Hypophyseal-Portal Concentrations of Growth Hormone-Releasing Factor and Somatostatin in Conscious Pigs: Relationship to Production of Spontaneous Growth Hormone Pulses (44222)

J. E. DRISKO,* T. D. FAIDLEY,† C. H. CHANG,* D. ZHANG,‡ S. NICOLICH,† D. F. HORA, Jr.,¶ L. MCNAMARA,* E. RICKES,* T. ABRIBAT,[a] R. G. SMITH,§ G. J. HICKEY*

Departments of Basic Animal Science Research, Branchburg Farm,† Biometrics Research,‡ Laboratory Animal Resources,¶ Biochemistry and Physiology,§ Merck Research Laboratories, Rahway, NJ; and Laboratory of Neuroendocrinology,[a] Notre Dame Hospital, Montreal, Quebec*

Abstract. **A method of collecting hypophyseal portal blood (HPB) in conscious pigs was used to show the relationship between GRF and somatostatin (SRIF) concentration and peripheral GH response. Six male castrate pigs (approximately 63 kg body weight) had HPB and jugular blood collected individually for an average of 175 min each. Twenty-seven spontaneous GH pulses were detected in the 1050 min of total HPB collection. Of the associations examined, the only significant finding was that GH pulse maxima occurred nonrandomly within periods of SRIF descent (63%; $P = 0.005$). Although 48% (13/27) of GH pulse maxima were associated with an ascent in portal GRF concentration, these associations were not determined to be nonrandom ($P = 0.14$). Only 7 of 27 (26%) GH pulse maxima were associated with an ascent in portal GRF concentration and a descent in SRIF concentration occurring simultaneously. A saline infusion given approximately 120 min after beginning blood collection resulted in an increase in SRIF pulse frequency and a decrease in GH-AUC and GRF-AUC. The cause of this saline effect is unknown, but it may have been related to acclimation of the pigs to the blood collection procedure. These data show the complexity of the relationship between SRIF and GRF concentrations and GH secretion and may indicate a close relationship with SRIF in GH pulse generation in the pig. In addition, these data support the hypothesis that, in the pig, mediation of GH release cannot be explained simply by antagonism between GRF and SRIF.** [P.S.E.B.M. 1998, Vol 217]

GH secretion has been found to be pulsatile in all species evaluated to date, including humans (1, 2), rats (3, 4), mice (5), sheep (6–8), and pigs (9, 10). Basal secretion of GH is believed to be regulated by the stimulatory action of GRF and the inhibitory action of somatotropin releasing inhibitory hormone (SRIF) (1, 2). Plotsky and Vale (3) have proposed a model where GH secretion is due to alternate tonicity of the pituitary by GRF and SRIF, although the GRF and SRIF changes upon which they based this theory were not measured in the same rats as those in which the GH hormone changes were measured. To date such an interaction has not been demonstrated conclusively in any species, and indeed the interaction of the two hypothalamic factors appears to vary with species and experimental design. Previously, hypophyseal-portal blood (HPB) collection in a conscious animal has only been possible using sheep (11). In conscious sheep, a significant increase in GRF was reported to be associated with a majority of GH peaks; however, no correlation with SRIF has been shown and a 1:1 relationship between changes in GRF, SRIF, and GH secretion has not been established (6–8). In

[1] To whom requests for reprints should be addressed at Department of Pharmacology, Merck Research Laboratories, P.O. Box 2000, R80Y-150, Rahway, NJ 07065. E-mail, jennifer_drisko@merck.com.
This study was fully funded by Merck and Company.

Received June 12, 1997. [P.S.E.B.M. 1998, Vol 217]
Accepted September 28, 1997.

0037-9727/98/2172-0188$10.50/0

anesthetized rats, direct measurement of HPB hormones showed a GH pulse associated with a concomitant rise in GRF and fall in SRIF (3). In humans given continuous infusions of hGRF (12), and in rats, using passive immunization techniques (4), GH pulses were hypothesized to be caused by GRF secretion coupled with SRIF withdrawal, as well.

We have recently developed an HPB collection model in the conscious pig (13). The purpose of the work described in this paper was to characterize GRF and SRIF concentrations in the conscious pig and to determine their relationship with the GH secretory profile.

Materials and Methods

Animals. Specific Pathogen Free, Yorkshire male castrate pigs, 4–5 months of age and weighing approximately 63 kg, were used. Animals were housed in AAALAC-accredited facilities in compliance with all applicable Federal, State and local regulations and in accordance with the *Guide for the Care and Use of Laboratory Animals,* NIH Publication No. 86-23, revised in 1985. All animals were subjected to a 12-hr light: 12-hr dark cycle beginning at 0700 hr. A commercially available swine diet (14% crude protein: Hunterdon Hog Mash, Agway, Flemington, NJ) was fed twice a day at 15 g/kg body weight. Water was provided free choice.

Surgical Procedures. Pigs were fasted for 18 hr prior to surgery. Animals were preanesthetized with Telazol (2.2 mg/kg, tiletamine HCL and zolazepam: Ft. Dodge Laboratories, Ft. Dodge, IA) given intramuscularly followed by mask induction of 4% isoflurane (Anaquest, Inc., Liberty Corner, NJ) in oxygen, using high flow techniques. Pigs were then endotracheally intubated, and anesthesia was maintained with 2%–3% isoflurane in oxygen in a semi-closed system. Isotonic saline was administered intravenously at a rate of 5 ml/kg/hr throughout surgery and during recovery from anesthesia.

Prior to cranial surgery, a double Micro-Renathane catheter, each 0.2 cm OD × 0.1 cm ID, (Braintree Scientific Inc., Braintree, MA) was surgically placed in one jugular vein. The catheters were connected by two Silastic cuffs (Dow-Corning, Midland, MI) that anchored them within the vein and kept the indwelling tips staggered approximately 1/2 inch. These catheters were used for venous access during the cranial surgery and for peripheral blood removal and heparinized saline infusion during the portal collection experiments. The dual catheter was exteriorized to the back of the neck, secured using the Chinese finger trap suture technique (14), and maintained in a fabric pouch glued to the skin.

Pituitary exposure was as previously described (13). Briefly, a transpalpebral eye exenteration was performed, and a burr hole was drilled caudal and slightly dorsal to the optic canal to expose the junction of the pituitary stalk and anterior pituitary. A premeasured double cannula similar to that described in sheep (11), but with a 5–10-mm Tygon tip (Norton Performance Plastics, Akron, OH), was inserted into the burr hole so that it rested approximately 1 mm from the pituitary. This device was cemented into place with dental acrylic (Paterson Dental, Carlstadt, NJ), and the palpebra was sutured together around the exposed cannula hubs. A heparinized (50 IU/ml) saline solution with 30 IU/ml Cefazolin (SoloPak Laboratories Inc., Elk Grove, IL) was flushed daily through one cannula and allowed to seep out of the other. This same solution was maintained within the cannulas between flushings. Portal collection occurred on Day 8 post surgery. Pigs weighed an average of 5.5 kg above preoperative weight by the time of portal collection.

Blood Collection Procedure. The collection method used was similar to that described in sheep (11) with exceptions noted. On the day of collection, the pig was placed in a Panepinto sling (Charles River Laboratories, Wilmington, MA). Whole animal heparinization began between 0830 hr and 1030 hr and was achieved by administration of heparin at 30,000–40,000 IU/bolus every 30 min for 120–180 min and maintained by a constant infusion of heparinized saline (1000 IU/ml) at 0.3 ml/min through the caudal jugular catheter. Jugular blood was removed at a similar rate through the rostral jugular catheter. One pig (Pig J) pulled out the heparin infusion line and therefore was given 20,000 IU of heparin at hourly intervals rather than a constant infusion of heparin. This same pig had to be tied into the sling to prevent escape prior to blood collection. Initial portal vessel lancing occurred 1 hr after initial heparinization for all pigs. Approximately 50% of the portal vessels were lanced through the portal cannula with a 16-gauge, 14-cm catheter stylette (Abbocath-T, AJ Buck, Owing Mills, MD). Experimental blood collection began when portal blood flow was established and stabilized, between 1130 hr and 1230 hr. HPB was removed at a rate of 0.06–0.08 ml/min. All pigs slept for a portion of the study.

Jugular and portal blood were collected continually and simultaneously into polypropylene tubes placed in a refrigerated fraction collector. Samples were pooled into 5-min portions. Aprotinin (Trasylol, Boehringer Mannheim, Indianapolis, IN) was added (500 KIU/tube) to chilled portal collection tubes prior to blood collection. Blood collection time (3–4 min) from blood vessel to collection tube for the jugular and portal blood was synchronized by either altering pump speed, shortening a blood line, or both. Approximately 12 in. of the portal blood collection line were submerged in ice to cool the blood before it reached the refrigerated fraction collector. Blood samples were maintained on ice and centrifuged at 30-min intervals. Harvested plasma was frozen immediately and stored at −70°C. Hematocrits were determined for jugular and portal blood samples at various time points during each experiment and used to correct portal samples for cerebrospinal fluid contamination, if present. Pituitary stalk lesions were verified on postmortem examination performed immediately at the conclusion of each experiment.

A 5-min saline infusion that served as a control for a

larger study, was administered approximately 120 min into each collection period.

Hormone Assays. Porcine GH (pGH) was measured in jugular plasma by a homologous assay system developed using reagents supplied by Dr. A. F. Parlow, Pituitary Hormones and Antisera Center, Harbor-University of California-Los Angeles Medical Center, Torrance, CA; (pGH for iodination and standards, USDA I-1; anti-pGH, AFP10318545). Intra-and interassay variations were 4.5% and 2.6%, respectively. The minimum level of detection of the GH assay was 0.45 ng/ml.

Porcine GRF(1–44)NH_2 was measured in unextracted portal plasma by a double antibody radioimmunoassay, with [^{125}I-Tyr^{10}] hGRF(1–44)NH_2 as tracer (Amersham, Oakville, Ontario), porcine GRF(1–44)NH_2 as standard (Peninsula, Belmont, CA), and polyclonal anti-human GRF(1–40)OH antibodies. The antiserum was raised in rabbits and previously characterized (15). The assay was conducted in a 20-m*M* phosphate buffer, pH 7.2, containing 0.1% BSA (RIA grade), 0.15 *M* NaCl, 10 m*M* EDTA, 0.1% sodium azide (all reagents from Sigma, St. Louis, MO) and 0.1% Tween 20 (Biorad, Richmond, CA). The 500-μl incubate consisted of standard (3 to 10,000 pg/tube) or unknown, antibody (final dilution 1/70,000) and tracer (10,000 cpm) in polypropylene tubes. The incubation was run for 20–24 hr at +4°C, then stopped by adding a second antibody (goat anti-rabbit antibody, Linco Research, St. Louis, MO) and incubating it for another 16–18 hr. The incubate was then centrifuged, the supernatants discarded, and the pellets counted for radioactivity.

For the GRF assay, the specific binding was 48.7 +/– 1.5% and the ED 50 was 131 +/– 4 pg/tube (means +/– SEM of 10 consecutive assays) at a final antiserum dilution of 1:70,000. Intra- and interassay coefficients of variation were 2.0% and 9.6%, respectively. The nonspecific binding in the assays was 5%. The recovery of exogenously added GRF was 91 ± 6% (mean ± SEM of six samples spiked with 30 or 300 pg GRF/tube). Results were not corrected for recovery. The threshold of sensitivity for the GRF assay was 50 pg/ml. In a previous experiment (not shown) parallelism between a serial dilution of normal (jugular) plasma supplemented with exogenous GRF and the standard curve was obtained.

SRIF was measured in unextracted portal plasma by radioimmunoassay, with [^{125}I-Tyr^{11}]SRIF 14 (Amersham, Oakville, Ontario) as tracer, SRIF 14 (Peninsula) as standard and polyclonal anti-SRIF 14 antibodies, previously characterized and used for SRIF assays (16). The assay was conducted in the same buffer as the GRF assay, without Tween 20. The 500-μl incubate was made of standard (2.5 to 320 pg/tube) or unknown, antibody (final dilution 1/100,000), and tracer (10,000 cpm) in polypropylene tubes. The incubation was run for 20–24 hr at +4°C, then stopped by adding 2 ml ice-cold pure ethanol, vortexing, incubating at +4°C for 15 min, then centrifuging the tubes. Supernatants were discarded, and pellets were counted for radioactivity.

For the SRIF assay, the specific binding was 43.4 ± 1.0%, and the ED 50 was 58 ± 2 pg/tube (means ± SEM of 10 consecutive assays) at a final antiserum dilution of 1:100,000. Intra- and interassay coefficients of variation were 2.7% and 11.2%, respectively, and the recovery of exogenously added SRIF 14 was 90 ± 5% (mean ± SEM of 6 samples spiked with 20 or 100 pg SRIF 14/tube). Results were not corrected for recovery. The threshold of sensitivity for the SRIF assay was 50 pg/ml. There was good parallelism between a serial dilution of portal plasma, to which aprotinin was added, and the standard curve. This was not the case with jugular plasma that had been collected without the addition of aprotinin. However, good parallelism was shown between a serial dilution of aprotinin-added jugular plasma and the standard curve.

Statistical Analysis. The following definitions were used for statistical interpretation. A pulse was comprised of an ascending period leading to a pulse maximum and a descending period after that maximum. A trough minimum was the single lowest point in a trough. A trough was the descending period leading to the trough minimum, the trough minimum, and the ascending period leading away from that minimum.

A baseline period averaging 175 min was examined in each of six pigs. The initial approach was to evaluate the general relationship between the concentrations of any two of the three hormones by cross-correlation coefficient analysis. The level of significance for a cross-correlation coefficient was determined by estimating the standard error for the cross-correlation coefficient. The standard error was given by $1/\sqrt{n-k}$, where n denotes the number of samples measured and k the particular lag (17). Cross-correlation coefficients within $\pm 2/\sqrt{n-k}$ were considered nonsignificant. Cross-correlations between the concentration of GH and the concentration of each of the other two hormones were calculated at zero lag (comparisons of simultaneous measurements) and lags of one and two samples (GH measurements were compared with measurements in GRF or SRIF that led by one or two samples).

The pulses present in each GH, GRF, and SRIF profile during the baseline period were determined by ULTRA (18). ULTRA is a model-independent method that uses significant differences between levels of adjacent points to identify significant changes. An increase from concentration A to B was considered significant if $(B - A)/B > 3CV$ and a decrease from concentration A to B was considered significant if $(A - B)/A > 3CV$ where CV is the intraassay coefficient of variation. A constant CV for individual animals was used across the ranges of concentrations in this study. A pulse was considered significant if both the ascending and descending periods met the requirements for significance. Nonsignificant changes occurring during a period of ascension or descension were ignored. The result was a clean profile in which only significant pulses re-

mained. A SRIF trough was considered significant if both the descending and ascending periods exceeded three times the *CV*.

The entire baseline was analyzed for GH, GRF, SRIF pulses, and SRIF troughs using the ULTRA software with the threshold of 3*CV*s. In addition to the identification of pulse maxima, ULTRA also gives the durations of pulses/troughs including the ascending and descending periods. GH pulse maxima were then compared to periods of ascent in GRF (including pulse maxima) and to periods of ascent (including pulse maxima) and descent (including trough minima) in SRIF. The number of observed coincident events in two hormones (e.g., GH pulse maxima matched with ascent in GRF) were then tested to determine if the event was a random occurrence. Given m events in hormonal Series A, n events in Series B, and z samples in each series, the number of coincident events x_d has a hypergeometric distribution with probability distribution function denoted by $\Pr(x_d)$. To assess the probability that at least the observed number of coincident events x could be attributed to chance alone, the formula $\Pr(X_d > x_d) = (1 - \sum_{i=0}^{d} \Pr(x_d))$ was used for calculation (17).

Because this baseline was a prelude to a larger experiment, a control saline infusion was present approximately 120 min into the baseline. Statistical analysis chosen required comparison of an equal time frame before and after this infusion. Therefore, to assess saline infusion effects, we compared frequency, amplitude, and duration of GH, GRF, and SRIF pulses in 10 samples (50 min) before and 10 samples (50 min) after saline infusion. GH, GRF, and SRIF areas under the curve (AUC) were also compared for the 50 min period before and after saline infusion.

Results

Secretory Patterns of GH, GRF, and SRIF. Jugular and hypophyseal portal samples were collected from six pigs in similar, but separate, collection experiments that lasted approximately 175 min each (Fig. 1) for a total of 1050 min. The six pigs in this study averaged 4.5 GH pulses with a range of 3–8, 8.8 GRF pulses with a range of 7–11, and 8.8 SRIF pulses with a range of 5–11 in each 175-min period. A total of 27 GH pulses were detected from jugular blood samples in the pooled 1050 min. GH pulse amplitudes ranged from 1.8–4.6 ng/ml and averaged 3.0 ng/ml. There were 53 GRF pulses identified in the pooled 1050 min. GRF pulse amplitudes ranged from 145–2748 pg/ml. The mean portal concentration of GRF was 435 pg/ml. There were 53 SRIF pulses identified in the pooled 1050 min. Their amplitudes ranged from 837–3016 pg/ml. The mean portal SRIF concentration was 1333 pg/ml. A total of 53 SRIF troughs were detected in the pooled 1050-min period. The mean interpulse duration, average time between pulse maxima for GH and GRF, was 32 min with a range of 10–85 min for GH and 19 min with a range of 10–40 min for GRF. For SRIF, the mean intertrough duration, average time between trough minima, was 17.7 min with a range of 10–55 min.

Relationship Between Changes in GH, GRF, and SRIF Concentrations. Interactions between GH pulse maxima and corresponding GRF and SRIF ascents and descents are shown in Table I. GH pulse maxima were always associated with a change in SRIF concentration immediately prior to or at the same time as the GH maximum. A GH pulse maximum occurred simultaneously with an ascent in GRF and a descent in SRIF only 26% (7/27) of the time. A GH pulse maximum occurred one sample after a simultaneous GRF ascent and SRIF descent only 19% (5/27) of the time. A simultaneous GH pulse maximum, GRF pulse maximum, and SRIF trough minimum never occurred.

Hypergeometric probability distribution analysis was performed to determine if the associations presented in Table I were nonrandom occurrances. SRIF was descending simultaneously with GH pulse maxima 63% (17/27) of the time (Table II), and hypergeometric analysis showed this to be a significant, nonrandom association. In individual analyses, GH pulse maxima significantly coincided with descending SRIF concentrations in four of the six pigs. GRF was ascending simultaneously with a GH pulse maximum 48.0% (13/27) of the time. However, hypergeometric analysis of all six pigs as a group failed to show that GH pulse maxima coinciding with GRF ascent was nonrandom. Individual analysis showed that only Pig J had a significant incidence of GH pulse maxima coinciding with ascending GRF concentrations. Furthermore, no nonrandom associations were seen for any other combinations (data not shown).

For GRF versus GH at zero lag, cross-correlation coefficients of the six pigs ranged in value between –0.148–0.506, only Pig J showed a significant positive correlation. At one sample lag, the correlation coeffients ranged between –0.119–0.392. At two sample lags, the range was 0.081–0.340. Pig N was the only animal to show a significant positive correlation at one and two sample lags.

For SRIF versus GH, cross-correlation coefficients at zero, one and two sample lags ranged from –0.285–0.282, –0.199–0.427, –0.093–0.247, respectively. Only Pig L at one sample lag showed a significant positive correlation.

Effects of Saline on Secretory Patterns and AUC of GH, GRF, and SRIF. Pulse amplitude and duration of GH, GRF, and SRIF pulses before and after saline infusion were not significantly different for each of the three hormones. Saline administration did not have a significant effect on GH or GRF pulse frequency; however, saline administration was associated with significantly more SRIF pulses. GH AUC was decreased (approximately 10%, $P = 0.06$) following saline infusion. GRF AUC was significantly decreased (approximately 14%, $P = 0.05$) after saline infusion. SRIF AUC was not significantly changed.

Discussion

The present study is the first to report HPB concentrations of GRF and SRIF in the conscious pig and to show a pulsatile pattern of release of these peptides in this species.

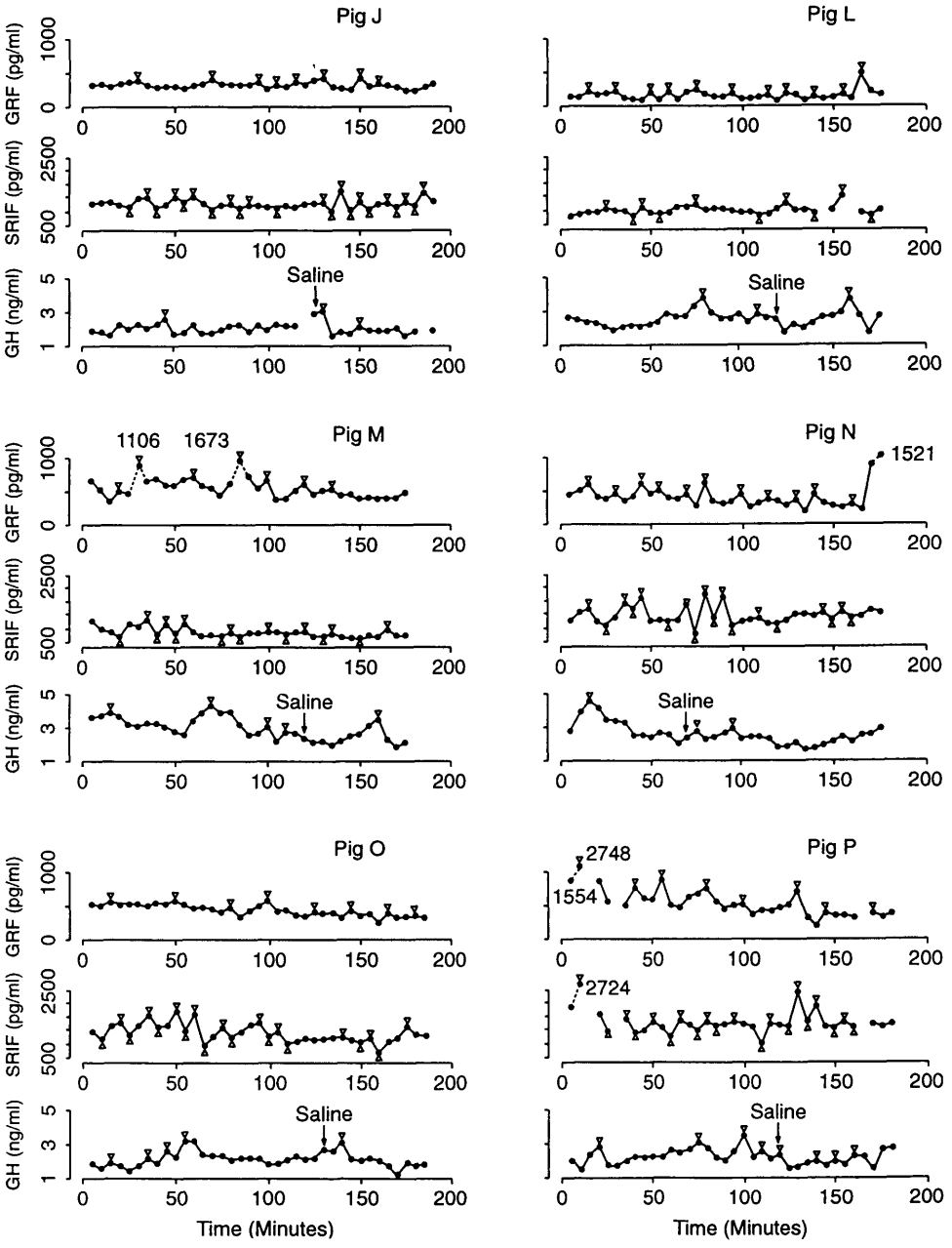

Figure 1. GRF and SRIF concentrations in HPB and GH concentrations in jugular blood of six conscious, castrated pigs. Each sample represents a 5-min continuous collection. The sampling period was approximately 175 min for each pig. Significant pulses and SRIF troughs, detected using ULTRA, are indicated by triangles (△, ▽, respectively). Missing samples are shown as breaks in the splined data points. Values above the Y-axis scale are connected by dotted lines.

Validation of the porcine HPB collection system was shown in other studies by the ability to detect CRF responses to LPS endotoxin administration (Drisko, JE *et al,* unpublished data). Previous to the present study, the only available conscious HPB collection model available was in the sheep. HPB peptide measurements and pulsatility patterns of GRF and SRIF secretion and associations with GH have been presented in that species (6–8).

Circulating GH concentrations are similar between pigs in this study (2.45 ± 0.59 ng/ml), castrated pigs of the same age with only jugular access (1.1 ± 0.2 ng/ml (10)), and sheep (4.6 ± 2.7 ng/ml, (6); 2.9 ± 0.6 ng/ml (7); 2.9 ± 2.2 ng/ml, (8)). However, there would appear to be marked differences between pigs and sheep in the reported HPB peptide concentrations as well as in the associations between GRF, SRIF, and GH pulse maxima. Porcine HPB GRF and SRIF concentrations (435 ± 249, 1333 ± 409 pg/ml, respectively) are much higher than concentrations reported in sheep (36.7 ± 15.3, 40.2 ± 13.2 pg/ml, respectively (6); 6.6 ± 1.4 pg/ml, 19.6 ± 1.6 pg/ml, respectively (7); 20.4 ± 6.7, 72 ± 33 pg/ml, respectively (8)). While there are differences in the assays among the studies, these differences may not explain the discrepancies between the pig and sheep data. The GRF and SRIF assays conducted in sheep plasma were more sensitive, < 20 pg/ml (6–8), than our assay, 50 pg/ml; however, the average concentrations of these peptides in pig portal plasma were well above our limits of detection. The porcine SRIF assay showed good parallelism in portal plasma and low nonspecific binding. The porcine GRF assay showed good parallelism with ex-

Table I. Association of GRF and SRIF Changes in Hypophyseal Portal Blood with Peripheral GH Pulse Maxima of Conscious Pigs[a]

Associations observed	Number occurring simultaneously with 27 GH pulse maxima	Number occurring one sample prior[b] to 27 GH pulse maxima
• GRF ascent; SRIF descent[c]	7	5
• GRF ascent; SRIF ascent	6	6
• GRF descent; SRIF descent	8	9
• GRF descent; SRIF ascent	3	3
• No change in GRF; SRIF descent	2	0
• No change in GRF; SRIF ascent	1	2
• GRF ascent; no change in SRIF	0	0
• GRF descent; no change in SRIF	0	0
• GRF pulse maxima; SRIF trough	0	0

[a] Blood was collected from six pigs for approximately 175 min each for a total of 1050 min.
[b] An ascent or descent in GRF or SRIF may be included in both columns; therefore, rows are not additive.
[c] Ascent and descent refer to significant ascending or descending slopes leading to pulse maxima or trough minima.

ogenously added GRF in jugular plasma and low nonspecific binding. The porcine GRF assay had lower intra- and interassay *CV*s than the sheep assays (2.0% and 9.6%, respectively for pig vs. 9% and 10%, respectively (6), 12.9% and 17.1%, respectively (7), and 12.5% and 10.6% respectively (8) for sheep). Intra- and interassay *CV* for the pig SRIF assay was comparable to the sheep assays (2.7 and 11.2%, respectively for pig, vs. 8 and 10%, respectively (6), 11.7% and 12.6%, respectively (7), and 7.1 and 11.2%, respectively (8) for sheep). Perhaps the increase in concentration of portal peptides is inversely related to the number of portal vessels. In our observations of portal vasculature, pigs have very few portal vessels compared to sheep.

The increase in pulse frequency of SRIF and the decrease in GH AUC and GRF AUC after saline infusion was surprising. Perhaps there was a saline infusion effect, but most likely the decreases in GH and GRF AUC were related to the pigs becoming more comfortable and less stressed as the experiment continued. All animals were acclimated to the sling 2–3 hr before the collection began and appeared calm at the time of collection. Isolation stress in sheep has been shown to cause an increase in GRF, SRIF, and GH release (19). Conversely, other stresses, such as ether stress, may cause a decrease in GH release in conscious rats (20) by increasing SRIF secretion to the anterior pituitary (21).

Pigs appear to have more frequent pulses of GRF, SRIF, and GH than do sheep. Frohman *et al.* (8) reported that in a 5-hr period, five sheep averaged 4.2 GRF peaks, 5.6 SRIF peaks, and 4.6 GH peaks. This suggests that GRF and SRIF pulses occur almost four times as often in pigs as in sheep, and GH pulses are about twice as frequent in pigs than in sheep. Dubreuil *et al.* reported GH pulse frequency similar to that seen in the sheep (22). However, in Dubreuil's study, discrete samples were taken every 20 min, and a more conservative approach to pulse detection was used. ULTRA, the pulse detection program used in the present study (provided by Dr. Eve Van Cauter, Department of Medicine, University of Chicago), and DETECT, the program used in the sheep studies, produced results that were found to be indistinguishable from each other based on simulation (23); therefore, differences in algorithms cannot be responsible for the variation between species reported here. The time of day and HPB collection model were similar for the sheep and pig studies. Gender and age differences may contribute to the differences in study results. Both Thomas' (7) and Frohman's (6) studies used ovariectomized adult ewes whereas Cataldi's study (8) used 8-month-old rams. Pigs in our study were approximately 20-week-old castrates. GH concentrations are similar between intact and castrated pigs at 26 weeks of age (10).

Sampling times may contribute to the differences observed between species, also. The shorter the sampling interval the more accurately the hypothalamic secretory events are monitored. Our pig data were collected using 5-min continuous sampling intervals, the sheep studies were conducted using 10-min continuous sampling intervals, and Plotsky and Vale's anesthetized rat model was conducted using 20-min continuous sampling intervals. The four-fold increase in GRF and SRIF pulses observed in pigs compared to sheep may be explained in part by dilution. However, even shorter sampling periods may be necessary to determine precise pulse patterns at the pituitary level, particularly if the changes are small. For instance, a small increase in either GRF or SRIF followed by a small decrease of that same peptide may register as no change in a sampling interval that included both of those events. Therefore, moment by moment sampling would be the most ideal in determining GRF pulse/SRIF trough relationship to GH pulse generation.

The classic premise of GH control is that GRF causes a release, and SRIF causes an inhibition of GH secretion (1, 2, 4). Plotsky and Vale inferred from an experiment, measuring immunoreactive GRF and SRIF in the anesthetized rat, that each GH pulse is initiated by either a mild decrease of SRIF followed by a pulsatile secretion of GRF into the portal circulation or a simultaneous decrease in SRIF and increase in GRF (3). The rats used were anesthetized with one of three different agents. SRIF and GH concentrations were dissimilar for each agent, and none was necessarily representative of the conscious state. In addition, GH measurements were made from a separate group of rats, not the

TABLE II. Hypergeometric Probability Distribution Analysis of GH Pulse Maxima, Ascending GRF, and Descending SRIF Concentrations Measured in HPB Collected from Conscious Pigs

Pig	Samples (z)	GH pulse maxima coinciding with ascending periods of GRF pulses			
		GH maxima (m)	GRF ascent[a] (n)	Matches (x_d)	P-value[b]
J	38	3	14	2	0.0431[d]
L	35	3	18	1	0.5221
M	35	5	12	2	0.2090
N	35	3	15	2	0.0695[e]
O	37	5	12	2	0.1816
P	36	8	16	4	0.2228
Total	216	27	87	13	0.1357

Pig	Samples (z)	GH pulse maxima coinciding with descending periods of SRIF troughs			
		GH maxima (m)	SRIFdescent[c] (n)	Matches (x_d)	P-value
J	38	3	14	0	0.7601
L	35	3	16	3	0[d]
M	35	5	16	4	0.0135[d]
N	35	3	14	2	0.0556[e]
O	37	5	13	1	0.5856
P	36	8	17	7	0.0008[d]
Total	216	27	90	17	0.0047[d]

Pig	Samples (z)	Ascending GRF periods coinciding with descending SRIF periods			
		GRFascent (m)	SRIFdescent (n)	Matches (x_d)	P-value
J	38	14	14	6	0.1746
L	35	18	16	6	0.8798
M	35	12	16	5	0.4946
N	35	15	14	4	0.8521
O	37	12	13	4	0.4127
P	36	16	17	8	0.2631
Total	216	87	90	33	0.7803

[a] P-value is calculated from $1 - \sum_{i=0}^{d} \Pr(x_d)$, where $\Pr(x_d)$ is the probability distribution function of hypergeometric distribution.
[b] GRF ascent = number of samples in the ascending period of the GRF pulse maxima.
[c] SRIF descent = number of samples in the descending period of the SRIF troughs.
[d] Denotes significance at $\alpha = 0.05$.
[e] Denotes significance at $\alpha = 0.1$.

same rats used for the GRF and SRIF measurements. In the present study, cross-correlation analysis failed to show either a consistent significant positive correlation of GH with GRF or a significant negative correlation of GH with SRIF. Furthermore, the sheep studies showed no significant correlation between GRF and SRIF (7, 8), or was not reported (6).

To further evaluate the relationship between GH secretion and portal concentrations of GRF and SRIF, the comparisons were broadened to look at GH pulse maxima as they related to areas of GRF ascent (including GRF pulse maxima) and SRIF descent (including trough minima). In addition, GRF ascents were compared with SRIF descents. GRF ascent was relatively independent from SRIF descent and not consistently matched with GH pulse maxima. A GRF ascension occurred with a GH pulse maximum 48% of the time in pigs in this study, but those events were not shown to be nonrandom. In two of the sheep trials, a significant relationship between GRF and GH was noted, but the occurrence of a simultaneous GH peak with a GRF peak was reported to be 38% by Frohman *et al.* (8) or 78% by Cataldi *et al.* (6). Even in Cataldi's study (6) 22% of the GH peaks were not associated with an increase in GRF. Thomas' study (7) showed an approximately equal distribution of GRF pulses coincident with or before, and coincident with or after, a GH pulse. Although a definite concordance between GRF and GH can be stated from those data, a cause and effect relationship cannot be determined.

GH pulse maxima are more consistently matched with SRIF descents in pigs than in sheep and rats. In pigs, 63% (17/27) of GH pulse maxima occurred with a nonrandom, simultaneous descent in SRIF. GH peaks in sheep were not

matched with SRIF troughs in one study (8), but were associated with decreasing concentrations of SRIF 62% (33/53) of the time in another study (6). In conscious rats equipped with a push-pull cannula into the median eminence, no correlation was shown between somatostatin levels and pharmacologically decreased growth hormone secretion (24). However, a pulse detection program was not used in that study. Means from the 15-min continuous sampling periods and change in means before and after treatments were evaluated using Student's *t* test.

The apparent independence of GH pulse maxima from GRF pulses suggests that the proposed model in the rat does not apply to the pig. This apparent independence of GH pulses from GRF pulses may be explained by some as yet undefined relationship between GRF and SRIF such as a receptor activation/inactivation loop modulated by these peptides and/or an additional factor(s) that triggers GH release (25). This speculation is consistent with a putative hypothalamic factor, the U factor postulated by Bowers *et al.* (26) to be involved with GH release in the rat. Bowers' hypothesis was based on the observation that GHRP, a synthetic GH secretagogue, synergistically releases GH in combination with GRF and that this synergism is probably not the result of inhibition of SRIF or release of endogenous GRF. The mechanism of the synergistic action of GHRP and GRF was not fully explained by the dual action of GHRP hypothalamic activation of GRF neurons and GHRP direct pituitary action. Supporting the hypothesis of a putative hypothalamic factor, Smith *et al.* (25) have reported synthetic GH secretagogues that bind to receptors, distinct from GRF receptors, in the hypothalamus and pituitary and that mediate GH release from the pituitary. These distinct receptors have recently been cloned in pig and human hypothalamus and pituitary (27).

In summary, even though GH concentrations are similar across species, pigs have high portal GRF and SRIF concentrations when compared to rats and sheep. The approximate 2:1 ratios of GRF pulses to GH pulses and SRIF troughs to GH pulses in the pig does not fit the hypothesis that a GH pulse is generated by a concomitant rise in GRF and fall in SRIF. Furthermore, a 1:1 relationship between these secretory peptides and GH secretion has not been shown in the sheep studies (6–8). The pig data suggest that an additional factor(s) influences GRF and SRIF associations and, hence, GH secretion. These data show the complexity of the relationship between GRF and SRIF concentrations and GH secretion and may suggest a close relationship of SRIF with GH pulse generation in the pig. Finally, results from this study indicate a marked species difference in pulsatility patterns between pigs and sheep, the only two species in which HPB from the conscious animal has been collected.

The authors wish to acknowledge Mr. Paul Cunningham and Dr. William Feeney for their assistance with the surgeries and animal care; and Mrs. Easter Frazier and Dr. Howard Chen for conducting the GH assays.

1. Hartman ML, Veldhuis JD, Thorner MO. Normal control of growth hormone secretion. Horm Res **40:**37–47, 1993.
2. Frohman LA, Downs TR, Chomczynski P. Regulation of growth hormone secretion. Front Neuroendocrinol **13:**344–405, 1992.
3. Plotsky PM, Vale W. Patterns of growth hormone-releasing factor and somatostatin secretion into the hypophyseal portal circulation of the rat. Science **230:**461–463, 1985.
4. Tannenbaum GS, Ling N. The interrelationship of growth hormone releasing factor and somatostatin in generation of the ultradian rhythm of growth hormone secretion. Endocrinology **115**(5):1952–1957, 1984.
5. Tamaki M, Sato M, Niimi M, Takahara J. Resistance of growth hormone secretion to hypoglycemia in the mouse. J Neuroendocrinol **7:**371–376, 1995.
6. Cataldi M, Magnan E, Guillaume V, Dutour A, Conte-Devolx B, Lombardi G, Oliver C. Relationship between hypophyseal portal GRF and somatostatin and peripheral GH levels in the conscious sheep. J Endocrinol Invest **17**(9):717–722, 1994.
7. Thomas GB, Cummins JT, Francis H, Sudbury AW, McCloud PI, Clarke IJ. Effect of restricted feeding on the relationship between hypophyseal portal concentrations of growth hormone (GH)-releasing factor and somatostatin, and jugular concentrations of GH in ovariectomized ewes. Endocrinology **128**(2):1151–1158, 1991.
8. Frohman LA, Downs TR, Clarke IJ, Thomas GB. Measurement of growth hormone-releasing hormone and somatostatin in hypothalamic-portal plasma of unanesthetized sheep: Spontaneous secretion and response to insulin-induced hypoglycemia. J Clin Invest **86:**17–24, 1990.
9. Dubreuil P, Pelletier G, Petitclerc D, Lapierre H, Couture Y, Morisset J, Gaudreau P, Brazeau P. Influence of age and sex on basal secretion of growth hormone (GH) and on GH-induced release by porcine GH-releasing factor (pGRF(1-29)NH2 in growing pigs. Domest Anim Endocrinol **4:**299–307, 1987.
10. Kraetzl WD, Schams D, Brem G. Secretory patterns of porcine growth hormone and insulin-like growth-factor-1 in growing pigs. Journal of Animal Physiology and Animal Nutrition **71:**1–14, 1994.
11. Caraty A, Locatelli A, Moenter SM, Karsch FJ. Sampling of hypophyseal portal blood of conscious sheep for direct monitoring of hypothalamic neurosecretory substances. In: Levine JE, Conn MP, Eds. Methods in Neurosciences, Pulsatility in Neuroendocrine Systems. San Diego, CA: Academic Press, Inc., Vol **20:**pp 162–183, 1993.
12. Vance ML, Kaiser DL, Evans WS, Furlanetto R, Vale W, Rivier J, Thorner MO. Pulsatile growth hormone secretion in normal men during a continuous 24-hour infusion of human growth hormone releasing factor (1-40). J Clin Invest **75:**1584–1590, 1985.
13. Drisko JE, Faidley TD, Hora DH Jr., Niebauer GW, Feeney W, Friscino BF, Hickey GJ. Transorbital approach to the porcine pituitary. J Invest Surg **9**(4):305–311, 1996.
14. Smeak DD. The Chinese finger trap technique for fastening tubes and catheters. J Am Anim Hosp Assoc **26:**215–218. 1990.
15. Brazeau P, Ling N, Wehrenberg WB, Jones KL, Guillemin R. Radioimmunoassay for hsomatrocrinin (human growth hormone releasing factor). In: Bizollan CA, Ed. Monoclonal Antibodies and New Trends in Immunoassays. New York: Elsevier Science Publishers, pp 247–252, 1984.
16. Brazeau P, Epelbaum J, Tannenbaum G, Rorstad O, Martin JB. Somatostatin: Isolation, characterization, distribution, and blood determination. Metabolism **27**(9):1133–1137, 1978.
17. Veldhuis JD, Johnson ML, Faunt LM, Seneto E. Assessing temporal coupling between two or among three or more neuroendocrine pulse trains. In: Levine JE, Ed. Methods in Neurosciences. San Diego, CA: Academic Press, Vol **20:**336–376, 1994.
18. Cauter EV. Estimating false-positive and false-negative errors in analysis of hormonal pulsatility. Am J Physiol **254**(Endocrinol Metab 17):E786–E794, 1988.
19. Cataldi M, Magnan E, Guillaume V, Dutour A, Suaze N, Mozzocchi

L, Conte-Devolx B, Oliver C. Acute stress stimulates secretion of GHRH and somatostatin into hypophysial portal blood of conscious sheep. Neurosci Lett **178:**103–106, 1994.
20. Aguila MC, Pickle RL, Yu WH, McCann SM. Roles of somatostatin and growth hormone-releasing factor in either stress inhibition or growth hormone release. Neuroendocrinology **54:**515–520, 1991.
21. Benyassi A, Gavalda A, Armario A, Arancibia S. Role of somatostatin in the acute immobilization stress-induced GH decrease in the rat. Life Sci **52:**363–370, 1993.
22. Dubreuil P, Lapierre H, Pelletier G, Petitclerc D, Couture Y, Gaudreau P, Morisset J, Brazeau P. Serum growth hormone release during a 60-hour period in growing pigs. Domest Animal Endocrinol **5**(2):157–164, 1988.
23. Urban RJ, Kaiser DL, Van Cauter E, Johnson ML, Vedhuis JD. Comparative assessments of objective peak-detection algorithms. II. Studies in men. Am J Physiol **254**(Endocrinol Metab 17):E113–E119, 1988.
24. Fukata J, Kasting NW, Martin JB. Somatostatin release from the median eminence of unanesthetized rats: Lack of correlation with pharmacologically suppressed growth hormone secretion. Neuroendocrinology **40:**193–200, 1985.
25. Smith RG, Pong S-S, Hickey GJ, Jacks T, Cheng K, Leonard R, Cohen CJ, Arena JP, Chang CH, Drisko J, Wyvratt M, Fisher M, Nargund R, Patchett A. Modulation of pulsatile GH release through a novel receptor in hypothalamus and pituitary gland. In: Conn PM, ed. Recent Progress in Hormone Research. Bethesda, Maryland: The Endocrine Society, Vol **51:**261–286, 1995.
26. Bowers CY, Sartor AO, Reynolds GA, Badger TM. On the actions of the growth hormone-releasing hexapeptide, GHRP. Endocrinology **128**(4):2027–2035, 1991.
27. Howard AD, Feighner SD, Cully DF, Arena JP, Liberator PA, Rosenblum CI, Hamelin M, Hreniuk DL, Palyha OC, Anderson J, Paress PS, Diaz C, Chou M, Liu KK, McKee KK, Pong S-S, Chaung L-Y, Elbrecht A, Dashkevicz M, Heavens R, Rigby M, Sirinathsinghji DJS, Dean DC, Melillo DG, Patchett AA, Nargund R, Griffin PR, DeMartino JA, Gupta SK, Schaeffer JM, Smith RG, Van der Ploeg LHT. A receptor in pituitary and hypothalamus that functions in growth hormone release. Science **273:**974–977, 1996.