Cholecystokinin and satiety in rats and rhesus monkeys[1-3]

James Gibbs and Gerard P. Smith

ABSTRACT When ingested food does not accumulate in the stomach or enter the small intestine, rats do not stop eating. Small amounts of food placed in the small intestine or intraperitoneal injections of the intestinal hormone cholecystokinin (CCK) elicit the full behavioral display of satiety in these sham-feeding rats. In rhesus monkeys, intravenous infusions of CCK produce large, dose-related reductions in meal size. In addition, gastric preloads of calorically trivial amounts of *l*-phenylalanine, but not *d*-phenylalanine, produce large reductions in meal size, suggesting that: *1*) endogenous CCK acts as a "satiety signal," and *2*) certain foods may be very efficient releasers of such a satiety signal. Whether the satiety effect of CCK is physiological in rats and monkeys or operates in humans has not been determined. *Am. J. Clin. Nutr.* 30: 758-761, 1977.

A physiological understanding of the mechanisms which determine the beginning or end of a normal meal does not exist. We chose the problem of satiety because it is difficult to predict the time a meal will begin, but it is certain that feeding will be quickly followed by the cessation of feeding. We began by asking two questions: Where does food generate satiety signals? What are those signals?

To begin to answer the first question, we observed the feeding behavior of rats provided with chronic gastric fistulas. When these rats eat a liquid food after overnight deprivation when the fistulas are closed, the food is tasted, swallowed, accumulates in the stomach, and rapidly begins to empty into the small intestine (1). When the fistulas are temporarily opened, the food is tasted and swallowed, but does not accumulate in the stomach and does not pass into the small intestine. The difference in the amount of gut surface exposed to the food stimulus between the two conditions produces a striking difference in behavior: when food does not accumulate in the stomach and does not pass into the small intestine, satiety does not occur (Fig. 1). Taste and other oropharyngeal stimuli, acting alone, do not elicit satiety. We conclude that the occurrence of satiety in the rat is critically dependent on an inhibitory reflex elicited by ingested food accumulating in the stomach, moving through the small intestine, or both (2).

Does the afferent limb of this inhibitory reflex originate in the stomach or intestine? We investigated this question by delivering liquid food to the small intestine while rats with open gastric fistulas were sham-feeding liquid food. Rats with gastric fistulas were each provided with a fine gauge polyethylene tube anchored in the first portion of the duodenum. After overnight food deprivation, while rats were rapidly and continuously sham-feeding, either saline or liquid food was infused at equivalent rates into the small intestine. On days when saline was infused, sham-feeding was unaffected; on days when liquid food was infused, there was a rapid, significant, and dose-dependent decrease in food intake. Results of such a test are seen in Figure 2. We believe that it is important that delivery of food to the small intestine not only stopped feeding, but

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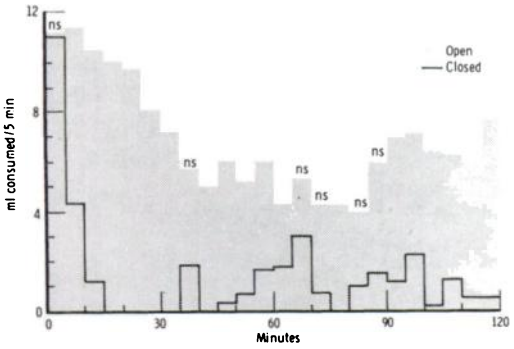

FIG. 1. Mean intake (in milliliters) of balanced liquid diet (no. 116 E.C., Grand Island Biological Co., Grand Island, N. Y., diluted to 25% strength) on a day when five rats with chronic stainless steel gastric fistulas ate liquid diet with gastric fistulas closed (*line*) and on the following day when they sham-fed for the first time with gastric fistulas open (*stippled area); ns* denotes those intervals during which sham intakes were not statistically larger than intakes with gastric cannulas closed ($P > 0.05$, matched-pairs t test, two-tailed).

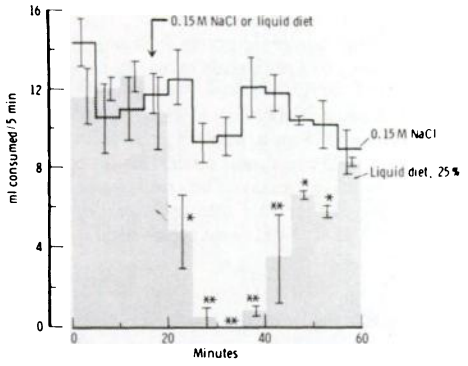

FIG. 2. Mean sham intake (in milliliters ± SEM) of liquid diet on a day when six rats received intestinal infusions of 6 ml of diluted liquid diet (1 ml/min, beginning at 17 min, *stippled area*) and on an adjacent day when they received 0.15 M NaCl at the same rate and time (*line*); $^{*}P < 0.05$, $^{**}P < 0.01$, matched pairs t test, two-tailed.

produced the rest of the typical behavioral sequence which characterizes normal satiety in the rat—a transient increase in activity (grooming and exploration), then apparent sleep (1). Because the entire behavioral sequence occurred in rats sham-feeding with open gastric fistulas, we conclude that food in the small intestine is sufficient to elicit satiety in the absence of any contribution from gastric distention.

The signal producing this "intestinal satiety" may be neural or hormonal. The plausible notion that gut hormones might act as satiety signals has been infrequently tested (3–6). We compared the satiety effect of four gut hormones which are available in relatively pure form—gastrin, cholecystokinin, secretin, and pancreatic glucagon. Each hormone was injected intraperitoneally into intact rats just before food presentation after an overnight food deprivation. A partially purified (20% w/w) preparation of cholecystokinin (CCK) produced large, dose-related suppressions of solid and liquid food intakes (7). Large doses of pentagastrin and gastrin, which are chemically similar to CCK, produced small suppressions of food intake. Secretin and pancreatic glucagon, which have different structures than CCK, had no effect on food intake.

The suppression of feeding produced by impure CCK was due to the CCK molecule and not to impurities in the preparation, because identical doses of the synthetic carboxyl-terminal octapeptide of CCK (a fragment with all of the biological activity of CCK) produced identical suppressions (7). Rats did not appear sick after injections of CCK, and CCK did not suppress drinking after overnight water deprivation. Two further experiments make it extremely unlikely that illness explains the inhibition of feeding behavior produced by CCK. First, behavioral ratings demonstrate that rats injected with CCK not only stop eating, but display the full behavioral sequence of normal satiety, including grooming and apparent sleep (8). Second, in sensitive tests of "bait shyness" designed to reveal any subclinical distress, rats did not learn an aversion to a novel taste paired with injections of impure CCK or the synthetic octapeptide, whereas they readily learned an aversion to a novel taste paired with injections of lithium chloride or apomorphine (7, 9).

When CCK is injected intraperitoneally into rats sham-feeding with gastric fistulas open, the results are strikingly similar to those obtained when food is infused into the small intestine of sham-feeding rats (compare Fig. 2 and Fig. 3). Both CCK and intestinal infusion suppress feeding in a dose-related manner (1, 10), and both elicit the entire behavioral sequence of satiety (1, 8).

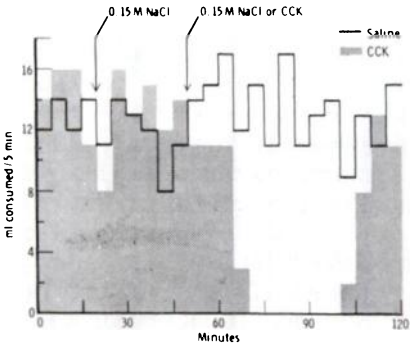

FIG. 3. Sham intake (in milliliters) of diluted liquid diet by one representative rat on a day when 20% (w/w) pure CCK (Gastrointestinal Hormone Research Unit, Karolinska Institutet, Stockholm, Sweden) was injected intraperitoneally in a dose of 40 Ivy dog U/kg of body weight (*stippled area*) and on an adjacent day when equivolumetric 0.15 M NaCl was injected (*line*).

All of the experiments above suggest the hypothesis that CCK, which is released into blood when food enters the small intestine, acts as a satiety signal. This hypothesis gains added plausibility by two further observations. First, CCK is released and is circulating within minutes after food contacts the duodenal mucosa in cats and dogs (11); thus, release is rapid enough to achieve short-term satiety at a meal. Second, CCK is released in proportion to the load (rather than the volume or concentration) of food contacting the duodenal mucosa (12), and it is intestinal load of food that appears to be the critical factor in eliciting intestinal satiety (1). We have not yet determined whether CCK is a physiological satiety signal. To do this, it will be necessary to demonstrate that enough endogenous CCK is released at a normal meal to elicit satiety. These measurements of CCK levels are in progress.

Recent experiments in rhesus monkeys (13) suggest that the satiety effect of CCK has therapeutic implications for humans whether CCK is finally determined to be a physiological signal for the control of food intake or not. Intravenous partially purified CCK caused large and dose-related suppressions of intake of a standard solid food after overnight food deprivation when it was infused into monkeys just before food presentation. An equivalent dose of the synthetic octapeptide produced an equivalent suppression. No tachyphylaxis or toxicity was observed in repeated CCK infusions. The results of one of these tests are shown in Figure 4. Three hours after CCK infusion, the suppression caused by CCK is as great as it was 15 min after infusion. Such a pro-

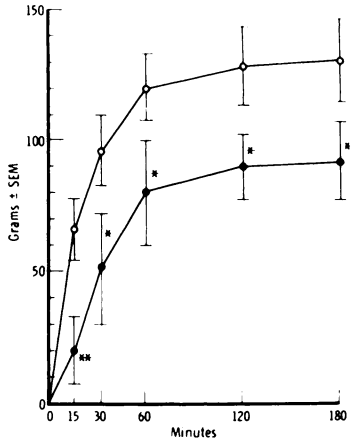

FIG. 4. Mean intake (in grams ± SEM) of solid food pellets (Teklad) by four rhesus monkeys on a day when they received an intravenous infusion of 20% pure CCK in a dose of 20 Ivy dog U/kg dissolved in 0.15 M NaCl during the 5 min immediately preceding food presentation at 0 min (*solid circles*) or on an adjacent day, when monkeys received an equivolumetric 0.15 M NaCl infusion at the same rate and time *(open circles)* $*P < 0.05$, $**P < 0.01$, t test, one-tailed.

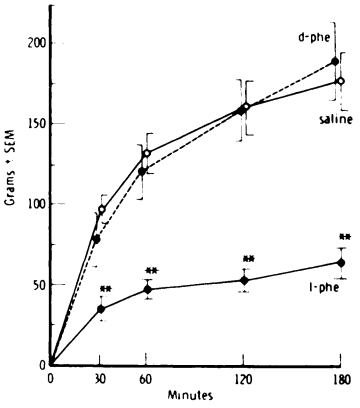

FIG. 5. Mean intake (in grams ± SEM) of pellets by nine rhesus monkeys on days when they received intragastric preloads of one isomer of phenylalanine (Nutritional Biochemicals) dissolved in 0.15 M NaCl (0.02 g/ml) in a dose of 1 g/kg delivered during the 15 min immediately preceding food presentation at 0 min. On an adjacent day a preload of equivolumetric 0.15 M NaCl was delivered at the same rate and time. $**P < 0.01$ t test, two-tailed.

longed action would be desirable in a therapeutic agent used to decrease food intake.

If endogenous CCK does act as a satiety signal, it should be possible to elicit satiety by releasing endogenous CCK from the intestine. Recent findings of Meyer and Grossman (14) allowed an indirect test of this prediction: they provided convincing evidence in dogs that intestinal perfusion of the *l*-isomer of phenylalanine (*l*-Phe) was a potent releaser of CCK but that perfusion of the *d*-isomer (*d*-Phe) had very little effect. If CCK is a satiety signal, and if *l*-Phe is a more potent releaser of CCK than is *d*-Phe, then gut preloads of *l*-Phe should produce a more potent suppression of food intake than do equivalent preloads of *d*-Phe. We tested this prediction by measuring the food intake of nine rhesus monkeys during 3 hr after an intragastric preload of isomers of phenylalanine or equivolumetric saline delivered over the 15 min just before food presentation after overnight food deprivation (Fig. 5). Preloads of *l*-Phe produced marked, rapid, and sustained suppressions of food intake; preloads of *d*-Phe did not suppress food intake. The lack of effect of this large (350-ml) preload of *d*-Phe suggests that volume, distention, and tonicity are not effective stimuli for suppressing food intake under these conditions. Rather, the differential effects of *l*- and *d*-Phe in suppressing feeding are consistent with their relative abilities to release CCK.

In terms of calories, *l*-Phe was a very efficient suppressor of feeding. At the dose of *l*-Phe shown in Figure 5, the mean total preload of 28 kcal produced a mean deficit of over 400 kcal at the end of the 3-hr feeding period. This observation raises the therapeutic possibility that certain foods which are relatively low in caloric content may be relatively potent in releasing a physiological satiety signal. We believe that the experiments reviewed here provide clear but not crucial evidence that CCK is such a signal.

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