Order Number 9029966

Flavor preferences and aversions conditioned by cholecystokinin

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City University of New York, 1990

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FLAVOR PREFERENCES AND AVERSIONS CONDITIONED BY CHOLECYSTOKININ

by

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A dissertation submitted to the Graduate Faculty in Psychology in partial fulfillment of the requirements for the degree of Doctor of Philosophy, The City University of New York.

1990

This manuscript has been read and accepted for the Graduate Faculty in Psychology in satisfaction of the dissertation requirement for the degree of Doctor of Philosophy.

March 8, 1990
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Abstract

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The ability of animals to avoid and approach foods based on the postingestive consequences of the foods is a well known phenomenon. In particular, studies using the Conditioned Flavor Preference (CFP) paradigm have demonstrated that rats learn preferences for arbitrary flavors associated with nutritive consequences. The present series of experiments examined whether cholecystokinin (CCK), an intestinal hormone that is a putative satiety agent, can mediate the CFPs produced by nutrients. Food-deprived rats were trained to consume a flavored solution (conditioned stimulus, CS+) which was paired with intraperitoneal injections of CCK octapeptide (unconditioned stimulus, US), and a different flavored solution (CS-) paired with saline injections. The conditioned response (CR), i.e., the flavor preference, was then measured in choice tests with the CS+ and CS- solutions. Several doses of CCK were investigated using test conditions and paradigms that were successful in producing flavor conditioning when a nutrient served as the US. In sham-feeding rats 2 and 4 μg/kg of CCK suppressed intake of flavored 8% Polycose but failed to condition a flavor preference. In rats real-feeding flavored saccharin solutions, CCK (0.125, 0.25, 0.5, 1, 2, and 4 μg/kg) suppressed intake in a dose-dependent manner, and conditioned both preferences and aversions depending on the dose. The two lower doses (0.125 and 0.25 μg/kg) failed to suppress CS+ intake during training and to condition either preferences or aversions during testing. At 0.5 and 1 μg/kg CCK had little effect on consumption but

conditioned a flavor preference. At 2 µg/kg CCK suppressed CS+ intake but failed to produce flavor conditioning. The highest dose (4 µg/kg) inhibited saccharin intake in a potent manner and conditioned an aversion to the CS+ solution. Cholecystokinin (0.5 µg/kg) was also observed to condition a flavor preference in rats real-feeding restricted amounts of an 8% Polycose solution. These findings are consistent with the hypothesis that CCK may mediate at least in part the CFPs produced by nutrients.

ACKNOWLEDGEMENTS

I wish to thank the members of my committee Dr. Kathleen V. Axen, Dr. Eric Heinemann, Dr. Ching-Tse Lee, and Dr. Gerard P. Smith because they did not give me a hard time, which could have interfered with the incomparable happiness of this critical experience in my life.

I am also grateful to Dr. Ackroff for being my private English dictionary and supporting me in moments of discouragement. I acknowledge the helpful comments and suggestions of Dr. Smith at the time of my oral defense. I have the greatest respect for his open-minded scientific approach. I am profoundly indebted to Dr. Lucas for the inestimable contribution of his always ludic, original and clever viewpoint.

Last but not least, I thank Dr. Sclafani, my adviser, who allowed me to work in his laboratory and provided me with intellectual guidance. His wise and brilliant thoughts and his human concern will always be a model to emulate for me. My highest goal is that my research will one day inspire in him an admiration comparable to the one that I have for his work.

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INTRODUCTION

Shortly after we begin to eat, we experience an internal state that we refer to as "being full" or "satisfied". The inhibition of eating that occurs after a meal is frequently referred to as satiety. The mechanisms that inhibit feeding after a meal has ended, however, may be different from the mechanisms that inhibit additional feeding toward the end of a meal. This distinction between the mechanisms of satiety, that is, the interval of noneating between two meals, and the mechanisms of satiation, that is, the process of terminating a meal, was first made by Le Magnen (1985). Satiety is a central state behaviorally characterized by the absence of feeding. Satiation is a process characterized by an inhibition of ongoing feeding.

Food in the gastrointestinal (GI) tract elicits a series of events (GI distension, GI hormone release, stimulation of GI chemoreceptors, etc.), some of which have been postulated as satiety signals that cause the termination of a meal. Although strictly speaking these signals should be called satiation signals (they participate in the termination of feeding rather than the maintenance of nonfeeding), I will refer to them as satiety signals to be consistent with usage in the literature. Putative satiety signals include gastric and upper duodenal distension and/or chemostimulation (Deutsch, 1978; Novin, Sanderson, and VanderWeele, 1974); preabsorptive and postabsorptive gut hormone release such as cholecystokinin, glucagon and insulin (Gibbs, Young, and Smith, 1973a; Langhans, Geary, and Scharrer, 1982; Langhans, Zieger, Scharrer, and Geary, 1982; VanderWeele, Haraczkiewicz, and Van Itallie, 1982); and accumulation of blood-borne factors or nutrients (Mayer, 1955; Russek, 1970). Some of these satiety signals may be neural in nature, mediated by the vagus and the splanchnic nerves, and some of them may be humoral.

Thus, a meal presumably generates multiple satiety signals originating from various preabsorptive (oral, gastric, duodenal) and postabsorptive sites. These signals may act in concert, and perhaps with a certain degree of redundancy, to convey satiation messages to

the brain, which ultimately makes the decision of when a meal should end.

The satiety signals often act to inhibit feeding before the ingested food can have any metabolic consequence. That is, they operate on a meal-to-meal basis, triggering short-term regulatory mechanisms of food intake. However, animals learn from the long-term consequences of a meal to adjust the short-term regulatory mechanisms (Deutsch and Tabuena, 1986). The work to be presented here is concerned with satiety signals and learning factors in general. In particular, I have examined the ability of one putative satiety signal, cholecystokinin, to modify behavior in the way the postingestive effects of some nutrients do.

SATIETY AND REINFORCEMENT

Satiety: satisfaction or discomfort?

The word satiety contains a semantic ambiguity that, although well tolerated by daily language, is troublesome for science. The etymology of the term satiety (from the Latin 'satietas'= satisfaction, and 'satis'= sad) and the Webster's dictionary's definition are equivocal: "1: the quality or state of being fed or gratified to or beyond capacity; 2: the revulsion or disgust caused by overindulgence or excess" (emphasis added). In spite of these two antithetic meanings, many daily life and scientific references to satiety (Rozin and Zellner, 1985; Booth, 1985, 1987; Kulkosky, 1985) frequently agree with the positive meaning of the term.

The assumption that satiety is a satisfying state may derive from the idea that satiety is a normal consequence of food ingestion, and food has positive reinforcing effects. This notion has its origin in the drive-reduction theory of reinforcement. According to this view, food is rewarding for a hungry animal because it reduces the strength of the hunger drive; the same food is not an effective reward for a sated animal because little drive is present. A pioneering attempt to investigate the relationship between satiation and reward is the

series of experiments reported by Kohn (1951) and Miller and Kessen (1952). In the first experiment, Kohn compared the effects of food consumed by mouth and food delivered via an intragastric (IG) catheter on the suppression of an instrumental response for food (hunger reduction). In the second experiment Miller and Kessen compared the ability of oral food and IG food to reinforce the learning of a simple habit (reward). Both stimuli suppressed the instrumental response for food and were effective rewards, but food by mouth was a more effective stimulus to produce both effects. Miller and Kessen interpreted their findings as evidence in support of the hunger-reduction hypothesis of reinforcement.

These experiments, however, did not prove that feeding termination and reinforcement were produced by the same postingestive action of food. Although they demonstrated that postingestive factors alone (intragastric feeding) were sufficient both to inhibit feeding and reinforce learning, they did not establish, that drive-reduction was a necessary condition for the postingestive reinforcement effect. In fact, recent evidence indicates that IG food is an effective reinforcer in nondeprived rats in which hunger drive is minimal (Elizalde and Sclafani, 1990; Sclafani and Nissenbaum, 1988). Furthermore, Sheffield and Roby (1950) have challenged the drive-reduction hypothesis of reinforcement using a stimulus that does not reduce any physiological need. They demonstrated that a non-nutritive solution such as saccharin served as an effective reward for hungry rats in an instrumental learning situation. This result suggests that, in Miller and Kessen's experiment, the sensory incentive properties of the oral stimulus in normal feeding could explain its greater reward value.

Miller and Kessen's experiment was one of the first demonstrations of the postingestive reinforcement effect. This experiment did not intend to isolate which gastric, intestinal or postabsorptive consequences of the food caused the end of the meal and whether this same signal(s) was responsible for the reward. As Figure 1 illustrates, food ingestion has multiple postingestive effects. It is well known that satiety is one of these

effects. The question that remained unanswered in Miller and Kessen's experiments is whether satiety served as the reward under their conditions. It is conceivable that some postingestive actions of food that do not produce satiety nevertheless can have positive reinforcing effects. This possibility has been directly explored using sham-feeding rats, that is, rats drinking a liquid food that drains out a gastric cannula. Food-deprived sham-feeding rats display little or no satiety, although a small amount of the ingested food may be absorbed. I have found that under some conditions the absorbed food, while it does not produce satiety, can have positive reinforcing effects (Perez and Sclafani, 1989).

The postingestive actions of food may also have negative effects, as is the case of foods that contain toxic agents. Also, under some conditions, the ingestion of nontoxic foods can have an aversive effect, particularly when consumption is excessive in volume or rate (Booth, 1985). Kulkosky (1985) has proposed the term "nimiety" (from the Latin 'nimietas'= excess, overabundance) to refer to the aversive state resulting from overfeeding, and suggests that we reserve the word satiety for the satisfying state that follows normal consumption of food. Although this distinction may eliminate the semantic ambiguity of the term "satiety", whether or not the postingestive actions of food that promote satiety, as opposed to nimiety, have positive reinforcing effects is an empirical question that has not been sufficiently investigated. Therefore, it is premature to conclude that the positive reinforcing effects of food are due to its satiating actions.

The Conditioned Flavor Preference paradigm

In Miller and Kessen's experiment (1952) the reward value of IG food was measured using a T-maze learning paradigm. More recent studies have used the conditioned flavor preference (CFP) paradigm to investigate the postingestive reinforcement effect.

In the traditional CFP paradigm, rats are trained to associate an arbitrary flavor cue (conditioned stimulus, CS+) with the unconditioned stimulus (US, e.g., IG nutrient infusion),

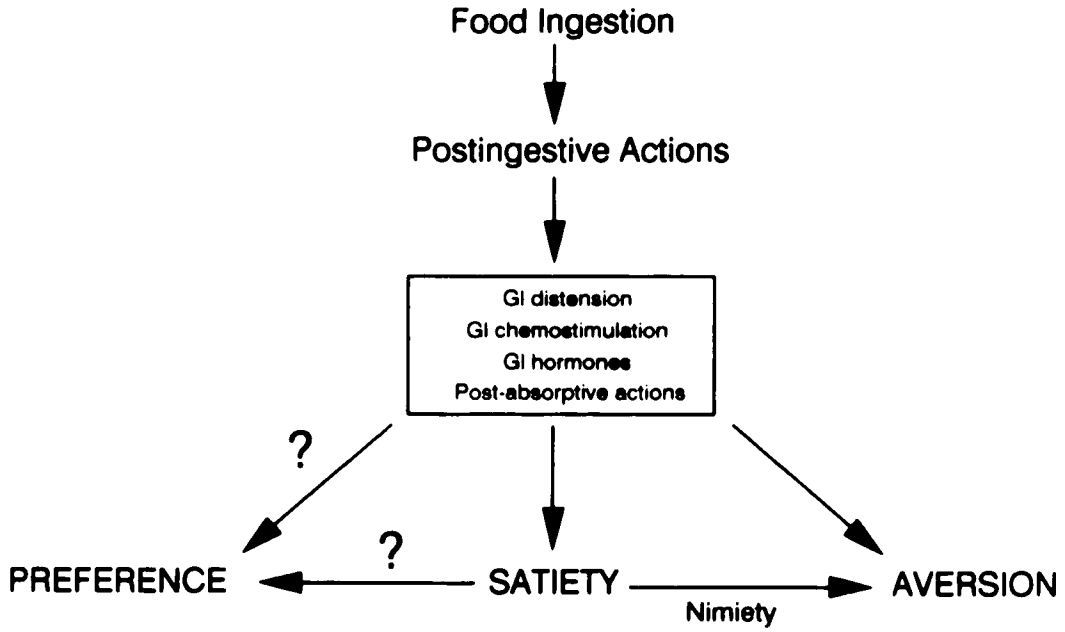

Figure 1. Schematic representation of the possible postingestive actions of food. Food ingestion can have negative reinforcing effects if the food contains a toxin or if the food is eaten in excess and produces nimiety. Food ingestion can also produce satiety and positive reinforcing effects. The question marks in the figure indicate that the relationship between satiety and positive reinforcement is not certain.

and another flavor (CS-) with the absence of the nutrient (e.g., IG water infusion). After repeated exposure to these pairings, the animals are offered a choice test between the two CS flavors. The conditioned response (CR), as measured in this choice test, is the relative intake of the CS flavors.

In the CFP experiment, the unconditioned response (UR) is not typically measured. Furthermore, according to Booth (1987), an UR is not necessary to prove that there was a learning effect. Any difference between the intake of the CS+ and CS- flavors in the choice test indicates that learning has occurred. The development of a preference for the nutrient-paired flavor (CS+), as compared to the control-paired flavor (CS-), is evidence that some aspect of the nutrient had beneficial or positive effects.

In CFP studies, a preference is sometimes interpreted as a "hedonic shift", that is, an acquired liking for the CS+ flavor. A hedonic shift implies that flavors that initially are not particularly attractive become palatable by virtue of their association with nutritive consequences. However, Rozin and Zellner (1985) have argued that preferences are not always based on liking, and thus a CFP is not necessarily evidence for a hedonic shift. For instance, the CS+ solution can be preferred not because the animal actually likes its flavor but because it anticipates the positive consequences of the associated nutrient. In human subjects "preferences" can be distinguished from "liking" using various rating scales but such techniques cannot be used with nonhuman subjects. However, the task of distinguishing between liking and preference in animals is not without hope. For instance, Grill and Norgren (1978) have used orofacial reactions to measure hedonic evaluation in rats.

Nutrient-based conditioned preferences and aversions

Many studies that have used the CFP paradigm have demonstrated that rats learn preferences for artificial flavors that have been associated with nutritive consequences (e.g., Bolles, Hayward, and Crandall, 1981; Booth, 1985; Capaldi, Campbell, Sheffer, and

Bradford, 1987; Puerto, Deutsch, Molina, and Roll, 1976; Elizalde and Sclafani, 1988, 1990). In some cases, flavor preferences have been observed when the nutrient is delivered by a non-oral route. For instance, rats develop preferences for flavors associated with IG infusions of fat (Lucas and Sclafani, 1989) and Polycose, a corn starch hydrolysate with very low sugar content (Elizalde and Sclafani, 1990; Sclafani and Nissenbaum, 1988; Nissenbaum and Sclafani, 1987). Other studies have reported flavor conditioning using IG proteins (Baker, Booth, Duggan, and Gibson, 1987); intravenous glucose (Mather, Nicolaïdis, and Booth, 1978), and hepatic-portal glucose (Tordoff and Friedman, 1986) (see Sclafani, 1990 for a review).

Not all the studies that have delivered the nutrient by non-oral routes have obtained CFPs. In particular, IG glucose infusions conditioned either no preferences, or even aversions in some studies (Deutsch, Molina, and Puerto, 1976; Puerto, Deutsch, Molina, and Roll, 1976; Koopmans and Maggio, 1978). It is conceivable that some aspects of the infusion procedure had aversive consequences that counteracted the positive effect of the nutrient. For instance, the hypertonic glucose used in these experiments may have produced GI discomfort (Booth, 1985). Similarly, when the infusions are excessive in rate and/or volume they may result in an abnormally large GI load (Sclafani, 1990), which may induce nimiety. It has also been observed that when the nutrients are not ingested by mouth they may not be normally processed by the GI system (see Booth, 1985, and Deutsch, 1987).

Recent studies by Sclafani illustrate how the postingestive actions of nutrients can have both positive and negative effects (Sclafani and Nissenbaum, 1988; Nissenbaum and Sclafani, 1987). In one experiment, two groups of rats received IG infusions of a fixed volume of a Polycose solution after access to the CS+ solution. One group infused with 8% Polycose displayed an immediate preference for the CS+ solution; another group infused with 32% Polycose initially avoided the CS+ solution and only after repeated training trials did it develop a CS+ preference. In another experiment, the volume of the IG infusion was

not fixed but was controlled by the rats; i.e., the rats' licking of the CS solution tubes turned on the infusion pumps. Under these conditions, IG infusions of either 8% or 32% Polycose produced immediate preferences for the CS+ flavor. Since the rats that controlled the size of the IG load were infused with less 32% Polycose than the rats that received a fixed amount, it may be that the initial aversion to the nutrient-paired flavor observed in the first experiment was due to discomfort produced by an excessive IG load.

Nissenbaum, Sclafani, Vigorito and Cassouto (1988) have also reported positive and negative effects using sham-feeding rats. They showed that when rats were trained to associate a flavor with a sham-fed 32% Polycose solution and a different flavor with a real-fed 32% Polycose solution, the rats initially tended to avoid the real-fed flavor in choice tests. Another group of rats, similarly trained and tested, but consuming an 8% Polycose solution displayed an immediate preference for the real-fed flavor. It appeared that, when real-fed, the more concentrated solution had an aversive component that interfered with its positive postingestive effects. The initial aversion to the real-fed flavor may have been an example of "nimiety", since the animals were food-deprived and consumed large amounts of this concentrated solution in short periods of time.

As reviewed above, many studies have demonstrated that the postingestive actions of food can have positive reinforcing effects. Few studies, however, have addressed the question of which specific postingestive action of food is responsible for this effect. There is one report of flavor preferences conditioned by intravenous injections of glucose (Mather, Nicolaïdis, and Booth, 1978). A site that has been postulated as a mediator of the postingestive reinforcement effect is the liver. Tordoff and Friedman (1986) reported flavor conditioning using hepatic-portal infusions of glucose. Deutsch and Wang (1977) reported that IG milk infusions produced a CPP in rats fitted with a pyloric cuff to prevent the passage of the IG infusion beyond the stomach. They concluded that nutrient "sensors" in the stomach were responsible for this effect, but Baker and Booth (1989) have challenged

this interpretation. They demonstrated that postgastric factors were not excluded in Deutsch and Wang's study.

Hormonal mediation of CFPs has also been investigated. In particular, Oetting and VanderWeele (1985) reported that small doses of insulin, sufficient to suppress sham-feeding, were able to induce a CFP in a sham-feeding paradigm. However, the same doses of insulin conditioned aversions in a real-feeding paradigm (VanderWeele and Deems, 1989). Thus, the importance of insulin in the conditioning of food preferences remains in doubt.

It is possible that the reinforcement signals, like the satiety signals, originate in multiple sites, involve a variety of pathways, and convey a redundant message to the brain. Much remains to be learned about the specific site(s) and the nature of the stimuli that are responsible for the postingestive reinforcement effect. In the experiments to be presented here, I have examined the contribution of the gut hormone cholecystokinin to the postingestive reinforcement effect. As discussed in the next section, CCK is one of the best-investigated putative satiety signals.

CHOLECYSTOKININ: ITS ROLE IN SATIETY AND REINFORCEMENT

Cholecystokinin and satiety

Cholecystokinin is a peptide that is released from intestinal cells in response to certain properties of food chyme: hydrogen ions, protein (and amino acids), fat, and magnesium and calcium ions (Mueller and Hsiao, 1978). The release of CCK in response to food, and the fact that CCK-secreting cells are concentrated mostly in the duodenum and proximal jejunum, suggested that CCK might act as the blood-borne factor that mediates satiety (Gibbs, Young, and Smith, 1973a).

The inhibitory effects of CCK on feeding are well documented. Gibbs, Young, and Smith (1973a) found that intraperitoneal (i.p.) injections of partially purified porcine CCK

and of CCK-8 (the synthetic C-terminal octapeptide of the molecule) produced a dose-dependent suppression of solid and liquid food intake in mildly food-deprived rats. The inhibition was specific for food because water consumption was unaffected by CCK and CCK-8 in water-deprived rats. The effect was on meal size rather than on the motivation to eat: CCK did not affect intake in the first 5 min of the test but reduced subsequent consumption. Maximal suppression occurred between 5-15 min postinjection, and the inhibitory effects began to disappear 30 min after the injection. Gibbs, Young, and Smith (1973b) replicated these results using sham-feeding rats. The threshold dose to suppress sham-feeding, however, was found to be four times higher than that required to suppress real-feeding. This difference was interpreted as being due to the summation of endogenous and exogenous CCK that takes place in real-feeding.

Although these results clearly demonstrate that CCK [1] inhibits feeding, they do not tell us whether the inhibition is due to satiety. Eating may be stopped for a variety of reasons without the animal being sated, e.g., the taste of the food was aversive, the animal developed an urge to drink or to explore, a predator appeared, the food produced gastrointestinal malaise, etc. Meal pattern analysis is one approach that has been used to differentiate between satiety and inhibitions of feeding due to other causes. Meal pattern is described in terms of two parameters, meal size (MS), and intermeal interval (IMI), that is the noneating period until the next meal. Ingestion of toxic substances will result not only in a smaller MS but also in an abnormally short IMI, indicating that the animal stopped eating for reasons other than satiety. Using this approach, Kraly, Carty, Resnick, and Smith (1978) found that CCK (1.5 μg/kg) was able to normalize the meal pattern of mildly food-deprived sham-feeding rats. When food-deprived for about 3 h, rats sham-

[1] Since most of the published research on CCK has used the synthetic octapeptide (CCK-8), beyond this point, and for the sake of brevity, I will use the abbreviation CCK to refer to CCK-8; when referring to any other form of the hormone, the specific adjective will be used.

feeding a palatable solution eat an abnormally large meal, take longer to rest after taking the meal, and have an abnormally short intermeal interval (IMI) as compared to real-feeding. Administration of CCK was shown to shorten the size of the meal and the latency to rest, and to increase the IMI.

Another argument to consider CCK as a satiety factor is its ability to elicit the sequence of behaviors (grooming, exploratory behaviors, and resting or sleeping) that normally follows the end of a meal and that has been used as a behavioral marker of satiety (Smith, Gibbs, and Young, 1974). Antin, Gibbs, Holt, Young, and Smith (1975) showed that CCK (2 μg/kg) elicited the behavioral sequence of satiety in both real- and sham-feeding rats.

Further evidence for the role of CCK in satiety is its ability to act in synergism with oral stimulation to inhibit food intake. Antin, Gibbs, and Smith (1977) showed that the ability of intraduodenal infusions of a liquid diet to suppress sham-feeding is dependent upon previous oral stimulation. They varied the time interval between the onset of sham-feeding and the onset of the intraduodenal infusion. The inhibition of intake was maximal when the infusions were administered 12 min after sham-feeding began; no inhibition occurred if the infusions were administered 12 min before sham-feeding began. Using the same paradigm, Antin, Gibbs, and Smith (1978) found that CCK (2 μg/kg) was able to mimic the effects of duodenal infusions, and had similar synergistic action with oral stimulation in the suppression of feeding.

The inhibitory effects of CCK on food intake have been confirmed in other species, including chickens, rabbits, pigs, sheep, rhesus monkeys and humans (see Smith, Gibbs, Jerome, Pi-Sunyer, Kissileff, and Thornton, 1981). Particularly important are the results obtained with humans because humans can be asked to make hedonic evaluations of the stimuli. Cholecystokinin reduced food intake in lean (Kissileff, Pi-Sunyer, Thornton, and Smith, 1981) and obese (Pi-Sunyer, Kissileff, Thornton, and Smith, 1982) humans without

producing side effects. In addition, CCK has been shown to decrease the perception of hunger elicited by the smell, sounds and sight of food that was being prepared in the room with the subject (Stacher, Bauer, and Steinringer, 1979). One interesting aspect of this inhibition of feeding in humans is that the subjects do not perceive that they have eaten less or that they are less satisfied by the test meal (Kissileff, Pi-Sunyer, Thornton, and Smith, 1981; Pi-Sunyer, Kissileff, Thornton, and Smith, 1982).

Taken together, the results of these experiments suggest that the inhibition of feeding produced by CCK may be the result of activating a physiological satiety mechanism. Unfortunately, many questions remain to be answered before we can accept or reject the satiety hypothesis. One problem is to obtain reliable measures of the endogenous release of CCK. There are many technical difficulties involved in the radioimmunoassay used to determine the physiological levels of circulating CCK. In addition, little is known about the catabolism or the rate of tissue uptake of CCK. Therefore, it remains unclear which doses of exogenous CCK have a physiological, as opposed to a pharmacological, effect. Furthermore, recent data suggest that circulating levels of CCK may not be the critical factor. Regarding its satiety effects, CCK may have paracrine and/or neuronal actions that are not mediated by circulating CCK (Calingasan, Ritter, Ritter, and Brenner, 1989).

Aversive effects of cholecystokinin?

The evidence described above supports the hypothesis that CCK plays a role in satiety. It has been argued, however, that CCK inhibits feeding by producing malaise (Deutsch and Hardy, 1977; Swerdlow, van der Kooy, Koob, and Wenger, 1983). To distinguish the non-specific inhibition of feeding due to malaise from a specific satiety effect, several investigators have used the conditioned flavor aversion (CFA) paradigm. In the traditional paradigm, a novel flavor (CS) is paired with a toxic treatment (US) such as LiCl injections, and water is paired with a control saline injection. When the animals

are given a choice between the CS and water, they will avoid (CR) the CS flavor after a single pairing. The CFA paradigm can also be applied using a discriminative procedure: the CS+ flavor is paired with LiCl and the CS- flavor is paired with saline (Deutsch and Hardy, 1977).

The CFA paradigm has become a classic technique to evaluate the effects of drugs. The avoidance of the drug-paired flavor in choice tests is taken as evidence of the aversive properties of the drug. However, this general interpretation should be taken cautiously because in some studies certain toxins have failed to condition aversions (Ionescu and Buresova, 1977; Gamzu, 1977; Goudie, 1979). Furthermore, as Kulkosky (1985) pointed out, any experimental treatment can be aversive when administered at sufficiently high intensity. For instance, some of the studies that have characterized CCK as a nausea-inducing agent (Deupree and Hsiao, 1987; Swerdlow, van der Kooy, Koob, and Wenger, 1983; Verbalis, McCann, McHale, and Stricker, 1986) have used high doses (up to 500 µg/kg!). There is no doubt that megadoses of CCK, or almost any other pharmacological agent, can have aversive consequences.

The question is whether "physiological" doses of CCK, sufficient to inhibit feeding, can serve as an effective US in a CFA paradigm. This question is difficult to answer, because the doses of exogenous CCK that induce "physiological" inhibitions of feeding have not been specified. Several studies have indicated that 0.25 µg/kg is a subthreshold dose, and that between 0.5-2 µg/kg CCK suppresses feeding in a dose-dependent manner by less than 50% (Gibbs, Young, and Smith, 1973a; Mueller and Hsiao, 1977, 1978; Le Sauter, Goldberg, and Geary, 1988). It is possible that doses of CCK that suppress feeding by more than 50% may be acting through pharmacological, rather than physiological, means.

The first studies that investigated CCK in a CFA paradigm reported that CCK (2 µg/kg) did not condition a saccharin aversion after a single pairing (Gibbs, Young, and Smith (1973a) or repeated pairings (Holt, Antin, Gibbs, Young, and Smith, 1974). LiCl-

and apomorphine-treated rats, developed saccharin aversions under similar conditions. A more recent study (Ervin and Teeter, 1986) reported that CCK (1-4 μg/kg) failed to condition saccharin aversions, but 8 μg/kg produced a mild aversion after a single saccharin-CCK pairing. In these experiments, the injections were administered after access to the CS solution, the animals were water-deprived, and intakes were measured in one-bottle tests.

These studies suggest that doses of CCK between 1 and 4 μg/kg do not act as effective USs in the CFA paradigm. However, Deutsch and Hardy (1977) have argued that the failure to observe a CCK-induced CFA in these studies may have been due to the use of an insensitive measure of aversion (one-bottle test) in conjunction with an extreme fluid deprivation condition. In these studies access to fluid was restricted to the 10 or 15 min test. It is conceivable that the rats may have overcome a possible aversion to saccharin in order to obtain fluid.

Deutsch and Hardy (1977), using a two-bottle test, reported that CCK (2 μg/kg) induced a flavor aversion after a single pairing. However, Kulkosky (1985) paired the same dose of CCK to saccharin and found that the slight decrease in saccharin preference (as measured versus water in two-bottle tests) observed after a single pairing completely recovered during six extinction trials. However, LiCl-treated rats did not extinguish the aversion to saccharin after as many trials. Note that in Deutsch and Hardy's experiment the CCK injections were administered after the test session, whereas in Kulkosky's experiment the injections preceded access to the solutions. Kulkosky (1985) argued that the sudden injection of the hormone after self-selected consumption may constitute a sufficiently novel stimulus to elicit neophobic avoidance. Thus, whether 2 μg/kg of CCK conditions aversions is a question that has not been clearly answered.

Some of the discrepancies found with 2 μg/kg of CCK can be explained in terms of design variables. For instance, this dose could be aversive if its administration produces a surprise, a physiological novelty. Alternatively, since we do not have appropriate

measures of physiological levels of endogenous CCK, we cannot exclude the possibility that 2 µg/kg is a "nonphysiological" dose, and that, under some experimental conditions, it may act as a nimiety signal. Although Kulkosky (1985) has defended the position that a dose of 2 µg/kg administered along with repeated training and testing does not have aversive effects, it remains to be specified which doses, and under which conditions, induce a state similar to physiological satiety as opposed to nimiety. Therefore, we have to be very cautious in our analysis of the aversive properties of a given experimental treatment, taking into consideration the dose and design used.

Positive reinforcing effects of cholecystokinin?

The reports that CCK can induce a CFA have raised questions concerning whether CCK is a true satiety signal. Booth (1985) has contended, however, that even if CCK produces conditioned aversions under some circumstances, this does not argue against the claim that it is a physiological satiety agent. If food, the natural stimulus for satiety, can also produce nimiety under certain conditions (hypertonic solutions, excessive intake rate or GI load, etc.), it is plausible that CCK could induce satiety or nimiety depending on the intensity of the signal (dose) used. In Booth's words, "voluntary satiety can be sufficiently extreme to condition an aversion". However, Booth has also argued that if CCK is a true physiological satiety signal then it should, under the appropriate conditions, be able to reinforce a CFP. This view is based on the assumption that satiety signals, by their nature, have positive reinforcing effects.

As it was discussed above, there are some indications that signals that contribute to the termination of a meal may be effective stimuli in a CFP paradigm (e.g., hepatic and intravenous effects of some nutrients). However, there is not enough evidence to support the notion that an inherent property of satiety signals is the ability to act as positive reinforcers. Therefore, further research is needed before we can accept the validity of

Booth's claim. Perhaps CCK does induce satiety, but not all satiety signals have positive reinforcing effects. It is conceivable that some postingestive consequences of food have satiety and positive reinforcing effects; others may have satiety effects but not positive reinforcing effects; and others may have positive reinforcing effects without involving satiety. Furthermore, there is the theoretical possibility that CCK may produce both satiety and positive reinforcing effects, but that these effects are independent.

The question of whether CCK can condition a flavor preference has been addressed in two preliminary reports. In one experiment, Mehiel (1989) reported that CCK (4 μg/kg) produced a mild flavor preference. However, these data were presented in abstract form and a full report has not been published. The preference produced by CCK was weak, emerged only after 20 CS+/US pairings, and was obtained under a rather uncommon food- and water-deprivation condition. Another experiment reported in abstract form found that CCK could condition odor preferences in rat pups 5-22 days of age. This effect, however, was not observed in 28-day old, or adult rats (Weller and Blass, 1988). The results of these experiments leave unresolved the question of whether CCK plays a major role in the CFPs produced by nutrients in adult animals.

RESEARCH STRATEGY

The studies to be presented here addressed the question of whether CCK plays a role in the postingestive reinforcement effect of nutrients. The research strategy that I adopted was to use test conditions and paradigms that were successful in producing flavor conditioning when a nutrient was the US (see Sclafani, 1990 for a review). In my experiments the effects of CCK were investigated in a feeding context. In particular the animals were food- rather than water-deprived. The rationale for that condition is that if CCK induces satiety, it should be a more effective reinforcement for a hungry than for a thirsty rat. In addition, CCK was administered during a feeding bout, rather than at the end

of the session. Since the animals had access to the CS+ solution after the CCK injections I was able to measure the feeding inhibitory effects of the hormone. Preliminary experiments explored different doses and conditioning procedures in an attempt to maximize the probability of obtaining positive results. The first doses investigated were 2 and 4 μg/kg since this is the most common dose range used in studies concerned with inhibition of feeding. Subsequent experiments examined lower doses (from 0.125 to 1 μg/kg).

To investigate the effects of exogenous CCK in isolation, the postingestive effects of food, including endogenous release of CCK, had to be minimized. To accomplish this, two strategies were used: either the animals were allowed to sham-feed a "real" food (a caloric solution) or they were offered a "sham" food (a noncaloric solution) to real-feed. Although the sham-feeding preparation does not completely prevent absorption (Sclafani and Nissenbaum, 1985a,b) nor gastric chemostimulation, it eliminates gastric distension and attenuates other postgastric actions of food (intestinal chemostimulation and hormone release, postabsorptive effects, etc.). By minimizing the inhibitory postingestive effects of food, this paradigm virtually eliminates postprandial satiety in food-deprived animals (Young, Gibbs, Antin, Holt, and Smith, 1974). In the real-feeding paradigm, GI distension is a potential satiety signal, but the use of a noncaloric solution minimizes other postingestive actions of nutritive solutions.

The CFP paradigm was used in all the experiments. This method involved training the animals to associate an arbitrary flavor (CS+) with CCK and a different flavor (CS-) with saline. These one-bottle training sessions also provide a measure of the feeding-inhibitory effects of CCK. In subsequent two-bottle choice tests the rats' relative preferences for the CS+ and the CS- solutions were assessed. In these latter tests a preference for the CS+ solution is evidence for a positive reinforcing effect of CCK.

Note that since the unconditioned response to CCK involves a suppression in food intake, it could be argued that the conditioned response to CCK should be a reduction in

food intake. According to this interpretation the rats should consume less not more of the CS+ solution in the two-bottle tests. In fact, nutrients have been observed to produce a conditioned "satiety" effect such that animals consume less of a flavor that is paired with a concentrated nutrient source compared to a flavor associated with a dilute nutrient source (Booth, 1972). This conditioned satiety effect has been observed in one-bottle tests, however, and it does not follow that similar results would be obtained in two-bottle tests. It is well known that rats, while they consume less of a concentrated nutrient source (e.g., 32% sucrose) than of a dilute source (e.g., 4% sucrose) in one-bottle tests, they display a preference for the concentrated nutrient source in two-bottle tests (Sclafani, 1987).

GENERAL METHODS

Subjects

The subjects were adult female, Sprague-Dawley rats (Charles River Laboratories, Wilmington, MA) between 13-15 week old at the beginning of the experiments. Upon arrival in the laboratory, the rats were individually housed in stainless steel cages in an air conditioned vivarium maintained at 21° C and under a 12:12-hr light-dark cycle. They were maintained on pelleted chow (Purina #5001) and tap water. A period of adaptation to the vivarium of at least 8 days elapsed before beginning the experiments.

Test solutions

The test solutions were prepared with Polycose® (Ross Laboratories, Columbus OH) or saccharin (Sigma Chemical Company, St. Louis, MO) dissolved in tap water. The concentrations were calculated on a weight/volume basis. These solutions were marked with two distinctive CS flavors. The CS flavors (0.05% unsweetened Kool Aid®; General Foods Corp., White Plains, NY) were the same in all the experiments. For half the subjects the CS+ was grape and the CS- cherry; for the other half the CS flavors were reversed. Previous work has shown that these two flavors are isohedonic to rats (Elizalde and

Sclafani, 1990). The CS solutions were offered in 50 ml graduated cylinders with metal drinking spouts. On training days, the solutions were presented in the center of the cage front. On test days, the solutions were presented to the left and right of the cage front. Intakes were recorded to the nearest 0.5 ml.

Procedure

Figure 2 illustrates the general training and testing procedure of a 5-day cycle. The temporal relationship between access to the CS solutions and the injections is shown in Figure 3 for each conditioning procedure. During the first four days of each cycle one-bottle tests were given. On alternate days, the rats were trained to associate one flavor (CS+) with intraperitoneal (i.p.) injections of CCK-8 (US) and a different flavor (CS-) with isovolemic saline injections. The hormone (kindly supplied by Hoffmann-La Roche Laboratories) was dissolved in saline so that a particular dose is delivered in 1 ml of saline. In this way, the amount of the hormone administered to each rat could be easily calculated on a volumetric basis. After four training sessions, the rats were given one or two days of two-bottle tests with the CS+ and the CS- solutions. In all two-bottle tests the right-left position of the two solutions alternated over test sessions. The details of this procedure are described with the individual experiments.

Before the beginning of the experiments, the rats were food-restricted and familiarized with the experimental conditions. The familiarization procedure lasted for a period of 8-10 days, during which the rats were exposed to the experimental routine before the CSs and US were introduced. During those days, the rats were placed in a room adjacent to the vivarium at about the same time every day, and allowed to consume a solution for a period of time equivalent to that of the experimental sessions. Except for experiment 1A, the rats were also familiarized with the two-bottle condition. These sessions were designed to expose the rats to the task of discriminating two different solutions. Finally the rats were familiarized with the injection procedure on the day prior to the

DAYS	CS	TREATMENT
	1-bottle Training	
1	CS-	Saline
2	CS+	CCK
3	CS-	Saline
4	CS+	CCK
	2-bottle Testing	
5	CS- vs. CS+	Saline

Figure 2. Description of a training (one-bottle) and testing (two-bottle) cycle. The CSs were Polycose or saccharin solutions flavored with grape and cherry Kool-Aid. Half of the rats had grape as CS+ and cherry as CS-; the other half had the reversed pairings. The one- and two- bottle sessions were 15-20 min in duration.

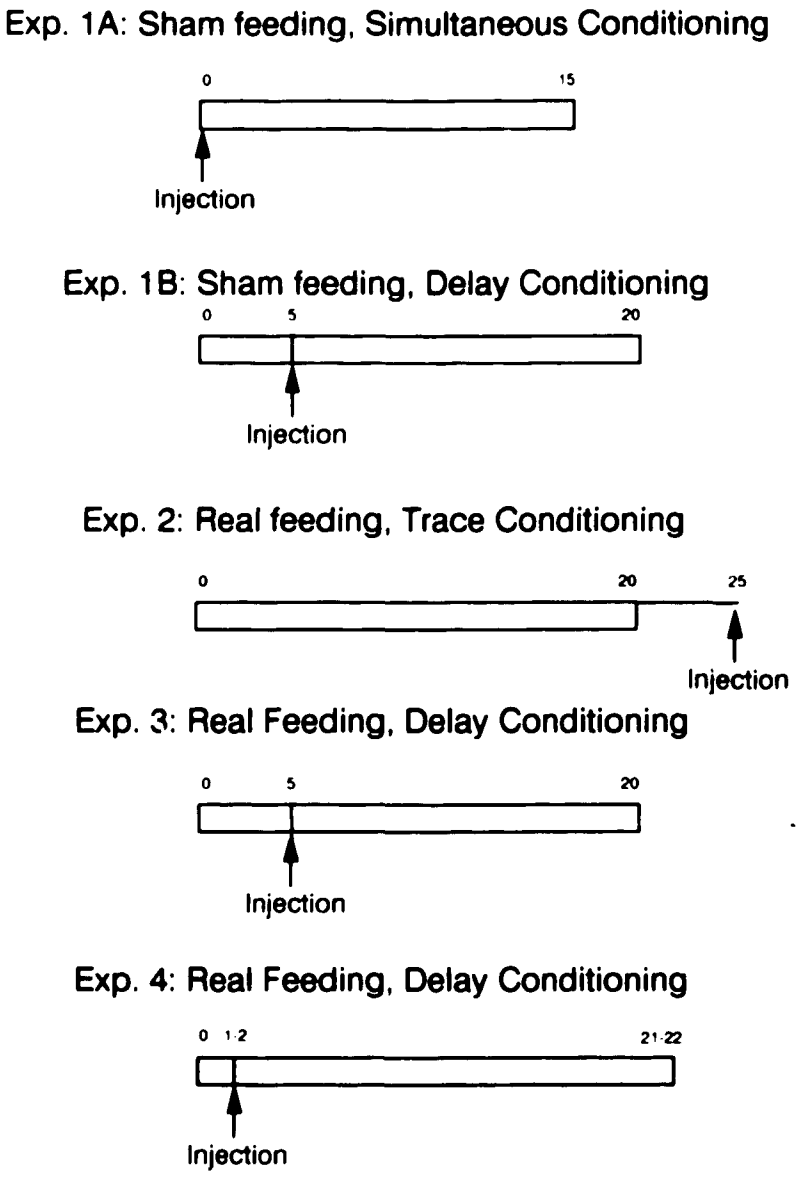

Figure 3. Schematic representation of the different paradigms and conditioning procedures used in the four experiments. The bars represent access to the flavored CS solutions. Note that in Experiment 4 the rats were prefed a fixed amount (2 ml) of CS solution which was consumed in 1-2 min. In the 20-min post-injection period the rats were given a restricted amount of the CS solution (50% of baseline).

commencement of formal training. To that end, the rats were offered an unflavored version of the solution to be used in the subsequent training phase, and injected with saline, mimicking the temporal sequence of a training day. In Experiment 1A, however, the rats had access to the CS- solution on that day. These data were not included in the statistical analysis.

After this familiarization procedure the rats were divided into two subgroups equated for solution intake on the last two days of one-bottle tests. For one subgroup grape was the CS+ flavor and cherry the CS- flavor; for the other subgroup the CS flavors were reversed. Water was always available ad libitum in the home cages. Daily food (a fixed ration) was distributed in the home cages 1-2 h after the test.

The duration of the sessions (15-20 min) was decided on the basis of the course of action of the hormone; the largest effect seems to occur between 5-15 min after feeding has begun, according to Gibbs, Young, and Smith (1973a).

Statistical Analysis

The intake data on training (one-bottle) and test (two-bottle) sessions were submitted to separate analysis of variance (ANOVA) with repeated measures on cycles and CS solutions. For the one-bottle data, the mean of the intakes on the two CS+ sessions and the mean of the intakes on the two CS- sessions were analyzed for each cycle. For the two-bottle data, the intakes of a single test were analyzed, except for Experiment 1A, where the average of the two choice tests were evaluated. When there was a significant CS solution x Cycle interaction, individual comparisons were evaluated with simple main effect and Newman-Keuls tests. The minimum criterion used for statistical significance was $p<0.05$.

In addition to absolute intakes, two other measures are reported: the percent suppression on training days (1-(CS+ intake/CS- intake) x 100), and the percent CS+ preference on choice-test days ((CS+ intake/(CS+ intake + CS-intake)) x 100). Note that

percent suppression is relative to CS- intake on one-bottle sessions whereas percent preference is relative to total (CS+ plus CS-) intake on two-bottle tests.

PART I: SHAM-FEEDING, REAL-FOOD

EXPERIMENT 1A

The first attempt to condition a flavor preference using CCK involved the sham-feeding paradigm and a procedure adapted from that of Nissenbaum et al. (1988). These investigators reported that rats acquired a preference for a flavored 8% Polycose solution that was real-fed over a differently flavored 8% Polycose solution that was sham-fed. In my experiments the rats were trained to sham-feed two different flavors of an 8% Polycose solution. One flavor was paired with CCK (4 μg/kg) and the other with saline. If the CFP for the real-fed flavor in the experiment of Nissenbaum et al. was mediated at least in part by CCK, the animals in my experiment should develop a preference for the CCK-paired flavor.

METHOD

The sham-feeding preparation

Fifteen rats were fitted with gastric cannulas, as described elsewhere (Sclafani and Nissenbaum, 1985b). Briefly, the rats were anesthetized with Chloropent, and the cannulas were implanted into the cardiac portion of the stomach along the greater curvature. The shaft of the cannula was passed through a stab wound in the abdominal wall. When the cannula is closed with a screw, the animal digests and absorbs the food normally. However, when the cannula is opened, the test solution drains out of the stomach as the animal feeds.

Before each session, the rats' cannulas were opened and the stomachs flushed with saline until they appeared empty of food. The rats were then placed in the experimental cages in a room adjacent to the vivarium, where they were exposed to the test procedure

described below. Drainage was collected in individual pans placed underneath the animals' cages. The patency of the cannulas was confirmed by recovering an amount of drainage fluid that equalled or exceeded the amount of solution consumed. After the session the rats were put back in their home cages and returned to the vivarium.

Test solutions

The test solutions used in this experiment were grape and cherry flavored 8% Polycose.

Procedure

The rats were allowed to recover from surgery for a 9-day period, after which food restriction and familiarization procedures were introduced. The 10 rats that completely recovered after this period were maintained at 90% of their postrecovery body weight (BW). Beginning on day 10 postsurgery, and following the procedure of Nissenbaum et al. (1988), the rats were familiarized with an 8% sucrose solution to real-feed for 15 min for 4 days, and to sham-feed for 6 more days. The rats were then tested with the CS- solution and given a saline injection before the 15 min session. Formal training began the next day (day 21 postsurgery).

The rats were trained and tested over three 6-day cycles (see Figure 2). On days 1 and 3, the CS- solution was paired with an injection of saline. On days 2 and 4, the CS+ solution was paired with an injection of CCK (4 μg/kg). On days 5 and 6, the rats were given two choice tests with the CS+ and the CS- solutions, alternating their left-right position daily. Saline was injected on these tests.

In this experiment the animals were injected immediately prior to access to the CS solutions for 15 min. Strictly speaking this procedure is a case of backward conditioning because the CS is presented a few seconds after the US; however, since the latency of the CCK effects is longer than that interval, it is more accurate to view this procedure as a case of simultaneous conditioning, where the US and the CS overlap for the 15 min session (see

Figure 3).

RESULTS AND DISCUSSION

The results of one-bottle and two-bottle tests are shown in Figure 4A and 4B, respectively. Overall, CCK inhibited Polycose intake during training ($F=10.98$, $df=1,9$, $p<.01$). The intake of the CS+ solution was stable over cycles while the intake of the CS- solution increased (Cycle x Solution $F=5.59$, $df=2,18$, $p<.05$). Individual comparisons revealed that the suppressions were significant during cycles 2 (37%, $p<.01$) and 3 (34%, $p<.05$). The lack of significance during the first cycle may be explained by a neophobic response to the novel CS flavors. Since the cycles start with the CS- solution (saline injection day), the low average CS- intake of the first cycle can be due to a neophobic reaction on the first day of the experiment. It is conceivable that the neophobic response to the novel CS+ flavor on the first CCK injection day may be less pronounced because of some generalization between the two CS flavors. This interpretation is further supported by the fact that, as Figure 4A indicates, the intake of the CS-solution significantly increased ($p<.01$) from the first to the second cycle whereas CS+ intake remained stable over the three cycles.

In the two-bottle tests, no reliable preference or aversion emerged, although a trend to avoid the CS+ solution was observed. The percent of total intake derived from the CS+ were 34%, 47% and 32%, for cycles 1 through 3, respectively. Inspection of individual data revealed that in cycle 3, half of the rats showed strong aversions to the CS+ solution (less than 5% of total intake as CS+); this subgroup of rats strongly suppressed Polycose intake during training in response to CCK (70% suppression). The other half of the rats did not show aversions to the CS+ (60% of total intake as CS+), and they did not suppress Polycose intake during training. The fact that the strong suppressions of intake observed in some rats were accompanied by potent aversions suggests that mechanisms other than satiety were involved.

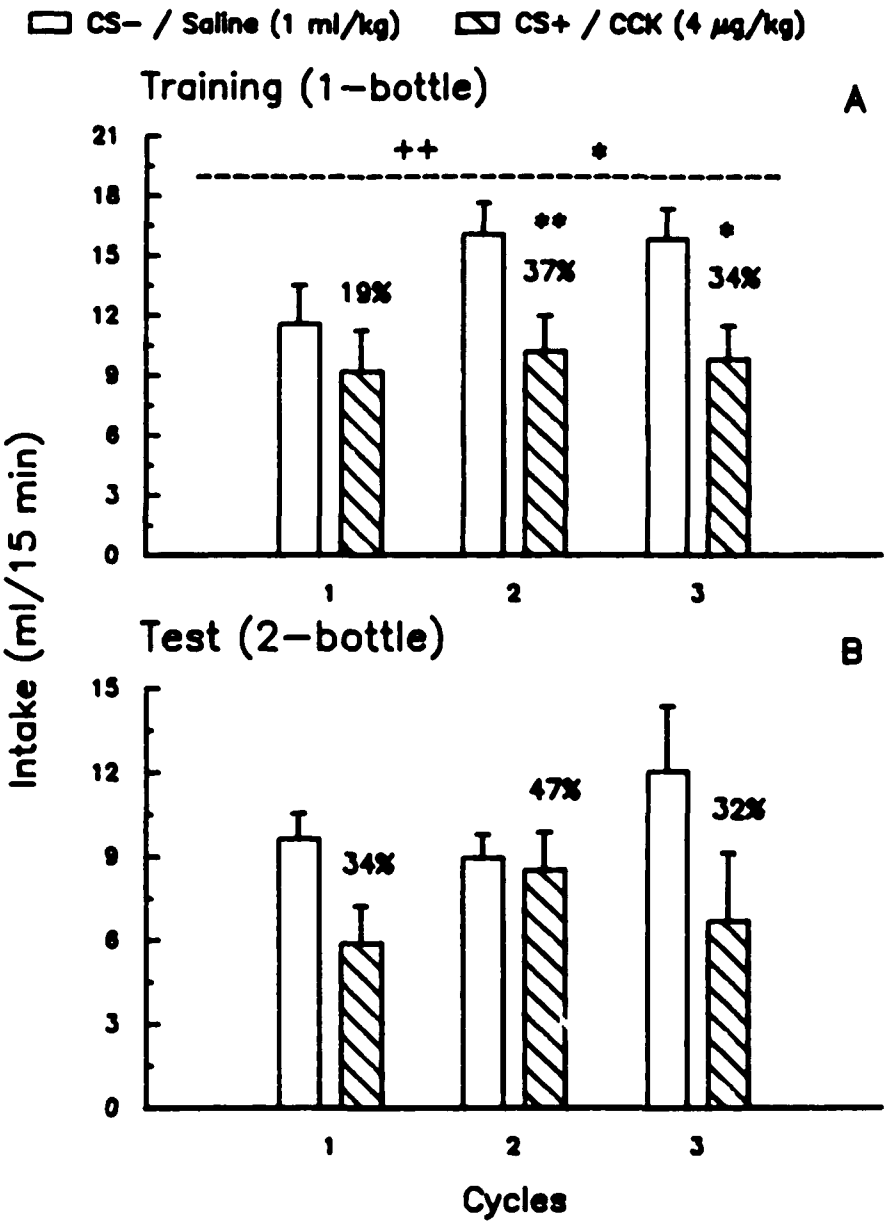

Figure 4. Experiment 1A. Simultaneous conditioning (4 mcg/kg CCK): sham-feeding of flavored 8% Polycose. Mean intake (+SE) of the CS+ and CS- solutions during 15 min in one-bottle (A) and two-bottle (B) sessions over 3 cycles. For each cycle the one- and two-bottle data are averages of two sessions. Percent CS+ suppressions (relative to CS- intake) are shown in A, and percent CS+ preferences (relative to total intake) in B. Significant CS solution effects are indicated by + ($p<0.05$) or ++ ($p<0.01$) above dashed line. Significant CS solution x Cycle interactions are indicated by * ($p<0.05$) or ** ($p<0.01$) above dashed line. In the latter case, significant differences between CS+ and CS- intakes at individual cycles are indicated by * ($p<0.05$) or ** ($p<0.01$) above the CS+ bar for that cycle.

EXPERIMENT 1B

The results of Experiment 1A suggested that 4 µg/kg of CCK might have been too a large dose for some rats. To minimize potential aversive effects of CCK, a lower dose (2 µg/kg) was used in Experiment 1B. To maximize the "satiety" effect of this dose the test paradigm was modified to include a short prefeeding period, that is 5 min access to the CS solutions prior to the injections. This strategy was adopted because oral stimulation prior to the CCK injections appears to enhance the inhibitory effects of the hormone (Antin et al., 1978). Several other procedural changes that were thought to improve the experimental conditions were introduced in this experiment. These changes are described below.

METHOD

Procedure

Twenty rats underwent gastric surgery as described above. The thirteen rats that had recovered after 12-14 days were food-restricted and familiarized with the experimental procedure by offering them an 8% sucrose solution to real-feed during four 15-min daily sessions, and to sham-feed for two sessions. Following this one-bottle familiarization, the rats were exposed to the two-bottle condition by giving them access to 8% and 4% sucrose solutions, for two sessions. The rats were then familiarized with the injection procedure as follows: they were allowed 5 min access to 8% plain Polycose followed by a saline injection and additional access to the solution for 15 min. Formal training began the next day with the CS solutions used in experiment 1A and a dose of CCK of 2 µg/kg.

Training and testing were similar to those in experiment 1A, except for the following modifications: First, 5-day cycles with a single two-bottle test per cycle were run. This change was introduced in an attempt to minimize the effects of extinction that presumably take place during two-bottle tests. Since in the two-bottle condition the animals have access to the CS+ solution without the effects of CCK, these sessions permit extinction to

occur. The left-right position of the CSs was alternated across cycles. Second, the stomachs were flushed after, as well as before, each training and test session, to prevent absorption of any Polycose remaining in the stomach after the session. Finally, the animals were maintained at 85% of their postrecovery BW.

The major change introduced in this experiment was the use a prefeeding paradigm, that is, the injections were preceded by 5 min, and followed by 15 min, of access to the CS solutions. This arrangement of stimuli is a case of delay conditioning (see Figure 3). Thus, the CS-US interval was 5 min (as measured from CS onset to US onset), and the CS overlapped with the US for 15 min. To ensure that the temporal sequence of events was identical for all the rats the presentation of stimuli was carefully controlled. Briefly, the rats were run in two squads of 6 and 7 rats, respectively. The CS solution was presented to the first rat and, after 30 sec had elapsed the solution was presented to the second rat. This sequence was repeated for all the rats, the first squad taking up 3 min and the second 3.5 min. When the first rat reached 5 min of consumption, it was taken out of the cage and, within 30 sec, injected, and returned back to the cage where the solution was available for an additional 15 min. This sequence was repeated for all the rats. Only the 15-min postinjection data are shown in the one-bottle tests.

In the two-bottle tests the rats were offered the CS+ and CS- solutions for 5 min, injected with saline, and then allowed access to the CS solutions for an additional 15 min. The statistical analysis of the CS+ and CS- intakes was based on the total 20 min period.

RESULTS AND DISCUSSION

The results of the one-bottle tests for the 15-min postinjection period are shown in Figure 5A. Overall, CCK suppressed Polycose intake during training ($F=12.36$, $df=1,9$, $p<.01$). However, there was a Cycle x Solution interaction ($F=4.34$, $df=3,27$, $p<.05$). Individual comparisons revealed that the suppressions were significant for cycles 1 (42%, $p<.01$), 2 (19%, $p<.05$) and 4 (22%, $p<.05$). The average magnitude of the inhibition

(23%) was slightly smaller than that obtained in Experiment 1A (30%).

The results of the two-bottle tests are shown in Figure 5B. The rats tended to consume slightly less CS+ than CS-, but statistical analysis revealed no significant differences. The percentages of total intake as CS+ ranged from 33% to 47% over the four cycles.

The extreme CS+ suppressions and aversions displayed by half the subjects in Experiment 1A were not observed in Experiment 1B. This might be due to the smaller dose of the drug used in the present experiment which may not have had the aversive effects of the higher dose. However, the possibility cannot be excluded that the priming paradigm reduced the likelihood of these aversive effects.

PART II: REAL-FEEDING, SHAM-FOOD

In Experiment 1, the two doses of CCK investigated suppressed sham-feeding but failed to reinforce a CFP. It might be that some component of real-feeding (e.g., gastric distension) may be necessary for a positive reinforcing effect of CCK to emerge. To provide a more natural feeding context, a real-feeding paradigm was used in Part II. However, to minimize the nutritive postingestive effects of the real-fed solution, the rats were offered a "sham" food, that is, a saccharin solution. Flavored saccharin solutions are effective CSs in producing flavor preferences when nutrients are the reinforcers (Sclafani, 1990).

Two experiments were conducted with different variations of the real-feeding paradigm. In one case, the delay conditioning procedure of Experiment 1B was used, except that the rats were real-feeding saccharin. In addition, a larger range of doses of CCK was investigated in this experiment. In the second case, I used a trace conditioning procedure based on a preliminary report by Mehiel (1989). These two strategies were investigated at the same time, but for the sake of clarity, the trace conditioning experiment is presented first.

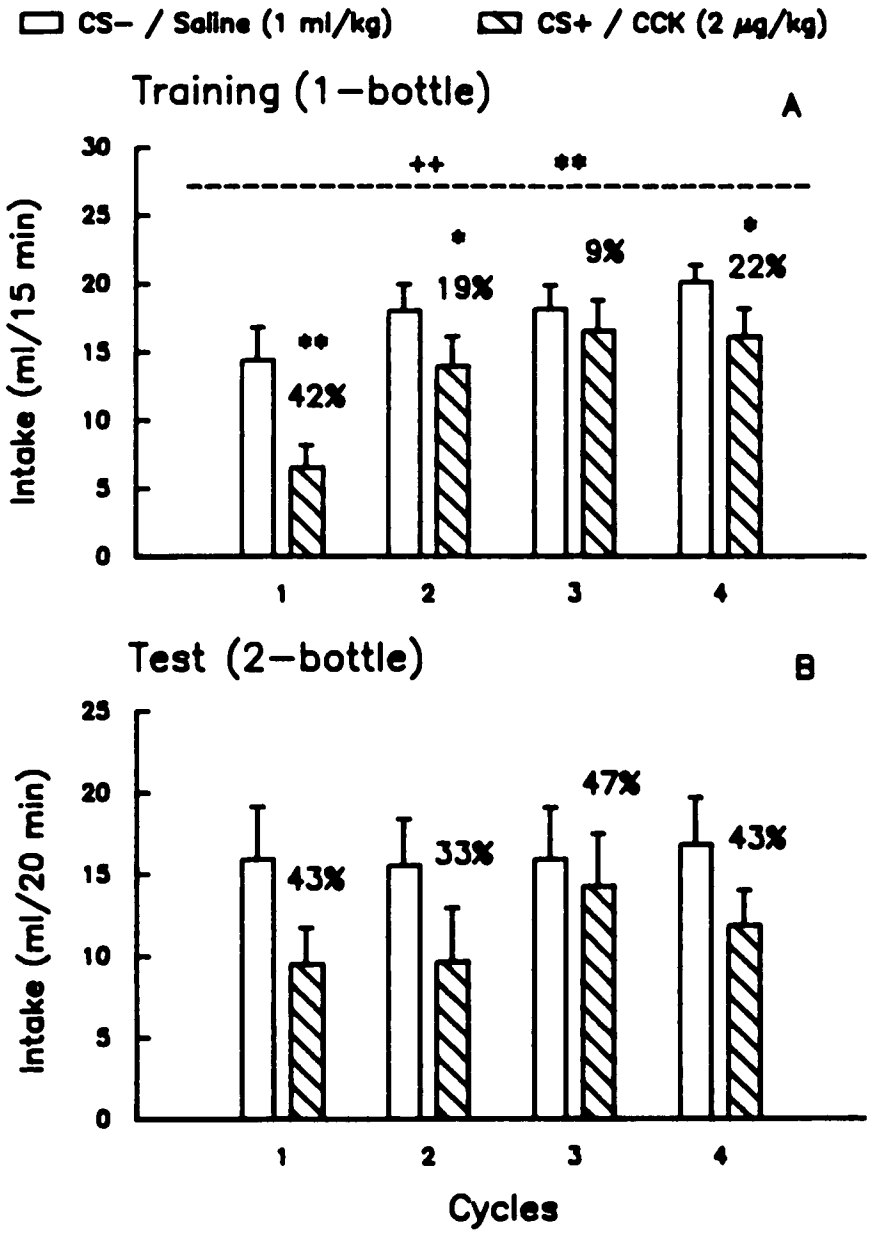

Figure 5. Experiment 1B. Delay conditioning (2 mcg/kg CCK): sham-feeding of flavored 8% Polycose. A. Mean intake (+SE) of the CS+ and CS- solutions and percent CS+ suppressions during the 15 min post-injection period in one-bottle sessions over 4 cycles. B. Mean intake (+SE) of the CS+ and CS- solutions and percent CS+ preferences during 20 min in two-bottle tests. The one-bottle data are averages of two sessions and the two-bottle data are the averages of one session. Statistical results indicated as in Figure 5.

EXPERIMENT 2

In this experiment, the 4 and 2 μg/kg CCK doses were examined in a real-feeding paradigm using a conditioning procedure adapted from Mehiel (1989). In Mehiel's experiment, thirsty rats were allowed to drink a flavored 0.2% saccharin solution for 40 min and 10 min later were injected with saline or CCK (4 μg/kg). This experiment is an example of trace conditioning because the US is presented after the CS has been terminated. After 10 pairings the rats were food deprived and offered a two-bottle test with the CS+ and CS- solutions. No preference for the CS+ flavor was observed. After 10 more training trials a second choice test was given. In this test the rats displayed a mild, but significant, preference for the CS+ flavor. No further testing was performed. Although from a learning point of view a trace procedure is poorer than a delay or a simultaneous procedure to reinforce learning (Mackintosh, 1974), the positive results obtained by Mehiel encouraged me to explore this method.

Except for the change in conditioning procedure, other parameters (length of the test, deprivation condition, etc.) remained similar to those used in all my experiments. Note that Mehiel used an atypical deprivation condition in that the animals were water-deprived on training days and food-deprived on testing days. The reason for training animals in a thirsty state and testing them in a hungry state is unclear. In my experiment, the animals were food- but not water-deprived both in training and testing sessions. In addition, in Mehiel's experiment the animals were offered the CS solutions for 40 min, and were injected with saline or CCK 10 min later. Thus, the CS-US interval was 50 min (from CS onset to US onset). In my experiment, the duration of the sessions was 20 min, followed by a 5-min delay and the appropriate injection, for a CS-US interval of 25 min. To the extent that conditioning is inversely related to the length of the CS-US interval, the shorter interval used in my experiment should produce stronger conditioning.

METHOD

Test solutions

The CS solutions were grape and cherry flavored 0.2% saccharin.

Procedure

The nineteen rats used in this experiment were maintained at 85% of their free-feeding BW. They were familiarized with the one-bottle condition by offering them 20-min daily access to a 0.2% saccharin solution for 7 days. Then they were familiarized with the two-bottle condition by offering them two saccharin solutions (0.2% and 0.1%) in two successive sessions. Two groups were then formed, matched for saccharin intake, which were randomly assigned to the 4 μg/kg (n=9) and 2 μg/kg (n=10) doses of CCK.

The rats were trained and tested over four 5-day cycles (see Figure 2). For each training trial, the rats were given access to the CS solutions for 20 min, and, following a 5-min delay, they received an injection of either saline or CCK (see Figure 3). After the injection, the rats remained in the experimental chamber for 30 min to allow the drug to have its effects without disruption. No injection was administered in two-bottle tests.

RESULTS AND DISCUSSION

The results for the 4 and 2 μg/kg doses are shown in Figures 6 and 7, respectively. Overall, neither dose suppressed saccharin intake in one-bottle tests. However, for the 4 μg/kg group, the intake of the CS- solution increased over cycles while CS+ intake remained stable (Cycle x Solution $F= 3.83$, $df=3,24$, $p<.05$). Individual comparisons revealed that CS+ intake was suppressed during cycle 3 (12% suppression, $p<.05$). For the 2 μg/kg group, significant suppressions were observed during cycles 2 and 4 (11%, $p<.01$) (Cycle x Solution $F=14.78$, $df=3,27$, $p<.001$). Note that, since the injections were administered after access to the CS solutions in the one-bottle sessions, any suppressions of the CS+ intake in these tests is a conditioned effect of the hormone. In fact, the inhibitions of CS+ intake emerged after the first cycle when presumably the rats had made

the CS-US associations.

In the two-bottle tests, the rats displayed a mild but significant aversion to the CS+ solution at both dose levels (4 μg/kg: $F=6.69$, $df=1,8$, $p<.05$; 2 μg/kg: $F=5.38$, $df=1,9$, $p<.05$). In the case of the 4 μg/kg dose, the percent CS+ intakes ranged from 31% to 40% (Figure 6B). For the 2 μg/kg dose the percent CS+ intakes ranged from 41% to 45% (Figure 7B).

These results show that CCK, at 2 and 4 μg/kg, rather than conditioning a flavor preference when a trace procedure was used, produced mild aversions. This contrasts with the CFP reported by Mehiel (1989). Which of the procedural differences between this experiment and Mehiel's is critical in generating the different results is not clear. One difference was the length of the CS-US interval. In Mehiel's experiment the CS-US interval was 50 min, whereas in the present experiment it was 25 min. From the point of view of classical conditioning, it is difficult to see why 50 min should be a more effective CS-US interval than 25 min. Another difference between Mehiel's and my experiment was the deprivation condition. The subjects in my experiment were food-deprived, whereas Mehiel's subjects were water-deprived during training and food-deprived during testing. Perhaps this unusual training and testing deprivation conditions contributed to his result. In any case, Mehiel's results should be taken cautiously: the effect was very weak, its replicability is questionable and the conditions under which it emerged are unusual.

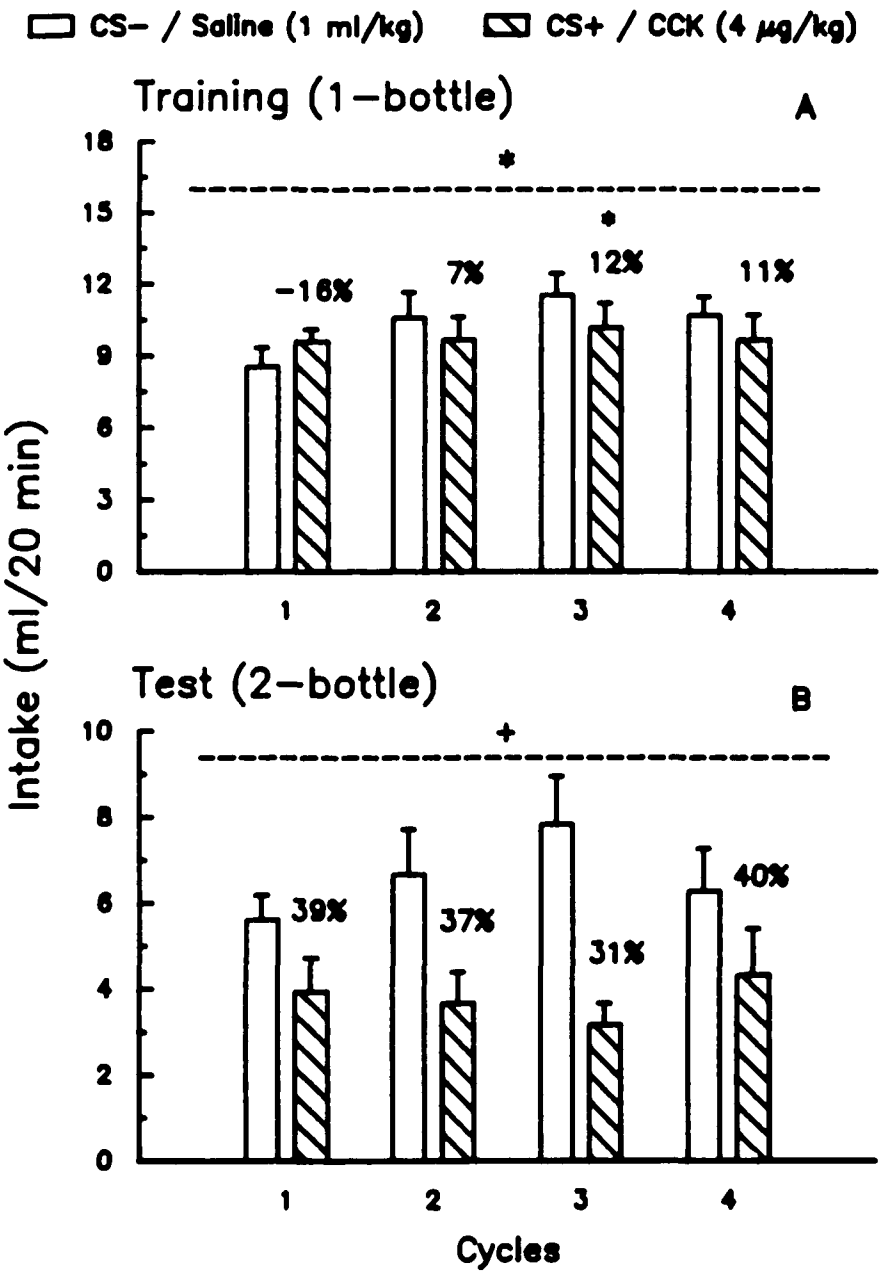

Figure 6. Experiment 2. Trace conditioning procedure (4 mcg/kg CCK): real-feeding flavored 0.2% saccharin. Mean intake (+SE) of the CS+ and CS- solutions during 20 min in one-bottle (A) and two-bottle (B) sessions over 4 cycles. Percent CS+ suppressions are shown in A, and percent CS+ preferences in B. Note that the injections were administered 5 min after the end of the one-bottle sessions. No injection was administered in two-bottle tests. Statistical results indicated as in Figure 4.

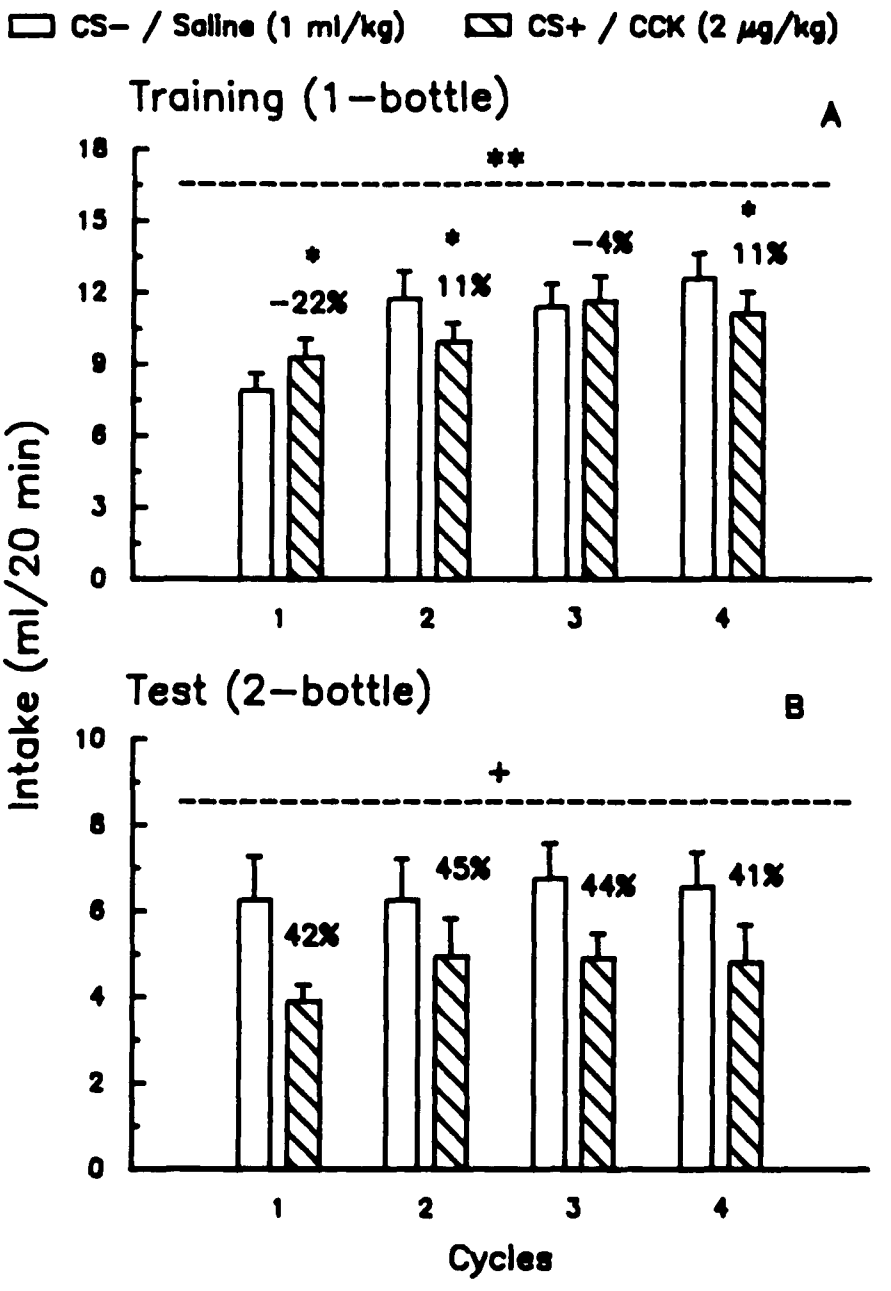

Figure 7. Experiment 2. Trace conditioning procedure (2 mcg/kg CCK): real-feeding flavored 0.2% saccharin. Mean intake (+SE) of the CS+ and CS- solutions during 20 min in one-bottle (A), and two-bottle (B) sessions over 4 cycles. Percent CS+ suppressions are shown in A and percent CS+ preferences in B. Note that the injections were administered 5 min after the end of the one-bottle sessions. No injection was administered in two-bottle tests. Statistical results indicated as in Figure 5.

EXPERIMENT 3

In this experiment, I also examined the real-feeding paradigm but, in this case, a delay conditioning procedure similar to that of Experiment 1B was used. In addition, a larger range of doses of CCK was investigated to determine whether positive results would be obtained with lower doses than those used in previous experiments. The results of experiment 1 and 2 indicated that 4 µg/kg, and perhaps 2 µg/kg, may have some aversive effects. In this experiment, therefore, I used CCK doses ranging from 4 to 0.125 µg/kg. The lower doses were not expected to inhibit ingestion but were included because, as discussed in the General Introduction, the relationship between satiety and positive reinforcement is uncertain. It is conceivable that if CCK has positive reinforcing effects, they may occur at doses that are lower than those required to suppress feeding.

METHOD

Procedure

The fifty-nine rats used in this experiment were maintained at 85% of their BW while feeding ad libitum. They were familiarized with the experimental conditions by offering them a 0.2% saccharin solution for 20 min for 5 days, and two-bottle tests with 0.2% and 0.1% saccharin for two days. They were then exposed to the injection procedure on the day prior to the commencement of formal training.

The flavored saccharin solutions were similar to those used in Experiment 2, and the delay conditioning procedure of Experiment 1B was employed (see Figure 3). Briefly, for each training trial, after 5 min (priming) access to a CS solution, the rats were injected, and then allowed to resume drinking for 15 more min. Only the 15-min postinjection data are shown in the one-bottle tests.

The rats were studied in six groups, each group with a different dose. Since it was not possible to run all six groups at the same time, they were distributed in three squads of two groups each. The rats were trained and tested in 5-day cycles (see Figure 2). The

4 µg/kg group (n=9) was run for three cycles. All the other groups (n=10) were run for four cycles, and received respectively 2, 1, 0.5, 0.25, and 0.125 µg/kg of CCK.

RESULTS AND DISCUSSION

The results for the 4 µg/kg group are shown in Figure 8. In the one-bottle sessions (Figure 8A) this dose suppressed saccharin intake in a potent manner in all three cycles ($F=85.40$, $df=1,8$, $p<.001$). The percent suppressions were 87%, 96%, and 87% for cycles 1 through 3, respectively. The intake of the CS- solution increased over time whereas CS+ intake remained low and stable throughout the experiment (Cycle x Solution $F=11.49$, $df=2,16$, $p<.001$). In the two-bottle tests Figure 8B), the rats displayed a strong aversion to the CS+ solution in all three cycles ($F=56.87$, $df=1,8$, $p<.001$). The percent CS+ intakes ranged from 10% to 20%.

This result confirms the impression obtained in sham-feeding rats given the 4 µg/kg dose (Experiment 1A). Whereas only half of the rats in Experiment 1A displayed strong inhibitions of intake in one-bottle sessions mirrored by strong aversions in two-bottle tests, all the rats in this experiment showed this pattern. This result suggests that the strong suppressions of intake induced by 4 µg/kg of CCK are probably due to aversions rather than satiety. The reason why this profile was more pronounced in this experiment than in Experiment 1A is unclear. Perhaps the delay conditioning procedure is more sensitive to detect these aversive effects, or the sham-feeding technique requires a higher dose for the appearance of aversions in all the rats. It is also possible that the greater palatability of the Polycose solution, as compared to the saccharin solution, counteracted the inhibitory effects of CCK.

Figure 9 illustrates the results of the 2 µg/kg dose. Overall this dose suppressed saccharin intake during training ($F=12.36$, $df=1,9$, $p<.001$). The suppressions were significant at cycles 1 (78%, $p<.001$)), and 2 (39%, $p<.001$) (Cycle x Solution $F=4.34$, $df=3,27$, $p<.05$). During cycles 3 and 4 CCK did not inhibit saccharin intake (-3% and 7%)

(negative values indicate that CS+ intake exceeded CS- intake). In the two-bottle tests, no reliable preference or aversion for the CS+ solution was apparent. Although the rats tended to avoid the CS+ solution during the first cycle (35% of total intake as CS+), this trend completely disappeared in successive cycles (59% and 57% of total intake as CS+ on cycles 3 and 4 respectively). Interestingly, in cycles 3 and 4 the absence of CCK-induced inhibition in one-bottle tests was mirrored by a trend to prefer the CS+ solution in two-bottle tests.

The results of the 1 μg/kg dose are shown in Figure 10. Overall, this dose produced mild suppressions of CS+ intake in the one-bottle tests ($F=15.36$, $df=1,9$, $p<.01$). The CS+ suppressions ranged from 7% to 29%. In the two-bottle tests, the rats tended to prefer the CS+ over the CS-, but there was no CS solution effect. There was, however, a Cycle x Solution interaction ($F=3.57$, $df=3,27$, $p<.05$), although the differences in CS+ and CS- intakes did not reach statistical significance at any individual cycle. Simple main effects tests indicated that CS+ intake increased over cycles ($p<.01$), whereas CS- intake remained stable. By cycles 3 and 4 the rats' CS+ preferences were 64%, and 59%, respectively.

The results of the 0.5 μg/kg dose are shown in Figure 11. This dose failed to suppress CS+ intake in the one-bottle tests. The percent CS+ suppressions ranged from -10% to 10%. In the two-bottle tests, there was a significant main effect of CS solution; overall the rats consumed more CS+ than CS- solution ($F=7.84$, $df=1,9$, $p<.05$). The CS+ preference was not very strong, however, and percent CS+ intakes ranged from 50% to 62%.

The results of the 0.25 μg/kg are shown in Figure 12. Overall, this dose failed to suppress saccharin intake in the one-bottle sessions. However, CS+ intake was mildly suppressed during cycle 3 ($p<.05$) (Cycle x Solution $F=4.06$, $df=3,27$, $p<.05$). The percent suppressions varied from -24% to 9%. This dose failed to produce a CS+ preference in the

two-bottle tests. The percent CS+ intakes varied from 39% to 53%.

The results of the 0.125 µg/kg doses are shown in Figure 13. This dose did not suppress CS+ intake in the one-bottle tests. Percent suppression scores ranged from -1% to 6%. Also, there were no differences between CS+ and CS- intakes in the two-bottle tests. The percent CS+ intakes ranged from 43% to 50%.

Figure 14 summarizes the percent CS+ suppressions and preferences obtained with all six doses of CCK; the data are presented as mean values over all cycles and the range of low to high percent intakes. With respect to the one-bottle results, the figure illustrates that the suppression in CS+ intake increased with dose from 0.125 to 2.0 µg/kg and then sharply increased at the 4.0 µg/kg dose. With respect to the two-bottle results, CCK treatment produced mild CS+ preferences at the 0.5 and 1.0, no preferences at the 0.125, 0.25, and 2.0 µg/kg doses, and a strong CS+ avoidance at the 4.0 µg/kg dose. Comparison of the two dose-response curves indicates that CCK reinforced flavor preferences only at doses (0.5 and 1.0 µg/kg) that produced minimal to mild suppressive effects on consumption. The sharp inflection in the dose-response curves at 4.0 µg/kg suggests that at this dose CCK produced nimiety or malaise rather than satiety.

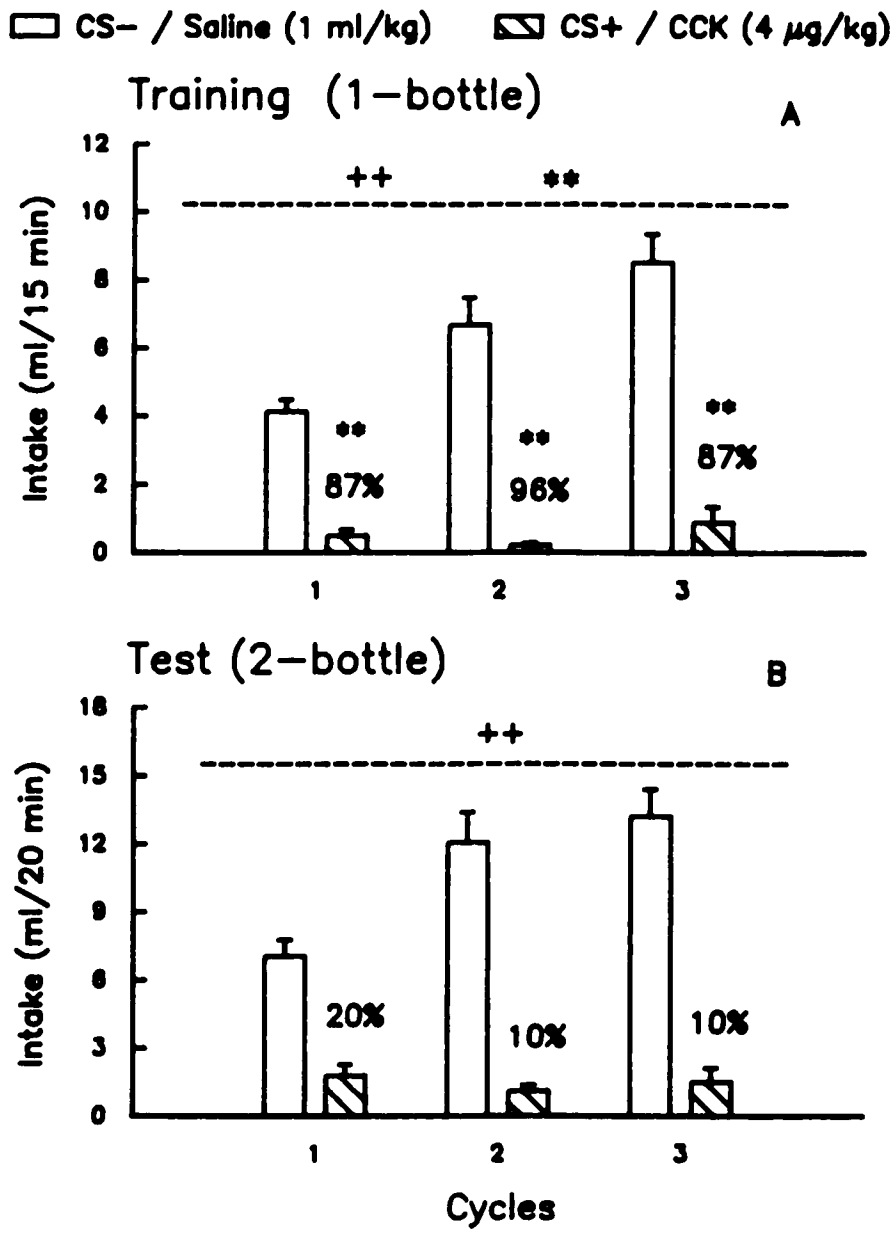

Figure 8. Experiment 3. Delay conditioning (4 mcg/kg CCK): real-feeding flavored 0.2% saccharin. A. Mean intake (+SE) of the CS+ and CS- solutions and percent CS+ suppressions during the 15 min post-injection period in one-bottle sessions over 3 cycles. B. Mean intake (+SE) of the CS+ and CS- solutions and percent CS+ preferences during 20 min in two-bottle tests. The one-bottle data are averages of two sessions and the two-bottle data are the averages of one session. Statistical results indicated as in Figure 4.

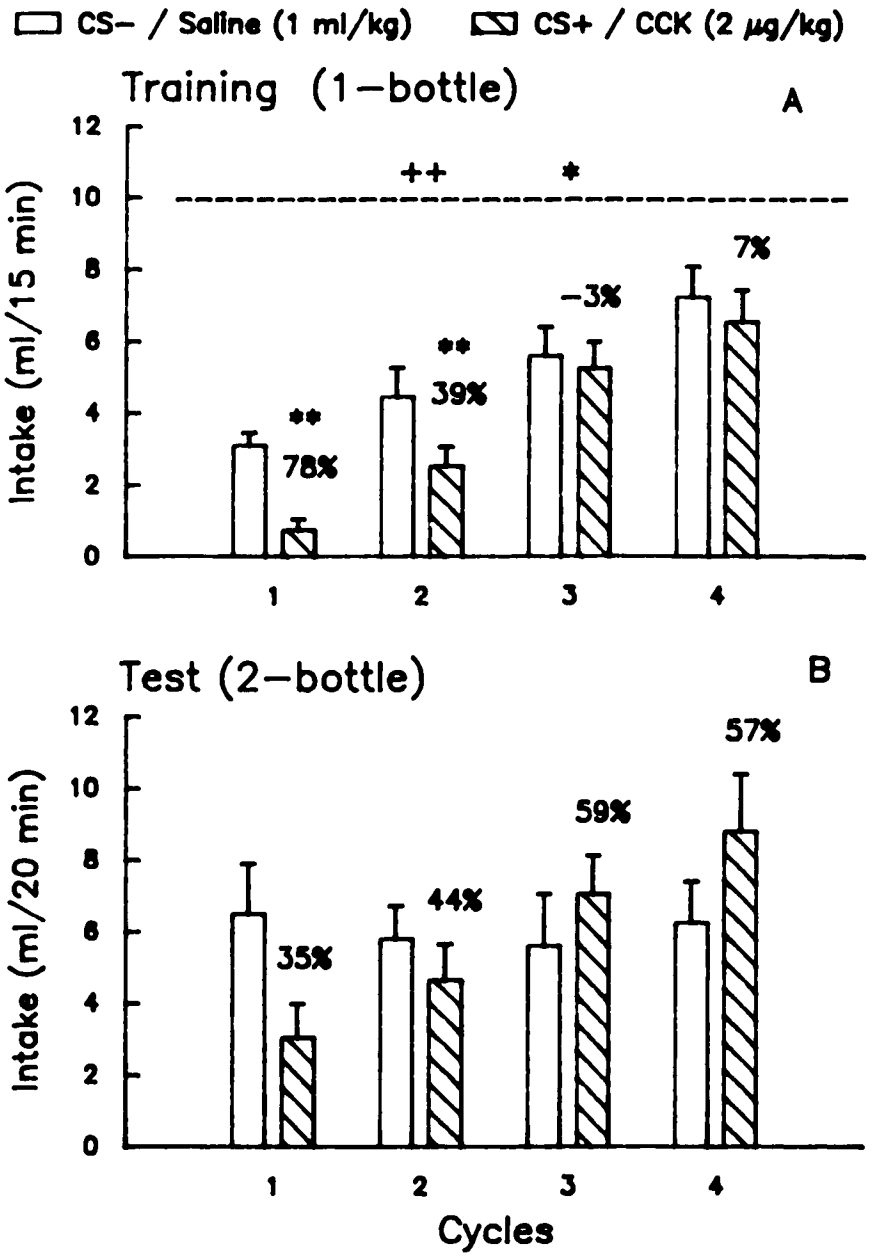

Figure 9. Experiment 3. Delay conditioning (2 mcg/kg CCK): real-feeding flavored 0.2% saccharin. A. Mean intake (+SE) of the CS+ and CS- solutions and percent CS+ suppressions during the 15 min post-injection period in one-bottle sessions over 4 cycles. B. Mean intake (+SE) of the CS+ and CS- solutions and percent CS+ preferences during 20 min in two-bottle tests. The one-bottle data are averages of two sessions and the two-bottle data are averages of one session. Statistical results indicated as in Figure 5.

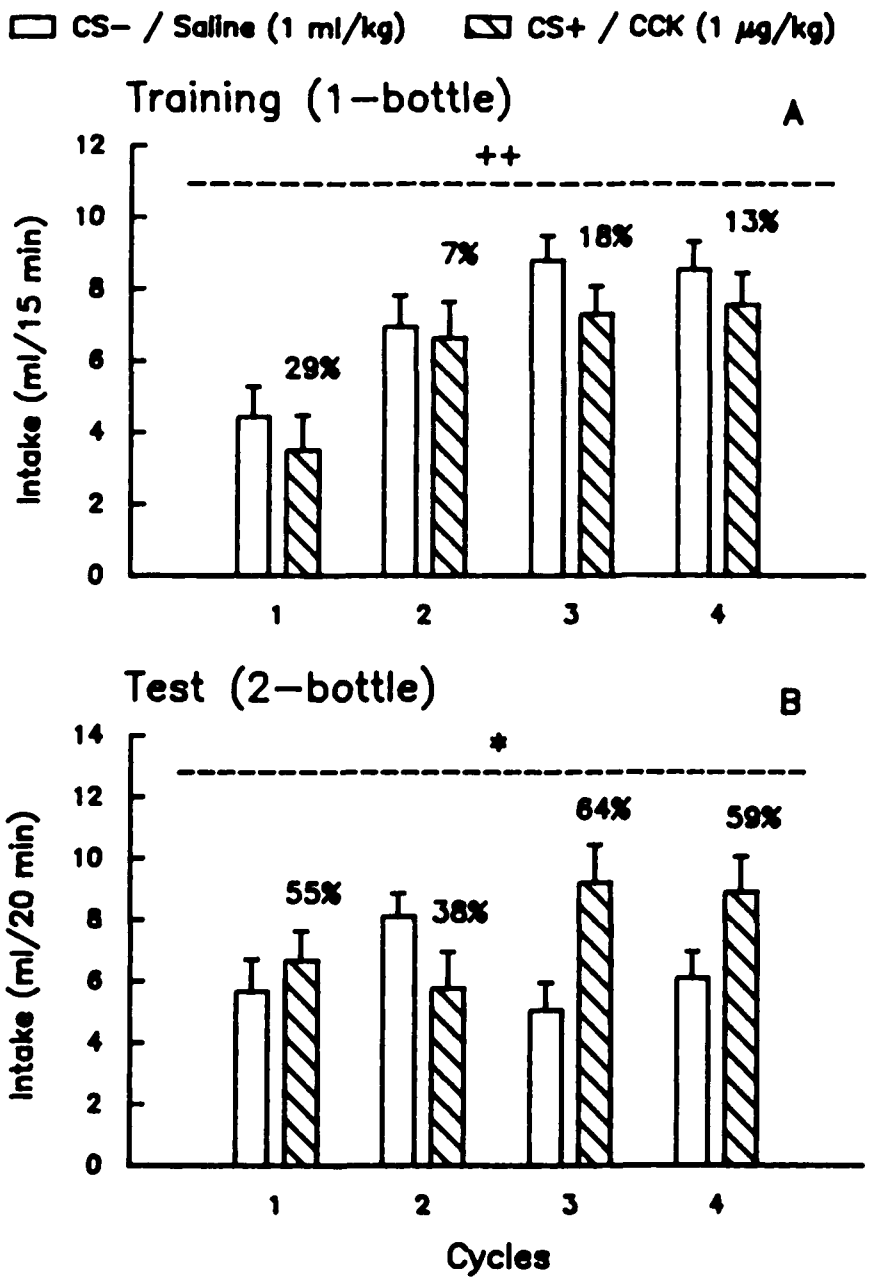

Figure 10. Experiment 3. Delay conditioning (1 mcg/kg CCK): real-feeding flavored 0.2% saccharin. A. Mean intake (+SE) of the CS+ and CS- solutions and percent CS+ suppressions during the 15 min post-injection period in one-bottle sessions over 4 cycles. B. Mean intake (+SE) of the CS+ and CS- solutions and percent CS+ preferences during 20 min in two-bottle tests. The one-bottle data are averages of two sessions and the two-bottle data are the averages of one session. Statistical results indicated as in Figure 4.

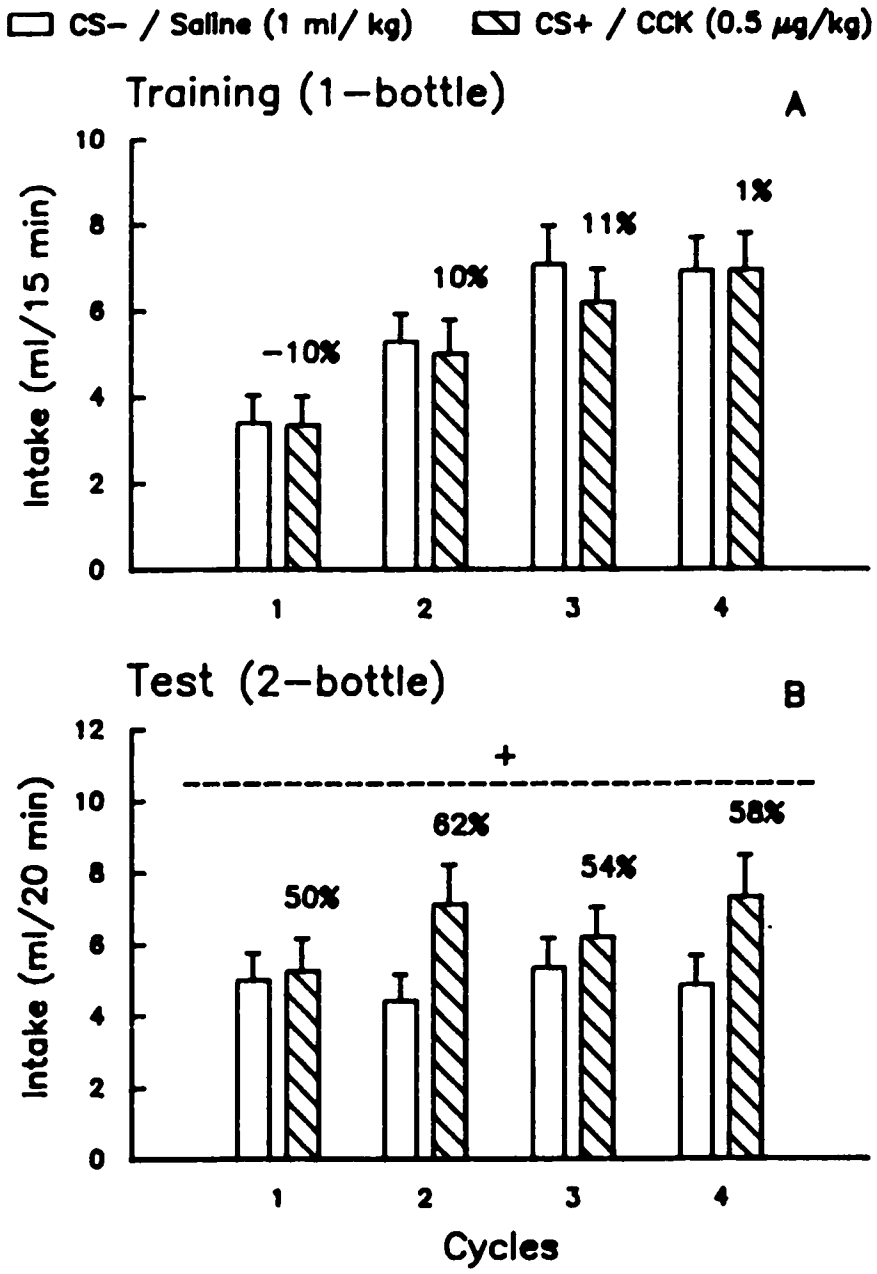

Figure 11. Experiment 3. Delay conditioning (0.5 mcg/kg CCK): real-feeding flavored 0.2% saccharin. A. Mean intake (+SE) of the CS+ and CS- solutions and percent CS+ suppressions during the 15 min post-injection period in one-bottle sessions over 4 cycles. B. Mean intake (+SE) of the CS+ and CS- solutions and percent CS+ preferences during 20 min in two-bottle tests. The one-bottle data are averages of two sessions and the two-bottle data are the averages of one session. Statistical results indicated as in Figure 5.

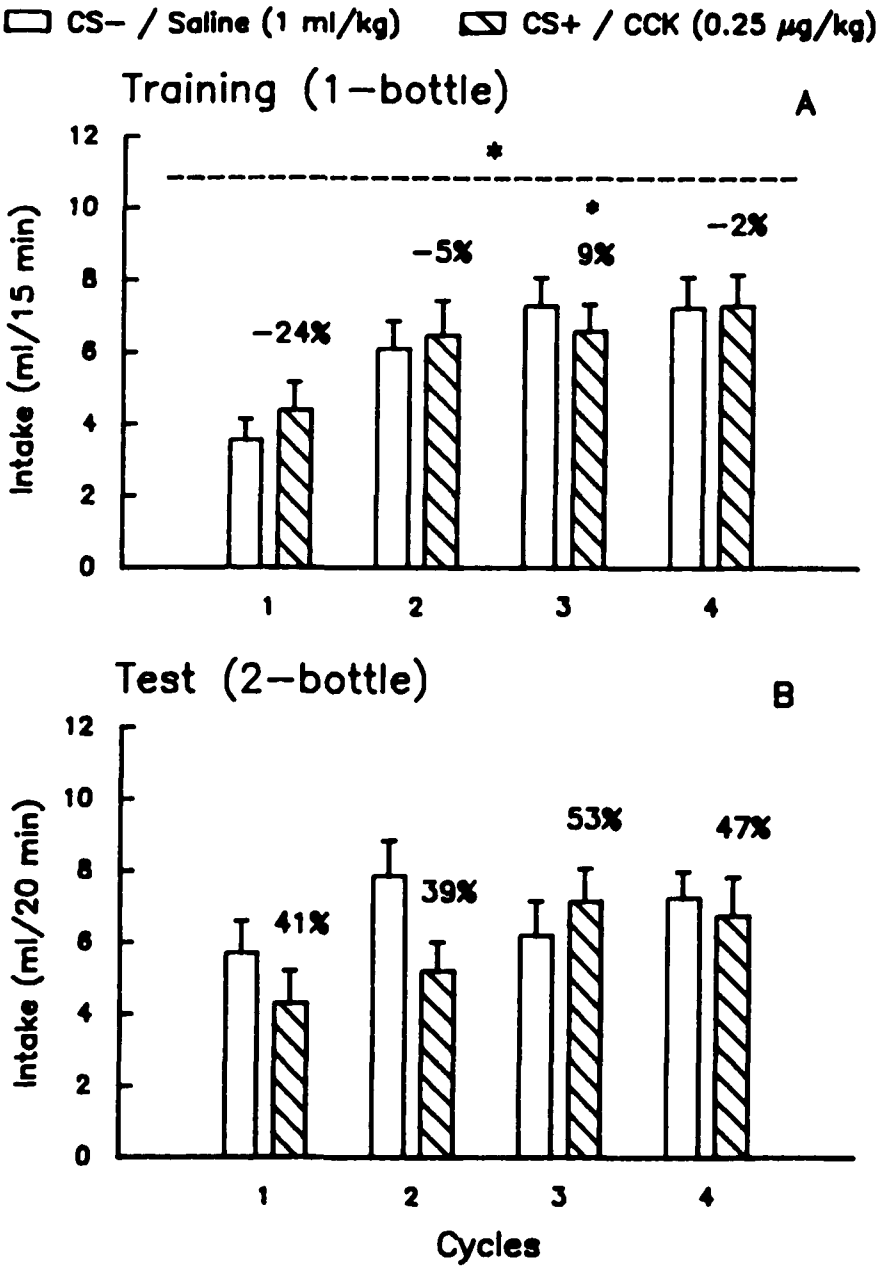

Figure 12. Experiment 3. Delay conditioning (0.25 mcg/kg CCK): real-feeding flavored 0.2% saccharin. A. Mean intake (+SE) of the CS+ and CS- solutions and percent CS+ suppressions during the 15 min post-injection period in one-bottle sessions over 4 cycles. B. Mean intake (+SE) of the CS+ and CS- solutions and percent CS+ preferences during 20 min in two-bottle tests. The one-bottle data are the averages of two sessions and the two-bottle data are the averages of one session. Statistical results indicated as in Figure 4.

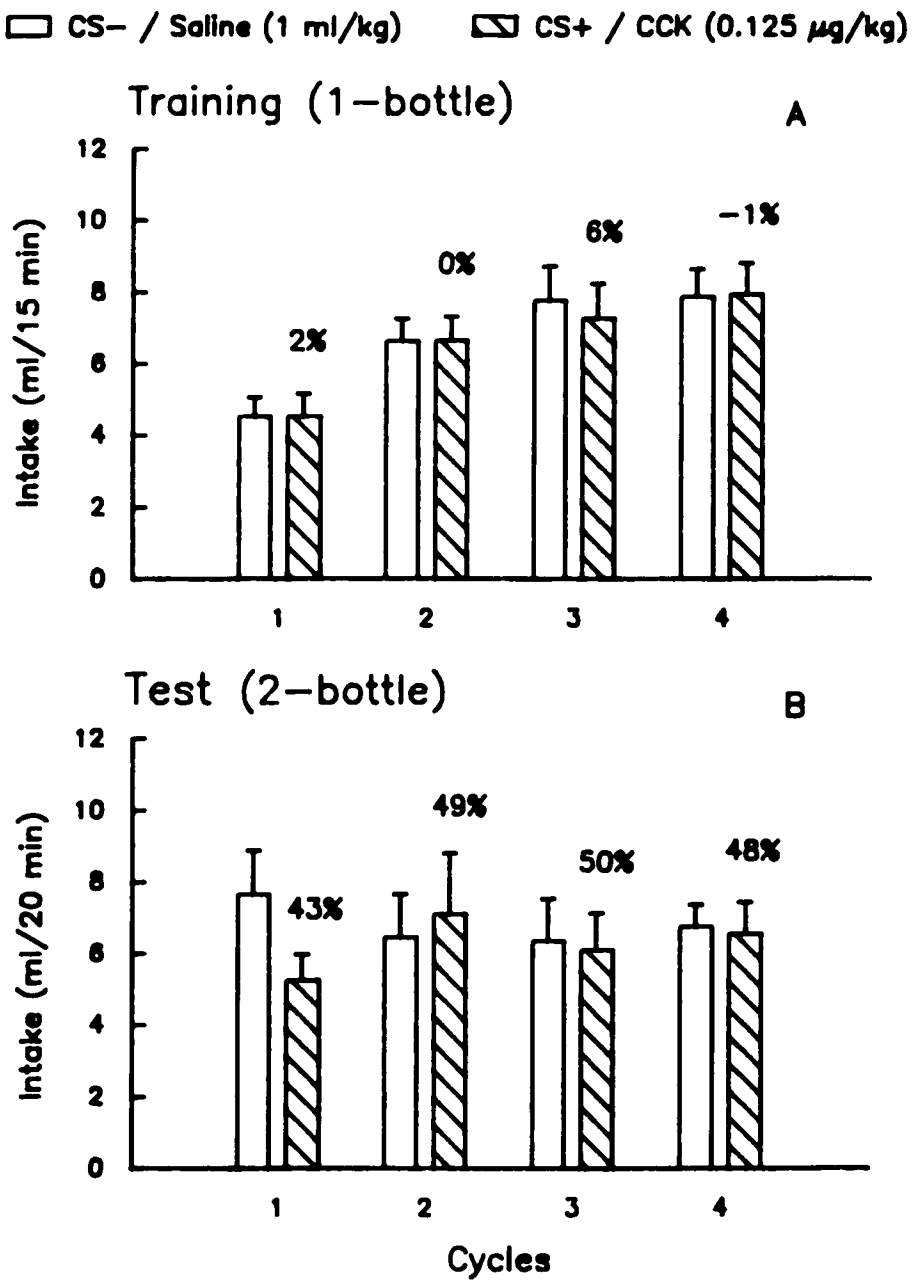

Figure 13. Experiment 3. Delay conditioning (0.125 mcg/kg CCK): real-feeding flavored 0.2% saccharin. A. Mean intake (+SE) of the CS+ and CS- solutions and percent CS+ suppressions during the 15 min post-injection period in one-bottle sessions over 4 cycles. B. Mean intake (+SE) of the CS+ and CS- solutions and percent CS+ preferences during 20 min in two-bottle tests. The one-bottle data are the averages of two sessions and the two-bottle data are the averages of one session. Statistical results indicated as in Figure 5.

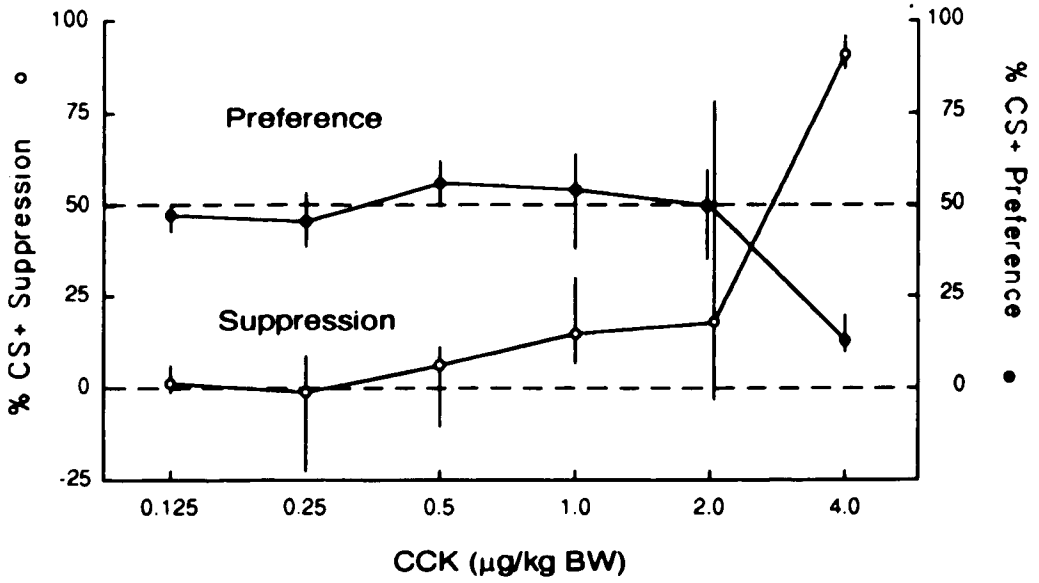

Figure 14. Summary of the six doses of CCK in Experiment 3. The percent CS+ suppression and percent CS+ preference scores are based on mean intakes over all cycles for each dose. The range of the mean group scores at each cycle is also shown. Note that a 0% suppression value indicates that CS+ and CS- intakes were equal in the 1-bottle tests. A 50% preference value indicates that CS+ and CS- intakes were equal in the 2-bottle tests.

PART III: REAL-FEEDING, REAL FOOD

EXPERIMENT 4

Experiment 3 revealed that mild flavor preferences can be conditioned by relatively low doses of CCK in rats real-feeding a saccharin solution. In this experiment I attempted to enhance the flavor preference effect by modifying the training paradigm. That is, the rats were trained to real feed a "real" food (Polycose) rather than a "sham" food (saccharin). Recent studies indicate that low doses of CCK act in synergism with the postingestive actions of carbohydrate solutions to inhibit food intake. In particular, CCK-induced feeding suppression is increased when the administration of the hormone is preceded by an oral, intragastric, or intraduodenal preload of a sucrose solution (Cox, 1986; 1989; Cox and Smith, 1987). It is possible that the reinforcing effect of CCK would also be enhanced if the administration of the hormone was associated with the ingestion of a carbohydrate solution rather than a saccharin solution.

The present experiment involved another modification of the training paradigm; during training the rats were given limited rather than unrestricted access to the CS solutions. The rationale for this change was to limit the satiating and reinforcing effects of the CS solutions. Note that in the prior experiments the CS solutions produced minimal postingestive effects either because they were sham-fed (Experiment 1) or because they were noncaloric (Experiments 2-3). In this experiment, however, the CS solutions would, by design, provide postingestive feedback. If the rats were allowed to feed the Polycose-containing CS solutions ad libitum then the reinforcing effects of Polycose (Elizalde and Sclafani, 1989, 1990; Sclafani and Nissenbaum, 1988) might mask the reinforcing effect of the CCK injections. Therefore, during the training sessions the rats were restricted to 50% of their ad libitum intake of the CS solutions. Also, to keep CS intakes limited, the rats were given a fixed preload (2 ml) of the solutions prior to the saline or CCK injections rather than a 5-min prefeeding period. These restrictions presumably left the rats

"unsatisfied" and more responsive to the reinforcing effects of the CCK injection.

METHOD

Test solutions

The CS+ and CS- solutions were grape and cherry flavored solutions of 8% Polycose.

Procedure

Eleven rats were used in this experiment. Since in this paradigm the test solution contributed to the rats' daily caloric intake, the animals were maintained at 90%, rather than 85%, of their ad libitum BW. With this deprivation level the daily chow ration (10-14 g) was similar to that used in Experiments 2 and 3.

The rats were familiarized with the one-bottle condition by offering them unflavored 8% Polycose solution 20 min/day for 5 days. They were then familiarized with the two-bottle procedure by giving them access to 8% and 1% Polycose solutions for two sessions. The animals were next given two additional one-bottle sessions with 8% Polycose. Each rat's mean intake in these last two sessions was taken as its baseline level. Finally, the rats were exposed to the injection procedure prior to formal training.

The rats were trained and tested over four 5-day cycles as described in previous experiments (see Figure 2). The conditioning procedure used in this experiment is illustrated in Figure 3. The rats were primed with 2 ml of the CS solutions, injected with saline or CCK, and then given access to restricted amounts of the CS solutions (50% of baseline). Although the animals consumed the solution within 10 min after the injection they were allowed to remain undisturbed in the experimental chamber for 20 min. In the two-bottle tests, the animals were injected with saline immediately before the 20 min session and were then given unlimited access to the CS+ and CS- solutions.

Since in the one-bottle tests access to the CS solutions was restricted, these tests did not provide information about the inhibitory actions of CCK. Therefore, following the

completion of the four training-testing cycles, additional tests were conducted. The animals were first given food ad libitum for 2 days to allow them to recover from the 1-month food deprivation. Food restriction was then applied to reach 90% of the new ad libitum BW. Following this, the rats were given four one-bottle tests similar to the training sessions except that, after the 2-ml preload and saline or CCK injections, the rats were allowed unlimited access to the CS solutions for 20 min.

RESULTS AND DISCUSSION

The results of Experiment 4 are shown in Figure 15. The data from the one-bottle training sessions were not analyzed because intakes of the CS solutions were restricted. In the two-bottle tests there was a main effect of CS solution with the rats consuming more CS+ than CS- ($F=5.82$, $df=1,10$, $p<.05$). Except for cycle 2, the rats preferred the CS+ solution with percent intakes ranging from 64% to 70%. The magnitude of the CS+ preference was larger than that obtained with the 0.5 µg/kg dose in Experiment 3, both in terms of the mean preference over the four cycles (61% vs. 56%) and the maximum preference obtained (70% vs. 62%). Statistical analysis, however, did not reveal significant differences in percent CS+ preferences between these two experiments.

In the feeding suppression test conducted at the end of the experiment CCK reduced flavored Polycose intake compared to the saline treatment (18.0 vs. 21.3 ml/20 min; $t=6.82$, $df=10$, $p<.001$). The percent CS+ suppression was 16%. This contrasts with the nonsignificant feeding suppression (10%) obtained with the 0.5 µg/kg dose in Experiment 3.

In summary, the 0.5 µg/kg dose of CCK conditioned a flavor preference in rats real-feeding an 8% Polycose solution. The magnitude of the preference was slightly but not reliably greater than that obtained in Experiment 3 with rats real-feeding a saccharin solution. These findings provide marginal support for the proposal that the positive reinforcing effects of CCK are enhanced by the postingestive actions of carbohydrates.

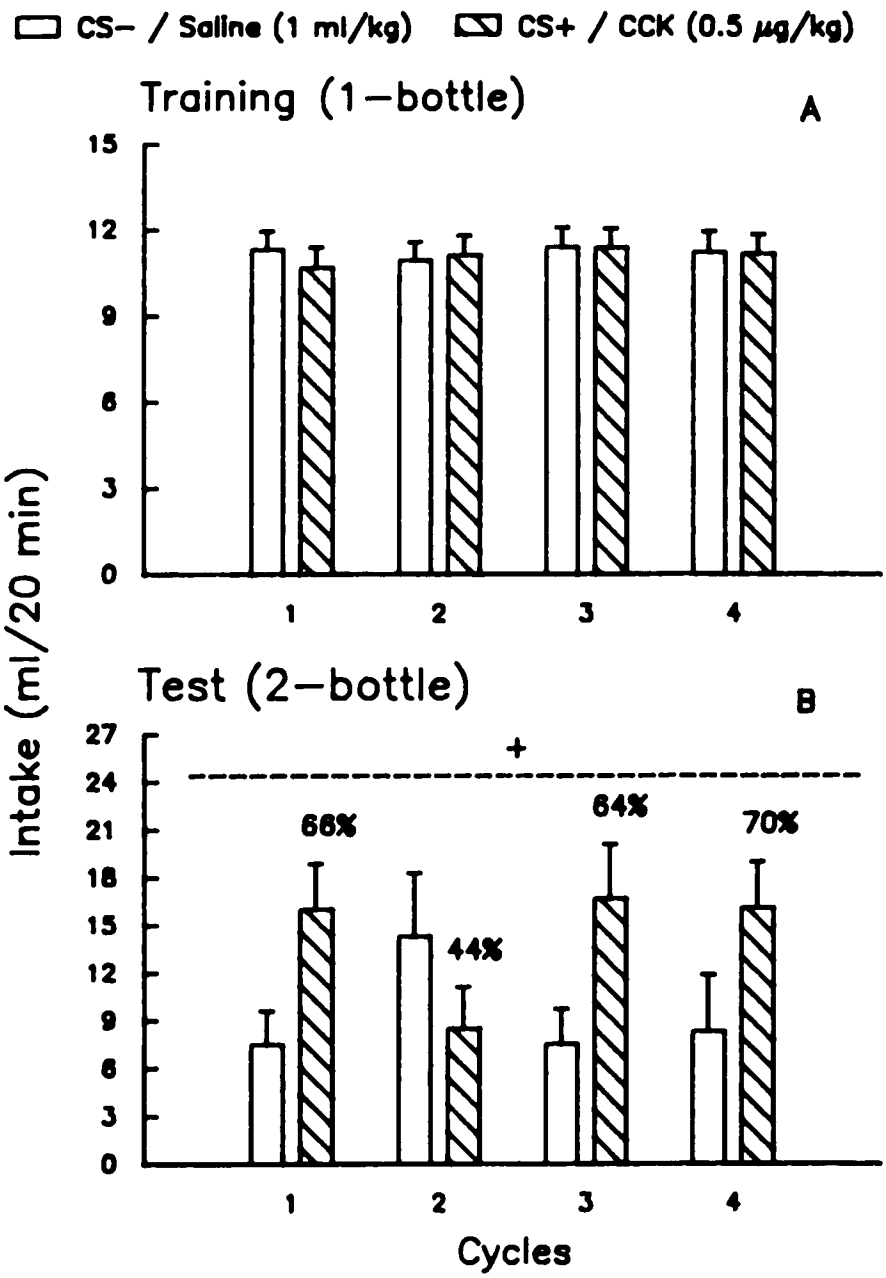

Figure 15. Experiment 4. Delay conditioning (0.5 mcg/kg CCK): real-feeding flavored 8% Polycose. Mean intake (+SE) of the CS+ and CS- solutions during 20 min post-injection in one-bottle (A), and two-bottle (B) tests over 4 cycles. The intakes of the CS solutions shown in A correspond to the limited amount given in the one-bottle sessions, after 2 ml priming. Percent CS+ preferences are shown in B. The one-bottle data are the averages of two sessions and the two-bottle data are the averages of one session. Statistical results indicated as in Figure 4.

GENERAL DISCUSSION

The experiments described above investigated whether CCK could play a role in the CFPs produced by nutrients. Different paradigms and test conditions that were effective when food was the US, as well as different doses of CCK, were explored. Several findings emerged from these experiments. In general, CCK was able to condition both preferences and aversions depending on the dose used. While the highest dose produced a dramatic inhibition of feeding and conditioned a strong aversion, lower doses that produced small or no inhibitory effects on feeding, conditioned a flavor preference. The conditioned preferences were not very large, but they were obtained in rats real-feeding either saccharin or Polycose solutions.

In these experiments I have confirmed previous results showing dose-dependent suppressions of intake with CCK treatment. Note, however, that in some experiments CCK appeared to lose its inhibitory effects over cycles. It is possible that with repeated training the rats habituated to the effects of the drug by developing either pharmacological or behavioral tolerance. In fact, Mineka and Snowdon (1978) have observed tolerance to the feeding inhibitory effects of CCK in hungry rats injected with CCK (1.75 and 2.5 μg/kg) on a daily basis. However, the tolerance disappeared when the rats were injected twice a week. Since the rats in my experiments were exposed to exogenous CCK in the absence of other postingestive actions of food they may have learned from this experience to disregard the exogenous CCK signal, and regulate their meals according to other satiation mechanisms. It is also possible that, since the rats were maintained at less than 100% of their normal BWs, the consequences of deprivation built up over cycles, and the rats may have adjusted their short-term regulatory mechanisms to cope with long-term challenges.

The 4 and 2 μg/kg doses suppressed intake whether the rats were real-feeding saccharin or sham-feeding Polycose (Experiments 1 and 3). The higher of these two doses conditioned potent aversions, suggesting that 4 μg/kg may be acting as a nimiety signal.

The lower dose did not produce either positive or negative conditioning. The reason for this latter result is unclear. Perhaps 2 µg/kg produced some aversive effects that interfered with any positive effect of CCK. Alternatively, it is conceivable that this dose induced a satiety state unaccompanied by positive reinforcing or aversive effects.

The 4 and 2 µg/kg doses were also examined in a trace conditioning procedure (Experiment 2). Both doses produced mild aversions. This result contrasts with the positive results reported by Mehiel using 4 µg/kg (1989). As discussed in Experiment 2, it is possible that the unusual deprivation condition used by Mehiel contributed to his positive result. Nevertheless, the preference he reported was a very weak effect, and its replicability is questionable. In my experiments, the trace conditioning procedure (Experiment 2) was poorer than the delay conditioning procedure (Experiment 3) in producing learning and detecting dose effects.

Taken together the results of the first three experiments, using different paradigms and test conditions, indicate that 4 µg/kg, and perhaps 2 µg/kg, of CCK may have aversive effects. The finding that flavor aversions were obtained under some test conditions and at some CCK doses but not others is consistent with the variable response reported in the literature (Gibbs, Young, and Smith, 1973a; Holt, Antin, Gibbs, Young, and Smith, 1974; Deutsch and Hardy, 1977; Ervin and Teeter, 1986).

The results of Experiments 3 and 4 indicate that at relatively low doses CCK can condition flavor preferences. Although significant, the flavor preferences were not very strong and emerged at doses (0.5 and 1 µg/kg) that produced only minimal suppressions of intake. Still lower doses of CCK (0.125 and 0.25 µg/kg) failed to suppress intake or condition preferences. These results indicate that the positive reinforcing effects of CCK are restricted to a narrow range of doses, and may not be dependent upon the hormone's satiety effects. It is conceivable that low doses (e.g., 0.5 to 1 µg/kg) of CCK mimic the physiological levels at the beginning of a meal. The initial release of CCK may signal the

arrival of food into the GI tract and this may have a positive reinforcing effect. Higher doses of CCK may mimic the levels of CCK towards the end of a meal. Perhaps, as endogenous CCK builds up it acts as a signal to terminate the meal, but looses its positive reinforcing effects. Still higher doses of CCK may produce nimiety or malaise and thereby have aversive effects.

The idea that CCK loses its positive reinforcing effect as its satiety effect increases is compatible with one proposed mechanism of action of CCK that involves "alliesthesia" (Cabanac, 1971). After a meal, human subjects report that the hedonic quality of taste stimuli is reduced as compared to the beginning of the meal. This decreased palatability of food is thought to facilitate the termination of a meal (Cabanac, 1971). It has been proposed that CCK is the physiological substrate of alliesthesia (Bartness and Waldbillig, 1984; Waldbillig and Bartness, 1982; Waldbillig and O'Callaghan, 1980), that is, CCK may act by reducing the palatability of the food. If this hypothesis is correct, the higher doses of CCK investigated in the present experiments may have reduced the palatability of the CS+ flavor thus interfering with the acquisition of a CFP.

The finding that exogenous CCK can condition flavor preferences at relatively low doses supports the view that the release of endogenous CCK may mediate, at least in part, the postingestive reinforcing effect of nutrients. Note that the maximum preferences obtained in the present study (62-70%) were slightly lower than those conditioned by IG Polycose infusions (71-77%) in other experiments using similar training procedures, but comparable to those obtained with IG fat infusions (61%, see Sclafani, 1990). Conceivably, CCK may be the primary mediator of fat-induced CFP's whereas other visceral signals may contribute to the reinforcing effects of carbohydrates. One way of investigating the role of endogenous CCK in the reinforcing effect of different nutrients is to use specific CCK blockers. In particular, it would be of interest to investigate whether CCK antagonists can block the acquisition of nutrient-induced CFPs.

Although the flavor preferences obtained in the present study were not very strong, it is possible that more robust preferences can be obtained with different training paradigms. In the case of nutrient-reinforced CFPs, the magnitude of the flavor preferences obtained varied as a function of the training conditions (see Sclafani, 1990). For instance, the maximum preference obtained with IG Polycose infusions using food deprived rats and short-term conditioning sessions was about 70%, but with nondeprived rats trained in 24 hr/day sessions preferences greater than CS+ 90% were obtained. In view of these latter results, the flavor conditioning effects of CCK should be examined using a long-term conditioning procedure.

In summary, relatively low doses of CCK were effective in conditioning flavor preferences in food-deprived rats. These doses had mild or no feeding inhibitory effect. Higher doses that produced moderate to large suppressions of intake failed to condition a flavor preference or, at the highest dose, conditioned an aversion. The fact that preferences were obtained with real- but not sham-feeding is probably due to the fact that only the two higher doses were investigated in the sham-feeding paradigm. The reinforcing effects of low doses of CCK in sham-feeding rats remain to be investigated. Further work with hormonal and neural signals generated by ingested food is needed to clarify the physiological mechanisms involved in the satiety and positive reinforcing effects of nutrients.

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