

AN INVESTIGATION OF THE PARAMETERS
OF CCK-INDUCED SATIETY

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PARAMETERS OF
CCK-INDUCED SATIETY

BY
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ABSTRACT

Two major aspects of CCK-induced satiety were examined in the food deprived sham-feeding rat: the kinetics of the inhibition of feeding produced by CCK and the interaction of CCK-induced satiety with oropharyngeal stimulation. Four results were presented regarding the kinetics of the CCK satiety effect. First, CCK administered coincident with the initiation of sham feeding inhibits feeding in a dose-related manner. Second, the increased efficacy of the higher dose of CCK delivered at the same time as sham feeding results from an increase in the size of maximum suppression and an increase in the time of suppression effected via a decreased latency to suppression. Third, CCK delivered 15 minutes before sham feeding begins has no effect on feeding. Fourth, the administration of a long-lasting derivative of CCK 15 minutes prior to the initiation of sham feeding can suppress intake. Two results were presented regarding the role of oropharyngeal stimulation in CCK-induced satiety. First, it was shown that oropharyngeal stimulation enhances the satiety action of CCK. Second, the amount and pattern of feeding inhibition produced by CCK are equivalent regardless of whether the peptide is administered coincident with, or some time after, the initiation of sham feeding. The implications of these results for the therapeutic use of this peptide and its role in producing satiety are discussed.

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GENERAL INTRODUCTION

The function of gut peptides has been viewed traditionally as the co-ordination of cellular processes involved with the digestion and absorption of ingested nutrients. Cholecystokinin (CCK) is a polypeptide produced, stored, and secreted by cells in mucosa of the duodenum and proximal jejunum (Buffa, Solica & Go, 1976; Polak et al., 1975). The CCK-secreting cell is elongate with an apical process extending into the lumen. These cells respond to changes in the chemical environment of the lumen and secrete CCK, the net effect of which is in the direction of restoring the chemical environment of the lumen to its original state (Makhlouf, 1974). CCK is released into the blood from such intraluminal stimuli as an ordinary meal and fat and amino acids are particularly potent stimuli for CCK release (Himeno et al., 1983).

Recently, it has been suggested that CCK is involved not only in the mechanisms of digestion but also in the control of feeding behavior. Most of the evidence that CCK is a satiety factor is based on observations that the administration of exogenous CCK reduces food intake. Gibbs, Young & Smith (1973a) were the first to demonstrate that partially purified CCK (20% pure, w/w) inhibited feeding when injected intraperitoneally into intact food-deprived rats. The inhibition of feeding was considerable and the magnitude of inhibition was a linear function of dose. But 10% pure CCK contains many impurities since it is

prepared from crude extracts of intestine. It may be these impurities, and not the CCK, which inhibit food intake. Experiments using pure CCK, the synthetic C-terminal octapeptide of CCK (CCK-8), suggests that this is not the case. CCK is a polypeptide and exists in five molecular forms, which contain 39, 33, 12, 8, or 4 amino acid residues on the C-terminal (Rehfeld, 1981). The octapeptide, CCK-8, is the smallest fragment which maintains the full spectrum of biological activities that the longer fragments possess (Rehfeld, 1981). The administration of CCK-8 and 20% pure CCK in equivalent doses (using the increase in dog gallbladder pressure as an index for comparing the biological activity of the two peptide preparations) produced identical inhibitions of food intake. This suggested that CCK was the substance in the 10% pure extract which inhibited feeding.

This initial demonstration that CCK inhibited feeding was produced by administering CCK to intact rats. However, satiety in the intact rat is a function of both the CCK administered and other satiety mechanisms. The most sensitive bio-assay of CCK's satiety-inducing effects is one where these other satiety mechanisms are absent or minimally active so that any inhibition of feeding observed would be a function of the peptide administered. Such a preparation is the food-deprived sham feeding rat. This rat is equipped with a chronic gastric cannula and sham feeding occurs when the rat ingests liquid diet because the diet drains immediately through the open fistula (Young, Gibbs, Antin, Holt & Smith, 1974). Under conditions of food deprivation, the sham feeding rat feeds continuously and never sates (Young et al., 1974). CCK administered to the sham feeding rat inhibits feeding in a

dose dependent fashion (Gibbs et al., 1973b). This demonstration is important for two reasons. First, it shows that the peptide is sufficient to inhibit food intake and produce satiety in a preparation which, in the absence of peptide administration, would feed continuously. Second, it demonstrates that CCK's inhibitory effects on feeding are not mediated through other mechanisms thought to be important in the control of feeding such as the accumulation of food in the gut or gastric distention. For example, it has been suggested that CCK suppresses food intake only indirectly by inhibiting gastric emptying and causing increased gastric distention (Moran & McHugh, 1982). However, the demonstration that CCK inhibits feeding in the sham feeding rat mitigates against this argument since the peptide is effective in this model where there is no gastric distention.

Spontaneous satiety in the rat is characterized by a predictable termination of feeding accompanied by a sequence of behaviors involving grooming, locomotion, rearing, and resting (Antin, Gibbs, Holt, Young and Smith, 1975). The administration of CCK is suggested to produce natural satiety since meals terminated by CCK are also accompanied by this satiety sequence in the intact (Antin et al., 1975) and sham feeding rat (Gibbs et al., 1973b). The demonstration that CCK elicits the behavioral sequence of satiety in the sham feeding rat suggests that CCK acting alone, or with some unidentified mechanism activated in the sham feeding rat, is sufficient to produce the behaviors characteristic of satiety.

Importantly, CCK appears to specifically inhibit feeding and does not reduce intake through a generalized inhibition of all

behaviors. CCK inhibits consumption of liquid food as effectively as it does solid food consumption (Gibbs et al., 1973a). This demonstrates that the peptide-induced reduction of feeding was not an impairment of the motor functions required to eat solid food. Furthermore, the inhibition of ingestion produced by CCK is specific for food. Administration of CCK, in doses which inhibit the consumption of liquid diet in food-deprived rats, does not inhibit the consumption of water in water-deprived animals (Gibbs et al., 1973a). Thus, in spite of the behavioral similarities between thirsty rats drinking water and hungry rats drinking liquid diet, CCK inhibits only the ingestion of food.

Other observations suggest that CCK's effects are specific to the inhibition of feeding. Body temperature is unaffected by the administration of CCK and rats maintain control intakes of liquid and solid food during 8 months of regular CCK injections (Gibbs et al., 1973a). Further, as one would expect from a signal mediating satiety, CCK exerts its effects in the later portions of a meal. The administration of CCK in rats (Gibbs et al., 1973a) and man (Kisseleff, Pi-Sunyer, Thornton & Smith, 1981) does not affect the initial rate of eating but results, simply, in the termination of the meal sooner than under control conditions.

CCK-induced suppression of feeding has been extended to a number of species including rabbits (Haupt, Anika & Wolff, 1978), pigs (Anika, Haupt & Haupt, 1981), sheep (Grofum, 1981), rhesus monkeys (Falasco, Smith & Gibbs, 1979; Gibbs, Falasco & McHugh, 1976), mice (McLaughlin & Baile, 1980; Parrot & Batt, 1980), and man (Kisseleff et al., 1981; Sturdevant & Goetz, 1976).

The suppression of food intake is not a sufficient condition to demonstrate that CCK elicits satiety since the peptide may inhibit feeding by making the subjects sick. The most compelling evidence that CCK suppresses feeding without inducing malaise comes from demonstrations in man. CCK, when administered to human subjects, reduces meal size by terminating the meal sooner without affecting the initial rate of eating (Kisseleff et al., 1981). Importantly, the administration of an extensive questionnaire showed that CCK-induced reduction of eating was not accompanied by reports of toxicosis or significant side effects. Taste aversion paradigms have also been used to assess if CCK produces its effects by inducing toxicosis. The results of these experiments are equivocal (Duetsch & Hardy, 1977; Holt, Antin, Gibbs, Young & Smith, 1974). However, the utility of this approach is questioned, however, since illness has been shown to be neither a necessary nor a sufficient condition for the formation of conditioned taste aversions. For example, conditioned taste aversions have been reported using agents as the unconditioned stimulus which are thought not to be toxic: Revusky et al., (1971) report a learned aversion using saline. Similarly agents known to be toxic such as strychnine and cyanide do not act as effective stimuli (Nachman & Hartley, 1975).

The administration of CCK clearly has behavioral effects and it might be expected, therefore, that its site of action was central. However, the available evidence suggests that its site of action is peripheral and is mediated by the gastric branches of the vagus. Vagotomy (Lorenz & Goldman, 1978) and selective gastric vagotomy (Smith,

Jerome, Cushin, Eterno & Simansky, 1981) block the satiety effect of peripherally administered CCK in the rat. Two further observations suggest that the gastric afferent vagus is the critical lesion which abolishes the CCK effect: i) administration of atropine methyl nitrate, which mimics the loss of vagal efferents, does not change the satiety effect of CCK (Smith et al., 1981), and, ii) a selective surgical efferent vagotomy eliminates the usual suppressive action of CCK on food intake (Smith, Jerome & Norgren, 1983). CCK released by a meal may produce its effects through an interaction with receptors on the vagus itself, rather than the structures which it innervates, since CCK receptors have been detected in the rat vagus (Zarbin, Wamsley, Innis & Kuhar, 1981). Presumably, this ligand-receptor interaction alters the activity of the vagus and initiate a sequence of events which culminates in satiety.

This thesis has two aims. The first is to characterize the time course of the CCK effect. This is presented in experiment 1. Information on the time course of the CCK effect is useful in understanding how CCK produces satiety and is essential for any consideration of therapeutic use of the peptide for the treatment of feeding disorders. The second aim is to investigate the role of oropharyngeal stimulation in the satiety produced by exogenous CCK. This is presented in experiment 2. The relationship between oropharyngeal stimulation and the satiety inducing effects of CCK is unclear. There is a suggestion that CCK is rendered more efficacious when its administration is preceded by oropharyngeal stimulation in the form of a small meal. Again, this information would be important in the therapeutic application of CCK.

The sham feeding rat was the preparation chosen for this study since the size and duration of the meal in the sham feeding rat are much larger than those in the intact rat. Since the meal size and duration are small in the intact rat compared to the sham feeding animal, absolute changes in response to peptide administration are sometimes difficult to detect with normal feeding. The error associated with reading the volume of these small meals may obscure the suppressive effects of the peptide, especially when delivered in small doses. Thus, the study of the time course of the CCK effect would be difficult in the intact rat since its meal duration and size are so restricted.

In contrast, the sham feeding rat feeds continuously and, hence, its meal size and duration are large. Further, sham feeding rats feed continuously over long observation periods and, thus, a stable baseline of feeding is available on the suppression of feeding in response to peptide administration may be evaluated. Of course, ultimately these data would be validated in the intact rat but the sham feeding rat is a more convenient preparation because of its large meal duration and size.

GENERAL METHODS

Subjects

Subjects in this study were Long-Evans hooded rats bred in the McMaster colony from breeding stock obtained from Blue Spruce Farms (Altamont, NY). Rats were housed individually in single hanging cages (Wahmann) in a room maintained at 26 degrees Celsius and on a 15:9 light:dark cycle. Water was available ad libitum and food was provided according to the protocol described below.

Surgery

Rats were 24-hour food deprived prior to surgery. Throughout the operation rats were anesthetized with 45 mg/Kg sodium pentobarbital (Somnotol, MTC). To implant the gastric cannula, the peritoneal cavity was exposed by making a midline incision extending approximately 4 cm caudally from the xiphisternum. One end of a stainless steel cannula (10.7 mm long; 8.5 mm o.d. x 7.9 mm i.d., flanged at both ends) was inserted into a stab wound along the forestomach and the wound was closed with two purse string sutures (5-0 silk). A collar of Marlex Mesh (2 cm x 2 cm, Bard Implants Division Maryland) was cemented to the shaft of the cannula so that it would fit snugly against the seosal surface of the stomach.

A stab wound was made in the left abdominal wall approximately 2 cm lateral to the midline incision. The muscle, but not the skin, was retracted medially and hemostats were inserted through the stab wound in the muscle wall into the peritoneal cavity. The hemostats were used to

grasp the cannula to exteriorize it through the muscle wall until the Marlex Mesh collar was flush against the peritoneum. A second Marlex Mesh collar was then force fit over the end of the cannula; this procedure effectively sandwiched the muscle wall between the two collars of Marlex Mesh. This procedure facilitated tissue fibrosis through the mesh which served to secure the cannula. the outer flange of the cannula was then exteriorized through the stab wound in the skin and the wound was closed using a purse string suture (3-0 silk). A stainless steel Allen screw was screwed into the cannula to keep it closed. The midline incision was closed by suturing the abdominal wall with 3-0 catgut and the skin was closed using wound clips. All rats were returned to their home cages and allowed to feed on Purina Rat Chow ad libitum for 3 weeks.

Since the sham feeding rat under a regimen of 17-hour food deprivation remains patent for only 3-4 months, three sets of animals were used for the experiments in this study. A summary of the sets of animals used in the specific experiments is presented in Table 1.

Apparatus and Procedure

Beginning three weeks after surgery rats were placed on a 17-hour food deprivation schedule; water was available ad libitum. To prepare the rat for a sham feeding test, it was removed from its home cage and its gastric fistula was opened by removing the Allen screw which was threaded into the cannula. Residual stomach contents were removed by lavaging the stomach through the fistula with warm tap water applied with a 10 cc syringe. A 19.1 mm long stainless steel collecting tube was then screwed into the cannula. A 15 cm plastic drainage tube

Table 1. Overview of subjects used for experiments in this thesis. For each experiment, the set of animals is indicated as well as the number of animals in the set and their mean (\pm 1SEM) body weight.

<u>experiment</u>	<u>set of animals</u>	<u>number of animals in set</u>	<u>weight (grams)</u>
1A	X	8	286 ± 20
1B	Y	9	282 ± 14
1C	Y	9	282 ± 14
2A	Z	7	338 ± 42
2B	X	8	286 ± 20
Appendix	Z	7	338 ± 42

ensheathed in a protective stainless steel spring was force fit onto the collecting tube. With this drainage system in place any liquid diet ingested orally drained freely out of the stomach and down the collecting tube by gravity flow.

All sham feeding tests took place with the rats housed in rectangular acrylic cages (21.5 cm long x 11 cm wide x 10 cm high, suspended on 20 cm high stilts). The floor of these cages was constructed of a sheet of acrylic with a 1.6 cm wide slot running longitudinally down the center to allow the collecting drainage tube to pass through the floor. The 10% sucrose solution to be sham fed was contained in graduated cylinders mounted on the outside of the front wall of the test cage. The licking spout of the graduated cylinder extended approximately 2.5 cm into the test cage through a hole in the front wall of the cage.

At the completion of the 60 minute test session, the collecting drainage tube was removed and the fistula was closed by replacing the Allen screw into the cannula shaft. The animal was returned immediately to its home cage and provided with Purina Rat Chow pellets for 6 hours.

Data Analysis

The effectiveness of CCK in suppressing feeding was assessed in two ways. First, the cumulative 30-minute intake on test days when CCK was administered was compared to intakes on control days when saline was administered. Second, the amount of feeding following the administration of CCK was expressed as a percent suppression relative to saline defined as:

$$\% \text{ suppression} = \frac{\text{amount eaten in CCK condition}}{\text{amount eaten in saline condition}} \times 100$$

Percent suppressions were calculated for each 3 minute time bin during the 30-minute sham feed.

The definition of suppression reflects an attempt to identify when the subjects intake in a CCK condition is less than that in corresponding salines. The parameters of suppression are defined below:

1) onset time of suppression: is the time measured from the onset of sham feeding until the beginning of the suppression for each animal. Suppression is defined as occurring when the average intake for two 3 minute time bins during the CCK trials plus the standard error of these trials is LESS than the average intake for the saline trials in the same two 3 minute time bins.

2) offset time of suppression: is the time measured from the initiation of sham feeding until the offset of suppression for each animal. The offset of suppression is defined as occurring when the average intake for two 3 minute time bins during the CCK trials is GREATER than or EQUAL to the average intake for the saline trials in the same two 3 minute time bins.

3) size of maximum suppression: is the largest percent suppression in any of the time bins.

4) time to maximum suppression: is the time, measured from the initiation of sham feeding until the time that maximum suppression occurs.

EXPERIMENT 1

INTRODUCTION

The purpose of experiment 1 is to characterize the time course of the CCK satiety effect. In spite of the tremendous volume of literature on the satiety-inducing effects of CCK, the time course of this effect is seldom reported and, in fact, poorly understood. Usually, the suppressive effects of CCK on feeding are measured by comparing the cumulative intake sometime after CCK administration to that after saline administration. For example, the ability of CCK to reduce eating has been expressed as suppressions (relative to saline) based on 15 minute (Gibbs et al., 1973a), 30 minute (Gibbs et al., 1973a), or 70 minute (Smith, Gibbs & Young, 1974) cumulative intakes. In the entire CCK and satiety literature there are only two studies which report any time course data at all. In one of the first reports of CCK's satiety action (Gibbs et al., 1973a), food intake following peptide or saline is reported in five consecutive 30 minute bins. In that same report, cumulative consumption of liquid diet 5, 10, 15, and 30 minutes following peptide or saline administration is presented. In the only other report of CCK which presents time course data, Smith et al., (1974) report the effects of CCK on the consumption of liquid diet in the sham feeding rat in 24 consecutive 5-minute time bins.

A comparison and interpretation of the little time course data available is difficult for three reasons. First, the bins of time through which the suppressive effects of CCK are assessed are not

uniform within or across studies. For example, Gibbs et al. (1973a) report suppressions following 5, 10, 15, or 30 minutes while Smith et al., (1974) report the suppressive effects of CCK using cumulative 70 minute intakes.

Second, the available information on the time course of the CCK effect is unsatisfying because the temporal resolution is poor. Reporting the effects of CCK using cumulative 30-minute intake (Gibbs et al., 1973a) might obscure important information about its effects during that interval itself. For example CCK may profoundly suppress food intake only during the first 5 minutes of that interval and, therefore, expressing CCK's effects using 30-minute cumulative intake might underestimate the peptide's potency. Further, the crudeness of the available time course data does not permit an analysis of whether animals compensate for a peptide-induced suppression by increasing intake in later time intervals. For example, it may be that CCK inhibits feeding profoundly for 5 minutes after its injection but that the subject compensates for this inhibition by increasing the amount eaten subsequently.

Finally, and most importantly, the available time course data are difficult to interpret because those data are based on experiments using 10% pure CCK purified from intestine. Since the CCK preparation used was 10% pure CCK, it follows that 90% of the extract contained unidentified agents, some of which may have had some impact on the degree of feeding. In fact, Lorenz, Kreielsheimer & Smith (1979) provide evidence that impure preparations of CCK contain a potent satiety producing contaminant. It is not clear, therefore, whether the

temporal parameters of CCK-induced satiety demonstrated with impure CCK are a function of CCK itself, some other agent(s) present in the extract, or an interaction of CCK with these hitherto unidentified factors.

Information on the time course of the CCK satiety effect is important for two major reasons: the possibility that CCK may be used clinically and that knowledge of the kinetics of CCK might provide insights into how CCK produces satiety.

The potential use of satiety-producing gut peptides for the treatment of eating disorders is imminent. Recently, there have been several experimental demonstrations which set the groundwork for clinical trials examining whether CCK can be used effectively to reduce or reverse the hyperphagia characteristic of obesity or bulimic episodes. Campbell and Smith (1983) report that the chronic administration of CCK-8 to obese Zucker rats result in a long-term reduction in daily caloric consumption and body weight level. With an eye towards therapeutic application, several investigators have examined the effect of CCK on food intake in humans (Kisseleff et al., 1981; Pi-Sunyer, Kisseleff, Thornton & Smith, 1981; Stacher, Steinrigher, Schmierer, Schneider & Winklehner, 1982; Sturdevant & Goetz, 1976). These studies confirm that CCK administration results in decreased food intake without accompanying toxic side-effects or malaise. Time course data are essential before considering the utility of gut peptides to modulate food intake. For example, it would be necessary to know when CCK begins to attenuate feeding, when maximal suppression of feeding occurs, what the duration of suppression is, or what the effects of the

dose of CCK are on these parameters. Ideally, in the therapeutic setting, one would like to time the administration of CCK so that the maximum satiety effects of CCK occurred during the meal itself and not sometime when the subject was not eating. Similarly, while it is known that increasing the dose of CCK decreases cumulative consumption (Gibbs et al., 1973a), it is not clear whether the increased suppression occurs through a more profound suppression occurring within the same time framework or an extension of the duration of the suppression. If increasing the dose of CCK decreases cumulative consumption by changing the duration but not the size of inhibition, for example, increasing the dose of CCK may be inappropriate for short meals in which the suppressive effects of the peptide might greatly outlast the meal itself. Thus, smaller doses of CCK would be chosen without any loss of efficacy and with the greatest minimization of potential side effects.

As well, information about the time course of the CCK effect may be useful to an understanding of how CCK in produces satiety. Two observations taken together suggest that the satiety effects produced by CCK are not strictly a function of the amount of biologically active circulating peptide. Recently, it has been shown that the half-life of biologically active CCK-8 in rat plasma is 17 minutes (Koulischer, Moroder & Deschodt-Lanckman, 1982). However, in the conventional test paradigm for the intact rat, CCK is administered 15 minutes before the presentation of food and satiety or suppression occurs 15 to 30 minutes after food presentation (Gibbs et al., 1973a). Hence, the maximum effects of the peptide are seen 30-45 minutes after CCK administration, when the biological activity of the peptide has been reduced to 1/4 or

less. Resolution of the relationship between the amount of satiety produced and the amount of circulating peptide requires fine-grained temporal analyses demonstrating when CCK actually exerts its satiety effect relative to the bout of feeding.

EXPERIMENT 1A

This experiment is the initial exploration of the kinetics of CCK-induced satiety and investigates the effects that increasing the dose of CCK has on the parameters of satiety. It is known that increasing the dose of CCK decreases cumulative 30 minute (Gibbs et al., 1973a) and 60 minute (Falasco et al., 1979; Lorenz et al., 1979) intakes in the sham feeding animal. The way in which this increased inhibition of eating is effected, however, is unknown; i.e., it is not clear which parameters of CCK-induced satiety are changed as a function of dose. For example, it is unclear whether the further suppression induced by increased CCK doses magnifies the duration or size of the suppression. This experiment identifies the parameters of suppression which are changed as dose increases.

METHOD

Chronic gastric cannulae were implanted in 8 rats weighing 286 +/- 20 grams at the time of surgery. Following a three week recovery period from surgery, rats were trained to lick reliably in the test cages under the conditions outlined below. This training period lasted approximately three weeks.

Rats were 17-hour food deprived for all sham feeding tests. Each test day, rats were placed in the sham feeding cage for a total of 60 minutes. During the last 30 minutes of this period subjects sham fed a 10% sucrose (w/v) solution. The liquid diet was presented in graduated cylinders and intake was recorded every 3 minutes. During the

30 minute period preceding the test sham fed rats were injected intraperitoneally (ip) at three times: 30 minutes before (T-30), 15 minutes before (T-15), and 0 minutes before (T0), the initiation of sham feeding. (The times of injection are always indicated with reference to the initiation of sham feeding so that, for example, an injection given at T-30 is delivered 30 minutes before the initiation of sham feeding).

On control days all three ip injections were .15 M saline. The saline injections at T-30 and T-15 minutes were .5 ml each. The saline injection at T0 was 1 ml. These injection times and sizes were necessary to equate the test conditions used in this experiment to those employed in subsequent studies and to control for the volume of ip injections used on CCK days. There were 6 control days. On test day, CCK-8 was injected at T0 (made up to a volume of 1 ml) and two saline injections, each of .5 ml were given at T-30 and T-15. Two doses of CCK-8 were used; 5.6 jg/kg or 11.2 ug/kg. Each of these two CCK doses was repeated three times. The order of test trials was arranged so that no subject ever received CCK-8 on two consecutive days. The CCK used in these experiments was the synthetic C-terminal octapeptide of cholecystokinin, CCK-8 (a kind gift of Dr. M. Ondetti, Squibb Laboratory for Medical Research Princeton, N.J.) The design of this experiment is summarized in Figure 1A.

RESULTS AND DISCUSSION

The effects of 5.6 and 11.2 ug/kg CCK-8 delivered at T0, i.e., coincident with the initiation of sham feeding are summarized in Figure 2 which presents the cumulative sham fed intakes on these test conditions and following saline. Initially, correlated t-tests

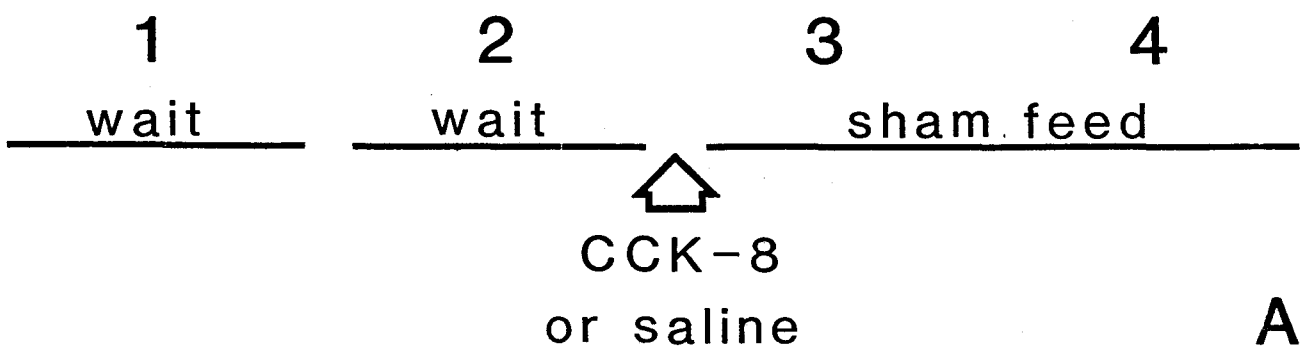
comparing the 30-minute intakes in the three test conditions were used to assess the inhibition of feeding produced by CCK-8. Both 5.6 ug/kg CCK-8, $t(7) = 3.38$ $p < .02$, and 11.2 ug/kg CCK-8, $t(7) = 3.28$ $p < .02$, significantly inhibited intake compared to saline control trials. Furthermore, 11.2 ug/kg CCK-8 produced a larger inhibition of feeding than did 5.6 ug/kg CCK-8, $t(7) = 2.47$ $p < .05$. Thus, both doses of peptide administered coincident with the initiation of sham feeding significantly inhibited eating and the amount of overall inhibition increased with dose.

The way in which this increased suppression with the larger dose of CCK-8 was produced was analysed by converting the absolute intakes on the two CCK-8 conditions to a percent suppression relative to saline for each of the time bins; these data are presented in Figure 3. On percent suppression graphs, "0" indicates no suppression with CCK-8, i.e., the intake in that time bin following peptide is identical to that with saline. Positive percent suppression indicates that the rats ate less with CCK-8 compared to saline. Negative percent suppression indicates that the intake for that time bin following CCK-8 was greater than after saline. The parameters of suppression (defined in General Methods) are extracted from these graphs and are summarized in Table 2. Increasing the dose of CCK-8 significantly increased the size of maximum suppression, $t(7) = 3.47$ $p < .02$. The time at which this maximum suppression occurred was not altered, $t(7) = .26$ $p > .8$, nor was the offset time of this suppression, $t(7) = .68$ $p > .05$. However, increasing the dose of CCK-8 decreased the onset time of suppression, $t(7) = 2.2$ $p < .1$, a comparison which just fails to attain significance.

Figure 1. Design of studies comprising Experiment 1. a) Experiment examining the effects of injection of 5.6 ug/kg CCK-8, 11.2 ug/kg CCK-8, or saline at time T0 on sham fed intake. b) Experiment examining the effects of injection of 5.6 ug/kg CCK-8, 11.2 ug/kg CCK-8, or saline at time T-15 on sham fed intake. c) Experiment examining the effects of injection of 5.6 ug/kg Ac-CCK-8, 11.2 ug/kg Ac-CCK-8, or saline at time T-15 on sham fed intake.

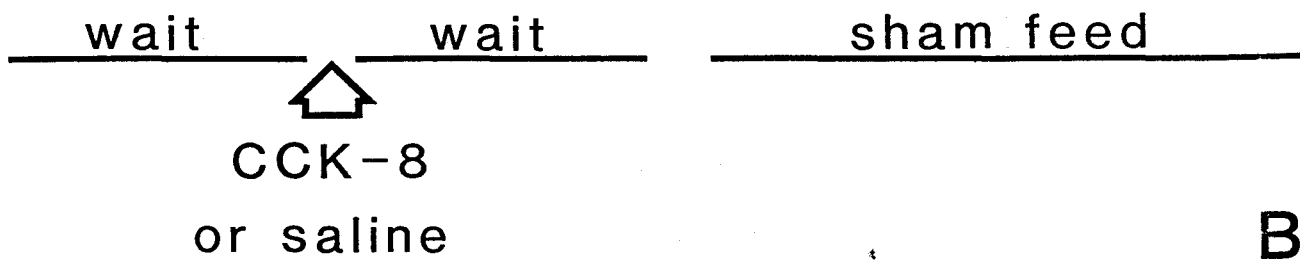
FIFTEEN MINUTE BLOCKS

experiment
1A



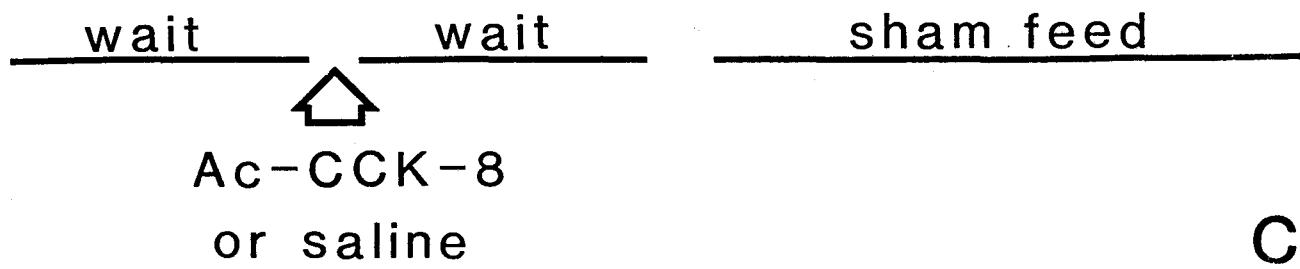
A

1B



B

1C



C

Figure 2. Group average cumulative sham fed intake following injection of either saline (), 5.6 ug/kg CCK-8 (), or 11.2 ug/kg CCK-8 () injected at time T0 min. Vertical lines represent 1 SEM.

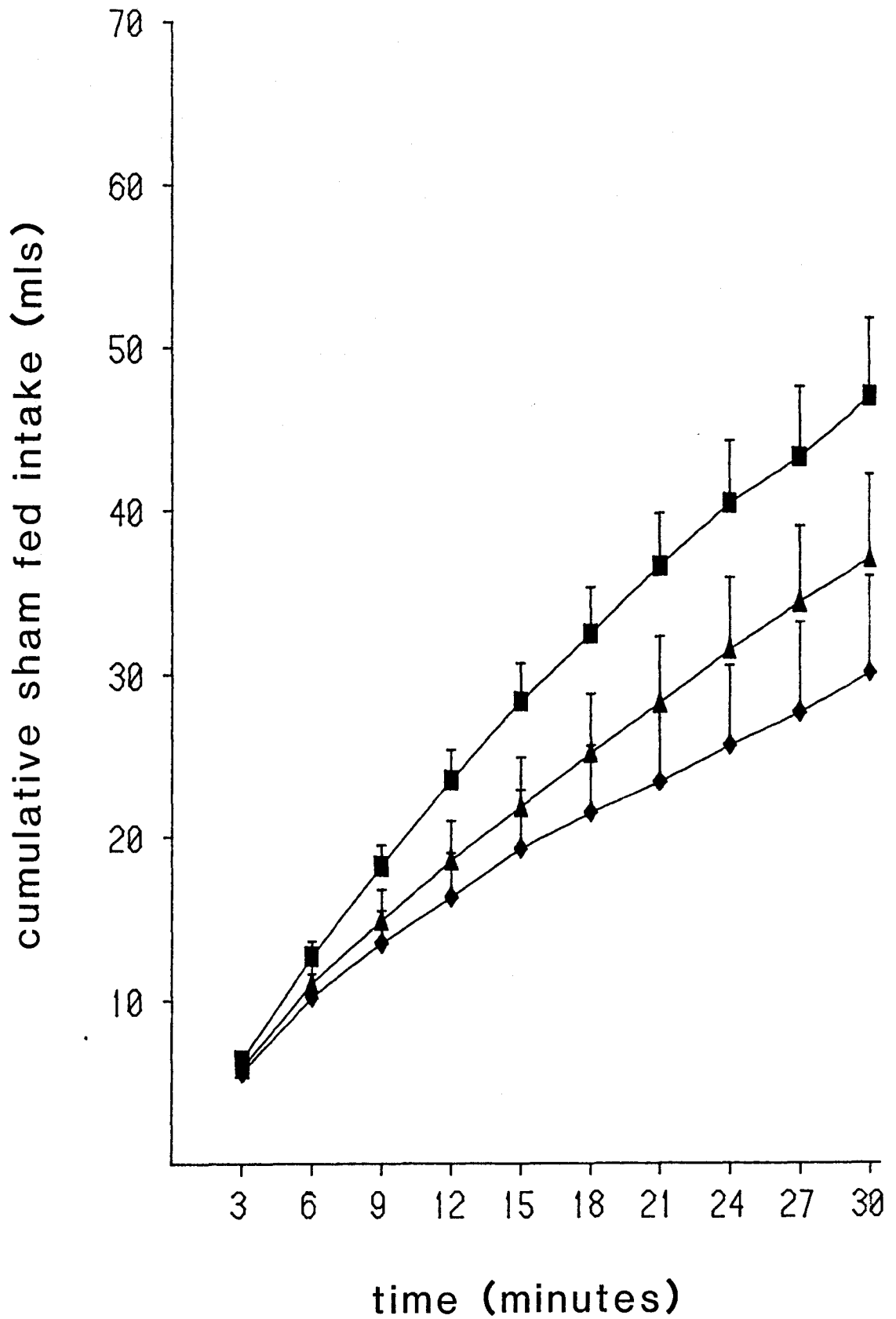
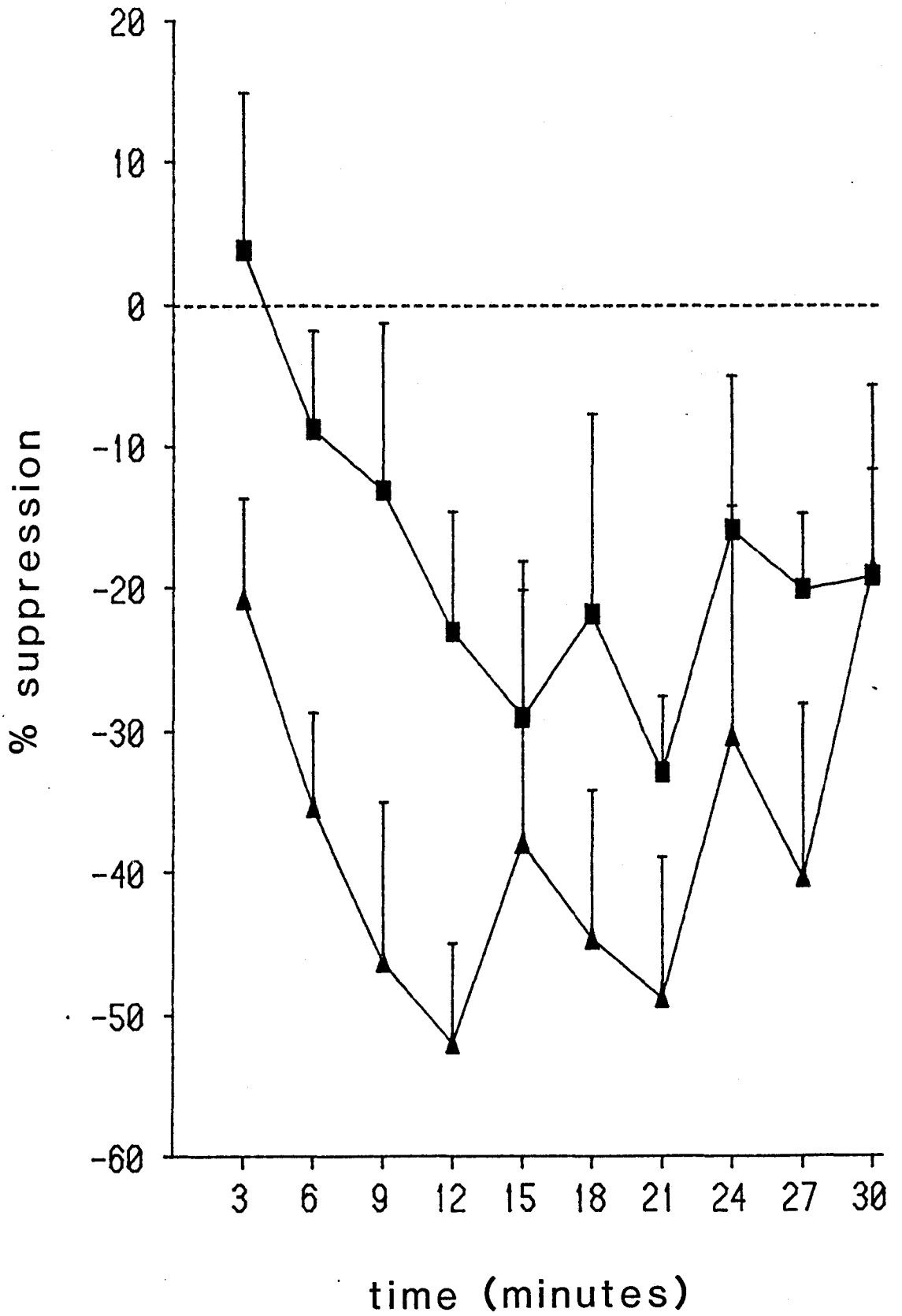


Figure 3. Group average percent suppressions produced by 5.6 ug/kg CCK-8 () and 11.2 ug/kg CCK-8 () relative to saline injections. Vertical lines represent 1 SEM.



These results demonstrate that an increase in the dose of CCK-8 decreases cumulative intake through an increase in the size of maximum suppression and a decrease in the onset time to suppression.

Table 2. Summary of Experiment 1 indicating the parameters of suppression (see General Methods for definitions) produced by the test conditions indicated.

peptide dose (ug/kg)	size (%) of maximum suppression	time (min) to maximum suppression	time (min) to onset of suppression	time (min) to offset of suppression
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5.6 CCK-8 @T0	52.1 ± 6.2	17.6 ± 2.2	8.6 ± 3.1	21.0 ± 3.5
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11.2 CCK-8 @T0	68.6 ± 8.4	18.4 ± 2.7	4.5 ± 1.6	25.1 ± 1.1
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11.2 Ac-CCK-8 @T-15	46.0 ± 8.3	16.3 ± 2.8	5.7 ± 2.0	17.7 ± 3.4
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EXPERIMENT 1B

Experiment 1A demonstrates that CCK-8 administered coincident with the initiation of sham feeding inhibits feeding and that the amount of inhibition increases with an increased dose of peptide. This experiment examines a second aspect of the kinetics of CCK. Specifically, it asks how soon before the initiation of a meal can CCK-8 be administered and still retain its satiety effect. This was examined by injecting 5.6 and 11.2 ug/kg CCK-8 15 minutes before sham feeding began.

METHOD

Chronic gastric cannulae were implanted in 9 rats weighing 282 +/- grams at the time of surgery. Each test day rats were prepared for sham feeding and injected ip with 5.6 or 11.2 ug/kg CCK-8 (made up to a volume of 1ml) at T-15. To remain consistent across experiments two injections of .15 M saline, each of .5 ml, were given at T-30 and T0. Each dose of CCK-8 was tested three times. On control days, all three injections were saline. Two saline injections of .5 ml given at T-30 and T0, and one of 1 ml at T-15. There are 7 control days. This design is summarized in Figure 1B.

RESULTS AND DISCUSSION

The effect of administering 5.6 or 11.2 ug/kg CCK 15 minutes before the initiation of sham feeding is summarized in Figure 4 which shows the cumulative sham fed intakes following the two doses of CCK-8 and saline. Correlated t-tests comparing the cumulative 30-minute

intakes in the 3 test conditions revealed that neither 5.6, $t(8) = .863$ $p > .2$, or 11.2 ug/kg CCK-8, $t(8) = 1.319$ $p > .2$, given 15 minutes prior to a meal significantly suppressed consumption compared to saline trials. Inspection of Figure 5, which presents the percent suppression of the CCK-8 conditions relative to the saline condition, confirms that neither dose of CCK-8 administered at T-15 reduced feeding.

These results demonstrate that CCK-8 administered 15 minutes before a sham feed is ineffective in reducing intake. There are two interpretations of the failure of CCK to suppress feeding under these conditions. One possibility is that, in this experiment, the satiety signal produced by CCK had no satiety effect since it occurred at a time removed from the bout of feeding. This suggests that the signal produced by CCK may not be interpreted as relevant to the suppression of feeding unless it is received continuously with feeding. Alternatively, CCK administered 15 minutes before the initiation of sham feeding may fail to inhibit eating since, by the time eating actually starts, the peptide has been degraded sufficiently so that circulating levels present at the time of the sham feed were insufficient to produce satiety. This latter interpretation is examined further in Experiment 1C.

Figure 4. Group average cumulative sham fed intakes following injections of saline (), 5.6 ug/kg CCK-8 (), and 11.2 ug/kg CCK-8 () at time T-15 min. Vertical lines represent 1 SEM.

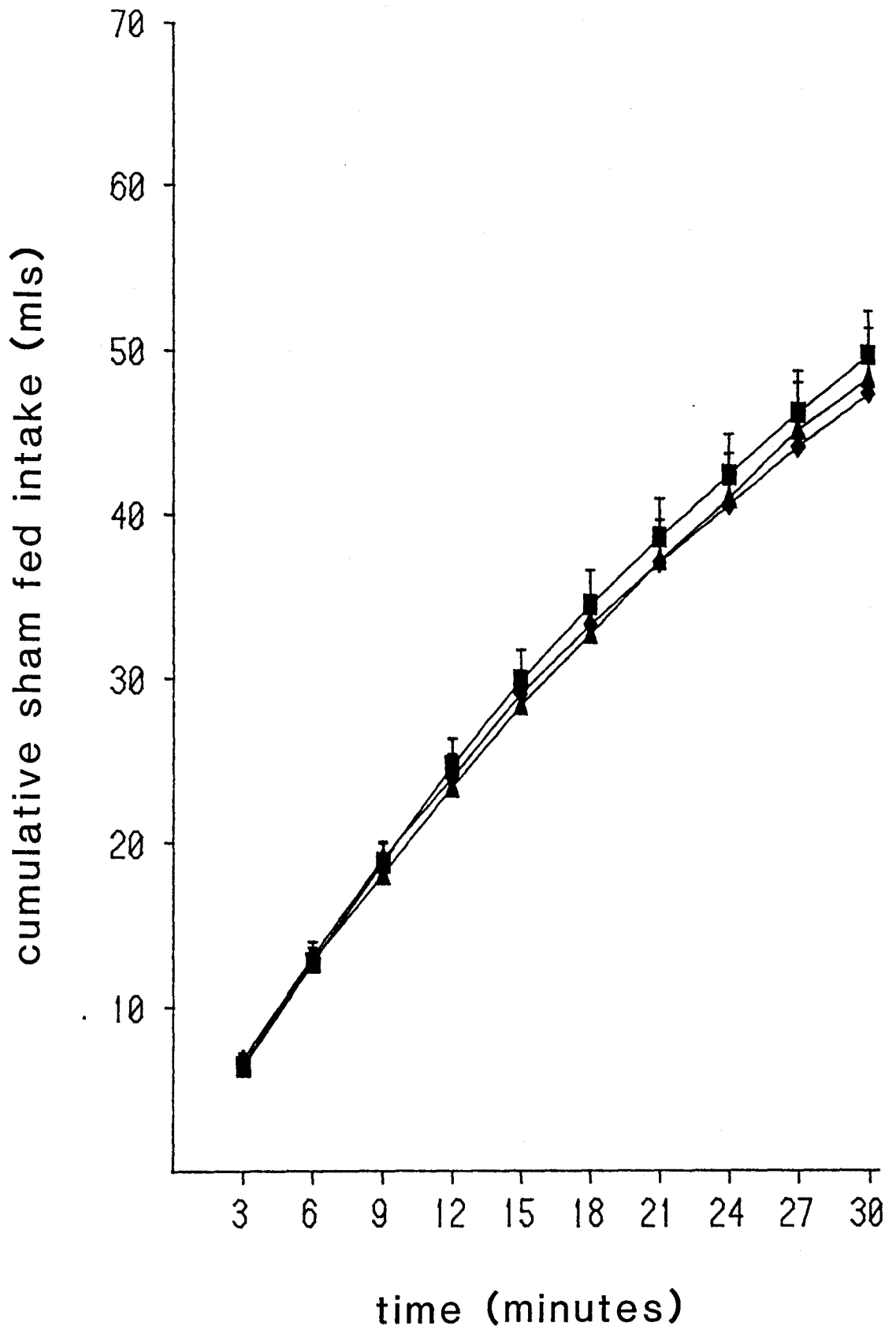
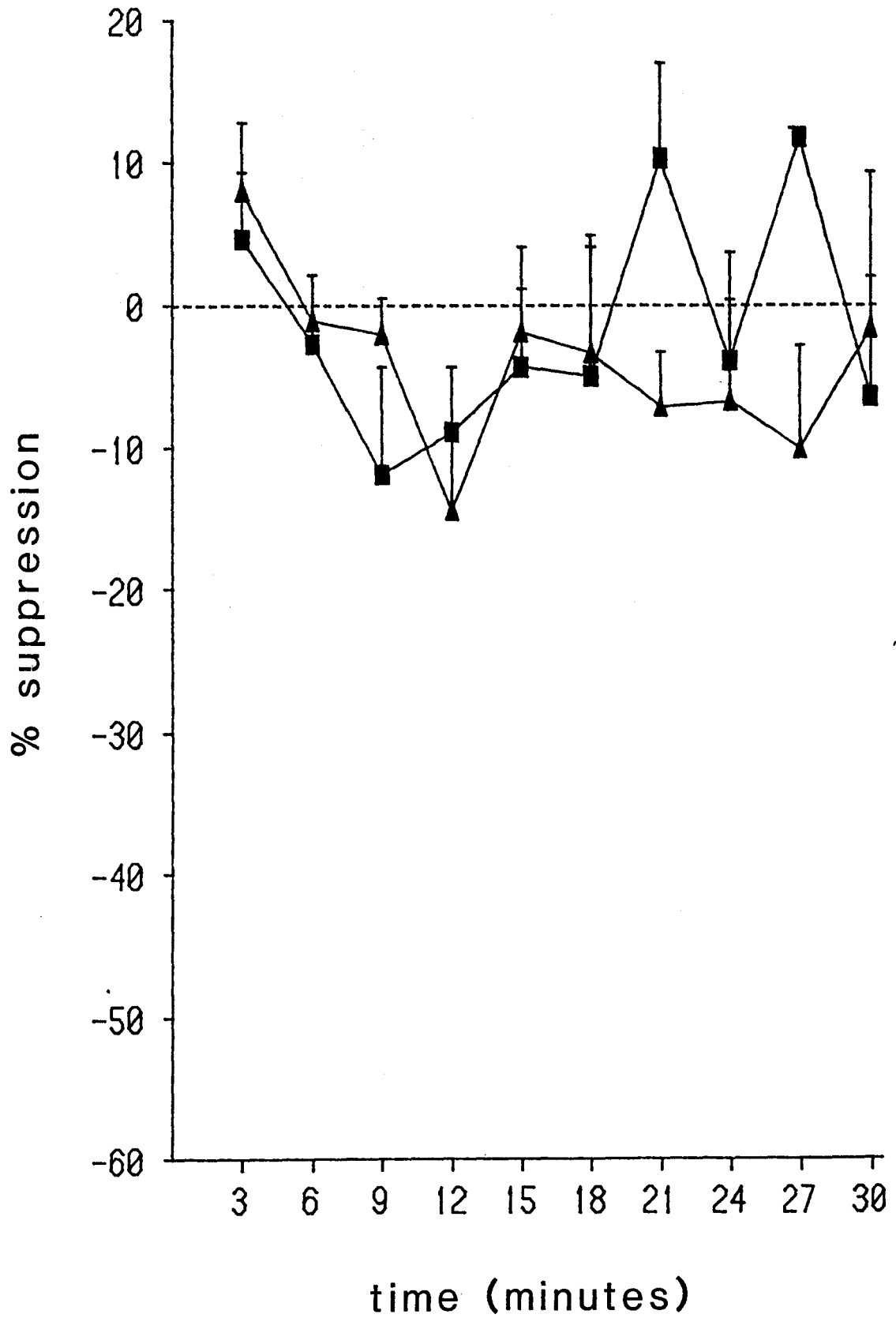


Figure 5. Group average percent suppressions representing the amount of suppression relative to saline produced by 5.6 ug/kg CCK-8 () and 11.2 ug/kg CCK-8 () injected at time T-15 min. Vertical lines represent 1 SEM.



EXPERIMENT 1C

The two previous experiments demonstrate that 5.6 and 11.2 ug/kg CCK-8 administered coincident with the initiation of sham feeding (T0) suppresses intake but that identical doses given 15 minutes before sham feeding (T-15) are ineffective in inhibiting ingestion. The failure of CCK-8 administered at T-15 to inhibit feeding may result because of peptide degradation so that the level of biologically active peptide present at the time of sham feeding was insufficient to inhibit feeding. This interpretation is tested in this experiment by using a derivative of CCK-8 which is more resistant to degradation than CCK-8.

The addition of an acetyl group to CCK-8 produces a variant of CCK-8 which is more resistant to degradation by peptidases present in tissues (Praisman, Fara, Praisman, & Berkowitz, 1982). This acetylated derivative of CCK-8 is called the acetyl-octapeptide of cholecystokinin (Ac-CCK-8). The relative longevity of Ac-CCK-8 to CCK-8 is well established in vitro. This was shown by incubating each peptide with various smooth muscle tissues. After 30 minutes, CCK-8 concentration (measured by radioimmunoassay) declined by 26% but Ac-CCK-8 concentration declined by only 0.8% (Praisman et al., 1982). Ac-CCK-8 retains all the biological activity and potency of CCK-8, since its dose-response curve is identical to CCK-8 using peptide-induced contractions of gallbladder as the bioassay. This study indicates a half-life for CCK-8 of greater than 30 minutes. However, in a different in vitro study, the half-life of CCK-8 in rat plasma is 17 minutes

(Koulischer et al., 1982). The half-life in plasma is the more appropriate measure since CCK-8's known functions associated with digestion have an endocrine mode of action. Unfortunately, demonstrations of half-life in vitro do not necessarily provide good estimates of half-life in vivo. Preliminary observations in our laboratory indicate that Ac-CCK-8 does have a prolonged satiety effect compared to CCK-8 in vivo. Specifically, the inhibition of intake in food-deprived sham feeding rats following the administration of Ac-CCK-8 was approximately twice as long as that produced by CCK-8 (see Appendix).

If the failure of CCK-8 to inhibit feeding when delivered at T-15 is because of an insufficient amount of biologically active peptide present at the time of the sham feed, then the administration of Ac-CCK-8 should be more likely to produce an inhibition of feeding under the same test conditions used before where CCK-8 was ineffective.

METHOD

Chronic gastric cannulae were implanted in 9 rats weighing 282 +/- grams at the time of surgery. Each test day rats were prepared for sham feeding and injected ip at T-15 with 5.6 or 11.2 ug/kg Ac-CCK-8 made up to a volume of 1 ml. To be consistent with the conditions of previous experiments, two .5 ml injections of .15 M saline were given at T-30 and T0. The 5.6 ug/kg Ac-CCK-8 at T-15 was tested 3 times, and the 11.2 ug/kg Ac-CCK-8 was tested 2 times. On control days, all three injections were saline. Two saline injections of .5 ml given at T-30 and T-0, and one of 1 ml at T-15. There are seven control days. This design is summarized in Figure 1C.

RESULTS AND DISCUSSION

Figure 6 presents the cumulative sham fed intakes following 5.6 ug/kg Ac-CCK-8, 11.2 ug/kg Ac-CCK-8, and saline administered 15 minutes before the initiation of sham feeding. Correlated t-tests comparing 30-minute intake in the 3 conditions revealed that 5.6 ug/kg Ac-CCK-8 did not significantly inhibit feeding relative to saline, $t(8) = 2.049$ $p > .05$. In contrast, 11.2 ug/kg Ac-CCK-8 significantly suppressed intake, $t(8) = 3.089$ $p < .02$, compared to saline.

The parameters of suppression associated with 11.2 ug/kg Ac-CCK-8 injected at T-15 were identified as before by converting the absolute intake in the Ac-CCK-8 condition to a percent suppression relative to saline for each of the time bins; these data are presented in Figure 7. The parameters of suppression (defined in General Methods) are extracted from these graphs for each rat. The maximum suppression

Figure 6. Group average cumulative sham fed intakes following injections of saline (), 5.6 ug/kg Ac-CCK-8 (), and 11.2 ug/kg Ac-CCK-8 () at time T-15 min. Vertical lines represent 1 SEM.

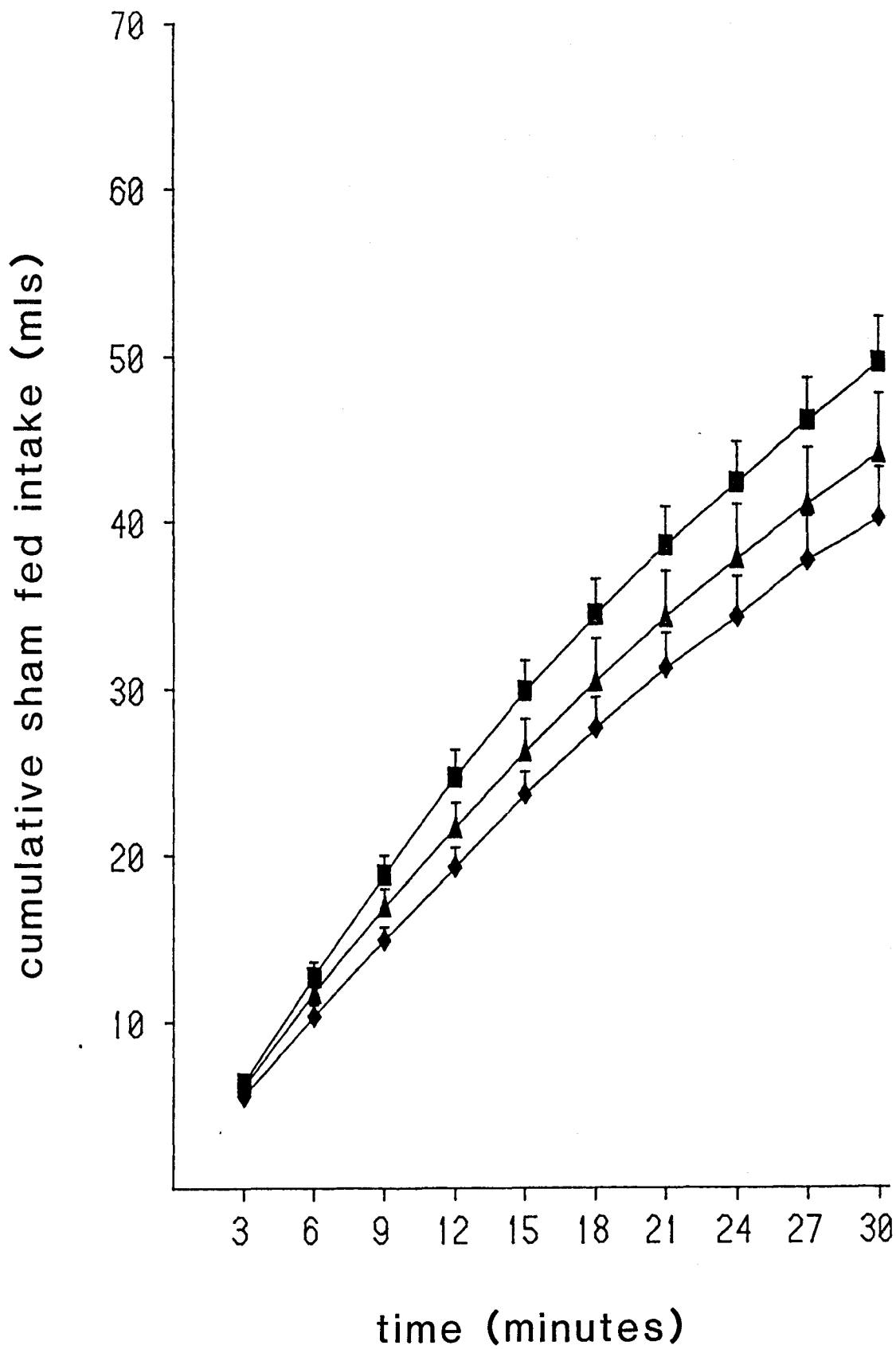
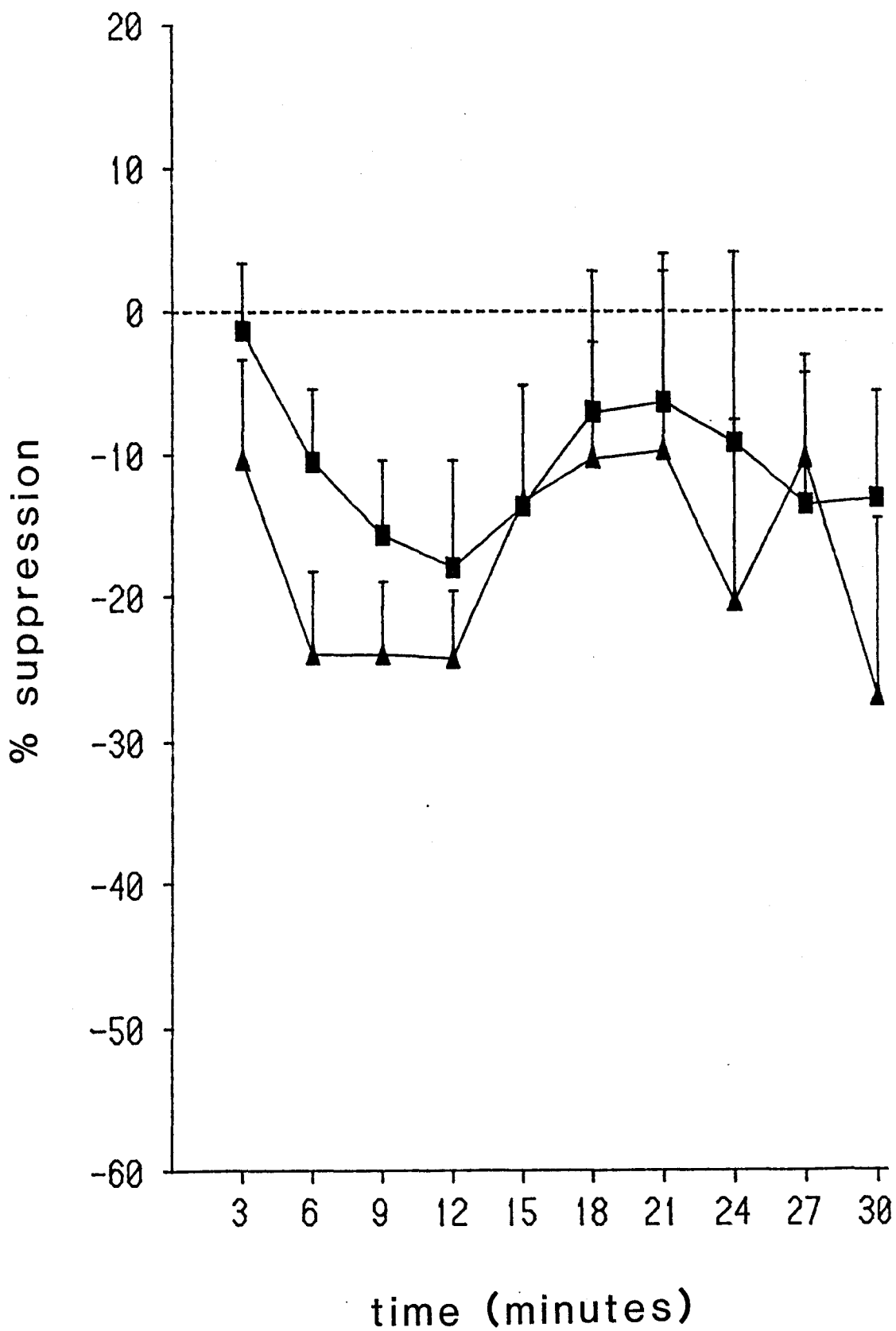


Figure 7. Group average percent suppressions representing the amount of suppression relative to saline produced by 5.6 ug/kg Ac-CCK-8 () and 11.2 ug/kg Ac-CCK-8 () injected at time T-15 min. Vertical lines represent 1 SEM.



of feeding was 46.0 +/- 8.3% and occurred 16.3 +/- 2.8 minutes after sham feeding was initiated. The suppression began (i.e., onset time) 5.67 +/- 2.0 min after the initiation of sham feeding, and had an offset time of 17.7 +/- 3.4 minutes.

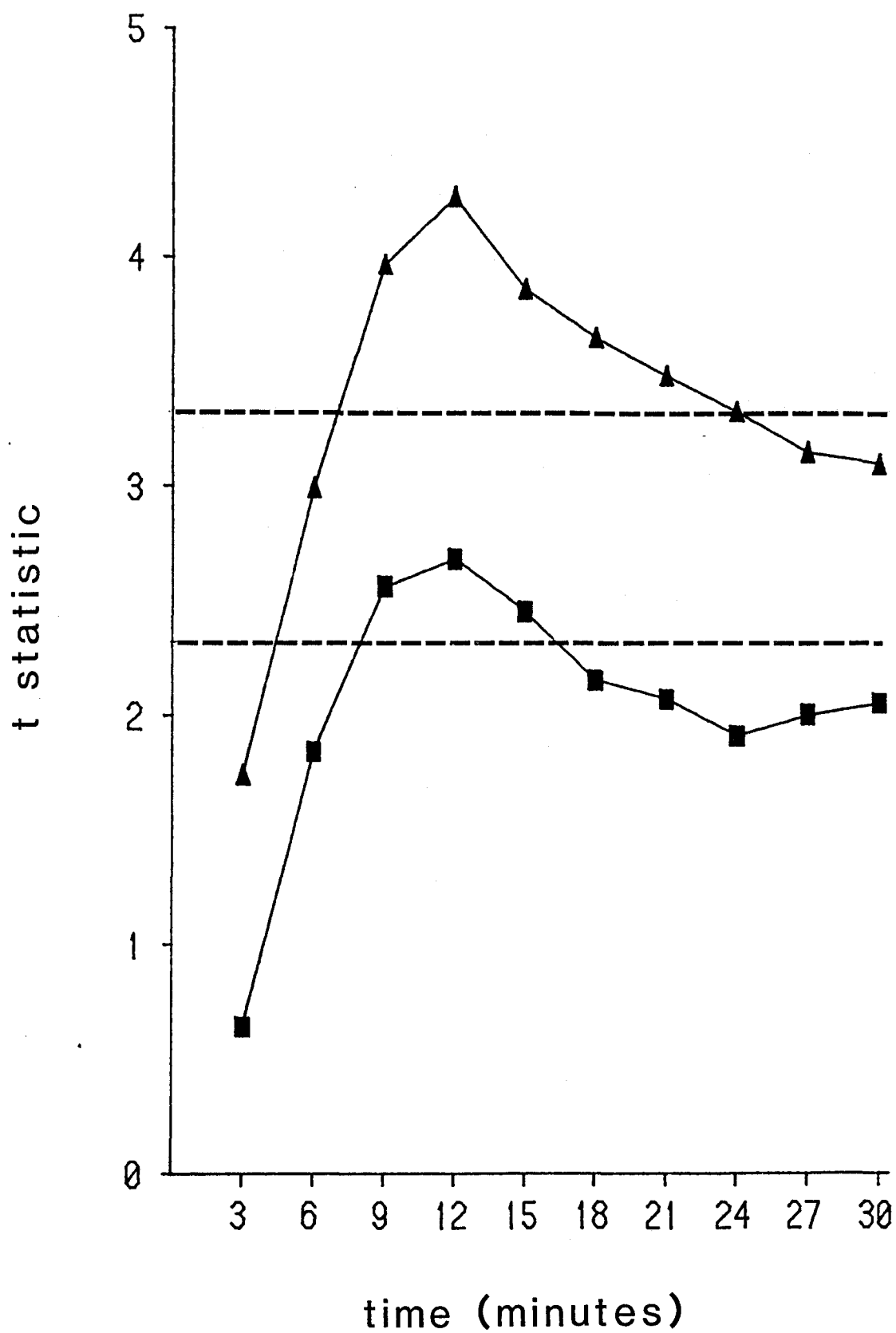
The parameters of suppression found with 11.2 ug/kg Ac-CCK-8 at T-15 were compared to the suppression found in experiment 1a with 11.2 and 5.6 ug/kg given at T0. A one-way analysis of variance was performed to compare each of the parameters of suppression in the 3 conditions. The size, $F(2,22) = 2.26$ $p > .1$, and time, $F(2,22) = .16$ $p > .25$, of maximum suppression were not significantly different. Similarly, the onset time, $F(2,22) = 1.44$ $p > .25$ and offset time, $F(2,22) = 1.65$ $p > .1$, did not differ significantly. These data are summarized in Table 2. Therefore, 11.2 ug/kg Ac-CCK-8 administered 15 min before the sham feed inhibited sham feeding and the parameters of the suppression produced were not different than those produced by 5.6 or 11.2 ug/kg given at the same time as the initiation of sham feeding.

These data provide an important illustration of the advantage of using time course data rather than cumulative consumption to evaluate effects of CCK on feeding. Figure 7, which shows the percent suppression of feeding after 5.6 and 11.2 ug/kg Ac-CCK-8 relative to saline, indicates that a small suppression following 5.6 Ac-CCK-8 does occur. However, a comparison of the 30-minute cumulative consumption following saline or 5.6 ug/kg Ac-CCK-8 shows that the two aren't significantly different and apparently 5.6 ug/kg Ac-CCK-8 did not suppress feeding. These apparently discrepant results illustrate that cumulative consumption data may conceal information which are revealed

by time course data. In fact, whether or not a significant difference is reported between the cumulative consumption following saline and peptide depends on when, during the meal, the consumption is measured. Correlated t-tests were performed to compare the cumulative consumption following saline and 5.6 or 11.2 ug/kg Ac-CCK-8 at each of the 10 3-minute time bins. The results of these t-tests are summarized in Figure 8 which presents the value of the t statistic corresponding to the difference in cumulative consumption following saline or Ac-CCK-8. These results demonstrate that a significant suppression occurred if cumulative consumptions were compared at certain times since the beginning of the meal but not at others. Hence, to assess whether the peptide inhibits feeding, time course data are preferred to cumulative consumption data. The use of cumulative consumption is a tool of poor sensitivity since its accuracy depends on when, during the course of the meal, it is applied.

This experiment has shown that the administration of Ac-CCK-8, a long-lasting derivative of CCK-8, at 15 minutes before the initiation of sham feeding suppresses intake. Therefore, the most parsimonious interpretation of the failure of CCK-8 give at T-15 to inhibit feeding demonstrated in previous experiments is that the CCK-8 had been degraded so that at the time of the initiation of sham feeding insufficient levels were present to inhibit feeding.

Figure 8. The value of the t-statistic (correlated t-tests) when the cumulative sham fed consumption following either 5.6 ug/kg Ac-CCK-8 () or 11.2 ug/kg Ac-CCK-8 () are tested against consumption on saline trials. Hatched horizontal bars indicated the .05 and .01 significance alpha levels (two-tailed) for the relevant number of degrees of freedom (8).



DISCUSSION OF EXPERIMENT 1

This experiment was designed to characterize the time course of the CCK satiety effect and identify several properties of the suppression of sham feeding produced by the administration of CCK-8. Four major results are presented. First, CCK-8 administered coincident with the initiation of sham feeding inhibits feeding in a dose-related manner. Second, the increased efficacy of the higher dose of CCK-8 delivered at the same time as sham feeding results from an increase in the size of maximum suppression and an increase in the time of suppression effected via a decreased latency to suppression. Third, CCK-8 delivered 15 minutes before sham feeding begins has no effect on feeding. Fourth, the administration of a long-lasting derivative of CCK-8 15 minutes prior to the initiation of sham feeding can suppress intake.

The derivative of CCK-8 with a prolonged satiety effect (Ac-CCK-8) inhibited feeding when injected 15 minutes before the sham feed, but CCK-8 did not. These data support, but do not prove, the interpretation that CCK-8's failure to affect feeding when injected at T-15 is because it is degraded sufficiently by the time feeding starts so that the circulating levels of the peptide are insufficient to inhibit feeding. This interpretation may be tested directly in a future study by measuring the plasma levels of CCK-8 corresponding to ip injections of CCK-8 which are threshold doses for the satiety effect. This would identify the plasma levels of CCK-8 which were sufficient for

the satiety effect. Using this procedure it may be shown whether, under test conditions in which CCK-8 failed to inhibit feeding (e.g., 11.2 ug/kg CCK-8 given at T-15), plasma levels of CCK-8 were below the threshold level necessary to reduce intake. Conversely, under test conditions in which CCK-8 does reduce intake (e.g., 11.2 ug/kg Ac-CCK-8 given at T-15), could be shown whether plasma levels of CCK-8 were above the threshold to induce satiety. Hence, this procedure would make the important distinction between the biological consequences of peptide administration (i.e., satiety) and the circulating levels of CCK-8.

The implications of these results for the therapeutic use of this peptide and its role in producing satiety are discussed in the General Discussion.

EXPERIMENT 2

INTRODUCTION

The second set of experiments in this thesis explores the role of oropharyngeal stimulation in CCK-induced satiety in the sham feeding rat.

Stimulation of the oropharynx by food stimuli has been shown to be important in producing satiety in certain circumstances. For example, Antin, Gibbs & Smith (1977) report that the infusion of food stimuli into the duodenum was ineffective in producing satiety unless the infusion was coincident with, or preceded by, oropharyngeal stimulation. Specifically, it is found that intestinal infusions delivered to food-deprived sham feeding rats 12 minutes prior to sham feeding failed to suppress eating. In contrast, when the intestinal infusion is preceded by, or is coincident with, oropharyngeal stimulation rats sated even with the same intestinal infusion parameters. Thus, oropharyngeal cues are important to the elaboration of intestinal satiety.

It has been suggested recently (Antin, Gibbs & Smith, 1978) that oropharyngeal cues also amplify CCK-induced satiety; this enhancement of CCK's satiety action by concurrent oropharyngeal stimulation is referred to as oropharyngeal synergy. This concept is based on a single experiment demonstrating that CCK's suppressive effects are increased the closer to the beginning of the meal CCK is injected, and that CCK injected after feeding has begun results in

greater suppression of feeding compared to when the peptide is injected coincident with meal initiation (Antin, Gibbs & Smith, 1978).

Two criticisms may be made of these data. First, in that study, the suppressive effects of CCK are evaluated beginning 12 minutes after peptide administration. However, what the rats do during that interval varies across conditions so that the effects of the different regimens of peptide administration may be confounded by differences in the rats' behavior during that interval. Second, this single study which supports the concept of oropharyngeal synergy uses 20% pure CCK, a non-pure form of CCK which is believed to contain a potent satiety-producing contaminant (Lorenz et al., 1979). It is interesting that although a replication of these findings supporting oropharyngeal synergy using pure CCK has never been published, the concept is ingrained sufficiently to have affected the design of subsequent studies assessing the effects of CCK on eating. For example, one study evaluating CCK's actions on eating in humans (Kisseleff et al., 1981) allows subjects a small snack before the injection of CCK and the introduction of the test meal. This small feed prior to the actual meal of interest is meant, presumably, to provide the necessary stimulation to enhance the effects of the CCK administered.

A conclusion about whether oropharyngeal stimulation enhances the suppressive effects of CCK has important consequences for the putative therapeutic use of this peptide as an appetite suppressant. For example, to ensure the maximum potency of the peptide, it may be necessary for the patient to eat a small meal sometime before he receives CCK.

EXPERIMENT 2A

The purpose of this experiment is to evaluate the importance of oropharyngeal stimulation in the satiety produced by exogenous CCK. The influence of oropharyngeal cues on CCK's action is assessed using the pure synthetic octapeptide of CCK (CCK-8).

METHOD

Chronic gastric cannulae were implanted in 7 rats weighing 338 +/- 42 grams at the time of surgery.

Each test day, rats were prepared for sham feeding as described previously and injected ip with either 5.6 ug/kg CCK-8 or equal volumes of saline. On some test days (Synergy days), the possibility for oropharyngeal synergy was allowed by permitting rats to sham feed 10% sucrose for 15 minutes prior to the CCK injection. On control days rats simply waited in the test cages for 15 minutes prior to CCK injection. Under both test conditions, rats waited in the test cages for 15 minutes after peptide or saline injection. then, a 30-minute sham feed was initiated. This design is summarized in Figure 9A. The order of test trials was arranged so that there were no consecutive test days with CCK. The results are based on a total of 4 trials at each of two saline conditions, and three trials at each of two CCK-8 conditions.

RESULTS AND DISCUSSION


Figure 10 shows the cumulative amount sham fed in all four test conditions. Even when injected with saline, a sham feed prior to

Figure 9. Design of studies in Experiment 2. a) Experiment examining the role of oropharyngeal stimulation on CCK-induced satiety. Injections of 5.6 ug/kg CCK-8 or saline are given at time T-15 min. b) Experiment examining whether a prior sham feed amplifies the satiety action of CCK (Prior S.F. condition) compared to injections of cck delivered without a prior sham feed (Control condition). All injections are administered at time T0, i.e., coincident with the initiation of the test sham feed. Dose of CCK-8 used was 5.6 ug/kg.

FIFTEEN MINUTE BLOCKS


CONDITION

	1	2	3	4
synergy	<u>sham feed</u>	<u>wait</u>	<u>sham feed</u>	
control	<u>wait</u>	<u>wait</u>	<u>sham feed</u>	


 CCK-8
 or
 saline

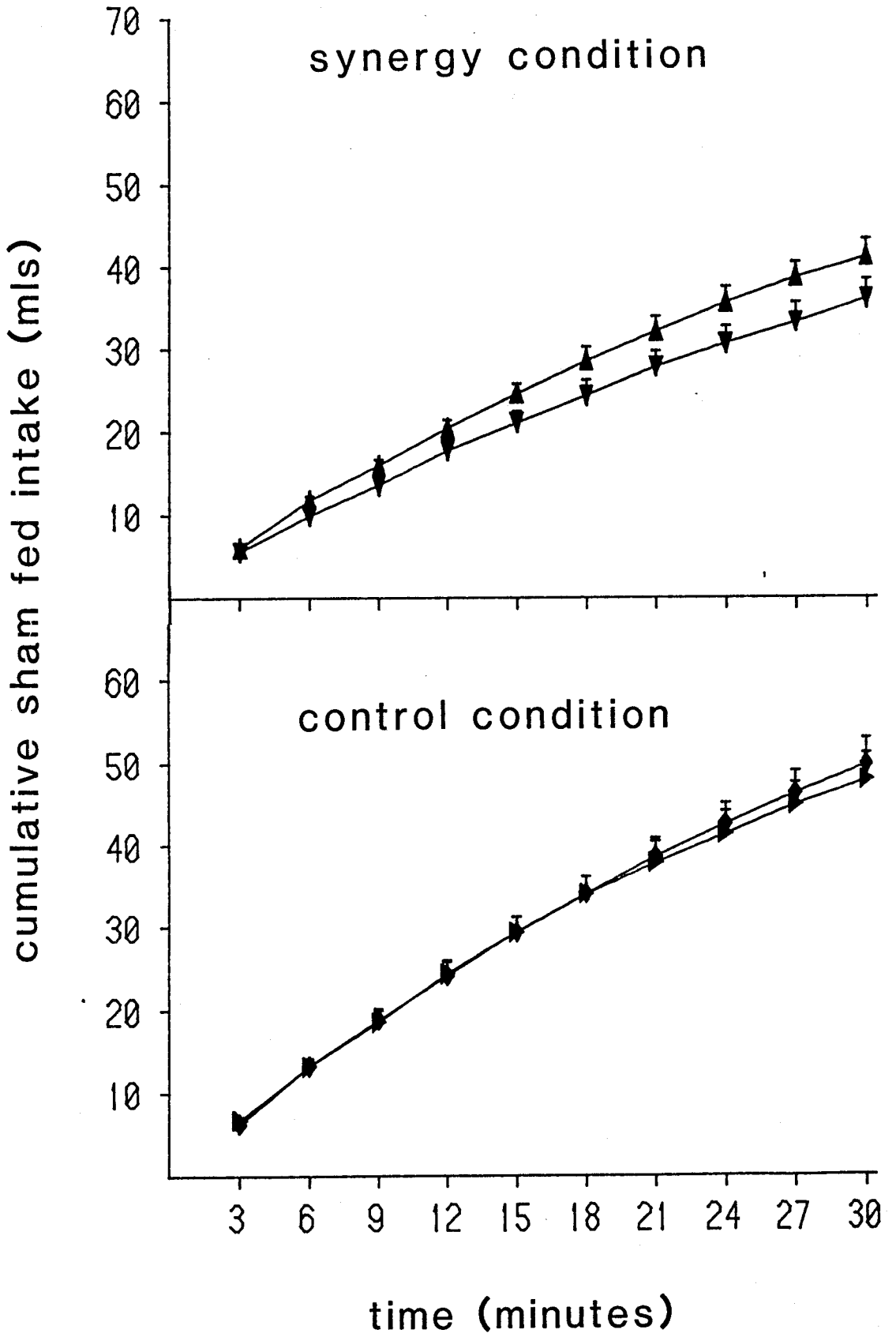
A

prior s.f.	<u>wait</u>	<u>sham feed</u>	<u>sham feed</u>
control	<u>wait</u>	<u>wait</u>	<u>sham feed</u>


 CCK-8
 or
 saline

B

Figure 10. Group average cumulative sham fed intakes for the 4 test conditions used in this experiment. Synergy condition: following saline () and 5.6 ug/kg CCK-8 (). Control condition: following saline () and 5.6 ug/kg CCK-8 (). All injections are given at time T-15 min. Vertical lines represent 1 SEM.



ip injection decreases the amount eaten during the test sham feed. Specifically, on Control trials (i.e., with no prior sham feed) rats ate 49.8 +/- 3.1 mls in the 30 minute test feed following saline but only 41.4 +/- 2.1 mls in the same period in the saline condition with a previous sham feed (i.e., Synergy days). Since the amount fed under Synergy and Control conditions following saline injection were different, it was necessary to compare with CCK-8 trials to saline trials in the same condition. More importantly, however, Figure 10 and statistical analyses demonstrate clearly that under the test conditions used here, pairing of oropharyngeal stimulation with CCK enhanced the CCK-induced inhibition of eating during the test sham feed. CCK-8 injected 15 minutes prior to the initiation of sham feeding without any accompanying oropharyngeal stimulation (Control days) had no effect on the amount eaten. A correlated t-test comparing cumulative 30 minute intake on saline and CCK trial indicated no significant difference, $t(6) = 1.38$, $p > .2$. In contrast, when CCK was administered with accompanying oropharyngeal stimulation (Synergy condition) it produced a significant reduction of feeding relative to saline trials, $t(6) = 3.55$, $p < .02$. The ability of CCK-8 to suppress eating only when paired with oropharyngeal stimulation under these test conditions is corroborated by comparing the percent of eating suppression produced by CCK-8 in the two test conditions. In the Control condition, CCK produced a 0.1% +/- 2.2% suppression relative to saline trials. In contrast, CCK-8 on Synergy days resulted in a 14.4 +/- 3.9% suppression. These results indicate that the ability of CCK-8 to suppress eating is enhanced when its administration is accompanied by oropharyngeal stimulation.

EXPERIMENT 2B

The results of the first experiment indicate that oropharyngeal stimulation enhances the satiety produced by exogenously administered CCK-8. In the conventional sham feeding experiment using CCK-8, rats are permitted to feed for some period of time prior to the CCK-8 injection. The suppressive action of CCK-8 is assessed by its effects on sham intake subsequent to the injections. It has been reported that this prior sham feed increases the efficacy of CCK's satiety-inducing effects (Antin et al., 1978). This experiment examines whether this peptide administration regimen is more efficacious than simply presenting CCK-8 coincident with the initiation of sham feeding. In both cases, CCK is administered coincident with the test meal upon which its satiety influences are evaluated. In one condition, however, a period of oropharyngeal stimulation precedes the peptide injection.

METHOD

Chronic gastric cannulae were implanted in 8 rats weighing 286 +/- grams at the time of surgery.

Each test day rats were prepared for sham feeding, placed in test cages, and allowed to wait for 15 minutes. On all days, rats were given a 30 minute test sham feed of 10% sucrose. Coincident with the initiation of this test feed, rats were injected ip with either 5.6 ug/kg CCK-8 or an equivalent volume of .15 M saline. Two test conditions were employed. In the first (Prior S.F. days), rats sham feed for 15 minutes prior to ip injection. In the second (Control

days), rats were left undisturbed for the 15 minutes prior to the ip injection. Results were based on 3 CCK-8 trials and 3 saline trials in each of the two test conditions. This design is summarized in Figure 9B.

RESULTS AND DISCUSSION

Figure 11 shows the group average cumulative intakes for the 4 test conditions. As in experiment 1, the sham feeding experience prior to the test meal affected the amount eaten during the test sham feed: animals ate 45.2 +/- 5.2 mls in 30 minutes on Control trials but only 29.0 +/- 4.4 mls on Prior S.F. saline days. Most importantly, however, separate correlated t-tests comparing the amount eaten following CCK and saline under each of the two test conditions revealed that CCK significantly reduced the amount sham fed both when it was injected with (Prior S.F. condition), $t(7) = 2.56$, $p < .05$, and without (Control condition), $t(7) = 3.10$, $p < .02$, a prior sham feed. Percent suppression scores were used to compare the amount of intake inhibition produced by CCK in the two test conditions. These results are presented in Table 3 and indicate that the two modes of CCK administration produced similar magnitudes and patterns of food intake inhibition.

Figure 11. Group average cumulative sham fed intakes for the 4 test conditions used. Prior S.F. Condition: following saline () and 5.6 ug/kg CCK-8 (). Control condition: following saline () and 5.6 ug/kg CCK-8 (). All injections are administered at time T0 min. Vertical lines represent 1 SEM.

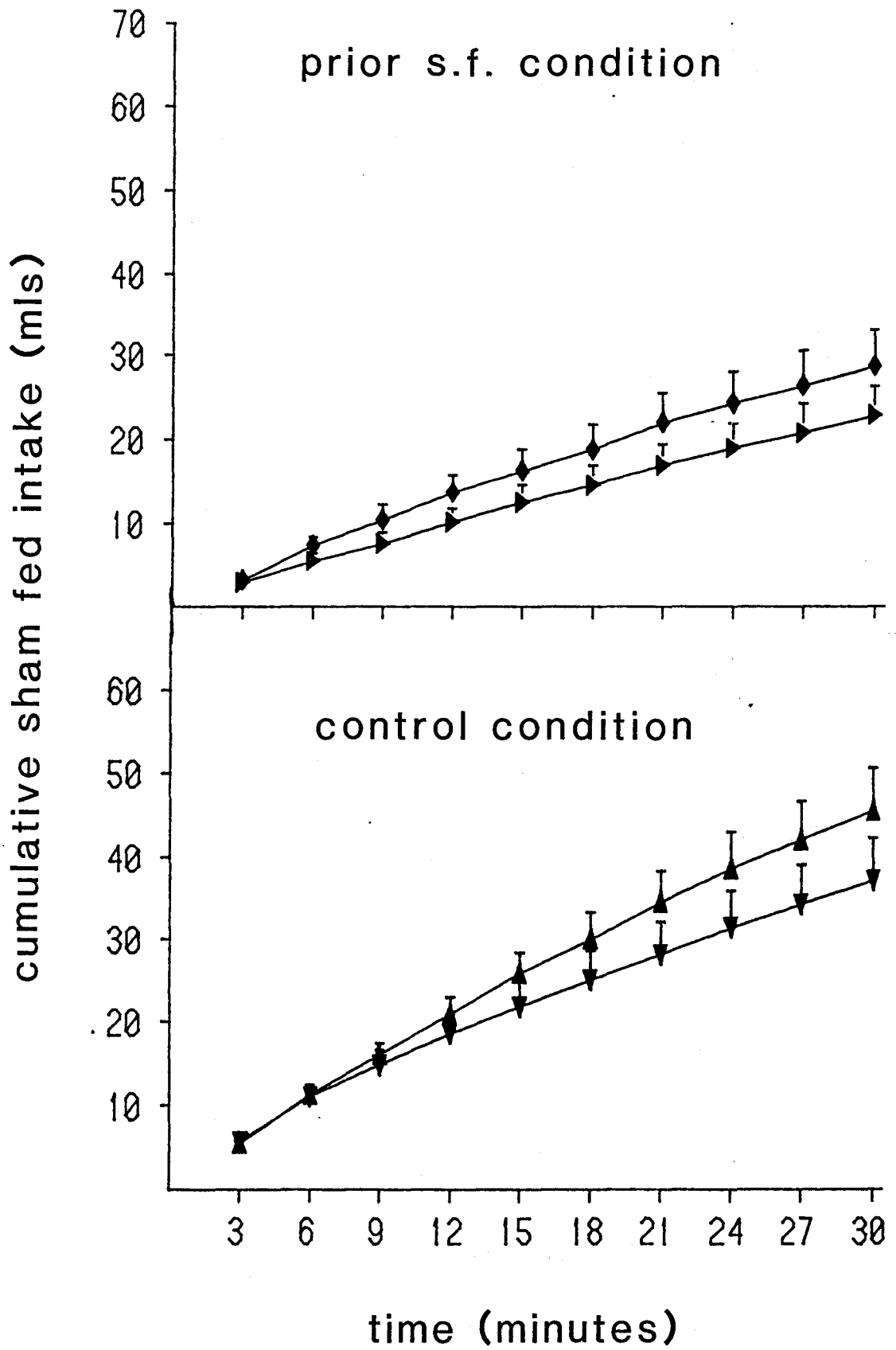


Table 3. Parameters of suppression (see General Methods for definitions) produced when 5.6 ug/kg CCK-8 is injected at time T0 min with either a prior sham feed (Prior S.F. condition) or no prior sham feed (Control condition).

	control condition	prior s.f. condition
size (%) of maximum suppression	52.1 ± 6.2	50.1 ± 7.7
time (min) to maximum suppression	17.6 ± 2.2	14.3 ± 2.6
time (min) to onset of suppression	8.6 ± 3.1	9.4 ± 3.3
time (min) to offset of suppression	21.0 ± 3.5	18.4 ± 2.7

DISCUSSION OF EXPERIMENT 2

This experiment was designed to assess the importance of oropharyngeal stimulation in the satiety produced by exogenously administered CCK. Two major findings are presented. First, oropharyngeal stimulation enhances the satiety action of CCK. This is shown most clearly in experiment 2a which demonstrates that CCK-8 has no effect on the amount eaten at a test meal begun 15 minutes after peptide injection. However, pairing the peptide injection with oropharyngeal stimulation does make a suppressive effect of CCK-8 apparent. Since the time period between peptide injection and eating, as well as what the rats do during that interval, are identical in the two conditions, the most parsimonious conclusion is that oropharyngeal stimulation interacts with CCK and amplifies its satiety effect. The second major finding is that the amount and pattern of eating inhibition produced by CCK are equivalent regardless of whether the peptide is administered coincident with, or some time after, the initiation of sham feeding.

These results corroborate some aspects of the original oropharyngeal synergy report but contradict others (Antin et al., 1978). The data of this experiment support an important role for oropharyngeal stimulation in enhancing CCK's effects. However, unlike Antin et al. (1978), it was not found that CCK was more efficacious administered after the initiation of sham feeding compared to injections coincident with the initiation of sham feeding. Several differences between the protocols of the two studies may account for this discrepancy. The most

noteworthy difference is that Antin et al. (1978) administered 20% pure CCK; in this experiment the synthetic pure octapeptide of CCK was used. The extract with 20% pure CCK is reported to contain another potent satiety agent(s) other than CCK (Lorenz et al., 1979). It is possible that this other agent present in the impure extract, and not the CCK itself, interacts with oropharyngeal stimulation in such a way as to reveal an increased enhancement when the impure peptide is injected following some prior period of sham feeding. A second possibility is that the difference in the way suppression is measured may account for the discrepancy. Antin et al., (1978) measured the suppressive effect of CCK beginning 12 minutes after peptide administration rather than immediately after peptide injection as is done in this study. However, even evaluating the data reported here by the method of Antin et al., indicates peptide given coincident with meal initiation is no less effective in producing satiety than CCK injected after some period of sham feeding.

GENERAL DISCUSSION

The aim of this thesis was to extend our understanding of the inhibition of feeding produced by exogenous administration of the gastrointestinal peptide cholecystokinin. Two major aspects of CCK-induced satiety were examined: the kinetics of the inhibition of feeding produced by CCK and the interaction of CCK-induced satiety with oropharyngeal stimulation. It was hoped that these studies would provide information relevant to an understanding of the mechanism by which CCK produces satiety and an assessment of the eventual utility or constraints of gut peptides in the therapeutic situation to treat feeding disorders.

The time course data or kinetics, have implications for both these issues. In terms of clinical significance the timing of peptide administration relative to the temporal parameters of the meal, such as meal initiation and termination, are important. Two points may be made which bear on the timing of CCK administration relative to the meal. First, CCK must be administered near the time that feeding begins or some time after eating has begun. It was shown in Experiment 2b that the peptide was equally effective in producing satiety when administered coincident with the initiation of feeding as when delivered after feeding had begun. Experiment 1b demonstrates clearly that the administration of CCK as little as 15 minutes before the initiation of feeding is ineffective in suppressing intake. This is a fortunate aspect of CCK's action since the subject does not have to perfectly

anticipate a meal so that the CCK could be taken the required amount of time before meal initiation. Instead the meal needn't be anticipated in advance since the peptide may be taken at the same time as the meal begins. Second, the kinetics data reveal that there is a fixed interval of time over which CCK suppresses feeding and the duration of this interval cannot be changed through changes in the dose of the peptide. Experiment 1a demonstrated that both the duration of suppression and the time at which the maximum suppression occurred, as measured from the beginning of the meal, were unaffected by changes in the dose of CCK. This also demonstrates that it would not be possible to deliver large doses of the peptide in order to suppress intake in subsequent meals but that a separate administration of CCK would have to precede each meal.

While increasing the dose of CCK does not affect the time that the suppression of feeding ends or the time that the maximum suppression occurs, it does affect the size of the maximum suppression. Hence larger doses of peptide would be useful in more profoundly inhibiting feeding in the meal immediately following its administration.

Finally observations of the time course of the CCK effect beyond the 30 minute testsham feed reveal that animals do not compensate for peptide-induced suppression by increasing their intake later during that meal. Campbell and Smith (1983) have shown that chronic administration of CCK-8 to obese Zucker rats results in a long-term reduction in body weight levels and caloric consumption. These two observations taken together suggest that subjects will not compensate for CCK-induced reductions in feeding either by eating more in that meal or by taking larger meals following repeated peptide administration.

The kinetics data also provide some insights into the mechanism of CCK in producing satiety. One possible conclusion from these data is that CCK functions as a signal to initiate the behavioral event satiety but that the duration of satiety is independent of the interaction of CCK with its receptor. Experiment 1a showed that some parameters of the CCK-induced suppression of feeding changed as a function of dose and others, such as the duration of suppression, were unaffected. These data are incompatible with the simple notion that all the parameters of CCK-induced satiety are a function of the number of receptors occupied by CCK since the duration of suppression did not change as the dose of peptide, and hence the number of occupied receptors, increased. The view that the parameters of CCK-induced satiety are proportional to the number of occupied receptors may account for the increase in the size of maximum suppression may be proportional to the number of receptors which are occupied. However, this view cannot account for the observation that the time at which maximum suppression occurs and the offset time of suppression do not change as dose is increased. Since an increase in the peptide's dose will increase the number of occupied receptors these parameters are not proportional to the number of occupied receptors. The most parsimonious interpretation of these data is that the strength of the satiety signal is proportional to the number of receptors which are occupied and once this signal is received it initiates the behavioral events which are satiety, and the duration of satiety is independent of the number of receptors occupied once the signal is received. This interpretation may be tested by the administration of a selective antagonist of CCK's

satiety inducing effects. The antagonist would be injected a few minutes after the delivery of CCK. Presumably this would allow CCK to interact with its receptor long enough to signal satiety and would then be displaced by the competitive antagonist. If it is true that satiety has a duration which is independent of the receptor event once it is signalled then the duration of satiety produced should be unaffected by the presence of the antagonist.

This study has also shown that the satiety produced by CCK interacts with a concomitant of feeding. The major finding of Experiment 2 is that oropharyngeal stimulation enhances the satiety produced by exogenous CCK. Two classes of explanation may be invoked to explain how oropharyngeal stimulation enhances the satiety-inducing effects of CCK. First, oropharyngeal stimulation might increase the strength of the event produced by CCK which signals satiety. For example, oropharyngeal stimulation might increase the number of CCK receptors occupied either by increasing the affinity of the receptor or by decreasing peptide degradation. As well, oropharyngeal stimulation might enhance the output of the receptor system by which CCK produces its effects. Alternatively, CCK might simply change the interpretation of the signal produced by CCK. Specifically, CCK's satiety signal, which appears to involve the gastric afferent fibre, may be interpreted as relevant to the cessation of feeding only in those cases when it is received coincidentally with oropharyngeal stimulation. The data reported here do not permit a discrimination between these two classes of explanation. In fact, ignorance about the location or action of CCK receptors make both of these explanations not directly testable.

More generally, the data reported here illustrate the advantages of evaluating the ability of CCK to reduce feeding using time course data rather than the convention in the literature of expressing CCK's satiety actions as suppressions (relative to saline) based on cumulative consumption of diet over long intervals, eg. 30 minutes. Time course data are superior to evaluating CCK's effects using cumulative intakes for two reasons. First, cumulative intakes obscure important information about CCK-induced reductions in eating. For example, it is widely reported by comparing cumulative intake of diet after saline to intake after CCK-8 that larger doses of peptide further suppresses feeding. But it has never been reported which parameters of the suppression are affected as dose increases. The analysis of time course data in this experiment has shown that a more profound suppression occurs through an increase in the size of the maximum suppression and a decrease in the onset time to suppression. Second, and most importantly, when comparing the cumulative consumption after saline versus CCK-8 injection whether or not a significant reduction in feeding is reported depends on the time, relative to the start of the meal, at which the cumulative consumptions are compared. This is dramatically demonstrated by the results of Experiment 1c where it was shown that 5.6 ug/kg Ac-CCK-8 injected at T-15 did not reduce intake when the cumulative 30-minute intake following saline was shown not to be significantly different than the cumulative consumption following Ac-CCK-8. However, a comparison of the 15-minute cumulative consumption following saline versus Ac-CCK-8 revealed that a significant reduction in intake had occurred. Inspection of Figure 7 which presents the time

course of suppression produced by Ac-CCK-8 reveals the reason for these apparently discrepant results: the suppression occurs early in the interval and is small compared to cumulative consumption at 30 minutes after meal initiation. Hence, the use of cumulative consumption to assess whether CCK inhibits feeding is a tool of poor sensitivity since its accuracy depends on when, during the course of the meal, it is applied.

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APPENDIX

The purpose of this experiment was to compare the duration of the satiety effect produced by CCK-8 and Ac-CCK-8.

METHOD

Chronic gastric cannulae were implanted in 7 rats weighing 338 +/- 42 grams at the time of surgery. Each test day rats were prepared for sham feeding and sham fed a 10% sucrose (w/v) for 60 minutes. Rats were injected ip at T0 with 5.6 ug/kg CCK-8, 5.6 ug/kg Ac-CCK-8, or .15 M saline. CCK-8 was tested 3 times, Ac-CCK-8 4 times, and saline injections were repeated 7 times.

RESULTS AND DISCUSSION

The effect of administering 5.6 ug/kg CCK-8, 5.6 ug/kg Ac-CCK-8 at the same time as the initiation of sham feeding is summarized in Figure 12 which shows the sham fed intake in each 3-minute time bin following CCK-8, Ac-CCK-8, and saline. Inspection of Figure 12 confirms that both CCK-8 and Ac-CCK-8 suppress feeding. More importantly, Figure 12 reveals that the duration of suppression produced by Ac-CCK-8 is approximately twice as long as that produced by the same dose of CCK-8.

Figure 12. Group average sham fed intakes for consecutive 3-min time bins throughout the test sham feed following injections of saline (), 5.6 ug/kg CCK-8 () or 5.6 ug/kg Ac-CCK-8 () injected at time T0 min. Vertical lines represent 1 SEM.

