For an unknown reason κ chains containing the Cys required for forming interdomain disulfide bonds are not expressed in the Basilea strain of rabbits (*20*).

Only a few cases of κ deficiency in humans have been reported, perhaps because κ deficiency does not have a drastic effect on the health of the individual—λ chains seem able to substitute effectively. The κ-deficient patient's immune response to a variety of antigens was normal (*1*). Furthermore, mice made κ-deficient by injection of antibodies to κ since birth mounted normal secondary, but defective primary, responses, suggesting that λ chains can substitute for κ chains after diversification by somatic mutation (*21*).

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Patterns of Growth Hormone–Releasing Factor and Somatostatin Secretion into the Hypophysial-Portal Circulation of the Rat

Abstract. *The interrelation between the secretion of two hypophysiotropic peptides, growth hormone–releasing factor (GRF) and somatostatin (SRIF), in the generation of episodic growth hormone (GH) secretion was inferred from direct measurements of immunoreactive GRF and immunoreactive SRIF concentrations in the hypophysial-portal plasma of the rat. Secretion of immunoreactive GRF was found to be episodic, with maximal concentrations present during periods of expected GH secretory episodes. Secretion of immunoreactive GRF was accompanied by a moderate reduction in portal plasma levels of immunoreactive SRIF. Passive immunoneutralization of SRIF was associated with increased concentrations of immunoreactive GRF in hypophysial-portal plasma. On the basis of these observations, it appears that each GH secretory episode is initiated by pulsatile secretion of immunoreactive GRF into the portal circulation, which is preceded by or is concurrent with a moderate reduction of inhibitory tone provided by portal immunoreactive SRIF. These experiments provide direct insights into central and adenohypophysial mechanisms by which GRF and SRIF interact to generate episodic secretion of GH.*

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Spontaneous secretion of growth hormone (GH) in the rat (*1*) and other species (*2*) is characterized by episodic, high-amplitude bursts separated by troughs of low-level secretion throughout each 24-hour period. The hypothalamic regulation of GH secretion appears to be mediated by two peptides, a recently identified stimulatory factor, growth hormone–releasing factor (GRF) (*3*), and an inhibitory peptide, somatostatin (SRIF) (*4*). These factors reach the anterior pituitary gland via the hypophysial-portal circulation after release from nerve endings in the zona externa of the median eminence (*5*). The central and adenohypophysial mechanisms by which GRF and SRIF interact to generate episodic secretion of GH are not well understood.

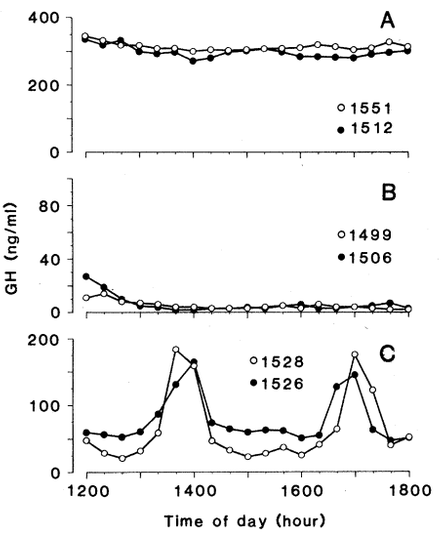

Fig. 1. Representative examples of GH secretory patterns in rats anesthetized with (A) pentobarbital, (B) urethan, or (C) ketamine: xylazine (see text for details).

Removal of the inhibitory influence of SRIF by passive immunoneutralization (*6*), electrolytic lesion of regions rich in SRIF perikarya (*7*), or hypothalamic cuts (*8*) increases the trough levels of GH. Withdrawal of exogenous SRIF is accompanied by rebound secretion of GH (*9*). Thus SRIF is a component of the dynamic GH release mechanism. Analyses of hypothalamic secretion of immunoreactive SRIF, based on the use of intracerebral push-pull perfusion sampling (*10*) and hypophysial-portal collection (*11*) techniques, have failed to convincingly establish the secretory dynamics of endogenous SRIF. To our knowledge, neither in vitro nor in vivo studies of GRF secretion have been reported. However, Wehrenberg *et al.* and others (*12*) have demonstrated blockade of episodic, but not of trough, GH secretion by passive immunoneutralization with specific antisera to synthetic GRF. This body of observations has led to the development of several hypothetical models of the GH regulatory process (*13*).

Our experiments were designed to probe the nature of hypophysiotropic regulation of GH secretion from the adenohypophysis. Participation of GRF and SRIF in the generation of episodic GH secretory patterns was inferred from changes in the hypophysial-portal concentrations of these peptides. Male rats (325 to 375 g) of the Sprague-Dawley strain (Charles River) were anesthetized with urethan (1.1 g per kilogram of body weight, intraperitoneally), pentobarbital (30 mg/kg intraperitoneally), or a mixture

Table 1. Effect of anesthetic agent on hypophysial-portal immunoreactive SRIF and systemic GH trough concentration in the rat.

Anesthetic agent	*n*	Immunoreactive SRIF (pg/ml)	GH (ng/ml)
Urethan	5	478 ± 49.2	3.6 ± 0.7
Ketamine:xylazine	5	164 ± 13.5	50 ± 6.9
Pentobarbital	5	61 ± 7.1	311 ± 28.4

of ketamine and xylazine (20:5 mg/kg intraperitoneally) with supplemental doses administered intravenously as necessary. Sampling and infusion catheters were inserted in the descending aorta and inferior vena cava via the femoral vessels. Animals were then placed in a stereotaxic frame, ventral surface up, for exposure of the infundibular region by a parapharyngeal approach (*14*). All points of pressure or incision were treated with local anesthetic to minimize pain. After surgery, the animals were removed from the stereotaxic frame and placed in a foam body cast to immobilize the head; a 60-minute postsurgical stabilization period was used. Body temperature was maintained at 37° ± 1.0°C throughout surgical and experimental procedures.

Before blood sampling was begun, rats were heparinized (400 IU, intravenously). Systemic blood samples were stored in cold polyethylene tubes containing 20 μl of a 100 m*M* EDTA solution. Portal samples were collected into a loop of polyethylene tubing immersed in an ice bath. After each collection period, blood was transferred to polyethylene tubes containing a solution (10 μl per 100 μl of blood) of 100 m*M* EDTA, 1000 $\times 10^3$ IU of Trasylol, and 10 m*M* Bacitracin. Samples were centrifuged, and plasmas were separated for storage at −20°C until assay.

Portal plasma samples were extracted on Bond Elut C18 cartridges (Analytichem, Harbor City, California) with 50 percent triethylammonium formate (TEAF) and 50 percent 2-propanol used for the elution of GRF (*15*) or 75 percent acetonitrile and 25 percent TEAF for the elution of SRIF. Recoveries of both peptides were >90 percent. Radioimmunoassay of serial dilutions of portal plasma extracts showed parallelism to authentic peptide standard dilutions. The minimum detectable concentrations of GRF and SRIF were 10 pg (1.43 fmol) and 2 pg (1 fmol), respectively. Intra- and interassay coefficients of variation were 8 percent and 13 percent for GRF, and 7 percent and 10 percent for SRIF, respectively.

Systemic blood samples were obtained from rats that had been prepared for portal collection to the point immediately prior to stalk transection; these blood samples were withdrawn from the arterial line at 20-minute intervals with simultaneous venous replacement of washed, resuspended red blood cells. Plasma GH levels, as determined by radioimmunoassay (*16*), are illustrated in Fig. 1, A through C. Episodic GH secretion, at levels four to five times the mean trough levels of 46 ± 10.7 ng/ml, occurred consistently between 1300 and 1500 hours and 1700 and 1800 hours in rats anesthetized with ketamine:xylazine (n = 7). The amplitude of these GH secretory episodes was moderately attenuated as compared to those in unanesthetized rats, while trough GH levels increased (*1, 6, 7*). Episodic GH secretion was not observed in rats anesthetized with urethan (n = 7) or pentobarbital (n = 6), an observation consonant with earlier reports (*17*).

Analysis of portal plasma samples, obtained from a separate series of rats in which the infundibular stalk had been transected and cannulated, yielded a nonlinear inverse relation between hypophysial-portal immunoreactive SRIF and systemic GH concentrations (Table 1). GRF-like immunoreactivity was undetectable in portal plasma obtained from rats anesthetized with urethan or pentobarbital. The mechanism or mechanisms by which these anesthetics interfere with the central regulation of GRF secretion is unknown. However, alterations in the activity of aminergic pathways (*18*) impinging on GRF-secreting neurons or an inhibitory effect of SRIF upon these peptidinergic cells (*19*) might be responsible.

In rats anesthetized with ketamine:xylazine, sequential 20-minute portal plasma samples revealed a consistent pattern of immunoreactive SRIF and immunoreactive GRF secretion. The presence of detectable immunoreactive GRF concentrations in portal plasma was normally observed only in collections obtained during periods of expected GH secretory activity. Mean portal immunoreactive GRF levels rose from <160 pg/ml to a mean peak concentration of 896 ± 65 pg/ml (n = 7) during one 20-minute collection period (Fig. 2A). Peak portal immunoreactive GRF concentration was comparable to the median effective concentration (EC_{50}) determined for synthetic rat GRF–stimulated GH secretion from cultured pituitary cells (*20*).

The mean immunoreactive SRIF concentration in portal plasma samples of rats anesthetized with ketamine:xylazine was 112 ± 9 pg/ml during periods of trough GH secretion. However, during

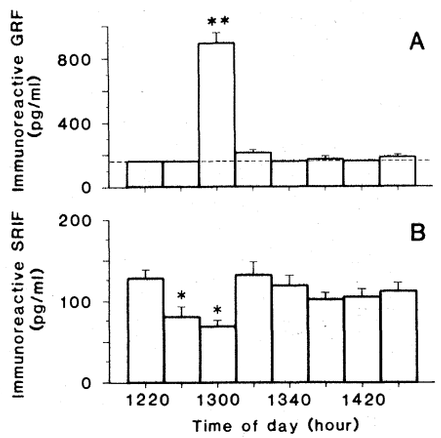

Fig. 2. Temporal profiles of immunoreactive GRF and immunoreactive SRIF concentrations in hypophysial-portal plasma of ketamine:xylazine–anesthetized rats. (A) Episodic secretion of immunoreactive GRF into hypophysial-portal plasma during a period of expected GH secretion (n = 7). The dashed line indicates the limit of detection (160 pg/ml). (B) Decrease in portal plasma concentration of immunoreactive SRIF during a period associated with an expected GH secretory episode (n = 7). Data were analyzed for significant overall effects by single factor analysis of variance corrected for repeated measures. Significant effects were evaluated by means of Duncan's new multiple range test. *, $P < 0.01$; **, $P < 0.001$ versus initial time sampled.

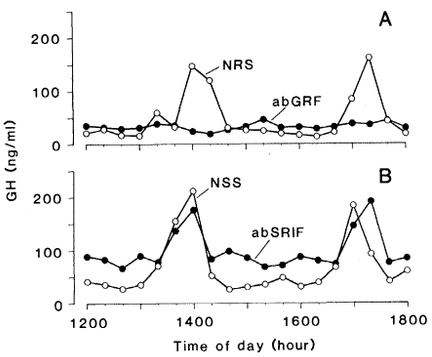

Fig. 3. Individual representative plasma GH profiles. (A) Suppression of episodic GH secretion after administration of antibody (ab) to GRF (antiserum rG71; 2.0 μl intracerebroventricularly; n = 5), but not normal rabbit serum (NRS; 2.0 μl intracerebroventricularly; n = 5). (B) Increased trough GH levels after passive immunoneutralization of SRIF with antibody (antiserum S-201, 2.0 μl intracerebroventricularly; n = 5), but not after administration of normal sheep serum (NSS; 2.0 μl intracerebroventricularly; n = 5).

collections coinciding with periods of expected GH secretory activity, hypophysial-portal plasma immunoreactive SRIF concentrations declined 37 ± 5 percent ($P < 0.05$) in two consecutive samples before returning to initial levels (Fig. 2B). Evidence of pulsatile immunoreactive SRIF secretion has been reported in rats bearing chronically implanted hypothalamic push-pull cannulas, although correlation with systemic GH levels was inconsistent (*10*).

Immunoneutralization of GRF activity by intracerebroventricular injection (*21*) of antiserum rG71 to synthetic rat GRF abolished episodic secretion of GH, whereas injection of normal rabbit serum was without effect (Fig. 3A). Portal plasma immunoreactive SRIF concentration and secretory pattern were unaffected by this treatment. These results suggest that hypophysiotropic SRIF secretion is independent of GRF modulation or that the site of GRF action on the SRIF system is inaccessible to the antiserum. Alternatively, administration of SRIF antiserum S-201 resulted in a mean 2.3-fold elevation of systemic trough GH concentration (32.1 ± 4.2 to 76.8 ± 6.9 ng/ml) with no significant alteration in peak episodic amplitude (Fig. 3B).

Intracerebroventricular administration of SRIF antiserum S-201, but not of normal sheep serum, was associated with a significant increase in the mean trough immunoreactive GRF concentration in hypophysial-portal plasma (Fig. 4). However, mean peak immunoreactive GRF amplitude was unaffected by SRIF antiserum S-201 (932 ± 88 pg/ml versus 794 ± 83 pg per milliliter of normal sheep serum). Electrical stimulation of the median eminence (*22*) in urethan-anesthetized rats consistently failed to evoke a significant increase of portal immunoreactive GRF levels in portal plasma. After systemic administration of SRIF antiserum S-486 (500 μl; $n = 4$), but not normal sheep serum (500 μl; $n = 4$), electrical stimulation was associated with a 9.5-fold increase of portal immunoreactive GRF to a mean level of 918 ± 103 pg/ml. These observations support the concept of central, SRIF-mediated inhibitory tone on pathways associated with the secretion of immunoreactive GRF.

In summary, we have developed an animal model in which hypophysiotropic regulation of episodic GH secretion may be directly examined. Our observations suggest the following: (i) hypophysial-portal immunoreactive SRIF and systemic GH concentrations are inversely correlated; (ii) immunoreactive GRF is secreted into the portal system in a strongly pulsatile fashion; (iii) secretion of immunoreactive SRIF into the portal circulation occurs in an oscillatory fashion that is 180° out of phase with respect to immunoreactive GRF secretion; and (iv) immunoreactive GRF secretion into the hypophysial-portal circulation occurs only in the presence of diminished immunoreactive SRIF secretion and thus may be directly or indirectly inhibited by immunoreactive SRIF. Within the constraints of our anesthetized rat model, it would appear that each GH secretory episode is initiated by a burst of immunoreactive GRF secretion into the hypophysial-portal system, which is preceded by or is concurrent with a moderate reduction of inhibitory tone normally maintained by immunoreactive SRIF. This pattern of secretion probably represents the dynamic interaction between SRIF and GRF at both the hypothalamic and the pituitary levels.

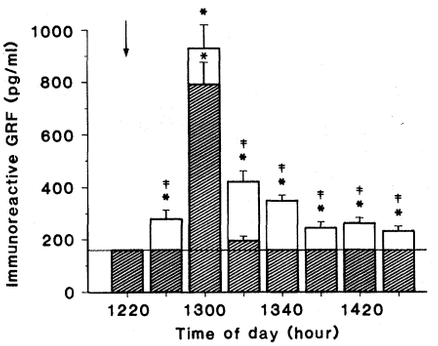

Fig. 4. Passive immunoneutralization of SRIF (open bars; SRIF antiserum S-201; 2.0 μl intracerebroventricularly; $n = 6$), but not treatment with normal sheep serum (striped bars; 2.0 μl intracerebroventricularly; $n = 5$), increased the trough hypophysial-portal immunoreactive GRF concentration without affecting the peak amplitude of the immunoreactive GRF pulse. The solid horizontal line represents the limit of detection (160 pg/ml); the arrow indicates the time of injection. Data were analyzed by means of multifactor analysis of variance corrected for repeated measures. Significant time ($P < 0.001$) and treatment ($P < 0.001$) effects were observed. Individual comparisons were made with Dunnett's test. *, $P < 0.01$ versus preinjection sample; ‡, $P < 0.01$ between treatment groups.

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22. Concentric bipolar stimulating electrodes (Rhodes Medical Electrodes) were placed in contact with the exposed region of the median eminence under microscopic control. Square-wave stimulation parameters were as follows: pulse duration, 0.2 msec; 100 μA; 60 Hz; 10 seconds "on"; 20 seconds "off" for a period of 45 minutes. Current and waveform were monitored on a storage oscilloscope throughout the stimulation.

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