Muscarinic M₁ receptor inhibition reduces gastroduodenal bicarbonate secretion and promotes gastric prostaglandin E₂ synthesis in healthy volunteers

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Abstract

The selective muscarinic M₁ receptor antagonist, pirenzepine, considerably stimulates duodenal mucosal bicarbonate secretion in the rat and increases gastric luminal release of prostaglandin E₂ (PGE₂) in humans. This study, therefore, looked at the effect of pirenzepine on bicarbonate secretion and luminal output of PGE₂ into the stomach and the duodenum of nine healthy volunteers using a new technique permitting simultaneous measurements. In the stomach modified sham feeding increased bicarbonate secretion from 382 (62) $\mu\text{mol/h}$ (mean (SEM)) to 959 (224) $\mu\text{mol/h}$ ($p < 0.02$). In the duodenum modified sham feeding and acid exposure (HCl 0.1 M; 20 ml; 5 min) of the duodenal bulb increased mucosal bicarbonate secretion from 191 (14) $\mu\text{mol/cm} \times \text{h}$ to 266 (27) $\mu\text{mol/cm} \times \text{h}$ ($p < 0.02$) and 634 (157) $\mu\text{mol/cm} \times \text{h}$ ($p < 0.01$), respectively. Pirenzepine (10 mg/h intravenously) reduced basal and vagally stimulated gastric and basal duodenal bicarbonate secretion by about 50% ($p < 0.03$). In the stomach, but not the duodenum, basal and vagally stimulated PGE₂ output increased significantly ($p < 0.05$) in response to pirenzepine. In conclusion, human gastroduodenal mucosal bicarbonate secretion is regulated by a pirenzepine sensitive mechanism, which is probably cholinergic. The rise in gastric PGE₂ output seen in response to M₁ receptor inhibition by pirenzepine suggests the existence of a feed back loop secondary to the decrease seen in bicarbonate secretion.

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Recent studies in animals and humans have focused on the ability of gastric and duodenal mucosa to actively secrete bicarbonate, which is considered an important contributor to the mucosal defence mechanisms directed against luminal acid.¹ Cholinergic components apparently play an important part in the processes governing active secretion of bicarbonate. Thus vagal stimulation increases human gastric^{2,3} and duodenal⁴ mucosal bicarbonate secretion, which is suppressed by the non-selective,

anticholinergic compound, atropine.^{3,4} On the other hand, the selective muscarinic M₁ receptor antagonist, pirenzepine, increases duodenal mucosal bicarbonate secretion in a dose dependent manner in rats,⁵ without influencing the increase in gastroduodenal bicarbonate secretion caused by vagal excitation in rats⁶ and in humans.³ These findings may reflect that the regulation of gastroduodenal mucosal bicarbonate secretion acts at several levels of nervous stimulation or involve other subtypes of muscarinic receptors than M₁ receptors.

Although the selective muscarinic M₁ receptor antagonist, pirenzepine, is a weak inhibitor of gastric acid secretion, healing rates of peptic ulcers obtained with pirenzepine are similar to those seen after treatment with a histamine H₂ receptor antagonist.⁷ Mechanisms other than acid inhibition may, therefore, promote ulcer healing by pirenzepine and a convincing gastroprotective effect of pirenzepine has also been shown.⁸ Besides stimulating duodenal mucosal bicarbonate secretion in the rat, pirenzepine has been shown to reduce the incidence of mucosal damage caused by various noxious agents,⁸ increase the content of gastric mucus,⁹ and promote mucosal blood flow.⁸ As previous studies have shown no influence of pirenzepine on gastroduodenal basal prostaglandin biosynthesis, endogenous prostaglandins are claimed not to participate in these effects.¹⁰⁻¹² On the other hand, we have previously shown that pirenzepine enhances gastric luminal prostaglandin E₂ (PGE₂) output in humans after vagal stimulation and luminal acidification,¹³ which are known stimulants of gastric and duodenal mucosal bicarbonate secretion, respectively.¹

As it is unresolved whether inhibition of muscarinic M₁ receptors may influence gastroduodenal mucosal bicarbonate secretion in humans by a prostaglandin dependent mechanism, we have performed simultaneous 'steady state' perfusions of the stomach and the duodenum in healthy volunteers to study the relation, if any, between gastric and proximal duodenal mucosal bicarbonate secretion and luminal output of PGE₂ during muscarinic M₁ receptor inhibition by pirenzepine.

Methods

SUBJECTS

Nine healthy volunteers, without a history of

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Figure 1: Six channel duodenal tube with a pear shaped balloon in the distal antrum and a button shaped balloon distal to the pylorus anchoring the duodenal segment. The third balloon occludes the distal part of the segment.

gastrointestinal disease or other medical diseases, participated in the study. Simultaneous gastric and duodenal perfusion studies were performed in all subjects (seven males, two females, median age 28 years, range 24–33). The study was carried out according to the Helsinki II Declaration and approved by the ethics committee of Copenhagen and Frederiksberg.

EXPERIMENTAL DESIGN

All nine healthy volunteers were studied twice with an interval of at least one week. The perfusions were performed in random order – that is, as one set of control perfusions and one set of experimental perfusions – in which 10 mg of pirenzepine (Boehringer Ingelheim, Vanløse, Denmark) was injected intravenously at the start of the investigation and followed by constant infusion at a rate of 10 mg/hour. To accomplish total inhibition of gastric acid secretion each subject was pretreated with a 60 mg oral dose of omeprazole (Astra A/S, Albertslund, Denmark) once daily for three days and 80 mg intravenously one hour before each set of perfusions. These included a 60 minute equilibration period followed by a 30 minute basal period, a 60 minute sham feeding period, and a 45 minute period after acidification of the isolated duodenal segment. Attempts to measure gastric bicarbonate secretion after acidification of the stomach failed, because it was impossible to remove all the instilled acid within 30 minutes.

SIMULTANEOUS GASTRIC AND DUODENAL PERFUSIONS

We used the method of isolating the duodenal bulb, originally developed by Isenberg *et al.*¹⁴ and further modified by Knutson *et al.*¹⁵ After an overnight fast a six channel tube (16 French, OD 5.3 mm, Kabi Pharmacia, Uppsala, Sweden) with three balloons was introduced orally. A Teflon coated guide wire (Amplatz Extra Stiff Wire Guide, OD 0.9 mm, William Cook Europe Ltd, Bjæverskov, Denmark) and the position of the tube was controlled by

fluoroscopy. Proximal to a distal tungsten weight two button shaped inflatable latex balloons isolated a 3 cm long segment of the proximal duodenum. A pear shaped balloon in the distal stomach anchored the tube against the pylorus (Fig 1). The gastric balloon was inflated with 30 ml of air and the duodenal balloons with 5–15 ml of air. A double lumen gastric tube (AN 10 Anderson Samplers Inc, Atlanta, GA, USA) was then placed in the distal antrum. The infusion port was located 8 cm proximal to the aspiration port. The isolated duodenal segment was perfused (Ivac 560, N C Nielsen, Glostrup, Denmark) with isotonic saline (2 ml/min; pH 7.0) using [⁵¹Cr]EDTA (10 µCi/l) as a non-absorbable marker. The stomach was similarly perfused with isotonic saline, containing phenol red (50 mg/l; pH 7.0), at a constant rate of 5 ml/min (LKB 2115 Multiperperex Pump, Bromma, Sweden). The effluents were collected from the stomach by intermittent suction (Pump AB, Einar Engell, Sweden) and from the duodenal segment by gravity drainage. During the perfusions saliva was continuously removed by dental suction. The efficacy of this method to avoid contamination with gastric saliva was tested in four subjects by measurement of amylase (used as marker of saliva) and bicarbonate concentrations in saliva and gastric effluents, respectively.

After the 60 minute equilibration period, gastric and duodenal effluents were collected at consecutive 15 minute intervals, except during modified sham feeding when gastric effluents were collected at five minute intervals. Modified sham feeding was performed using chewing gum (five pieces of fruit gum for 15 minutes).¹⁶ For acidification of the duodenal segment 20 ml of 100 mM HCl plus 54 mM NaCl were instilled into the segment for five minutes and then removed by gentle flushing with NaCl for 10 minutes. In all subjects pH had returned to neutral values when sampling was resumed.

ANALYTICAL PROCEDURES

The concentration of bicarbonate in 100 µl aliquots from gastric and duodenal effluents was measured in triplicate (Corning 965 Carbon Dioxide Analyzer, Corning Ltd, Halstead, England).¹⁵ Before analysis the samples were gassed with carbon dioxide free nitrogen for five minutes to remove dissolved carbon dioxide. The Corning analyser was calibrated daily against 1.0, 2.5, and 5.0 mM NaHCO₃.

Samples for measurement of PGE₂ were neutralised immediately and, like samples for measurements of trypsin, stored at –20°C until analysis. PGE₂ was measured in the gastric and duodenal effluents by a radio-immunological method validated by gas chromatography mass spectrometry as previously described in detail.^{17,18} To determine the contamination of the test segment with pancreatic juice, trypsin was measured in the duodenal effluents by a microscale modification of the procedure described by Johnson *et al.*¹⁹ based on the rate at which N^α-benzoyl-

Simultaneous measurements of basal gastric and duodenal fluid transport rates, pH, and recovery of the non-absorbable markers, phenol red, [^{51}Cr]EDTA, and trypsin, during control conditions and after pirenzepine administration (10 mg/h intravenously) in healthy volunteers (mean (SEM); $n=9$)

	Fluid output (ml/h)		pH		Phenol (%)		[^{51}Cr]EDTA (%)		Trypsin (%) duodenal
	Gastric	Duodenal	Gastric	Duodenal	Gastric	Duodenal	Gastric	Duodenal	
Control	36 (5)	21 (2)	6.9 (0.1)	7.5 (0.1)	87 (2)	1.3 (0.3)	1.5 (0.4)	86 (1)	1.0 (0.2)
Pirenzepine	28 (5)	8 (1)*	6.8 (0.1)	7.2 (0.1)*	89 (2)	0.8 (0.2)	1.5 (0.4)	97 (2)*	0.8 (0.1)

* $p<0.05$ compared with control.

L-arginine-*p*-nitroanilide hydrochloride (Merck, Darmstadt, Germany) was hydrolysed by a standard solution of porcine trypsin (Sigma, St Louis, MO, USA). The reaction was performed in microtitration plates (Nunc, Roskilde, Denmark) and was measured spectrophotometrically (Bio-Kinetics reader EL 312e, Bio-Tek Instruments, Winooski, VT, USA) at 405 nm against a substrate water blank. Amylase was determined by an enzymatic, colorimetric method using maltoheptaoside (Merck, Darmstadt, Germany) as a substrate.

Activities of [^{51}Cr] and concentrations of phenol red were determined in both gastric and duodenal effluents and served as markers for determination of fluid transport and contamination of the respective test segment. Activities of [^{51}Cr] were measured by gamma-spectrometry (Model 1185, Searle Nuclear Chicago Division, Chicago, IL, USA). Concentrations of phenol red were measured spectrophotometrically at 560 nm after alkalisation (pH of about 11) by a 4+1 dilution with a 0.5 M Na_3PO_4 solution.

CALCULATIONS AND STATISTICS

Rates of bicarbonate secretion and PGE_2 output were calculated as the mean of the two 15 minute values obtained during the basal periods. Similarly, the results obtained after stimulation were calculated as the mean of the three five minute values obtained in the stomach and the first 15 minute value obtained in the duodenum after start of modified sham feeding or the mean of the three 15 minute values obtained in the duodenum after luminal acidification.

All results were expressed as mean (SEM). The normality assumption for this model was tested with the Kolmogorov-Smirnov statistics and the Levene statistics were used to test for equal variance.²⁰ For some of the end points, the normality assumption was violated. Thus, the Wilcoxon signed rank test was performed to corroborate the parametric analysis test. p Values from the Wilcoxon test were reported. Values of $p<0.05$ were considered significant.

Results

VALIDATION OF METHODS

Pretreatment with omeprazole resulted in nearly complete suppression of gastric acid secretion – that is, pH >6.6 . A significant reduction ($p<0.05$) in duodenal pH values was seen during pirenzepine experiments (Table).

The Table lists the mean recoveries of the non-absorbable markers, phenol red and

[^{51}Cr]EDTA, from the gastric and duodenal effluents, respectively. Only a small amount of the gastric contents entered the duodenal segment, as estimated by the appearance of phenol red in the duodenal effluents – that is, 1.1 (0.3)% of phenol red in the gastric infusate, and 0.06 ml/min contaminating the duodenum. Similarly, the appearance of the duodenal marker, [^{51}Cr]EDTA, in the gastric effluents equalled 1.5 (0.4)%, roughly 0.03 ml/min. The amount of salivary bicarbonate contaminating the stomach was 2.3 (0.8)% ($n=4$) of total gastric bicarbonate content. The amount of swallowed saliva did not increase after sham feeding ($p>0.05$). The content of trypsin in the duodenal effluents was roughly 1.0 (0.1)% of the average minimum trypsin output from the pancreas.²¹

STOMACH

Bicarbonate secretion

During control conditions the basal rate of bicarbonate secretion into the stomach was 382 (62) $\mu\text{mol/h}$. Modified sham feeding for 15 minutes increased ($p<0.02$) bicarbonate secretion to 959 (224) $\mu\text{mol/h}$ with a peak response occurring 10 minutes after start of sham feeding (Fig 2A).

Pretreatment with pirenzepine did not change fluid transport significantly ($p>0.05$; Table), but inhibited ($p<0.03$) the basal rate of bicarbonate secretion to 195 (56) $\mu\text{mol/h}$. The response to modified sham feeding was reduced to 413 (96) $\mu\text{mol/h}$ ($p<0.02$) by pirenzepine compared with control conditions (Fig 2A).

Luminal PGE_2 output

During control conditions the basal luminal PGE_2 output was 56 (6) ng/h. Modified sham feeding increased ($p<0.05$) PGE_2 output to 133 (32) ng/h with a peak response occurring only five minutes after start (Fig 2B).

Pretreatment with pirenzepine raised ($p<0.05$) the basal output of PGE_2 to 92 (11) ng/h. Also the response to modified sham feeding was significantly ($p<0.05$) increased (223 (52) ng/h) by pirenzepine compared with control conditions (Fig 2B).

DUODENUM

Bicarbonate secretion

During control conditions the basal rate of mucosal bicarbonate secretion into the duodenal segment was 191 (14) $\mu\text{mol/cm}\times\text{h}$.

Figure 2: Effect of pirenzepine (10 mg/h intravenously) on basal and stimulated (SH=modified sham feeding) gastric bicarbonate secretion (A) and luminal output of PGE₂ (B) in healthy volunteers (mean (SEM); n=9).

Modified sham feeding and acid exposure of the test segment increased bicarbonate secretion to 266 (27) $\mu\text{mol}/\text{cm} \times \text{h}$ ($p < 0.02$) and 634 (157) $\mu\text{mol}/\text{cm} \times \text{h}$ ($p < 0.01$), respectively (Fig 3A).

Pretreatment with pirenzepine reduced basal fluid transport significantly ($p < 0.02$; Table) and reduced ($p < 0.01$) the basal rate of mucosal bicarbonate secretion to 81 (15) $\mu\text{mol}/\text{cm} \times \text{h}$. A significant response to modified sham feeding was not seen. The acid stimulated increase in bicarbonate secretion to 375 (67) $\mu\text{mol}/\text{cm} \times \text{h}$ was insignificantly ($p > 0.05$) different from that seen during control conditions (Fig 3A).

Luminal PGE₂ output

During control conditions the basal luminal PGE₂ output was 2.1 (0.4) $\text{ng}/\text{cm} \times \text{h}$. Modified sham feeding did not influence PGE₂ output significantly ($p > 0.05$), while acid exposure of the test segment increased ($p < 0.05$) PGE₂ output to 9.0 (3.3) $\text{ng}/\text{cm} \times \text{h}$ (Fig 3B).

Pretreatment with pirenzepine caused no significant ($p > 0.05$) changes either in basal or stimulated PGE₂ output compared with control conditions (Fig 3B).

Discussion

The results of this study clearly show that M₁ receptor blockade by pirenzepine inhibited basal mucosal bicarbonate secretion, both in the stomach and in the duodenum, while modified sham feeding stimulated bicarbonate secretion was inhibited only in the stomach. The results also show that pirenzepine increased basal and vagally stimulated PGE₂ release only in the stomach. These differences in response between the stomach and the duodenum might be explained by differences in pathways mediating stimulation. In contrast, the data could not support our hypothesis that muscarinic M₁ receptor inhibition may increase gastroduodenal mucosal bicarbonate secretion by a prostaglandin dependent mechanism.

The basal gastric bicarbonate secretion rates seen in this study during control perfusions were in the same order of magnitude as those previously reported.²² We determined the gastric bicarbonate content by using a Corning 965 Carbon Dioxide Analyzer, which has previously been shown to provide the same high degree of accuracy as back titration. In comparison with the pH-pCO₂ method (using the Henderson-Hasselbalch formula) and the osmolality-H⁺ method, back titration determines bicarbonate secretion in the acid suppressed stomach more accurately.^{15 22} Also the results of our measurements of proximal duodenal mucosal bicarbonate secretion were similar to those obtained by others.^{14 15}

Similarly, the luminal output of PGE₂ into the stomach and the duodenum seen during control conditions in this study was not different from that previously reported.^{18 23-25} We measured PGE₂ in the perfusates, rather than the synthetic capacity or the content of PGE₂ in mucosal biopsy specimens, because this approach is less traumatising and, therefore, minimises non-specific PGE₂ formation.²⁶

Previous experiments in the rat have shown a dose dependent, stimulatory effect, or no effect, of pirenzepine on duodenal mucosal bicarbonate secretion.^{5 6} Surprisingly, the results of this study clearly show that muscarinic M₁ receptor inhibition by pirenzepine in humans caused a reduction, rather than an increase, in bicarbonate secretion. Species differences in cholinergic stimulatory and inhibitory patterns²⁷ and the finding that pirenzepine may penetrate the blood brain barrier of rats, but not humans, might explain the discrepancy.⁵ On the other hand, it has previously been reported that atropine, but not

Figure 3: Effects of pirenzepine (10 mg/h intravenously) on basal and stimulated (SH=modified sham feeding; HCl=acid load) proximal duodenal mucosal bicarbonate secretion (A) and luminal output of PGE_2 (B) in healthy volunteers (mean (SEM); $n=9$).

pirenzepine, significantly decreased basal and vagally stimulated gastric bicarbonate secretion in humans.³ The pirenzepine doses chosen were, however, only 5–20 $\mu\text{g}/\text{kg}$ and equivalent to those of atropine on a weight to weight basis. As pirenzepine has only one tenth to one eighth of the potency of atropine on a weight to weight basis,²⁸ the doses were probably insufficient for eliciting a response.

In this study we gave pirenzepine in a dose, which was about 10 times higher and similar to that recommended for parenteral application for clinical use.

The findings in rats and in humans have led to the hypothesis that gastroduodenal mucosal bicarbonate secretion is regulated by M_2 , rather than M_1 , subtypes of muscarinic receptors. The results obtained in this study suggest that human basal and vagally stimulated gastroduodenal mucosal bicarbonate secretion is regulated by a pirenzepine sensitive mechanism, which is probably cholinergic and less discriminating than previously proposed. Muscarinic M_1 receptors are believed to be located predominantly on intramural postganglionic neurons and to mediate the effects of ganglionic transmitters through the release of stimulatory substances, such as acetylcholine.²⁹ The finding that gastric bicarbonate, but not fluid output, was reduced suggests that pirenzepine affects an ion exchange component of the bicarbonate transport process. The acid stimulated increase in duodenal mucosal bicarbonate secretion, which is probably of non-cholinergic origin,⁴ was unaffected by pirenzepine. More likely candidates as mediators of duodenal bicarbonate secretion after luminal acid are VIP and PGEs .³⁰

The cholinergic regulation of human gastroduodenal PGE_2 formation seems to be more complex than previously imagined. Vagal stimulation induced by insulin hypoglycaemia or sham feeding is known to enhance gastric luminal PGE_2 output.^{3 13 31} A similar increase in vagally stimulated, gastric luminal PGE_2 output was seen in this study. Although atropine failed to change this response (Mertz-Nielsen *et al*, unpublished data), suggesting that cholinergic pathways do not increase prostaglandin synthesis, we have confirmed our previous finding that muscarinic M_1 receptor blockade with pirenzepine significantly increases gastric PGE_2 output in humans, both during basal conditions and after vagal stimulation.¹³ In addition, we have previously seen that muscarinic M_1 receptor inhibition increases gastric PGE_2 synthesis during acid exposure.¹³ It may be speculated, therefore, that muscarinic M_1 receptor blockade permits the activation of other stimulatory muscarinic receptors or facilitates non-cholinergic stimulation by modulation of sympathetic ganglia or autoreceptors. Thus, an association between stimulation of muscarinic M_2 or M_3 receptors, or both and prostaglandin synthesis, determined as increased cyclic guanine monophosphate and 6-keto-prostaglandin $\text{F}_{1\alpha}$ concentrations, has also been seen in aortic endothelial and vascular smooth muscle cells.³² Another, possibly more likely, interpretation would be that a feed back loop, secondary to the decrease seen in bicarbonate secretion, results in promotion of prostaglandin synthesis through stimulation of receptors distinctly separated from those sensitive to atropine. In the duodenum, neither vagal stimulation nor muscarinic M_1 receptor inhibition affected luminal PGE_2 output significantly.

In conclusion, muscarinic M₁ receptor inhibition by pirenzepine apparently inhibits the cholinergic mechanism entailed in gastroduodenal mucosal bicarbonate secretion in humans. This potentially weakens the mucosal defence by reducing the pH gradient from the lumen to the surface epithelial cells. Muscarinic M₁ receptor inhibition also enhances gastric mucosal release of prostaglandins, which have potent inhibitory effects on the release of proinflammatory mediators from, for example, mast cells.³³ On theoretical grounds, such effects are potentially beneficial and may contribute to the gastroprotective effects of pirenzepine previously reported.

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