Insulin-Like Growth Factors: A Role in Growth Hormone Negative Feedback and Body Weight Regulation via Brain

Abstract. *Intracerebroventricular administration of ILA's, a preparation enriched in insulin-like growth factors, caused a marked decrease in growth hormone secretory episodes and in body weight associated with reduced food intake over 24 hours. Central injection of insulin and bovine serum albumin had no such effects. These findings suggest that insulin-like growth factors play a role in growth hormone negative feedback and body weight regulation at the level of the central nervous system.*

It is currently believed that central nervous system (CNS) control of growth hormone (GH) secretion is achieved by way of the complex interaction of two hypothalamic hormones—a GH-releasing factor, GRF, and a GH-release inhibiting factor, somatostatin (*1*). In addition, we have demonstrated that GH itself can regulate its own secretion via a "short-loop" negative feedback system at the level of the CNS (*2*). While the regulation of most anterior pituitary hormones by specific peripheral target gland hormones occurs by way of a classic "long-loop" feedback mechanism (*3*), such a mechanism has not been apparent for GH.

Although GH does not stimulate the secretion of a specific endocrine product from a distinct endocrine target organ, it has been postulated that many of the growth-promoting peripheral actions of GH are mediated by the somatomedins (*4*). Somatomedin is a general term for a family of peptides, under GH control, that exhibits growth-promoting and insulin-like activities on several tissues (*5*). Several somatomedins have been identified as insulin-like growth factors (IGF's) (*6*); however, the role of these substances in GH regulation is unknown. In the study described here we assessed the involvement, at a CNS level, of ILA's, a preparation enriched in IGF's (*7*), in feedback regulation of pulsatile GH secretion. Because there appears to be a relation between IGF's and nutritional status (*8*), we also examined the effects of the central administration of ILA's on food intake and body weight. Our results indicate that IGF's participate in a GH negative feedback system at the level of the brain, and suggest that this family of peptides plays a role in maintenance of body weight and nutritional homeostasis at a CNS level.

We studied two preparations of ILA's that had been purified according to their insulin-like activity in a placental insulin radioreceptor assay (RRA) (*7, 9*). The potency of ILA's is reported as nanogram equivalents of porcine insulin as determined in the insulin RRA. Sephadex-ILA's were prepared from acid-ethanol extracts of Cohn fraction IV-4 (*7*) and were enriched in neutral to slightly acidic IGF's. Carboxymethyl cellulose (CMC)-ILA's refers to ILA's that were further purified by CMC ion exchange chromatography and contained primarily neutral IGF material. Both preparations were utilized on the basis of their biologic potency in the insulin RRA. Sephadex-ILA's were administered at a dose of 106 ngEq in 10 μl of normal saline; the dose of CMC-ILA's was approximately equivalent at 112 ngEq/10 μl.

Adult male Sprague-Dawley rats (275 to 350 g) were implanted with intracerebroventricular and intracardiac venous cannulas as described (*2*). After surgery the animals were placed directly in isolation test chambers and given free access to Purina Rat Chow and tap water until their body weights returned to preoperative levels. During this time (usually 1 week) the rats were handled daily to minimize any stress associated with handling on the day of the experiment.

In the first experiment, with five groups of rats, food was removed and a blood sample was obtained at 1000 hours; immediately afterward, 10 μl of Sephadex-ILA's (N = 4) or CMC-ILA's (N = 6) was injected slowly over 15 seconds into the left lateral brain ventricle. Three control groups received, respectively, 10 μl of normal saline (N = 8); bovine serum albumin (BSA; Sigma) in a dose equivalent to the protein content of the CMC-ILA's, 27 μg (N = 6), or porcine insulin (Connaught Laboratories), 10 μg (N = 7). Blood samples (0.45 ml) were withdrawn every 15 minutes for periods of 6 hours (1000 to 1600 hours) from all rats. All blood samples were immediately centrifuged and the plasma separated and stored at −20°C for subsequent radioimmunoassay of GH and insulin (*10*). To prevent hemodynamic disturbance, we resuspended the red blood cells in 0.25 ml of isotonic saline and returned them to the animal after removal of the next blood sample. Analysis of variance for repeated measures, followed by Scheffé's test (*11*), was used to compare data for significant differences between groups.

Intracerebroventricular administration of normal saline did not alter the typical

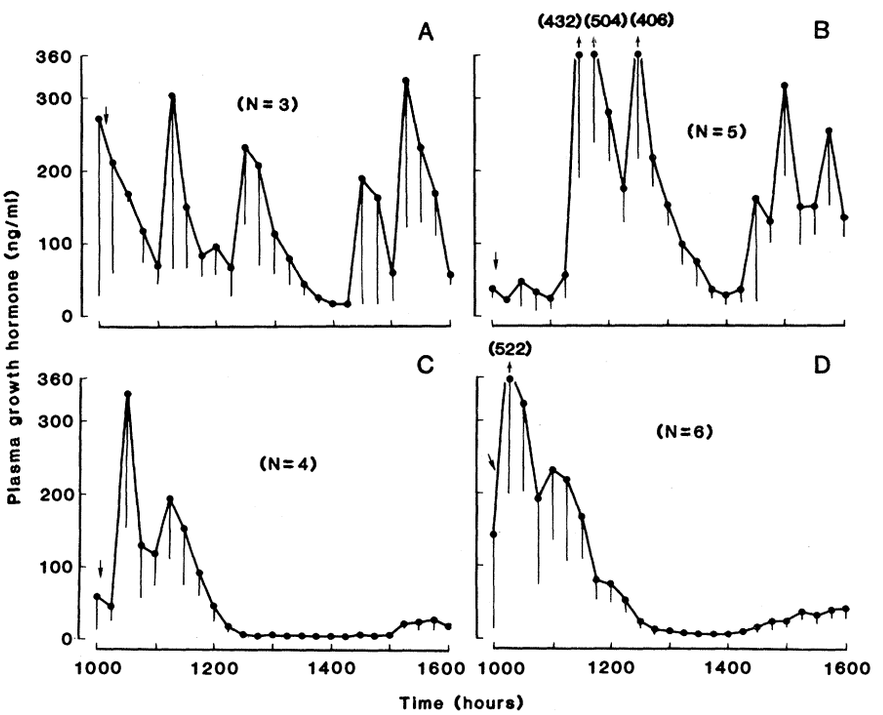

Fig. 1. Effect of intracerebroventricular administration of (A and B) normal saline, (C) Sephadex-ILA's, and (D) CMC-ILA's on mean plasma GH concentrations over 6 hours. Central administration of the ILA preparations suppressed the amplitude of GH secretory bursts after about 2 hours and plasma GH remained suppressed for up to 6 hours after injection. Arrows indicate time of injection; vertical lines represent standard errors of the mean. The number of animals in each group is shown in parentheses.

Table 1. Plasma GH and insulin concentrations 2 to 6 hours after the intracerebroventricular administration of ILA's. Results are expressed as means (± standard error of the mean).

Group	*N*	Plasma GH (ng/ml)	Plasma insulin (ng/ml)
Normal saline	8	146.1 ± 16.4	0.51 ± 0.06
ILA's (CMC preparation; 112 ngEq/10 μl)	6	26.9 ± 6.5*	0.42 ± 0.04
ILA's (Sephadex preparation; 106 ngEq/10 μl)	4	15.2 ± 2.9*	0.94 ± 0.26
BSA (27 μg/10 μl)	6	104.5 ± 16.5	0.43 ± 0.06
Porcine insulin (10 μg/10 μl)	7	108.2 ± 6.0	0.86 ± 0.14

*Significantly different from all other groups, $P < .01$, as determined by analysis of variance and Scheffé's test for multiple comparisons.

Table 2. Effects of central administration of ILA's on food intake and body weight. Results are expressed as means (± standard error of the mean).

Group	*N*	Change over 24 hours		
		Food intake (g)	Body weight	
			Grams	Percentage
Normal saline	5	37.0 ± 3.6	+4.6 ± 0.8	+1.3
ILA's (106 ngEq/10 μl)	5	12.6 ± 2.6*	−29.6 ± 2.8*	−8.1*
BSA (27 μg/10 μl)	4	31.2 ± 2.7	+2.2 ± 1.8	+0.7
Porcine insulin (10 μg/10 μl)	5	28.6 ± 0.7	+1.3 ± 0.7	+0.4

*Significantly different from all other groups, $P < .01$, as determined by analysis of variance and Scheffé's test for multiple comparisons.

pulsatile pattern of GH secretion previously observed in normal, freely moving rats (*12*). Two major episodes of GH secretion were evident during a 6-hour sampling period, with most peak GH values exceeding 500 ng/ml (Fig. 1, A and B). Central administration of both preparations of ILA's suppressed the amplitude of the GH secretory bursts after about 2 hours, and plasma GH remained suppressed for up to 6 hours after injection (Fig. 1, C and D). Peak GH values did not exceed 90 ng/ml (range 13 to 90 ng/ml) during the latter half of the sampling period, which was in contrast to the normal saline–treated animals whose peak GH values ranged from 230 to 764 ng/ml during this time.

Analysis of the time course of the effect of ILA's revealed no significant difference in mean plasma GH concentrations between ILA-treated and saline-treated groups during the first 2 hours after injection. However, mean plasma GH concentrations were significantly depressed during the remaining 4-hour sampling period (Table 1). The finding that the ability to suppress GH release was very similar for the CMC-ILA's and Sephadex-ILA's despite an approximately threefold greater purity of the former suggests that the biological activity inheres in the ILA's.

The time course of the suppressive effect was further assessed by sampling animals 24 hours after the administration of ILA's. Restoration of high-amplitude GH secretory bursts was evident at this time indicating that the duration of the suppression was between 6 and 24 hours. The specificity of the GH response to ILA's is indicated by the findings that neither BSA, a protein control, nor porcine insulin, another growth factor not directly stimulated by GH, significantly altered plasma GH concentrations (Table 1). Furthermore, the ILA preparations had no significant effect on plasma insulin concentrations (Table 1). These data suggest that IGF's modulate the hypothalamic-pituitary GH axis.

In a second study we used four groups of rats (350 to 425 g) to assess the role of ILA's in nutritional regulation. Over a 24-hour period we measured the intake of pelleted Purina Rat Chow and the changes in body weight after the intracerebroventricular administration of ILA's (106 ngEq/10 μl), normal saline, BSA (27 μg/10 μl), or porcine insulin (10 μg/10 μl). Central administration of ILA's reduced food intake to approximately one-third that of the normal saline controls, whereas both BSA and porcine insulin had no significant effect (Table 2). Body weight decreased in parallel to the reduction in food intake. Rats that received ILA's showed a loss in body weight (8 percent), whereas rats in the control groups gained weight (Table 1). The behavior of the animals was observed for 6 hours after injection, and we found no deficits in motor coordination. Furthermore, plasma prolactin (*13*), a sensitive monitor of stress in the rat (*14*), did not increase significantly in the ILA-injected rats (mean prolactin concentration over 6 hours, 1.24 ± 0.13 ng/ml, compared with 2.22 ± 0.35 ng/ml in the controls; not significant by Student's *t*-test).

To determine whether the anorectic effect of the centrally administered ILA's was due to illness or aversive consequences, we used a conditioned aversion paradigm that provides a measure of the aversive effects of drugs or other treatments (*15*). Additional rats were given access to tap water for only 20 minutes per day for five consecutive days. On day 6, immediately after their first access to a 0.2 percent sodium saccharin solution, the rats received an intraperitoneal injection (20 ml/kg) of either 0.15*M* lithium chloride ($N = 6$) or sodium chloride ($N = 5$). We observed a 57 percent reduction in drinking of the saccharin solution 48 hours after injection of lithium chloride (6.6 ± 1.9 ml compared with 15.3 ± 2.3 ml; by Student's *t*-test, $P < .05$). An attempt to use centrally administered ILA's as the aversive stimulus was unsuccessful. Rats that received ILA's (106 ngEq/10 μl) ($N = 5$) after their first exposure to saccharin subsequently drank amounts of this solution that were not statistically different from the amounts consumed by animals injected intracerebroventricularly with normal saline ($N = 6$) (12.1 ± 2.7 ml compared with 14.7 ± 1.6 ml; $t = .85$, $P > .2$). Thus the ILA-induced reduction in appetite appears not to be the result of illness or aversive effects of the peptide, but to be a more specific effect of the preparation.

Our results indicate that IGF's, or somatomedins, may participate in a GH negative feedback system at the level of the CNS. The same effect was not seen with BSA or a large dose of porcine insulin. Clinical observations are consistent with the hypothesis that IGF's play a role in GH negative feedback. In Laron dwarfism, where somatomedin generation is lacking, increased concentrations of GH are present (*16*), whereas in kwashiorkor, low concentrations of somatomedin coexist with high concentrations of GH (*17*).

The mechanism whereby IGF's inhibit GH release is of interest. Studies by Berelowitz *et al.* (*18*) show that a somatomedin, SM-C, directly stimulates somatostatin release from rat hypothalami in culture. Their findings, together with the results presented here, suggest that IGF's are involved in a GH negative feedback loop at a CNS level via the inhibitory mechanism of hypothalamic somatostatin release. These observations do not exclude the possibility that

IGF's have a simultaneous inhibitory effect on GRF. Furthermore, in view of recent reports demonstrating that centrally administered peptides rapidly enter peripheral blood (*19*), it is possible that intracerebroventricularly administered ILA's also feed back at the pituitary level by transport from cerebrospinal fluid to anterior pituitary.

In our experiments, central injection of ILA's caused a diminution in body weight associated with decreased food intake, but large doses of insulin did not have a similar effect, suggesting that the response is mediated by somatomedin receptors rather than insulin receptors in the CNS. This finding is not at variance with that of an earlier study (*20*) suggesting a role for centrally administered insulin in the control of appetite since, in that study, animals received insulin infused centrally over a 14-day period and showed no significant effects after 24 hours. Since many studies indicate that somatomedins are depressed in undernutrition (*8, 17*), our findings raise the possibility that IGF's participate in the regulation of feeding and appetite and suggest that IGF's should be further investigated in human obese states. Our observations should be confirmed with more highly purified somatomedins before the IGF's are included in the ever-growing list of putative satiety factors (*21*).

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Trypsin Inhibition by Tapeworms: Antienzyme Secretion or *p*H Adjustment?

Abstract. *The tapeworm* Hymenolepis diminuta *releases proteins that inhibit trypsin activity. These proteins may be either antienzymes or nonspecific macromolecules that interfere with trypsin. Saline solutions with initial* p*H values ranging from 5.5 to 10.0 were all acidified to* p*H 5.0 by tapeworms. If the initial* p*H was lower than 5.0, it was raised. Because trypsin activity is inhibited at* p*H 5.0, this intestinal parasite can protect itself from digestion by regulating its environmental* p*H or releasing trypsin inhibitors, or both.*

It is not clear how intestinal parasites such as tapeworms live and thrive in an environment of hydrolytic enzymes without being digested. One hypothesis is that they produce and release hydrolase inhibitors—the so-called antienzymes (*1*). We report that *Hymenolepis diminuta* releases proteins that appear to inhibit trypsin activity. These proteins could be antienzymes or nonspecific macromolecules that interfere with trypsin activity or assays. This tapeworm can also regulate the *p*H of its environment to about 5.0 by excreting organic acids. Because trypsin activity is minimal at *p*H 5.0, this endoparasite can protect itself from trypsin digestion by acidifying its environment. Indeed, the rat intestine is more acidic when *Hymenolepis* is present (*2*).

Tapeworms were obtained from young rats infected in the laboratory and washed in buffered Krebs-Ringer saline (*3*). Groups of six to eight worms were incubated at 37°C in 10 ml of saline for 15 minutes, transferred to 5 ml of fresh saline for further incubation at 37°C for various periods, and removed. Replicate samples of the conditioned saline were pooled (total volume, 15 ml), and the *p*H was readjusted to 7.4 (All *p*H adjustments were made by appropriate additions of HCl or NaOH.) Samples of the pooled saline were used for measuring protein (by the Lowry method) and trypsin inhibitory activity, with azoalbumin used as the substrate for trypsin (*4*). The buffer, tris(hydroxymethyl)aminomethane maleate, was replaced isosmotically with NaCl when unbuffered saline was used.

Buffered saline (initial *p*H 7.4) conditioned with tapeworms for 2 hours contained both Lowry-positive material (95 μg/ml) and trypsin inhibitory activity. Trypsin digestion of azoalbumin was 16 percent less in conditioned saline than in control saline. Both Lowry-positive material and trypsin inhibitory activity appeared during the second 30 minutes of incubation and increased progressively for up to 2 hours. Dialysis (cutoff, 3500 daltons) of conditioned saline adjusted to *p*H 7.4 against 3 liters of buffered saline at *p*H 7.4 and 4°C rapidly removed Lowry-positive material. After 5 hours of dialysis 65 percent of the Lowry-positive material was removed; a maximum of 75 percent was removed after 16 hours. However, even after 48 hours of dialysis there was no significant decrease in trypsin inhibitory activity in the nondialyzable fraction. After a 5-minute exposure of this fraction to 95°C, 50 percent of the