pain was relieved when the dorsal root was inactivated by a local anesthetic (*5*). The phenomenon was termed recurrent sensibility. Thus it seems clear in both animals and humans that ventral root stimulation leads to pain and that interruption of dorsal root conduction abolishes that pain.

In 1894 Sherrington (*6*) noted a few sensory myelinated ventral root fibers and suggested that these were the morphological basis of recurrent sensibility. The suggestion was not accepted, however, because large fibers are thought not to carry nociceptive information. In recent years it has been shown that there are many unmyelinated ventral root afferents and that most of these carry nociceptive information (*7*). Evidence was obtained to indicate that some of the unmyelinated ventral root fibers enter the spinal cord directly through the ventral root (*8, 9*). These fibers may well be responsible for the failure of dorsal rhizotomy to relieve pain, and the observations imply that it is impossible to provide sensory denervation by dorsal rhizotomy alone (*10*).

However, fibers that enter the spinal cord directly through the ventral root would not explain the pain elicited by stimulating the distal stump of a cut ventral root and abolished by inactivating the fibers in the dorsal root. Thus there may be other types of afferent fibers in the ventral root. The present study provides, as far as we are aware, the first physiological data at the single-unit level showing that ventral root afferents can modify the activity of neurons in the dorsal horn. Furthermore, measurements of latency indicate that the afferent information entering the spinal cord is carried by unmyelinated fibers. Since we find, in confirmation of earlier work, that interruption of conduction in the dorsal root abolishes the phenomenon, it would seem that the information ultimately enters the spinal cord through the dorsal root. Thus some of the unmyelinated fibers that have recently been discovered in the ventral root are presumably the fibers carrying the noxious information to the spinal cord through the dorsal root and are thus the explanation of recurrent sensibility.

There seem to be two populations of ventral root afferents. First, there are those that enter the spinal cord directly through the ventral root. These probably explain the failure of dorsal rhizotomy to relieve pain, and their presence blurs the previously perceived clear separation of function of the spinal roots. Second, there are the ventral root afferents that enter the spinal cord through the dorsal root. These might be further subdivided into those with receptive fields in the meninges on the ventral side of the spinal cord (*11*) and those whose fibers loop into the ventral root and then enter the dorsal root (*12*). It remains an important task to determine the proportions of each and to see whether different functional modalities are transmitted by the different groups.

J. M. CHUNG
K. H. LEE
K. ENDO
R. E. COGGESHALL
Marine Biomedical Institute and *Departments of Anatomy* and *Physiology and Biophysics, University of Texas Medical Branch, Galveston 77550*

References and Notes

1. C. W. Anderson and M. R. Cushman, *J. Neurosci. Methods* **4**, 435 (1981).
2. J. M. Chung, D. R. Kenshalo, Jr., K. D. Gerhart, W. D. Willis, *J. Neurophysiol.* **42**, 1354 (1979).
3. F. Magendie, *J. Physiol. Exp. Pathol.* **2**, 276 (1822); R. E. Coggeshall, *Physiol. Rev.* **60**, 716 (1980).
4. F. Magendie, *J. Physiol. Exp. Pathol.* **2**, 366 (1822).
5. R. Frykholm, J. Hyde, G. Norlen, C. R. Skoglund, *Acta Physiol. Scand.* **29** (Suppl. 106), 455 (1953).
6. C. S. Sherrington, *J. Physiol. (London)* **17**, 211 (1894).
7. R. E. Coggeshall, J. D. Coulter, W. D. Willis, *J. Comp. Neurol.* **153**, 39 (1974); G. L. Clifton, R. E. Coggeshall, W. H. Vance, W. D. Willis, *J. Physiol. (London)* **256**, 573 (1976); R. E. Coggeshall and H. Ito, *ibid.* **267**, 215 (1977).
8. C. W. Maynard, R. B. Leonard, J. D. Coulter, R. E. Coggeshall, *J. Comp. Neurol.* **172**, 601 (1977); T. Yamamoto, K. Takahashi, H. Satomi, H. Ise, *Brain Res.* **126**, 350 (1977); A. Hiura, *Fukushima J. Med. Sci.* **28**, 77 (1982).
9. J. C. Longhurst, J. H. Mitchell, M. B. Moore, *J. Physiol. (London)* **301**, 467 (1980); P. E. Voorhoeve and J. Nauta, in *Advances in Pain Research and Therapy*, J. J. Bonica *et al.*, Eds. (Raven, New York, 1983), p. 105.
10. R. E. Coggeshall, M. L. Applebaum, M. Fazen, T. B. Stubbs III, M. T. Sykes, *Brain* **98**, 157 (1975).
11. C.-J. Dalsgaard, M. Risling, C. Cuello, *Brain Res.* **246**, 168 (1982).
12. M. Risling and C. Hildebrand, *J. Neurol. Sci.* **56**, 219 (1982).
13. Supported by NIH grants NS 10161 and 11255 and a grant from the Moody Foundation to R.E.C. Also supported by NIH grant NS 18830 and grants from the Pearl and Aaron Forman Research Fund and Texas Heart Association to J.M.C.

12 August 1983; accepted 23 September 1983

Inhibition of Gastric Acid Secretion in Rats by Intracerebral Injection of Corticotropin-Releasing Factor

Abstract. *Intracisternal injection of ovine corticotropin-releasing factor (CRF) into the pylorus-ligated rat or the rat with gastric fistula resulted in a dose-dependent inhibition of gastric secretion stimulated with pentagastrin or thyrotropin-releasing hormone. When injected into the lateral hypothalamus—but not when injected into the cerebral cortex—CRF suppressed pentagastrin-stimulated acid secretion. The inhibitory effect of CRF was blocked by vagotomy and adrenalectomy but not by hypophysectomy or naloxone treatment. These results indicate that CRF acts within the brain to inhibit gastric acid secretion through vagal and adrenal mechanisms and not through hypophysiotropic effects.*

Neuropeptides originally characterized from the hypothalamus because of their ability to regulate pituitary hormone secretion—thyrotropin-releasing hormone (TRH), luteinizing hormone-releasing hormone (LHRH), and somatostatin—have been implicated in various regulatory processes in addition to their specific hypophysiotropic action (*1*). For example, TRH and somatostatin act both peripherally and within the brain to regulate gastrointestinal functions (*2*). Vale *et al.* used ovine hypothalami to characterize a 41–amino acid peptide called corticotropin-releasing factor (CRF), which stimulates the secretion of corticotropin and β-endorphin in vitro and in vivo (*3*). We showed that CRF injected into the cisterna magna or the lateral hypothalamus inhibits basal and pentagastrin-stimulated gastric acid secretion in rats through modulation of the autonomic nervous system.

Male Sprague-Dawley CD rats weighing 200 to 250 g were given free access to Purina Laboratory Chow and tap water and were housed under conditions of controlled temperature and lighting. All experiments were performed in rats deprived of food for 24 hours but with free access to water until the beginning of treatment. Ovine CRF was synthesized by the solid-phase method and purified by high-performance liquid chromatography (HPLC) after cleavage and deprotection by hydrofluoric acid (*4*). The peptide in lyophilized form was freshly dissolved in 0.9 percent saline just before each experiment. Animals were lightly anesthetized with ether for injection of CRF into the cisterna magna and were anesthetized with urethane (1.25 g/kg, intraperitoneally) for injection of CRF bilaterally into the lateral hypothalamic area or frontal cortex (*5*). Gastric secretions were collected by means of a 2-hour pylorus ligation or a short-term gastric fistula (*6*). Gastric secretions ob-

tained from pylorus-ligated rats were centrifuged, the volume was measured, and the concentration of acid was determined by titration with 0.1*M* NaOH to *p*H 7.0 on an automatic titrator (Radiometer, Copenhagen). In the gastric fistula method, rats were anesthetized with urethane, the esophagus and pylorus were ligated, and a double-lumen cannula was inserted into the stomach. The gastric lumen was flushed with two 5-ml boluses of 0.15*M* NaCl and one 5-ml bolus of air at the end of each 10-minute period. Acid output was determined by titration of the flushed perfusate.

Intracisternal injection of CRF resulted in a dose-dependent inhibition of gastric acid output in the pylorus-ligated rat (Table 1), and in the rat with gastric fistula when gastric acid secretion was stimulated by intravenous infusion of pentagastrin or by intracisternal injection of TRH (Fig. 1). The inhibitory action of CRF was long-lasting since over 85 percent decrease in gastric acid output was still observed 2 hours after a single 2.3-nmole dose of CRF (Table 1 and Fig. 2). In contrast to the inhibitory action of CRF, hpGRF-(1–40), the synthetic replicate of the growth hormone–releasing factor characterized from human pancreatic tumor that had caused acromegaly (*7*), had no effect on gastric secretion when injected intracisternally (0.2 to 2.2 nmole) in pylorus-ligated rats.

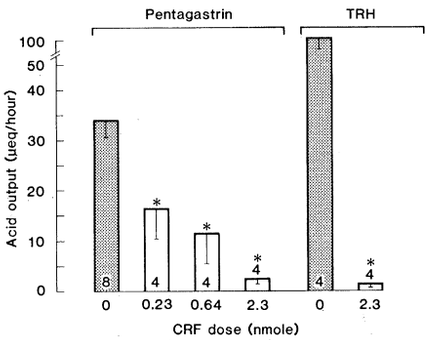

Fig. 1. Inhibitory effect of intracisternally administered CRF on gastric secretion stimulated by pentagastrin and TRH. Rats with gastric fistula received an intracisternal injection of saline (10 μl) or CRF and immediately afterward, pentagastrin (16 μg/kg per hour) was infused through the femoral vein for 2 hours, or TRH (2.8 nmole) was injected as a bolus intracisternally. Acid output in microequivalents of H^+ per hour [mean ± standard error (S.E.M.)] represents the total response for the 6- to 10-minute collection periods after pentagastrin infusion or TRH injection. The number of animals for each treatment regimen is indicated at the bottom of each bar. For pentagastrin-treated rats, comparisons of CRF dose to control were computed with Dunnett's procedure for multiple comparisons after a one-way analysis of variance. For TRH-treated rats, CRF was compared to control with a *t*-test. (Both original data and data transformed by taking square roots to stabilize variances were analyzed.) $*P < 0.05$.

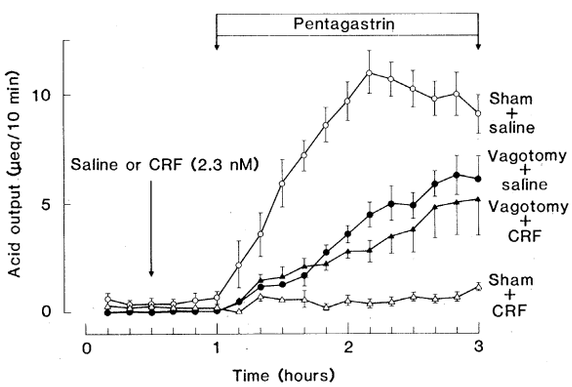

Fig. 2. Gastric response to intracisternally administered CRF in vagotomized rats. Rats with gastric fistulas were vagotomized or given sham operations. One hour later, saline or CRF was injected intracisternally. All animals were infused through the femoral vein with pentagastrin (16 μg/kg per hour). For each animal, total acid output after the pentagastrin injection was computed. These total outputs were analyzed by a one-way analysis of variance and then by contrasts using the Bonferroni method for multiple comparisons. (Data were analyzed in the original scale and after a square root transformation to stabilize variances.) Each point represents the mean ± S.E.M. of eight rats (five for the sham + CRF group).

Table 1. Inhibitory effects of intracisternal injection of CRF on gastric acid output in pylorus-ligated rats and influence of hypophysectomy and adrenalectomy. Rats deprived of food for 24 hours were given sham operations or were hypophysectomized 9 days before the experiment or adrenalectomized just before CRF injection. Each animal was injected intracisternally with saline (10 μl) or CRF (0.1, 0.2, or 2.3 nmole); the pylorus was then ligated, and 2 hours later the animal was decapitated. The number of animals is given in parentheses. Values are means ± S.E.M.

Operation	Gastric acid output (μeq of H^+ per 2 hours)			
	After CRF			After saline
	0.1 nmole	0.2 nmole	2.3 nmole	
None	379 ± 55 (5)	284 ± 70* (5)	88 ± 21* (5)	511 ± 55 (6)
Sham hypophysectomy			76 ± 23* (7)	495 ± 102 (7)
Hypophysectomy			41 ± 11* (7)	120 ± 21 (7)
Sham adrenalectomy			59 ± 38 (4)	226 ± 61 (5)
Adrenalectomy			405 ± 73 (5)	353 ± 131 (4)

$*P < 0.05$ by *t*-test for the CRF-treated animal compared with its saline control (for the first row by contrast following a significant one-way analysis of variance). Results were the same whether analysis was done on the original data or on data transformed by taking square roots to stabilize variances.

Because CRF injected intravenously is also a potent inhibitor of gastric acid secretion in rats and in dogs and because there is evidence of rapid appearance of intracerebroventricularly administered neuropeptides in the circulation (*8*), the action of CRF on the central nervous system was further ascertained by administration of the peptide directly into specific brain sites. Corticotropin-releasing factor (0.2 to 0.9 nmole) injected bilaterally into the lateral hypothalamus, an important diencephalic region concerned with regulation of gastric secretion in rats (*9*), elicited a dose-dependent suppression of gastric acid secretion stimulated by pentagastrin. A significant decrease in gastric acid output (78 percent the first hour and 67 percent the second hour) was induced by a 0.9-nmole dose of CRF injected into the lateral hypothalamus but did not occur when the CRF was injected into the dorsomedial frontal cortex. An extensive hypothalamic as well as extrahypothalamic distribution for CRF-like immunoreactivity has been observed in various species, with highest concentrations of CRF occuring in the hypothalamus (*10*). In addition, specific binding of ^{125}I-labeled CRF to cell membranes prepared from several ovine brain areas has been reported (*10*). These observations provide a neuroanatomic substrate and support the concept that CRF could modulate gastric secretion by initially acting within the brain.

The neurohumoral mechanisms through which intracisternal CRF inhibits acid secretion are unrelated to its regulatory action on the pituitary-adrenocortical axis, since the decreased gastric acid output in hypophysectomized rats was further inhibited by CRF injection (Table 1). The inhibitory effect of CRF is not mediated through a decrease in the release of gastrin (which stimulates gastric acid secretion), because intracisternal injection of CRF results in an

elevation of plasma gastrin (*11*). Several pieces of evidence indicate that CRF acts through modulation of the autonomic nervous system. Vagal tone has a critical role in gastric acid secretion elicited by pylorus ligation, intracisternal injection of TRH, or intravenous infusion of pentagastrin (*2*, *12*) (Fig. 2). We demonstrated that CRF markedly suppressed acid secretion produced under these conditions (Table 1 and Fig. 1) and that vagotomy reversed the inhibitory action of CRF (Fig. 2).

Corticotropin-releasing factor acts within the brain to cause an increase in sympathetic outflow leading to hyperglycemia (*13*). Adrenalectomy just before the injection of CRF completely reversed both the gastric (Table 1) and the hyperglycemic (data not shown) response to CRF in pylorus-ligated rats. These results show that intracisternal injection of CRF, unlike intravenous administration (*8*), decreases gastric acid secretion through vagal and adrenal-dependent mechanisms. This contrasts with the inhibition of gastric acid secretion induced by intracisternally administered bombesin, which is unmodified by vagotomy or by adrenalectomy just before treatment (*6*). In that respect, some specific neuropeptides may be used as new chemical probes to further elucidate the various neurohumoral mechanisms involved in brain-gut interaction.

Although the physiological role of CRF has yet to be elucidated, the findings that CRF mimics the autonomic and endocrine response to stress (*3*, *13*) and that it alters gastric secretion in a manner similar to that of various stressors (*14*) suggest that CRF may have a role in the pathophysiologic gastric response to stress.

YVETTE TACHÉ
YOSHIAKI GOTO
MARK W. GUNION
Center for Ulcer Research and Education, VA Wadsworth and UCLA Medical Center, Los Angeles, California 90073
WYLIE VALE
JEAN RIVER
MARVIN BROWN
Salk Institute for Biological Studies, La Jolla, California 92037

References and Notes

1. M. Brown, J. Rivier, W. Vale, *Endocrinology* **104**, 1709 (1979); M. Brown *Diabetologia* **20**, 299 (1981).
2. S. R. Bloom *et al.*, *Lancet* **1974-II**, 1106 (1974); J. R. Smith, T. R. La Hann, R. M. Chesnut, M. A. Carino, A. Horita, *Science* **196**, 660 (1977); J. Morley, J. H. Steinback, E. J. Feldman, T. E. Solomon, *Life Sci.* **26**, 1059 (1979). Y. Taché, W. Vale, M. Brown, *Nature (London)* **287**, 149 (1980); Y. Taché, W. Vale, J. Rivier, M. Brown, *Regul. Peptides* **1**, 307 (1981).
3. W. Vale, J. Spiess, C. Rivier, J. Rivier, *Science* **213**, 1394 (1981); J. Spiess, J. Rivier, C. Rivier, W. Vale, *Proc. Natl. Acad. Sci. U.S.A.* **78**, 6517 (1981); C. Rivier, M. Brownstein, J. Spiess, J. Rivier, W. Vale, *Endocrinology* **110**, 272 (1982); C. Rivier, J. Rivier, W. Vale, *Science* **218**, 377 (1982).
4. W. Marki, J. Spiess, Y. Taché, M. Brown, J. Rivier, *J. Am. Chem. Soc.* **103**, 3778 (1981); J. Rivier, J. Spiess, C. Rivier, W. Vale, *International Symposium on HPLC of Proteins and Peptides* (Washington, D.C., 1981), p. 25.
5. Coordinates used for microinjections into the dorsolateral hypothalamic area were anterior posterior, −2.4; lateral, ±1.9; and dorsoventral, −7.8. Coordinates for microinjections into the dorsomedial frontal cortex were anterior posterior, +4.0; lateral, ±2.0; and dorsoventral, −0.7. The cannulas (28 g, stainless steel) were lowered into position bilaterally and simultaneously, and 1 μl of saline or 0.20 to 0.90 nmole of CRF was expelled over 2.0 minutes; after another 2 minutes the cannulas were slowly withdrawn. Pentagastrin (16 μg/kg per hour) was then infused into the femoral vein, and gastric acid secretion was monitored every 10 minutes. At the end of the experiment, the brains were removed and fixed in 10 percent Formalin. Frozen sections at 50 μm were mounted and stained with thionine for microscopic examination (five to seven animals per treatment).
6. Y. Taché, W. Vale, J. Rivier, M. Brown. *Proc. Natl. Acad. Sci. U.S.A.* **77**, 5515 (1980); M. Ligumsky, Y. Goto, H. Debas, T. Yamada, *Science* **219**, 301 (1982).
7. J. Rivier, J. Spiess, M. Thorner, W. Vale. *Nature (London)* **300**, 276 (1982); R. Guillemin, P. Brazeau, P. Böhlen, F. Esch, N. Ling, W. Wehrenberg, *Science* **218**, 585 (1982).
8. Y. Taché, Y. Goto, H. Debas, J. Walsh, W. Vale, J. Rivier, *Gastroenterology* **84**, 1329 (1983); E. Passaro, H. Debas, W. Oldendorf, T. Yamada, *Brain Res.* **241**, 355 (1982).
9. A. Misher and F. P. Brooks, *Am. J. Physiol.* **211**, 403 (1966); Y. Osumi, S. Aibara, M. Fujimara, *Life Sci.* **31**, 2485 (1982); Y. Taché, C. Grijalva, M. Gunion, J. Walsh, D. Novin, *ibid.*, p. 2485.
10. L. W. Swanson, P. E. Sawchenko, J. Rivier, W. Vale, *Neuroendocrinology* **36**, 165 (1983); J. Cote, M. Lavoie, P. Poyet, F. Labrie, N. Barden, *Soc. Neurosci. Abstr.* **8**, 110 (1982). As deduced from the present knowledge of normal CRF concentration in the rat hypothalamus [3 pmole per milligram of protein; K. Hashimoto *et al.*, *Life Sci.* **32**, 1001 (1983)] and from CRF release into the rat hypophyseal portal blood [105 pmole; D. Gibbs and W. Vale, *Endocrinology* **11**, 1418 (1982)], the efficient dose used in our study for local intrahypothalamic injection appears pharmacologic. However, ovine hypothalamic CRF-(1–41) differs in seven residues from rat CRF-(1–41), leading radioimmunoassay to underestimate rat CRF by a factor of 2 (W. Vale, J. Spiess, J. Rivier, unpublished results); hence, ovine hypothalamic CRF may have a reduced biological potency when tested in rat hypothalamus as compared with the native molecule.
11. A. Soll and J.Walsh, *Annu. Rev. Physiol.* **41**, 35 (1979); Y. Taché and J. Rivier, *Soc. Neurosci. Abstr.* **8**, 287 (1982).
12. D. Brodie, *Am. J. Dig. Dis.* **11**, 231 (1966); R. Hakanson, J. Hedenbro, G. Liedberg, F. Sundler, S. Vallgreen, *J. Physiol. (London)* **305**, 139 (1980).
13. M. Brown, L. Fisher, J. Rivier, J. Spiess, C. Rivier, W. Vale, *Life Sci.* **30**, 207 (1982); *Regul. Peptides* **4**, 107 (1982); L. Fisher *et al.*, *Endocrinology* **111**, 928 (1982).
14. R. Menguy, *Am. J. Dig. Dis.* **5**, 911 (1960); O. A. Brodie, R. W. Marshall, O. M. Moreno, *Am. J. Physiol.* **202**, 812 (1962); D. A. Brodie and L. S. Valitski, *Proc. Soc. Exp. Biol. Med.* **113**, 998 (1963); P. O. Schwille, R. Putz, R. Thun, W. Schellerer, G. Draxler, G. Lang, *Acta Hepato-Gastroenterol.* **26**, 259 (1977); P. O. Schwille, W. Englehardt, U. Wolf, E. Hanisch, *Horm. Metab. Res.* **13**, 710 (1981).
15. Supported by the NIH grant AM30110 and Medical Research Council of Canada grant MA6836. We thank Dr. J. E. Elashoff for statistical evaluation of the data, G. Samuel and D. Mai-Tam for technical assistance, and S. Heil for help with manuscript preparation of the manuscript.

15 February 1983; revised 28 June 1983

Normalization of Spiroperidol Binding in the Denervated Rat Striatum by Homologous Grafts of Substantia Nigra

Abstract. *Transplantation of embryonic substantia nigra into the adult rat brain decreases the motor asymmetry that is produced by dopamine receptor supersensitivity after a unilateral lesion of the substantia nigra. The authors report that this effect of transplantation is specific to grafts of substantia nigra. They also report that, in conjunction with the decrease in motor asymmetry, these grafts cause postsynaptic dopaminergic binding sites to return to normal density as measured by tritiated spiroperidol autoradiography. Thus, in animals with brain lesions, grafts of substantia nigra produce a long-term alteration in the functional status of host brain cell receptors that is associated with a reduction in the behavioral deficit.*

Brain tissue transplantation has been used in several central nervous system areas to correct genetically produced hormone deficiencies or to reverse the effects of lesions (*1–5*). For example, unilateral lesions of the rat substantia nigra eliminate the ipsilateral dopaminergic innervation to the striatum, causing supersensitivity of dopaminergic receptors (*6–8*). It is believed that this supersensitivity is brought about by an increase in receptor density rather than by an increase in receptor affinity. When animals with these lesions are given apomorphine, a dopamine receptor agonist, they rotate away from the lesioned side, presumably because apomorphine stimulates the supersensitive striatum more than the intact side (*8*). Grafting fetal substantia nigra (*2–4*) or adult adrenal medulla (*5*) to the denervated striatum decreases this rotation effect and increases the concentrations of dopamine in parts of the striatum adjacent to the graft (*4*). We now report that grafts of other brain areas do not reduce apomorphine-induced turning. Furthermore, in adjacent areas of the striatum, grafts of fetal substantia nigra restore dopamine receptor density to normal levels, as measured by light microscopic autoradiography with [^{3}H]spiroperidol.

We lesioned the right sustantia nigra of 41 Sprague-Dawley albino rats with stereotaxic injections of the neurotoxin 6-hydroxydopamine. We then screened