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Peripheral Injections of Bombesin and Cholecystokinin Affect Dietary Self-Selection in Rats[1]

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AVERY, D. D. AND M. LIVOSKY. *Peripheral injections of bombesin and cholecystokinin affect dietary self-selection in rats.* PHARMACOL BIOCHEM BEHAV **25**(1) 7–11, 1986.—Patterns of dietary self-selection were examined in adult female rats following peripheral injections of either bombesin (BBS) (6, 10, 14 and 16 μg) or cholecystokinin octapeptide (CCK-8) (0.75, 1.5, 2.25, and 3.0 μg). Animals were food deprived for 18 hours and then offered three isocaloric diets (protein, carbohydrate, and fat) following injections of peptides. Each subject received each of 4 doses of both peptides in a within-subjects design. All doses of BBS decreased total food intake and fat intake 30 minutes following injections. Also at this time period the two highest doses suppressed carbohydrate intake, while protein was unaffected. Cumulative intake at one hour revealed that total intake remained suppressed. The two highest doses continued to suppress carbohydrate intake, while only the 14 μg dose continued to suppress fat intake. Additionally protein was now significantly suppressed by all doses. The three highest doses of CCK-8 produced a decrease in total food intake and fat intake 30 minutes after injections. By one hour, only total intake remained suppressed but only with administration of the highest dose. Results are interpreted as providing support for the notion that BBS and CCK are physiological satiety signals and that they maintain unique functions in regulating food intake.

Bombesin Cholecystokinin Dietary self-selection Isocaloric diets

THE role of neuropeptides in the control of behavior has received considerable attention over the past two decades [13]. Two of these peptides, bombesin (BBS) and cholecystokinin (CCK), have been implicated in the control of feeding behavior. BBS, a tetradecapeptide originally derived from amphibian skin, has been shown to be widely distributed in vertebrate tissue [3,4]. CCK, a polypeptide of 33 amino acids, was first identified as a stimulant of gall-bladder contractions [10] and has since been considered to play a major role in the feeding process. The presence of BBS and CCK in both the central nervous system and the gastrointestinal tract, coupled with their ability to decrease food intake in a number of different species and under varying conditions, has led investigators to postulate a gut-brain axis, whereby feeding is mediated by peptide-directed brain activity [1, 5, 9]. In this process, BBS and CCK are viewed as physiological satiety signals. An alternative explanation, however, is that administration of BBS and/or CCK, either peripherally or centrally, leads to a condition of malaise, not satiety, which manifests itself in decreased feeding behavior [6–8]. Efforts have continued to provide evidence for or against either of these positions.

In addition, BBS has been shown to modulate the release of CCK from the gut [2,12]. It is possible, then, that BBS functions only as a signal for CCK, with CCK serving as the actual putative satiety signal. A number of physiological differences between the peptides have been noted, however. Specific and separate binding sites have been identified for BBS and CCK in pancreatic tissue [16], and it is possible to eliminate the effects of CCK-induced hypophagia, but not BBS-induced hypophagia, by severing the vagus nerve [18,19]. Such information suggests that different modes of operation characterize BBS and CCK, although they have yet to be elucidated.

A useful paradigm for the examination of both the quantitative and qualitative aspects of feeding is one which provides access to isocaloric diets in a self-selection design. The well-documented ability of animals to select and qualitatively monitor nutrient intake is at the basis of this paradigm [15,17]. A number of studies have looked at the effects of drugs on specific macronutrient selection and have identified shifts in consumed nutrients following administration of particular substances [11,14]. Calisher (unpublished doctoral dissertation) examined the effects of centrally administered BBS and CCK-8 on dietary self-selection. Rats received central injections of BBS and CCK-8 in varying doses. With moderate doses of BBS, a selective suppression of fat intake was observed at two and six hours following injections. A suppression of protein intake two hours after injections was also noted. The largest dose of CCK-8 yielded a suppression

[1]This research was a portion of a master's thesis submitted to Colorado State University.

TABLE 1
SELF-SELECTION DIETARY COMPONENTS*

Protein		Carbohydrate		Fat	
960 g	Vitamin-free Casein (ICN Pharmaceuticals, Cleveland, OH)	580 g	Corn Starch (Teklad Test, diets, Madison, WI)	404 g	Hydrogenated Vegetable Fat (Crisco)
				22 g	Safflower Oil (Hollywood Health Foods)
40 g	U.S.P. XIV Salt Mixture (ICN Pharmaceuticals)	280 g	Dextrin (Teklad Test Diets)		
		100 g	Commercial-grade Sucrose	267 g	Alphacel Non-nutritive Bulk (ICN Pharmaceuticals)
22 g	Vitamin Diet Fortification Mixture (ICN Pharmaceuticals)	40 g	U.S.P. XIV Salt Mixture (ICN Pharmaceuticals)	267 g	Petroleum Jelly
				40 g	U.S.P. XIV Salt Mixture (ICN Pharmaceuticals)
		22 g	Vitamin Diet Fortification Mixture (ICN Pharmaceuticals)	22 g	Vitamin Diet Fortification Mixture (ICN Pharmaceuticals)

From "Increased fat consumption induced by morphine administration in rats," by R. Marks-Kaufman. *Pharmac; Biochem Behav 16:* 950, 1982.
*Each dietary component has a caloric density of 3.8 kcal/g.

of fat intake for the entire six hours. In a study that compared the self-selection patterns of CCK-8 with lithium chloride [20], CCK-8 was again shown to reduce fat intake. The investigators also found that carbohydrate consumption was lowered, while protein consumption was spared, in their young (70-day-old) male rats. The demonstrated pattern of suppression by lithium chloride, a known toxic substance, was opposite that shown for CCK-8.

The following experiments were conducted to examine the role of peripheral injections of BBS and CCK-8 on the self-selection process. Determination of a selective suppression of macronutrients (protein, carbohydrate, and fat) would lend further support for the notion that the two peptides function as physiological satiety signals and do not decrease food intake by making animals ill. Additionally, if distinct patterns of macronutrient selection are evident, they can then be compared and contrasted. Differential selectivity produced by BBS and CCK would provide further evidence of their unique functions in the feeding process.

METHOD

Subjects

Fourteen female Sprague-Dawley rats (Charles River Strain), weighing between 250 and 300 grams at the onset of the study, were housed in individual hanging wire cages in a temperature-controlled (23±1°C) room maintained on a 12:12 light/dark cycle. Ad lib access to dietary components was provided except for the 18-hour deprivation period prior to injection. Tap water was continuously available.

Peptide Preparation

CCK-8 (a gift from the Salk Institute, La Jolla, CA) and BBS (Sigma Chemical Company, St. Louis, MO) were obtained in powder form and reconstituted with sterile distilled water. Peptides were diluted in 0.9% normal saline for administration intraperitoneally. Injection volumes ranged from 0.25 to 0.40 ml. Injection doses of BBS were: 6, 10, 14 and 16 μg/kg body weight. Doses of CCK were: 0.75, 1.5, 2.25, and 3.0 μg/kg body weight. Each subject received each dose and a control injection of saline.

Diets

The self-selection diet consisted of three separate rations—protein, carbohydrate, and fat. Specific components of each diet are listed in Table 1. All diets had a caloric density of 3.8 kcal/g. Rations were presented in individual plastic feeding cups fastened to the front of each subject's cage. Location of the cups was varied randomly each day, and a block of wood was placed in each cage to prevent excessive tooth growth.

Procedure

Animals were screened for acceptance of the diets. Subjects who consumed consistent and sufficient amounts of all three components (greater than an average of 2.0 g per day) were included in the experiment. A six day baseline period under free-feeding conditions was conducted, followed by 13 days of habituation to the 18-hour deprivation period. A control injection of saline was administered to each animal as their first injection. Animals were then randomly assigned to receive BBS or CCK initially, where they were given injections of each of the four doses. This was followed by injections of all four doses of the alternate peptide. Specific doses were randomly determined on the day of injection. Dose sequence for the group receiving BBS first and CCK-8 second was: (BBS) 6, 10, 16, 14 μg/kg, (CCK) 0.75, 1.5, 3.0, and

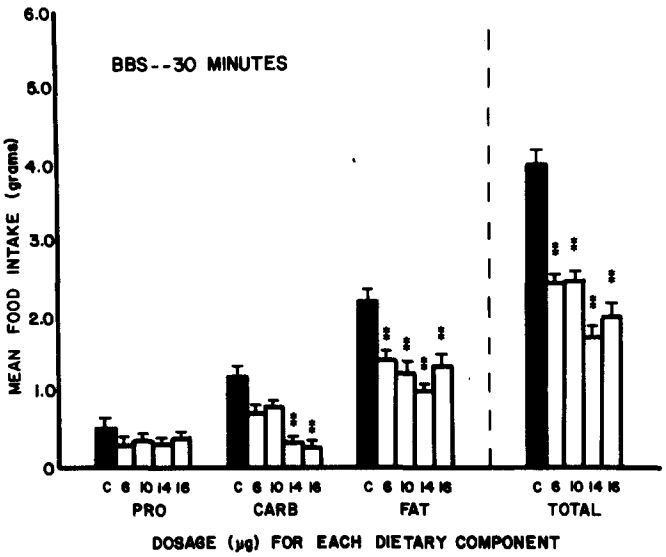

FIG. 1. Mean (+S.E.M.) protein, carbohydrate, fat and total food intake during the 30-minute period following peripheral injections of bombesin (6 μg, 10 μg, 14 μg, and 16 μg) and saline control. *$p<0.05$ vs. saline control; **$p<0.01$ vs. saline control.

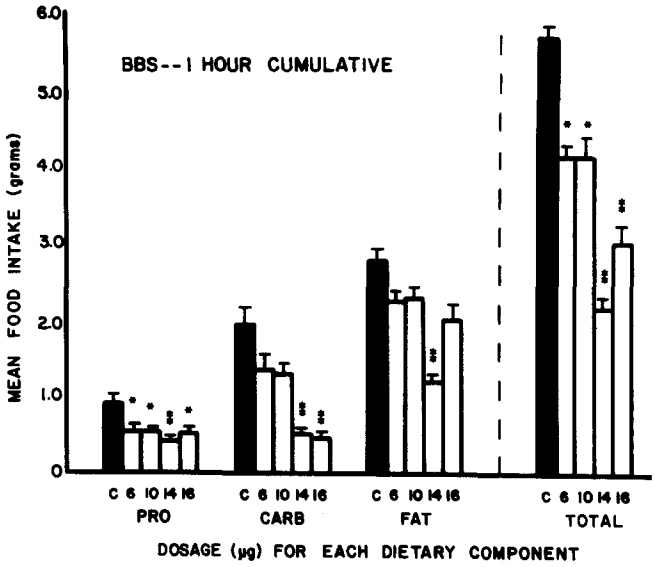

FIG. 2. Mean (+S.E.M.) protein, carbohydrate, fat and total food intake during the 1-hour cumulative period following peripheral injections of bombesin (6 μg, 10 μg, 14 μg, and 16 μg) and saline control. *$p<0.05$ vs. saline control; **$p<0.01$ vs. saline control.

2.25 μg/kg. For the subjects receiving CCK-8 initially, followed by BBS, the sequence of doses was: (CCK-8) 2.25, 0.75, 1.5, 3.0 μg/kg, (BBS) 14, 6, 10 and 16 μg/kg. Injections were separated by two recovery days. Food consumption on each second recovery day was checked against baseline levels (ANOVA, $p>0.05$). Subsequent injections were not administered unless the amount of macronutrients consumed on recovery days did not differ significantly from baseline.

Following injection, subjects were immediately placed in their home cages, where a premeasured amount of each of the three diets was available. Amount consumed was measured at 30, 60, and 120 minutes, and at 6 hours after injection. Food spillage was collected on mats underneath each subject's cage and deducted from the appropriate measure of dietary component.

Data Analysis

A within-subjects multivariate analysis of variance was performed with the three dependent variables (protein, carbohydrate, and fat) for BBS and CCK. There were six levels of dose (baseline, control injection, and four treatment doses). Separate analyses were conducted at each time period. In addition, cumulative analyses at one, two and six hours were performed. Both interval and cumulative measures were taken to determine the peptides' temporal effectiveness and to examine any alterations in dietary preference. Diets were examined separately and also in combination to assess both the amount of specific diet eaten and total amount of food consumed at each time period. Post hoc analyses were conducted using the Newman-Keuls procedure. Significance of peptide effects was evaluated against control injection measures.

RESULTS

Bombesin

Total food intake 30 minutes following injections of all doses of BBS showed a significant univariate value, $F(5,65)=27.97$, $p<0.01$. A significant decrease in food intake was obtained. With the use of Hotelling's criterion (a special type of multivariate analysis in which at least two groups comprise the independent variable), the combined dependent variables were significantly affected by the independent variable, $F(15,185)=10.86$, $p<0.01$, during this 30 minute period. Significant univariate values were found for protein, carbohydrate, and fat. Subsequent post hoc analyses revealed a selective suppression at this time period. With the carbohydrate diet, the two highest doses (14 and 16 μg) significantly decreased intake ($p<0.01$), and for fat, all doses significantly suppressed fat intake ($p<0.01$).

Between 30 and 60 minutes, the two highest doses produced a significant suppression of the protein diet ($p<0.05$). No other significant results occurred during this time.

Between 60 and 120 minutes, total food intake was significantly affected, $F(5,65)=5.67$, $p<0.01$; all doses except the 14 μg dose produced a significant increase in total food consumption. Multivariate significance was observed at this time period for the separate diets, $F(5,65)=3.1$, $p<0.01$. In the univariate analysis, however, only protein, $F(5,65)=2.5$, $p<0.05$, and carbohydrate, $F(5,65)=4.7$, $p<0.01$, showed significant overall F values. Post hoc testing indicated differences only in the carbohydrate diet. The three highest doses significantly increased food consumption ($p<0.01$, 16 μg dose; $p<0.05$, 10 and 14 μg doses).

At six hours following injection, only the 14 μg dose continued to effect total food intake. With injections of this dose, food consumption showed a significant increase ($p<0.01$). Similarly, the 14 μg dose continued to effect the fat dietary component, producing significantly greater food intake ($p<0.05$) 6 hours after injection.

In the one hour cumulative analysis, all doses produced a significant suppression of food intake ($p<0.01$ for the 14 and 16 μg doses; $p<0.05$ for the 6 and 10 μg doses). Multivariate significance was obtained for dose, $F(15,185)=11.66$,

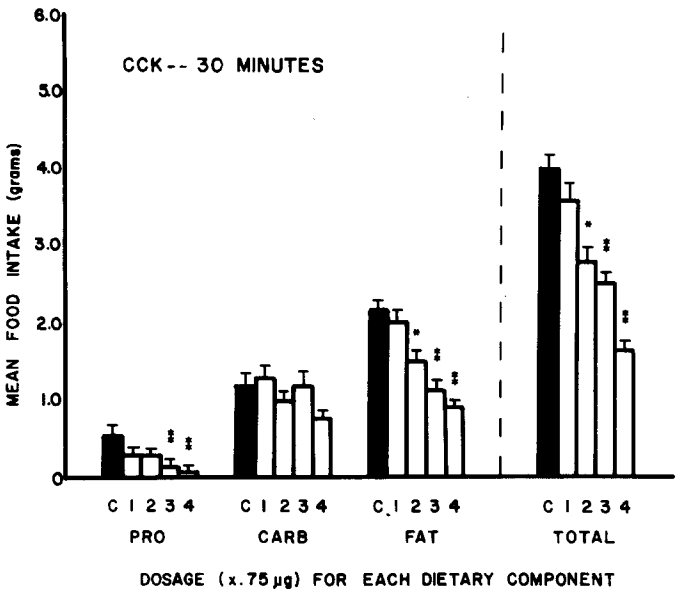

FIG. 3. Mean (+S.E.M.) protein, carbohydrate, fat and total food intake during the 30-minute period following peripheral injections of cholecystokinin (0.75 µg, 1.5 µg, 2.25 µg, and 3.0 µg) and saline control. *$p<0.05$ vs. saline control; **$p<0.01$ vs. saline control.

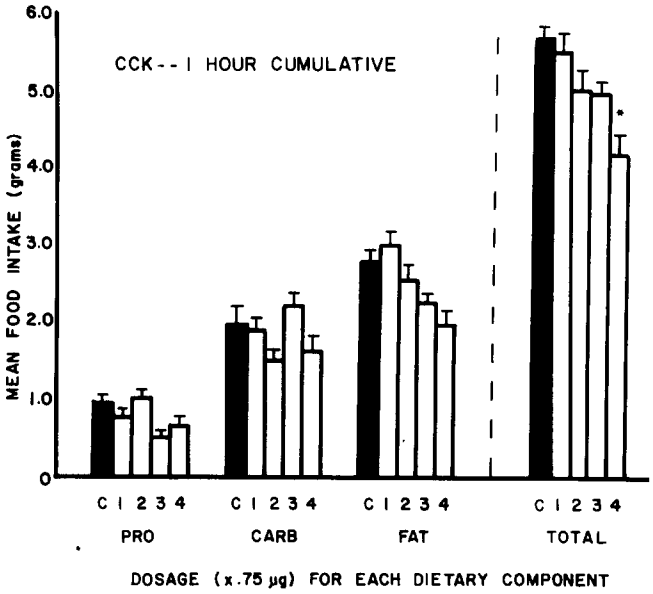

FIG. 4. Mean (+S.E.M.) protein, carbohydrate, fat and total food intake during the 1-hour cumulative period following peripheral injections of cholecystokinin (0.75 µg, 1.5 µg, 2.25 µg, and 3.0 µg) and saline control. *$p<0.05$ vs. saline control; **$p<0.01$ vs. saline control.

$p<0.01$, at this time period. Separate univariate analyses of each diet indicated that protein, $F(5,65)=7.3$, $p<0.01$, carbohydrate, $F(5,65)=19.15$, $p<0.01$, and fat, $F(5,65)=9.5$, $p<0.01$, were significantly affected. Post hoc analyses for protein showed that all treatment doses produced a significant decrease in food intake ($p<0.05$ for 6, 10 and 16 µg doses; $p<0.01$ for the 14 µg dose). For carbohydrate, both the 14 and 16 µg doses yielded a significant decrease in consumption of this macronutrient ($p<0.01$). In the fat diet, the 14 µg dose produced a significant decrease in food intake ($p<0.01$).

Cumulative data at two hours showed a significant overall value, $F(5,65)=13.1$, $p<0.01$. The post hoc analysis showed that the 14 µg dose significantly suppressed intake of total food at this time period. No other significant differences were noted.

No significant differences were noted with respect to cumulative intake six hours after injection.

Cholecystokinin Octapeptide

For the 30 minute period following injection, the univariate value for total food intake was significant, $F(5,65)=19.92$, $p<0.01$. The three highest doses ($p<0.01$, 2.25 and 3.0 µg doses; $p<0.05$, 1.5 µg dose) produced a significant decrease in food intake. Making use of Hotelling's criterion, the combined dependent variables were shown to be significantly affected by the independent variable, $F(15,185)=7.87$, $p<0.01$. Significant univariate values were found for protein, $F(5,65)=7.9$, $p<0.01$, carbohydrate, $F(5,65)=4.29$, $p<0.01$, and fat, $F(5,65)=12.15$, $p<0.01$. In the post hoc analysis, the two highest doses significantly suppressed intake of protein ($p<0.01$). For the carbohydrate diet, no significant differences were found. In the fat component, the three highest doses significantly suppressed fat intake ($p<0.01$, 2.25 and 3.0 µg doses; $p<0.05$, 1.5 µg dose).

Between 30 and 60 minutes following injections, no significant differences were found.

Between 60 and 120 minutes, the highest dose (3.0 µg) significantly increased total food intake ($p<0.01$), but no other differences were noted. At six hours, no significant differences occurred.

For the one hour cumulative period, a significant univariate value for total intake was obtained, $F(5,65)=11.54$, $p<0.01$. The 3.0 µg dose significantly suppressed total food intake ($p<0.05$). No other significant differences at this time period were noted. Also, cumulative intake at two and six hours revealed no significant differences among total intake or any of the dietary components.

DISCUSSION

The results support the prediction that peripheral injections of BBS and CCK suppress food intake in food deprived rats and that the suppression occurs selectively. The major effects are of a relatively short duration, most potent during the first hour. This is consistent with a peripheral mode of administration, where injected substances enter general circulation and are subject to the degradation and breakdown processes of normal metabolism. Both peptides suppressed overall food intake, a result consistent with a number of previous studies [1, 5, 7, 9, 18, 19]. BBS selectively suppressed fat and carbohydrate consumption at 30 minutes and protein after one hour. In comparison, CCK-8 administration reduced fat and total intake in a dose-dependent manner 30 minutes following injections. The two highest doses were also effective in reducing the amount of protein consumed at 30 minutes. This difference in selective suppression may indicate the existence of distinct and separate mechanisms of

action and, hence, unique roles for BBS and CCK in the feeding process.

The increase in food consumption that occurred between early and late intervals presumably reflects an attempt on the part of the animal to ingest the needed amounts of nutrients (satiate) following the deprivation period. Typically, food consumption is greatest during the first 30 minutes that food becomes available. The subjects appeared to be compensating for the induced suppression by consuming more than baseline levels during the latter parts of the test period. This, again, points to the relatively short time of effectiveness of peripherally administered BBS and CCK-8.

The selective decrease of macronutrients evident here may be interpreted as providing additional evidence that BBS and CCK are physiological satiety signals and do not decrease food intake by producing malaise, as it would be unlikely that illness could generate a particular pattern of decreased consumption. Rather, suppression would be expected to occur randomly across all dietary components. These results are in agreement with those of Calisher (unpublished doctoral dissertation) who found selective suppression of macronutrients following central injections of BBS and CCK-8. BBS, when administered centrally, decreased protein consumption up to two hours following injections. Similarly, intraperitoneal injections of BBS decreased protein consumption during the first hour after injection. Centrally administered BBS also resulted in an increase in the cumulative carbohydrate intake six hours after injection. Central injections of the highest dose of CCK-8 resulted in a decrease in fat consumption that persisted throughout the six hours. The three highest doses of CCK-8, when given peripherally, reduced consumption of the fat diet for 30 minutes in what appears to be a dose-dependent manner. These results are consistent with the available information on CCK-8 as a stimulator of gall-bladder contractions and with the speculation that it may be specific for fat metabolism [20]. In addition, the results of this research are in agreement with the VanderWeele *et al.* [20] study with respect to CCK-8's effectiveness in lowering consumption of a fat diet. VanderWeele *et al.* [20] did not, however, find a decrease in protein consumption at any time during his investigation. Major differences between the two studies, such as peptide dose, diet composition, and age and maturational level of the subjects, may explain this inconsistency.

The existence of patterns of suppression among animals allowed to choose available macronutrients may reflect some underlying metabolic regulation. In addition, while the exact manner in which BBS and CCK exert their influence remains unspecified, the current results provide justification for continuing to search for their physiological mode of operation.

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