autoimmunity. There is evidence that CTLs recognize a peptide from a self heat shock protein and that these cause tissue destruction during mycobacterial infection (*11*).

Third, one could speculate that the relative ease in the production of CTLs against randomly selected self peptides could be related to positive thymic selection. It is a paradox of T cell development that T cells are positively selected for the recognition of complexes of self MHC and foreign peptide in the absence of foreign peptide (*12*). As a way out of this dilemma, altered MHC molecules or special peptides presented by thymic epithelium have been discussed (*1, 13*). Our data raise a third possibility, that the CTLs specific for virtually nonphysiologic self peptide sequences have been selected for on thymic epithelial cells by presentation of these peptides. A mechanism for production of peptides through a novel type of RNA not under conventional transcription control (*14*) or thymus-specific proteases could initiate the presentation of self peptides on thymic epithelium that would be nonphysiologic elsewhere in the immune system, thereby mimicking a universe of foreign peptides.

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23. We thank J. Klein for his support, P. Bohley for helpful discussions, A. Lopez for providing mouse hemoglobins, H. Wagner and B. Frangoulis for critically reading the manuscript, S. Faath for technical assistance, and L. Yakes for preparing the manuscript. Supported by Sonderforschungsbereich 120.

31 May 1989; accepted 8 February 1990

Endogenous Cholecystokinin Reduces Feeding in Young Rats

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The hypothesis that endogenous cholecystokinin (CCK) released from the small intestine during feeding causes satiety was tested in rat pups, 9 to 12 days old. Intragastric administration of soybean trypsin inhibitor, a procedure that releases CCK from the small intestine, decreased the subsequent intake of a test meal. This effect was reversed by prior treatment with MK-329, a selective antagonist of CCK at alimentary-type CCK (CCK-A) receptors. Thus, endogenous, small intestinal CCK can cause satiety in the neonatal rat and this effect involves CCK-A receptors.

CHOLECYSTOKININ (CCK) IS A PEPtide that is found in the periphery, where it acts locally and hormonally, and in the central nervous system, where it acts as a neuromodulator and a neurotransmitter. The peptide has diverse effects, both gastrointestinal (for example, on gastric motility, pancreatic and biliary secretion, and gastric emptying) and behavioral (for example, on feeding and sleeping). During development, CCK exerts trophic effects on the gastrointestinal tract (*1*) and influences learning and stress responsivity (*2, 3*). This report focuses on the hypothesized satiating effect of endogenous CCK released from the small intestine in young (preweanling) rats.

In 1973, we hypothesized that CCK released from the small intestine by food ingested during a meal was part of the mechanism that terminated the meal and elicited postprandial satiety (*4*). Subsequent work has shown that peripherally administered CCK reduces feeding in rats, from birth through adulthood (*5–6*), and in many other species, including humans (*7*). Three observations suggest that the satiating effect of peripherally administered CCK in adult rats is mediated by CCK-A (alimentary) receptors (*8*) outside the blood-brain barrier: (i) peripherally administered CCK does not penetrate the blood-brain barrier (*9*); (ii) the relative potency of CCK-8, desulfated CCK-8, CCK-4, and gastrin for inhibiting food intake (*6, 10*) is similar to the relative potency of these peptides for binding to the CCK-A receptor, but not for binding to the brain-type receptor (CCK-B) (*8*); and (iii) MK-329, a potent and selective antagonist for CCK-A receptors (*11*), administered in low doses decreases the satiating effect of peripherally administered CCK-8 (*12–14*).

We tested this hypothesis further by investigating whether endogenous CCK released from the small intestine can inhibit milk intake in 9- to 12-day-old rats by acting on CCK-A receptors.

Pregnant Sprague-Dawley rats (Taconic Farms, Germantown, New York) were obtained 1 week before delivery, housed indi-

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vidually, and given free access to food and water. Their offspring were studied at 9 to 12 days of age (*15*). Pups were taken from their nest, voided, the urethral meatus was closed with cyanoacrylate glue, and they were weighed. They were then rapidly infused intragastrically through PE-50 tubing with 1 ml of isotonic saline or soybean trypsin inhibitor (STI) (Sigma) (100 mg in 1 ml of saline), a potent stimulus for the release of CCK from the small intestine in adult rats (*16*). They were then reweighed and housed in a group at 33°C. After 10 min the pups were transferred to individual containers at room temperature, and 5 min later were placed in a warm (36° to 38°C) and humid terrarium. Each pup was tested by being placed on a paper towel (Kimwipes, Kimberly-Clark) soaked with 4 ml of milk diet (commercially available Half and Half, at 36° to 38°C) on the floor of a 1-liter Nalgene beaker (*17*). The test lasted for 30 min (1 ml of diet was added at 18 to 25 min); after the test, the pups were dried and weighed. Milk intake was expressed as the percent of body weight gained during the test (%BWG).

The administration of STI reduced the %BWG significantly: control (saline-infused) groups gained (average ± SEM reported throughout) 1.28 ± 0.19% and 1.54 ± 0.39%, whereas groups given STI gained 0.69 ± 0.17% and 0.27 ± 0.17% in two replications, respectively ($n = 8$, $P < 0.05$; $n = 7$, $P < 0.02$). When data from both replications were combined and analyzed by analysis of variance (ANOVA), there was no statistically significant effect of gender ($F < 1$) or interaction between gender and infusion ($F < 1$).

To determine if the inhibition produced by STI was due to endogenous CCK acting on CCK-A receptors, we treated new groups of pups with MK-329, a potent and selective antagonist of CCK-A receptors (*11*). Pups were removed briefly from the nest, weighed, administered MK-329 or vehicle (*18*) intraperitoneally, and returned to the nest and the dam for 80 to 90 min. When pups were taken out again, there were no significant group differences in body weight gain. From this point on, the experiment was identical to that described above, with rats receiving an infusion of either saline or STI (*19*). Initially, we used MK-329 at a dose of 1 mg/kg. As in the former two experiments, STI reduced %BWG (0.64 ± 0.15%) compared to saline (2.25 ± 0.32%) in rats that had been previously treated with vehicle ($n = 18$, $P < 0.01$) (Fig. 1). Pretreatment with MK-329 (1 mg/kg) reversed this effect so that the intake produced by MK-329 plus STI was not significantly different from MK-329 alone (Fig. 1) [$F(2,29)$, $P < 0.001$; Duncan's test $P < 0.05$]. Lower doses (*20*) of MK-329 (500 and 100 μg/kg) did not reduce the inhibitory effect of STI (*21, 22*), and a larger dose of MK-329 (2 mg/kg) had the same effect as 1 mg/kg (*23*).

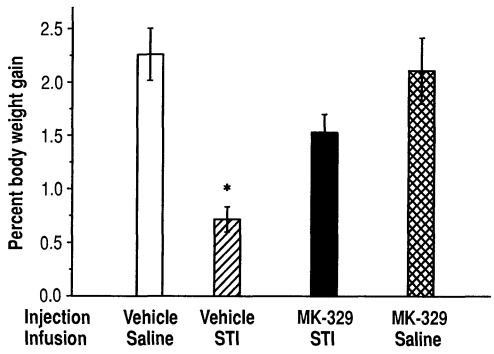

Fig. 1. The effects of treatment with MK-329, a selective antagonist of CCK-A receptors, on STI-induced suppression of feeding. Intake of a 30-min meal is expressed as percent of body-weight gain (%BWG). Intragastric infusion of STI decreased feeding (diagonal hatching, $n = 24$) compared to saline controls (open bar, $n = 20$). Pretreatment with MK-329 (1 mg/kg) (filled bar, $n = 24$) reversed STI-induced low levels of ingestion. Although the reversal by MK-329 appears to be incomplete, there was no significant difference between the intakes after MK-329 plus STI and after MK-329 plus saline. Note that MK-329 did not significantly increase %BWG in the absence of STI (cross-hatched bar, saline-infused rats, $n = 8$). *Significantly different from (i) the vehicle-saline group in two replications ($P < 0.05$) and (ii) the groups treated with MK-329 [$F(2,53) = 13.35$; $P < 0.001$; Duncan's test $P < 0.05$]. The groups treated with MK-329 were not significantly different from each other (Duncan's test). Data shown for the three left-hand groups are averaged for two replications.

The efficacy of MK-329 for reversing the effect of STI indicates that the inhibitory effect of STI involves endogenous CCK acting at CCK-A receptors. It is relevant to this interpretation that CCK-A receptors have been reported to be present in preweanling rats (*24*) and that they apparently mediate the decreased intake produced by the peripheral administration of exogenous CCK-8 (*6*).

The dose of MK-329 required to reduce the satiating effect of endogenous CCK released by STI, however, is much larger than the dose required to block the satiating effect of peripherally administered CCK in adults (*12–14*) or to block the binding of CCK to CCK-A receptors (*11–14*). In fact, such large doses of MK-329 antagonize the binding of CCK to CCK-B receptors (*11*) so that our results could be interpreted as evidence that blockade of both CCK-A and CCK-B receptors is necessary for effective antagonism of the satiating effect of endogenous CCK released from the small intestine.

Thus, further investigation of the involvement of CCK-B receptors in the antagonism we observed with MK-329 is required, particularly in light of the report by Dourish *et al.* (*25*) of the efficacy of an antagonist of CCK-B receptors to block the inhibitory effect of prefeeding on food intake in adult rats. This large effect of the CCK-B receptor antagonist is not explained by available evidence concerning the peripheral administration (and, presumably, the peripheral release as in this experiment) of CCK-8 in adult rats (*9–14*) or in preweanling rats (*6*) that were tested under experimental conditions similar to ours.

Although MK-329 reversed the inhibitory effect of the STI pretreatment, it did not increase milk intake when given alone (Fig. 1), suggesting that endogenous CCK was not involved in the control of milk intake under these conditions. This differs from some of the results with MK-329 in adult rats (*13, 26*), but not with others (*14, 27*). We conclude that more work is required to delineate the conditions under which endogenous CCK limits meal size in rats of all ages.

In summary, there are two major results of this experiment. First, intragastric administration of STI, a procedure that releases endogenous CCK from the small intestine, decreased milk intake significantly in 9- to 12-day-old rats. Second, the inhibition of milk intake was reversed by an antagonist of CCK-A receptors. These results show that endogenous CCK released from the small intestine can inhibit the size of a test meal (*4*). The results also provide evidence that CCK-A receptors at an unidentified site, presumably outside the blood-brain barrier, are involved in this effect. Because a relatively large dose of MK-329 was required to reverse the effect of STI, CCK-B receptors may also be involved, and further work is required to evaluate the relative contributions of the two types of CCK receptors. It remains to be determined whether the same results with STI can be obtained at other ages and under other conditions of diet and deprivation (*28*).

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15. Pups were chosen randomly (excluding runts). No more than one subject in each experimental group was drawn from a single litter. Average weights (±SD) were 21.67 ± 2.14 g (9-day-old rats), 24.5 ± 2.99 g (10-day-old rats), 26.29 ± 2.6 g (11-day-old rats), and 30.24 ± 2.73 g (12-day-old rats).
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19. The first batch of STI we used was reported to be 2.3 times as potent as the batch used for subsequent experiments (Sigma). Accordingly, pups received adjusted doses of 230 mg in the standard 1-ml infusion. There were 12 pups per treatment group in all the following experiments with the exception of the two saline-infused groups in the initial experiment (n = 8 per group).
20. The vehicle used for lower doses of MK-329 was that described above (*18*) diluted 1:1 or 1:9 with isotonic saline.
21. Mean body weight gains for vehicle plus STI versus MK-329 plus STI were 1.20 ± 0.29% versus 1.67 ± 0.37% and 1.30 ± 0.28% versus 1.09 ± 0.24% after treatment with MK-329 at 500 and 100 μg/kg, respectively. Note that the %BWG of vehicle plus STI-fed control groups used in these experiments did not differ significantly ($P > 0.29$).
22. The effects of treatment with MK-329 (100 and 500 μg/kg) were evaluated simultaneously from the same pool of litters. These treatments did not change %BWG compared to the appropriate controls ($P > 0.50$).
23. The effect of a higher MK-329 dose (2 mg/kg) was evaluated in an additional experiment with other pups. Rats first treated with vehicle and then infused intragastrically with STI gained less (0.79 ± 0.18%) than rats infused with saline (2.27 ± 0.35%) ($P < 0.01$). Treatment with MK-329 significantly reversed STI inhibition of feeding both at 1 mg/kg (1.50 ± 0.26%) and at 2 mg/kg (1.57 ± 0.23%) ($P < 0.05$). There was no significant difference in the effectiveness of the two doses.
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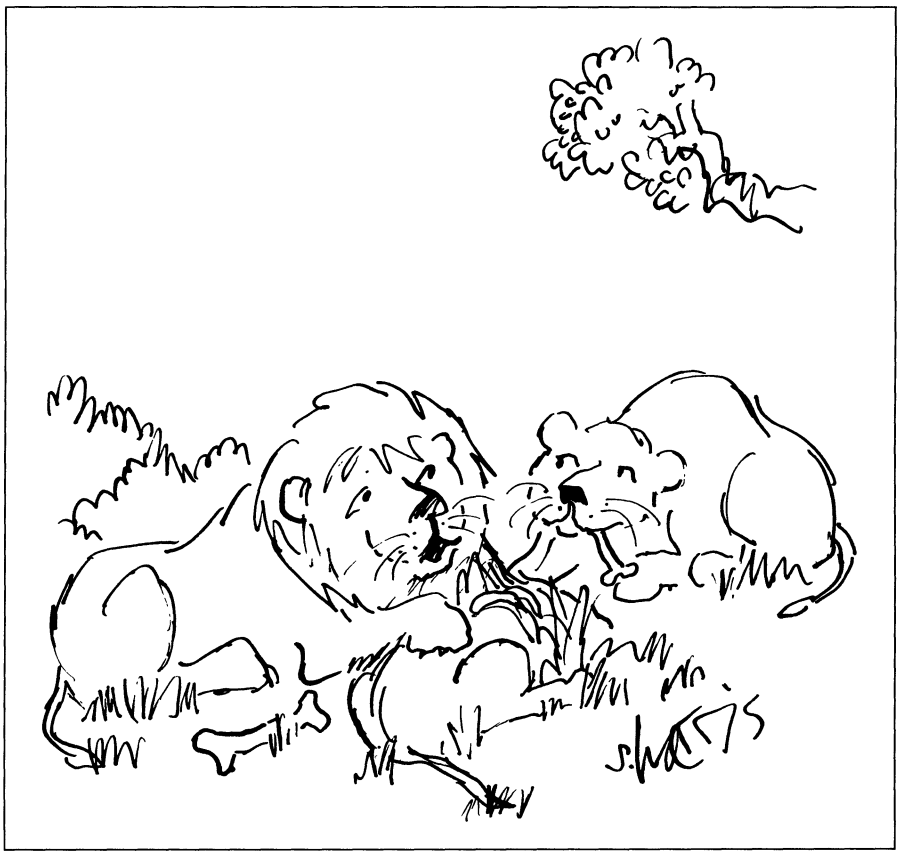
23 August 1989; accepted 1 February 1990



"A lovely day, a good meal, and, thank heaven, no guilt."