5-Hydroxytryptaminergic Receptor-Stimulated Growth Hormone Secretion Occurs Independently of Changes in Peripheral Somatostatin Concentration (43881)

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

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

Abstract. **Effects of activation or blockade of 5-hydroxytryptaminergic receptors on concentrations of growth hormone and somatostatin in serum were studied in Holstein steers (mean ± SEM: 159 ± 8 days of age; 160 ± 9 kg of body wt). A pelleted diet was available *ad libitum* between 1000 and 1200 hr each day. Blood was sampled from a cannula in a jugular vein. Peak concentration of growth hormone in serum within 1 hr before feeding (12.8 ± 3.8 ng/ml) was greater than peak concentration within 1 hr after removal of feed (3.3 ± 1.0 ng/ml). In contrast, concentration of somatostatin in plasma did not change from 1 hr before feeding through 1 hr after removal of feed (47.3 to 43.0 ± 4.5 pg/ml). Relative to saline-injected controls, activation of 5-hydroxytryptaminergic receptors with quipazine (0.05, 0.1, and 0.2 mg/kg body wt, iv) increased the area under growth hormone response curves 5.5- to 25-fold before and after feeding. In another experiment, injection of 0.1 mg of quipazine/kg body wt at 1300 hr increased the concentration of growth hormone in serum 7.8-fold compared with controls, but had no effect on concentration of somatostatin in plasma. Relative to water-injected controls, blockade of 5-hydroxytryptaminergic receptors with cyproheptadine (0.2 mg/kg body wt, sc) decreased the area under growth hormone response curves for at least 110 min before feeding (71%) and after removal of feed (69%).**

The data support the hypothesis that 5-hydroxytryptaminergic receptors are involved in stimulation of pulsatile growth hormone secretion in meal-fed cattle. Lack of a change in concentration of somatostatin in plasma with respect to time of meal-feeding or after injection of the 5-hydroxytryptaminergic-receptor agonist quipazine suggests that 5-hydroxytryptaminergic receptor-stimulated growth hormone secretion is likely mediated within the central nervous system, rather than by meal-induced changes in peripheral secretion of somatostatin. [P.S.E.B.M. 1995, Vol 209]

Neuropeptides and monoamine neurotransmitters regulate secretion of growth hormone from somatotropes in the anterior pituitary gland (1, 2). Data showing that intravenous administration of anti-growth hormone-releasing hormone (GHRH) serum blocks pulsatile secretion of growth hormone provide direct support for the hypothesis that pulses in GHRH cause pulses in growth hormone secretion (3–4). In addition, Frohman *et al.* (5) reported that approximately 70% of pulses in growth hormone

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

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secretion occurred coincident with, or immediately after, pulses in GHRH release in sheep. Although troughs in secretion of growth hormone occurred coincident with pulses in somatostatin (SRIF) in hypophysial-portal blood of rats (6), Frohman *et al.* (5) reported that increased SRIF release into hypophysial-portal blood did not correlate with troughs in concentration of growth hormone, nor did troughs in SRIF release correlate with pulses in growth hormone release in sheep.

In addition to secretion from the hypothalamus, SRIF is secreted from D cells in the pancreas and from cells in the stomach and small intestine (7, 8). Thus, the possibility exists the SRIF of peripheral origin may affect secretion of growth hormone. In cattle, the concentration of growth hormone in serum is higher and more pulsatile before meal-feeding, declines throughout feeding, and remains low for 1–2 hr after eating (9–11). We speculated that higher concentrations of growth hormone in serum before meal-feeding resulted from increased GHRH release coincident with low SRIF tone while the opposite was true after feeding (11).

Serotonergic receptors in the hypothalamus may affect secretion of growth hormone by altering the synthesis or release of either GHRH or SRIF (12–14). In rats, stimulation of 5-hydroxytryptaminergic (5-HT) receptors is associated with increased GHRH release and increased growth hormone release (12–14). In steers, Sartin *et al.* (15) showed that stimulation of 5-HT receptors with quipazine increased release of growth hormone, whereas the 5-HT-receptor antagonist cyproheptadine suppressed release of growth hormone.

Our objective was to determine if activation or blockade of 5-HT receptors affected concentrations of growth hormone or SRIF in serum from a jugular vein before and after meal-feeding in cattle. Data presented support the hypothesis that 5-HT receptors are involved in stimulation of pulsatile growth hormone secretion in meal-fed cattle without affecting concentration of SRIF in peripheral plasma.

Materials and Methods

General. Holstein steer calves were housed in rooms maintained at 20° ± 1°C (four steers per room). Lights came on at 0300 hr and went off at 2100 hr.

Steers were fed a pelleted diet (18% crude protein, 19.6% acid detergent fiber; Countrymark Cooperative, Indianapolis, IN) balanced to meet nutrient requirements (16). 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. Body weight gains averaged 1.03 kg per day.

A catheter was inserted into a jugular vein of each calf at least one day before each experiment. A solution of 3.5% sodium citrate in sterile water was used as an anticoagulant in catheters. Blood samples (6 ml unless otherwise specified) were collected at 5-, 10-, or 20-min intervals. Samples were allowed to clot at room temperature and then were stored at 4°C. The following day, blood was centrifuged at 1500*g* for 25 min. Serum was transferred to polypropylene tubes and frozen at −20°C. Concentration of growth hormone in serum was determined with a radioimmunoassay using rabbit anti-ovine growth hormone primary antibody obtained from the NIDDK in Rockville, MD (Appendix A). Intra- and interassay coefficients of variation were 10% and 12%, respectively.

A commercially available radioimmunoassay kit validated for extraction (2 ml plasma) and determination of SRIF concentration in human plasma was used (Peninsula Laboratories, Belmont, CA). Immunoreactive SRIF appears to be indistinguishable among vertebrates (17). The intraassay coefficient of variation was 9.9%. Recovery of SRIF added to a plasma sample before extraction was 97%.

Concentrations of Growth Hormone and SRIF Before and After Feeding. Blood samples were collected from six meal-fed steers (mean ± SEM: 123 ± 6 days of age; 118 ± 8 kg body wt) on a single sampling day. Ten milliliters of blood were withdrawn into chilled syringes between 0800 and 1300 hr. Four of the 10 ml of blood were placed in glass tubes at room temperature, allowed to clot, and serum was assayed for concentration of growth hormone. The remaining 6 ml were transferred to chilled glass tubes containing 6 mg of ethylenedinitrilio tetraacetic acid (J. T. Baker Corp., Phillipsburg, NJ) and 3000 kallikrein inactivator units of aprotinin (A6279, Sigma Chemical Co., St. Louis, MO). Tubes were stoppered, inverted three times, and placed on ice. Within 2 hr of collection, blood samples collected for plasma were centrifuged at 1600*g* for 15 min at 4°C. Plasma was transferred to polypropylene tubes and stored at −80°C before determination of SRIF concentration.

Effect of Quipazine (5-HT Agonist) on Concentrations of Growth Hormone in Serum Before and After Feeding and on SRIF After Feeding. Seven steers (134 ± 8 days of age; 161 ± 10 kg body wt) were assigned to one of four treatments on each of four sampling days in one complete- and one incomplete-Latin square design. Quipazine dimaleate (Research Biochemicals Inc., Natick, MA) was diluted in 0.9% sterile saline (10 mg/ml) before iv injection. A dose of 0.2 mg/kg body wt was used because increases in concentrations of growth hormone in a preliminary experiment were similar with doses of 0.1, 0.2, and 0.4 mg of quipazine/kg body wt compared with vehicle-injected controls (data not shown). Treatments were factorial combinations of time of injection relative to time of

feeding (before or after) and substance injected (saline or quipazine). Thus, blood samples were collected from three or four steers between 0700 and 1000 hr, and from remaining steers between 1200 and 1500 hr. Treatments were injected immediately after the 0800 or the 1300 hr blood sample was collected. One day separated sampling days.

In the experiment described above, we observed a sharp increase in concentration of growth hormone at 1310 hr in response to quipazine. However, a coincidental increase in growth hormone pulsatility occurred for controls. Thus, the objective of a second experiment was to determine effects of stimulation of 5-HT receptors with quipazine at lower doses and at an earlier time relative to the end of feeding on concentrations of growth hormone in serum. Accordingly, 11 steers (179 ± 12 days of age; 188 ± 10 kg body wt) were randomly assigned to one of six treatments on four sampling days. Treatments were vehicle-injected controls, 0.05, and 0.1 mg of quipazine/kg body wt. Blood samples were collected between 0700 and 1000 hr and between 1100 and 1400 hr. Saline or quipazine was injected immediately after the 0800 or the 1200 hr blood sample was collected.

The objective of a third experiment was to determine if quipazine affected concentrations of SRIF in plasma. Six steers (183 ± 9 days of age; 206 ± 10 kg body wt) were used in a single-reversal experimental design with two treatments and two sampling days. Ten milliliters of blood were withdrawn into chilled syringes at 20-min intervals between 1200 and 1400 hr and dispensed into separate tubes for growth hormone and SRIF analyses as described previously. Sterile saline or 0.1 mg of quipazine/kg body wt was injected iv immediately after the 1300 hr blood sample was collected. Concentration of SRIF was determined in two blood samples for each steer on each sampling day: the sample collected immediately before injection of sterile saline or 0.1 mg of quipazine/kg body wt at 1300 hr and the sample collected at 1320 hr.

Effect of Cyproheptadine (5-HT Antagonist) on Concentrations of Growth Hormone in Serum Before and After Feeding. Eight steers (178 ± 11 days of age; 190 ± 10 kg body wt) were arranged in two replicates of a 4 × 4 Latin square design. Treatments within square were 2 × 2 factorial combinations of injection time (before or after feeding) and substance injected (water or cyproheptadine). Blood samples were collected between 0700 and 1000 hr and between 1100 and 1400 hr. On each sampling day, two steers received injections of water, and two received injections of cyproheptadine immediately after the 0800 hr blood sample was collected. Of the remaining four steers, two received injections of water and two received injections of cyproheptadine immediately after the 1200 hr blood sample was collected. Cyproheptadine (Sigma Chemical Co., St. Louis, MO) was diluted in sterile water (5 mg/ml) before sc injection (0.2 mg/kg body wt). The 0.2 mg of cyproheptadine/kg body wt dose was used because 0.025, 0.05, and 0.5 mg of cyproheptadine/kg body wt equally suppressed concentrations of growth hormone in a preliminary dose-response experiment in serum before feeding (data not shown).

Statistical Evaluation. Areas under growth hormone response curves were calculated using the trapezoidal rule. For the first experiment with quipazine, response area before feeding was calculated using concentrations of growth hormone in serum between 0810 and 0920 hr and response area after feeding was calculated using concentrations of growth hormone in serum between 1310 and 1420 hr. For the second experiment with quipazine, response area after feeding was calculated using concentrations of growth hormone in serum between 1210 and 1300 hr. For the experiment with cyproheptadine, response area before feeding was calculated using concentrations of growth hormone in serum between 0820 and 1000 hr and after feeding using values between 1220 and 1400 hr. Analysis of variance was performed for each experiment using Statistical Analysis System software (18). Main effect terms in statistical models were treatment, steer, and day as appropriate (19). Treatment means for areas under curves and single point estimates of growth hormone and SRIF responses were compared with F tests. Probability values ≤ 0.05 were considered significant.

Results

Concentration of Growth Hormone and SRIF Before and After Feeding. Concentration of growth hormone averaged 4 ng/ml between 0800 and 0900 hr, increased to 12 to 15 ng/ml between 0940 and 1020 hr, and decreased rapidly throughout the time that feed was available (Fig. 1A). Concentration of growth hormone averaged 2.8 ng/ml between 1200 and 1300 hr. Concentration of SRIF averaged 42 pg/ml of plasma between 0900 and 1300 hr, and concentrations were similar among time points (Fig. 1B).

Effect of Quipazine on Concentration of Growth Hormone in Serum Before and After Feeding and on Concentration of SRIF After Feeding. Relative to saline-injected controls, 0.2 mg of quipazine/kg body wt increased area under growth hormone response curves before (0810 to 0920 hr) and after (1310 to 1420 hr) feeding (Fig. 2). Similarly, 0.05 and 0.1 mg of quipazine/kg body wt increased area under growth hormone response curves compared with controls (Fig. 3). Within each dose, the increase in area under the response curve in comparison with controls was similar before and after feeding.

Relative to saline-injected controls, concentration

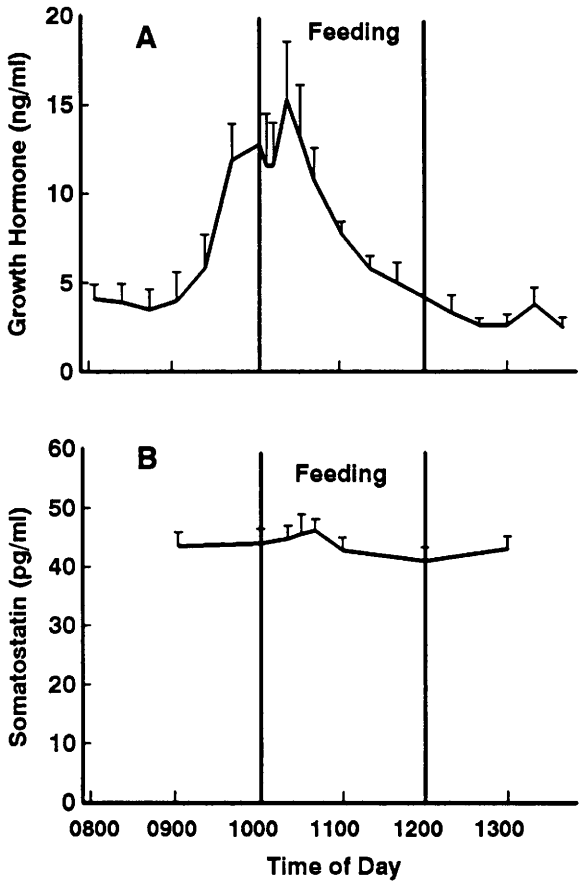

Figure 1. Concentrations of growth hormone (A) and SRIF (B) in peripheral blood before and after meal-feeding.

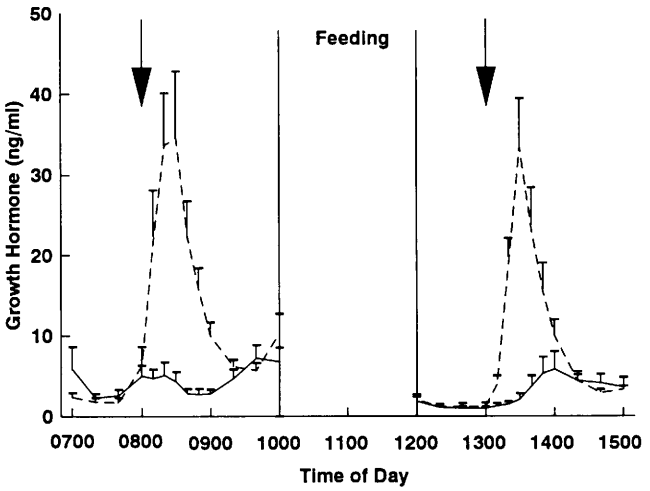

Figure 2. Concentrations of growth hormone in serum for saline-injected control (——) and 0.2 mg of quipazine (– – – –)/kg body wt treatments before and after meal-feeding. Arrows point to last sample before injections (0800 and 1300 hr). Areas under growth hormone response curves (ng/ml · min) before (0810 to 0920 hr) and after (1310 to 1420 hr) feeding averaged 262 and 263, respectively for saline-injected controls; and 1378 and 1118, respectively, for 0.2 mg quipazine/kg body wt treatment (n = 7, SE = 162).

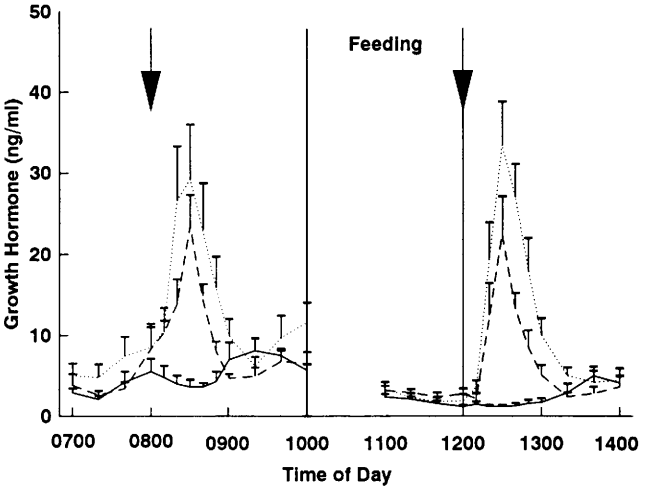

Figure 3. Concentrations of growth hormone in serum for saline-injected control (——), 0.05 (– – – –), and 0.1 mg (. . . . . .) of quipazine/kg body wt treatments before and after meal-feeding. Arrows point to last sample before injections (0800 and 1200 hr). Areas under growth hormone response curves (ng/ml · min) before (0810 to 0900 hr) and after (1210 to 1300 hr) feeding averaged 197 and 39, respectively for saline-injected controls; 682 and 639, respectively, for 0.05 mg quipazine/kg body wt treatment; and 902 and 980, respectively, for 0.1 mg quipazine/kg body wt treatment (n = 7, SE = 116).

of growth hormone in serum increased 7.8-fold within 20 min after injection of 0.2 mg of quipazine/kg body wt (Table I). In contrast, concentration of SRIF in plasma did not change significantly after injection of either quipazine or sterile saline.

Effect of Cyproheptadine on Concentration of Growth Hormone in Serum Before and After Feeding. Compared with controls, injection of cyproheptadine reduced area under growth hormone response curves before and after feeding (Fig. 4). The decrease was similar before and after feeding.

Discussion

Increased concentration of growth hormone in serum following stimulation of 5-HT receptors could re-

Table I. Concentrations of Growth Hormone and SRIF in Blood from a Jugular Vein Following iv Injection of Saline or Quipazine After Feeding[a]

Parameter	Treatment	
	Control	Quipazine[b]
n	6	6
Growth hormone (ng/ml)		
Before injection (1300 hr)	1.6 ± 0.5	1.0 ± 0.3
After injection (1320 hr)	2.0 ± 1.1	17.5 ± 3.6[c]
Somatostatin (pg/ml)		
Before injection (1300 hr)	20.5 ± 3.2	30.2 ± 5.1
After injection (1320 hr)	24.8 ± 3.8	34.1 ± 3.1

[a] Values are means ± SEM.
[b] Dose was 0.1 mg of quipazine/kg body wt.
[c] $P < 0.0001$ for concentration of growth hormone before versus after injection of quipazine.

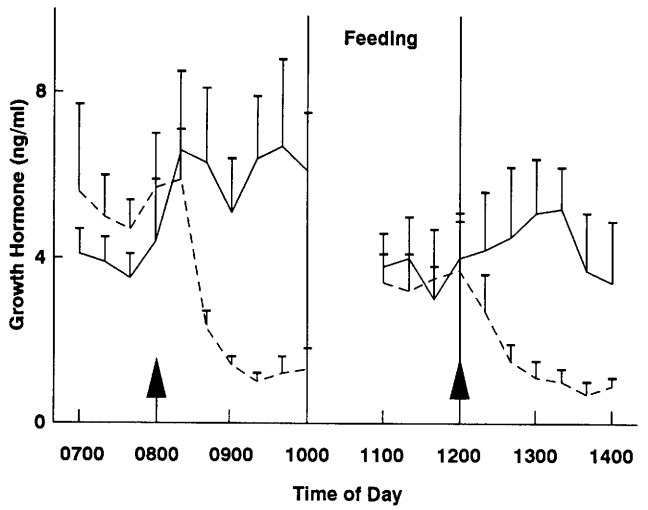

Figure 4. Concentrations of growth hormone in serum for water-injected control (——) and 0.2 mg of cyproheptadine/kg body wt (– – – –) treatments before and after meal-feeding. Arrows point to last sample before injections (0800 and 1200 hr). Areas under growth hormone response curves (ng/ml · min) before (0820 to 1000 hr) and after (1220 to 1400 hr) feeding averaged 660 and 404, respectively, for water-injected controls; and 190 and 124, respectively, for 0.2 mg cyproheptadine/kg body wt treatment (n = 8, SE = 82).

sult from increased GHRH release, decreased SRIF release, or a combination of both. In rats, passive immunization with anti-GHRH serum blocked 5-HT-receptor-mediated increases in growth hormone release (4, 12). Conversely, stimulation of 5-HT receptors has been reported to inhibit (20), to have no effect (21), and to increase (22) SRIF release. Thus, in rats, increased GHRH release probably mediates increased release of growth hormone following stimulation of 5-HT receptors.

In contrast, experiments with cattle suggest that SRIF of peripheral origin may be associated with release of growth hormone. For example, intracarotid infusion of 500 μg of SRIF over 30 min into Holstein bulls (232 kg body wt) decreased concentration of growth hormone in serum (23). In addition, Svennersten *et al.* (7) reported that concentration of SRIF in serum from a jugular vein tended to be increased at 20 min after meal-feeding in lactating dairy cows. However, data from the current experiments do not support the notion that SRIF of peripheral origin affects release of growth hormone. For example, concentration of SRIF in plasma from a jugular vein was similar at all times relative to time of meal-feeding while concentrations of growth hormone varied between 4 and 15 ng/ml. Further, despite a 7.8-fold increase in concentration of growth hormone in serum within 20 min after quipazine injection, concentration of SRIF in plasma from a jugular vein was unchanged. Thus, increased release of growth hormone before feeding or following stimulation of 5-HT receptors with quipazine was not due to decreased concentration of SRIF in peripheral blood.

The interrelationship between α_2-adrenergic and 5-HT pathways in regulation of growth hormone secretion has been studied in rats (14, 24). Conway *et al.* (14) utilized 5-HT antagonists, α_2-adrenergic agonists, and neurotoxin-induced lesions of the arcuate nucleus to establish that α_2-adrenergic receptor-mediated increases in growth hormone secretion required intact 5-HT terminals in the arcuate nucleus. We previously demonstrated that stimulation of α_2-adrenergic receptors with clonidine increased growth hormone secretion before but not after feeding (11). In the present experiments, stimulation of 5-HT receptors increased growth hormone release both before and after meal-feeding. Thus, if the relationship between α_2-adrenergic and 5-HT receptors in cattle is similar to that observed for rats, the previously observed (11) inability of clonidine to stimulate growth hormone secretion after meal-feeding may have resulted from reduced activity of 5-HT neurons.

Arnold and Fernstrom (22) reported that administration of anti-SRIF serum into a jugular vein of rats blocked 5-HT antagonist-induced decreases in growth hormone release. Thus, when cyproheptadine was injected, increased SRIF release may have mediated suppression of pulsatile growth hormone secretion before feeding and basal growth hormone secretion after feeding.

In conclusion, stimulation of 5-HT receptors with quipazine increased concentrations of growth hormone in serum at doses of 0.05, 0.1, and 0.2 mg/kg body wt. Conversely, blockade of 5-HT receptors with 0.2 mg of cyproheptadine/kg body wt decreased concentrations of growth hormone in serum. These data support the hypothesis that 5-hydroxytryptaminergic receptors are involved in stimulation of pulsatile growth hormone secretion in meal-fed cattle. Lack of a change in concentration of SRIF in plasma with respect to time of meal-feeding or after injection of the 5-HT receptor agonist quipazine suggests that 5-HT receptor-stimulated growth hormone secretion is likely mediated within the central nervous system, rather than by meal-induced changes in peripheral secretion of SRIF.

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Appendix A

Growth Hormone Assay. Recombinantly derived bovine growth hormone (U-72104; The Upjohn Co., Kalamazoo, MI) was used as radiolabeled antigen and as standard. The chloramine-T method was used to iodinate bovine growth hormone. Sequential additions to a vial containing 5 μg of growth hormone and 20 μl of distilled-deionized water (pH 10.0) were 25 μl of 0.5 *M* phosphate buffer (pH 7.5), 10 μl of $Na^{125}I$ (1 mCi; Amersham, Arlington Heights, IL), and 25 μl of chloramine-T (3 μg/μl; Sigma, St. Louis, MO). After addition of chloramine-T, the mixture was allowed to react for 2 min. Fifty microliters of sodium-metabisulfite (2.5 μg/ul; J. T. Baker Chemical Co., Phillipsburg, NJ) was added to stop the chloramine-T reaction. One minute after sodium-metabisulfite addition, the reaction mixture was diluted with 100 μl of buffer (1 g KI plus 16 g sucrose per 100 ml distilled-deionized water) and transferred to a 1 cm × 16 cm P-60 polyacrylamide gel column (Bio-Rad Laboratories, Richmond, CA) previously equilibrated with phosphate buffer (0.05 *M*, pH 7.5) and 2% bovine serum albumin (BSA, A3059; Sigma). Elution of 1 ml fractions (into tubes containing 1 ml of 2% BSA) with 0.05 *M* phosphate buffer (pH 7.5) yielded two distinct peaks. Eluate from the first peak (fractions 4 and 5), containing ^{125}I-growth hormone, was diluted to a concentration of 0.011 μCi per 100 μl with 1% BSA (pH 7.0) and used in radioimmunoassays.

Normal rabbit serum (Gibco Laboratories, Madison, WI) was diluted 1:400 in 0.01 *M* phosphate buffered saline (PBS) containing 0.05 *M* ethylenedinitrilio tetraacetic acid (NRS-EDTA, pH 7.0). Antiserum to ovine growth hormone (NIDDK-anti-oGH-2, stored frozen at a 1:100 dilution) was diluted to a concentration of 1:20,000 in 1:400 NRS-EDTA. Assay buffer was 1% BSA in 0.01 M PBS (pH 7.0). Standards (0.025 ng/μl) or samples (4–400 μl) plus 1% BSA to a total volume of 500 μl, antiserum (100 μl, 1:20,000 dilution), and radiolabeled growth hormone (100 μl, 0.011 μCi) were added to 12 × 75-mm glass tubes, vortexed, and incubated 24 hr at 23°C (room temperature).

The precipitating antibody was ovine-anti-rabbit gamma globulins (Antibodies, Inc., Davis, CA) diluted to a concentration of 1:400 in a 5% solution of polyethylene glycol-8000 (Mallinckrodt Chemical Co., Paris, KY). Each tube received 500 μl of precipitating antibody solution and was incubated for 1 hr at 23°C. Tubes were then centrifuged at 1600 × g for 30 min at 4°C. The supernatant was decanted and the radioactivity in the pellet was counted in a gamma counter.

Binding in tubes containing no growth hormone was between 30 and 40%, with nonspecific binding between 1% and 3% of the total counts added. The linear portion of the standard curve was between 0.1 and 10.0 ng per tube. Increasing volumes (4 to 400 μl) of a pool of bovine serum depressed binding parallel to the standard curve. Recovery of 1–5 ng of added bovine growth hormone to tubes containing 150 or 250 μl bovine serum was 106% ± 1%, and 100.5% ± 1%, respectively. Assay sensitivity was 0.1 ng/tube. Cross-reactivity of NIDDK-anti-oGH-2 antibody was 1.4% with USDA-bPRL-B1 at a concentration of 300 ng/tube, 1.4% at 30 ng NIH-bTSH-B7, 1.4% at 500 ng USDA-bFSH-B1, and 1.4% at 50 ng NIH-bLH-B8 per tube.