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Specificity of Cholecystokinin Satiety Effect: Reduction of Food but not Water Intake[1]

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MUELLER, K. AND S. HSIAO. *Specificity of cholecystokinin satiety effect: reduction of food but not water intake.* PHARMAC. BIOCHEM. BEHAV. 6(6) 643–646, 1977. – The C-terminal octapeptide of cholecystokinin (CCK) was injected into water-deprived rats to observe the effect on water and milk intake. The doses used were 0, 20, and 40 Ivy dog units/kg. The data indicated that milk intake was suppressed in a dose-related fashion within 2–4 min of injection but water intake was not suppressed. This satiety hormone is thus specific to food intake.

C-terminal octapeptide of cholecystokinin Water intake Liquid diet intake Water deprivation Satiety effect

CHOLECYSTOKININ (CCK) has recently been suggested to function as a short-term satiety-inducing hormone [1, 7, 8, 9, 10, 16, 19]. As chyme enters the duodenum, certain of its chemical properties – acidity, protein content and fat content – stimulate the release of CCK [2,14], which enters the bloodstream and presumably interacts with satiety centers in the brain [3] to terminate the meal.

Although a blood-borne satiety-inducing factor had been hypothesized earlier, clear evidence was not found until the blood of a satiated rat was thoroughly mixed with the blood of a hungry rat, resulting in a 50% reduction of food intake in the hungry rat [4]. The duodenal origin of the satiety inducing factor was shown with the duodenal infusion of varying substances resulting in satiety induction [16, 17, 19, 20] and with sham feeding studies in which rats with open gastric fistulas appeared satiated only when the fistulas were closed or CCK was administered [9]. CCK appears to be the active component of enterogastrone, a hormone derived from hog duodenum, which was shown to reduce food intake in mice [18].

A physiological satiety-inducing hormone would be expected to affect only ingestive behaviors. This criterion serves to eliminate the possibility that observed suppressions in intake may be due to general physio-behavioral effects such as sedation due to a tranquilizer or stimulation due to amphetamine. In order to explain a rat's normal feeding pattern a satiety-inducing hormone should be specific not only to ingestive behaviors in general but to food intake alone. Drinking normally occurs at the close of a meal – after the release of the hypothesized satiety-inducing factor. Because water intake is associated with the occurrence of satiety, the specificity of CCK has been investigated by observing the effect of exogenous CCK on water consumption. If water consumption – which is so behaviorally similar to liquid diet consumption – is unaffected by CCK, the likelihood that CCK suppresses food intake because of general physio-behavioral effects is very low.

The specificity of the suppression of intake induced by CCK is not clear. CCK has been observed to suppress water intake as well as food intake in mice [15]. On the other hand, it has been reported to reduce food intake in rats with either unchanged water intake or increased water intake [8,10]. If the temporary suppression of food intake caused by CCK is the result of a nonspecific mechanism interfering with consummatory behaviors a short-term satiety-inducing factor would have to be sought elsewhere.

The present study was undertaken to clarify the specificity of CCK with respect to food and water intake. If CCK is a nonspecific agent interfering with general consummatory activities different doses of the C-terminal octapeptide of CCK (biologically active part of CCK) should disrupt water intake as well as food intake in a dose-related fashion. If, on the other hand, CCK does function specifically as a short-term satiety signal for food intake, water intake should be unaffected by administration of the C-terminal octapeptide of CCK while liquid diet intake should be suppressed in a dose-related fashion.

METHOD

The animals were eight adult male Holtzman rats obtained from the Holtzman Company. The animals were housed individually and maintained on Wayne Laboratory

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pellets and tap water. Their body weight ranged from 370 to 470 g per rat throughout the experiments. The laboratory was lit on an artificial 12 hr light-dark cycle; training and testing occurred 2 hr before the end of the light cycle.

The animals were trained for four days to consume their daily water requirements in 30 min. Water was presented in 25 ml cylinders which were graduated to 0.1 ml and fitted with metal drinking spouts. Food was always available except during the 30 min training sessions and testing sessions. Thus, at the time of drinking the rats were approximately 23-1/2 hr water-deprived.

During testing 0.5 ml of either 0, 20, or 40 Ivy dog units/kg (U/kg) of the CCK-octapeptide was administered intraperitoneally. Each animal received a total of four injections (0, 0, 20, and 40), in counter-balanced orders. The daily sequences for the four injections were 0–20–0–40, 0–40–0–20, 20–0–40–0 and 40–0–20–0. Two control sessions were used to assess the possible effect of repeated treatments and also served to minimize the carry-over effect of a CCK treatment to the following CCK treatment. Two rats were randomly assigned to each sequence. The C-terminal octapeptide of CCK (SQ 19844, Squibb and Sons, Inc.) was stored in 0.5 N sodium bicarbonate and refrigerated. It was diluted for use with normal saline.

Experiment 1

Immediately following injection the animals were presented with 25 ml of room temperature tap water. Intake was recorded every 2 min until 14 min since injection had elapsed. Final intake was recorded at 30 min and water was removed.

Experiment 2

After Experiment 1 the same rats were used for Experiment 2. Immediately following injection the animals were presented with 25 ml of Similac infant formula with iron, diluted according to the manufacturer's instructions. Drinking tubes were refilled as necessary. Intake was recorded every 2 min until 14 min since injection had elapsed. Final intake was recorded after 30 min when Similac was removed and a 16 min water period was allowed to insure that the animals drank enough liquid for proper hydration. A two day habituation period to the liquid diet was allowed before data was collected. Experiment 2 was carried out in exactly the same manner as Experiment 1 with the exception of the replacement of water with liquid diet and the 16 min water recovery period.

RESULTS

Experiment 1

Analyses of variance were performed to compare cumulative mean water intake at successive 2-min intervals for all four condition means (2 control, 20 U/kg and 40 U/kg). When the overall condition difference was found to be significant Duncan's Multiple Range Test [5] was performed to compare the four means individually. The condition difference was significant, $F(3,21) = 3.9$, $p < 0.05$, only at the 4-min interval when the 20 U/kg intake mean was significantly higher than the means of the rest of the conditions. The difference was 1.5 ml or 2% of the control value. At no time were the two control means significantly different from each other. Figure 1 presents the cumulative mean water intake at successive 2-min intervals for the baseline (pooling the data from the two control sessions) and each of the two CCK treatments.

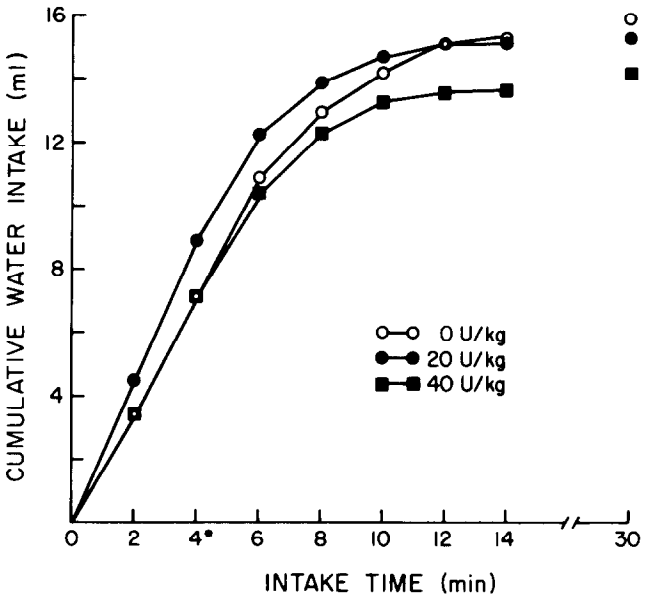

FIG. 1. Mean cumulative intake of water at 2-min intervals as a result of injection of 0 (Control), 20 or 40 Ivy dog units/kg of cholecystokinin. *Indicates that the means are significantly different ($p<0.05$): the 20 U/kg intake mean is significantly different from the rest.

Experiment 2

Analyses of variance were performed to compare cumulative mean liquid diet intake at successive 2-min intervals for all four condition means (2 control, 20 U/kg and 40 U/kg). Significant differences were indicated for the four conditions at all intervals except at the first interval of 2-min. Duncan's Multiple Range tests showed that at no time were the two control conditions significantly different from each other, but the control means were always significantly different from the two experimental means. The difference between 20 U/kg and 40 U/kg was significant at 10 min and later intervals. Figure 2 presents the cumulative mean intake of the liquid diet at successive 2-min intervals for the baseline (pooling the data from the two control sessions) and the two CCK treatments.

Mean intake of water during the recovery period was 4.5, 7.0, 7.8 and 10.1 ml, respectively, for the control Session 1, control session 2, 20 U/kg, and 40 U/kg. Analysis showed that the 40 U/kg mean was significantly greater than the three other means with no difference among the three.

DISCUSSION

The results indicate that the synthetic C-terminal octapeptide of CCK, which contains the biologically active portion of the molecule [14] does not suppress water intake, but clearly suppresses intake of liquid diet. The findings that under CCK treatments rats drank as much

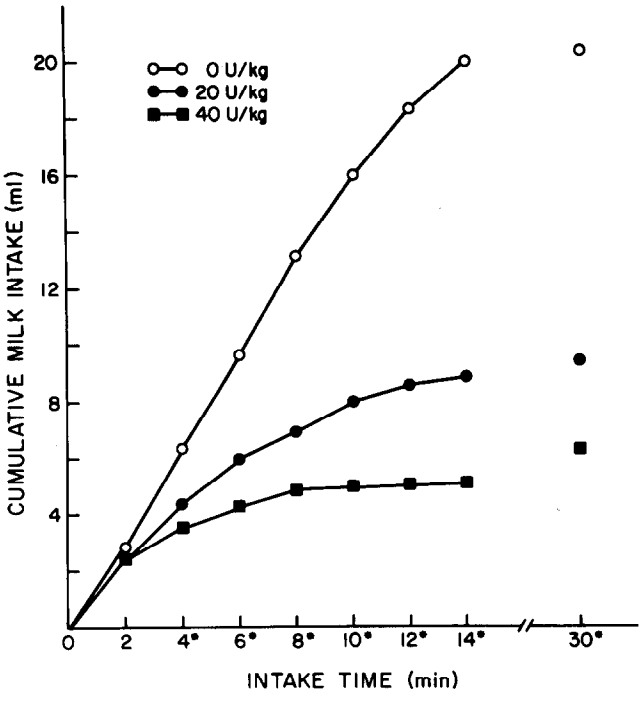

FIG. 2. Mean cumulative intake of milk at 2-min intervals as a result of injection of 0 (Control), 20 or 40 Ivy dog units/kg of cholecystokinin. *indicates that the means are significantly different ($p<0.05$): Up to 8 min 20 and 40 U/kg intake means are not significantly different, but each of them is different significantly from the control, however, after 10 min 20 and 40 U/kg intake means are significantly different from each other and also from the control intake.

water as the control and that the rats drank water during the water recovery period after the intake of liquid diet indicate that the animals were not generally depressed by CCK. Thus, the CCK suppressive effect is specific to food intake. However, it should be noted that CCK treatments did have some sedative effect. We observed that the animals generally laid down after CCK treatments. However, the sedative effect was so mild that animals could be aroused by a gentle blow of air and it did not affect water intake. A similar sedative effect was reported in terms of EEG responses as a result of CCK [6], however, fat infused into the duodenum resulted in the same EEG responses presumably due to the release of endogenous CCK. Thus, mild sedation may be a segment of the satiety syndrome rather than an effect which interferes with the ingestive response. We also observed that CCK treatments induced defecation. It is not clear whether defecation is also a part of satiety in rats, but CCK is known to induce loose stools in humans [21].

The animals were water-deprived when they were given water (Experiment 1) or milk (Experiment 2). Water deprivation is known to inhibit food intake and hydration to disinhibit it [13]. Thus, water-deprived rats are both thirsty and hungry and milk may be ingested as food. Apparently, when ingesting food the blood level of CCK is interpreted as a satiety signal by the hungry animal, probably at the ventromedial hypothalamic area [3], but is not when the animal is ingesting water.

The effect of CCK, suppressing food intake and inducing other satiety-related responses, has been amply documented, particularly in rats [1, 7, 9]. However, one study [11] showed no such effect in rats. Their result may have been due to the low dose used (12 Crick-Harper-Raper units or 3 U/kg) or to the slow rate of administration of the hormone (0.05 ml/min for 10 min).

Contrary to the effect of CCK on food intake the effect of CCK on water intake has been inconclusive. A strong suppressive effect of CCK in both food and water intake in C3H mice has been reported [15]. In 10 min the control mice drank about 0.5 ml and those given CCK (1 U/mouse or 40 U/kg) about 0.1 ml with a great deal of variation. The authors reported an ill effect of CCK and suggested that suppression may be due to this general effect. We did not observe any serious ill effects and we question whether this effect may be due to some unique factors in the experimental situation with inbred mice. Also the low amount of intake with a great deal of individual variation in the mouse may make the data susceptible to some uncontrolled experimental error.

A hypothesis that some chemical properties of chyme entering the duodenum stimulate the release of CCK, which interacts with the brain to induce satiety, is an attractive one. It parallels a hormonal hypothesis in water intake that hypovolemia may act on the kidney to activate the renin-angiotensin system and that angiotensin II interacts with the subfornical organ to induce water intake [12].

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