Hepatic-Portal and Cardiac Infusion of CCK-8 and Glucagon Induce Different Effects on Feeding

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STRUBBE, J. H., J. G. WOLSINK, A. M. SCHUTTE, B. BALKAN AND A. J. A. PRINS. Hepatic-portal and cardiac infusion of CCK-8 and glucagon induce different effects on feeding. PHYSIOL BEHAV 46(4) 643-646, 1989.—In order to compare effects of circulating CCK-8 and glucagon on food intake, rats were provided with a permanently implanted catheter in the right atrium. Another cannula was implanted into the hepatic-portal vein by a new technique. After a standard fasting period graded loads of CCK-8 and glucagon were infused via these catheters during refeeding. Intracardiac glucagon and CCK loads dose-dependently suppressed meal size. Intraportal infusion of glucagon caused similar suppression compared to intracardiac administration. This may indicate a minor role of the liver as a target for the suppression of feeding by glucagon. In contrast, intraportal infusion of CCK-8 did not reduce food intake. The results indicate that CCK-8 is removed or inactivated by the liver. It is suggested that CCK-8 acts locally on vagal nerve endings to exert its suppressive action on food intake.

Food intake Satiety CCK-8 Glucagon Liver Hepatic portal vein

CHOLECYSTOKININ and glucagon are proposed to be satiety signals in the control of food intake (5, 10, 11, 25). Since CCK is secreted by the intestines and glucagon mainly by the pancreas they reach the liver via the hepatic-portal vein. To test the satiety hypothesis, CCK and glucagon should depress food intake when they are infused into the portal vein. Recently some doubt raised whether CCK acts via this route, as intraportal infusion of CCK-8 caused very little suppression of food intake in rats (7,8). In many studies in this field glucagon and CCK-8 were administered by intraperitoneal injection (4, 5, 10, 11). Although after intraperitoneal injection these hormones are taken up rapidly into the portal circulation they may exert their suppressive effects by a direct influence on the serosal side of the intestines (7,18). Some evidence exists that in rats intraportally injected glucagon is effective in suppressing food intake (12,25), probably via afferent vagal pathways (13).

However, the comparison with infusion in the general circulation is needed to shed more light on the satiety effects of CCK-8 and glucagon and the possible site of action. Therefore graded loads of glucagon and CCK-8 were infused during feeding after moderate fasting periods via permanently implanted cannulas into the right atrium and hepatic portal vein in rats.

METHOD

Animals and Housing

Male Wistar rats (350-400 g) were kept in a sound attenuated room on a light-dark regime (LD 12:12). For practical reasons lights were on from 0-12 hr and off from 12-24 hr. The rats were housed individually in perspex cages. Food pellets of rat chow (Hope Farms, The Netherlands) were available ad lib unless otherwise stated. Room temperature was thermostatically controlled at 21 ± 0.2°C.

Cannulation Technique

The rats were provided with a jugular vein catheter with the tip in the right atrium (20). During the same surgery procedure, the rats were provided with a permanent cannula for infusion into the portal vein. During the last decade we developed this new surgical technique. This technique has three main advantages over other techniques (16, 17, 23, 24) described in the past: 1) disturbances to intestinal blood flow are minimal because only a very small part of the cannula is inserted in the widest part of the portal vein, 2) cannula longevity is enhanced such that cannulas can be used for at least one month for blood sampling and for at least 2 months for infusion, 3) no special stitch techniques as used in microsurgery are required. In the following paragraphs this technique will be described shortly.

The preparation of the cannula. A silastic medical-grade tubing (Dow Corning, Midland, MI, Silastic 620-135, i.d. 0.51 mm, o.d. 0.94 mm) of 22 cm is beveled at one end to a point. At a distance of 1 mm from the tip a hole was cut with a sharp cut 20 ga needle. Two rings of silicon glue were placed at 10 mm and at
40 mm from the cannula tip. Cannulas were sterilized before surgery.

**Surgery**

The sterilized cannula was filled with heparin solution in saline (500 U/ml). Under ether anesthesia the ventral abdominal wall was exposed. The hairs at the place of the incision, i.e., 2–4 cm caudal to the xiphoid process, were removed. The skin was sterilized with a chlorhexidine solution. A midline incision of about 3 cm was made and the duodenal loop was turned, so that the portal vein was exposed. The venous part of the superior mesenteric vein between the entrance of the gastroduodenal and superior pancreatic-duodenal vein was clamped very shortly (1–2 min). With a 20 ga needle a hole was made in the middle of this venous compartment. Immediately thereafter the cannula tip was inserted and pushed downstream till the silicon ring touched the vein. The clamps were removed and the cannula tip was situated in the main stream of the portal vein. A piece of silk thread (7-0, Ethicon Perma Hand Seide) was fixed just behind the silicon ring. The loose ends of the thread were slipped under the vein and tied in such a way that the blood stream was not hindered. The second silicon ring in the cannula was fixed onto the abdominal wall. This ring prevents tension on the just implanted cannula. The cannula is inserted under the skin and drawn to the skull where it is connected to a stainless steel tubing (20 ga). Subsequently, the cannula is anchored onto the skull with dental cement and filled with 50% w/v polyvinylpyrrolidon (mol.wt. 25,000, Merck) in a heparine solution 500 IU/ml as described earlier (20). The rats were allowed to recover for about a week before they were used in the experiments.

**Feeding Behavior**

Rats were enabled to gnaw food through vertical stainless steel bars situated in front of a food hopper. Spillage was collected in an undertray attached to the food hopper. Each food hopper’s weight was sampled by a programmed microprocessor every tenth of a second. Every second the mean weight was calculated and stored on a magnetic cassette tape. From this a computer retrieved the size and timing of meals. An Esterline Angus event recorder was used to monitor feeding behavior directly, thereby obtaining a visual check on the computer data. Feeding sequences of at least 1 minute which were separated from each other by nonfeeding intervals longer than 15 minutes were considered as separate meals.

**Experimental Procedures**

Access to food was restricted by means of an automatic horizontally sliding door situated in front of a food hopper. The rats were deprived of food over the light phase and in addition over the first 2 hr of the dark phase. Immediately after opening of the doors they ate a large meal of fairly constant size and duration of about 20 minutes. The effect of CCK and glucagon infusions was tested on this meal. Previous work has shown that at this time there is minimal interaction with the circadian control of feeding behavior (22). Moreover, this method leaves the meal pattern in the other part of the night unaffected. Therefore, this method is less disturbing for a rat than the often used overnight fast.

**Infusions**

In order to prevent disturbances in the experiment by handling the animals or by entering the experimental room, the rats were connected with the pump 4 hr before the end of the deprivation. This connection occurred by attaching a long polyethylene tubing on the catheters ending on the skull. The tubing was kept tight by a counterweight. A small swivel joint prevented torsion of the tubing and a stainless steel spring around the tubing protected it against biting (21). The door in front of the food hopper and the infusion pump were activated simultaneously. The rats were habituated to the infusion procedure for about a week with saline infusions (0.9% NaCl w/v) until they ate a constant amount of food during the first meal after deprivation. Intraperitoneal and intracardiac infusions of glucagon and saline were randomly given to the same animals (n = 6). Thereafter, the same approach was used for CCK and saline (n = 6) in the same animals.

**Solutions.** The hormones were dissolved in saline 0.9% NaCl w/v. In order to prevent binding of the hormones to the walls of the infusion system, we added 0.1% w/v bovine serum albumin to the solution. The rate of infusion was 0.075 ml/min. Glucagon (NOVO) infusions of 75, 150 and 300 µg/kg/hr were given intravenously and 30, 75, 150 and 300 µg/kg/hr via intraportal routes. Intravenous infusions of CCK-8 (sulphated, Sigma) of 1.0, 1.5, 3.0, 9.0 and 15 µg/kg/hr and portal infusions at a rate of 3.0, 9.0, and 15 µg/kg/hr were given. All loads were tested twice.

After these experiments the rats were subjected to another surgery. A small incision in the abdominal wall was made in order to localize the portal vein cannula. At that time we had a visual check on the proper functioning of the portal vein cannula. By giving a saline injection into all cannulas we had a check on possible leakage of these cannulas. In the experimental group no leakage was observed. In some cases blood could be withdrawn. This cannula was cut and the part connected with the portal vein was tied off, so that the remaining part was suitable for intraperitoneal infusion. After recovery from surgery intraperitoneal infusions of saline and CCK (9 µg/kg/hr) were given.

**Data Analysis**

MANOVA was applied to all data with dose as covariant. Dose response relations were tested by a Spearman rank correlation. As individual differences contributed substantially to the total variance, statistics on the drug and dose effects are applied to the percentages of the control values, i.e., food consumed during infusion of saline. Spearman Rank correlation and paired t-test two-sided were applied to the means of the two tests for all animals, doses and routes tested.

**RESULTS**

MANOVA on the complete dataset revealed a high variance in individual intakes and interactions between animal and route of administration as well as the hormone given. For that reason all intakes were recalculated as percentages of the mean of two control meals (i.e., during saline infusion) taken by each individual animal nearest in time to that particular experiment. Mean food intake during saline infusions of the glucagon test amounted to 4.9 ± 0.2 g and 5.3 ± 0.2 g (n = 12) for intracardiac and intraportal administration, respectively. For the CCK-8 experiment these values amounted to 6.1 ± 0.4 g and 5.0 ± 0.3 g (n = 12), respectively. During glucagon infusion all doses caused significant suppression of food intake [MANOVA: F(1,69) = 72, p < 0.001] (Fig. 1). Spearman rank correlation between dose and percentage of the food taken relative to that taken during saline infusion was significant for both infusion routes via the jugular vein (n = 24,
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FIG. 1. Satiety effect of graded loads of glucagon expressed as percentage of control intake when infused into the right atrium (●) or into the portal vein (○) (mean ± SEM, n = 6).

FIG. 2. Satiety effect of graded loads of CCK-8 expressed as percentage of control intake when infused into the right atrium (●) or into the portal vein (○) (mean ± SEM, n = 6).

There was a dose-dependent suppression of food intake when CCK-8 was administered into the right atrium. The correlation between CCK-8 dose and percentage of food intake was significant (n = 36, r = −0.8, p < 0.001) (Fig. 2). In contrast, when CCK-8 was infused into the portal vein no dose-response effect was found (n = 24, r = 0.2, p = 0.27) nor was any significant effect seen of the drug with a paired t-test between intake in grams during saline infusion and portal infusion of the higher doses (Fig. 2), i.e., for 9 μg/kg/hr (n = 6, p = 0.27) and for 15 μg/kg/hr (n = 6, p = 0.7), or for relative intakes with the same doses: 9 μg/kg/hr (n = 6, p = 0.6) and 15 μg/kg/hr (n = 6, p = 0.80). There was a significant suppression of food intake after intraperitoneal administration of 9 μg/kg/hr CCK-8, resulting in 52 ± 6% of the intake during saline infusion (n = 6, p < 0.01). Total daily food intake was not affected by any of the treatments. The reason for this is probably the short half-life of the hormones. During the rest of the night-time there is enough time to compensate for the relatively small reductions of food intake.

DISCUSSION

These experiments clearly show that glucagon caused similar suppression during intraportal and intracardiac infusion, whereas CCK-8 only reduced food intake when administered into the general circulation or into the abdominal cavity. For obvious reasons the liver will sense much higher plasma glucagon concentrations when glucagon is administered in the portal vein compared with infusion into the general circulation. Yet there is no difference in effect. This may indicate that a functional target for the suppressive effects of glucagon could be outside the liver. In this respect it has been reported that intraportal glucagon is a potent stimulus for insulin secretion (26). Since insulin may have satiety effects (15), it could be a potential mediator of glucagon-induced inhibition of feeding (26). There is also evidence that vagal afferents are involved in glucagon-induced suppression of food intake in chickens (9) and rats (13). It has also been reported that suppression of food intake by intraperitoneal glucagon injections was blocked by selective denervation of the liver, suggesting that the target for the suppressive effects of glucagon on food intake is located in the liver (4). Further experiments are needed using blood sampling and plasma assaying of glucagon to establish whether glucagon reaches physiological levels during the infusions.

The absence of any reduction when CCK-8 was intraportally infused suggests that intraportal CCK-8 may not suppress feeding under normal physiological conditions. In contrast, the present study also shows that intraperitoneal administration caused strong suppressions of food intake, thereby confirming others (5, 7, 8). This may indicate that CCK-8 given via this route may act on intraabdominal receptors or probably on the serosal side of the intestines or stomach. There is indeed much evidence that CCK-8 inhibits stomach emptying rate (1, 18) and activates intestinal motility (18). Moreover, cutting branches of the vagus nerve to the stomach decreased CCK-8 induced anorexia (19). It is very likely that intravenously given CCK-8 acts on the same place although other areas like the brain and vagus nerve are also potential candidates (14, 28, 29). But how does CCK-8 act when it is not passing the liver in an active form? One possibility is a local paracrine action as suggested by others (7, 8). It is also possible that CCK-8 is released as a hormone to the portal circuit and reaches by way of diffusion or via arteriovenous connections in the intestines the target areas for CCK. There is some evidence that the liver clearance of the larger hormone CCK-33 is slower than that of CCK-8 (2, 6), which may result in low peripheral levels of CCK-8. Therefore, intraportal CCK-33 may be more effective than the octapeptide. CCK-8 has been found in significant quantities in plasma (3), but the precise origin is difficult to ascertain (27). Considering the available evidence the most likely possibility is that CCK-8 acts locally on vagal sensory nerve endings to exert its suppressive action on food intake.

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