Efferent pathways in the reflex control of gastric emptying in rats

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Forster, E. R., T. Green, and G. J. Dockray. Efferent pathways in the reflex control of gastric emptying in rats. Am. J. Physiol. 260 (Gastrointest. Liver Physiol. 23): G499-G504, 1991.—Previous studies have established that acid, hypertonic, or protein-rich liquid test meals delay gastric emptying by reflex pathways involving the extrinsic innervation of the gut. To characterize the efferent pathways involved in these reflexes, we have studied the emptying of liquid test meals in control rats and in rats after celiac ganglionectomy, pyloroplasty, and treatment with guanethidine or 6-hydroxydopamine, and in rats with circulating vasoactive intestinal polypeptide (VIP) antibodies. The results suggest that the action of hypertonic solutions on gastric emptying requires an intact celiac ganglion, that acid requires an intact pylorus, and that the action of protein-rich meals is suppressed by VIP antibodies. Sympathetic adrenergic neurons do not apparently mediate the gastric emptying of any of these solutions. The results suggest that there are at least three different reflexes by which the different components of a mixed meal might control gastric emptying. The results are also consistent with the idea that vagovagal reflexes mediate the action of protein-rich solutions on gastric emptying in rats.

cholecystokinin; acid; osmolarity

The composition of liquid test meals determines the rate at which they empty from the stomach. Among the factors that delay emptying of liquid meals are their content of protein or fat, their pH, viscosity, and osmolality (15). We recently reported (11) that in conscious gastric fistula rats the delay in gastric emptying of protein-rich solutions (peptone) was mediated in part by the release of the intestinal hormone cholecystokinin (CCK). The action of peptone was substantially reduced in animals that had been treated as neonates with the sensory neurotoxin capsaicin (9), suggesting that extrinsic small diameter afferents might mediate the effects of CCK. Acid and hyperosmolar solutions do not release CCK, but the delayed emptying caused by these solutions was also reduced in capsaicin-treated rats, suggesting that they too activate reflexes involving small diameter afferents. Although this evidence supports the idea of extrinsic reflex control of gastric emptying, the relevant afferent mechanisms remain poorly understood.

It is generally thought that the emptying of liquids depends on the difference in pressure between the stomach and duodenum and the resistance to flow across the pylorus. CCK contracts the pylorus and also decreases intragastric pressure in the body of the stomach, both of which could lead to delayed gastric emptying. In anesthetized rats, CCK relaxes the body of the stomach by reflexes that have a vagal afferent arm and both vagal and sympathetic adrenergic afferent components (19). It is now well established that vagal stimulation releases vasoactive intestinal polypeptide (VIP) from inhibitory motor neurons in the stomach and that VIP relaxes gastric muscle (7). It seems possible therefore that CCK might control gastric emptying by reflex release of VIP or norepinephrine or both. The pylorus has often been thought to be important for control of emptying of acid (1), but whether extrinsic reflexes are involved is uncertain; moreover the reflex pathways that might mediate emptying of hypertonic solutions are also unknown. In the present study, we examined the importance of the sympathetic innervation, VIP, and the pylorus in control of the gastric emptying of protein-rich meals and of acid and hyperosmolar solutions. Antagonists to VIP that are suitable for in vivo use are not yet available; in the past, however, we have inhibited the actions of other peptides released from peripheral neurons by means of immunonutralization (14). We used a similar approach in this study to block the action of endogenous VIP by immunizing rats with a VIP-protein conjugate. The sympathetic innervation of the stomach is provided by the celiac ganglion, so we examined emptying patterns in rats after removal of the celiac ganglion or after adrenergic blockade by guanethidine or 6-hydroxydopamine. The results indicate that there are at least three different pathways by which liquid test meals delay gastric emptying.

METHODS

Animals. The present results were obtained using male Wistar rats. In preliminary studies on the effects of celiac ganglionectomy, male Sprague-Dawley rats were used; because the results were similar to those obtained from Wistar rats only the latter are presented here. At the time the gastric fistula was installed the rats weighed 220-280 g. They were housed individually and were kept on a 12-h light-dark cycle. Solid food was withdrawn overnight before experiments or preparative surgery, but access to water and to a glucose drink was allowed ad libitum.

Surgery. Anesthesia was induced with halothane and maintained with pentobarbitone sodium (60 mg/kg ip). In all rats a small Gregory cannula was installed in the

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body of the stomach using aseptic conditions as previously described by Dimaline et al. (5). Animals were allowed at least 3 wk to recover from the operation before experiments. In one group of animals (n = 5) an indwelling catheter was installed into the jugular vein under pentobarbitone anesthesia, and 24 h later the rats received intravenous guanethidine (see below).

**Experimental treatments.** Six types of experiment were carried out. The emptying of standard liquid test meals was studied before and after treatment of rats with guanethidine (n = 5), or 6-hydroxydopamine (n = 5, 100 mg/kg in saline ip daily for 3 days before experiments). Guanethidine was given intravenously via an indwelling catheter (5 mg/kg in saline) immediately before experiments, and this treatment is referred to as acute guanethidine administration. In a further group of animals (n = 7) subcutaneous injections of guanethidine (50 mg/kg in saline) were given 5 days a week for 3 wk starting 7 days after birth. This treatment causes irreversible sympathectomy and is referred to as chronic guanethidine treatment (12). The success of 6-hydroxydopamine and chronic guanethidine treatment was subsequently verified by determination of catecholamines in stomach extracts using high-performance liquid chromatography (HPLC) and electrochemical detection (see below). In a fourth group of rats (n = 5) the celiac ganglion was removed under pentobarbitone anesthesia 2–3 wk before installation of the gastric cannula. The success of the celiac ganglionectomy was subsequently verified by showing that calcitonin gene-related peptide (CGRP), which is a marker for the afferent fibers that pass through the ganglion to supply the stomach (23), was absent in gastric biopsies taken after the experiments. A fifth group of rats (n = 7) was given a pyloroplasty to allow at least 3 wk to recover from the operation before experiments. In a group of animals (n = 5) an indwelling catheter was installed into the jugular vein under pentobarbitone anesthesia, and 24 h later the rats received intravenous guanethidine (see below).

**Gastric emptying studies.** The rate of gastric emptying was examined using methods similar to those previously described (3, 11). Test meals (3 ml) containing phenol red (60 mg/l) as a nonabsorbable dilution marker were instilled into the gastric fistula, and gastric emptying was determined from the volume and phenol red concentrations recovered at times from 40 s to 10 min later; phenol red concentration was determined from absorption at 550 nm as described by Debas et al. (3) and Green et al. (11). In all groups of animals the emptying of hyperosmolal mannose (0.9 M) was studied in celiac-ganglionectomized rats. The osmolarity of solutions was adjusted to 300 mosM (except hyperosmolal saline and mannose), and pH was adjusted to 7.0 (except HCl). Routinely, at least 30 min was allowed between different emptying trials. As in our previous work (9, 11), peptone was studied alone and after preloading with 3 ml peptone instilled for 5 min before the emptying trial.

**Statistics.** Results are expressed as means ± SE, and comparisons are made by t test.

**RESULTS**

In control gastric fistula rats, the emptying of peptone, acid, and hyperosmolar solutions was similar to that described previously (9, 11). In each case, there was rapid emptying over a period of 1–2 min after instilling the solutions, but thereafter they delayed emptying compared with physiological saline (Fig. 1). As previously noted, the emptying of peptone was further delayed by administration of an additional 3 ml of peptone 5 min before the peptone-emptying trial.

**Celiac ganglionectomy.** Removal of the celiac ganglion virtually abolished the action of 0.45 M NaCl in delaying gastric emptying. A second hyperosmolar solution (mannose), which delayed emptying in control rats, also failed to delay emptying in celiac-ganglionectomized rats. Evidently, therefore, both electrolyte and nonelectrolyte hypertonic solutions regulate gastric emptying by mechanisms depending on an intact celiac ganglion (Fig. 2). In contrast, the rate of emptying of peptone, and 50 mM HCl, in celiac-ganglionectomized animals was comparable to that in control rats (Fig. 3).

**Sympathetic blockade.** To determine whether the effects of celiac ganglionectomy might be due to the loss of the sympathetic adrenergic innervation of the stomach.
ach, several procedures were used to lesion adrenergic neurons. Guanethidine and 6-hydroxydopamine were given acutely immediately before experiments, and in addition, guanethidine was given chronically to produce long-lasting sympathectomy. There was no significant difference in the inhibition of gastric emptying caused by hyperosmolal solutions in these groups of rats compared with controls. Similarly, the effects of acid, and of peptone, were not significantly different in intact animals and in animals pretreated with guanethidine or 6-hydroxydopamine (Table 1). The efficacy of treatment was established by assay of corpus muscle norepinephrine concentrations; we and others (17, 19) have previously found gastric muscle norepinephrine concentrations of 300–450 ng/g, and in the present study control rats had similar concentrations. In contrast, chronic guanethidine-treated rats had 60.2 ± 10.0 ng/g (n = 7), and in 6-hydroxydopamine-treated rats, the concentra-
TABLE 1. Volume of liquid test meals emptied in 5 min in intact rats and in rats treated with 6-hydroxydopamine or guanethidine

<table>
<thead>
<tr>
<th>Liquid Test Meal</th>
<th>Intact</th>
<th>6-Hydroxydopamine</th>
<th>Chronic Guanethidine</th>
<th>Acute Guanethidine</th>
</tr>
</thead>
<tbody>
<tr>
<td>300 mosmol/kg saline</td>
<td>3.03±0.21</td>
<td>2.52±0.22</td>
<td>2.55±0.13</td>
<td>2.63±0.10</td>
</tr>
<tr>
<td>900 mosmol/kg saline</td>
<td>2.28±0.17</td>
<td>2.05±0.26</td>
<td>2.02±0.12</td>
<td>2.15±0.11</td>
</tr>
<tr>
<td>50 mM HCl</td>
<td>1.65±0.05</td>
<td>2.01±0.23</td>
<td>1.75±0.12</td>
<td>1.74±0.11</td>
</tr>
<tr>
<td>Peptone</td>
<td>2.32±0.18</td>
<td>1.90±0.30</td>
<td>1.88±0.17</td>
<td>2.21±0.10</td>
</tr>
<tr>
<td>Preload peptone</td>
<td>1.41±0.18</td>
<td>1.45±0.28</td>
<td>1.60±0.20</td>
<td>1.64±0.10</td>
</tr>
</tbody>
</table>

Values are means ± SE. Volume (in ml) of 3-ml test meal emptied in 5 min is shown. Rats received 6-hydroxydopamine (100 mg/kg ip daily for 3 days) or guanethidine, either 50 mg/kg sc (chronic) for 15 days or 5 mg/kg iv (acute).

DISCUSSION

The results of the present study provide insight into the mechanisms by which different types of liquid test meals influence gastric emptying in conscious rats. They suggest that the action of hyperosmolal saline, but not
acid or peptone, requires an intact celiac ganglion. Peptone, but not acid or hyperosmolar saline, would appear to act through a mechanism that is at least in part mediated by VIP, whereas acid acts by a mechanism requiring an intact pylorus. None of the liquid test meals would appear to require a sympathetic adrenergic input to influence gastric emptying in conscious rats. Taken together with findings reported previously (9-11), the results suggest that there are at least three different reflex pathways by which liquid test meals regulate gastric emptying in the conscious rat.

It is established that protein-rich meals (peptone) release the intestinal hormone CCK, and the results of several studies (3, 4, 8, 11, 13, 21) indicate the action of CCK on gastric emptying is likely to be physiological. In previous studies (9, 20), it has been shown that lesion of primary afferent neurons by capsaicin reverses the action of peptone and of CCK on gastric emptying, suggesting the involvement of an autonomic reflex. The evidence from electrophysiological studies in anesthetized rats (2, 7, 18) suggests that the site of action of CCK may be vagal afferent fibers that also subserves gastric mechanoreceptor functions; it seems possible that CCK acts directly on vagal afferents because binding sites have been demonstrated on the vagal trunk by autoradiography (16, 24). Stimulation of gastric vagal mechanoreceptors is known to cause reflex relaxation of the stomach, so it is not surprising that CCK also decreases intragastric pressure (7). In anesthetized rats this action of CCK depends on the integrity of both vagal and sympathetic efferent pathways and probably involves vagal nonadrenergic noncholinergic relaxation of the corpus, as well as adrenergic sympathetic relaxation (19). However, the action of endogenous CCK on gastric emptying in conscious rats appears to require neither an intact celiac ganglion nor an adrenergic input; the physiological significance of the sympathetic effects of CCK in anesthetized rats is therefore doubtful. The data as a whole indicate that endogenous CCK controls emptying by a vagovagal reflex. Several studies over the last decade have indicated that VIP is a mediator of vagally induced relaxation of gastric smooth muscle and is released by activation of vagovagal reflexes (6). Neutralization of endogenous VIP by circulating antibodies reduced the inhibitory effect of peptone on the rate of gastric emptying, indicating that VIP might be the final mediator of reflexes caused by CCK. The VIP-immunized rats still responded somewhat to peptone, and it is not yet clear whether the residual response reflects incomplete neutralization of endogenous VIP, or (perhaps more plausibly) the existence of other mediators. The latter could include peptides histidine isoleucine/valine (PHI or PHV-42), which are biosynthetically related to VIP (6), purinergic transmitters, or depression of tonically active vagal cholinergic neurons.

There is an abundance of CCK receptors in the region of the pyloric sphincter (22), and it is known that CCK contracts the pylorus by both direct effects on smooth muscle and indirectly by release of norepinephrine. Even so, there was no change in the rate of emptying of peptone after pyloroplasty. This suggests that the pylorus is not important in mediating the control of emptying by protein-rich liquid test meals. In contrast, the action of acid was partially reversed by pyloroplasty. Cannon (1) suggested that the pylorus may be an important effector for the action of acid in controlling gastric emptying, and our data are consistent with this. We have previously shown (9) that the effect of acid on gastric emptying in rats is partially reduced after capsaicin treatment, suggesting a role for the extrinsic afferent innervation. We also showed that the effects of acid were seen after contact with only gastric and proximal duodenum mucosa, indicating that they were exerted in the stomach or proximal duodenum. Celiac ganglionection had no effect on the rate of emptying of acid, indicating that spinal afferents do not have a role in acid inhibition of gastric emptying in rats. The extrinsic pathway is therefore likely to be vagal, but whether there is reflex vagal control of the pylorus remains to be clarified.

The action of hyperosmolar saline on gastric emptying is known to require an intact extrinsic afferent innervation. We have shown that the effects of hyperosmolar solutions on gastric emptying are reversed by celiac ganglionection. Adrenergic blockade by 6-hydroxydopamine or guanethidine had no effect on the gastric emptying of hyperosmotic solutions, indicating that the sympathetic adrenergic innervation is not important here. The action of hyperosmolar solutions are, however, likely to be mediated by extrinsic afferent fibers (either splanchnic afferents or vagal afferents from the intestine) that pass through the celiac ganglion. The relevant pathway might therefore be vagovagal or splanchnic-vagal; either way, the action of hyperosmolar solutions would appear not to have a VIPergic component, nor is an intact pylorus required. The efferent mechanisms are therefore distinguishable from those mediating the effects of acid and peptone. One possibility is that hyperosmolar solutions reflexly relax the body of the stomach by suppressing cholinergic tone that depends on a vagal excitatory input.

The present data indicate that a number of different reflexes can be elicited in response to different meal types. The data from the current study combined with that from previous reports (9-11) are shown in Table 2. Each meal type acts by a separate reflex pathway to regulate gastric emptying. The data are consistent with the following: protein releases CCK that acts via a vagovagal reflex using VIP as a final mediator; acid also acts via a vagovagal reflex, but its actions are directed at the pylorus; hyperosmolar solutions act via intestinovagal afferents, and presumably by vagal afferents that do not release VIP and do not act at the pylorus. During digestion of a mixed meal, all these pathways are likely to be activated.

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