Effects of M1 and CCK antagonists on latency of pancreatic amylase response to intestinal stimulants

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Enhancements of the early amylase response to intestinal nutrients were measured in six conscious dogs with gastric and duodenal cannulas. Secretin (164 pmol·kg⁻¹·h⁻¹·iv) was given to provide a flow of pancreatic juice of ~1 drop/s. Amylase activity was measured in each drop before and after rapid intravenous injection of caerulein (7.4 pmol/kg) or intraduodenal injection of L-tryptophan (1 mmol), sodium oleate (3 mmol), and HCl (3 mmol). All experiments were repeated in the presence of the M1 receptor antagonist telenzepine (81 nmol·kg⁻¹·h⁻¹·iv) and the cholecystokinin (CCK) receptor antagonist L-364718 (0.1 mg/kg·iv). Latency of amylase response (time between injection of stimulant and sustained increase in amylase activity greater than mean + 3 SD of prestimulatory activity) to tryptophan (17 ± 7 s; n = 6) and oleate (16 ± 6 s; n = 6) was significantly (P < 0.05) shorter than to caerulein (28 ± 4 s) and HCl (120 ± 47 s). Telenzepine significantly increased the latency of amylase response to tryptophan and oleate by >10-fold but not the latency to caerulein or HCl. L-364718 abolished the amylase response to all stimulants. These findings indicate that the early amylase response to intraduodenal tryptophan and oleate is mediated by a neural enteropancreatic reflex ending on M1 receptors rather than by hormone release. However, the activation of (possibly vagal) CCK receptors is essential to run the reflex. The early amylase response to intraduodenal HCl is probably mediated by the release of CCK into the blood circulation.

Telenzepine (>30 s) was significantly longer than the observed latency of amylase response to the intestinal nutrients (<20 s). Thus the early pancreatic enzyme response to intestinal amino acids and fatty acids is not likely to be mediated by the release of hormones but rather by a neural mechanism. Furthermore, truncal vagotomy and atropine both increased the latency to the intestinal stimulants 10-fold but had no effect on the latency to intraportal CCK. On the basis of these experimental findings, the authors concluded that an enteropancreatic reflex mediates the early pancreatic enzyme response to intestinal stimulants and that the reflex is cholinergic and vagovagal (17). Studies on the latency of the pancreatic fluid secretory response suggest that the early pancreatic fluid responses to intestinal oleate and tryptophan are also mediated, at least in part, through a vagovagal, cholinergic enteropancreatic reflex (15).

Follow-up studies using stepwise extrinsic denervation of the pancreas ruled out possible splanchnic pathways of enteropancreatic reflexes (19). Celiac and superior mesenteric ganglionectomy did not alter the pancreatic protein response to intestinal tryptophan. Truncal vagotomy significantly reduced the protein response to low loads of tryptophan. Additional ganglionectomy did not cause a further reduction of the already reduced pancreatic protein response to tryptophan. Atropine significantly reduced the pancreatic protein response to low loads of tryptophan before but not after truncal vagotomy (19). These data suggest that, in dogs, the pancreatic protein response to intraduodenal tryptophan is, at least in part, mediated by long, cholinergic enteropancreatic reflexes with both afferent and efferent fibers running within the vagus nerves (16). An anatomic basis for these reflexes was provided by the demonstration of projections of enteric neurons to the pancreatic ganglia in rats (4, 5).

In recent years, the development of specific antagonists for muscarinic and CCK receptors has offered a new approach to the understanding of neurohormonal interactions in the regulation of pancreatic exocrine secretion. Studies on the effect of the specific cholinergic M1 receptor antagonist telenzepine indicate that at least a significant portion of the cholinergic fibers of...
the vagovagal enteropancreatic reflex end on muscarinic receptors of subtype M1 (7). Although CCK is considered to be the most important hormone stimulating pancreatic enzyme secretion, recent evidence has challenged the hypothesis that, under physiological conditions, CCK causes pancreatic enzyme secretion only by acting on CCK receptors on pancreatic acini (8, 20, 21). CCK appears also to work through a vagovagal mechanism (9, 12). Kirchgessner and Liu (4) suggested from recent anatomic, electrophysiological, and neurochemical investigations that CCK may act on CCK receptors located on vagal afferent nerves, which in turn elicit a vagovagal reflex to stimulate pancreatic secretion.

In the present study, we wished to functionally investigate the role of cholinergic receptors of subtype M1 and of CCK receptors in the enteropancreatic reflex. Therefore, we studied the effects of the M1 receptor antagonist telenzepine (3) and the CCK receptor antagonist L-364718 (2) on the latency of amylase response to different intraduodenal and intravenous stimulants in conscious dogs. Parts of these results have been published in abstract form (13).

MATERIALS AND METHODS

Animal preparation. Six foxhounds of either sex, weighing 28–34 kg, were fitted with chronic gastric and duodenal fistulas according to Thomas (see Ref. 10). The duodenal cannula was placed opposite the main pancreatic duct, and the accessory duct was ligated. The surgical procedure was performed under general anesthesia induced by propofol (4 mg/kg iv) 30 min after premedication with acepromazine (0.02 mg/kg im) and maintained after endotracheal intubation by inhalation of halothane-N₂O.

Plan of experiments. Studies were started no sooner than 3 wk after the operation. Dogs were fasted 18 h before each experiment but had free access to water. On each experimental day, a glass cannula was inserted into the main pancreatic duct and connected to a 20-cm length of polyethylene tubing through which pancreatic juice was collected into ice-chilled tubes. The total dead space (including both glass cannula and polyethylene tubing) was measured before each experiment but had free access to water. On each experimental day, a glass cannula was inserted into the main pancreatic duct, and the accessory pancreatic duct was ligated. The surgical procedure was performed under general anesthesia induced by propofol (4 mg/kg iv) 30 min after premedication with acepromazine (0.02 mg/kg im) and maintained after endotracheal intubation by inhalation of halothane-N₂O.

A background infusion of 164 pmol · kg⁻¹ · h⁻¹ of secretin (SEKRETOLIN, Hoechst, Frankfurt/Main, Germany) containing 0.1% canine serum albumin (18) was given to provide a flow of pancreatic juice of ~1 drop/s. Just before each experiment, average drop size was determined by collecting and weighing 15 drops. The average drop size for all experiments was 24 ± 0.6 mg. In earlier studies, there was no change in drop size when dog pancreatic juice of varying protein concentrations was perfused through the tubing with a syringe pump (18); we assume that this is also valid for the present in vivo studies.

Each drop of pancreatic juice was collected from 30 s before until 60 s after the beginning of a rapid intravenous or intraduodenal injection of a stimulant. The drop rate was relatively constant (0.97 ± 0.05 drop/s), and no difference was seen in the 30 s before and the 60 s during and after injection of a stimulant. After drop-by-drop collection of pancreatic juice for 90 s, minute-by-minute collections were continued for 10 min.

On separate days, all experiments were repeated for 10 min after intravenous injection of 81 nmol/kg telenzepine (kindly provided by Byk Gulden, Konstanz, Germany) and 0.1 mg/kg L-364718 (kindly provided by Merck Sharp & Dohme, Munich, Germany). These doses have been proven to be effective for inhibiting pancreatic enzyme secretion in dogs without causing side effects such as alteration of the heart rate (7, 9, 11).

Stimulants. The following stimulants were given as intraduodenal (id) injections in a volume of 20 ml over a 5-s interval (15, 17): 1) L-tryptophan (1 mmol, pH 7.0; Serva, Heidelberg, Germany), 2) sodium oleate (3 mmol, pH 9.4; Riedel-de Haën, Seelze, Germany), 3) HCl (3 mmol, pH 0.9; Merck, Darmstadt, Germany), and 4) NaCl (0.15 M, pH 7.0). All solutions were made up on the day of the experiment. Osmolality was adjusted to 305 ± 4 mmol/kg by adding NaCl as needed. Caerulein (7.4 pmol/kg; TAKUS, Pharmacia, Erlangen, Germany) was dissolved in 6 ml of 0.15 M NaCl and injected intravenously as rapidly as possible, usually in ~3 s.

Because the latency to intraportal CCK was not significantly different from that to intravenous CCK (17), in the present study we chose the easier peripheral intravenous access rather than intraportal injection.

Measurements. In each drop and in each 1-min collection, amylase activity (U/ml) was determined spectrophotometrically (Uvikon 930, Kontron Instruments, Eching, Germany) using an enzymatic test kit (α-amylase EPS, Boehringer, Mannheim, Germany).

Calculations. The latency of the amylase secretory response was defined as the time elapsing between beginning of injection of the stimulant and a sustained increase in amylase activity of the pancreatic juice greater than the mean ± 3 SD of the prestimulatory amylase activity. The reported data reflect the subtraction of the time required by the pancreatic juice to traverse the dead space of the collecting system. This time was calculated for each experiment by using the average drop size, determined as described above, and the average flow rate.

In the minute-by-minute collection periods, amylase output per minute was calculated from the amylase activity and volume of each 1-min sample (measured to the nearest 0.1 ml). The control value for amylase output per minute was calculated from the amylase output in the 30-s drop-by-drop period before the stimulant was given.

The statistical analysis was carried out by a repeated-measures analysis of variance followed by paired t-test with α-correction according to Bonferroni (SPSS, Chicago, IL). Differences were considered significant if P < 0.05.

RESULTS

Relative efficiency of stimulants. The stimulants were nearly identically efficient in stimulating amylase release. The highest maximal amylase activity (2,301 ± 155 U/ml) was evoked by caerulein (7.4 pmol/kg iv). The maximal amylase response to tryptophan (1 mmol id) was 99.5%, to oleate (3 mmol id) was 94.5%, and to HCl (3 mmol id) was 91.3% that of caerulein (Fig. 1).

Latency of amylase response. Figure 1 shows the change in amylase activity in the pancreatic juice in response to rapid intravenous injection of 7.4 pmol/kg caerulein and to intraduodenal injection of 1 mmol tryptophan and 3 mmol oleate in representative experiments in the same dog. A significant and sustained increase in amylase activity greater than the mean ± 3 SD of prestimulatory activity occurred in the experiment with caerulein after 27 s, with tryptophan after 17 s, and with oleate after 18 s.
Table 1 summarizes the results for the latency of amylase response to the intraduodenal stimulants and to intravenous caerulein in six dogs. The mean latencies of the responses to the intraduodenal stimulants tryptophan (17 ± 7 s) and oleate (16 ± 5 s) were similar and were significantly (P < 0.05) shorter than the latency to intravenous caerulein (28 ± 4 s). The mean latency of amylase response to intraduodenal HCl (120 ± 47 s) was about seven times longer than the latency to intraduodenal tryptophan or oleate and about four times that of intravenous caerulein. Intraduodenal injection of NaCl did not elicit any significant amylase response.

**Effect of telenzepine.** Intravenous injection of 81 nmol/kg of the M1 receptor antagonist telenzepine 10 min before intraduodenal injection of tryptophan or oleate significantly increased the latency of amylase response by >10-fold but had no significant effect on the latency of response to intraduodenal HCl and to intravenous caerulein (Table 1).

The longer-term effects of telenzepine on the amylase response are shown in Figs. 2–4. Telenzepine significantly reduced the total 10-min amylase response to intraduodenal tryptophan (Fig. 2), oleate (Fig. 3), and HCl (Fig. 4).

**Effect of L-364718.** Intravenous injection of 0.1 mg/kg of the CCK receptor antagonist L-364718 10 min before the intravenous injection of caerulein (Fig. 5) or intraduodenal injection of tryptophan (Fig. 2), oleate (Fig. 3), or HCl (Fig. 4) abolished the total 10-min amylase response to all of these stimulants.

**DISCUSSION**

The major findings of the present study can be summarized as follows: 1) the latency of pancreatic amylase response to intraduodenal tryptophan and intraduodenal oleate was significantly shorter than to intravenous caerulein and to intraduodenal HCl; 2) telenzepine significantly increased the latency of amylase response to tryptophan and oleate by >10-fold; 3) telenzepine did not alter the latency of amylase response to caerulein or HCl; and 4) L-364718 abolished the amylase response to all given stimulants.

In the present study, we wished to functionally investigate the role of cholinergic receptors of subtype M1 and of CCK in the enteropancreatic reflex. Therefore, we mimicked the experimental conditions of the study of Singer et al. (17), providing strong evidence in favor of an enteropancreatic reflex in dogs. As Singer et al. found, we have found a significantly more rapid pancreatic enzyme response to intraduodenal bolus injection of tryptophan and oleate than to intravenous bolus injection of the CCK analog caerulein. Inasmuch as we gave a large, continuous background infusion of secretin and a large dose of caerulein, the experimental conditions should have been optimal for the most rapid possible response to caerulein. Even under these optimal conditions, however, the latencies of the responses to intraduodenal tryptophan and oleate were significantly shorter than the latency of the response to intravenous bolus injection of the CCK analog caerulein, indicating that the initial amylase response to these intraduodenal stimulants cannot be mediated by the release of hormones such as CCK. This finding confirms the earlier results of Singer et al. (17) indicating that the early pancreatic enzyme response to intestinal amino acids and fatty acids is not likely to be mediated by

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**Table 1. Effect of telenzepine (81 nmol/kg iv) and L-364718 (0.1 mg/kg iv) on latency of pancreatic amylase response to intravenous and intraduodenal stimulants**

<table>
<thead>
<tr>
<th>Conditions</th>
<th>Caerulein, 7.4 pmol/kg iv</th>
<th>L-Tryptophan, 1 mmol id</th>
<th>Sodium Oleate, 3 mmol id</th>
<th>HCl, 3 mmol id</th>
<th>NaCl, 0.15 M, 20 ml id</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>28 ± 4</td>
<td>17 ± 7†</td>
<td>16 ± 5†</td>
<td>120 ± 47*</td>
<td>&gt;10 min</td>
</tr>
<tr>
<td>Telenzepine</td>
<td>30 ± 5</td>
<td>178 ± 116‡</td>
<td>208 ± 121‡</td>
<td>123 ± 66*</td>
<td>&gt;10 min</td>
</tr>
<tr>
<td>L-364718</td>
<td>&gt;10 min</td>
<td>&gt;10 min</td>
<td>&gt;10 min</td>
<td>&gt;10 min</td>
<td>&gt;10 min</td>
</tr>
</tbody>
</table>

Latency values (in s, unless otherwise stated) are means ± SE; n = 6 dogs. id. **Intraduodenal. †P < 0.05 vs. caerulein; ‡P < 0.05 vs. HCl; §P < 0.05 vs. control.**
the release of hormones but rather by a neural enteropancreatic reflex. Such enteropancreatic reflexes may be critical for obtaining a rapid pancreatic response as chyme begins flowing into the duodenum, with humoral mechanisms providing a sustained subsequent stimulus (16).

The actions of vagotomy and atropine, which both increased the latency to the intestinal stimulants 10-fold but had no effect on the latency to CCK (17), support the concept of a cholinergic vagovagal enteropancreatic reflex. Possible splanchnic (extravagal) pathways of cholinergic enteropancreatic reflexes have been ruled out, because celiac and superior mesenteric ganglionectomy did not alter the pancreatic protein response to intestinal tryptophan (19). An anatomic basis for these reflexes was provided by demonstrating projections of enteric neurons to the pancreatic ganglia in rats (4, 5). Electrophysiological investigations have shown that mucosal stimuli such as pressure and glucose depolarize submucosal primary afferent neurons, which transmit the signal to enteropancreatic neurons in the myenteric plexus. Intrapancreatic ganglia appear to be the primary targets of the enteropancreatic innervation (4).

Telenzepine, a synthetic pirenzepine analog, is a specific anticholinergic drug with high affinity for neural cholinergic M1 and low affinity for non-M1 receptors (3). It has been found to inhibit pancreatic exocrine response to intestinal tryptophan in dogs (11, 12). As previously shown for the nonspecific antimuscarinergic drug atropine (17), in the present study intravenous injection of telenzepine 10 min before intraduodenal injection of tryptophan or oleate also increased the latency of amylase response by >10-fold. These similar effects of atropine and telenzepine indicate that the effect of atropine on the latency of amylase response is mediated by muscarinic receptors of subtype M1 and that such receptors are part of the enteropancreatic reflex pathway. The expression of telenzepine-sensitive (M1) receptors by rat pancreatic acinar cells has recently been demonstrated (14). The finding of Schmid et al. (14) that telenzepine inhibits enzyme
secretion of acinar cells stimulated with carbachol supports the hypothesis that telenzepine may interrupt the enteropancreatic reflex at the acinar level.

The latency of pancreatic enzyme response to intraduodenal HCl was clearly different from that of intraduodenal oleate or tryptophan. Similar to the study of Singer et al. (17), the mean latency of amylase response to intraduodenal HCl was about seven times longer than the latency to intraduodenal tryptophan or oleate and about four times that of intravenous caerulein. In addition, it was affected neither by nonspecific anticholinergic treatment (atropine, vagotomy; Ref. 17) nor by the specific M1 antagonist telenzepine (present study). We interpret these findings as indicating that hormone release is a major component of the early enzyme response to intraduodenal HCl, and we suggest that these long latencies are caused by the time required to build up sufficient blood concentrations of the hormone to reach a threshold for amylase response. Because in the present study the amylase response to HCl was abolished by the CCK receptor antagonist L-364718, we conclude that the early pancreatic amylase response to intraduodenal HCl is mainly mediated by humoral CCK acting at acinar CCK receptors. This concept, however, does not rule out some neural (M1 receptor) participation in the enzyme response, as indicated by the depressed overall amylase response to HCl after administration of telenzepine.

Although CCK is considered to be the most important hormone stimulating pancreatic enzyme secretion, more recent investigations have challenged the hypothesis that, under physiological conditions, CCK causes pancreatic enzyme secretion only by acting on CCK receptors on pancreatic acini (8, 20, 21). On the one hand, the CCK receptor antagonist L-364718 depressed the canine pancreatic protein response to tryptophan even after truncal vagotomy (9), indicating that, at least in dogs, endogenously released CCK acts in part as a classic humoral factor independent of the vagal nerves. On the other hand, there exists good
Experimental evidence that in humans (1) and rats (6) all action of physiological doses of CCK on pancreatic enzyme output is vagally mediated. In the present study, 0.1 mg/kg of the CCK receptor antagonist L-364718 totally abolished the early amylase response to intraduodenal bolus injection of tryptophan and oleate. This finding suggests that the CCK receptor antagonist L-364718 interrupts the enteropancreatic reflex pathway for telenzepine and oleate, indicating that in dogs activation of CCK receptors is essential for the function of the enteropancreatic reflex.

With regard to this observation, the question emerges of where the interaction between CCK and the cholinergic system in the dog occurs. First, CCK and cholinergic nerves may interact at the level of the acinar cell, inasmuch as the threshold of cholinergic input conditions the pancreatic secretory response of the acinar cell to humoral-acting CCK. However, this hypothesis is incompatible with the observations that in dogs vagotomy, atropine, and telenzepine do not alter the magnitude and latency of pancreatic enzyme response to exogenous CCK or caerulein (Refs. 12, 17; present study). Second, recent anatomic, electrophysiological, and neurochemical investigations (4) suggest that CCK may act on CCK receptors located on vagal afferent nerves, which in turn elicit a vagovagal reflex to stimulate pancreatic secretion.

Schmid et al. (14) observed that telenzepine did not affect enzyme response of isolated rat pancreatic acini to exogenous CCK. This is in accordance with the finding that telenzepine does not significantly alter the magnitude (9, 12) or the latency (present study) of pancreatic enzyme response to exogenous caerulein in vivo. Furthermore, it confirms the hypothesis that the interruption of the enteropancreatic reflex caused by CCK receptor antagonists in vivo does not arise at the acinar (postsynaptic) level but rather at vagal CCK receptors.

In conclusion, the present study demonstrates in dogs a physiological role for both cholinergic M1 and CCK receptors in the enteropancreatic reflex mediating the initial pancreatic enzyme response to intraduodenal amino acids and fatty acids. In particular, it shows experimental evidence that the early amylase response to intraduodenal tryptophan and oleate is mediated by a neural enteropancreatic reflex ending on M1 receptors rather than by hormone release. However, the activation of (probably vagal) CCK receptors is essential to run the reflex. The early amylase response to intraduodenal HCl is probably mediated by the release of CCK into the circulation.

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REFERENCES