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EVIDENCE FOR BOTH HISTAMINE H_1- AND H_2-RECEPTORS IN THE GASTRIC VASCULATURE OF THE CAT

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1 Experiments have been done to study the gastric vascular response to histamine given intra-arterially in the cat.

2 In experiments utilising pump perfusion of the stomach at constant flow rates, rapid intra-arterial injections of histamine elicited dose-dependent vasodilatation. The dose-response curve to histamine was displaced to the right by mepyramine and further to the right by mepyramine plus cimetidine. Cimetidine alone did not displace the histamine dose-response curve. This interaction between histamine and histamine antagonists is very similar to the interaction observed in other vascular beds.

3 Intra-arterial infusions of histamine also caused vasodilatation with increased gastric blood flow, measured with an electromagnetic flow probe. Mepyramine reduced the immediate increase in blood flow during each infusion, although responses in the later stages of the infusions were unaltered. Cimetidine had no effect on the immediate response but reduced sustained responses to histamine. Treatment with mepyramine and cimetidine was required to abolish histamine responses.

4 Infusions of 2-(2-aminoethyl) pyridine and dimaprit also increased gastric blood flow.

5 These results indicate the involvement of both H_1- and H_2-receptors in histamine-induced gastric vasodilatation. There appears to be a time-base in the interaction between histamine and vascular histamine receptors; H_1-receptor responses preceding H_2-receptor responses.

Introduction

Numerous studies have shown that histamine increases gastric blood flow (see review by Lanciault & Jacobson, 1976). These studies have usually been made in conjunction with studies on histamine-induced acid secretion and have required specialized techniques and conditions not appropriate to study vascular responses in other tissues and organs. Thus, the most common technique involves the clearance of aminopyrine or other basic substances between plasma and the acid contents of the stomach (e.g. Jacobson & Chang, 1969; Reed & Smy, 1971) or measurement of vessel diameter by microscopy (e.g. Guth & Smith, 1978). In many of these studies histamine has been given intravenously which, in addition to causing changes in gastric blood flow, also produced marked systemic haemodynamic changes and other non-gastric responses which may modify the local vascular responses within the stomach.

The experiments described in this paper were designed to allow measurement of the local gastric vascular responses to histamine and to characterize the role of histamine receptors in this response by means of techniques suitable for studies in other parts of the peripheral circulation and to allow comparison of findings in the stomach with findings in other tissues and organs.

Methods

Cats, body weight 2.5 to 6.0 kg, were deprived of food overnight but allowed free access to water. Anaesthesia was induced and maintained by intraperitoneal injection of sodium pentobarbitone, 50 mg/kg. The trachea was cannulated. Catheters were tied into the right femoral artery to measure blood pressure and into one femoral and one brachial vein for drug administration.

The circulation of the cat stomach is complex but is derived principally from the coeliac artery. The coeliac artery was approached via a right transverse incision following the line of the ribcage. A length of artery was cleared, taking care not to damage the many nerve plexi in the area. All blood vessels supplying the spleen were ligated as closely as possible to the organ.

Two preparations were used to study gastric vascular responses to histamine.

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Perfusion at constant flow

The animals were given heparin, 1000 iu/kg intravenously. Blood was withdrawn from one carotid artery and pumped, by means of a roller pump, at constant flow rate through the stomach via a catheter tied into the coeliac artery. In these experiments, the hepatic artery was tied and a tight ligature placed around the pyloric end of the stomach to eliminate any blood supply to the stomach from the superior mesenteric circulation. On completion of the surgery, the pump flow rate was set to provide perfusion pressure equal to systemic arterial blood pressure and fixed for the duration of the experiment. Blood flow rate remained constant during changes in perfusion pressure. Perfusion pressure was measured with a Statham P23A transducer in the circuit between the pump and the coeliac artery. Histamine was administered into the perfusion circuit immediately before the pump in a volume of 10 μl/kg. This preparation is very similar to that used previously to characterize vascular histamine receptors in the femoral and superior mesenteric vasculature in cats (Flynn & Owen, 1975). Preliminary studies showed that injections of histamine elicit vasodilatation without any detectable secretion of acid.

Measurement of gastric blood flow with an electromagnetic flow-probe

A tightly fitting electromagnetic flow probe was placed around the coeliac artery between the aorta and the hepatic artery. The hepatic artery was tied leaving sufficient length to allow retrograde cannulation of the artery for local administration of drugs. Histamine and histamine-like agonists were administered by slow intra-arterial infusion through this cannula at a flow rate of 0.16 ml/min. In preliminary experiments, acid secretion could not be detected until about 20 min after starting an infusion of histamine. No secretion was ever detected during a 10 min infusion.

Measurement of acid secretion

The stomach was cannulated via the pyloric sphincter and the animal laid in a position to permit efficient drainage of fluid from the lumen into glass collecting vials. On completion of experiments, the position of the cannula and the efficiency of drainage were determined. After initial experiments had indicated no acid secretion during the histamine treatments used, the pyloric sphincter was not cannulated in subsequent experiments to measure blood flow changes.

Drugs

The following drugs were used: mepyramine maleate (an H_1-receptor antagonist), cimetidine (an H_2- receptor antagonist), histamine acid phosphate (BDH), 2-(2-aminoethyl) pyridine hydrochloride (an H_1-receptor agonist, Durant, Ganellin & Parsons, 1975) and dimaprit (an H_2-receptor agonist, Parsons, Owen, Durant & Ganellin, 1977).

Administration of histamine antagonists

In both preparations, histamine antagonists were administered intravenously; mepyramine by slow injection and cimetidine by continuous intravenous infusion.

Each of the drugs except cimetidine was dissolved in 0.9% w/v NaCl solution (saline). Cimetidine was dissolved in a small volume of 0.1 N HCl neutralized by addition of 0.1 N NaOH and made up to volume with saline.

Statistics

Dose-response relationships and displacements of dose-response curves after receptor blockade were examined by an analysis of variance.

Results

Perfusion at constant flow

Histamine caused vasodilatation over the approximate dose-range 1×10^{-11} to 1×10^{-8} mol/kg. The threshold dose for vasodilatation varied from 1×10^{-11} mol/kg in 4 of 8 preparations to 1 ± 10^{-9} mol/kg in the least responsive preparation. In all experiments, the response to histamine was dose-dependent. Treatment of animals with mepyramine, 1.25×10^{-5} mol/kg, reduced responses to histamine and caused parallel displacement of the histamine dose-response curve to the right with a dose-ratio of 26.3 (13.3 to 52.6, 95% confidence limits). Subsequent treatment with cimetidine, 2×10^{-6} mol kg^{-1} min^{-1}, thus providing both H_1- and H_2-receptor blockade, caused further parallel displacement of the histamine dose-response curve with a dose ratio in excess of 1000 as shown in Figure 1a.

Treatment with cimetidine alone 2×10^{-6} mol kg^{-1} min^{-1}, had no significant effect on the histamine dose-response curve, dose-ratio 1.1 (0.4 to 3.7, 95% confidence limits) but when mepyramine, 1.25×10^{-5} mol/kg, was also administered, a large parallel displacement of the histamine dose-response curve was obtained with a dose-ratio in excess of 1000 as shown in Figure 1b.

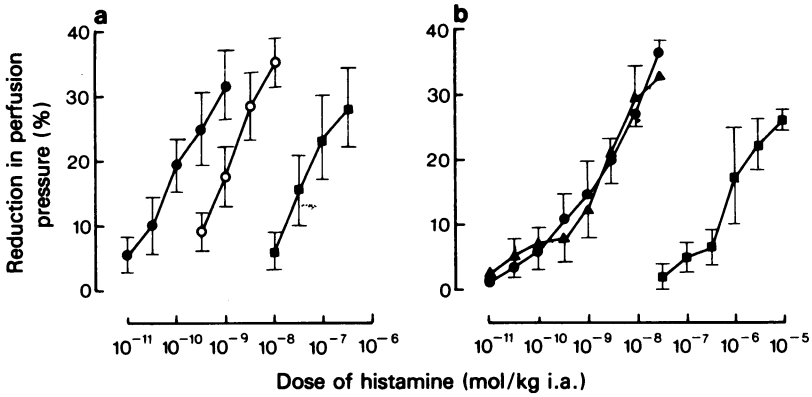

Figure 1 Constant flow perfusion of cat gastric vasculature. (a) Displacement of control dose-response curve to histamine (●) by mepyramine, 1.25×10^{-5} mol/kg (○), and further displacement by mepyramine plus cimetidine, 2×10^{-6} mol kg^{-1} min^{-1} (■). (b) Cimetidine, 2×10^{-6} mol kg^{-1} min^{-1} (▲) does not displace the histamine dose-response curve (●) whereas cimetidine plus mepyramine, 1.25×10^{-5} mol/kg (■) causes a large displacement. Values are means; vertical lines show s.e.mean. $n = 5$.

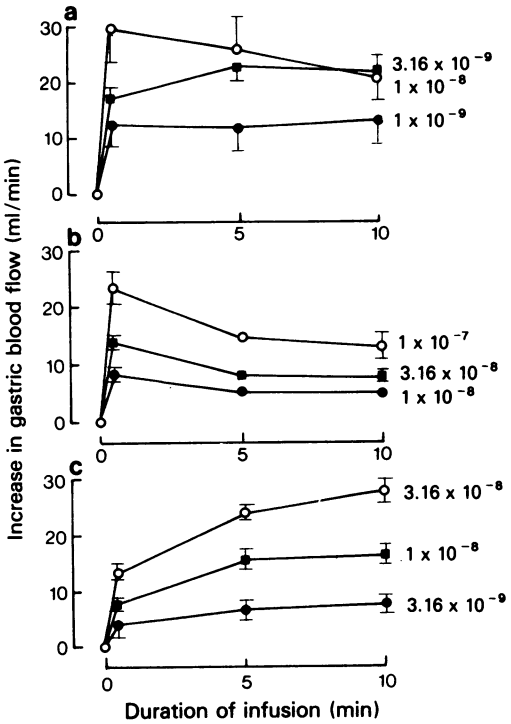

Figure 2. Measurement of gastric blood flow. Intra-arterial infusions of 3 doses each of histamine (a, $n = 8$), 2-(2-aminoethyl)pyridine (b, $n = 5$) and dimaprit (c, $n = 5$) cause dose-dependent increases in gastric blood flow. Doses, in mol kg^{-1} min^{-1} are shown on the figures. Note the different time-course of the response to each agonist.

Electromagnetic flow probe study

Intra-arterial infusions of histamine, 1×10^{-9}, 3.16×10^{-9} and 1×10^{-8} mol kg^{-1} min^{-1} caused large dose-dependent increases in gastric blood flow (Figure 2a). The response to the largest dose of histamine was not well maintained because in the later part of the infusion there was usually a fall in blood pressure, presumably because sufficient histamine was passing through the tissue to elicit a systemic response. Calculation of gastric resistance showed a peak fall of 74% 30 s after the start of the infusion. At the end of the infusion, resistance had still fallen 64%, indicating that the decline in blood flow during the later stages of the largest infusion was due mainly to the fall in blood pressure.

Treatment with mepyramine, 6.25×10^{-6} mol/kg, significantly ($P < 0.0005$) reduced the increase in blood flow at the start of histamine infusions i.e. 30 s after the start of the infusion, but had little effect later in the infusion (Figures 3 and 4). In contrast, cimetidine, 2×10^{-6} mol kg^{-1} min^{-1}, had no effect on the immediate response to histamine but did significantly ($P < 0.001$) reduce the sustained response (Figures 3 and 4). Despite significant modification of the histamine response by either antagonist alone, a substantial response persisted after either H_1- or H_2-receptor blockade. However, the response to histamine was abolished by treatment with both mepyramine and cimetidine (Figures 3 and 4).

Infusion of either 2-(2-aminoethyl) pyridine or dimaprit caused dose-dependent increases in blood flow (Figures 2 and 5). The response to dimaprit was relatively slow in onset but very well sustained and

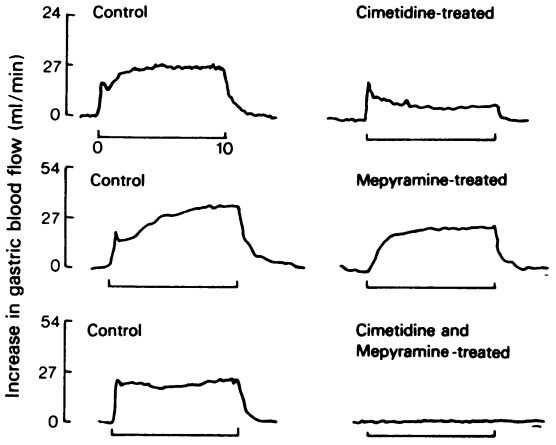

Figure 3. Measurement of gastric blood flow. Histamine receptor blockade modifies the response to histamine, 3.16×10^{-8} mol kg^{-1} min^{-1}. Top panel: treatment with cimetidine, 2×10^{-6} mol kg^{-1} min^{-1} had little effect on the immediate response to infusion of histamine but substantially reduced the response later in the infusion; control on the left. Middle panel: treatment with mepyramine, 6.25×10^{-6} mol/kg, reduced the immediate response to histamine but had little effect on the sustained response; control response on the left. Lower panel: treatment with cimetidine, 2×10^{-6} mol kg^{-1} min^{-1}, and mepyramine, 6.25×10^{-6} mol/kg, abolished the response to histamine, control response on the left.

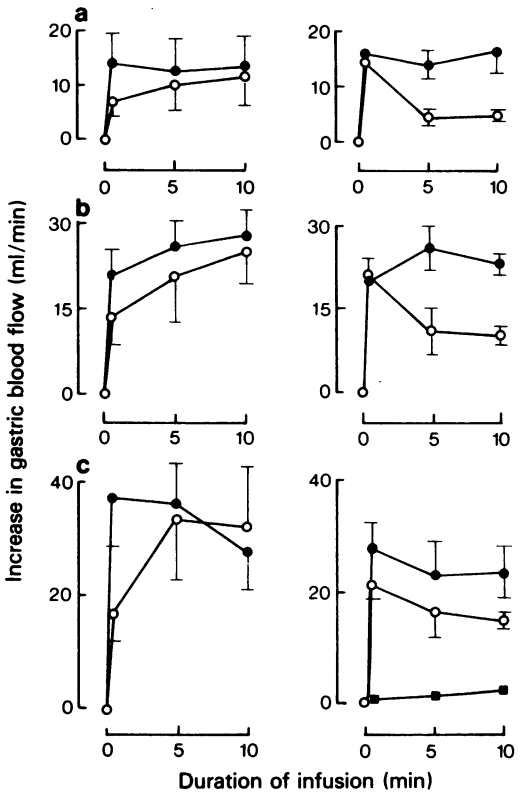

Figure 4 Measurement of gastric blood flow. Modification of responses to histamine by treatment with mepyramine, 6.25×10^{-6} mol/kg, shown on the left and by cimetidine, 2×10^{-6} mol kg^{-1} min^{-1}, shown on the right. Infusion rates of histamine were 1×10^{-9} (top) 3.16×10^{-9} (middle) and 1×10^{-8} mol kg^{-1} min^{-1} (bottom panel). Mepyramine consistently reduced the initial peak response to histamine but had little subsequent effect. Cimetidine had little or no effect on the initial response, but significantly reduced the sustained response. Control responses (●); responses after receptor blockade (○). The lower right hand panel shows abolition of the histamine responses by mepyramine plus cimetidine (■). Values are means; vertical lines show s.e.mean. $n = 4$ for mepyramine experiments and $n = 3$ for cimetidine experiments.

very similar to the response to histamine in mepyramine-treated cats. 2-(2-Aminoethyl) pyridine produced a large initial response i.e. within 30 s of the start of the infusion, but the increase in blood flow declined in the later parts of the infusion, similar to the response to histamine in cimetidine-treated cats.

Discussion

The present experiments provide clear evidence that the gastric vasodilator response to histamine involves both H_1- and H_2-receptors. The nature of the modification of histamine responses by histamine receptor antagonists in the stomach was very similar to the modification previously observed in other peripheral vascular beds by comparable techniques (Flynn & Owen, 1975). The vasculature perfused was most of the stomach plus a small region in the omentum and pancreas. Differential responses within parts of the stomach could not be separated with the techniques used.

Studies with bolus injections of histamine in pump-perfused preparations have shown that mepyramine displaces the histamine dose-response curve to the right in a variety of vascular beds including cat femoral and superior mesenteric vasculature (Flynn & Owen, 1975). The maximum displacement of the histamine dose-response curve in the cat femoral and superior mesenteric vasculature was obtained with mepyramine, 2.5×10^{-6} mol/kg, providing dose-ratios of approximately 10 and 16 respectively (Flynn

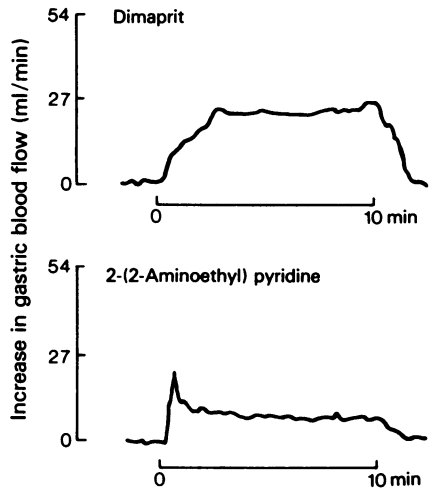

Figure 5 Measurement of gastric blood flow. The time-course of the response to infusion of dimaprit, 3.16×10^{-8} mol kg^{-1} min^{-1} and 2-(2-aminoethyl)pyridine, 1×10^{-7} mol kg^{-1} min^{-1}.

& Owen, 1975). Larger doses of mepyramine did not cause any further displacement of the histamine dose-response curve but did ensure adequate H_1-receptor blockade for the entire duration of the experiment. In the present study, mepyramine was used at a single dose, 1.25×10^{-5} mol/kg, in order to achieve prolonged H_1-receptor blockade, and displaced the histamine dose-response curve with a dose-ratio of 26.3 i.e., a little larger than the dose-ratio obtained in other vascular beds. It has not been directly established that this dose-ratio represents the maximum displacement by H_1-receptor blockade. However, because the dose-response curve to histamine is displaced so far to the right by cimetidine in mepyramine-treated animals, it would appear likely that the dose of mepyramine is fully adequate for H_1-receptor blockade. Thus, these studies provide evidence of H_1-receptors associated with gastric vasodilatation. Cimetidine alone did not displace the dose-response curve to injections of histamine, although in animals treated with mepyramine, cimetidine was highly effective in further displacing the histamine dose-response curve. Again this pattern of interaction between histamine and histamine receptor antagonists is similar to that reported in other peripheral vascular beds. This effect of cimetidine provides evidence of vasodilatation associated with H_2-receptors.

The modification of the gastric vasculature response to infusions of histamine showed that the interaction between histamine and antagonists varies with time. The immediate response to histamine was reduced in every animal by mepyramine. Cimetidine did not reduce the immediate response, but did reduce the sustained response, particularly at the lower histamine infusion rates. Mepyramine did not reduce the sustained response to histamine. Responses to histamine throughout the duration of each infusion were abolished by mepyramine plus cimetidine. Thus these studies also provide clear evidence that both H_1- and H_2-receptors contribute to gastric vasodilatation.

The studies with the selective receptor agonists also provide evidence of both receptors associated with vasodilatation. The time-course of the response to the agonists was also consistent with the idea that H_1-receptor responses are rapid in onset but poorly maintained whereas H_2-receptor responses are slow in onset but better maintained. A time base in the interaction between histamine and histamine vascular receptors has been observed in dog mesentery (Pawlik, Tague, Tepperman, Miller & Jacobson, 1977) and in the total peripheral circulation in cats (Harvey & Owen, unpublished observations). The nature of this time base requires clarification but the pattern observed in the stomach in these experiments is not unique to the stomach.

There is little reason to doubt that the H_1-receptor part of the response is a direct vascular effect of histamine, whereas it is far more difficult to distinguish whether the H_2-receptor part of the response is a direct vascular effect of histamine, secondary to the increased metabolic requirements during histamine-induced acid secretion or whether both mechanisms contribute to the total response. The remarkable similarity in the interaction between histamine, whether given by injection or infusion, and histamine receptor antagonists in the gastric vasculature and that previously observed in other vascular beds e.g. femoral and mesentric vasculature in cats (Flynn & Owen, 1975) and mesenteric vasculature in dogs (Pawlik *et al.*, 1977), where there is no evidence that H_2-receptor vasodilatation would function via metabolic needs suggest the presence of H_2-receptors on gastric blood vessels. Thus, the H_2-receptor component of histamine-induced vasodilatation probably reflects both dilatation due to the presence of vascular H_2-receptors in addition to vasodilatation to sustain the increased activity of parietal cells. The contribution of each part to the total response has not been determined. Further support for this claim is derived from the split microscopy study of Guth & Smith (1978). This technique requires topical application of histamine and vasodilatation involving both H_1- and H_2-receptors occurs without evidence of acid secretion.

Previous studies in which clearance techniques were used to measure mucosal flow have claimed that histamine H_2-receptor antagonists in doses which prevent acid secretion also prevent the increase in

gastric mucosal flow (e.g. Holton & Curwain, 1973; Reed, Smy, Venables & Harris, 1973; Konturek, Tasler, Obtulowicz & Rehfeld, 1974; Reed & Sanders, 1975; Main & Whittle, 1976). The greater effectiveness of H_2-receptor blockade reported in clearance studies relative to our own results may reflect differences in technique and route of administration. In an attempt to clarify these mechanisms, our experimental protocol was designed to allow studies of blood flow responses prior to detectable secretion of acid. This design should favour analysis of direct vascular effects although a metabolic component cannot be totally excluded.

Thus, Archibald, Moody & Simons (1975) have provided evidence that the clearance of aminopyrine into the gastric fluid is, in part, dependent on the secretory status of the stomach despite attempts to minimise the importance of this. Consequently, the method accurately assesses blood flow during secretion but may underestimate blood flow when secretion is prevented. The possibility therefore exists that in the presence of H_2-receptor blockade, when secretion is antagonized, blood flow is underestimated and the vascular effects of the antagonist consequently overestimated.

The major difference between local and systemic administration may be reflected particularly in terms of the fall in blood pressure caused by secretory doses of histamine given intravenously (e.g. Durant, Duncan, Ganellin, Parsons, Blakemore & Rasmussen, 1978). Thus, when blood pressure is lowered, gastric blood flow can only be maintained if some vasodilatation occurs. This problem can be avoided by local administration of histamine which can elicit large local responses without changing blood pressure. Studies made using systemic administration of histamine without knowledge of blood pressure may fail to recognise the vasodilatation needed to maintain blood flow as pressure is reduced. Further consequences of systemic administration of histamine may include release of catecholamines from chromaffin tissue, an H_1-receptor phenomenon (Emmelin & Muren, 1949). Catecholamines usually cause powerful gastric vasoconstriction (Lanciault & Jacobson, 1976) and may oppose direct vasodilatation. Thus, H_1-receptor vasodilatation in the stomach may be opposed by H_1-receptor mediated release of catecholamines. This would again be avoided by local administration of histamine.

In conclusion, evidence has been presented for the existence of both H_1- and H_2-receptors associated with gastric vasodilatation. The H_2-receptor response probably consists of both direct vasodilatation and an indirect component due to increased parietal cell activity.

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