the pattern of innervation to the limb. Thus, they have found that upper arm regenerates are derived mainly from the posterior and ventral sectors of the stump, in which the major nerve trunks are concentrated. A nonuniform distribution of nerves in the blastema could therefore account for the nonequivalence of regenerates arising from double anterior and double posterior half limbs. This possibility cannot be ruled out, for blastemas derived from those limbs are heavily innervated throughout [see also (*2*)]. Why then, do both double anterior and double posterior hindlimb stumps not form normal regenerates? The most likely answer is that the halves of symmetrical hindlimbs in some way interact with one another in an inhibitory fashion so that each is prevented from regenerating other than its original prospective significance. This hypothesis is supported by the fact that double posterior half limbs allowed to heal for 32 days before amputation produce fewer tarsals and toes than do those which healed for only 10 to 14 days.

Although the idea of differential contributions to the blastema by different stump sectors is consistent with the results reported here, it is not consistent with inability of both double anterior and double posterior upper arms of adult newts to undergo distal transformation (*2*). However, we cannot discount the possibility that positional information may be arranged or generated in different ways in forelimbs and hindlimbs, in limbs of different genera, or in embryonic and postembryonic limbs. For example, Carlson (*7*) has shown that amputation after 180° displacement of tissues relative to one another in the upper arm of *A. mexicanum* results in about 80 percent of the regenerates having multiple digits, whereas the same experimental manipulations in the hindlimb result in multiple digits in only 10 percent of the cases (*8*). It would be instructive in this regard to do a comparative study which might lead to a more unified concept of the process of pattern formation and regulation in amphibian appendages.

DAVID L. STOCUM

Department of Genetics and Development, University of Illinois, Urbana 61801

References and Notes

1. V. French, P. Bryant, S. Bryant, *Science* **193**, 969 (1976).
2. S. Bryant, *Nature (London)* **263**, 676 (1976).
3. As currently conceived for upper arm regeneration, the polar coordinate model does assume an unequal spacing of positional values in the circular sequence which, however, places more than 50 percent of the sequence in the anterior half of the limb [S. Bryant and L. Iten, *Dev. Biol.* **50**, 212 (1976)]. This spacing pattern is necessary to account for the fact that supernumerary limbs formed after 180° rotation of a blastema with respect to its stump arise only in the anteroventral and dorsoposterior quadrants of the limb. It is possible that the spacing pattern is reversed in the hindlimb of *A. mexicanum* or *A. tigrinum*.
4. R. Goss, *J. Morphol.* **100**, 547 (1957).
5. D. Stocum, *Dev. Biol.* **18**, 457 (1968).
6. M. Singer, E. Ray, A. Peadon, *Folia Biol. (Krakow)* **12**, 347 (1964).
7. B. Carlson, *Dev. Biol.* **47**, 269 (1975).
8. ———, personal communication.
9. T. Steen, *J. Exp. Zool.* **167**, 49 (1968).
10. P. Weiss, *Wilhelm Roux Arch. Entwicklungsmech. Org.* **194**, 359 (1925).
11. E. Francis, *The Anatomy of the Salamander* (Oxford Univ. Press, London, 1934).
12. I thank Drs. J. B. Nardi and D. L. Nanney for their helpful discussion and critical evaluation of the mauscript. Supported by grant BMS 71-01579 A 01 from National Science Foundation.

10 November 1977; revised 1 February 1978

Cholecystokinin Inhibits Tail Pinch–Induced Eating in Rats

Abstract. *Peripheral administration of the COOH-terminal octapeptide of cholecystokinin in doses from 1 to 100 micrograms per kilogram of body weight (0.25 to 25.0 micrograms per rat) significantly antagonized tail pinch–induced eating in rats, an animal model for stress-induced human hyperphagia. Centrally administered cholecystokinin was effective only in high doses (3 micrograms into the cerebral ventricle). The finding that the minimal effective dose of cholecystokinin in suppressing stress-induced appetitive behavior is smaller after peripheral than central administration suggests that the peptide is acting on peripheral, as opposed to central nervous system, substrates.*

Antelman and associates (*1*, *2*) and others (*3*) have reported that mild tail pinch reliably induces eating in sated rats. This is particularly interesting in view of observations that eating behavior in both humans and animals increases during stress (*4*). We now report that administration of the active core (COOH-terminal octapeptide) of cholecystokinin (CCK) (*5*), an intestinal peptide hormone reported to induce satiety (*6*, *7*), antagonizes tail pinch–induced ingestive behavior in rats.

The tail pinch method (*1*, *2*) was used in our experiments. Naive adult male Sprague-Dawley rats weighing about 250 g were screened by placing them in a stainless steel surgical bowl (33.8 cm in diameter) in the presence of 5 g of rat chow (45-mg pellets, Noyes) and applying a 2-minute tail pinch about 5 cm from the tail tip with a 25-cm surgical hemostat insulated at the tips with foam rubber. This procedure produced a positive response in 66 percent of animals tested; approximately the same response rate was obtained by applying a pressure of 70 to 100 pounds per square inch (psi) with a calibrated pressure cuff. Positive responders were defined as those that exhibited continuous chewing and eating during the 2-minute test period. Nonresponders were excluded from further experimentation and responders were used on subsequent days as follows. In experiments examining the effects of peripheral peptide administration on tail pinch–induced ingestive behavior, rats were injected intraperitoneally with various doses of the COOH-terminal octapeptide of CCK (*8*) or vehicle (0.9 percent NaCl, *p*H 7.5). Five minutes later each rat was placed in a surgical bowl as described above and a continuous 10-minute mild tail pinch was applied. At the end of the test period, the amount of food consumed was calculated. In experiments on the effects of central administration of CCK on tail pinch–induced eating, rats with implanted lateral ventricular cannulae (1.4 mm lateral and 0.5 mm posterior to the bregma and 3.5 mm from the top of the skull) (*9*) were treated with CCK or vehicle (10 μl) and tested 5 minutes later as described above. Other experiments were performed to determine the effects of CCK administration on tail pinch–induced gnawing behavior. In these studies rats received either CCK or vehicle intraperitoneally and were placed in a surgical bowl containing wood chips. Gnawing behavior was quantified by counting jaw movements

Table 1. The effect of intracerebroventricular administration of cholecystokinin on tail pinch-induced eating in rats. Rats were injected through lateral ventricular cannulae with 10 μl of vehicle (0.9 percent NaCl, *p*H 7.5) or CCK dissolved in vehicle; 5 minutes later they received a 10-minute continuous tail pinch. Mean food consumed by control rats was 1.82 ± 0.19 g. Student's *t*-test (two-tailed) was used to compute statistical differences. Although the total number of control animals is shown, computation of statistical differences was made between experimental and control groups ($N \geq 6$) for each experiment. Food consumption is given as mean percentage of control ± standard error of mean; N.S., not significant.

Treatment	*N*	Food consumed (% of control)	*P*
Vehicle	30	100.0 ± 10.0	
CCK, 0.3 μg	6	92.4 ± 19.1	N.S.
CCK, 0.5 μg	13	72.2 ± 12.9	N.S.
CCK, 1.0 μg	6	59.6 ± 19.0	N.S.
CCK, 3.0 μg	8	22.16 ± 6.9	< .01

0036-8075/78/0519-0793$00.50/0

during the tail pinch. In all experiments an observer, unaware of the treatment given, recorded food consumption or gnawing behavior during the testing period.

Peripheral administration of the COOH-terminal octapeptide of CCK (1.0 to 100 μg per kilogram of body weight) produced a dose-dependent reduction in tail pinch–induced ingestive behavior (Fig. 1). In contrast, peripheral administration of bradykinin (100 μg/kg, intraperitoneally), an endogenous nonapeptide of molecular weight similar to that of the COOH-terminal octapeptide of CCK, had no effect on tail pinch–induced eating. During the 10-minute tail pinch seven bradykinin-treated rats consumed 1.25 ± 0.17 g (mean ± standard error of mean); seven control rats consumed 1.55 ± 0.21 g ($P > 0.1$). Peripherally administered CCK in a dose that maximally abolished tail pinch–induced eating (100 μg/kg) had no effect on tail pinch–induced gnawing behavior; six CCK-treated animals exhibited 2125 ± 354 gnawing movements, and six control animals exhibited 1851 ± 308 gnawing movements.

Central administration of the COOH-terminal octapeptide of CCK significantly reduced tail pinch–induced eating only when a high dose (3 μg) was given (Table 1).

These results demonstrate that the active core of CCK produces a dose-dependent antagonism of tail pinch–induced eating. This intestinal peptide hormone, which is released when food enters the gut (*10*), suppresses eating in intact rats (*11*), in rats with open gastric fistulas (*12*), and in intact rhesus monkeys (*13*). In humans an impure CCK preparation either inhibits or stimulates food intake (*14*), depending on the dose and route of administration. Furthermore, CCK does not inhibit water intake in rats (*15*). Cholecystokinin does not induce satiety in rats by inducing illness; this has been demonstrated by use of the bait-shyness paradigm (*16*). The hormone brings about release of insulin and glucagon (*17*), but the subsequent changes in blood sugar probably do not account for the observed satiety effect since glucagon administration does not mimic the effects of CCK (*18*).

Although several endogenous peptides exert direct effects on the central nervous system (*19*), the results of intracerebroventricular administration of CCK (Table 1) indicate that this peptide probably does not act directly on the brain, since the dose of centrally administered CCK needed to suppress feeding

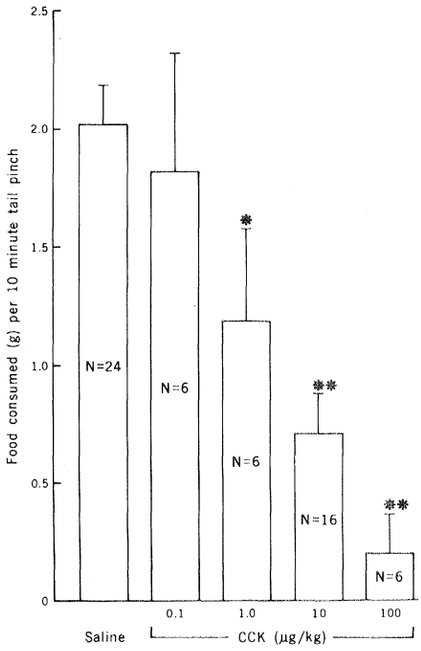

Fig. 1. The effect of intraperitoneally administered CCK on tail pinch–induced eating in rats. Rats were injected with 0.9 percent NaCl (*p*H 7.5), or CCK (0.1 to 100 μg/kg). Five minutes later they were given a 10-minute tail pinch (70 to 100 psi). Significance was determined by Student's *t*-test, two-tailed; $*P < .05$; $**P < .001$; *N*, number of animals. The thin bars indicate standard error of mean.

(3 μg) is greater than the minimal anorexic dose for peripheral administration (1 μg/kg or 0.25 μg per rat). The effect observed at this high dose may be due to leakage of administered CCK from the cerebrospinal fluid into the peripheral circulation. These results are of particular interest in view of a recent hypothesis (*20*) that hunger and satiety can be explained on the basis of peripheral biochemical and physiological processes of energy metabolism. This hypothesis contrasts with the traditional view that ingestive behavior is primarily modulated by central nervous system structures (*4*). Immunohistochemical and radioimmunoassay data have recently provided evidence for the presence of CCK-like peptides in the brains of rats, pigs, and dogs (*21*). Thus it is possible that CCK released from brain may modulate satiety mechanisms.

Tail pinch–induced eating has been shown to be dependent on the nigroneostriatal dopamine system (*1*). The data reported here do not contradict those results but indicate that CCK, which appears to act through peripheral mechanisms, can also modify this response.

The finding that peripherally administered CCK inhibits ingestive behavior in the tail pinch paradigm, which is considered to be an animal model of stress-induced eating (*22*), suggests that this peptide (or an analogue that is resistant to biological degradation and therefore perhaps longer-acting) may be useful in the therapy of human stress-induced hyperphagia. This possibility is heightened by the small peripheral doses that were effective in these experiments, as little as 1 μg/kg.

CHARLES B. NEMEROFF
ALBERT J. OSBAHR III, GARTH BISSETTE
GLORIA JAHNKE, MORRIS A. LIPTON
ARTHUR J. PRANGE, JR.
Biological Sciences Research Center, Department of Psychiatry, and *Neurobiology Program, University of North Carolina School of Medicine, Chapel Hill 27514*

References and Notes

1. S. M. Antelman and H. Szechtman, *Science* **189**, 731 (1975); _____, P. Chin, A. E. Fisher, *Brain Res.* **99**, 319 (1975).
2. N. E. Rowland and S. M. Antelman, *Science* **191**, 310 (1976).
3. E. J. Mufson, S. Balagura, W. Riss, *Brain Behav. Evol.* **13**, 154 (1976); G. F. Koob, P. J. Fray, S. D. Iversen, *Science* **194**, 637 (1976).
4. L. D. Lytle, in *Nutrition and the Brain*, R. J. Wurtman and J. J. Wurtman, Eds. (Raven, New York, 1977), p. 1.
5. In this report the COOH-terminal octapeptide of the CCK molecule, which contains all the known biological and behavioral activity of the parent compound (*6*), is sometimes referred to as CCK. One microgram of the octapeptide is equivalent to 22 Ivy dog units.
6. G. P. Smith, J. Gibbs, R. C. Young, *Fed. Proc. Fed. Am. Soc. Exp. Biol.* **33**, 1146 (1975); G. P. Smith and J. Gibbs, *Pharmacol. Biochem. Behav.* **3** (Suppl. 1), 135 (1975).
7. I. L. Bernstein, E. C. Lotter, J. C. Zimmerman, *Physiol. Behav.* **17**, 541 (1976).
8. The peptide was provided by M. Ondetti, Squibb Laboratories, Princeton, N.J.
9. J. F. R. Konig and R. A. Klippel, *The Rat Brain: A Stereotaxic Atlas of the Forebrain and Lower Parts of the Brain Stem* (Williams & Wilkins, Baltimore, 1973).
10. G. M. Makhloof, *Gastroenterology* **67**, 159 (1974).
11. J. Gibbs, R. C. Young, G. P. Smith, *Nature (London)* **245**, 323 (1975).
12. _____, *J. Comp. Physiol. Psychol.* **84**, 488 (1973).
13. J. Gibbs and G. P. Smith, *Am. J. Clin. Nutrit.*, in press.
14. R. A. L. Sturdevant and H. Goetz, *Nature (London)* **261**, 713 (1976).
15. K. Mueller and S. Hsiao, *Pharmacol. Biochem. Behav.* **6**, 643 (1977).
16. J. Holt, J. Antin, J. Gibbs, R. C. Young, G. P. Smith, *Physiol. Behav.* **12**, 497 (1974).
17. M. I. Grossman, in *Methods in Investigative and Diagnostic Endocrinology*, S. A. Berson and R. S. Yalow, Eds. (Elsevier, New York, 1973), vol. 2B, p. 1080.
18. A. V. Schally, T. W. Redding, H. W. Lucien, J. Meyer, *Science* **157**, 210 (1967).
19. A. J. Prange, Jr., C. B. Nemeroff, M. A. Lipton, G. R. Breese, I. C. Wilson, in *Handbook of Psychopharmacology*, L. L. Iversen, S. D. Iversen, and S. H. Snyder, Eds. (Plenum, New York, 1978), vol. 13.
20. M. I. Friedman and E. M. Stricker, *Psychol. Rev.* **83**, 409 (1976).
21. G. J. Dockray, *Nature (London)* **264**, 568 (1976); E. Straus, J. E. Muller, H.-S. Choi, F. Paronetto, R. S. Yalow, *Proc. Natl. Acad. Sci. U.S.A.* **74**, 3033 (19 → J. E. Muller, E. Straus, R. S. Yalow, *ibid.*, p. 3035.
22. M. B. Wallach, M. Dawber, M. McMahon, C. Rogers, *Pharmacol. Biochem. Behav.* **6**, 529 (1977).
23. Supported by NIH grants NS-05722, HD-03110, and a career scientist award to A.J.P. (MH-22536). We thank V. Jerrett for her help in preparation of this manuscript.

1 August 1977; revised 16 December 1977