distinguished from patients who are not responsive to neuroleptic treatment and who may have different or additional pathophysiology (*26*). Because DBH is found only in noradrenergic neurons and not in dopaminergic neurons, these findings emphasize the need to include central noradrenergic systems in the study of schizophrenia (*27*).

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References and Notes

1. H. Y. Meltzer and S. M. Stahl, *Schizophr. Bull.* **2**, 19 (1976).
2. P. Seeman and T. Lee, *Science* **188**, 1217 (1975); I. Creese, D. R. Burt, S. H. Snyder, *ibid.* **192**, 481 (1976).
3. B. M. Angrist, G. S. Sathananthan, S. Gershon *Psychopharmacologia* **31**, 1 (1973); D. S. Janowsky, M. K. El-Yousef, J. M. Davis, H. J. Sekerke, *Arch. Gen. Psychiatry* **28**, 185 (1973).
4. S. Kaufman and S. Friedman, *Pharmacol. Rev.* **17**, 71 (1965); B. K. Hartman, *J. Histochem. Cytochem.* **21**, 312 (1973); O. H. Viveros, L. Argueros, R. J. Corvett, *Life Sci.* **7**, 608 (1968).
5. R. M. Weinshilboum, N. B. Thoa, D. G. Johnson, I. J. Kopin, J. Axelrod, *Science* **174**, 1349 (1971).
6. R. M. Weinshilboum and J. Axelrod, *ibid.* **173**, 931 (1971).
7. D. J. Goldstein and L. X. Cubeddu, *J. Neurochem.* **26**, 193 (1976); W. P. DePotter, *J. Physiol. (London)* **258**, 26P (1976).
8. W. P. DePotter, R. W. DePotter, F. H. DeSmett, *Neuroscience* **5**, 1969 (1980).
9. L. Wetterberg, H. Aberg, S. B. Ross, O. Froden, *Scand. J. Clin. Lab. Invest.* **30**, 283 (1972); F. Lamprecht, R. Andres, I. J. Kopin, *Life Sci.* **17**, 749 (1975).
10. S. B. Ross, L. Wetterberg, M. Hyrhed, *Life Sci.* **12**, 529 (1973); T. Ogihara, C. Nugent, S. Shen, *J. Lab. Clin. Med.* **85**, 566 (1975).
11. B. Shopsin, L. S. Freedman, M. Goldstein, S. Gershon, *Psychopharmacologia* **27**, 11 (1972); D. L. Dunner, C. K. Cohn, R. M. Weinshilboum, R. J. Wyatt, *Biol. Psychiatry* **6**, 115 (1973); H. Y. Meltzer, H. W. Cho, B. J. Carroll, P. Russo, *Arch. Gen. Psychiatry* **33**, 585 (1976); E. S. Markianos, I. Mystrom, H. Reichel, N. Matussek, *Psychopharmacology* **50**, 259 (1976); L. E. Delisi, C. D. Wise, S. G. Potkin, S. Zaleman, B. H. Phelps, W. Lovenberg, R. J. Wyatt, *Biol. Psychiatry* **15**, 899 (1980).
12. T. Okada, T. Ohta, T. Shinoda, T. Kato, K. Ikuta, T. Nagatsu, *Neuropsychobiology* (Switzerland) **2**, 139 (1976); K. Fujita, K. Marata, R. Teradaira, H. Beppu, T. Nagatsu, T. Kato, *J. Neurochem.* **29**, 1141 (1977).
13. P. Lerner, F. K. Goodwin, D. P. van Kammen, R. M. Post, L. F. Major, J. C. Ballenger, W. Lovenberg, *Biol. Psychiatry* **13**, 685 (1978).
14. C. D. Wise and L. Stein, *Science* **181**, 344 (1973); R. J. Wyatt, M. A. Schwartz, E. Erdelyi, J. D. Barchas, *ibid.* **187**, 368 (1975).
15. R. L. Spitzer, J. Endocott, E. Robins, *Am. J. Psychiatry* **132**, 1187 (1975).
16. W. E. Bunney and D. A. Hamburg, *Arch. Gen. Psychiatry* **9**, 280 (1963).
17. All lumbar punctures were nontraumatic and the CSF was free of blood, as determined by the cell counts. To further minimize any blood contamination as well as any possible gradient for DBH, we studied the CSF from each lumbar puncture from a pooled sample of the 16th to the 28th cubic centimeter. The CSF was collected in polypropylene tubes, put into ice immediately, frozen on dry ice within 30 minutes, and then stored in liquid nitrogen. All specimens were assayed within 3 days of the lumbar puncture. Cerebrospinal fluid DBH activity was determined by a modification (*13*) of the radioenzymatic method of P. B. Molinoff, R. Weinshilboum, and J. Axelrod [*J. Pharm. Exp. Ther.* **178**, 425 (1971)]. No significant endogenous inhibition of DBH activity in the CSF was noted. All CSF samples were assayed in duplicate. The coefficient of variation was 8 percent within runs and 13 percent between runs. Recovery of added DBH was always greater than 95 percent.
18. D. E. Sternberg, D. P. van Kammen, P. Lerner, J. C. Ballenger, R. M. Post, W. E. Bunney, *Arch. Gen. Psychiatry*, in press.
19. T. J. Crow, *Br. Med. J.* **280**, 66 (1980).
20. M. S. Buchsbaum, D. L. Murphy, R. D. Coursey, C. R. Lake, M. G. Ziegler, *J. Psychiatr. Res.* **38**, 387 (1979).
21. R. G. Heath, W. Nesselhof, M. P. Bishop, L. W. Byers, *Dis. Nerv. Syst.* **26**, 99 (1965); L. F. Major, P. Lerner, J. C. Ballenger, G. L. Brown, F. K. Goodwin, W. Lovenberg, *Biol. Psychiatry* **14**, 337 (1979).
22. R. L. Sack and F. K. Goodwin, *Arch. Gen. Psychiatry* **31**, 649 (1974).
23. E. Hartmann, *Psychopharmacology* **49**, 1 (1976); E. Hartmann and M. Keller-Teschke, *Biol. Psychiatry* **14**, 455 (1979).
24. N. E. Anden and K. Fuxe, *Br. J. Pharmacol.* **43**, 747 (1971).
25. I. J. Kopin, S. Kaufman, H. Viveros, D. Jacobowitz, C. R. Lake, M. G. Ziegler, W. Lovenberg, F. K. Goodwin, *Ann. Intern. Med.* **85**, 211 (1976).
26. E. C. Johnstone, T. J. Crow, C. D. Frith, *Acta Psychiatr Scand.* **57**, 305 (1978); D. R. Weinberger, L. B. Bigelow, J. W. Kleinman, S. T. Klein, J. E. Rosenblatt, R. J. Wyatt, *Arch. Gen. Psychiatry* **37**, 11 (1980).
27. I. J. Farley, K. S. Price, E. McCullough, J. H. N. Deck, W. Hordynski, O. Hornykiewicz, *Science* **200**, 456 (1978); E. D. Bird, E. G. Spokes, L. L. Iversen, *ibid.* **204**, 93 (1979); C. R. Lake *et al.*, *ibid.* **207**, 331 (1980); D. E. Sternberg, D. P. van Kammen, C. R. Lake, J. C. Ballenger, S. R. Marder, W. E. Bunney, *Am. J. Psychiatry* **138**, 1045 (1981).

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Reduced Leucine-Enkephalin–Like Immunoreactive Substance in Hamster Basal Ganglia After Long-Term Ethanol Exposure

Abstract. *Golden Syrian hamsters were placed individually in cages with three drinking bottles—one empty, one containing water, and the third containing water and ethanol. Control hamsters received water only. After 1 year the experimental hamsters showed a significantly lower concentration of leucine-enkephalin–like immunoreactive substance in the basal ganglia than the control hamsters. This finding indicates that the action of ethanol involves endogenous peptidyl opiates.*

The effect of ethanol has been linked with opioids (*1*), and ethanol has been shown to alter neuronal functions, such as the synthesis, release, and degradation of certain neurotransmitters (*2*). Furthermore, ethanol has been described as effectively interfering with the synthesis of brain peptides (*3*). Concentrations of the peptidyl opiates β-endorphin and enkephalins, which are putative neurotransmitters, neuromodulators, or hormones (*4*), have been altered by short- and long-term treatments with various drugs (*5*). Evidence from animal and human studies indicates that ethanol and narcotic drugs interact at similar endogenous peptidyl opiate sites (*6*) and that these drugs could induce euphoria via the endorphinergic system (*7*). We now report that long-term ethanol consumption in hamsters significantly reduces the concentration of an enkephalin-like immunoreactive substance in the basal ganglia. This finding further supports the involvement of peptidyl opiates in the action of ethanol.

In our experiment we used the alcohol-preferring Syrian hamster (*Mesocricetus auratus*) (*8*). Experimental animals ($N = 10$) were placed individually in cages with three drinking bottles. One bottle was empty, the second contained water, and the third contained a solution of water and 5 percent ethanol (*9*). Control animals ($N = 10$) were placed individually in cages in which they had access to water only. After 1 month the concentration of ethanol was increased to 10 percent and kept there for the remaining 11 months of the experiment. The animals were maintained on a photoperiod with 12 hours of light and room temperature (23° to 25°C) throughout the experiment. Consumption of water and ethanol was measured daily.

At the end of the 12-month period, eight experimental and five control hamsters (*10*) were anesthetized with ether and killed by perfusing the brain through the left cardiac ventricle with a 2.5 percent paraformaldehyde Sorensen-buffer (*p*H 7.4) for approximately 10 minutes or until the liver color turned a grayish white.

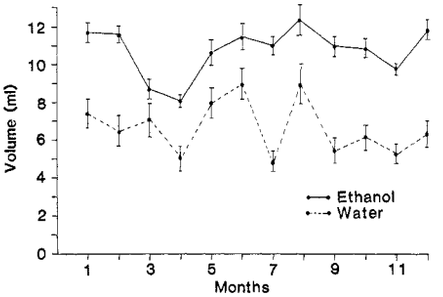

Fig. 1. Consumption of ethanol and water by the eight surviving experimental hamsters over the 1-year period. Values are means ± standard errors.

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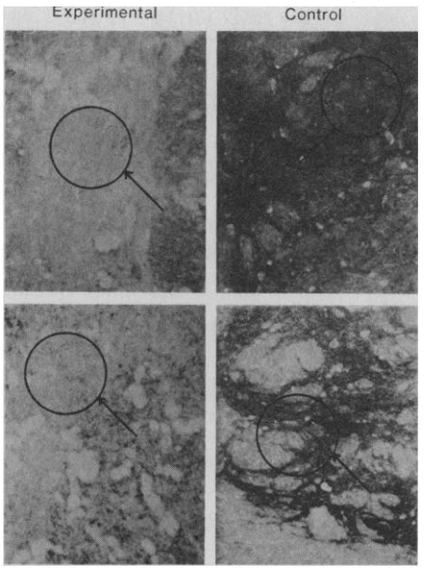

Fig. 2. Brain sections showing relative densities of leucine-enkephalin immunoreactive substance in basal ganglia of hamsters that consumed ethanol (left) and control hamsters (right). Each picture represents a different animal. The circled areas are recommended for comparison.

After perfusion the brains were dissected out and immersed in the same fixative overnight at 4°C. After several washes in 0.1*M* phosphate buffer, the forebrains were dehydrated in ascending grades of alcohol and xylene and embedded in paraffin. Serial frontal sections 5 mm thick were cut, mounted on glass slides, and placed in an incubator at 37°C overnight.

We then used an immunoperoxidase staining procedure (*11*) with antiserum to leucine-enkephalin (*12*). The preparation and specificity of the primary antiserums have been described by Miller *et al.* (*13*). Dilution at 1:100 gave optimal specific staining. After primary incubation, the slides were washed in phosphate-buffered saline (PBS) for 3 minutes, incubated for 10 minutes with antiserum to rabbit gamma globulin (1:100), washed as before with PBS, and incubated for 20 minutes with peroxidase-antiperoxidase (PAP) (1:50). After being washed in PBS, the sections were immersed in 200 ml of 0.05*M* tris (*p*H 7.6) containing 150 mg of diaminobenzidine tetrahydrochloride and 15 μl of 3 percent hydrogen peroxide. The sections were then washed with tris and PBS, counterstained with methyl green, and mounted with Permount. Specificity of the immunoreactivity was established by incubating serial sections with primary antiserums absorbed with leucine-enkephalin or with unabsorbed antiserums.

Figure 1 shows the monthly mean preference ratios for the experimental hamsters (average fluid consumption, 8.52 g/kg per day). As expected, these animals preferred ethanol over water across the 12-month period [$F(13, 143) = 18.23$, $P < .001$]. Although control-group water consumption is not shown in Fig. 1, it ranged from 35.23 ± 1.25 ml per month at the beginning of the experiment to 50.22 ± 3.51 ml per month, at maturity ($N = 5$).

Examples of stained basal ganglia from the hamsters are shown in Fig. 2. Qualitatively, the density of leucine-enkephalin immunoreactivity is much higher in the control ganglia than in the experimental ganglia. All 13 animals exhibited this difference.

Past studies demonstrated that long-term treatment with opiates significantly reduces endogenous peptidyl opiates (*14*) and that long-term ethanol treatment decreases enkephalin activity (*15*) and depresses either methionine-enkephalin in striatum, medulla, pons, and midbrain or β-endorphin in the intermediate and posterior lobes of the rat pituitary (*16*). Although other studies did not show that morphine (*17*) or ethanol (*18*) alters the level of methionine or leucine-enkephalin in brain tissue, our finding of a marked reduction in basal ganglia leucine-enkephalin in hamsters drinking ethanol for a 12-month period is in full agreement with the former studies (*15, 16*) and also suggests occupancy of opiate receptors by ethanol or a metabolite. Interpretation of these results tend to support in part the negative feedback theory for agonist (opiate-like)-induced inhibition of neuronal enkephalin synthesis (*19*). Alternatively, the ethanol-induced reduction of enkephalin-like material may simply be due to the neurotoxic effects of ethanol (*20*).

These results are evidence for the putative role of the peptidyl opiate system in mediating the long-term effects of ethanol (*21*). Additional research along these lines may provide important information concerning the mechanisms underlying alcohol-seeking behavior (*22*).

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References and Notes

1. K. Blum, A. H. Briggs, M. Hirst, M. G. Hamilton, S. F. Elston, K. Verebey, in *Alcohol Tolerance and Dependence*, H. Rigter and J. Crabbe, Eds. (Elsevier–North Holland, Amsterdam, 1980), p. 371.
2. H. Kalant, in *Proceedings of the European Society of Neurochemistry*, V. Neuhoff, Ed. (Weinheim, New York, 1978), vol. 1, p. 317; S. Liljequist, in *ibid.*, p. 359.
3. J. Ellinghoe, in *Psychopharmacology: A Generation of Progress*, M. A. Lipton, A. DiMascio, R. F. Killam, Eds. (Raven, New York, 1978), p. 1653.
4. A Beaumont and J. Hughes, *Annu. Rev. Pharmacol. Toxicol.* **19**, 245 (1979).
5. V. Hollt, R. Przewlocki, A. Herz, *Life Sci.* **23**, 1057 (1978); J. S. Hong, H.-Y. T. Yang, W. Fratta, E. Costa, *J. Pharmacol. Exp. Res.* **205**, 141 (1978); T. Duka, V. Hollt, R. Przewlocki, D. Wesche, *Biochem. Biophys. Res. Commun.* **85**, 1119 (1978); R. Przewlocki, V. Hollt, T. Duka, A. Kleber, C. Gramsch, I. Haarmann, A. Herz, *Brain Res.* **174**, 357 (1979).
6. J. M. Hiller, L. M. Angel, E. J. Simon, *Science* **214**, 468 (1981).
7. D. H. Catlin, K. K. Hui, H. H. Loh, C. H. Li, in *Advances in Biochemistry and Psychopharmacology*, E. Costa and M. Trabucchi, Eds. (Raven, New York, 1978), p. 341; L. Stein and J. D. Belluzzi, in *ibid.*, p. 299; K. Verebey and K. Blum, *J. Psychedelic Drugs* **11**, 305 (1979); E. Wei and H. Loh, *Science* **193**, 1262 (1976); W. K. K. Ho, H. L. Wen, N. Ling, *Neuropharmacology* **19**, 117 (1980); J. E. Greenwald, R. H. Fertel, L. K. Wong, R. D. Schwarz, J. R. Bianchine, *Fed. Proc. Fed. Am. Soc. Exp. Biol.* **38**, 379 (1979); M. G. Hamilton, M. Hirst, K. Blum, *Life Sci.* **25**, 2205 (1979); M. G. Hamilton and M. Hirst, *Substance Alcohol Actions/Misuse* **1** (No. 2), 121 (1980).
8. A. Arvola and O. Forsander, *Nature (London)* **191**, 819 (1961). The hamsters weighed 51 to 60 g initially (age, 1 month) and 125 to 150 g after 1 year.
9. R. D. Meyers and B. Holman, *Psychon. Sci.* **6** (No. 5), 235 (1966). We decided not to utilize a controlled caloric supplement like Sustacal because we wanted to mimic closely the typical alcoholic seeking alcohol in a uncontrolled environment.
10. During the year seven of the hamsters died from either respiratory infection or an undetermined cause.
11. M. Sar, W. E. Stumpf, R. J. Miller, K.-J. Chang, P. Cuatrecasas, *J. Comp. Neurol.* **182**, 17 (1978).
12. The antiserum was provided by R. J. Miller, Department of Pharmacology, University of Chicago. Leucine-enkephalin is now seen as a more important putative neurotransmitter than methionine-enkephalin because it is more opioid. Leucine-enkephalin, not methionine-enkephalin, enhances the morphine-releasing effect of naloxone in vivo and in vitro in the corpus striatum of mice. [J. L. Vaught, T. Kitano, A. E. Takemori, *Mol. Pharmacol.* **19**, 236 (1981).]
13. R. J. Miller, K. J. Chang, B. Cooper, P. Cuatrecasas, *J. Biol. Chem.* **253**, 531 (1978).
14. V. Hollt, R. Przewlocki, A. Herz, *Life Sci.* **23**, 1057 (1978); G. Ungar, A. L. Ungar, D. H. Malin, D. Sarantakis, *Psychoneuroendocrinology* **2**, 1 (1977).
15. F. S. Labella, in *Drug Dependence and Alcoholism*, A. Schecter, Ed. (Plenum, New York, 1980), pp. 1079–1110.
16. R. Schultz, M. Wuster, T. Duka, A. Herz, *Psychopharmacology* **68**, 221 (1980); R. Schultz, *Naunyn-Schmiedeberg's Arch. Pharmacol.* **308**, 124 (1979).
17. S. R. Childers, R. Simanton, S. H. Snyder, *Eur. J. Pharmacol.* **46**, 289 (1977); P. Wesche, V. Hollt, A. Herz, *Naunyn-Schmiedeberg's Arch. Pharmacol.* **301**, 75 (1977); J. Shani, R. Azor, B. A. Weissman, *Neurosci. Lett.* **12**, 319 (1979); W. Fratta, H.-Y. T. Hong, *Nature (London)* **268**, 452 (1977).
18. S. Ryder, E. Straus, C. S. Lieber, R. S. Yalow, *Peptides* **2**, 223 (1981).
19. A. Goldstein, *Alcohol and Opiates: Neurochemical and Behavioral Mechanisms*, K. Blum, Ed. (Academic Press, New York, 1977), p. 397. Alcohol addicts have three times less β-endorphin and significantly more adrenocorticotropic hormone in cerebrospinal fluid than normal volunteers (A. R. Genazzini, G. Nappi, F. Facchinetti, G. L. Mazzella, D. Parrini, E. Sinforiani, F. Petraglia, F. Savoli, *Clin. Endocrinol. Metab.* in press).
20. C. J. Golden, B. Graber, I. Blose, R. Berg, J. Coffman, S. Bloch, *Science* **211**, 508 (1981).
21. J. S. Hong, E. Majchrowicz, W. A. Hunt, J. C.

Gillin, *Substance Alcohol Actions/Misuse* **2** (No. 4), 233 (1981); H. L. Altshuler, P. E. Phillips, D. A. Feinhandler, *Life Sci.* **26**, 679 (1980); D. H. Ross, I. Geller, R. J. Hartmann, *Proc. West. Pharmacol. Soc.* **19**, 326 (1976).
22. K. Blum, A. H. Briggs, S. F. A. Elston, L. DeLallo, *Substance Alcohol Actions/Misuse* **1** (No. 2), 255 (1980). In experiments in which radioimmunoassay was used to investigate the interrelatedness between peptidyl opiates and ethanol-seeking behavior, we found a significant negative correlation between the amount of ethanol consumed in four different inbred strains of mice and methionine-enkephalin content in whole brains [K. Blum, A. H. Briggs, S. F. A. Elston, L. DeLallo, *Toxicol. Eur. Res.* **3** (No. 5), 261 (1981)].
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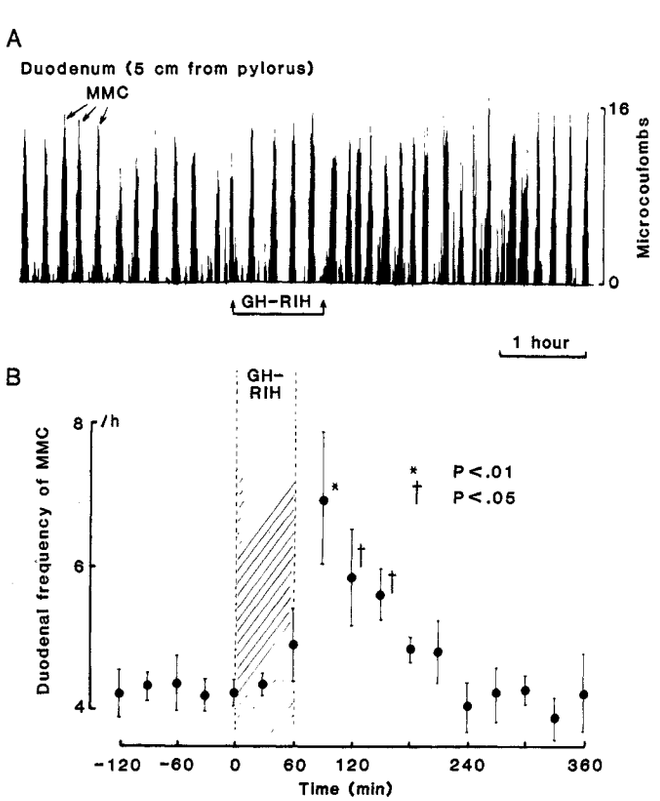
Central Regulation of Intestinal Motility by Somatostatin and Cholecystokinin Octapeptide

Abstract. *When injected continuously into the lateral ventricles of the rat, somatostatin increased the frequency of the migrating myoelectric complexes of the small intestine in a dose-related manner. A significant increase was obtained at a dose as low as 0.066 picomole per minute. In contrast, cholecystokinin octapeptide decreased the frequency of the migrating myoelectric complex of the small intestine or disrupted this pattern when injected into the lateral ventricle at rates of 0.073 to 0.23 picomole per minute. These findings support the hypothesis that somatostatin and cholecystokinin octapeptide act on central nervous system structures that are involved in the control of intestinal motility.*

Many peptides in the brain also occur in the gut. Biologically active peptides such as somatostatin and cholecystokinin octapeptide (CCK-OP), which are secreted from endocrine cells of the gastrointestinal tract, have also been identified in afferent fibers of the vagus nerves (*1*). There is evidence that CCK-OP is a satiety factor in the rat (*2*) and sheep (*3*); CCK-OP has been shown to both originate and act in the brain (*3, 4*). Somatostatin has been found in the cerebrospinal fluid (CSF) and in several areas of the brain in larger amounts than in peripheral blood (*5*), but no experiments have been performed on its role in feeding behavior.

These two neuropeptides modify the pattern of intestinal motility when they are injected intravenously. The motility of the small intestine is organized in many species in cyclical sequences of contractions occurring regularly in the duodenum and propagated through the entire small intestine. Each contraction is associated with a burst of potentials, and the recording of the electrical activity shows the presence of migrating myoelectric complexes (MMC) consisting of irregular spiking activity followed by a short period of intense activity sometimes called regular spiking activity (*6, 7*). This motor sequence progressively migrates to the ileum propelling the digestive contents at a mean rate of 15 cm/min. Successive MMC are separated by periods of quiescence. In fasted rats these MMC last 12 to 15 minutes and occur in the duodenum at about 20-minute intervals (*7*).

Systemic infusion of somatostatin increases the frequency of the MMC in the dog and reduces gastric activity (*8, 9*); in contrast, CCK-OP stimulates antroduodenal motility and disrupts the MMC pattern in the dog (*10*). Intracerebroventricular administration of somatostatin can stimulate gastric secretion (volume, titrable acidity, and pepsin), and it has been proposed that this peptide acts initially within the brain, stimulating afferent vagal fibers to cause the increase in gastric secretion via a cholinergic mech-



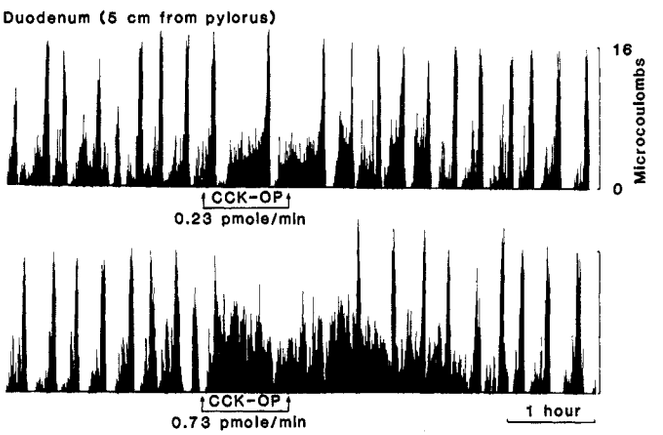


Fig. 1 (left). Influence of intracerebroventricular administration of somatostatin (*GH-RIH*) on the frequency of the migrating myoelectric complex (*MMC*) of the small intestine in a fasted rat. (A) Integrated record of electrical activity of the duodenum obtained by continuous 20-second summation of spikes collected from intramuscular electrodes. The period of somatostatin infusion (0.2 pmole/min) is indicated by the vertical arrows. The interval between two consecutive MMC shortened after somatostatin infusion. (B) Frequency of duodenal MMC (mean ± standard error, $N = 12$) measured during consecutive 30-minute periods. Somatostatin infusion (0.2 pmole/min) significantly increased the frequency of MMC. Fig. 2 (right). Comparative effects of two doses of CCK-OP administered intracerebroventricularly on the pattern of MMC of the small intestine in a fasted rat. The duration of the MMC cycle increased at the lower rate of infusion while at the higher rate (0.73 pmole/min) the MMC was disrupted in a manner similar to that seen after a meal.

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