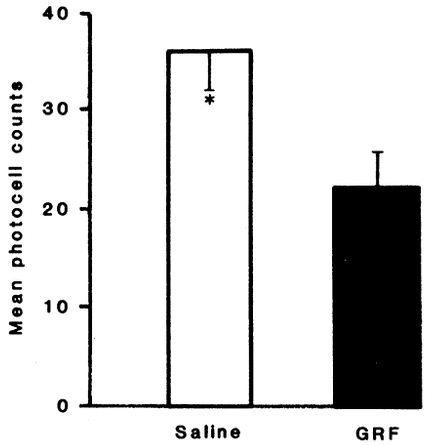

Fig. 2. Mean locomotor responses in rats receiving an intracerebroventricular injection of GRF (10 μg) or saline. Locomotion was measured by counting the number of photocell beam interruptions over sequential 5-minute periods for a total of 4 hours. GRF administration caused a significant decrease ($P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

of hGRF used in my original study (*5*). As shown in Table 1, the amino acid composition of this putative hGRF peptide was found to be incompatible with the known structure of hGRF (*7*), but matched perfectly the amino acid composition of ovine (o) corticotropin-releasing factor (CRF) (*8*). Through a series of biochemical and immunologic studies, we established that this particular batch of hGRF consisted of a mixture of oCRF (~ 95%) and true hGRF (~3–5%) (*5*). How such a curious mixture occurred remains a mystery.

The combination of the two peptides readily explains my earlier results (*1*), which are in agreement with the known GH-inhibiting (*9*), hyperglycemic (*10*), and behaviorial (*11*) effects of centrally administered CRF. The available evidence indicates that the major effect of GRF administered to the brain, in high doses, is to stimulate pituitary GH release, most probably by leakage of the peptide from the cerebral ventricles into the vessels of the hypophyseal portal system. However, the possibility that GRF secretion may be subject to regulation by means of a negative feedback system within the central nervous system cannot be discounted in view of the existing evidence that icv administration of low doses of GRF exerts inhibitory effects on spontaneous GH secretion (*2*).

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

REFERENCES

1. G. S. Tannenbaum, *Science* **226**, 464 (1984).
2. M. D. Lumpkin, paper presented at the 65th Annual Meeting of the Endocrine Society, 8 to 10 June 1983, San Antonio, TX (Abstr. 540); 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); M. D. Lumpkin, W. K. Samson, S. M. McCann, *Endocrinology* **116**, 2070 (1985).
3. N. Ling *et al.*, *Proc. Natl. Acad. Sci. U.S.A.* **81**, 4302 (1984).
4. J. Spiess, J. Rivier, W. Vale, *Nature (London)* **303**, 532 (1983).
5. G. S. Tannenbaum, M. van der Rest, T. R. Downs, L. A. Frohman, *Endocrinology* **118**, 1246 (1986).
6. F. J. Vaccarino, F. E. Bloom, J. Rivier, W. Vale, G. F. Koob, *Nature (London)* **314**, 167 (1985).
7. R. Guillemin *et al.*, *Science* **218**, 585 (1982); J. Rivier *et al.*, *Nature (London)* **300**, 276 (1982).
8. W. Vale *et al.*, *Science* **213**, 1394 (1981).
9. C. Rivier and W. Vale, *Endocrinology* **114**, 2409 (1984).
10. M. R. Brown *et al.*, *Endocrinology* **111**, 928 (1982).
11. R. E. Sutton, G. F. Koob, M. Le Moal, J. Rivier, W. Vale, *Nature (London)* **297**, 331 (1982).

27 January 1986; accepted 17 March 1986