Oxytocin Secretion in Response to Cholecystokinin and Food: Differentiation of Nausea from Satiety

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Administration of cholecystokinin (CCK) to rats caused a dose-dependent increase in plasma levels of the neurohypophyseal hormone oxytocin (OT). The OT secretion was comparable to that found in response to nausea-producing chemical agents that cause learned taste aversions. The effect of CCK on OT secretion was blunted after gastric vagotomy, as was the inhibition of food intake induced by CCK. Food ingestion also led to elevated plasma OT in rats, but CCK and aversive agents caused even greater OT stimulation. Thus, after administration of large doses of CCK, vagally mediated activation of central nausea pathways seems to be predominantly responsible for the subsequent decrease in food intake. Despite their dissimilar affective states, both nausea and satiety may activate a common hypothalamic oxytocinergic pathway that controls the inhibition of ingestion.

SYSTEMIC ADMINISTRATIO' OF CHOlecystokinin (CCK) inhibits food intake in many species, including *Homo sapiens* (*1*). Intact afferent vagal fibers are essential for full expression of the inhibitory effects of CCK on food intake in rats (*2*). This requirement, along with CCK's ability to delay gastric emptying and potentiate activity of brainstem neurons in response to gastric distension (*3*), suggests that CCK may act synergistically with local gut factors, as well as with other hormones, to produce satiety. However, controversy continues concerning the potential role of toxicity, or nausea, in the effect of CCK on food intake. Like agents that produce illness in animals, CCK can produce learned taste aversions when administered under certain conditions (*4*). Furthermore, CCK causes emesis in some species (*5*), and inhibition of food intake by CCK in rats is attenuated by antiemetic agents (*6*). Thus, the effect of CCK on food intake may represent a nonspecific response to nausea rather than specific activation of central satiety pathways (*7*).

We have recently found that chemical agents that cause learned taste aversions in rats stimulate dose-dependent secretion of the neurohypophyseal hormone oxytocin (OT), but not vasopressin (AVP) (*8*). OT secretion could be provoked either directly by activation of central chemoreceptors or indirectly by vagal afferents, and it occurred at doses effective at producing taste aversions. However, our data did not indicate a causal role for peripheral OT, because administration of synthetic OT did not produce taste aversions, and immunoneutralization of circulating OT did not attenuate subsequent acquisition of taste aversions. Thus, when other stimuli to OT secretion are absent, plasma OT levels represent a relatively quantifiable marker of activation of central nausea pathways in the rat. Accordingly, we have now examined the effects of CCK on food intake and neurohypophyseal secretion to better define the mechanisms by which this peptide inhibits eating.

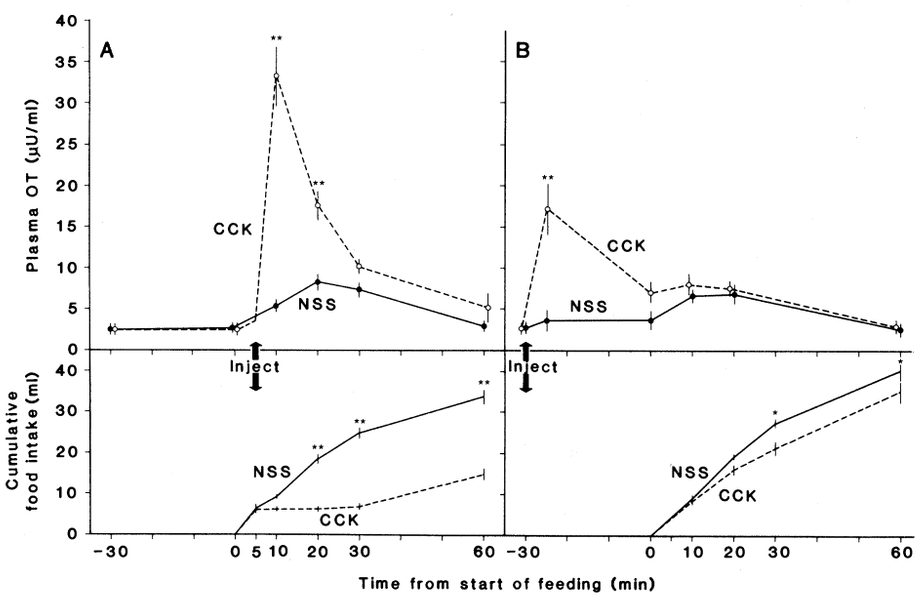

Fig. 1. Plasma OT response to food intake and to CCK administered during and before feeding. Adult rats (n = 12) were trained to a twice-daily schedule of access to liquid formula (AIN-76, Bio-Serv, Frenchtown, NJ, diluted to a ratio of 3:1 with tap water to yield 0.75 kcal/ml). All studies were done during the first (morning) period and food was limited to 10 ml per 10-minute period to avoid rapid increases in plasma osmolality. Blood was sampled from jugular catheters at regular intervals. (A) Plasma OT concentrations and cumulative feeding volumes after intraperitoneal injection of either CCK (10 μg/kg) or an equivalent volume of 0.9% NaCl (NSS) given 5 minutes after the start of feeding. (All rats received both agents on successive days in a crossover design.) (B) A repeat study of the same rats; this time the injections were given 30 minutes before the onset of the feeding period. Results are expressed as means ± standard errors (*$P < 0.05$, **$P < 0.01$; analysis of variance for repeated measures between groups).

OT concentrations in response to CCK (Table 1) were of similar magnitude to those in response to chemical agents causing taste aversions (*8*). Statistically significant OT secretion occurred in response to CCK whether administered by intraperitoneal or intravenous injection. A doubling of the zero-dose OT level was attained after administration of a 1.0-μg/kg dose of CCK, the minimum associated with reduction of food intake in rats. Plasma AVP levels were not affected by any dose of CCK and remained at basal levels (1.3 ± 0.2 pg/ml). High-performance liquid chromatography of plasma extracts from CCK-treated rats confirmed that >90% of the immunoreactive OT coeluted at the retention time of synthetic OT and was well separated both from other neurohypophyseal peptides and from CCK. These results are therefore consistent with CCK activation of central nausea pathways in rats.

To ascertain the relation between feeding behavior and OT secretion, we also studied these variables simultaneously during physiological (feeding) and pharmacological (exogenous CCK administration) conditions. Figure 1A shows the effects of CCK and of normal saline solution (NSS) administered shortly after the onset of a meal. Rats injected with NSS continued to feed rapidly and manifested a gradual but statistically significant threefold increase in plasma OT peaking at ~20 minutes, after which their intake

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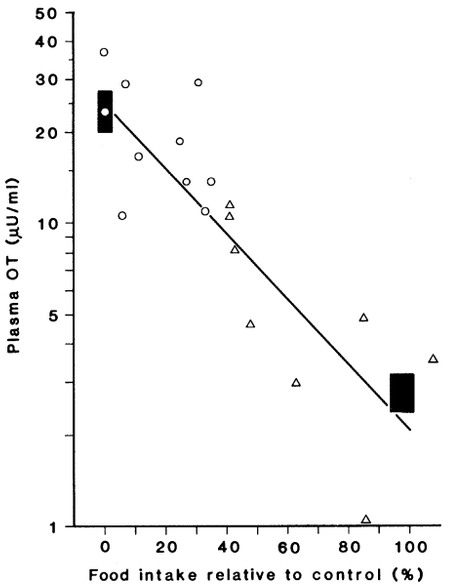

Fig. 2. Relation between plasma OT (measured 5 minutes after injection of CCK) and feeding behavior (measured for 20 minutes after the same dose of CCK), expressed as a percentage of food ingested after an injection of NSS in the same animal. Symbols represent individual rats with selective gastric vagotomies (△) and control rats with incomplete vagotomies (○). Plasma OT levels were logarithmically transformed before the regression analysis. The line relating food intake to plasma OT was defined by the equation: log plasma OT = (−1.078 ± 0.014) times the percentage of food intake + 1.419. The relation was statistically significant (t test, $P < 0.001$). The shaded areas show the OT and feeding responses of normal (intact) rats (means ± standard errors) after intraperitoneal administration of CCK (top left) and NSS (bottom right).

slowed and plasma OT decreased to fasting levels. Neither plasma osmolality nor AVP levels increased significantly during feeding. These results therefore suggest that OT secretion can also occur in response to satiety, if the rat has not experienced nausea during the brief period of feeding. However, the increases in plasma OT observed even during rapid feeding were modest, generally <10 μU/ml. In contrast, administration of CCK at a dose of 10 μg/kg caused feeding to cease abruptly and OT to increase markedly. Plasma OT levels after CCK (Fig. 1) were greater than those observed in food-deprived rats (Table 1), which suggests that the effects of feeding and CCK administration on OT secretion are additive. By 30 minutes, plasma OT decreased into the range of the NSS-injected rats, and thereafter the food intakes of both groups did not differ significantly.

Because the effect of CCK on food intake is relatively short-lived (*9*), we next injected either CCK or NSS 30 minutes before the feeding period. After NSS injection, food intakes and OT levels were identical to those observed when NSS was injected during a meal. As expected, plasma OT increased immediately after injection of CCK, but then quickly returned to basal values. During the subsequent feeding period, OT levels were not significantly different from those after NSS injection, and there was little inhibition of food intake. Thus, regardless of whether CCK was injected during or before a meal, plasma OT predicted feeding behavior in that food intake was inhibited when OT levels were markedly elevated but proceeded normally once they returned to lower ranges.

In the final experiment, we studied rats after selective gastric vagotomy to determine whether the effects of CCK on OT secretion, like those on food intake (*2*), would be attenuated. Control animals subjected to sham operations had OT responses to intraperitoneally injected CCK that were identical to those of intact rats, whereas completely vagotomized rats had blunted responses (Table 1). After vagotomy, feeding showed an inverse exponential relation to peak OT secretion after an intraperitoneal CCK dose of 100 μg/kg (Fig. 2). This close association between OT secretion and attenuation of feeding, even after large doses of CCK, provides further evidence that OT secretion reflects vagally mediated activation of central nervous system pathways involved in the control of food intake.

All oxytocinergic neurons in the brain originate in the hypothalamus, for the most part in the paraventricular and supraoptic nuclei (*10*). Therefore, neurohypophyseal stimulation of OT secretion probably represents a final step during activation of central nausea pathways originating from vagal afferent neurons in the brainstem. Food ingestion also leads to elevated plasma OT in rats (*11*), which is consistent with activation of central satiety pathways as another stimulus to pituitary OT secretion. However, there are important quantitative differences in the degree of OT secretion seen with each stimulus: aversive agents caused much greater OT stimulation than was observed during feeding in rats. Consequently, the magnitude of OT stimulation can be useful for differentiating between aversion and satiety. When markedly elevated plasma OT follows large doses of CCK, aversive effects must be predominantly responsible for the associated changes in feeding behavior because food intake alone does not produce such large elevations. On the other hand, when more modest increases in plasma OT are produced by smaller doses of CCK, the situation is less clear. This phenomenon could result from lesser activation of central nausea pathways, similar to the results with other chemical agents which at smaller doses can reduce food intake without producing learned taste aversions (*12*). Alternatively, CCK may act both as a satiety agent at lower doses and as a nausea-producing agent at higher doses (*13*).

Because a continuum of OT secretion occurs in response to both satiating and nausea-producing stimuli, and because both satiety and nausea share the behavioral effect of reducing food intake, they may also share a common central pathway mediating inhibition of ingestion. This is not to equate nausea and satiety, because each obviously has different affective components that are undoubtedly mediated by separate neural pathways. In this regard, we view increased plasma OT simply as a marker of the disinclination to eat, which reflects activation of common central oxytocinergic pathways

Table 1. Plasma OT (means ± standard errors) after administration of CCK to intact and vagotomized rats that had been deprived of food. Synthetic sulfated carboxyl-terminal CCK octapeptide (Peninsula Laboratories, Belmont, CA) was dissolved in 5% dextrose to yield the indicated amounts per kilogram of body weight when injected in volumes of 1.0 ml (intravenously, over 1 minute) or 4.0 ml (intraperitoneally). Sprague-Dawley male albino rats, weighing 200 to 300 g, were injected with each dose on successive days, and blood was sampled from indwelling jugular catheters at predetermined times of peak plasma OT levels after administration of CCK (intravenous, 2 minutes; intraperitoneal, 5 minutes). Plasma AVP and OT were measured by specific radioimmunoassays (*8*). Rats were intraperitoneally injected with CCK 1 to 2 weeks after selective gastric vagotomy. Completeness of vagotomy was verified by the absence of gastric acid secretion 60 minutes after hypoglycemia induced by the subcutaneous injection of insulin (3 U/kg). CCK injection produced a significant OT response in all groups ($P < 0.01$, analysis of variance for repeated measures within groups), but only the completely vagotomized group had an OT response significantly different from that observed in the intact rats ($P < 0.01$, analysis of variance for repeated measures between groups).

Group	CCK dose (μg/kg)				
	0	0.1	1	10	100
Intact rats					
Intravenous (n = 12)	2.4 ± 0.3	2.7 ± 0.4	5.9 ± 0.8	11.3 ± 0.8	19.3 ± 2.2
Intraperitoneal (n = 8)	1.3 ± 0.3	1.6 ± 0.4	3.0 ± 0.3	13.8 ± 4.0	23.9 ± 3.8
Vagotomized rats					
Incomplete (n = 10)	1.9 ± 0.2			14.5 ± 1.9	21.2 ± 3.0
Complete (n = 8)	2.1 ± 0.4			2.1 ± 0.3	5.9 ± 1.4

that lead to inhibition of ingestive behavior but are independent of the dissimilar affective states associated with nausea and satiety.

Although the effects of CCK on plasma OT resemble those of various chemical agents, it is provocative that CCK is an endogenous peptide found in gut and brain rather than an exogenous toxin. CCK immunoreactivity has been colocalized in many hypothalamic oxytocinergic neurons (*14*). Our results therefore also imply that multiple vagally mediated stimuli converge in the hypothalamus to cause secretion of CCK as well as OT. Together with the finding that ablation of the paraventricular nucleus can lead to hyperphagia and obesity in rats (*15*), these observations support suggestions that this hypothalamic nucleus plays an important role in the control of feeding behavior and related autonomic functions (*16*) and, further, that brain OT, CCK, or both are involved in this regulatory system.

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Synthetic Diamond as a Pressure Generator

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Synthetic diamond crystals were used as opposed anvils whose small culet surfaces were driven into contact with each other to generate high static pressures. Pressures in excess of 68 gigapascals (680 kilobars) were achieved, as determined from the fluorescence line of a ruby fragment sandwiched between the diamonds.

DURING THE LAST DECADE remarkable progress has been made in high-pressure science. Static pressures higher than 250 GPa (2.5 Mbar) have become attainable with single-crystal gem-quality diamonds used as opposed anvils (*1*). Measurements of the physical properties of condensed matter can now be made with diamond anvils (*2, 3*), sometimes with geophysical implications (*4, 5*).

The diamond-anvil technique was developed in the late 1950's (*6*), but its use did not become widespread until the feasibility of using the ruby fluorescence line for in situ pressure measurement was established (*7*). The ruby fluorescence scale was calibrated against the equation of state for NaCl to 19.5 GPa (*8*) and later to 29.1 GPa (*9*), and against the equation of state for heavy metals up to 100 GPa on the basis of shock compression data (*10*). The pressures reached with diamond anvils [to 100 GPa (*11*), 170 GPa (*12*), and 250 GPa (*1*)] were estimated from extrapolations of these calibrations, although the ruby scale becomes ambiguous above 185 GPa where the fluorescence line weakens and disappears (*1*).

A reproducible method for generating stable, very high static pressures is of great importance. The advantages of selecting diamonds as the anvil material have been discussed (*13–15*). Important factors are size, type, color, orientation, inclusions, and impurities. Beveling is also important, especially at extremely high pressures where plastic deformation is observable around the anvil surface (*12*). Some ideas for designing diamond anvils have been derived from stress analyses based on the use of the finite element method (*16, 17*).

All the aforementioned experiments have been carried out with natural gem-quality diamond used as the anvil material. Synthetic carat-sized diamond has recently become available (*18*); single crystals have been grown under prolonged high pressure and temperature inside a large-volume apparatus, and it now is possible to make anvils from synthetic diamond. Some advantages derived from the use of synthetic diamond instead of natural diamond as the anvil material are as follows: (i) imperfections are fewer and can be controlled, (ii) the concentration of nitrogen can be controlled so as to improve the strength (*19*), (iii) the pressure-temperature conditions for preparing diamond crystals can be determined, and (iv) suitable crystals can be obtained repeatedly.

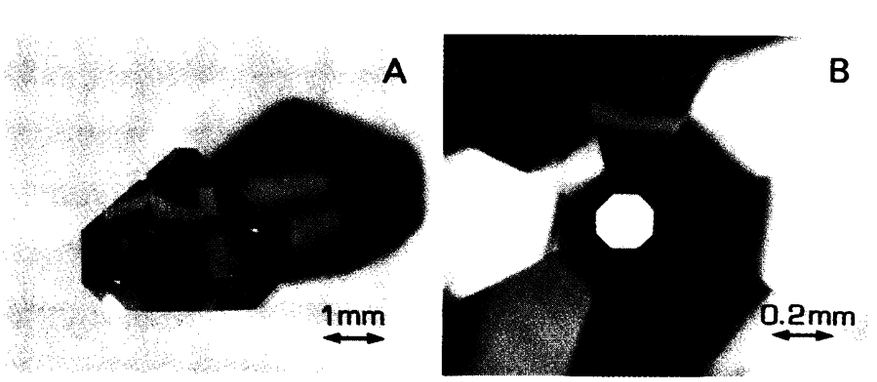

Fig. 1. Side view (A) and top view (B) of the synthetic diamond anvil.

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