Role of bradykinin in the hyperaemia following acid challenge of the rat gastric mucosa

†Gábor Pethő, *Milana Jocič & †Peter Holzer

*Department of Experimental and Clinical Pharmacology, University of Graz, A-8010 Graz, Austria and †Department of Pharmacology, Medical University of Pécs, H-7643 Pécs, Hungary

1 This study examined whether the hyperaemia following acid challenge of the rat gastric mucosa involves bradykinin, a peptide formed in response to tissue injury.

2 Gastric mucosal blood flow in urethane-anaesthetized rats was assessed by the hydrogen gas clearance method. Infusion of a bradykinin solution (10 μM) into the gastric wall augmented gastric mucosal blood flow by a factor of 2.3, an effect that was prevented by the bradykinin B_2 antagonist Hoe-140 (icatibant; 100 $\mu\text{mol kg}^{-1}$, i.v.).

3 I.v. injection of bradykinin (20–60 nmol kg^{-1}) caused a 2.3–3.5 fold increase in blood flow through the left gastric artery as measured by the ultrasonic transit time shift technique. The hyperaemic effect of bradykinin in this gastric artery was also prevented by Hoe-140 (100 $\mu\text{mol kg}^{-1}$, i.v.).

4 Gastric acid backdiffusion was evoked by perfusing the stomach with 15% ethanol, to break the gastric mucosal barrier, in the presence of luminal acid. Depending on the concentration of acid (0.05 and 0.15 M HCl), this procedure increased gastric mucosal blood flow by a factor of 1.6–2.8 and caused formation of gross damage in 1.5–3% of the glandular mucosa. Hoe-140 (100 $\mu\text{mol kg}^{-1}$, i.v.) failed to alter the moderate vasodilatation seen in the presence of 0.05 M HCl but significantly ($P < 0.05$) attenuated the marked hyperaemia and enhanced the gross mucosal damage observed in the presence of 0.15 M HCl.

5 These data show that bradykinin is able to enhance gastric mucosal blood flow via activation of B_2 receptors. It appears as if this kinin is formed during severe acid challenge of the rat gastric mucosa and participates in the hyperaemic reaction to gastric acid backdiffusion.

Keywords: Bradykinin; bradykinin antagonist; Hoe-140 (icatibant); bradykinin B_2 receptors; gastric blood flow; acid-evoked gastric hyperaemia; gastric mucosal damage

Introduction

Acid backdiffusion through a disrupted gastric mucosal barrier leads to a rise of gastric mucosal blood flow, a reaction that limits acid damage to the surface of the mucosa (Whittle, 1977; Bruggeman *et al.*, 1979; Holzer *et al.*, 1991). The hyperaemic reaction to acid back-diffusion in the rat gastric mucosa is to a large extent mediated by sensory neurones (Holzer *et al.*, 1991; Raybould *et al.*, 1992) that cause arteriolar dilatation via release of calcitonin gene-related peptide (Li *et al.*, 1992) and formation of nitric oxide (Lippe & Holzer, 1992). It is not yet known, however, how acid influx into the gastric mucosa activates this neurogenic vasodilator mechanism. Histamine, which has been implicated in the acid-evoked hyperaemia of the canine gastric mucosa (Bruggeman *et al.*, 1979), does not seem to play a role in the acid-challenged mucosa of the rat stomach (Holzer *et al.*, 1991). Although hydrogen ions themselves can stimulate sensory neurones in the gastric mucosa (Geppetti *et al.*, 1991), other mediators generated during tissue damage need also to be taken into account.

Tissue injury, ischaemia and low pH are known to activate tissue and plasma kallikreins to form bradykinin and other kinins (Hall, 1992; Dray & Perkins, 1993). Newly formed bradykinin acts locally close to its site of production on a wide variety of tissues including blood vessels and sensory nerve fibres. Thus, the kinin has been found to dilate the coeliac and mesenteric arteries *in vitro* (Ritter *et al.*, 1989; Santiago *et al.*, 1993), to augment blood flow through the superior mesenteric artery of the rat (Berguer *et al.*, 1993) and to excite visceral afferents in several species (Longhurst *et al.*, 1984; Stein *et al.*, 1986; Holzer, 1992; Holzer-Petsche, 1992; Geppetti, 1993; Griesbacher, 1994). Both the splan-

chnic vasodilator and sensory nerve stimulant actions of bradykinin are mediated by bradykinin B_2 receptors as revealed by the use of receptor subtype-selective antagonists (Holzer, 1992; Holzer-Petsche, 1992; Berguer *et al.*, 1993; Santiago *et al.*, 1993; Griesbacher, 1994). The aim of the present study was to test whether bradykinin participates in the gastric hyperaemic reaction to mucosal acid challenge. This question was addressed in two stages. Firstly, we examined whether bradykinin is able to enhance blood flow in the rat stomach. Secondly, we tested whether Hoe-140 (icatibant), a potent, selective and long-acting bradykinin B_2 antagonist (Hock *et al.*, 1991; Lembeck *et al.*, 1991; Wirth *et al.*, 1991) modifies the rise of gastric mucosal blood flow in response to acid backdiffusion.

Methods

Animal preparation

All experiments of this study were approved by the Ministry of Science and Research of the Federal Republic of Austria. Female Sprague-Dawley rats, weighing 180–220 g, were fasted for 20 h but had free access to water. After the induction of anaesthesia with urethane (1.5 g kg^{-1} , s.c.) the rats were fitted with a tracheal cannula to facilitate spontaneous respiration and to allow for the administration of hydrogen. Mean arterial blood pressure was recorded from a cannula in the right carotid artery by means of a pressure transducer (model ISOTEC; HSE, March-Hugstetten, Germany) and displayed on a chart recorder (model SERVOGOR 464; ABB-GOERZ, Vienna, Austria). In some experiments the blood pressure signal was fed into a heart ratemeter (model MINO; HSE, March-Hugstetten, Germany) with digital

¹ Author for correspondence.

readout of the heart rate. A catheter in the left jugular vein was used for the continuous infusion of saline (1.5 ml h^{-1}), to avoid dehydration, and for the i.v. injection of drugs. The stomach, exposed by a midline laparotomy, was fitted with an inflow cannula placed in the forestomach and an outflow cannula inserted through the pylorus (Holzer *et al.*, 1991). The experimental media (kept at room temperature) were perfused through the stomach at a rate of $0.7\text{--}0.8 \text{ ml min}^{-1}$. The body temperature of the animals was kept at 37°C by means of a heated table.

Blood flow measurements

Gastric mucosal blood flow was measured with the hydrogen clearance technique. The washout of hydrogen gas was estimated by a needle-type platinum electrode inserted from the serosa into the basal portion of the gastric corpus mucosa and positioned at the submucosal border of the muscularis mucosae (Holzer *et al.*, 1991). The method is based on the principle that the oxidation of molecular hydrogen at the surface of a platinum electrode generates electrons and that the resulting current is proportional to the hydrogen concentration at the site of the electrode (Leung *et al.*, 1984). In the present experiments, hydrogen (3%) was added to the air which the rats were breathing until the current tracing reached a plateau. When 15 min later the external hydrogen source was removed, the current gradually fell due to removal of hydrogen by blood flow. The rate of hydrogen clearance from the tissue is related to the magnitude of tissue blood flow (Leung *et al.*, 1994) which was calculated with a computer programme (Livingston *et al.*, 1989) and expressed as $\text{ml min}^{-1} 100 \text{ g}^{-1}$. It is evident that the measurement of blood flow with this method was discontinuous, as the experimental protocol involved alternating 15 min periods of saturation, and desaturation, of the tissue with hydrogen gas.

Blood flow in the left gastric artery was recorded continuously with the ultrasonic transit time shift technique which measures the net volume of blood flow with a factory-calibrated sensor (Barnes *et al.*, 1983; Burton & Gorewit, 1984). To this end, the terminal segment of the left gastric artery close to its entry into the stomach was separated from the surrounding tissue over a length of 4–5 mm under a binocular microscope. A perivascular ultrasonic flow probe (model 1RB, Transonic, Ithaca, New York, U.S.A.) was placed around the left gastric artery and connected to a small animal flowmeter (model T106, Transonic) which calculated the volume of blood flow (ml min^{-1}) and fed the signal into a chart recorder (Holzer *et al.*, 1993).

Determination of gross mucosal damage

Gross damage to the gastric glandular mucosa was determined from photographs of the stomachs pinned flat on a board and quantified by computerized planimetry. The area covered by visible haemorrhagic lesions (defined as gross injury) was measured with a digitizing tablet and the software SigmaScan (Jandel, Corte Madera, California, U.S.A.). Gross injury was evaluated by an observer unaware of the experimental treatment. The area covered by gross injury was expressed as a percentage of the total area of the glandular mucosa of the rat stomach.

Experimental protocols

An equilibration period of 45 min was allowed before the experiments were initiated. Three studies were conducted. In Study 1, the effect of local administration of bradykinin on gastric mucosal blood flow was examined. To this end an injection needle (outer diameter 0.3 mm) was inserted in the gastric corpus wall and positioned at the submucosal border of the muscularis mucosae at a distance of 0.5–1 mm from the platinum electrode. The needle, which was connected to an infusion pump, was placed in the gastric wall 1 h before

application of the test substances. Bradykinin was administered by intramural infusion ($3 \mu\text{l min}^{-1}$) of a $10 \mu\text{M}$ solution for a period of 5 min immediately before a hydrogen desaturation curve was recorded. The vasodilator effect of bradykinin was determined by comparison of the bradykinin-evoked hyperaemia with that induced by the vehicle (saline) infused at a rate of $3 \mu\text{l min}^{-1}$.

The effect of bradykinin, injected i.v. at 15 min intervals, on blood flow in the left gastric artery was investigated in Study 2. The experiments were started by priming the animals with i.v. injections of bradykinin (20 nmol kg^{-1}) until the hyperaemic response to the kinin became stable (Holzer *et al.*, 1993). Then the dose-dependency of the haemodynamic effect of bradykinin was tested by recording the responses to 20 and 60 nmol kg^{-1} bradykinin. Thereafter the bradykinin antagonist Hoe-140 ($100 \mu\text{mol kg}^{-1}$) was administered i.v. followed by further injections of 60 nmol kg^{-1} bradykinin. All drugs were given at a volume of 1 ml kg^{-1} , the vehicle being saline.

Study 3 was carried out to investigate the influence of Hoe-140 ($100 \mu\text{mol kg}^{-1}$) on the increase in gastric mucosal blood flow caused by gastric acid backdiffusion. The basic experimental protocol used for this purpose was evaluated in previous studies (Holzer *et al.*, 1991; Holzer & Lippe, 1992). The experiments were begun by perfusing the stomach with 0.05 M hydrochloric acid (HCl) in 0.1 M saline (first set of experiments) or with 0.15 M HCl in water (second set of experiments). Saline and water, respectively, were used for preparing the gastric perfusion media in order to achieve solutions of comparable ionic strength and osmolarity (0.15 M). After perfusion of the stomach with these media for 1 h, acid backdiffusion was induced by adding ethanol (15%, wt/wt) to the acid media and continuing the gastric perfusion for another hour. Measurements of gastric mucosal blood flow were taken during the periods 45–30 min and 15–0 min before, and 15–30 min and 45–60 min after, the induction of gastric acid backdiffusion. Hoe-140 or its vehicle (saline, 1 ml kg^{-1}) was injected i.v. 30 min before the gastric perfusion with ethanol in acid was begun.

Substances

The saline solution used here was 0.15 M NaCl. Urethane (Fluka, Buchs, Switzerland) was dissolved in water at a concentration of 25% (wt/wt). Bradykinin (Bachem, Bubendorf, Switzerland) and Hoe-140 (icatibant; Hoechst, Frankfurt am Main, Germany) were dissolved in saline (1 mM). Further dilutions, if necessary, were also made with saline. HCl (1 M) and ethanol (100%) were obtained from Merck (Darmstadt, Germany).

Statistics

All data are presented as mean $\pm$ s.e.mean. Statistical evaluation of the results was performed with the Kruskal-Wallis H test or the Mann-Whitney U test, where applicable. Probability values $P < 0.05$ were regarded as significant.

Results

Effect of intramural bradykinin on gastric mucosal blood flow (Study 1)

Since it was found that intramural infusion of $1 \mu\text{M}$ bradykinin failed to augment gastric mucosal blood flow consistently ($n = 3$), a ten fold higher concentration of the kinin ($10 \mu\text{M}$) was used in this study. This concentration of bradykinin resulted in a 2.3 fold increase in blood flow through the gastric mucosa, whereas the vehicle (saline) infused intramurally at a rate of $3 \mu\text{l min}^{-1}$ failed to enhance gastric mucosal blood flow significantly (Figure 1). The hyperaemic response to bradykinin was transient and seen

only during the gastric mucosal blood flow measurement period immediately following the intramural infusion of the kinin. Mean arterial blood pressure (88 ± 3 mmHg, $n = 10$) did not change in response to intramural vehicle or bradykinin (data not shown).

I.v. injection of Hoe-140 ($100 \mu\text{mol kg}^{-1}$, 30 min before the kinin) virtually abolished the vasodilator effect of intramural bradykinin ($10 \mu\text{M}$), and the bradykinin-induced hyperaemia observed after Hoe-140 was indistinguishable from the

insignificant hyperaemia caused by intramural saline (Figure 1). Neither basal gastric mucosal blood flow (Figure 1) nor mean arterial blood pressure (91 ± 2 mmHg, $n = 7$) was significantly altered by Hoe-140. I.v. injection of saline (1 ml kg^{-1}), the vehicle for Hoe-140, had no effect on these parameters and failed to influence the rise of gastric mucosal blood flow elicited by intramural bradykinin (Figure 1).

Effect of i.v. bradykinin on blood flow in the left gastric artery (Study 2)

I.v. injection of bradykinin (20 and 60 nmol kg^{-1}) led to a dose-related increase in blood flow through the left gastric artery, a dose-related increase in heart rate, and a dose-related decrease in mean arterial blood pressure (Figure 2 and Table 1). These cardiovascular effects of bradykinin were transient and abated within 3–5 min (Figure 2). After injection of Hoe-140 ($100 \mu\text{mol kg}^{-1}$, i.v.) the effects of bradykinin (60 nmol kg^{-1}) on blood flow in the left gastric artery, mean arterial blood pressure and heart rate were practically abolished (Figure 2 and Table 1) for a period of 1 h, the longest period examined. Injection of saline (1 ml kg^{-1} , i.v.), the vehicle for Hoe-140, did not affect any of the cardiovascular responses to bradykinin under study ($n = 3$).

Effect of Hoe-140 on the rise of gastric mucosal blood flow due to acid backdiffusion (Study 3)

Hoe-140 was tested in two series of experiments in which the stomachs were perfused with either 0.05 or 0.15 M HCl. The hyperaemia ensuing after the induction of acid backdiffusion by adding ethanol (15%) to the acid perfusion medium depended on the concentration of HCl. Acid backdiffusion from a medium with 0.15 M HCl caused a 4 fold ($P < 0.01$) higher increase in gastric mucosal blood flow than acid backdiffusion from a medium with 0.05 M HCl (Figures 3 and 4). In contrast, the formation of gross haemorrhagic lesions in the gastric corpus mucosa was not significantly related to the HCl concentration in the perfusion medium, although on average the injury seen after acid backdiffusion

Figure 1 Effect of intramural bradykinin (BK, $10 \mu\text{M}$) or its vehicle (VH, saline, infused at a rate of $3 \mu\text{l min}^{-1}$) on gastric mucosal blood flow (GMBF) in untreated rats and in rats that had received an i.v. injection of Hoe-140 ($100 \mu\text{mol kg}^{-1}$) or its vehicle (Veh, saline, 1 ml kg^{-1}) 30 min before the administration of bradykinin. Bradykinin or its vehicle was infused for a period of 5 min immediately before a measurement of gastric mucosal blood flow was taken. The graphs show the basal (B) values of gastric mucosal blood flow that were recorded 15 min before, and the gastric mucosal blood flow values recorded immediately after, the intramural infusion of bradykinin or its vehicle. The values shown are mean with the s.e.mean and the number of rats (n) indicated at the base of the columns. * $P < 0.01$ versus respective basal values; † $P < 0.05$ versus respective value in the vehicle-treated rats.

Figure 2 Recording of the effect of i.v. injected bradykinin (BK, doses in nmol kg^{-1} indicated in parentheses) on heart rate (HR), mean arterial blood pressure (MAP) and blood flow in the left gastric artery (BF/LGA) before, and 30 min after, the i.v. injection of Hoe-140 ($100 \mu\text{mol kg}^{-1}$).

Table 1 Effect of i.v. bradykinin (BK) on blood flow in the left gastric artery (BF/LGA), mean arterial blood pressure (MAP) and heart rate (HR): dose-dependency and antagonism by Hoe-140 (100 $\mu\text{mol kg}^{-1}$)

Agonist	Antagonist	BF/LGA (ml min ⁻¹)	MAP (mmHg)	HR (min ⁻¹)
None (basal parameters)	None	0.54 $\pm$ 0.04 (17)	99 $\pm$ 1.7 (17)	465 $\pm$ 5.0 (13)
BK (20 nmol kg ⁻¹)	None	+0.71 $\pm$ 0.12 (9)	-34.8 $\pm$ 2.7 (9)	+22.3 $\pm$ 3.2 (7)
BK (60 nmol kg ⁻¹)	None	+1.33 $\pm$ 0.20 (8)	-42.0 $\pm$ 2.0 (8)	+33.0 $\pm$ 2.9 (6)
BK (60 nmol kg ⁻¹)	Hoe-140	+0.14 $\pm$ 0.05 (6)*	-0.4 $\pm$ 0.3 (6)*	+1.4 $\pm$ 1.1 (6)*

The basal parameters were recorded immediately before the i.v. injection of bradykinin. The effects of bradykinin are expressed as increments (labelled with +) or decrements (labelled with -) of the parameters under study (peak values minus basal values). Hoe-140 (100 $\mu\text{mol kg}^{-1}$) was injected i.v. 30 min before the effect of bradykinin was tested. The figures shown are mean $\pm$ s.e.mean, with the number of rats (*n*) indicated in parentheses. **P* < 0.01 versus the effect of bradykinin (60 nmol kg⁻¹) measured in the absence of Hoe-140.

Figure 3 Effect of gastric acid backdiffusion in the presence of 0.05 (a,b) or 0.15 M (c,d) HCl on gastric mucosal blood flow (GMBF) in rats treated either with Hoe-140 (100 $\mu\text{mol kg}^{-1}$) or its vehicle (saline, 1 ml kg⁻¹). Hoe-140 or its vehicle was injected i.v. 30 min before acid backdiffusion was induced by adding ethanol (EtOH, i.g. = intragastrically, 15%) to the acidified gastric perfusion media. The 4 columns in each graph show gastric mucosal blood flow measured 45–30 min (A) and 15–0 min (B) before, and 15–30 min (C) and 45–60 min (D) after, induction of gastric acid backdiffusion. The values shown are mean with the s.e.mean. The number of rats (*n*) in each group was 8–10. **P* < 0.01 versus respective values in the vehicle-treated rats.

from 0.15 M HCl was double the injury seen after exposure to 0.05 M HCl (Figure 4). Mean arterial blood pressure was not affected by the HCl concentration in the gastric perfusion medium and did not change after induction of gastric acid backdiffusion (data not shown).

The effect of Hoe-140 (100 $\mu\text{mol kg}^{-1}$ injected i.v. 30 min before the induction of acid backdiffusion) on the acid-evoked hyperaemia in the gastric mucosa depended on the HCl concentration in the gastric perfusion medium. At a concentration of 0.05 M HCl, Hoe-140 failed to alter significantly the hyperaemia and the formation of gross mucosal damage induced by gastric acid backdiffusion (Figures 3 and 4). Conversely, the rise of gastric mucosal blood flow elicited in the presence of 0.15 M HCl was significantly attenuated by Hoe-140 as compared with the

acid-evoked hyperaemia seen in rats injected with the vehicle (saline). The inhibitory effect of Hoe-140 on the hyperaemic response to acid backdiffusion in the presence of 0.15 M HCl was associated with enhanced formation of gross mucosal damage (Figure 4). Basal gastric mucosal blood flow as well as mean arterial blood pressure before and after gastric acid backdiffusion were not influenced by Hoe-140 (data not shown).

Discussion

The present data demonstrate that bradykinin increases blood flow in the rat stomach and plays a role in the gastric hyperaemic response to backdiffusing acid. The evidence for

Figure 4 Increment of gastric mucosal blood flow (Δ GMBF) and formation of gross mucosal damage in response to gastric acid backdiffusion in the presence of 0.05 (a,b) or 0.15 M (c,d) HCl in rats treated either with Hoe-140 ($100 \mu\text{mol kg}^{-1}$) or its vehicle (saline, 1 ml kg^{-1}). Hoe-140 or its vehicle (Veh) was injected i.v. 30 min before gastric acid backdiffusion was induced by adding ethanol (15%) to the acidified gastric perfusion media. The increment of gastric mucosal blood flow was measured during the period 15–30 min, and gross damage was quantified 60 min, after the induction of gastric acid backdiffusion. The values shown are mean with the s.e.mean. The number of rats (n) in each group was 8–10. * $P < 0.01$ versus respective values in the vehicle-treated rats; # $P < 0.01$ versus respective values in rats whose stomachs were perfused with 0.05 M HCl.

this conjecture was obtained in three experimental stages. In the first study it was shown that bradykinin administered by local infusion into the gastric wall is able to enhance gastric mucosal blood flow. The vasodilator effect of intramural bradykinin implies that formation of this kinin in the gastric tissue is expected to facilitate blood flow through the gastric mucosa. The concentration of intramural bradykinin ($10 \mu\text{M}$) required to augment gastric mucosal blood flow may seem high, but it is necessary to take into account the fact that the kinin was infused at a rate of $3 \mu\text{l min}^{-1}$ (30 pmol min^{-1}) in order to avoid the infusate volume affecting blood flow. It is possible that small amounts of bradykinin may be degraded rapidly within the gastric wall.

In the second study it was found that bradykinin given i.v. increases blood flow in the left gastric artery, one of the major arteries supplying the stomach, in spite of an appreciable decrease in mean arterial blood pressure. Bradykinin thus can facilitate gastric blood flow independently of whether it is administered extra- or intravascularly, an issue that was of interest to know in view of the possible extra- and intravascular formation of bradykinin in the injured gastric mucosa. The vasodilator effects of both intramural and i.v. bradykinin were short-lived, which is indicative of a rapid breakdown of the kinin by proteolytic enzymes (Hall, 1992; Geppetti, 1993). Another property common to the bradykinin-induced increases of blood flow in the left gastric artery and gastric mucosa was their susceptibility to blockade by Hoe-140. Since this compound is a selective bradykinin B_2 receptor antagonist (Hock *et al.*, 1991; Wirth *et al.*, 1991) it follows that the dilator effect of bradykinin in the gastric vascular system is exclusively mediated by this type of

bradykinin receptor. This conclusion is in keeping with the finding that the dilator response of the mesenteric artery to bradykinin is also brought about by activation of B_2 receptors (Berguer *et al.*, 1993; Santiago *et al.*, 1993).

Unlike the vasodilator effect of intramurally infused bradykinin, the gastric hyperaemic response to i.v. injected bradykinin was associated with transient hypotension and tachycardia. It is unlikely that the rise of blood flow through the left gastric artery was secondary to the effects of bradykinin on blood pressure and heart rate, because hypotension occurring in the absence of gastric vasodilatation is expected to lower blood flow through the stomach. This is the case with another hypotensive peptide, substance P, which fails to increase gastric mucosal blood flow (Holzer & Guth, 1991) and after i.v. injection decreases both blood pressure and blood flow through the left gastric artery (Holzer *et al.*, 1994).

In the third study Hoe-140 was used to examine a possible role of bradykinin in the gastric mucosal hyperaemia evoked by acid backdiffusion. The action of this drug is long-lasting (Hock *et al.*, 1991; Lembeck *et al.*, 1991; Wirth *et al.*, 1991; Holzer, 1992; Santiago *et al.*, 1993), which makes this compound a suitable tool for studying the pathophysiological implications of bradykinin acting via B_2 receptors. The dose of Hoe-140 used here ($100 \mu\text{mol kg}^{-1}$) has previously been established as effective and specific in antagonizing various responses to bradykinin (Lembeck *et al.*, 1991; Holzer, 1992; Holzer-Petsche, 1992). Its specificity (Hock *et al.*, 1991; Lembeck *et al.*, 1991; Wirth *et al.*, 1991; Holzer, 1992; Holzer-Petsche, 1992; Santiago *et al.*, 1993) was therefore not tested further, especially after it was found to be without

influence on the rise of gastric mucosal blood flow induced by gastric perfusion with 15% ethanol in 0.05 M HCl.

When, however, the intraluminal concentration of HCl was raised to 0.15 M, Hoe-140 was able to reduce significantly the hyperaemia evoked by exposure of the gastric mucosa to ethanol. It has previously been shown that the gastric mucosal hyperaemia seen under these conditions results from acid backdiffusion into the mucosal tissue (Holzer *et al.*, 1991). Ethanol is used to disrupt the gastric mucosal barrier and it is only after exposure to combined ethanol and HCl that gastric mucosal blood flow increases to a significant extent (Holzer *et al.*, 1991). The present study provided further support for this reasoning by showing that the gastric vasodilatation is closely related to the intraluminal concentration of acid. The acid-evoked hyperaemia plays a physiological role in preventing deep mucosal erosions which present themselves as haemorrhagic lesions at the macroscopic level (Holzer *et al.*, 1991).

The inhibitory effect of Hoe-140 on the hyperaemia seen after barrier disruption in the presence of 0.15 M HCl, but not 0.05 M HCl, indicates that endogenous bradykinin contributes to the acid-evoked rise of blood flow through the gastric mucosa only under conditions of severe acid

challenge. The relevance of hyperaemia for gastric mucosal protection is reflected by the finding that attenuation of the acid-evoked vasodilatation by Hoe-140 resulted in enhanced lesion formation. The increase in gastric mucosal blood flow seen after acid backdiffusion is accomplished by a reflex-like neural mechanism (Holzer *et al.*, 1991; Holzer & Lippe, 1992; Raybould *et al.*, 1992), and further studies are necessary to establish whether bradykinin itself acts as a vasodilator mediator or whether it acts by stimulating neurones involved in the hyperaemic response. Either mechanism is conceivable, given that the kinin is able to dilate splanchnic blood vessels directly. (Ritter *et al.*, 1989; Berguer *et al.*, 1993; Santiago *et al.*, 1993) and to stimulate visceral afferent neurones (Longhurst *et al.*, 1984; Stein *et al.*, 1986; Holzer, 1992; Holzer-Petsche, 1992; Geppetti, 1993; Griesbacher, 1984).

This work was supported by the Austrian-Hungarian Foundation, the Jubiläumsfonds of the Austrian National Bank (grants 4207 and 4905) and the Austrian Science Foundation (grant P9473-MED). The authors are grateful to Professor B.A. Schölkens, Hoechst AG, Frankfurt am Main, Germany, for the kind donation of Hoe-140 and to Wolfgang Schluet for organizational assistance.

References

- BARNES, R.J., COMLINE, R.S., DOBSON, A. & DROST, C.J. (1983). An implantable transit time ultrasonic blood flow meter. *J. Physiol.*, **345**, 2P–3P.
- BERGUER, R., HOTTENSTEIN, O.D., PALEN, T.E., STEWART, J.M. & JACOBSON, E.D. (1993). Bradykinin-induced mesenteric vasodilation is mediated by B₂-subtype receptors and nitric oxide. *Am. J. Physiol.*, **264**, G492–G496.
- BRUGGEMAN, T.M., WOOD, J.G. & DAVENPORT, H.W. (1979). Local control of blood flow in the dog's stomach: vasodilatation caused by acid back-diffusion following topical application of salicylic acid. *Gastroenterology*, **77**, 736–744.
- BURTON, R.G. & GOREWIT, R.C. (1984). Ultrasonic flowmeter uses wide beam transit-time technique. *Med. Electron.*, **15**, 68–73.
- DRAY, A. & PERKINS, M. (1993). Bradykinin and inflammatory pain. *Trends Neurosci.*, **16**, 99–104.
- GEPPETTI, P. (1993). Sensory neuropeptide release by bradykinin: mechanisms and pathophysiological implications. *Regul. Pept.*, **47**, 1–23.
- GEPPETTI, P., TRAMONTANA, M., EVANGELISTA, S., RENZI, D., MAGGI, C.A., FUSCO, B.M. & DEL BIANCO, E. (1991). Differential effect on neuropeptide release of different concentrations of hydrogen ions on afferent and intrinsic neurons of the rat stomach. *Gastroenterology*, **101**, 1505–1511.
- GRIESBACHER, T. (1994). Blood pressure reflexes following activation of capsaicin-sensitive afferent neurones in the biliopancreatic duct of rats. *Br. J. Pharmacol.*, **111**, 547–554.
- HALL, J.M. (1992). Bradykinin receptors: pharmacological properties and biological roles. *Pharmacol. Ther.*, **56**, 131–190.
- HOCK, F.J., WIRTH, K., ALBUS, U., LINZ, W., GERHARDS, H.J., WIEMER, G., HENKE, S., BREIPOHL, G., KÖNIG, W., KNOLLE, J. & SCHÖLKENS, B.A. (1991). Hoe 140 a new potent and long-acting bradykinin-antagonist: *in vitro* studies. *Br. J. Pharmacol.*, **102**, 769–773.
- HOLZER, P. (1992). Reflex gastric motor inhibition caused by intraperitoneal bradykinin: antagonism by Hoe 140, a bradykinin antagonist. *Peptides*, **13**, 1073–1077.
- HOLZER, P. & GUTH, P.H. (1991). Neuropeptide control of rat gastric mucosal blood flow. Increase by calcitonin gene-related peptide and vasoactive intestinal polypeptide, but not substance P and neurokinin A. *Circ. Res.*, **68**, 100–105.
- HOLZER, P., LIPPE, I.T.H. (1992). Gastric mucosal hyperaemia due to acid back-diffusion depends on splanchnic nerve activity. *Am. J. Physiol.*, **262**, G505–G509.
- HOLZER, P., LIPPE, I.T.H., JOCIC, M., WACHTER, CH., ERB, R. & HEINEMANN, A. (1993). Nitric oxide-dependent and -independent hyperaemia due to calcitonin gene-related peptide in the rat stomach. *Br. J. Pharmacol.*, **110**, 404–410.
- HOLZER, P., LIVINGSTON, E.H. & GUTH, P.H. (1991). Sensory neurons signal for an increase in rat gastric mucosal blood flow in the face of pending acid injury. *Gastroenterology*, **101**, 416–423.
- HOLZER, P., WACHTER, CH., JOCIC, M. & HEINEMANN, A. (1994). Vascular bed-dependent roles of the peptide CGRP and nitric oxide in acid-evoked hyperaemia of the rat stomach. *J. Physiol.* (in press).
- HOLZER-PETSCH, U. (1992). Blood pressure and gastric motor responses to bradykinin and hydrochloric acid injected into somatic or visceral tissues. *Naunyn-Schmied. Arch. Pharmacol.*, **346**, 219–225.
- LEMBECK, F., GRIESBACHER, T., ECKHARDT, M., HENKE, S., BREIPOHL, G. & KNOLLE, J. (1991). New, long-acting, potent bradykinin antagonists. *Br. J. Pharmacol.*, **102**, 297–304.
- LEUNG, F.W., GUTH, P.H., SCREMIN, O.U., GOLANSKA, E.M. & KAUFFMAN, G.L. (1984). Regional gastric mucosal blood flow measurements by hydrogen gas clearance in the anesthetized rat and rabbit. *Gastroenterology*, **87**, 28–36.
- LI, D.-S., RAYBOULD, H.E., QUINTERO, E. & GUTH, P.H. (1992). Calcitonin gene-related peptide mediates the gastric hyperaemic response to acid back-diffusion. *Gastroenterology*, **102**, 1124–1128.
- LIPPE, I.T.H. & HOLZER, P. (1992). Participation of endothelium-derived nitric oxide but not prostacyclin in the gastric mucosal hyperaemia due to acid back-diffusion. *Br. J. Pharmacol.*, **105**, 708–714.
- LIVINGSTON, E.H., REEDY, T., LEUNG, F.W. & GUTH, P.H. (1989). Computerized curve fitting in the analysis of hydrogen gas clearance curves. *Am. J. Physiol.*, **257**, G668–G675.
- LONGHURST, J.C., KAUFMAN, M.P., ORDWAY, G.A. & MUSCH, T.I. (1984). Effects of bradykinin and capsaicin on endings of afferent fibers from abdominal visceral organs. *Am. J. Physiol.*, **247**, R552–R559.
- RAYBOULD, H.E., STERNINI, C., EYSSELEIN, V.E., YONEDA, M. & HOLZER, P. (1992). Selective ablation of spinal afferent neurons containing CGRP attenuates gastric hyperemic response to acid. *Peptides*, **13**, 249–254.
- RITTER, J.M., DOKTOR, H.S. & CRAGOE, E.J. (1989). Actions of bradykinin and related peptides on rabbit coeliac artery rings. *Br. J. Pharmacol.*, **96**, 23–28.
- SANTIAGO, J.A., OSEI, S.Y. & KADOWITZ, P.J. (1993). Inhibitory effects of Hoe 140 on vasodilator responses to bradykinin in the mesenteric vascular bed of the cat. *Eur. J. Pharmacol.*, **236**, 315–318.

- STEIN, R.D., GENOVESI, S., DEMAREST, K.T. & WEAVER, L.C. (1986). Capsaicin treatment attenuates the reflex excitation of sympathetic activity caused by chemical stimulation of intestinal afferent nerves. *Brain Res.*, **397**, 145–151.
- WHITTLE, B.J.R. (1977). Mechanisms underlying gastric mucosal damage induced by indomethacin and bile salts, and the actions of prostaglandins. *Br. J. Pharmacol.*, **60**, 455–460.
- WIRTH, K., HOCK, F.J., ALBUS, U., LINZ, W., ALPERMANN, H.G., ANAGNOSTOPOULOS, H., HENKE, S., BREIPOHL, G., KÖNIG, W., KNOLLE, J. & SCHÖLKENS, B.A. (1991). Hoe 140, a new potent and longacting bradykinin-antagonist: *in vivo* studies. *Br. J. Pharmacol.*, **102**, 774–777.

(Received May 5, 1994

Revised June 13, 1994

Accepted July 15, 1994)