served with opioids might be explained by their ability to interact with the various receptor classes. The ability of naloxonazine to effectively decrease salivation and penile discharge as well as analgesia without affecting the other signs of dependence supports this concept. In addition, the dissociation by naloxonazine of receptor mechanisms mediating analgesia from those involved with many aspects of physical dependence raises the possibility of synthesizing selective analgesics with little dependence liability.

GEOFFREY S. F. LING
JANET M. MACLEOD
SHAY LEE
STEPHEN H. LOCKHART
GAVRIL W. PASTERNAK*

Laboratory of Neuro-Oncology and *Laboratory of Biostatistics, Memorial Sloan-Kettering Cancer Center,* and *Departments of Neurology* and *Pharmacology, Cornell University Medical College, New York 10021*

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4. Under enflurane anesthesia, a cannula connector pedestal (Plastic Products) was affixed to each rat's cranium with two 1-mm mounting screws and plastic cement. For drug administration, a metal-jacketed catheter was attached to the pedestal and connected to the jugular vein cannula. The other end of the catheter was passed through the top of the cage, where it was attached to a swivel (Alice King Chatam Medical Arts). An infusion line was passed through the swivel to a multichannel peristaltic infusion pump (Manostat).
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7. Twelve quantal and five graded signs were observed. The quantal signs were ear blanching, irritability, ptosis, tachypnea, teeth chattering, salivation, penile discharge, diarrhea, defecation, exophthalmos, tearing, and rhinorrhea. The graded signs were wet-dog shakes, exploratory rearing, self-stimulation, escape attempts, and writhing. Irritability is defined as struggling or screaming when handled during weighing. Defecation is a formed stool. Penile discharge is a red watery fluid. The remaining quantal and graded withdrawal signs have been detailed elsewhere [G. S. F. Ling, N. S. Tappe, C. E. Inturrisi, *Life Sci.* **34**, 683 (1984); W. R. Martin, A. Wikler, C. G. Eades, F. T. Prescor, *Psychopharmacologia* **4**, 247 (1963); D. G. Teiger, *J. Pharmacol. Exp. Ther.* **190**, 408 (1974); J. R. Weeks, *Science* **138**, 143 (1962); T. Akera and T. M. Brody, *Biochem. Pharmacol.* **17**, 675 (1968); M. Nozaki, *Psychopharmacologia* **47**, 225 (1976); E. Wei, H. H. Loh, E. L. Way, *J. Pharmacol. Exp. Ther.* **184**, 398 (1973); J. Blasig, A. Hertz, K. Reinhold, S. Ziegelgansberger, *Psychopharmacologia* **33**, 19 (1973); E. Wei, *ibid.* **28**, 35 (1973); T. J. Cicero and E. R. Meyer, *J. Pharmacol. Exp. Ther.* **184**, 404 (1973); M. Nozaki, T. Akera, C. H. Lee, T. M. Brody, *ibid.* **192**, 506 (1975)].
8. A comparison of the proportion of animals exhibiting each of the 12 quantal and 5 graded withdrawal signs between groups was made with the Fisher Exact Test. The frequency of graded signs among groups was compared with the Kruskal-Wallis test. As a nonparametric test, this procedure is not dependent on underlying distribution assumptions. Pairwise multiple comparisons were performed with multiple two-sample tests adjusted by the Bonferroni inequality.
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11. We thank K. Spiegel, J. Posner, R. Houde, C. E. Inturrisi, and R. Hawks for their advice and assistance with these studies. This work was supported by grant DA 002615 from the National Institute on Drug Abuse and grant PDT 169 from the American Cancer Society. G.S.F.L. is supported by fellowship DRG-564 from the Damon Runyon–Walter Winchell Cancer Fund. G.W.P. is a recipient of a Teacher Investigator Award (NS 00415) from the National Institute of Neurological and Communicative Disorders.
* To whom requests for reprints should be addressed.

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Growth Hormone–Releasing Factor: Direct Effects on Growth Hormone, Glucose, and Behavior via the Brain

Abstract. *Intracerebroventricular administration of human pancreatic growth hormone–releasing factor caused a dose-dependent inhibition of growth hormone secretion, elevated plasma glucose concentrations, and produced marked behavioral and motor effects. Immunoneutralization with antiserum to somatostatin did not reverse the suppression of growth hormone. These findings suggest that hypothalamic growth hormone–releasing factor may regulate its own neurosecretion through an "ultrashort-loop" negative feedback mechanism and may have important neurotransmitter and neuromodulatory functions in the brain.*

Peptides with high growth hormone (GH)–releasing activity were recently isolated from two human pancreatic islet cell tumors (*1*). Synthetic replicates of these peptides are potent and specific stimulators of pituitary GH release when administered systemically (*2*) and are indistinguishable in biological activity from the GH-releasing factor (GRF) present in human and rat hypothalamus (*3*). There is evidence that the hypothalamic releasing and inhibiting hormones not only regulate endocrine function of the adenohypophysis but also exert nonendocrine actions in the central nervous system (CNS) (*4*).

I report that intracerebroventricular administration of the 44-amino-acid peptide, human pancreatic GRF (hpGRF), severely suppresses GH release, elevates plasma glucose concentrations, and produces marked behavioral and motor effects. The findings suggest that hypothalamic GRF may regulate its own neurosecretion through an "ultrashort-loop" feedback mechanism and that it may, in addition to its endocrine role as a hypophysiotropic hormone, regulate glucose and behavior by direct action on the brain.

Adult male Sprague-Dawley rats (300 to 350 g) were implanted with intracerebroventricular and intracardiac venous cannulas (*5*). After surgery the animals were placed in isolation chambers (lights on between 0600 and 1800 hours) and given unlimited Purina Rat Chow and 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 test. In the first experiment, with six groups of rats, a baseline blood sample was obtained at 1000 hours; immediately afterward the rats were administered 10 μl of hpGRF at various doses or normal saline through the left lateral ventricle of the brain. The hpGRF had been synthesized by solid-phase techniques (*6*) and diluted in normal saline to attain concentrations of 10, 5, 2.5, 1.25, and 0.5 μg per 10 μl.

Blood samples were withdrawn 5 minutes after the injection and subsequently every 15 minutes for 6 hours (1000 to 1600 hours). All samples were immediately centrifuged and the plasma was separated and stored at −20°C for subsequent assay of GH, prolactin, and glucose (*7*). The apparatus used allowed the animals to behave freely during the removal of blood samples. After the injections, behavior and motor control were continuously monitored through a one-way observation port. The results were evaluated by analysis of variance for

repeated measures and by linear regression analysis (*8*).

Figure 1 illustrates the effects of various doses of hpGRF on mean plasma GH concentrations over 6 hours. Saline-treated rats ($n = 5$) exhibited the typical pulsatile pattern of GH secretion (*9*), with most individual peak GH values exceeding 600 ng/ml. Central administration of 10 μg of hpGRF ($n = 5$) sharply suppressed the amplitude of GH surges almost immediately, and plasma GH concentrations remained severely suppressed for up to 6 hours (mean 6-hour plasma GH concentration: 23.8 ± 3.2 and 141.1 ± 21.2 ng/ml for experimental and control animals, respectively; $P < 0.001$). The suppressive effect was dose-dependent, with near normal GH concentrations and secretion patterns being observed after administration of 0.5 μg (Fig. 1). There was a log-linear relation between mean 6-hour plasma GH and hpGRF concentration described by the equation $y = -9.12x + 103.75$ ($r = -0.91$; $P < 0.01$). Specificity of the GH response to hpGRF is indicated by the finding that hpGRF (10 μg) had no significant effect on plasma prolactin. Further evidence for specificity is that central administration of a large amount of protein or of another hormone (insulin) not directly involved in GH regulation does not significantly alter pulsatile GH secretion (*10*).

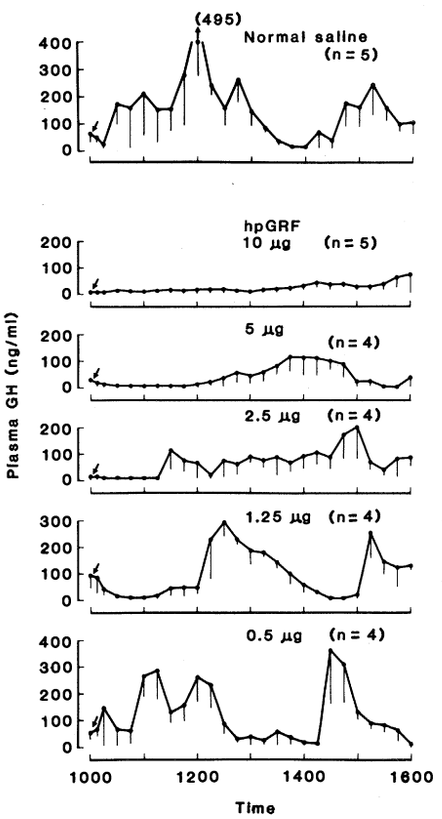

Fig. 1. Effect of intracerebroventricular administration of various doses of hpGRF on GH secretion. The peptide caused a dose-dependent suppression in the amplitude of spontaneous bursts of GH secretion. Arrows indicate times of injection; vertical lines represent standard errors of the mean. The number of animals in each group is shown in parentheses.

One mechanism whereby centrally administered GRF might cause GH suppression by the pituitary would be stimulation of the hypothalamic GH release–inhibiting factor, somatostatin (*11*), into the hypophyseal portal circulation. To examine this possibility, we used the technique of passive immunization with a specific antiserum to somatostatin (*12*). Two groups of five rats each were administered 10 μg of hpGRF through the brain and then 1 ml of antiserum to somatostatin or normal sheep serum intravenously. The antiserum failed to restore the amplitude of the GH surges or to significantly alter the suppressed plasma GH profile over 6 hours. It is unlikely that inadequate immunoneutralization was responsible for the lack of reversal of GH surges, since analysis of the plasma of antiserum-treated rats (*12*) revealed that significant titers of antibody to somatostatin were present for the entire 6-hour period (mean binding of a 1:100 dilution of plasma 1 minute before and 15 minutes, 3 hours, and 6 hours after treatment with antiserum to somatostatin, 1.3 ± 0.2, 46.6 ± 5.9, 45.8 ± 2.3, and 40.2 ± 0.3 percent, respectively).

It appears therefore that increased release of somatostatin is not the mechanism whereby hpGRF mediates suppression of GH. Rather, the results suggest that hypothalamic GRF inhibits its own neurosecretion through an ultrashort-loop negative feedback mechanism, thus removing GRF-induced stimulation of GH release—although the possibility that hpGRF causes the release of another substance that inhibits GH cannot be dismissed. The presence of recurrent inhibition in the tuberoinfundibular system, whereby axon collateral terminals of peptidergic neurosecretory neurons terminate directly or indirectly on their cells of origin to inhibit their firing, is well documented (*13*). This could provide the pathway for ultrashort-loop feedback of GRF, a concept originally postulated for hypothalamic releasing factors by Motta *et al.* (*14*) and recently proposed for somatostatin (*15*). Such a mechanism might function in the mediation of the phasic or rhythmic nature of the GH secretory profile, in which bursts of GH secretion are evident only at distinct intervals and plasma GH concentrations are undetectable between surges (*9*). Disruption of this feedback regulation of GRF could play a role in the pathogenesis of pituitary disorders (such as acromegaly) in which patients exhibit elevated plasma GH (*16*).

Compared to baseline and saline control values, there was a consistent and significant elevation of plasma glucose 30 minutes after injection of 10 and 5 μg of hpGRF (Fig. 2). This relative hyperglycemia appeared to peak 3.5 hours after treatment. The mean 6-hour plasma glucose concentrations for the groups given 10 and 5 μg of hpGRF (170.5 ± 9.6 and 161.3 ± 4.2 mg/dl, respectively) were significantly ($P < 0.05$) greater than that of the saline-treated controls (147.7 ± 1.8 mg/dl). Plasma glucose was also elevated (although not significantly) after intracerebroventricular injection of the lower doses of hpGRF, and a dose-response relation was established between mean 6-hour plasma glucose and hpGRF dose ($y = 3.35x + 139.78$; $r = 0.96$, $P < 0.01$). The action of hpGRF on the CNS to produce hyperglycemia could be mediated by humoral or neural efferent mechanisms, as has been shown for other neuropeptides (*17*); however, the role of GRF in physiological regulation of glucose metabolism remains to be established.

Marked behavioral and motor effects were observed after injection of hpGRF, particularly the higher doses. Within 5 minutes the animals became more active and were sniffing, grooming themselves, and rapidly exploring the test cage. After 2 hours the animals were running around the cage, rearing, and attempting to climb the walls. Their stools were watery

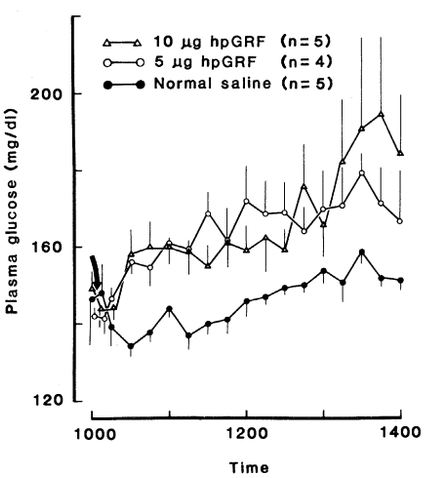

Fig. 2. Development of hyperglycemia in response to intracerebroventricular administration of 10 and 5 μg of hpGRF. Arrow indicates time of injection; vertical lines represent standard errors of the mean. The number of animals in each group is shown in parentheses.

and vocalization was excessive. This pattern of behavior, characteristic of a fear response, was in striking contrast to that observed in saline-injected rats, who slept for almost the entire 6-hour observation period. Specificity of the behavioral response to hpGRF is indicated by the finding that it differs widely, both in time course and pattern, from that described after administration of other hypothalamic releasing and inhibiting peptides, including somatostatin, into the CNS (*4*).

While the doses of hpGRF that I used were large, it is difficult to estimate what percentage of the peptide actually reaches particular neuronal sites when administered through the cerebrospinal fluid. Furthermore, the doses were in the range of those used in most previous studies of actions of hypothalamic peptides in the CNS (*4*). In view of the lack of effect of hpGRF on plasma prolactin—a sensitive monitor of stress in the rat (*18*)—it is unlikely that the hpGRF-induced responses were due to nonspecific stress. It is also unlikely that any of the effects reported here were mediated systemically by leakage from the brain, since similar doses of hpGRF administered peripherally fail to affect either plasma glucose or behavior and have opposite effects on plasma GH (*19*). Our findings support the hypothesis that hpGRF exerts direct actions in the brain that are independent of its effects at the level of the pituitary gland.

Involvement of the CNS in glucoregulation has been recognized since the classic observation of Bernard (*20*). In particular, the ventromedial hypothalamus has been implicated as a critical CNS locus for carbohydrate metabolism by a variety of techniques; stimulation of this region elevates plasma glucose whereas lesions facilitate insulin secretion (*21*). It is also widely believed that the ventromedial hypothalamus is a major center for integration of emotional behavior. Electrical stimulation of the ventromedial nucleus produces an affective defense response in cats, and rats display fear-related behaviors consisting of vocalization, rearing, and escape attempts (*22*), behaviors remarkably similar to those we observed in response to central injection of hpGRF. Immunohistochemical studies have revealed the presence of hpGRF-immunoreactive neurons in arcuate and ventromedial nuclei of the primate and rat hypothalamus (*23*), neural loci consistent with physiological findings on hypothalamic regulation of GH secretion (*24*). In addition, hpGRF-immunoreactive fibers were found projecting to several regions of the hypothalamus outside the characteristic termination sites on median eminence portal capillaries (*25*). It is possible that GRF is the neural substrate subserving all these functions and plays an important neurotransmitter or neuromodulatory role in the basal hypothalamus to coordinate the neuroendocrine, visceral, and behavioral responses of the organism.

GLORIA SHAFFER TANNENBAUM
Neuroendocrine Research Laboratory, McGill University–Montreal Children's Hospital Research Institute, and *Departments of Pediatrics* and *Neurology and Neurosurgery, McGill University, Montreal, Quebec H3H 1P3, Canada*

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Adriamycin-Induced DNA Damage Mediated by Mammalian DNA Topoisomerase II

Abstract. *Adriamycin (doxorubicin), a potent antitumor drug in clinical use, interacts with nucleic acids and cell membranes, but the molecular basis for its antitumor activity is unknown. Similar to a number of intercalative antitumor drugs and nonintercalative epipodophyllotoxins (VP-16 and VM-26), adriamycin has been shown to induce single- and double-strand breaks in DNA. These strand breaks are unusual because a covalently bound protein appears to be associated with each broken phosphodiester bond. In studies in vitro, mammalian DNA topoisomerase II mediates DNA damage by adriamycin and other related antitumor drugs.*

Because of the clinical importance of adriamycin in the treatment of many common tumors, extensive studies have been performed to determine the possible antitumor mechanism or mechanisms of adriamycin and other related antitumor anthracyclines (*1, 2*). Adriamycin binds tightly to DNA and interferes with many DNA-related functions such as DNA replication and RNA synthesis (*1, 2*). It has been shown that, when linked to agarose beads, adriamycin can exert its cytotoxic effect without entering cells (*3*). Adriamycin can also be reduced to a semiquinone radical that damages macromolecules such as DNA and cell mem-