Dual effects of somatostatin analog octreotide on gastric emptying during and after intragastric fill

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Smedh, U., J. M. Kaplan, E. Björkstrand, and K. Uvnäs-Moberg. Dual effects of somatostatin analog octreotide on gastric emptying during and after intragastric fill. Am. J. Physiol. 277 (Regulatory Integrative Comp. Physiol. 46): R1291–R1296, 1999.—The effect of the somatostatin analog agonist octreotide (Oct) on gastric emptying of 12.5% glucose during and after intragastric infusion was examined in nondeprived rats equipped with stainless steel gastric fistulas. The rate of intragastric infusion (1.0 ml/min) and the volumes delivered (6 or 12 ml) were within the ranges typically observed in rats normally ingesting the same stimulus. In experiment 1, a dose-related suppression of glucose emptying during 12-min infusions was obtained in response to Oct (0.0014, 0.014, 0.14, and 1.4 nmol/kg sc) injected 60 min before the test. The highest dose tested yielded a 37% suppression of glucose solute emptying during fill. In experiment 2, the suppression of emptying during fill induced by Oct (1.4 nmol/kg) was reversed by 10 or 40 µg/kg of the somatostatin antagonist cyclo(7-aminohexanoyl-Phe-Trp-Lys-Thr-[Bzl]). The antagonist did not by itself affect emptying. Experiment 3 showed that the suppression of emptying obtained with 0.14 and 1.4 nmol/kg Oct had disappeared when the gastric sample was withdrawn 36 min after the termination of 12-min glucose infusions. Experiment 4 showed that the Oct-induced reductions in emptying during 6- and 12-min infusions, in fact, were reversed within 6 min after infusion offset. The point of transition between suppressed and increased emptying did not depend on time from injection or from infusion onset but was linked to the offset of the intragastric infusion regardless of its duration. The present findings support the notion that separable mechanisms govern gastric emptying during vs. after gastric fill.

gastric fill; rat; SMS 201–995

Somatostatin is a peptide that acts on multiple targets throughout the body, including the gastrointestinal (GI) tract. For the digestive system, as for virtually all other organs, somatostatin actions have been generally characterized as inhibitory. For example, somatostatin strongly inhibits secretion of a number of GI (e.g., cholecystokinin, gastrin, motilin, GI peptide) and pancreatic (e.g., insulin, glucagon) hormones and reduces gastric acid secretion, blood flow, and intestinal absorption of various nutrients, including glucose. The literature is equivocal, however, with respect to the effects of somatostatin receptor activation on gastric emptying. Bloom et al. (1) first showed that somatostatin infused intravenously in humans reduced gastric emptying as assessed by scintigraphy. By contrast, gastric emptying of 25% glucose in humans was enhanced during prolonged intravenous somatostatin administration (8, 9), but the effect was promptly reversed on somatostatin infusion offset (8). A scintigraphic study in rats also showed increased emptying of a noncaloric load in response to somatostatin (4). Much of the recent work on somatostatin receptor activation has focused on the synthetic analog agonist octreotide (Oct), which contains the four-amino acid residue essential for the biological activity of somatostatins. Oct does not cross the blood-brain barrier and has a considerably longer half-life (~120 min) than somatostatin (~1.5 min) in humans as well as in rats, enabling administration by the subcutaneous route. In one study, Oct first enhanced and later inhibited emptying of a mixed meal in humans (23). However, enhancements (7, 23) as well as inhibition (3, 16, 19) of gastric emptying after intravenous or subcutaneous administration of Oct have been reported.

Here we explore effects of somatostatin receptor activation on gastric emptying during and after intragastric delivery of glucose in the rat. Almost all studies have focused on emptying in the postprandial period and, as such, may have failed to capture systematic effects of the peptide that may be specific to the stomach filling period. Recent work suggests that different mechanisms control emptying of nutritive fluids during and after fill and demonstrates that a given treatment may selectively influence emptying in one or the other phase (13, 14). Emptying of caloric fluids in the postfill period appears to conform well to the principle of feedback control (18), whereby solute emptying rate remains relatively stable, despite variations in stomach volume and stimulus concentration. Solute emptying rate tends to be considerably higher during than after fill and, moreover, varies broadly as functions of stimulus concentration and the rate of fluid delivery. Rapid emptying during fill cannot be characterized as an “initial rush” before feedback control is enabled, insofar as it is sustained until an intragastric infusion is terminated, regardless of infusion duration and how much has cumulatively emptied. Supporting the notion of different control mechanisms for the two phases are results of a recent study in which the effects of gastric branch vagotomy on glucose emptying were shown to be entirely confined to the period during the fill (13). The effects of the various GI peptides on the different phases of gastric emptying have yet to be distinguished.

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In experiment 1, the effects of Oct dose on gastric emptying during intragastric infusion of 12.5% glucose were measured in freely moving, nondeprived rats. In experiment 2, we assessed the ability of the somatostatin analog antagonist cyclo(7-aminohexanoyl-Phe-\text{D-Trp-Lys-Thr[BzI]}) (CPP-1) to block the Oct effect on emptying during fill (5, 6, 15, 22). One might expect, given the long half-life of Oct, that the effects during and after fill would be in the same direction (enhancement or suppression). Experiment 3 showed that this was not the case; an inhibition during fill gave way to an enhancement of gastric emptying after fill. In experiment 4, we therefore evaluated two competing hypotheses: that the point of transition depends on time per se (from infusion onset or from injection) or, alternatively, that the transition is linked specifically to the termination of the intragastric infusion regardless of its duration and regardless of the amount cumulatively emptied from the stomach.

The intragastric infusions were delivered at a rate (1.0 ml/min) typical of the rat's rate of voluntary ingestion of the same solution from a drinking spout, and the amounts delivered (6 or 12 ml) are within the range of meal sizes observed in such short-term intake tests (10, 11, 21). Also, the basal emptying profiles for orally and intragastrically delivered glucose do not significantly differ (12, 14). The intragastric infusion protocol thus allows effective experimental control of the parameters of stimulus delivery while simulating conditions of normal fluid ingestion in the rat.

MATERIALS AND METHODS

Animals

Male Sprague-Dawley rats, weighing 325 ± 20 g on the day of arrival (B & K, Sollentuna, Sweden), were housed individually under conditions of controlled temperature (20 ± 1°C) and illumination (lights on 7 PM–7 AM). Pelleted food (Ewos R34) and tap water were provided ad libitum. All rats were handled daily from the day of arrival. The experiments were approved by the local ethical committee for experiments in animals.

Surgery

After overnight food deprivation, rats were anesthetized with chloral hydrate (400 mg/kg ip) and received gastric cannula implants under semisterile conditions. After laparotomy, the stomach was exposed and two concentric purse-string sutures were sewn in the fundus along the major curve. The stomach was then opened, and the stainless steel fistula was inserted and secured with double ligatures. The outer part of the fistula was then exteriorized through a puncture in the left lateral side of the abdominal wall and skin. Finally, the midline incision was closed, and each rat received a subcutaneous injection of 10 ml of saline in the neck to provide for postoperative fluid requirements. The animals were allowed 9 days of recovery before the start of habituation training. The rats showed no signs of infection, (e.g., wound infection, loss of weight) at any time during the present study.

Procedure

The experiments were performed between 2 and 7 PM, with each rat tested in the same daily time slot. The experiments were performed with the rats placed in rectangular test chambers (base 25 × 20 cm, height 30 cm) with wire mesh floors. The animals were unrestrained throughout the experimental testing sessions. Sixty minutes before drug or vehicle injection, the fistula was opened and gastric contents were removed by gentle lavage with warm tap water. Just before testing, the fistula was again opened and fitted with a tube connected to an infusion pump (Harvard Pump 22, B & K). At the start of the test session, the pump was turned on and a 12.5% glucose solution was infused intragastrically for 6 or 12 min at a rate of 1 ml/min. The tube was then clamped and cut, and the residual volume was withdrawn by careful aspiration immediately or 6–36 min later, as described in detail below. Immediately after gastric evacuation, the stomach was rinsed with 5.0 ml of distilled water. The volume of the primary sample was measured, and its glucose concentration was determined by the glucose oxidase method (GOD-PAP test, Merck, Darmstadt, Germany) with a Shimadzu UV-210A double-beam spectrophotometer. Absorbances were recorded at a wavelength of 510 nm. Any glucose in the rinse return was measured by the same procedure, and the estimates for gastric glucose and volume were adjusted accordingly. The estimate for glucose solute (g) emptied was taken as the difference between the amount infused and gastric glucose recovered. Volume was expressed as total volume retained, including intragastric secretion volumes.

Drugs

Oct (100 µg/ml; Sandostatin, Sandoz) was diluted in sterile saline and freshly prepared on each experimental day. Drug or saline as a vehicle was injected (0.5 ml/kg sc) in the right hindleg 40 min (experiment 2) or 60 min (experiments 1, 3, and 4) before the start of the intragastric infusion.

The somatostatin receptor antagonist CPP-1 (Sigma Chemical) was diluted in sterile saline and frozen. Aliquots were defrosted on each experimental day and discarded after use. Drug or saline vehicle was injected (0.5 ml/kg sc) in the left hindleg 40 min before the start of the intragastric infusion.

Habituation Training

Beginning 9 days after surgery, rats received a series of four daily habituation training sessions. During each training session, rats received a saline vehicle injection and a 12-min intragastric glucose infusion, as described above, after which gastric contents were removed.

Experimental Design

Test sessions were run every 2nd day (experiment 1) or every 3rd day (experiments 2–4) to minimize potential carryover effects of Oct. In each experiment, each rat was tested once under each condition, and condition presentation order was counterbalanced across rats.

Experiment 1. The dose-response profile of Oct on gastric emptying during intragastric fill was examined with four doses of Oct (1.4, 0.14, 0.014, and 0.0014 nmol/kg body wt) and saline as a vehicle. The highest dose chosen, 1.4 nmol/kg, corresponds to 1.43 µg/kg, within the clinical dose range used in humans. Peptide or vehicle was administered 60 min before a 12-min intragastric infusion. The gastric contents were immediately removed after the end of the infusion.

Experiment 2. The specificity of the Oct-induced suppression of emptying during fill was explored via treatment with the somatostatin antagonist CPP-1. Forty minutes before the start of 12-min intragastric infusions, rats received injections of CPP-1 (10 or 40 µg/kg ip) or vehicle in the left leg followed immediately by injection of vehicle or Oct (1.4 nmol/kg) in
Regardless of its duration (6 or 12 min), and emptying is related to time per se vs. the offset of the infusion followed by post hoc tests, as indicated in RESULTS, provided Statistical Analysis with samples withdrawn immediately or 6 min later, so that emptying after fill can be derived by subtraction from the during-fill estimate.

Experiment 3. The effect of Oct on gastric emptying during and after intragastric fill was examined. Vehicle or 1.43 or 0.143 nmol of octreotide was administered, and, as in the previous experiment, the rats received a 12-min intragastric infusion of glucose. Intragastric contents were removed either immediately after infusion offset, to derive an estimate of emptying during fill, or 36 min later, so that emptying after fill can be derived by subtraction from the during-fill estimate.

Experiment 4. Results of experiment 3 showed a suppressive effect of Oct on emptying during fill that was completely reversed by the time the 36-min postinfusion sample was withdrawn. This experiment addressed 1) whether the inhibitory effect of Oct was uniformly expressed in both 6-min segments of a 12-min infusion, 2) whether the suppression of emptying is related to time per se vs. the offset of the infusion regardless of its duration (6 or 12 min), and 3) whether the reversal of the effect occurred rapidly in the postfill period (i.e., within 6 min of infusion offset). Rats received injections of Oct (1.4 nmol/kg) or vehicle before each of four test conditions. They received 6- or 12-min intragastric infusions, with samples withdrawn immediately or 6 min later.

Statistical Analysis

The data were analyzed with repeated-measures ANOVA followed by post hoc tests, as indicated in RESULTS, provided the ANOVA showed an overall significant effect. Differences were considered significant at $P < 0.05$.

RESULTS

A dose-related suppression of glucose emptying during fill was obtained in response to Oct treatment [repeated-measures ANOVA, $F(4,28) = 3.905, P < 0.05$; Fig. 1A]. The post hoc Dunnett's test showed that the two highest doses of Oct significantly suppressed emptying of glucose solute relative to vehicle values ($P < 0.05$). A comparable overall result was obtained when emptying was expressed in terms of volume retained in the stomach [$F(4,28) = 5.475, P < 0.01$], except the three highest doses yielded a significant increase vs. vehicle baseline ($P$ values $< 0.05$; Fig. 1B).

In experiment 2, repeated-measures ANOVA revealed an overall effect of drug [$F(5,35) = 3.161, P > 0.05$]. Subsequent post hoc comparisons (Newman-Keuls test) showed a significant suppression of solute emptying during fill induced by 1.4 nmol/kg Oct ($P < 0.05$). Both doses of CPP-1 blocked the effect of Oct ($P$ values $< 0.05$ vs. control and $P$ values $< 0.05$ vs. Oct treatment), but CPP-1 did not by itself affect emptying in the doses studied ($P$ values $> 0.05$ vs. control; Fig. 2).

The overall two-factor repeated-measures ANOVA performed on results from experiment 3 revealed a significant effect of both drug [$F(2,12) = 7.057, P < 0.01$] and infusion condition [$F(1,6) = 465.3, P < 0.005$], but there was no significant two-factor interaction [$F(2,12) = 2.381, P = 0.134$]. Post hoc tests (Newman-Keuls) showed significant reduction of emptying during the 12-min fill period by both Oct doses tested (0.14 and 1.4 nmol/kg vs. vehicle, $P < 0.05$ and $P < 0.01$, respectively), thereby replicating results of experiment 1.

Fig. 2. Antagonism of octreotide-induced inhibition of gastric emptying by pretreatment with somatostatin antagonist cyclo(7-aminohexanoyl-Phe-d-Trp-Lys-Thr[Bzl]) (CPP-1). Octreotide (1.4 nmol/kg) or saline and saline or CPP-1 (10 or 40 µg/kg sc) were administered 40 min before start of a 12-min intragastric glucose infusion. Intragastric samples were collected immediately after infusion. Values are means ± SE; $n = 8$. Octreotide (II) suppressed solute emptying compared with control (I): *$P < 0.05$. CPP-1 at 10 (V) or 40 µg/kg (VI) blocked effect of octreotide on glucose emptying ($P < 0.05$ vs. I) but did not by itself affect emptying.

Fig. 1. Glucose solute emptied (A) and volume retained (B) during 12-min intragastric infusion of 12.5% glucose as a function of octreotide dose. Values are means ± SE; $n = 8$. Octreotide dose vs. vehicle baseline: *$P < 0.05$; **$P < 0.01$.
There was no difference between Oct and vehicle injection conditions on glucose emptied, however, when the samples withdrawn 36 min after infusion offset were evaluated. It would appear, then, that the Oct-induced suppression of emptying during fill was compensated for by more rapid emptying in the postfill period (Fig. 3).

The two-factor repeated-measures ANOVA for experiment 4 revealed a significant effect of drug \(F(1,7) = 63.59, P < 0.001\) and infusion condition \(F(3,21) = 57.75, P < 0.001\), as well as a two-factor interaction \(F(3,21) = 8.720, P < 0.001\). Post hoc Newman-Keuls tests account for the pattern of results obtained and address the three issues named in MATERIALS AND METHODS. Oct significantly reduced emptying relative to vehicle baseline for samples withdrawn immediately after 6- and 12-min infusions (\(P < 0.05\) and \(P < 0.01\), respectively). In addition, the Oct-induced suppression of emptying, expressed as a ratio against vehicle, for the 6-min sample point (6:6 condition) did not differ from that for the 12-min sample point (12:12 condition paired 2-tailed t-test). This suggests that the Oct-induced suppression of emptying was uniformly expressed in the 6- to 12-min during-fill interval. For both infusion durations, however, there was no longer a difference in glucose solute emptied between Oct and vehicle conditions when the sample was withdrawn 6 min after infusion offset. Of particular interest was a comparison between results of the sample taken immediately after the 12-min infusion and the sample taken 6 min after offset of the 6-min infusion. Despite a common sample time in relation to the infusion onset, there was a strong Oct-induced inhibition of emptying in the former (\(P < 0.01\)) but not in the latter case (compare 6:12 and 12:12 conditions in Fig. 4). This contrast indicates that the point of transition from initial inhibition to increased emptying did not depend on time per se (either from OCT administration or from infusion onset) but, rather, on the termination of the intragastric infusion regardless of its duration.

**DISCUSSION**

Subcutaneous injections of Oct induced a reliable dose-related suppression of glucose emptying during intragastric fill. The highest doses tested, 0.14 and 1.4 nmol/kg, suppressed glucose solute emptying by 30 and 37%, respectively. Injection of the somatostatin analog antagonist CPP-1 (10 and 40 µg/kg body wt) did not by itself affect gastric emptying but completely blocked the emptying effect of the highest Oct dose tested. Although it cannot be stated with certainty which receptor subtype may underlie the Oct effects obtained here, the results do suggest that the effect is somatostatin receptor mediated. Interestingly, the high doses of CPP-1, when administered alone, neither enhanced nor suppressed emptying during fill. These findings taken together may be consistent with the suggestion that glucose emptying during fill is not influenced by endogenous somatostatin in nondeprived rats under the present conditions. This suggestion must be viewed with some caution, however, given that CPP-1 has been reported to possess weak partial agonistic properties when administered in high doses (6).

The suppression of glucose emptying by Oct reported here agrees with certain studies in which somatostatin was intravenously delivered (1, 20). On the other hand, more recent studies with Oct, the synthetic somatostatin analog agonist, have reported accelerated (7, 23) as well as diminished (3, 16) emptying. The contrasting results may well be attributable to different species tested (i.e., rat vs. human), differences in the test stimulus, route and rate of stimulus delivery, or other conditions.
methodological differences. The temporal aspect of the Oct effect here raises another possible explanation for such contrasting results. The inhibitory effect of Oct was entirely located within the period during which the stomach was filling. The effect would have been entirely overlooked had gastric samples been withdrawn even shortly after infusion offset (see below), and there is strong reason to suspect that one commonly used method for assessment of gastric emptying, radionuclide scintigraphy, could not possibly capture effects on emptying during fill. The basic logic of the scintigraphic method entails the assumption that no emptying occurs during the loading period; that is, 100% of the load is taken as present within the stomach for the first reading. The assumption may be inappropriate for measurement of emptying of solid or semisolid foods, given the lag phase in the emptying of these stimuli. It is not valid, however, for fluids such as glucose, where substantial portions of the load empty during fill (2, 14, 17). The direct sampling methods such as the one used in the present study are more suitable for evaluating treatments that may yield different effects in the fill and postfill periods.

Experiment 3 showed that the Oct effect had disappeared by the sample withdrawn 36 min after the termination of the intragastric infusion, indicating that the inhibition of emptying during fill was offset by a more rapid emptying in the postfill period. It does not appear that the more rapid emptying was sustained throughout the 36-min postfill period. Experiment 4 showed no difference between Oct and vehicle injection conditions in the amount emptied for samples withdrawn 6 min after infusion offset. It appears, then, that there are three phases of Oct action on glucose emptying in the rat: a suppression during fill and an increase in the early postfill period before emptying rate is finally normalized relative to the vehicle profile. The postload actions are reminiscent of those described in a number of previous studies of the gastric emptying effects of somatostatin receptor activation (7–9, 23).

The present results reinforce the suggestion (12–14) that separable mechanisms control gastric emptying during vs. after fill. Further work is needed to establish the mechanisms underlying the functional results reported here. Such mechanistic studies, whichever parameters are highlighted (e.g., gastric tone, volume, intragastric and duodenal pressure, pyloric action), should be structured to uncover salient changes that attend the termination of the fill period. Experiment 4 showed clearly that the transition from slow to rapid emptying is not time locked to the injection or to the start of the intragastric infusion. The transition point was instead linked to the termination of the infusion, regardless of when the infusion was interrupted. Thus the emptying suppression obtained for the 6- and 12-min infusions was reversed completely for the samples taken 6 min later. For samples withdrawn a fixed 12 min after infusion offset, moreover, the suppressive effects of Oct treatment were expressed only when glucose delivery was sustained throughout the 12-min period. The during- vs. after-fill distinction was important for an adequate characterization of somatostatin receptor-induced effects and may well be relevant for the interpretation of other hormonal influences on the emptying of nutritive fluids.

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