Cholecystokinin satiety involves CCK<sub>A</sub> receptors perfused by the superior pancreaticoduodenal artery

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**METHOD**

**Experiment 1**

Experiment 1 compared the effectiveness of SPD artery and jugular vein administration of a single dose of the CCK<sub>A</sub> receptor antagonist devazepide (courtesy of Merck Sharpe & Dohme Research Laboratories) for increasing sucrose intake when given alone or in conjunction with intraperitoneal administration of CCK octapeptide (CCK-8).

Adult male Sprague-Dawley rats (~450 g at surgery) were individually housed and maintained on Teklad pellets. Room lights were on from 7 AM to 7 PM. Feeding tests, 15 min in duration, were conducted between 3 and 5 PM after 6 h of food deprivation. The test diet was 30% (0.88 M) sucrose.

Experimental design. Rats were randomly assigned to two experimental groups receiving polyurethane catheters in the SPD artery (SPD group) or the right external jugular vein (IV group). Each rat underwent four feeding tests in a 2 x 2 design: each test included intravascular injection of 20 μg/kg of devazepide or vehicle and intraperitoneal injection of 2 μg/kg of CCK-8 (Sigma Chemical) or saline. Tests were run on consecutive days, and the order of the tests was randomized.

Surgery and adaptation to test procedures. Rats were initially adapted for ~3 wk to handling, the deprivation schedule, and consumption of 30% sucrose from graduated drinking tubes. They then underwent surgery for implantation of SPD artery or jugular vein catheters constructed from polyurethane tubing (Microrenchathane, Braitree Scientific, MRE033, 0.014 in. ID, 0.033 in. OD). Tips of arterial catheters were tapers to approximately one-third of their original diameter by pulling after immersion in hot sesame oil. On the day of surgery, each rat received a subcutaneous injection of the antibiotic sulfamethoxazole-trimethoprim and was anesthetized with pentobarbital sodium (42 mg/kg ip). Nine rats received SPD artery catheters according to the following protocol. The common hepatic artery was exposed and temporarily ligated, and the gastroduodenal artery was punctured at its junction with the common hepatic artery. The catheter was inserted and threaded into the SPD artery ~0.5 cm past the gastroepiploic artery (9). It was fixed in place, and the puncture wound was sealed by application of cyanoacrylate glue to the point of entry. In addition, silk suture was glued to the catheter 3 cm from this point, the catheter was looped, and the suture was sewn to muscle of the pylorus. The cephalic end of the catheter was threaded out of the abdominal cavity and then subcutaneously to an exit on the dorsal surface of the neck, where it was secured to underlying muscles with silk suture. In another six rats, catheters were inserted into the right external jugular vein and secured as described above. Catheters were filled with a solution of 40% glucose and 100 U/ml heparin (11) and capped with monofilament fishing line. They were subsequently flushed daily with 0.5 ml of normal saline followed by 0.05 ml of the glucose-heparin solution. Beginning 1 wk after surgery, rats were adapted to test procedures (described below) for ~2 wk.

Experimental protocol. Final group sizes were six rats in the SPD group and six in the IV group. Before each test, each rat first received an intravascular injection of 20 μg/kg of devazepide; duodenum; endogenous cholecystokinin; rat; satiety

A large body of evidence indicates that the reduction in food intake resulting from systemic administration of cholecystokinin (CCK) results from the action of this peptide on type A CCK (CCK<sub>A</sub>) receptors within the abdomen and, more specifically, within the upper gastrointestinal (GI) tract (6, 15, 24). However, although it is well established that CCK<sub>A</sub> antagonists such as devazepide can increase intake (12, 13, 16), there is little evidence that endogenous CCK reduces intake by action in this region. Moreover, some reports have suggested that the mechanisms of action of the endogenous peptide may not be identical to those engaged by CCK after systemic administration (15, 19, 23). For example, Ebenezer and Baldwin (8) obtained evidence suggesting that satiety produced by endogenous CCK involves action on central receptors only (but see Ref. 2). The primary aim of the present experiments was, therefore, to address the hypothesis that endogenous CCK reduces intake by action on CCK<sub>A</sub> receptors in the upper GI tract, in particular within tissue perfused by the superior pancreaticoduodenal (SPD) artery, which supplies the proximal duodenum (9). Additional tests were conducted to determine whether parallel evidence could be obtained concerning the action of exogenous CCK.
devazepide (from a stock solution containing 50 µg/ml devaze- 
epide in 0.5% carboxymethylcellulose) or vehicle followed by 
0.05 ml of the glucose-heparin solution. Immediately after-
ward, 2 µg/kg of CCK-8 or normal saline was injected 
intraperitoneally and the rat was placed in a test cage with 
30% sucrose available for 15 min. On completion of testing, 
rats in the SPD group were anesthetized and received arterial 
injections of 0.20 ml of 10% methylene blue. In all six rats 
with patent SPD artery catheters, this procedure resulted in 
a dense accumulation of dye within an area ~1–2 cm long in 
the proximal duodenum, as well as the adjacent portion of the 
pancreas. In one-half of the rats, dye was also seen in the 
stomach; a patch much fainter than that in the duodenum 
extended for several millimeters along the greater curvature 
within the antrum. The peritoneal cavity was also inspected 
for evidence of leakage of dye into the cavity; across experi-
ments 1–3, such leakage was observed in three rats. In one 
case the catheter had ruptured before the point of entry. In 
the other two cases there was obvious backleakage of dye out 
of the artery, apparently because the catheter tip was oc-
ccluded. For rats in the IV group a functional test of catheter 
placement was performed; 0.15 ml (9 mg) of pentobarbital 
sodium was injected through the jugular catheters. In all 
cases, rats exhibited immediate ataxia (25).

Data analysis. Within-group comparisons were performed 
using dependent-samples t-tests. An independent-groups t-
test on devazepide-vehicle difference scores was used to 
compare devazepide’s effects on sucrose intake in the SPD 
and IV groups. In each case the test was one-tailed, because 
the effect of interest was an increase in intake produced by 
devazepide or an increase in the potency of devazepide in the 
SPD group.

Experiment 2

With use of a range of doses of devazepide bracketing that 
used in the first study, experiment 2 compared the effective-
ness of SPD artery and jugular vein administration for 
increasing sucrose intake. In addition, the effect of a single 
dose of CCK-8 by these two routes was compared.

Procedures. Twelve rats were randomly assigned to receive 
SPD artery catheters, and six received jugular vein catheters. 
Surgical procedures were as described for experiment 1, 
except a ligature was placed around the SPD artery catheter 
immediately distal to the gastroepiploic artery. Although the 
SPD artery is occluded by this procedure, its target tissues 
are subsequently supplied by flow from the inferior pancreati-
coduodenal artery, which anastomoses with the SPD artery 
(9). During each surgical procedure, infusion of saline into the 
SPD artery catheter produced blanching of tissue in the 
proximal duodenum. The tissue immediately returned to its 
normal color on termination of the infusion.

All rats were tested in the nondeprived state. Two sets of 
intake tests were performed. The first set consisted of six 
tests, in which rats received injections of devazepide (8, 20, 
and 50 µg/kg) or vehicle into the SPD artery or jugular vein on 
alternating days. Concentration of devazepide for the three 
doses was 32, 80, and 200 µg/ml, respectively, so that injection 
volume was 0.25 ml/kg in all tests. Order of doses was 
randomized. The second set consisted of two tests, in which 
rats received intravascular injections of 20 µg/kg of devazepide (0.25 ml/kg in a concentration of 80 
µg/ml or vehicle in randomized order. Order of injection sites 
(SP动脉 or jugular vein) was also randomized. Procedures 
were otherwise identical to those described for experiment 2. 
At the end of the experiment, five rats were found to satisfy 
the previously described criteria with regard to SPD artery 
and jugular vein catheters.

Data analysis. Dependent-samples t-tests were used for the 
following comparisons: 1) sucrose intake after SPD artery 
or jugular vein devazepide administration compared with 
tests with vehicle injection, 2) devazepide-vehicle difference 
scores in SPD artery and jugular vein injection conditions 
(equivalent to a test of the drug × injection site interaction), 
and 3) intake after vehicle administration into the SPD 
artery vs. the jugular vein. As described previously, compari-
sions 1 and 2 were one-tailed.

Experiment 4

Experiment 4 tested the effectiveness of devazepide for 
increasing sucrose intake when delivered intraduodenally. 
After injection of methylene blue into the SPD artery, dye is 
occasionally observed within the lumen of the GI tract. This 
observation raises the possibility that SPD artery injections 
of devazepide are effective by virtue of action on GI receptors 
distant from the site of delivery. If this is so, the effects of the 
doses of devazepide used in experiment 2 should be replicated 
when those doses are given intraduodenally. Pilot observa-
tions with intraduodenal administration of methylene blue 
showed that dye was very rapidly diluted in the luminal 
contents and distributed throughout most of the small intes-
tine. Thus intraduodenal administration of devazepide should
result in low concentrations of the antagonist acting over an extensive portion of the GI tract.

**Procedures.** Six adult, male Sprague-Dawley rats underwent surgery for implantation of polyurethane catheters into the duodenum. Catheters were inserted 0.5 cm distal to the pylorus, as described previously (5). Procedures for testing the effectiveness of 8, 20, and 50 µg/kg of devazepide were identical to those used in experiment 2. At the end of the study, rats were anesthetized and given injections of 0.25 ml/kg of 10% methylene blue. In five animals, dye was rapidly (within 10 s) distributed throughout much of the small intestine. In the remaining rat, the catheter had pulled out of the duodenum. Data from this animal were discarded. Effectiveness of each dose of devazepide for increasing intake was analyzed using the multiple-comparison procedure described for experiment 2.

**RESULTS**

**Experiment 1**

In the SPD group, 20 µg/kg of devazepide given alone elevated intake by an average of 19% compared with vehicle-injection tests \(t(5) = 3.73, P < 0.01\); Fig. 1). By contrast, there was no indication of a parallel effect in the IV group \(t(5) = -1.67, P > 0.50\). Devazepide's effect was significantly larger in the SPD than in the IV group \(t(10) = 2.77, P < 0.01\).

In tests with intraperitoneal injection of CCK-8, rats consumed significantly more sucrose when also receiving devazepide through the SPD artery than when receiving vehicle \(t(5) = 2.84, P < 0.01\). However, clear evidence of antagonism of the effect of exogenous CCK-8 would require that devazepide's effect be significantly larger in these tests than when the antagonist was given alone (15). Although Fig. 1 suggests a trend in that direction, it was not significant \(t(5) = 1.03, P > 0.25\). Devazepide had no effect in the IV group \(t(5) = 0.97, P > 0.25\).

**Experiment 2**

In the SPD group, all doses of devazepide produced significant increases in sucrose intake of approximately equal magnitude [Fig. 2; \(t_1(13) = 2.15, 2.90, \text{and } 2.40\), respectively, all \(P < 0.05\)]. In the IV group, only the largest dose was effective [for 8 µg/kg: \(t_2(13) = 1.04, P > 0.50\); for 20 µg/kg: \(t_2(13) = 0.35, P > 0.25\); for 50 µg/kg: \(t_2(13) = 2.42, P < 0.05\)]. Comparison of difference scores in the two groups by pooling across doses revealed significantly greater effectiveness of devazepide when it was administered into the SPD artery \(t(10) = 2.39, P < 0.05\).
Injection of 1 µg/kg of CCK-8 (Fig. 3) reliably suppressed intake in the SPD group \[(t(4) = 2.81, P < 0.05)\] but not the IV group \[(t(5) = 0.15, P > 0.25)\]. The effect of CCK-8 was significantly larger after injection into the SPD artery than into the jugular vein \[(t(9) = 2.24, P < 0.05)\].

**Experiment 3**

As in experiments 1 and 2, 20 µg/kg of devazepide increased intake on injection into the SPD artery \[(t(4) = 3.25, P < 0.025)\] but not the jugular vein \[(t(4) = -0.17, P > 0.50;\ Fig. 4\]. Moreover, arterial administration resulted in significantly greater effectiveness of devazepide than venous delivery \[(t(4) = 3.87, P < 0.01)\]. Although the difference in mean baseline intakes on SPD and IV tests was substantially smaller (9%) than in experiments 1 and 2 (cf. Fig. 4 and Figs. 1 and 2), the decrease on SPD vehicle-injection tests was statistically reliable \[(t(4) = 3.67, P < 0.05)\].

On administration of methylene blue into the SPD artery, dye was most heavily concentrated in the proximal duodenum and head of the pancreas, but spread of dye into the stomach was observed in three of five rats, as observed previously. Because in all experiments the extent and density of this spread were typically very modest compared with that in the duodenum, it is unlikely that gastric stimulation was critical for observed effects of SPD artery infusions. To further address this issue, data were pooled from tests utilizing 20 µg/kg of devazepide, the dose common to experiments 1–3. In those eight rats without encroachment of dye onto the stomach, devazepide significantly increased sucrose intake \[(t(7) = 2.58, P < 0.05)\]. The magnitude of this effect was not different from that in the nine rats in which such spread did not occur \[(t(15) = 0.49, P > 0.50)\].

**Experiment 4**

None of the doses of devazepide used here affected intake after intraduodenal administration (all \(P > 0.25;\ Fig. 5\).

**DISCUSSION**

In three experiments, devazepide was more potent for increasing sucrose intake when injected into the SPD artery than when administered into the right external jugular vein. In experiment 1, SPD artery injection of 20 µg/kg of this antagonist increased intake on tests with and without concurrent intraperitoneal injection of CCK-8 but was ineffective in the group with jugular vein catheters. In experiment 2, in which a range of doses of devazepide were tested, only the highest dose of devazepide administered into the right external jugular vein affected consumption, but all doses were effective after arterial delivery. Because the
SPD effect size was approximately equal across doses, it is likely that the threshold dose is <8 µg/kg, the lowest dose tested. Moreover, this apparent maximal effect, also very similar to the increases observed in experiments 1 and 3, was somewhat smaller than maximal effects of devazepide reported by other investigators using intraperitoneal or intravenous administration (12, 13). These observations suggest that the SPD artery injections were targeting and saturating a specific subpopulation of the type A receptors involved in CCK satiety. This experiment also provided preliminary evidence that potency of CCK-8 for reducing sucrose intake is enhanced by SPD artery administration. Experiment 3 demonstrated the SPD-IV difference in devazepide’s effectiveness in a within-subjects design. Thus the between-groups differences observed in experiments 1 and 2 cannot be dismissed as artifacts of possible unintended differences between animals with and without SPD artery catheterization, such as altered blood flow to the duodenum.

Consistent with textbook descriptions of the SPD arterial bed as the proximal duodenum and head of the pancreas (9), methylene blue injection resulted in high concentrations of dye within the first 1–2 cm of the duodenum and an adjacent area of similar size in the pancreas. In approximately one-half of the rats in experiments 1–3, a small amount of dye was also seen in the antrum, typically a patch that extended for several millimeters and was much fainter than in the duodenum. However, statistical analyses of data from subgroups with and without gastric involvement suggested that such encroachment was of little or no consequence for the observed results. Admittedly, this and other conclusions regarding the site(s) of action of devazepide and CCK-8 in this study are based on the assumption that the distribution of methylene blue after SPD artery administration provides a reasonable approximation of the distribution of the infusates administered during feeding tests. Care was taken to equalize volume and rate of injections during tests and the subsequent catheter assessments. However, definitive mapping of the spread of devazepide and CCK-8 would require injections of radiolabeled forms of these substances.

Experiment 4 provided a clear demonstration that the effectiveness of devazepide administered into the SPD artery was not duplicated by intraduodenal injection. Thus arterial delivery to that region apparently provides greater access of devazepide to the critical receptors than does intraluminal administration. Further research is needed to explicature this difference, but the outcome of this study unambiguously rules out diffusion into the intestinal lumen and subsequent spread as important for the effectiveness of devazepide after SPD artery injection.

The preceding considerations strongly suggest that the observed effects of devazepide injected into the SPD artery, as well as CCK-8, resulted from action within the classically defined SPD arterial bed. Thus these results support the involvement of CCKA receptors within the proximal duodenum and/or the adjacent portion of the pancreas in the inhibition of intake produced by exogenous and endogenous CCK. The role of pancreatic CCKA receptors in mediating physiological actions of CCK is well known (7). There has been little attention paid to the possibility that the pancreas is a target organ in CCK satiety, although the greater potency of devazepide after SPD than after intraduodenal injection is consistent with a pancreatic, as opposed to a duodenal, site of action. On the other hand, additional support for a duodenal action has come from reports of activation by CCK-8 of vagal afferent fibers innervating the duodenum (1, 21) and indirectly from evidence of a nonendocrine mode of action of the endogenous peptide (17, 18). The precise nature of the mechanism involved in the hypothesized duodenal satiating action of CCK is still speculative. For example, the mode of action may be paracrine after release from mucosal cells. This possibility appears to be inconsistent, however, with data from Ritter and colleagues (3, 4, 27). Their results suggest that the differential effectiveness of various nutrients for engaging the nonendocrine satiety mechanism on duodenal infusion exhibits a pattern distinct from that for stimulating endocrine CCK release. Alternatively, the relevant CCK-containing cells may be neurons (18), although immunohistochemical studies have indicated a paucity of CCK-containing neuronal somata in the duodenum (9, 20). Furthermore, it has not been estab-

Fig. 5. A: mean 15-min intake of 30% sucrose by rats in experiment 4 with intraduodenal catheters (n = 5). B: devazepide-vehicle difference scores; error bars, SE. Rats received injections of devazepide (filled bars) or vehicle (open bars).
lished that entry of chyme into the duodenum stimulates release of neuronal CCK.

Much of the previous work on the site(s) of action of endogenous CCK has investigated the more general question of whether receptors involved in CCK satiety are peripheral and/or central (7). The conclusion from the current experiments that at least some of these receptors are located in the abdomen is at odds with a report by Ebenezer and Baldwin (8); food intake was not significantly affected by intraperitoneal administration of the antagonist 2-naphthalesulfonyl-L-aspartyl-2-(phenethyl) amide, which is presumably unable to cross the blood-brain barrier. Devazepide, which can freely enter the central nervous system, was effective under the same test conditions. This contrast led the authors to conclude that CCK does not inhibit intake by a peripheral action (or by action on central neurons in areas with high permeability of the blood-brain barrier). On the other hand, a similar experiment by Brenner and Ritter (2) obtained evidence of endogenous CCK’s action on receptors outside the blood-brain barrier; sucrose intake was increased by the peptide CCK antagonist t-boc-Tyr(SO$_3^-$)-Nle-Gly-d-Trp-Nle-Asp-a-2-phenylethyl amide. The reasons for the discrepancies among these studies are not apparent, in part because of the numerous procedural differences. It would be of interest, therefore, to compare the effectiveness of the different antagonists when injected into the SPD artery.

Sucrose intake on vehicle-injection tests was significantly reduced in SPD tests compared with jugular vein tests in experiments 2 and 3 and showed a substantial, but nonsignificant, trend in that direction in experiment 1. The magnitude of this difference was especially large in between-groups designs, i.e., in experiments 1 and 2, averaging 35% in both cases. It was substantially smaller (9%) in experiment 3, which was a within-subjects design. These results suggest that this reduction was for the most part a consequence of the SPD artery catheter implantation and to a lesser degree a result of administration of vehicle and/or the glucose-heparin mixture into the SPD artery. With regard to the effect of the surgical procedure, rats appeared healthy and gained weight after implantation of SPD artery catheters. However, weight gain from surgery until the start of behavioral testing was significantly lower in rats in the SPD groups of experiments 1 and 2 than in those with jugular vein catheters only (P < 0.05). Of concern for interpretation of the current results is the possibility that the greater effectiveness of devazepide in increasing sucrose intake when injected into the SPD artery was an artifact of reduced baseline intake compared with jugular vein tests. This alternative explanation cannot be ruled out. However, the observation that the magnitude of the increase in intake produced by devazepide administered into the SPD artery was no smaller in experiment 3 than in experiments 1 and 2, in which the baseline difference was almost fourfold larger, makes this alternative appear less plausible. The basis of the small within-subjects difference in baseline intakes in experiment 3 is unknown, but injection of vehicle or the glucose-heparin mixture may have suppressed intake by stimulating CCK release. If this is the case, the observed increases in intake produced by devazepide administered into the SPD artery may reflect antagonism of this effect. Such an interpretation would not be problematic for the major conclusions of this study if the released CCK acted locally. Admittedly, a role for circulating CCK is also possible. However, previous studies in the rat have indicated that increases in plasma CCK in the range stimulated by food intake or GI infusions are not associated with decreases in food intake (4, 15, 17). Thus it appears unlikely that the present results are explicable in terms of changes in circulating CCK unless control injections into the SPD artery resulted in very high plasma levels.

In summary, these experiments suggest that the SPD arterial bed contains CCK$_A$ receptors mediating the satiating action of this peptide. The results are consistent, therefore, with the hypothesis of local action of duodenal CCK in inhibiting intake.

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