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14. Experiments where the human serum concentrations were more than 50 percent are not reported because they produced agglutination of SRBC (despite repeated absorptions). However, purified human IgG at 4 mg/ml produced complete inhibition of lysis of EA-G but not of EAC-G.
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16. Antigen and antiserum were provided by Dr. Roy Hopfer. Soluble *Candida albicans* antigens were prepared by mechanically disrupting the organisms and discarding insoluble particles by centrifugation and filtration through a 0.22-μm Millipore filter. Rabbit antiserum was obtained after repeated immunizations with the antigen preparation in complete Freund's adjuvant. The rabbit antiserum was inactivated by heat and absorbed with SRBC before use. The antigen preparation was used at a 1 : 20 dilution, which was optimal for obtaining precipitation lines in counterelectrophoresis.
17. Spleen cells attached to plastic petri dishes treated with poly-L-lysine [J. C. Kennedy and M. A. Axelrod, *Immunology* **20**, 253 (1971)], were incubated with ^{51}Cr-labeled target cells (E, EA-G, EA-M, EAC-M, EAC-G) for 1 hour at 37°C in a rocking platform. Each petri dish was then washed three times with saline and immersed three more times in a beaker with saline. The target cells that remained attached to the spleen cells were lysed with distilled water, and radioactivity was measured in a well-type gamma counter [J. C. Scornik and B. Drewinko, *J. Immunol.* **115**, 1223 (1975)].
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Antiserum to Somatostatin Prevents Stress-Induced Inhibition of Growth Hormone Secretion in the Rat

Abstract. *Plasma growth hormone levels fall and remain low for several hours after stress in the rat. When antiserums to somatostatin are administered to rats prior to stress, growth hormone secretory pulses are partially restored. The results provide evidence that circulating somatostatin plays a prominent role in stress-induced inhibition of growth hormone secretion in the rat.*

Growth hormone (GH) secretion in the rat is characterized by pulsatile or episodic release (*1*). In freely behaving, nonstressed male rats surges of GH secretion occur at intervals of 3.2 to 3.4 hours, reaching plasma concentrations of 300 to 400 ng/ml (*2*). Between bursts, levels of GH are undetectable (< 1 ng/ml). The surges in GH are entrained to the light-dark cycle with major secretory episodes occurring at 1030 to 1230 hours and at 1330 to 1500 hours when lights turn on at 0600 hours (*2, 3*). Pulsatile GH surges are blocked by administration of somatostatin (GH-release inhibiting hormone), a tetradecapeptide isolated from the hypothalamus (*4*), and by bilateral lesions placed in the hypothalamic ventromedial nuclei (*1*).

Stress in the rat results in acute inhibition of GH secretion (*5*). The mechanism of this suppression is unknown, although it is hypothesized to be mediated by the hypothalamus (*5*). We now report that stress suppresses pulsatile GH release for at least 5 hours and that antiserum to somatostatin administered prior to stress partially restores the GH secretory pattern.

Male Sprague-Dawley rats (300 to 350 g) were prepared with permanent indwelling intra-atrial catheters and adapted to small isolation boxes to permit repeated blood sampling without disturbance to the animal (*1*). This experimental procedure is important since even minor stress, such as handling, inhibits GH secretion (*1, 6*). Cage adaptation was carried out by housing the animals in the isolation chambers for a period of 48 to 72 hours prior to sampling. All animals had reached their presurgical body weight before study. Each rat was given free access to water and Purina rat chow; the light-dark (LD) cycle was 12 : 12, with lights on from 0600 to 1800 hours. Blood samples (0.4 ml) were taken every 15 minutes from 0930 to 1500 hours to encompass two GH secretory episodes. The plasma was separated and frozen; red blood cells were resuspended in physiologic saline and returned to the animal after the removal of the next sample. This technique prevented a fall in hematocrit and allowed multiple samples to be taken without hemodynamic disturbance. Plasma GH was measured in duplicate samples by radioimmunoassay using materials supplied by the National Institute of Arthritis, Metabolism, and Digestive Diseases.

To develop a model to study the effects of stress on GH secretion, two different stressful conditions were used. In the first, the effect of an intraperitoneal injection was evaluated. Six normal rats were sampled for 5 hours (1000 to 1500 hours) on two separate occasions. Three of the animals were first sampled without handling and three were injected with 0.5 ml of physiological saline intraperitoneally at 0830 hours. The sequence was reversed 2 to 4 days later. The area encompassed by a single GH secretory episode was calculated by planimetry. In order to standardize the determination, a period of 3.5 hours was taken from the onset of each secretory episode. Both saline-injected (Fig. 1A) and control groups had a normal pulsatile pattern of GH release. However, animals that were injected had a significantly lower, mean integrated GH level ($P < .05$) compared to the noninjected group (62.9 ± 7.2 compared to 105.6 ± 12.5 ng/ml per 3.5 hours) (*7*) (Table 1). These results demonstrate that a minor stress can alter the subsequent secretion of GH without completely abolishing the pulsatile pattern.

In a second experiment, the effect of a more severe stress on GH secretion was assessed. After the baseline blood samples were obtained, each animal was removed from its isolation cage at 1000 hours, placed in a large water bath at 37°C and forced to swim for 30 minutes. Each rat was then returned to the isolation cage and sampled for 3.5 to 5.0 hours. Six control rats showed complete suppression of pulsatile GH secretion for up to 5.0 hours after stress, confirming previous reports (*5, 6*) that acute stress causes inhibition of GH in the rat and demonstrating that this suppression is due to abolition of pulsatile GH release.

If the GH suppression to stress is mediated by somatostatin, we hypothesized that its effects might be blocked or diminished by administration of a large dose of antiserum to somatostatin. Two different antiserums to somatostatin were used. One antiserum (AS 1) was generated in normal rabbits by injecting somatostatin bound to thyroglobulin, and the second (AS 2) by injecting somatostatin bound to bovine serum albumin. At a dilution of 1 : 2000 both antiserums bound more than 50 percent of ^{125}I-labeled tyrosine-1 somatostatin and at a dilution of 1 : 300 they bound more than 95 percent. The antiserums were specific for somatostatin and showed no cross-reactivity to thyrotrophin releasing hormone, lute-

Table 1. Integrated plasma GH levels in nonstressed, saline-injected animals and in stressed rats injected with antiserum to somatostatin.

Experimental group	N	Serum GH (ng/ml per 3.5 hours)*
Nonstressed	6	105.6 ± 12.5†
Saline-injected	6	62.9 ± 7.2‡
Stressed + normal rabbit serum	6	7.6 ± 1.3
Stressed + antiserum to somatostatin	6	25.5 ± 5.5§

*Derived by calculating area under secretory curve by planimetry. †Mean ± S.E.M. ‡$P < .05$ compared to nonstressed rats. §$P < .01$ compared to controls treated with normal rabbit serum; $P < .005$ compared to saline-injected rats.

inizing hormone releasing hormone, and various other small peptides. Three rats were given 1.0 ml each of AS 1 and three were given 1.0 ml of AS 2 intravenously through the cannulae at 0830 hours. Control rats received normal rabbit serum. Each animal was placed in the swimming tank from 1000 to 1030 hours and blood samples were taken every 15 minutes from 0930 to 1500 hours.

Plasma GH levels were low (< 10 ng/ml) in all animals prior to stress. Each of the six animals given antiserums showed a partial restoration of pulsatile GH secretion to above baseline levels within 15 minutes to 2.5 hours after stress (Fig. 1C). In contrast, the serum control group showed complete suppression of GH throughout the sampling period (Fig. 1B). Over the 3.5-hour period, the mean integrated GH levels of animals given the antiserums were significantly greater ($P < .01$) than that of the control serum group (25.5 ± 5.5 as compared to 7.6 ± 1.3 ng/ml). The mean integrated GH levels of the group treated with antiserums to somatostatin were significantly lower ($P < .005$) than those of the saline-injected group (Table 1).

Somatostatin is present not only in the hypothalamus, but also in extrahypothalamic brain regions and in pancreas, stomach, and intestine (*8*). Administration of pharmacologic doses of somatostatin inhibits GH in animals (*9*) and man (*10*) and also suppresses glucagon, insulin, secretin, and gastrin secretion (*11*). To qualify as a hormone, the peptide must be demonstrated to have physiologic effects and to circulate in the blood. Our findings that two different antiserums partially restore the pulsatile pattern of GH secretion after stress provide compelling evidence that somatostatin plays a prominent role in stress-induced inhibition of GH in the rat and suggest that this effect is due to circulating somatostatin. The fact that plasma GH levels before stress were not increased by the antiserum indicates that normal low levels of GH observed between bursts of secretion are not due to somatostatin. We believe this suggests that normal pulsatile surges of GH are due to hypothalamic release of GH releasing factor.

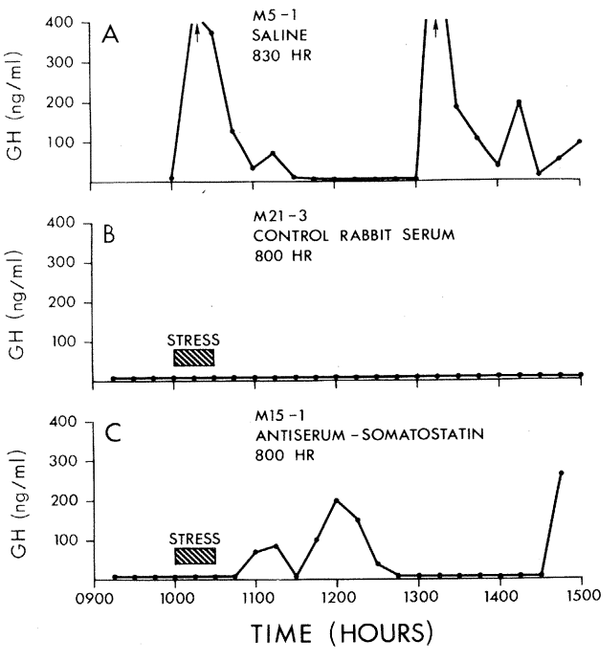

Fig. 1. Growth hormone secretion in individual unanesthetized rats. (A) Plasma GH levels show typical pulsatile pattern in saline-injected rat. (B) Stress causes complete suppression of pulsatile GH secretion in animals first treated with normal rabbit serum. Growth hormone levels remain low (< 10 ng/ml) for 4.5 hours after stress. (C) Partial restoration of GH pulses in rats treated with antiserum to somatostatin. Two distinct GH surges are evident: 15 minutes and 225 minutes after stress. Values of plasma GH are expressed in terms of the rat GH reference preparation No. 1 supplied by National Institute of Arthritis, Metabolism, and Digestive Diseases.

Note added in proof: Two short communications that also indicate an effect of antiserums to somatostatin on GH secretion have appeared after submission of this report. Ferland *et al.* (*12*) reported that plasma GH levels in the rat rose two- to threefold after injection of antiserum to somatostatin without any effect on the pulsatile pattern. Arimura *et al.* (*13*) found significantly higher levels of plasma GH 30 minutes after electroshock in animals first treated with antiserum to somatostatin.

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Sibling Species in the Marine Pollution Indicator *Capitella* (Polychaeta)

Abstract. *Electrophoretic patterns for eight enzyme loci clearly distinguish six sibling species in the well-known pollution indicator worm,* Capitella capitata. *Unlike sibling species of* Drosophila, *the* Capitella *species have virtually no alleles in common. Close examination has revealed only slight morphological differences between species, while life histories and reproductive modes are very distinct. The* Capitella *species are ideally suited for genetic and evolutionary studies.*

The polychaete worm *Capitella capitata* has been regarded as an excellent indicator of pollution or environmental disturbance (*1*). This so-called cosmopolitan species has been found in every part of the world where marine benthic studies have been conducted. Our results show that it is, in fact, a complex of as many as six sibling species.

We have examined life histories, reproductive modes, and morphologies of this worm, and the electrophoretic patterns for the following enzymes: phosphohexose isomerase (PHI), xanthine dehydrogenase (XDH), phosphoglucomutase (PGM), isocitrate dehydrogenase (IDH-1 and IDH-2), malate dehydrogenase (MDH-1 and MDH-2), and α-glycerophosphate dehydrogenase (α-GPDH) (*2*). Samples of five *Capitella* populations in the vicinity of Woods Hole, Massachusetts, and two populations from Gloucester, Massachusetts, collected at intervals for more than a year, consist of six sibling species. Although allelic variation occurs within each species, we have not detected any gene exchange between species. Table 1 lists morphological and life history features of the six species. It is apparent that the morphological differences are slight and that there is some overlap in life histories. However, considering the eight genetic loci listed above, only two alleles are found in common between any pair of species—the monomorphic PGM in species I and Ia, and the monomorphic MDH-1 in species II and IIa (*3*). More limited results on two additional loci (a single esterase and a single leucine aminopeptidase locus) also show no alleles in common between the species. In Table 1 the species have been numbered according to the relative electrophoretic mobilities of their most common PHI alleles, with bovine serum albumin (1.0) as a standard: species I (0.48); Ia (0.50); II (0.29); IIa (0.36); III (0.18); and IIIa (0.16). In Table 2 the mobilities of all PHI alleles relative to species I PHI (the most common allele) in a tris-maleate buffer system are given. There are no alleles in common between any two species.

The individuals of all six species have been maintained in the laboratory in standing seawater at constant temperature, and azoic mud was provided as food. Species I and II are ideal laboratory animals in that they have a short generation time (30 to 40 days at 20°C, depending on the abundance of food); we have now carried them through many generations. The genetic systems at the eight loci have been confirmed for these two species by comparing the electrophoretic mobilities of enzymes in parents and offspring from a number of crosses. All the differences between species were consistent, whether the individuals were brought directly from the field or reared under constant laboratory conditions for several generations. We have not found any evidence for environmen-

Table 1. Features of morphology and life history in six sibling species of *Capitella*. There are three thoracic setigers with capillary setae in species III [compare, *Capitellides jonesi* Hartman 1959 (*14*)], and seven in the other species. In the formulas for teeth above the main fang of the hooded, abdominal hooked setae, the first row of teeth is immediately above the fang. Abbreviations: P, protandrous; ♂, male; ♀, female; ☿, hermaphrodite.

Species	Head		Tail		Teeth above fang		Weight of worms* (mg)	Sex	Eggs (*6*)		Larvae in plankton
	Prostomium	Peristomium	Shape	Dorsal cleft	Row	Formula			Diameter (μm)	No./brood	
I	Broad, triangular	Short	Plain	Absent	3 2 1	3 to 5 5 5	3 to 12	♂,♀,P☿†	260 by 180	30 to 400	Several hours
Ia	Sharply conical	Partly fused	Plain	Absent	3 2 1	0 to 5 4 to 6 5	10.1	♂,♀,?☿	75	200 to 2000	Several days
II	Long, triangular	Partly fused	Lobed	Present	3 2 1	0 to 3 3 to 4 4	12.0	♂,♀,P☿	230	30 to 400	6 to 24 hours
IIa (*15*)	Like II	Like II	Plain	Absent			11.9				
III	Broad, triangular	Distinct, very long	Flared	Present	3 2 1	3 to 4 4 to 5 4 to 5	1 to 4	P☿	50	200 to 1000	≤ 2 weeks
IIIa	Broad, triangular	Partly fused	Lobed	Present			1.7	♂,♀	250	30 to 50	None

*Average wet weight. †Occasional self-fertilization.