

# $\alpha_2$ -Adrenergic Receptor-Mediated Regulation of Growth Hormone Secretion in Meal-Fed Holstein Steers (43669)

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**Abstract.** Effects of activation or blockade of  $\alpha_2$ -adrenergic receptors on serum growth hormone concentrations were studied in Holstein steers (115  $\pm$  4 days of age; 112  $\pm$  4 kg body wt). A pelleted diet was available *ad libitum* for 2 hr each day. Serum growth hormone concentrations were greater for 80 min immediately before feeding compared with 80 min immediately after removal of feed. Relative to saline-injected controls, activation of  $\alpha_2$ -adrenergic receptors with clonidine (2  $\mu$ g/kg body wt iv) before feeding rapidly increased serum growth hormone concentrations, but clonidine had no effect when administered after feeding. Compared with vehicle-injected controls, blockade of  $\alpha_2$ -adrenergic receptors with either idazoxan (20 mg/kg body wt sc) or yohimbine (5 mg/kg body wt sc) decreased serum growth hormone concentrations before as well as after feeding. Feeding abolished the stimulatory  $\alpha_2$ -adrenergic receptor-mediated increase in growth hormone secretion. Our data support the hypothesis that  $\alpha_2$ -adrenergic receptor stimulation is an essential event mediating pulsatility of growth hormone secretion before feeding and is required to maintain basal concentrations after feeding.

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Hypothalamic growth hormone-releasing hormone (GHRF) stimulates, and somatostatin (SRIF) inhibits, the secretion of growth hormone from the anterior pituitary gland (1, 2). Monoaminergic neurotransmitters, primarily norepinephrine, regulate the release of GHRF and SRIF into hypophysial portal blood (3, 4). In rats, inhibition of norepinephrine synthesis or blockade of  $\alpha_2$ -adrenergic receptors reduces concentrations of growth hormone in plasma and eliminates spontaneous pulses of growth hormone (3, 5, 6). Conversely, activation of central  $\alpha_2$ -adrenergic receptors stimulates secretion of growth hormone (6, 7).

Timing of pulses in concentration of growth hormone in serum of cattle with continuous access to feed occurs unpredictably (8). However, restricting the time that feed is available each day to 2 hr induces peaks in

serum growth hormone concentration during the 1–2 hr before feeding. Concentration of growth hormone in serum declines throughout feeding and remains low for 1 to 2 hr after eating (9, 10). Thus, the meal-fed steer offers a unique opportunity to study neuroendocrine regulation of growth hormone secretion under markedly different physiological conditions within the same animal in a relatively short time frame. We hypothesize that meal-feeding-induced changes in the secretory pattern of growth hormone in steers may represent a coordination of hypothalamic GHRF and SRIF secretion, possibly mediated by central noradrenergic neurons.

Our objective was to determine whether activation or blockade of  $\alpha_2$ -adrenergic receptors affected serum growth hormone concentrations before and after meal feeding. The results suggest that feeding inhibits spontaneous and  $\alpha_2$ -adrenergic receptor-mediated activation of growth hormone secretion.

## Materials and Methods

**General.** Holstein bull calves were castrated at 6 weeks of age. Steers were housed in individual stalls in environmentally controlled rooms (four steers/room). Temperature of animal rooms was 20  $\pm$  1°C. Lights came on at 0300 hr and went off at 2100 hr.

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Animals were fed a pelleted diet (18% crude protein and 19.6% acid detergent fiber; Countrymark Cooperative, Indianapolis, IN) balanced to meet nutrient requirements (11). Body weight gains were in excess of 1 kg/day. Bloat Guard (5–10 g; SmithKline Beecham Animal Health, Westchester, PA) was added to feed each day to prevent bloating. To adapt the animals to meal feeding, the time allowed for eating was reduced gradually over 14 days. By Day 14, feed was available only between 1000 and 1200 hr. The 2-hr meal-feeding pattern was continued for a minimum of 10 days before an experiment began. Water was available at all times.

Indwelling jugular catheters were inserted aseptically 15–20 hr before blood collection (12). Blood samples (6 ml) were collected at 20-min intervals within each experiment. After collection of each blood sample, 3–5 ml of 3.5% sodium citrate was injected into the jugular catheter to maintain patency. Blood samples were allowed to clot at room temperature and were refrigerated overnight. Whole blood was centrifuged at 1500g for 25 min. Serum was harvested and frozen at  $-20^{\circ}\text{C}$  preceding growth hormone analysis (13). Intra- and interassay coefficients of variation were 9.3% and 16.8%, respectively. Body weights of steers were recorded 1 day before each experimental day. Drugs to be injected were solubilized in sterile vehicle within 1 hr of use.

**Concentrations of Growth Hormone in Serum before and after Feeding.** Blood samples were collected between 0700 and 1500 hr from eight steers ( $97 \pm 3$  [mean  $\pm$  SE] days of age;  $93 \pm 4$  kg body wt) on 2 days with 1 day of rest between sample days.

**Effect of Activation of  $\alpha_2$ -Adrenergic Receptors (Clonidine) on Serum Growth Hormone Concentrations before and after Feeding.** Twelve meal-fed steers ( $106 \pm 2$  days of age;  $99 \pm 2$  kg body wt) were used in a  $4 \times 4$  Latin square design of steer and day of experiment. Clonidine (Sigma Chemical Co., St. Louis, MO) was diluted in 0.9% saline (50  $\mu\text{g}/\text{ml}$ ) before intravenous injection (2  $\mu\text{g}/\text{kg}$  body wt). Treatments within square were  $2 \times 2$  factorial combinations of time relative to feeding (before or after) and drug treatment (saline or clonidine). Thus, three steers received saline, and three received clonidine at 0810 hr each day of the experiment. Blood samples were collected between 0700 and 1000 hr. Of the remaining six steers, three received saline and three received clonidine at 1310 hr, and blood samples were collected between 1200 and 1500 hr. Sampling days were separated by 2 days of rest.

The 2- $\mu\text{g}/\text{kg}$  body wt dose of clonidine was based on a preliminary study (data not shown) in which 0.4, 2, and 10  $\mu\text{g}$  clonidine/kg body wt induced a dose-responsive increase in the concentration of growth hormone in serum of calves and increased the duration of side effects (labored breathing, inability to stand). Com-

pared with vehicle-injected controls, the 2- $\mu\text{g}/\text{kg}$  body wt dose induced a significant increase in the concentration of growth hormone in serum with a minimum duration of side effects (approximately 1 hr).

**Effect of Blockade of  $\alpha_2$ -Adrenergic Receptors (Idazoxan and Yohimbine) on Serum Growth Hormone Concentrations before Feeding.** Sixteen steers ( $98 \pm 4$  days of age;  $104 \pm 4$  kg body wt) were used in a completely randomized design. The specific  $\alpha_2$ -adrenergic receptor antagonist idazoxan (Sigma) was diluted in water (150 mg/ml). Eight steers received water and eight received idazoxan (20 mg/kg body wt sc) at 0730 hr. Blood samples were collected between 0700 and 1500 hr. The dose of idazoxan was identical to that used in rats (14).

The objective of an additional experiment was to determine whether yohimbine (Sigma), a less costly, more readily available, albeit less specific  $\alpha_2$ -adrenergic receptor antagonist, could be substituted for idazoxan in future experiments. Sixteen steers ( $136 \pm 7$  days of age;  $130 \pm 5$  kg body wt) were used in a single reversal experimental design. Yohimbine was diluted in water (15 mg/ml) before subcutaneous injection (5 mg/kg body wt) at 0730 hr. Treatments were water and yohimbine. Blood samples were collected between 0640 and 1200 hr. One day of rest separated sampling days.

In a preliminary study, doses of 2.5, 5, and 10 mg yohimbine/kg body wt were tested for suppression of concentrations of growth hormone in serum of calves. The 5-mg/kg body wt dose was chosen because it suppressed growth hormone concentrations in serum with a minimum duration of side effects (labored breathing, inability to stand) (approximately 1 hr).

**Effect of Blockade of  $\alpha_2$ -Adrenergic Receptors (Yohimbine) on Serum Growth Hormone Concentrations after Feeding.** Sixteen steers ( $139 \pm 4$  days of age;  $132 \pm 5$  kg body wt) received water or yohimbine (5 mg/kg body wt sc) at 1210 hr in a single reversal experimental design. Blood samples were collected between 0700 and 1500 hr. Two days of rest separated sampling days.

**Statistical Evaluation.** Areas under serum growth hormone response curves were calculated using the trapezoidal rule. Unless otherwise specified, areas before feeding were calculated between the first sample taken after drug or vehicle injection and 1000 hr. Similarly, areas after feeding were calculated between the first sample taken after drug or vehicle injection and 1500 hr. Because clonidine appeared to exert immediate and longer-term effects on concentrations of growth hormone in serum before and after feeding, response areas for this experiment were arbitrarily divided into two equal components before feeding (0820–0900 hr and 0920–1000 hr) and two equal components after feeding (1320–1400 hr and 1420–1500 hr).

Analysis of variance was performed for each exper-

iment. Main effects in statistical models were treatment, steer, and day as appropriate for each experiment. Treatment means were compared with F tests. Probabilities  $\leq 0.05$  were considered statistically different.

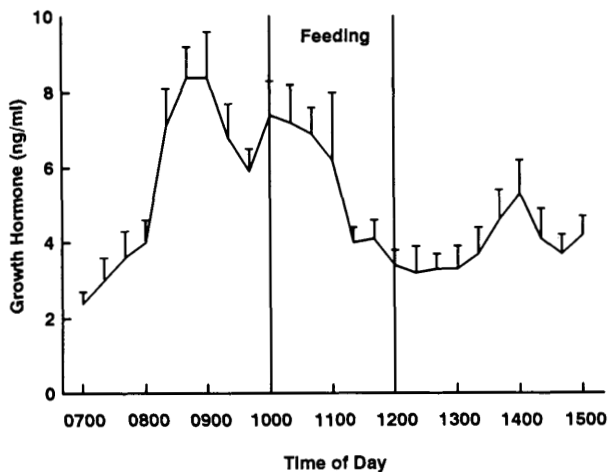
## Results

**Baseline Serum Growth Hormone Concentrations for Meal-Fed Steers.** Serum growth hormone concentrations were greater before feeding (0820–0940 hr) compared with after feeding (1220–1340 hr) (Fig. 1).

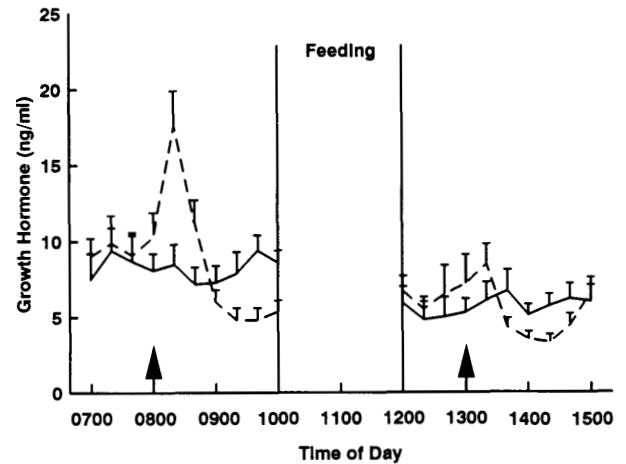
**Serum Growth Hormone Concentrations after Activation of  $\alpha_2$ -Adrenergic Receptors before and after Feeding.** Relative to saline-injected controls, clonidine given before feeding (0810 hr) increased the area under the growth hormone response curve between 0820 and 0900 hr, whereas clonidine injection after feeding (1310 hr) did not affect the area under the growth hormone response curve between 1320 and 1400 hr (Fig. 2).

In addition to the immediate effect, clonidine also had a delayed inhibitory effect on serum growth hormone concentrations before and after feeding. Specifically, the area under the clonidine-induced growth hormone response curves eventually decreased below that of saline-injected steers between 0920 and 1000 hr and between 1420 and 1500 hr. The decreases were similar between 0920 and 1000 and 1420 and 1500 hr.

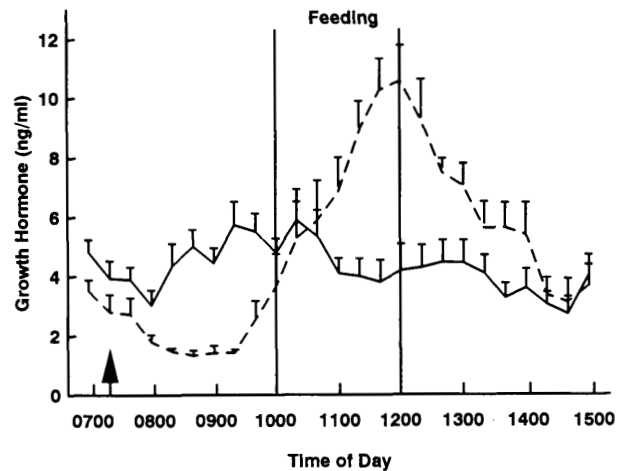
**Serum Growth Hormone Concentrations after Blockade of  $\alpha_2$ -Adrenergic Receptors before and after Feeding.** Compared with controls, the specific  $\alpha_2$ -adrenergic receptor antagonist idazoxan suppressed the area under the growth hormone response curve before feeding (Fig. 3). Following a nadir at 0920 hr, serum growth hormone concentration steadily increased to a peak at 1200 hr in idazoxan-treated steers.



**Figure 1.** Serum growth hormone concentrations (mean  $\pm$  SE) in meal-fed Holstein steers ( $n = 16$  observations, eight steers on 2 sampling days/point).



**Figure 2.** Serum growth hormone concentrations (mean  $\pm$  SE) in meal-fed Holstein steers injected with sterile saline (solid line) or the  $\alpha_2$ -adrenergic receptor agonist clonidine (dashed line) ( $2 \mu\text{g}/\text{kg}$  body wt iv) at 0810 and 1310 hr ( $n = 12$  observations, 12 steers on 4 sampling days/point). Arrows point to last sample before injections.



**Figure 3.** Serum growth hormone concentrations (mean  $\pm$  SE) in meal-fed Holstein steers injected with sterile water (solid line) or the  $\alpha_2$ -adrenergic receptor antagonist idazoxan (dashed line) ( $20 \text{ mg}/\text{kg}$  body wt sc) at 0730 hr ( $n =$  eight observations, eight steers on 1 sampling day/point). Arrow points to last sample before injection.

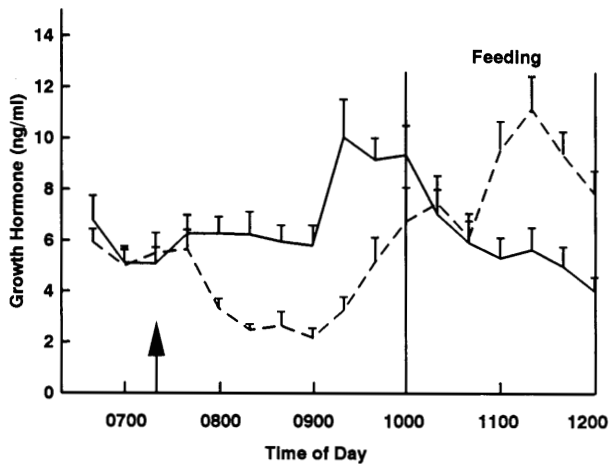
Growth hormone concentrations peaked at 0920 hr for water-injected controls (Fig. 3).

Compared with controls, injection of yohimbine suppressed the area under the growth hormone response curve before feeding (Fig. 4). Concentrations of growth hormone in serum reached a nadir at 0900 hr and then increased to a peak at 1120 hr. In contrast, peak serum growth hormone concentrations for water-injected controls occurred at 0920 hr.

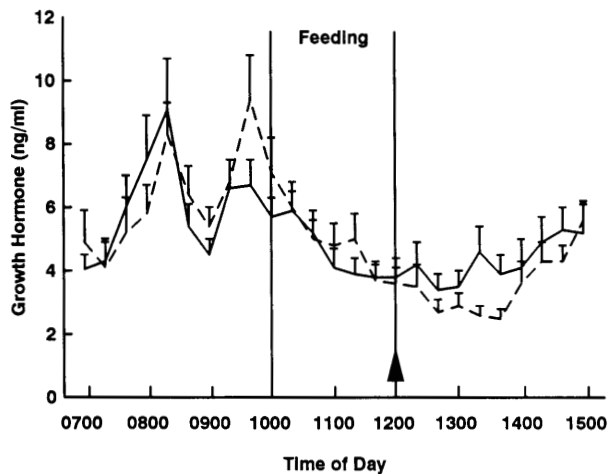
Compared with controls, inhibition of  $\alpha_2$ -adrenergic receptors with yohimbine after feeding reduced the serum growth hormone response curve area between 1220 and 1500 hr (Fig. 5).

## Discussion

Growth hormone pulses are the consequence of increased release of GHRF occurring together with



**Figure 4.** Serum growth hormone concentrations (mean  $\pm$  SE) in meal-fed Holstein steers injected with sterile water (solid line) or the  $\alpha_2$ -adrenergic receptor antagonist yohimbine (dashed line) (5 mg/kg body wt sc) at 0730 hr ( $n = 16$  observations, eight steers on 2 sampling days/point). Arrow points to last sample before injection.



**Figure 5.** Serum growth hormone concentrations (mean  $\pm$  SE) in meal-fed Holstein steers injected with sterile water (solid line) or the  $\alpha_2$ -adrenergic receptor antagonist yohimbine (dashed line) (5 mg/kg body wt sc) at 1210 hr ( $n = 16$  observations, eight steers on 2 sampling days/point). Arrow points to last sample before injection.

decreased release of SRIF, while troughs in concentration of growth hormone in serum occur when SRIF release is elevated and GHRF release is minimal (15, 16). Our data support the theory that meal feeding induces an organization of endogenous GHRF and SRIF secretion patterns which are manifested as spontaneous peaks (before feeding) and as a trough (immediately after feeding) in serum growth hormone concentrations (9, 10). It is unlikely that time of day, instead of time of feeding, accounts for these results because pulses of growth hormone in cattle with free access to feed occur randomly (8). Furthermore, the growth hormone response to meal feeding at different times of day among studies is virtually identical (9, 10, and present study).

In the rat, stimulation of  $\alpha_2$ -adrenergic neurons induces growth hormone secretory pulses (4, 17). In the present study utilizing steers, stimulation of  $\alpha_2$ -adrenergic receptors with clonidine before feeding rapidly increased serum growth hormone concentrations. Increased GHRF release or decreased SRIF release could have mediated clonidine-induced increases in serum growth hormone concentrations before feeding. Passive immunization of rats with GHRF antibodies suppresses the stimulatory effects of clonidine on growth hormone release, thereby supporting the hypothesis that stimulation of  $\alpha_2$ -adrenergic receptors increases GHRF release (18).

Clonidine did not stimulate growth hormone release after feeding. The possibility that  $\alpha_2$ -adrenergic receptor stimulation with clonidine increased SRIF release after feeding is unlikely since this clearly was not the case before feeding. Alternatively,  $\alpha_2$ -adrenergic receptor stimulation may have increased GHRF release after feeding, but somatotropes may have been nonresponsive. Moseley *et al.* (9) reported a 40-fold greater response of growth hormone concentrations in serum when a GHRF challenge was given before feeding compared with after feeding. However, in sham-fed steers, GHRF induced similar, large increases in concentrations of growth hormone in serum before and after sham feeding (9).

While the effects of feeding on hypothalamic SRIF release are unstudied, peripheral SRIF concentrations increased within 5 min and remained elevated for 1 hr after feeding in lactating cows (19). The growth hormone-releasing effects of all growth hormone secretagogues, including GHRF, are blocked by SRIF-mediated inhibition of adenylate cyclase activation and reduction of intracellular  $Ca^{2+}$  through G-protein-linked receptor mechanisms (20). Thus, if feeding increased SRIF concentrations in the present study, then it is unlikely that a clonidine-induced increase in GHRF release could increase growth hormone release from somatotropes. An additional unstudied possibility is that SRIF inhibited  $\alpha_2$ -adrenergic-stimulated GHRF release.

A delayed decrease of similar magnitude before as well as after feeding followed the immediate response to clonidine injections. This delayed decrease in serum growth hormone concentrations probably was not due to depletion of pituitary stores of growth hormone, since clonidine did not induce a pulse in serum growth hormone concentrations after feeding. Alternatively, clonidine-induced hypothalamic GHRF release may in turn have triggered increased SRIF release via an ultra-short feedback loop (4). In support of this hypothesis are data showing that GHRF augments the release of SRIF from isolated median eminence (3), and that intracerebroventricular administration of GHRF increases SRIF release (21, 22).

Inhibition of  $\alpha_2$ -adrenergic receptors with idazoxan or yohimbine decreased growth hormone concentrations in serum regardless of time administered relative to meal feeding. Injection of antibodies to SRIF did not reverse the inhibitory effects of yohimbine on serum growth hormone in rats (23), suggesting that reduced GHRF release, and not increased SRIF secretion, mediates inhibitory  $\alpha_2$ -adrenergic receptor effects on serum growth hormone concentrations.  $\alpha_2$ -Adrenergic antagonists block postsynaptic  $\alpha_2$ -adrenergic receptors and increase release of norepinephrine from presynaptic terminals (24). The eventual increase in serum growth hormone concentrations following idazoxan and yohimbine injections may represent a diminution of postsynaptic blockade, thereby restoring stimulatory postsynaptic adrenergic-mediated GHRF release. These data, coupled with our finding that yohimbine inhibits release of growth hormone before and after feeding, support the hypothesis that  $\alpha_2$ -adrenergic neurons tonically stimulate GHRF secretion.

In conclusion, the stimulatory  $\alpha_2$ -adrenergic increase in growth hormone secretion before feeding was abolished after feeding. Collectively, these data support the hypothesis that  $\alpha_2$ -adrenergic receptor stimulation is an essential event mediating pulsatility of growth hormone secretion before feeding and is required to maintain basal concentrations after feeding.

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