also not significant. Both paranoid and nonparanoid groups were hospitalized on identical wards and were chronically ill for comparable time periods, so that chronicity and environmental factors did not account for the differences in PEA excretion seen in these two groups (*23*). Patient cooperation in urine collection is always a methodological problem, and the adequacy of collection can never be assured. Nevertheless, even with urine volumes that may have been inadequate, we found an increase in urinary PEA in schizophrenic patients.

Since monoamine oxidase (MAO), a major enzyme in PEA degradation, is decreased in the platelets of some chronic schizophrenics—perhaps, in particular, those with paranoid symptoms (*24*)—it is attractive to speculate that such a reduction might represent a decreased capacity to metabolize PEA and thereby lead to its accumulation. Demish *et al.* (*25*), in the only published study in which PEA was used as substrate for MAO, reported reduced MAO for the paranoid subgroup only (12.1 nmole per milligram of protein per hour versus 16 for normal subjects).

The finding of increased PEA in urine of paranoid chronic schizophrenics offers some indication that PEA may be an endogenous amphetamine. Nonetheless, results must be viewed with caution. Although PEA readily crosses the blood-brain barrier, the relationship of urinary 24-hour PEA excretion and circulating brain concentrations of PEA is unknown. The relationship between PEA excretion and changes in clinical state has not yet been studied. Most importantly, although much is known about amphetamine's ability to produce a paranoid psychosis and of the similarities of PEA to amphetamine in animal models, the potential psychotomimetic effects of PEA in humans are yet to be explored.

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19. Urine was collected into refrigerated containers with 5 ml of 10 percent EDTA and stored at − 20°C until analyzed. In the analyses, 0.2-ml portions of the urine were mixed with 1 ml of 0.5 M phosphate buffer. Deuterated PEA was added as internal standard and the mixture was immediately extracted into ethyl acetate. The PEA was then converted to a pentafluoropropionyl derivative with 100 percent pentafluoropropionyl imidazole (PFPI) in ethyl acetate (25 μl). The extracted PEA was heated at 70°C for 10 minutes and cooled; 5 μl of 10 percent dry methanol was added, and the mixture was reheated at 70°C for 10 minutes. This last step was necessary to remove excess PFPI. Gas chromatography was carried out on an 0.8″ (outside diameter) column packed with 1 percent SE 54 + 0.5 percent OV + 0.2 percent OV_{210}. A quadrupole gas chromatograph mass spectrometer (Finnegan 3200) focused on ions of 104 and 107 atomic mass units to detect PEA and [$^2H_4$]PEA, respectively (*14*).
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21. Consecutive 12-hour urine collections were begun at 9 a.m. No difference was observed between the morning and evening collections. Suzuki and Yagi (*17*) collected consecutive 8-hour urine samples from three normal subjects and found increased PEA excretion in the samples taken from 4 p.m. to midnight.
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Cholecystokinin Octapeptide: Continuous Picomole Injections into the Cerebral Ventricles of Sheep Suppress Feeding

Abstract. *Cholecystokinin octapeptide decreased food intake in a dose-related manner when injected continuously into the lateral cerebral ventricles of sheep that had been deprived of food for 2, 4, 8, or 24 hours. In sheep deprived of food for 2 hours, as little as 0.01 picomole per minute suppressed feeding 35 percent 1 hour after beginning injection. Pentagastrin also decreased feeding in the 2-hour group, but only at a much higher dose range. Secretin had no effect. These findings support the hypothesis that cholecystokinin octapeptide acts on central nervous system structures that are involved in control of food intake.*

The peptides in the gastrointestinal (GI) tract likely play some role in the control of food intake under normal conditions. They are secreted in response to the quality and quantity of ingested food, enter the bloodstream, and can be transported to the brain to act as signals to regulatory mechanisms. In the past few years, evidence has been accumulating for a role of cholecystokinin (CCK, 33 amino acids) as a satiety factor.

Originally it was hypothesized that CCK released by the GI tract acted on receptors that mediate the food intake response (*1*). The central nervous system (CNS) has been considered to be the probable site for these receptors. It was shown that the ventromedial hypothala-

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mus (VMH) selectively bound radioactively labeled caerulein (the decapeptide amide of CCK) after intraperitoneal administration, whereas the lateral hypothalamus (LH) did not (*2*).

Recently, CCK and several of its derivatives, such as CCK octapeptide, have been found in cerebrospinal fluid (CSF) and in large amounts in selective areas of the brain of several species, including human beings (*3*), sheep (*4*), pigs (*5*), and rabbits (*6*). Gastrin (G_{17}, G_{34}) has been found in human CSF (*3*) and in the anterior and posterior pituitary of the pig, which is devoid of CCK activity. The role in satiety of CCK released from the GI tract has therefore become less clear. There are at least three lines of evidence suggesting that CCK or CCK octapeptide originating in the CNS rather than the gastrointestinal tract plays the major role in food intake control. First, compared to the amounts normally found in other species (*8*) only large, systemically administered amounts of CCK or its derivatives effectively inhibit feeding in the rat (*9*) or pig (*10*). Second, the concentration of CCK in human CSF is more than ten times that measured in plasma in individuals who have fasted and is more than five times the concentration measured after eating (*3*). Third, the brains of obese mice of the *ob/ob* genotype have significantly lower amounts of CCK octapeptide than those of their lean littermates (*OB/-*) (*11*). In this report, we provide further evidence that CCK octapeptide is a potent inhibitor of feeding when administered centrally.

Castrated male sheep (wethers) were prepared for long-term experimentation with bilateral stainless steel cannulas directed toward the lateral ventricles (LV's). The sheep were given fresh food and allowed to eat for 1 hour prior to each period of food deprivation. After the sheep were deprived of food for 2, 4, 8, or 24 hours, synthetic CSF (*12*) with one of several concentrations of a peptide (CCK octapeptide, pentagastrin, or secretin) was injected continuously into the LV's for 3 hours at the rate of 0.1 ml/min. Treatment with CCK octapeptide (0.010 to 40.8 pmole/min) or pentagastrin (64 to 1020 pmole/min) was determined by randomized Latin-square designs. Food intake was measured at various intervals during the injection period and 1 hour after the end of injection.

Cholecystokinin octapeptide was extremely effective in reducing food intake by the sheep. For all food-deprivation periods, CCK octapeptide reduced intake in a dose-related manner. In sheep denied food for 2 hours, [a normal between-meals interval in this species (*13*)], as little as 0.01 pmole/min suppressed feeding 35 percent ($P < .03$, analysis of variance) during the first hour of treatment. A dose of 0.159 pmole/min reduced feeding 50 percent ($P < .03$) during the first 15 minutes; 1 hour after the injection ended, feeding was still reduced by 47 percent ($P < .006$) (Fig. 1). Doses greater than 0.159 pmole/min suppressed feeding at least 85 percent during the injection period. The longer the period of food deprivation, the smaller the effect of CCK octapeptide on food intake: after 3 hours at 0.64 pmole/min, feeding by sheep deprived of food for 2, 4, 8, and 24 hours was reduced by 95, 48, 34, and 19 percent, respectively, and at 2.5 pmole/min, by 100, 89, 60, and 22 percent, respectively (Table 1). Although the latter dose completely inhibited feeding in the 2-hour group when injected into the LV, it had no effect when administered as a continuous intravenous injection for 3 hours. The injection of CCK octapeptide into the LV had no effect on rectal temperature. Evidence that CCK octapeptide specifically affected food intake is that sheep deprived of water rather than food for 2 hours did not decrease water intake when injected intraventricularly for 3 hours at the rate of 0.64 pmole/min but did decrease food intake.

Table 1. Food intake (grams) during the 3 hours of injection of CCK octapeptide (0.1 ml/min) into the lateral ventricles of sheep. Data are expressed as means ± standard errors.

Hours food was withheld	Carrier (synthetic CSF)	CCK octapeptide (pmole/min)	
		0.638	2.55
2	124 ± 20	11 ± 7*	0 ± 0*
4	202 ± 45	105 ± 34†	22 ± 18†
8	294 ± 69	194 ± 79	140 ± 48†
24	437 ± 60	356 ± 106	340 ± 126

*$P < .01$. †$P < .05$.

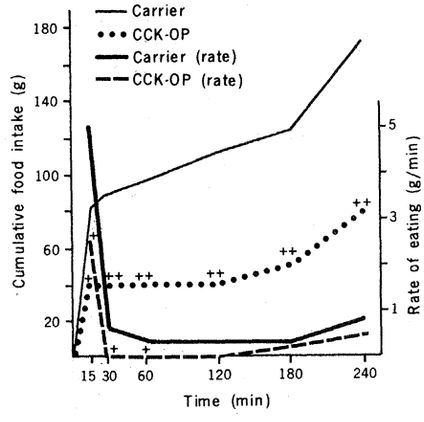

Fig. 1. Cumulative food intake and rate of eating of sheep deprived of food for 2 hours and injected with CCK octapeptide (CCK-OP; 0.159 pmole/min) into the lateral ventricles. The carrier was synthetic CSF. Values for sheep injected with CCK octapeptide are significantly different from control values at + ($P < .05$) and ++ ($P < .01$).

Pentagastrin (the COOH terminus pentapeptide of gastrin that has all the biological activity of gastrin but which is structurally similar to CCK octapeptide) also suppressed feeding when injected into the LV of sheep deprived of food for 2 or 4 hours, but the dose range was several hundred times that of CCK octapeptide. In the 2-hour group, CCK octapeptide (40.8 pmole/min) suppressed feeding 100 percent ($P < .001$) during the first hour of the injections, but a similar dose of pentagastrin (65 pmole/min) suppressed feeding by only 56 percent ($P < .01$). Food intake by the 4-hour group was reduced by 56 percent ($P < .01$) during the first hour only by the largest dose of pentagastrin (1040 pmole/min); whereas as little as 2.55 pmole of CCK octapeptide per minute decreased food intake by 90 percent ($P < .01$) for 1 hour. Although pentagastrin decreased food intake, injections produced behaviors, such as foot-stamping and vocalization, not normally associated with satiety in sheep. These behaviors were not exhibited during the CCK octapeptide injection. Intravenous injections of pentagastrin (1040 pmole/min) in the 2-hour group had no effect. Secretin, another GI hormone (27 amino acids, structurally unrelated to CCK and gastrin), had no effect on food intake when injected into the LV at a dose comparable to the largest pentagastrin dose (1000 pmole/min).

The three peptides were also tested in rats with long-term implants of cannulas into the LV. In the first experimental condition, rats were exposed to a cycle of 12 hours of light and 12 hours of darkness and were given food only while the lights were on (leftover food was removed after lights out). After they were deprived of food for 12 hours, continuous injections (1 μl/min) were made into the LV for 3 hours with solutions in synthetic CSF of CCK octapeptide, pentagastrin, or secretin. In the second condition, rats were maintained in constant light, with food and water freely available. After being deprived of food for 2 hours, they were injected in the same manner.

None of the peptides had any effect on food intake in the rats we tested, even though the doses were very much greater than those used on the sheep when ex-

pressed in picomoles per kilogram per minute (for sheep, 0.00033 pmole/kg-min; for rats, 1.36 pmole/kg-min). Even after food deprivation of only 2 hours in either lighting condition, CCK octapeptide did not affect feeding. However, food intake has been reduced in rats deprived of food for 4 hours by bolus intraventricular injection of caerulein (80, 200, and 300 pmole/kg), which has been shown to decrease food intake in rats when administered systemically (*2*); thus, rats appear to be considerably less sensitive than sheep to the CNS-mediated inhibition of feeding by CCK octapeptide or pentagastrin.

In the past decade the effects of CCK and its derivatives on feeding behavior have received much attention. Although rats have been the principal focus of these studies, a reduction of food intake following systemic administration of the hormone has been demonstrated for several other species (*9*). It has been recognized that not only do CCK and CCK octapeptide exist in the brain and CSF, but when injected there they can have specific effects. For example, caerulein injected as a bolus into the LV of rats at much larger doses than in our experiment inhibited feeding, as did microinjections of caerulein into the VMH but not the LH (*1*); CCK octapeptide injected into various areas of rat brain caused electrophysiological changes (*13*). It has been found that obese mice of the *ob/ob* genotype had significantly reduced concentrations of cerebral cortical CCK octapeptide compared to their lean littermates (*OB/-*), suggesting that brain CCK octapeptide may play a role in the genesis of obesity (*11*). Innis *et al.* (*14*) have reported that in rat brain, high concentrations of CCK octapeptide cells are located in the hypothalamus—particularly the dorsomedial area. This also supports a role of CCK octapeptide in control of food intake. Concentrations of CCK of 1 nmole per gram of tissue (wet weight) have been found in the telencephalic gray matter of human brains. This amount is at least ten times greater than those reported so far for other hormonal peptides, releasing factors, or release-inhibition factors, a finding that is consistent with the neurotransmitter role that has been suggested for CCK octapeptide (*3*).

Our use of a prolonged slow rate of injection of extremely low concentrations of these peptides is probably a more accurate test method from a physiological point of view than methods in which bolus injections of high concentrations are used. The specificity of the effect of CCK octapeptide for food intake rather than water intake and the lack of effect of systemic injections at much larger doses suggest that CCK octapeptide acts on CNS structures involved in food intake control. The fact that CCK octapeptide was more effective in sheep deprived of food for the normal intermeal interval (2 hours) than in sheep deprived of food for longer periods suggests a physiological role for this peptide in their control of food intake. The decrease in food intake produced by pentagastrin injection could be explained by the structural similarity between that portion of the gastrin molecule and the active portion of the CCK molecule. This explanation can be supported in that (i) pentagastrin was effective only at much higher concentrations (several hundred times those of CCK octapeptide) despite the fact that, peripherally, gastrin is approximately 1/20 as potent as CCK in its effect on gallbladder contraction (*15*), and (ii) these doses of pentagastrin resulted in abnormal behaviors. In this study, as in others, secretin had no effect on food intake.

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Stimulation of Abducens Nucleus Supports Classical Conditioning of the Nictitating Membrane Response

Abstract. *The acquisition and terminal performance of a classical conditioning group compared with a control group indicated that extension of the nictitating membrane elicited by direct electrical stimulation of the abducens nucleus was successfully conditioned to a previously neutral stimulus. The conditioning so obtained was associative and not due to such nonassociative factors as sensitization, pseudoconditioning, or alteration in base-rate responding.*

One of the fundamental goals of the neurosciences is to specify the neural pathways involved in learning. Following the pioneering studies of Loucks (*1*), a number of investigators (*2*) have reported that electrical stimulation of cortical motor neurons could serve as unconditioned stimuli (US's) in the classical conditioning of movements in response to a variety of conditioned stimuli (CS's). However, there are "many vagaries in the appearance of conditioned responding" (*3*) when stimulation of cortical motor centers serve as the US. Thus, even after extensive training, conditioning is not reliably obtained, and when obtained it is at a low level. In addition, conditioned responses (CR's) often occur in response systems different from that of the unconditioned response (UR). Finally, there has been a failure to demonstrate that CR's, when they do occur, are due to associative rather than nonassociative factors (*2*, *3*).

Recently, a number of investigators (*4*-*7*) have come to recognize the unique advantages of the classically conditioned rabbit nictitating membrane response (NMR) for investigating the neural processes involved in learning. The conditioning parameters have been well specified, and there is essentially no contribution of nonassociative factors to the CR (*8*). In addition, both conditioned and unconditioned NMR's—retraction of the eyeball, via the retractor bulbi muscle, and passive extension of the membrane over the globe—result from activation of the abducens motor neurons (*4*, *6*). Thus, electrical stimulation of the abducens nu-

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