metabolites. Our estimates of B_{max} included model correction for brain as well as plasma metabolites, with calculated values found to be similar to measurements by high-performance liquid chromatography (*3, 7*). When ^{18}F-labeled NMSP was used in rodents (*9*), concentrations of metabolites in the caudate nucleus and cerebellum were quite small. In the same study the unchanging striatal radioligand mass over time suggested similar inferences about low concentrations of [^{11}C]NMSP metabolites in brain.

Administering double or multiple injections of low and much higher (blocking) doses of neuroleptic drugs makes it possible to measure absolute receptor density (B_{max}). The slope of the line relating the caudate-cerebellar ratio to time is useful as a preliminary index for the generation of hypotheses, especially in low receptor density states. To collaborate this, we note that according to our absolute density measurements, aged normal subjects over 58 years of age have B_{max} values which are approximately half that of young normal subjects (*7*). This is compatible with our results with Ca/Cb slope (*2*).

DEAN F. WONG
HENRY N. WAGNER, JR.
ROBERT F. DANNALS
JONATHAN M. LINKS
Divisions of Nuclear Medicine and Radiation Health Sciences, Johns Hopkins Medical Institutions, Baltimore, MD 21205-2179
MICHAEL J. KUHAR
Departments of Neuroscience, Psychiatry, and Pharmacology and Therapeutics Johns Hopkins Medical Institutions, Baltimore MD 21205-2179
ALBERT GJEDDE
Medical Physiology A, Panum Institute, University of Copenhagen, Copenhagen, Denmark 2200

REFERENCES

1. H. N. Wagner, Jr., *et al.*, *Science* **221**, 1264 (1983).
2. D. F. Wong *et al.*, *ibid.* **226**, 1393 (1984); *ibid.* **227**, 1156 (1985).
3. D. F. Wong *et al.*, *J. Nucl. Med.* **26**, 52 (1985); *Soc. Neurosci. Abstr.* **11** (part 1, no. 139.1), 455 (1985); *Proc. IV World Congr. Biol. Psychiatr.*, in press.
4. M. J. Kuhar, L. C. Murrin, A. T. Malouf, N. Klemm, *Life Sci.* **22**, 203 (1978); M. Baudry, M. P. Martres, J. C. Schwartz, *ibid.* **21**, 1163 (1977); V. Hollt, A. Czlonkowski, A. Herz, *Brain Res.* **130**, 176 (1977); P. Laduron and J. Leysen, *Biochem. Pharmacol.* **26**, 1003 (1977); V. Hollt and P. Schubert, *Brain Res.* **151**, 149 (1978); S. Bischoff, H. Bittiger, J. Kraus, *Eur. J. Pharmacol.* **68**, 305 (1980).
5. C. Crone, *J. Physiol. (London)* **181**, 103 (1965); A. Gjedde, *Brain Res. Rev.* **4**, 237 (1982).
6. F. J. Ehlert and W. I. Yamamura, in *Aging*, S. J. Enna and B. Beer, Eds. (Raven, New York, 1983), vol. 23, pp. 73–77; J. Enna and R. Strong, in *ibid.*, vol. 17, pp. 133–142; J. A. Joseph *et al.*, *Life Sci.* **29**, 575 (1981); L. J. Thal *et al.*, *Brain Res.* **192**, 185 (1980); A. DeBlasi and T. Mennini, *ibid.* **242**, 361 (1982); J. K. Marquis *et al.*, *Biochem. Pharmacol.* **30**, 1876 (1981); M. Meino *et al.*, *Brain Res.* **202**, 488 (1980); C. H. Misra *et al.*, *Life Sci.* **27**, 526 (1980); J. Marcusson, *Clin. Neuropharmacol.* 7 (suppl. 1), 22 (1984); J. A. Severson *et al.*, *J. Neurochem.* **39**, 1623 (1982); J. A. Severson and C. E. Finch, *Brain Res.* **210**, 201 (1980).
7. D. F. Wong, A. Gjedde, H. N. Wagner, Jr., *J. Cereb. Blood Flow Metab.* **6**, 137 (1986); D. F. Wong *et al.*, *ibid.*, p. 147.
8. M. Titler *et al.*, *J. Neuroscience*, in press; P. Seeman *et al.*, *Science* **225**, 728 (1985).
9. C. Arnett *et al.*, *Life Sci.* **36**, 1359 (1985).
10. D. F. Wong *et al.*, *Psychopharm. Bull.* **21**, 595 (1985); *J. Nucl. Med.* **26**, 52 (1985); *ibid.*, p. 107.

27 November 1985; accepted 2 January 1986

Effects of Growth Hormone–Releasing Factor in the Brain

THE ABILITY OF GROWTH HORmone–releasing factor (GRF) directly to stimulate release of pituitary growth hormone (GH) is now well established (*1*). Intravenous (*2*), subcutaneous, and intranasal (*3*) routes of administration are known to be effective. The report by Tannenbaum (*4*) on the effects of intracerebroventricular administration of GRF on plasma GH and glucose concentrations and behavior is presented as evidence that this neuropeptide might have direct effects within the central nervous system, these being the inhibition of pituitary GH secretion, presumably by a GRF negative feedback effect on GRF itself, and the stimulation of behavioral activity. Our own observations of the effects of intracerebroventricular administration of GRF in rats are not consistent with those of Tannenbaum. Indeed, they support the argument that GRF, at the doses used, stimulates GH secretion and inhibits behavioral activity.

We have reached these conclusions using experimental models that are identical or similar to those used by Tannenbaum (*4*). They can be summarized as follows: approximately 2 weeks before experimentation, rats were surgically prepared with stainless steel guide cannulas aimed at the lateral cerebral ventrical. In addition, some animals were prepared with stainless steel recording electrodes in the calvarium over the sensorymotor and occipital cortices (*5*). Other animals were prepared with intravenous catheters in the right external jugular vein (*6*). The rats were then adapted to individual isolation recording chambers. Intracerebroventricular injections of 10 μg of human GRF (1–44)NH_2 (*7*) in saline or of saline alone were given over 30 seconds with a Hamilton syringe. To monitor the effects of GRF on plasma GH concentrations, an initial blood sample was drawn at 1000 hours, followed by the intracerebroventricular injection. Sequential blood samples were then drawn at 30-minute intervals for the next 6 hours. To monitor the effects on levels of arousal, electroencephalogram (EEG) and behavioral recordings were made before, during, and up to 120 minutes after the injections of saline or GRF. EEG analysis was made by (i) calculating the Fourier transform of 4-second continuous epochs from 0 to 127.75 Hz in Y4 Hz increments, (ii) determining the power spectra of the epochs, (iii) further compressing the data to six frequency bands, and (iv) calculating the mean power density (*8*). Behavioral analysis was made by measuring locomotor activity during the normally active (nocturnal) period. Recordings were made in cages with two infrared photocell beams. Activity was quantified by totaling photocell beam interruptions for 5-minute epochs for 4 hours after the intracerebroventricular injection.

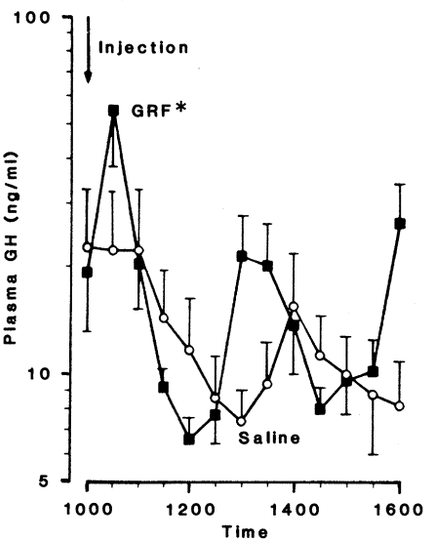

Fig. 1. Effect of intracerebroventricular administration of GRF (10 μg) or saline on GH secretion in conscious, freely moving male rats. The neuropeptide caused a significant increase ($P < 0.01$) in plasma GH concentration 30 minutes after administration. Each data point represents the mean for eight animals. Vertical lines represent the standard error of the means. GH concentrations are expressed in units of the NIH reference preparation GH-RP-2. As we used it, the preparation was ten times more potent than the reference preparation used by Tannenbaum (*4*). When corrections for this factor are made, the GH concentrations in the two studies are seen to be virtually identical.

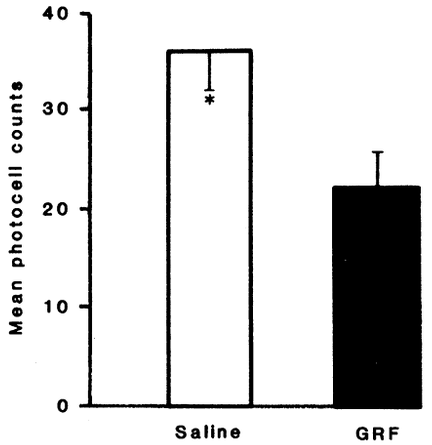

Fig. 2. Mean locomotor responses in rats receiving an intracerebroventricular injection of GRF (10 μg) or saline. Locomotion was measured by counting the number of photocell beam interruptions over sequential 5-minute periods for a total of 4 hours. GRF administration caused a significant decrease ($P < 0.05$) in locomotor activity. Bars represent the means of 15 animals. Vertical lines represent the standard error of the means.

The intracerebroventricular administration of 10 μg of GRF resulted in a significant increase ($P < 0.01$) in circulating plasma GH concentrations 30 minutes after injection (Fig. 1). In addition, neither GRF nor saline altered the 3-hour pulsatile pattern of spontaneous GH secretion. EEG recordings indicated that the time to onset of slow wave sleep was significantly less ($P < 0.05$) in the GRF-treated rats (9 ± 1 minute) than in the saline-treated ones (15 ± 2 minutes). Similarly, the amount of time spent in slow wave sleep was significantly greater in the GRF-treated rats (24 ± 4% versus 13 ± 4%, $P < 0.05$). Intracerebroventricular GRF also significantly decreased ($P < 0.05$) mean locomotor activity (Fig. 2).

Our results demonstrate that administration of GRF in the lateral ventricles of the brain increases plasma GH concentrations and does not alter the normal pulsatile pattern of GH secretion. This neuropeptide also clearly increases slow wave sleep and decreases locomotor activity, which indicates a decrease in the arousal state, consistent with findings in humans and several animal species in which GH release is associated with sleep (*9*). Our observations are consistent with the preliminary observations of Katakami and Frohman (*10*) and McCann *et al.* (*11*). These investigators reported that large intracerebroventricular doses of GRF caused a significant increase in plasma GH concentrations. The former group also reported that very low doses of GRF (0.01 μg) caused a decrease in plasma GH concentrations.

Clinical descriptions of the comportment of acromegalics by physicians are also consistent with our findings of decreased locomotion after administration of GRF (*12*). One additional comment concerning the dose of GRF holds true regardless of which results are ultimately confirmed. Isolation of rat GRF from the hypothalamus (*13*) indicates that there is approximately 100 to 500 pg of GRF per hypothalamus. Thus, the doses of GRF administered in the present study and in Tannenbaum's study (*4*) are on the order of 10^4 to 10^5 times greater than the total hypothalamic content. Although one can argue that the amount of GRF that actually reaches a particular neuronal site is unknown, the dilution factor is probably not on the order of 10^4 to 10^5. We believe it is unlikely that GRF regulates its own neurosecretion through an "ultrashort-loop" negative feedback mechanism, but suggestions that GRF may have important neurotransmitter or neuromodulator roles within the brain are indeed open.

WILLIAM B. WEHRENBERG*
Laboratories for Neuroendocrinology, Salk Institute, Post Office Box 85800, San Diego, CA 92138
CINDY L. EHLERS
Scripps Clinic Research Foundation, 10666 North Torrey Pines Road, La Jolla, CA 92037

REFERENCES AND NOTES

1. R. Guillemin *et al.*, *Science* **218**, 585 (1982); J. Rivier *et al.*, *Nature (London)* **300**, 276 (1982); F. S. Esch *et al.*, *Biochem. Biophys. Res. Commun.* **109**, 152 (1982).
2. M. O. Thorner *et al.*, *Lancet* **1983-I**, 24 (1983); W. B. Wehrenberg *et al.*, *Biochem. Biophys. Res. Commun.* **109**, 382 (1982).
3. W. S. Evans *et al.*, *J. Clin. Endocrinol. Metab.* **57**, 1081 (1983); M. O. Thorner *et al.*, paper presented at the 7th International Congress of Endocrinology, 1 to 7 July 1984, Quebec City, Canada (Abstr. 2310).
4. G. S. Tannenbaum, *Science* **226**, 464 (1984).
5. C. L. Ehlers, T. K. Reed, S. J. Henriksen, *Neuroendocrinology* **42**, 467 (1986).
6. W. B. Wehrenberg *et al.*, *Biochem. Biophys. Res. Commun.* **109**, 562 (1982).
7. N. Ling *et al.*, *Proc. Natl. Acad. Sci. U.S.A.* **81**, 4302 (1984).
8. C. L. Ehlers and J. W. Havstad, *Psychopharm. Bull.* **18**, 43 (1982).
9. D. C. Parker *et al.*, *Clin. Endocrinol. Metab.* **29**, 871 (1969); Y. Takahashi, D. M. Kipnis, W. H. Daughaday, *J. Clin. Inv.* **47**, 2097 (1968); D. C. Parker *et al.*, *Endocrinology* **91**, 1462 (1972); F. Kimura and M. Kawakami, *Neuroendocrinology* **33**, 276 (1981).
10. H. Katakami and L. A. Frohman, paper presented at the 7th International Congress of Endocrinology, 1 to 7 July 1984, Quebec City, Canada (Abstr. 1231).
11. S. M. McCann *et al.*, paper presented at the 7th International Congress of Endocrinology, 1 to 7 July 1984, Quebec City, Canada (Abstr. S.28).
12. M. Bleuler, *J. Nerv. Ment. Dis.* **113**, 497 (1951).
13. J. Spiess, J. Rivier, W. Vale, *Nature (London)* **303**, 532 (1983); P. Bohlen *et al.*, *Biochem. Biophys. Res. Commun.* **125**, 1005 (1984).

* Present address: Department of Health Sciences, School of Allied Health Professions, University of Wisconsin, Milwaukee, WI 53201.

19 April 1985; accepted 12 August 1985

Response: The discordance between the results in my report (*1*) of the effects of human (h) growth hormone–releasing factor (GRF) in the brain and the results of Wehrenberg and Ehlers can be attributed to the fact that our two laboratories were using different preparations of hGRF. Indeed, the observation of Wehrenberg and Ehlers that a high dose of hGRF administered centrally stimulates growth hormone (GH) release had already reported by two other groups (*2*).

It was precisely to resolve this discrepancy in the literature that I undertook a critical reexamination of the effects of intracerebroventricular (icv) administration of a high dose (10 μg) of GRF in the rat using both the same GRF peptide employed in my original study, designated hpGRF-44-NH_2 (*3*), and the more physiologic rat (r) GRF peptide (*4*). The findings indicated that, while the putative hGRF peptide continued to cause a dramatic suppression of spontaneous GH secretion, consistent with what I had reported, the icv injection of 10 μg of rGRF produced an acute stimulation of plasma GH (*5*), similar to that observed by others who had used hGRF (*2*). Moreover, rGRF icv did not alter glycemia or behavior (*5*), in agreement with another report demonstrating no significant effect of icv-administered rGRF on locomotor activity (*6*).

These results led me to question whether the peptide used in my original study was similar to that used by the other groups. Therefore, in collaboration with two other laboratories (those of L. A. Frohman and M. van der Rest), I undertook a detailed chemical characterization of the preparation

Table 1. Amino acid composition of putative hGRF peptide.

Amino acid	Putative hGRF peptide*	hGRF†	oCRF†
Asp	4.3	4	4
Thr	2.0	1	2
Ser	2.9	4	3
Glu	6.5	7	7
Pro	2.1	0	2
Gly	0.6	3	0
Ala	4.0	5	4
Val	1.1	1	1
Met	0.5	1	1
Ile	1.9	2	2
Leu	7.8	5	8
Tyr	0.5	2	0
Phe	0.9	1	1
His	1.9	0	2
Lys	1.9	2	2
Arg	1.9	6	2

*Expressed as residues per 41 residues. †Residues per reference molecule (*7*, *8*).