–releasing hormone (GHRH) and somatostatin (SRIF) (1). In turn, GHRH and SRIF regulate secretion of growth hormone from the anterior pituitary gland (1, 2). Although 5-hydroxytryptaminergic (5-HT) or α_2-adrenergic receptor-agonists do not stimulate secretion of growth hormone from somatotropes of rats (3, 4). In sheep, a 5-HT receptor-agonist also had no ef-

fect, whereas an α_2-receptor-agonist inhibited secretion of growth hormone directly from somatotropes (5). Analogous experiments have not been conducted in cattle. Thus, our first objective was to determine if 5-HT or α_2-adrenergic receptor agonists directly affect secretion of growth hormone from bovine somatotropes.

Stimulation of either 5-HT or α_2-adrenergic receptors increased secretion of growth hormone in cattle (6–8). In rats, α_2-adrenergic receptor–stimulated secretion of growth hormone required intact 5-HT terminals within the hypothalamus (9, 10), but the relationships between these stimulatory pathways in other species has not been determined. Therefore, our second objective was to determine the relationships between 5-HT and α_2-adrenergic receptors in the regulation of growth hormone secretion in cattle.

Materials and Methods

Effects of Quipazine (5-HT Receptor Agonist), Clonidine (α_2-Adrenergic Receptor Agonist), and GHRH on Secretion of Growth Hormone from Anterior Pituitary Cells *In Vitro*. The objective was to determine if either quipazine or clonidine directly stimulated secretion of growth hormone from anterior pituitary cells *in vitro*. Growth hormone–releasing hormone was used as a positive control to establish that cells contained growth hormone and were responsive. Anterior pituitary glands from cattle were collected at slaughter and enzymatically dispersed as descried previously (11). Briefly, 1-mm^3 pieces of adenohypophyseal tissue were digested in a solution of calcium-magnesium-free Hanks' Balanced Salt Solution (CMF-HBSS) and 0.3% collagenase (Type I) for 45 min at 37°C. After enzymatic digestion, the tissue was triturated 20 times by passage through a 10-ml serological pipette. The resulting cell suspension was filtered through sterile, presoaked gauze and then centrifuged (400 *g* for 5 min) to recover the cells. Cells were washed three times with CMF-HBSS and one time with Dulbecco's modified Eagle's medium with low glucose (<1000 mg/l) containing 1% newborn calf serum, 25 m*M* HEPES, 10 U penicillin/ml, 10 μg streptomycin/ml, and 0.25 μg fungizone/l. This mixture is hereafter referred to as DMEM. Cells were then suspended in DMEM at a concentration of 10^6 cells/ml. After dispersion and resuspension, cell viability was greater than 90% as determined by trypan blue exclusion. All culture media and supplements were purchased from GIBCO Laboratories (Grand Island, NY). Collagenase was obtained from Worthington Biochemicals (Freehold, NJ).

The perifusion system of Hassan *et al.* (12) was modified for use in this experiment. The barrels of 3-ml syringes (Becton Dickinson, Franklin Lakes, NJ) served as perifusion chambers. Rubber serum stoppers were placed on the tips of syringes and a layer of sterile glass wool was inserted to retain Cytodex I beads (Pharmacia Biotech, Piscataway, NJ). Syringes were packed by gravity to a volume of 0.5 ml with sterile Cytodex I beads, which were swollen in 0.9% NaCl. Aliquots (1 ml) of the cell suspension in media were pipetted into perifusion chambers. Syringes were then filled to the 3-ml mark with DMEM and placed in a humidified atmosphere of 95% O_2-5% CO_2 at 37°C for 24 hr.

After the initial incubation, perifusion chambers were connected to peristaltic pumps and perifused with DMEM, gassed with 95% O_2-5% CO_2, at a flow rate of 0.15 ml/min for 320 min. After the first 120 min of perifusion, samples of effluent media were collected every 20 min during a 200-min experimental period. In one experiment, six perifusion chambers containing anterior pituitary cells were assigned to each of the following treatments: control containing media alone, positive control consisting of media plus 10^{-8} *M* GHRH, or media containing 10^{-8}, 10^{-6}, or 10^{-4} *M* quipazine dimaleate (Research Biochemicals, Inc., Natick, MA). In another experiment, six perifusion chambers containing anterior pituitary cells were assigned to each of the following treatments: control containing media alone, positive control consisting of media plus 10^{-8} *M* GHRH, or media containing 10^{-8}, 10^{-6}, or 10^{-4} *M* clonidine hydrochloride (Sigma Chemical Co., St. Louis, MO). Treatments were perifused between 40 and 60 min of the experimental period. Between 120 and 140 min of the experimental period, chambers were perifused with DMEM containing 60 mM KCl to test cell viability. Samples of media were frozen (−20°C) and later, concentrations of growth hormone were measured by radioimmunoassay as described previously (8). Intraassay coefficient of variation for concentration of growth hormone was 7.5%.

General Protocol for Experiments with Calves. Holstein steer calves were housed in rooms maintained at 20° ± 1°C (four steers per room) with 18 hr of light and 6 hr of dark each day and fed a pelleted diet (18% crude protein, 19.6% acid detergent fiber; Countrymark Cooperative, Indianapolis, IN) balanced to meet nutrient requirements (13). Beginning at 8 to 10 weeks of age, steers were adapted to meal-feeding over a 14-day period. Thereafter, steers had access to feed between 1000 and 1200 hr daily. Water was available at all times.

A catheter was inserted into a jugular vein of each calf at least 1 day before each experiment. A solution of 3.5% sodium citrate in sterile water was used as anticoagulant in catheters. Blood samples (6 ml) were collected at 20-min intervals before feeding (0700 to 0940 hr). Samples were allowed to clot at room temperature and then were stored at 4°C. Blood was centrifuged the following day at 1500 *g* for 25 min. Serum was transferred to polypropylene tubes and frozen at

−20°C. Concentrations of growth hormone in serum were determined by radioimmunoassay as described previously (8). Intraassay coefficient of variation was 7.4%.

Steers were assigned to treatments in completely randomized designs. For experiments with more than one sampling day, either two or three nonsampling days separated sampling days.

Effects of Quipazine on Secretion of Growth Hormone *in Vivo*. We showed previously that 0.2 mg quipazine/kg body wt increased concentration of growth hormone in serum of steers injected before or after feeding (8). The objective was to identify a dose of quipazine that maximally increased 5-HT receptor-stimulated secretion of growth hormone. Twelve meal-fed steers (170 ± 6 days of age; 170 ± 4 kg body wt [mean ± SEM]) were sampled on 2 days. Quipazine was diluted in 0.9% sterile saline (10 mg/ml). Treatments were vehicle-injected control, 0.2, 0.4, and 0.6 mg of quipazine per kg body wt. Either vehicle or quipazine was injected iv immediately after a blood sample was collected at 0820 hr.

Effects of Clonidine on Secretion of Growth Hormone *in Vivo*. We showed previously that 2 μg clonidine/kg body wt increased concentration of growth hormone in serum of steers injected before feeding (7). The objective of this experiment was to identify a dose of clonidine that maximally increased α_2-adrenergic receptor-stimulated secretion of growth hormone before feeding. Fourteen meal-fed steers (173 ± 7 days of age; 173 ± 6 kg body wt) were sampled on a single day. Clonidine was diluted in 0.9% sterile saline (350 μg/ml). Steers were randomly assigned to the following treatments: vehicle-injected control or 8- or 16-μg clonidine/kg body wt. Either vehicle or clonidine was injected iv immediately after collection of a blood sample at 0820 hr.

Effect of Concurrent Administration of Quipazine and Clonidine on Secretion of Growth Hormone. The objective was to determine if concurrent activation of 5-HT and α_2-adrenergic receptors additively increased secretion of growth hormone. Doses of quipazine and clonidine that maximally increased 5-HT receptor– and α_2-adrenergic receptor–stimulated secretion of growth hormone were used. Seventeen steers (198 ± 7 days of age; 194 ± 6 kg body wt) were sampled on 3 days. Treatments were: vehicle-injected control, 0.2 mg quipazine/kg body wt, 8 μg clonidine/kg body wt, and concurrent administration of 0.2 mg of quipazine plus 8 μg clonidine/kg body wt. Treatments were injected immediately after a blood sample was collected at 0820 hr. For the treatment in which quipazine and clonidine were injected together, both drugs were diluted simultaneously in the same vial and then injected together.

Effect of Pretreatment with Yohimbine or Idazoxan (α_2-Adrenergic Antagonists) on Quipazine-Induced Secretion of Growth Hormone. The objective was to determine if blockade of α_2-adrenergic receptors affected the magnitude of subsequent 5-HT receptor–stimulated secretion of growth hormone. Fourteen steers (139 ± 6 days of age; 148 ± 4 kg body wt) were sampled on 3 days. Yohimbine hydrochloride (Sigma) was diluted in sterile water (30 mg/ml). Treatments were factorial combinations of vehicle or 5 mg yohimbine/kg body wt injected sc immediately after a blood sample was collected at 0740 hr followed by vehicle or 0.2 mg quipazine/kg body wt injected iv immediately after a blood sample was collected at 0820 hr. Previous work showed that 5 mg yohimbine/kg body wt suppressed secretion of growth hormone (7).

The same objective was addressed in a second experiment using the more selective α_2-adrenergic receptor antagonist, idazoxan. Eight steers (97 ± 3 days of age; 101 ± 4 kg body wt) were sampled on 3 days. Idazoxan hydrochloride (Sigma) was diluted in sterile water (150 mg/ml). Treatments were factorial combinations of vehicle or 20 mg idazoxan/kg body wt injected sc immediately after a blood sample was collected at 0740 hr followed by vehicle or 0.2 mg quipazine/kg body wt injected iv immediately after a blood sample was collected at 0820 hr. Previous work showed that 20 mg idazoxan/kg body wt suppressed secretion of growth hormone (7).

Effect of Cyproheptadine (5-HT Receptor Antagonist) on Clonidine-Induced Secretion of Growth Hormone. The objective was to determine if blockade of 5-HT receptors with cyproheptadine affected the magnitude of clonidine-induced secretion of growth hormone. Seventeen steers (177 ± 7 days of age; 177 ± 6 kg body wt) were sampled on 3 days. Cyproheptadine hydrochloride (Sigma) was diluted in sterile water (5 mg/ml). Treatments were combinations of vehicle or 0.2 or 1.0 mg cyproheptadine/kg body wt injected sc immediately after collection of a blood sample at 0740 hr followed by vehicle or 8 μg clonidine/kg body wt injected iv immediately after collection of a blood sample at 0820 hr. Previous work showed that 0.2 mg cyproheptadine/kg body wt suppressed secretion of growth hormone in cattle (8).

Statistical Analyses. For all experiments, areas under growth hormone curves (ng · ml^{-1} · min) were calculated using the trapezoidal rule and statistically analyzed (14). For *in vitro* experiments, areas under curves for samples collected at 20 and 40 min of the experimental period (basal) were used as covariates for the analysis of areas under curves for samples collected between 60 and 120 min of the experimental period. To achieve homogeneous variances among treatments, areas under curves were transformed to $\log_{10}$ before analysis (15). Depolarization-stimulated (60 m*M* KCl) secretion of growth hormone was calcu-

lated as the average of concentrations in media samples collected at 140 and 160 min of the experimental period. Comparisons between basal and depolarization-stimulated secretion of growth hormone were determined using Student's paired t test (15).

For experiments with live calves, effects of receptor agonists and antagonists were evaluated using areas under curves for 1 hr after injection of drugs. Where appropriate, areas under growth hormone curves were transformed to $\log_{10}$ before statistical analysis to achieve homogeneous variances among treatments (15). Terms in statistical models were steer, day, and treatment as appropriate (15). Differences among treatment means were determined with either orthogonal contrasts or Bonferroni t tests. Probability values ≤ 0.05 were considered to be significant. In addition to values for areas under curves, mean concentrations of growth hormone in media and serum for each treatment at each sampling time are presented in figures because the units and scale are more readily comprehensible.

Results

Effects of Quipazine, Clonidine, and GHRH on Secretion of Growth Hormone from Dispersed Anterior Pituitary Cells. Compared with perifusion of media alone, media containing either quipazine (10^{-8}, 10^{-6}, 10^{-4} M) or clonidine (10^{-8}, 10^{-6}, 10^{-4} M) had no effect on secretion of growth hormone from dispersed anterior pituitary cells (Fig. 1, A and B, respectively). In contrast, perifusion of media containing GHRH (10^{-8} M) increased secretion of growth hormone compared with perifusion of media alone ($P < 0.05$). For all treatments, perifusion of media containing 60 mM KCl increased ($P < 0.05$) concentration of growth hormone in samples collected at 140 and 160 min compared with basal secretion (mean of samples collected at 20 and 40 min), indicating that cells remained viable throughout the experimental period.

Effect of Quipazine on Secretion of Growth Hormone *in Vivo*. Quipazine doses of 0.2, 0.4, and 0.6 mg/kg body wt each similarly increased secretion of growth hormone above that of vehicle-injected control (Fig. 2) ($P < 0.05$).

Effect of Clonidine on Secretion of Growth Hormone *in Vivo*. Clonidine doses of 8 and 16 μg/kg body wt each similarly increased secretion of growth hormone above that of vehicle-injected control (Fig. 3). ($P < 0.05$).

Effects of Concurrent Administration of Quipazine and Clonidine on Secretion of Growth Hormone. Compared with vehicle-injected control, clonidine and quipazine alone similarly increased secretion of growth hormone (Fig. 4) ($P < 0.05$). However, secretion of growth hormone in response to concurrent administration of clonidine and quipazine was greater

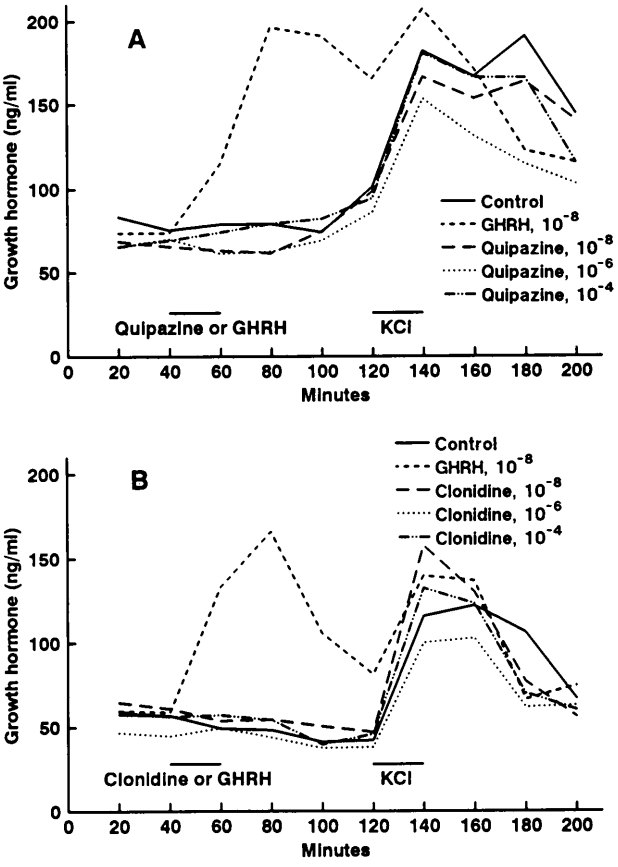

Figure 1. Effects of GHRH, quipazine, and clonidine on basal and KCl-stimulated secretion of growth hormone from dispersed pituitary cells. (A) Mean areas under growth hormone curves (ng · ml^{-1} · min, 60 to 120 min): 169, 5522, 744, 106, and 403 ± a pooled SEM of 402 (n = 6) for control, GHRH, 10^{-8}, 10^{-6}, and 10^{-4} M quipazine, respectively. (B) Mean areas under growth hormone curves (ng · ml^{-1} · min, 60 to 120 min): −808, 4899, −571, −218, and −765 ± a pooled SEM of 482 (n = 6) for control, GHRH, 10^{-8}, 10^{-6}, and 10^{-4} M clonidine, respectively. Negative values reflect declines in secretion of growth hormone. Horizontal lines indicate times when treatments were perifused.

($P < 0.05$) than that for either clonidine or quipazine injected alone.

Effect of Pretreatment with Yohimbine or Idazoxan on Quipazine-Induced Secretion of Growth Hormone. Compared with vehicle-injected control, quipazine alone increased secretion of growth hormone in both experiments (Fig. 5 and 6) ($P < 0.05$). Also, yohimbine and idazoxan each suppressed basal and quipazine-induced secretion of growth hormone ($P < 0.05$).

Effect of Cyproheptadine on Clonidine-Induced Secretion of Growth Hormone. Compared with vehicle-injected control, clonidine increased secretion of growth hormone (Fig. 7) ($P < 0.05$). In addition, cyproheptadine (0.2 and 1.0 mg/kg body wt doses) decreased concentration of growth hormone in serum at 0820 hr compared with vehicle-injected control ($P < 0.05$), but had no effect on the magnitude of clonidine-induced secretion of growth hormone.

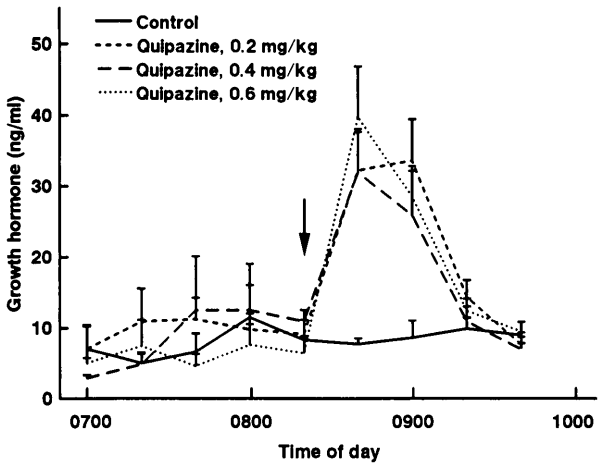

Figure 2. Effect of quipazine on secretion of growth hormone *in vivo*. Values are expressed as means ± SEM. Arrow points to last sample before injection (0820 hr). Mean areas under growth hormone curves (ng · ml^{-1} · min, 0840 to 0940 hr) were 782, 1176, 986, and 1382 ± a pooled SEM of 134 (n = 5 or 6) for vehicle-injected control, 0.2, 0.4, and 0.6 mg quipazine/kg body wt treatments, respectively.

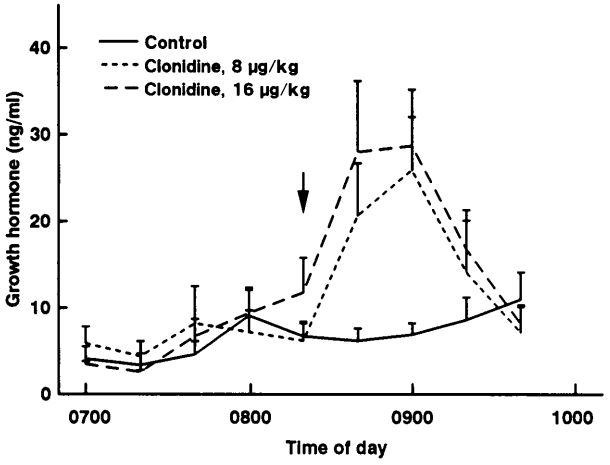

Figure 3. Effect of clonidine on secretion of growth hormone *in vivo*. Values are expressed as means ± SEM. Arrow points to last sample before (0820 hr) injection. Mean areas under growth hormone curves (ng · ml^{-1} · min, 0840 to 0940 hr) were 483, 1079, and 1272 ± a pooled SEM of 276 (n = 5 or 6) for vehicle, 8 µg clonidine, and 16 µg clonidine/kg body wt treatments, respectively.

Discussion

5-Hydroxytryptaminergic and α_2-adrenergic receptors regulate rates of synthesis and secretion of GHRH and SRIF, and thus regulate secretion of growth hormone (1, 2). We showed that stimulation of 5-HT or α_2-adrenergic receptors increased secretion of growth hormone, whereas blockade of 5-HT or α_2-adrenergic receptors decreased secretion of growth hormone (7, 8). Our current observations that activation of 5-HT or α_2-adrenergic receptors stimulated secretion of growth hormone in steers is in agreement with previous reports (6–8), and support the hypothesis that these types of receptors are important in the

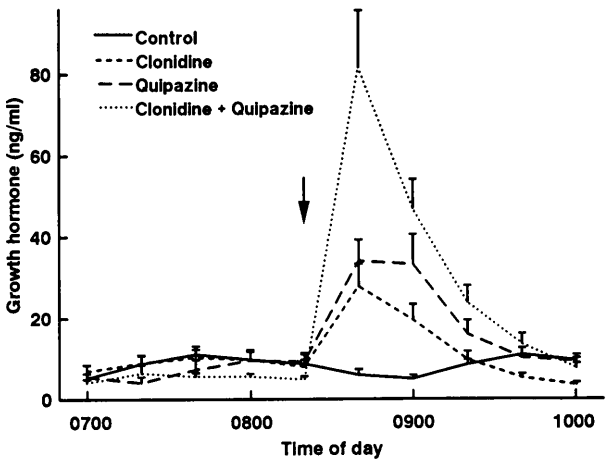

Figure 4. Effects of concurrent administration of clonidine and quipazine on secretion of growth hormone. Dose of clonidine = 8 mg/kg body wt; dose of quipazine = 0.2 mg/kg body wt. Values are expressed as means ± SEM. Arrow points to last sample before (0820 hr) injection. Mean areas under growth hormone curves (ng · ml^{-1} · min, 0840 to 0940 hr) were 439, 914, 1425, and 2359 ± a pooled SEM of 246 (n = 10) for vehicle, 8 µg clonidine/kg body wt, 0.2 mg quipazine/kg body wt, and 8 µg clonidine/kg body wt plus 0.2 mg quipazine/kg body wt treatments, respectively.

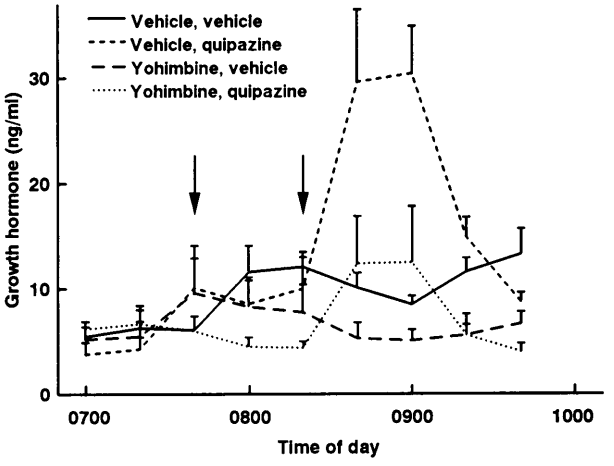

Figure 5. Effect of yohimbine on quipazine-induced secretion of growth hormone. Dose of quipazine = 0.2 mg/kg body wt; dose of yohimbine = 5 mg/kg body wt. Values are expressed as means ± SEM. First arrow (0740 hr) points to last sample before vehicle or yohimbine injection, and second arrow (0820 hr) points to last sample before vehicle or quipazine injection. Mean areas under growth hormone curves (ng · ml^{-1} · min, 0840 to 0940 hr) were 568, 1252, 410, and 558 ± a pooled SEM of 108 (n = 10 or 11) for vehicle-vehicle, vehicle-quipazine, yohimbine-vehicle, and yohimbine-quipazine treatments, respectively.

regulation of growth hormone secretion in cattle. Because 5-HT and α_2-adrenergic receptor agonists did not stimulate secretion of growth hormone directly from the bovine somatotropes, the aminergic mechanisms involved are undoubtedly located within the brain.

Results from our experiments demonstrate that two mechanisms mediate 5-HT receptor-stimulated secretion of growth hormone in cattle: one that is inde-

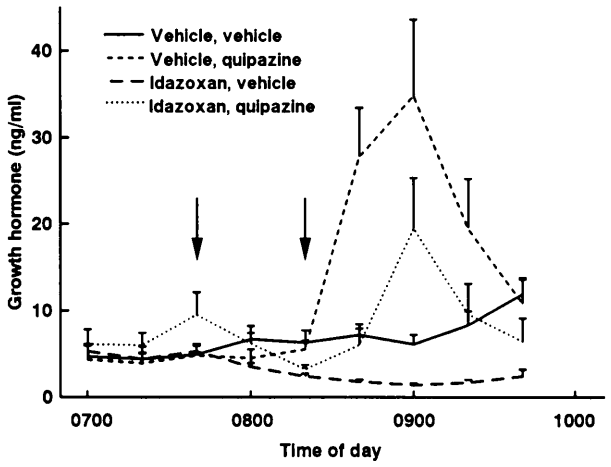

Figure 6. Effect of idazoxan on quipazine-induced secretion of growth hormone. Dose of quipazine = 0.2 mg/kg body wt; dose of idazoxan = 20 mg/kg body wt. Values are expressed as means ± SEM. First arrow (0740 hr) points to last sample before vehicle or idazoxan injection and second arrow (0820 hr) points to last sample before vehicle or quipazine injection. Mean areas under growth hormone curves (ng · ml^{-1} · min, 0840 to 0940 hr) were 553, 1468, 194, and 686 ± a pooled SEM of 221 (n = 5 or 6) for vehicle-vehicle, vehicle-quipazine, idazoxan-vehicle, and idazoxan-quipazine treatments, respectively.

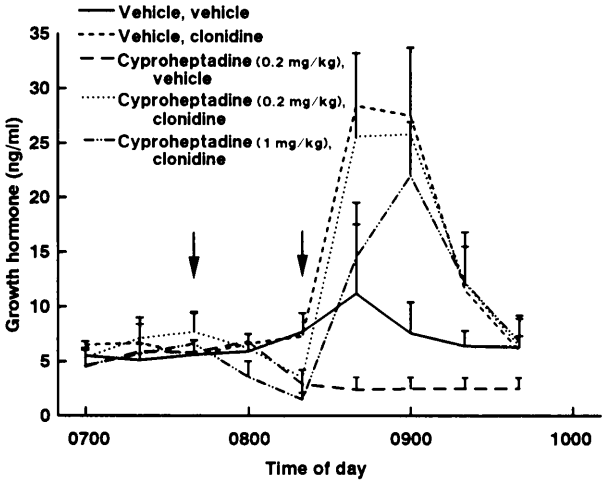

Figure 7. Effect of cyproheptadine on clonidine-induced secretion of growth hormone. Dose of clonidine = 8 μg/kg body wt. Values are expressed as means ± SEM. First arrow (0740 hr) points to last sample before vehicle or cyproheptadine injection, and second arrow (0820 hr) points to last sample before vehicle or clonidine injection. Mean areas under growth hormone curves (ng · ml^{-1} · min, 0840 to 0940 hr) were 591, 1218, 363, 1087, and 1002 ± a pooled SEM of 177 (n = 7, 8, or 9) for vehicle-vehicle, vehicle–8 μg clonidine/kg body wt, 0.2 mg cyproheptadine/kg body wt–vehicle, 0.2 mg cyproheptadine/kg body wt–8 μg clonidine/kg body wt, and 1.0 mg cyproheptadine/kg body wt–8 μg clonidine/kg body wt treatments, respectively.

pendent of α_2-adrenergic receptors, and another that is dependent on α_2-adrenergic receptors. The hypothesis that 5-HT receptor-stimulated secretion of growth hormone is independent of α_2-adrenergic receptors is supported by our observation that simultaneous administration of quipazine and clonidine, at doses that individually maximized secretion of growth hormone, additively increased secretion of growth hormone. If α_2-adrenergic receptors mediate 5-HT receptor–stimulated secretion of growth hormone, then quipazine and clonidine should not have additively increased secretion of growth hormone. Further support for the concept that one mechanism of 5-HT–stimulated secretion of growth hormone is independent of α_2-adrenergic receptors is the fact that quipazine stimulates secretion of growth hormone in the face of either yohimbine or idazoxan, although the magnitude was smaller than that observed for quipazine alone.

On the other hand, evidence that 5-HT receptor–stimulated secretion of growth hormone is dependent on α_2-adrenergic receptors is provided by our observations that blockade of α_2-adrenergic receptors with either yohimbine or idazoxan partially suppresses quipazine-stimulated secretion of growth hormone. Decreased responsiveness to the 5-HT receptor agonist quipazine may be due to suppression of tonic α_2-adrenergic receptor–mediated basal secretion of growth hormone.

Our results also demonstrate that α_2-adrenergic receptor–stimulated secretion of growth hormone occurs independent of 5-HT receptors. Specifically, blockade of 5-HT receptors with cyproheptadine had no effect on clonidine-induced secretion of growth hormone. If α_2-adrenergic receptor–stimulated secretion of growth hormone was dependent on 5-HT receptors, then blockade of 5-HT receptors should have prevented α_2-adrenergic receptor–stimulated secretion of growth hormone. Our results are in contrast to results from experiments with rats, in which cyproheptadine, at lower doses than those used in the current experiment, blocked clonidine-induced secretion of growth hormone (9, 10).

One hypothesis, which integrates these results, is that GHRH mediates the stimulatory effects of α_2-adrenergic receptors on secretion of growth hormone, whereas GHRH and an additional growth hormone secretagogue, possibly thyrotropin-releasing hormone (TRH), mediate 5-HT receptor–stimulated secretion of growth hormone. Simmons *et al.* (16) showed that clonidine increased secretion of GHRH from perifused bovine hypothalamic slices, which supports the first portion of this hypothesis. The effects of stimulation of 5-HT receptors on secretion of GHRH have not been reported for cattle. However, Chen and Ramirez (17) showed that stimulation of 5-HT receptors increased secretion of TRH in rats, and others (18, 19) showed that TRH increased secretion of growth hormone in cattle.

In summary, current results suggest that mechanisms mediating monoaminergic regulation of secretion of growth hormone are located within the hypothalamus. Furthermore, two mechanisms may mediate

5-HT receptor-stimulated secretion of growth hormone in cattle: one that is independent of α_2-adrenergic receptors and another that is dependent on α_2-adrenergic receptors, possibly *via* regulation of basal secretion of growth hormone. Finally, α_2-adrenergic receptor–stimulated secretion of growth hormone occurs independently of 5-HT receptors in cattle.

The gifts of anti-serum to ovine growth hormone from the National Institute for Diabetes, Digestive, and Kidney Diseases in Rockville, MD; recombinant bovine growth hormone and growth hormone–releasing hormone from The Upjohn Company, Kalamazoo, MI; and bovine pituitary glands from Sheldon's Packing House, Ovid, MI, are greatly appreciated.

1. Müller EE. Neural control of somatotropic function. Physiol Rev **67**:962–1053, 1987.
2. Frohman LA, Downs TR, Chomczynski P. Regulation of growth hormone secretion. Front Neuroendocrinol **13**:344–405, 1992.
3. Kato Y, Matsushita N, Katakami H, Imura H. Brain serotonin and the secretion of prolactin and growth hormone. Clin Endocrinol (Tokyo) **28**:625–630, 1980.
4. Alba-Roth J, Losa M, Spiess Y, Schopohl J, Müller OA, Von Werder K. Interaction of clonidine and GHRH on GH secretion *in vivo* and *in vitro*. Clin Endocrinol **30**:485–491, 1989.
5. Soyoola ED, Burgess MF, Bird RC, Kemppainen RJ, Williams JC, Sartin JL. Neurotransmitter receptor agonists regulate growth hormone gene expression in culture ovine pituitary cells. Proc Soc Exp Biol Med **207**:26–33, 1994.
6. Sartin JL, Kemppainen RJ, Marple DN, Carnes R, Dieberg G, Oliver EH. Effects of parachlorophenylalanine, quipazine and cyproheptadine on growth hormone and adrenocorticotropin secretion in steers. Dom Anim Endocrinol **4**:33–41, 1987.
7. Gaynor PJ, Chapin LT, Lookingland KJ, Tucker HA. α_2-Adrenergic receptor–mediated regulation of growth hormone secretion of meal-fed Holstein steers. Proc Soc Exp Biol Med **204**:318–322, 1993.
8. Gaynor PJ, Lookingland KJ, Tucker HA. 5-Hydroxytryptaminergic receptor-stimulated growth hormone secretion occurs independently of changes in peripheral somatostatin concentration. Proc Soc Exp Biol Med **209**:79–85, 1995.
9. Conway S, Richardson L, Speciale S, Moherek R, Mauceri H, Krulich L. Interaction between norepinephrine and serotonin in the neuroendocrine control of growth hormone release in the rat. Endocrinology **126**:1022–1030, 1990.
10. Aulakh CS, Hill JL, Lesch KP, Murphy DL. Functional subsensitivity of 5-hydroxytryptamine$_{1C}$ or alpha$_2$adrenergic heteroreceptors mediating clonidine-induced growth hormone release in the Fawn-Hooded rat strain relative to the Wistar rat strain. J Pharm Exp Ther **262**:1038–1043, 1992.
11. Padmanabhan V, Enright WJ, Zinn SA, Convey EM, Tucker HA. Modulation of growth hormone-releasing factor-induced release of growth hormone from bovine pituitary cells. Dom Anim Endo **4**(4):243–252, 1987.
12. Hassan HA, Merkel RA. Perifusion model system to culture bovine hypothalamic slices in series with dispersed anterior pituitary cells. In Vitro Cell Dev Biol **30A**:435–442, 1994.
13. National Research Council. Nutrient Requirements of Dairy Cattle (6th rev ed). Washington, DC: National Academy Press, 1989.
14. SAS Institute. SAS User's Guide: Statistics, Version 5 Edition. Cary, NC: SAS Institute, Inc., 1985.
15. Sokal RR, Rohlf FJ. Biometry. San Francisco: WH Freeman and Company, 1981.
16. Simmons CR, Chapin LT, Lookingland KJ, Tucker HA. Alpha$_2$-adrenergic receptors stimulate secretion of growth hormone releasing hormone (GHRH) from perifused bovine hypothalamic slices. (abstract) J Anim Sci **73**(Suppl 1):216, 1995.
17. Chen YF, Ramirez VD. Serotonin stimulates thyrotropin-releasing hormone release from superfused rat hypothalami. Endocrinology **108**:2359–2366, 1981.
18. Bourne RA, Tucker HA, Convey EM. Serum growth hormone concentrations after growth hormone or thyrotropin releasing hormone in cows. J Dairy Sci **60**:1629–1635, 1977.
19. Kesner JS, Convey EM, Davis SL. Bovine serum hormone concentrations after thyroprotein and thyrotropin releasing hormone. J Anim Sci **44**(5):784–790, 1977.