British Journal of Pharmacology (1996) 119, 1525-1532

Tachykinin inhibition of acid-induced gastric hyperaemia in the rat

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1 Primary afferent neurones releasing the vasodilator, calcitonin gene-related peptide, mediate the gastric hyperaemic response to acid back-diffusion. The tachykinins neurokinin A (NKA) and substance P (SP) are located in the same neurones and are co-released with calcitonin gene-related peptide. In this study we investigated the effect and possible role of tachykinins in the acid-evoked gastric vasodilatation in urethane-anaesthetized rats.

2 Gastric acid back-diffusion, induced by perfusing the stomach with 15% ethanol in the presence of 0.05 M HCl, increased gastric mucosal blood flow by 60-90%, as determined by the hydrogen clearance technique. NKA and SP (0.14-3.78 nmol min^{-1} kg^{-1}, infused intra-aortically) inhibited the gastric mucosal hyperaemic response to acid back-diffusion in a dose-dependent manner, an effect that was accompanied by aggravation of ethanol/acid-induced macroscopic haemorrhagic lesions.

3 The inhibitory effect of NKA (1.26 nmol min^{-1} kg^{-1}) on the acid-induced gastric mucosal vasodilatation was prevented by the tachykinin NK_2 receptor antagonist, MEN 10,627 (200 nmol kg^{-1}) but left unaltered by the NK_1 receptor antagonist, SR 140,333 (300 nmol kg^{-1}) and the mast-cell stabilizer, ketotifen (4.6 μmol kg^{-1}).

4 Under basal conditions, with 0.05 M HCl being perfused through the stomach, NKA (1.26 nmol min^{-1} kg^{-1}) reduced gastric mucosal blood flow by about 25%, an effect that was abolished by SR 140,333 but not MEN 10,627 or ketotifen.

5 SR 140,333, MEN 10,627 or ketotifen had no significant effect on basal gastric mucosal blood flow nor did they modify the gastric mucosal hyperaemic reaction to acid back-diffusion.

6 The effect of NKA (1.26 nmol min^{-1} kg^{-1}) in causing vasoconstriction and inhibiting the vasodilator response to acid back-diffusion was also seen when blood flow in the left gastric artery was measured with the ultrasonic transit time shift technique.

7 Arginine vasopressin (AVP, 0.1 nmol min^{-1} kg^{-1}) induced gastric mucosal vasoconstriction under basal conditions but was unable to inhibit the dilator response to acid back-diffusion.

8 These data show that NKA has two fundamentally different effects on the gastric circulation. Firstly, NKA reduces gastric blood flow by activation of NK_1 receptors. Secondly, NKA inhibits the gastric hyperaemic response to acid back-diffusion through an NK_2 receptor-mediated mechanism. These two tachykinin effects appear to take place independently of each other since they are mediated by different receptors. This concept is further supported by the inability of AVP to mimic tachykinin inhibition of the gastric vasodilator response to acid back-diffusion.

Keywords: Acid back-diffusion; gastric mucosal blood flow; gastric mucosal lesions; left gastric artery; neurokinin A; substance P; tachykinin receptors.

Introduction

Acid back-diffusion through a damaged gastric mucosal barrier causes a marked increase in blood flow through the gastric mucosa (Whittle, 1977; Holzer *et al.*, 1991) and large extramural arteries supplying the stomach such as the left gastric artery (Holzer *et al.*, 1994). This hyperaemia which reduces acid injury to the mucosa appears to be brought about by perivascular fibres of extrinsic primary afferent neurones (Holzer *et al.*, 1991; Raybould *et al.*, 1992). Calcitonin gene-related peptide (CGRP) is supposed to be the prime mediator since it is localized in and released from primary afferent neurones in the stomach (Sternini *et al.*, 1987; Green & Dockray, 1988; Holzer *et al.*, 1990), since it is a potent vasodilator in the gastric microcirculation (Holzer & Guth, 1991; Holzer *et al.*, 1993) and since CGRP receptor blockade inhibits the gastric vasodilator response to acid back-diffusion (Li *et al.*, 1992; Holzer *et al.*, 1994).

The tachykinins substance P (SP) and neurokinin A (NKA) are likewise abundantly present in primary afferent neurones of the stomach (Green & Dockray, 1988) and co-released with CGRP upon nerve stimulation (Kwok & McIntosh, 1990). In the skin, SP plays multiple roles in neurogenic inflammation (Brain, 1996), causing plasma protein extravasation (Lembeck & Holzer, 1979) through activation of NK_1 receptors (Lembeck *et al.*, 1992) and reducing CGRP-induced vasodilatation via release of mast cell proteases (Brain & Williams, 1988). The significance of tachykinins in the gastric vasculature which expresses receptors for these peptides (Mantyh *et al.*, 1988) is less well studied. Unlike CGRP, SP and NKA fail to increase blood flow in the rat gastric mucosa (Holzer & Guth, 1991). On the contrary, SP has been found to reduce the hyperaemia caused by electrical stimulation of the vagus nerve (Yokotani & Fujiwara, 1985) or capsaicin-evoked stimulation of afferent neurones (Grønbech & Lacy, 1994). SP has furthermore been shown to aggravate ethanol-induced damage of the rat gastric mucosa (Karmeli *et al.*, 1991) whereas NKA and NK_2 receptor-selective analogues protect the gastric mucosa from ethanol injury (Evangelista *et al.*, 1989; Stroff *et al.*, 1996).

While these data suggest that tachykinins are of relevance for the regulation of gastric circulation and mucosal integrity,

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little is known about the precise effects of tachykinins on the gastric microcirculation, their possible implication in the acid-evoked gastric hyperaemia and the nature of the tachykinin receptors in the vascular system of the stomach. The present study was designed, therefore, to re-examine the effects of tachykinins on basal blood flow in the rat gastric mucosa, to study their influence on the neurogenic vasodilator response to gastric acid back-diffusion and acid-induced mucosal injury, to characterize the pertinent tachykinin receptors with receptor-selective antagonists, and to test for a possible implication of endogenous tachykinins in the control of gastric circulation.

Methods

Surgical procedure

All experiments in this study were approved by the Federal Ministry of Science and Research of the Republic of Austria. Female Sprague-Dawley rats, weighing 180–220 g, were fasted for 20 h but were allowed free access to water. After the induction of anaesthesia with urethane (1.5 g kg^{-1}s.c.) the rats were placed on a heated table, to maintain rectal temperature at 37 °C, and fitted with a tracheal cannula, to facilitate spontaneous respiration and to allow for the administration of hydrogen. An arterial cannula, inserted through the left carotid artery, was advanced into the descending aorta and connected to a pressure transducer (ISOTEC; HSE, March-Hugstetten, Germany). The amplified signal from the pressure transducer was fed into a personal computer via an analog-digital converter. Mean arterial pressure (MAP) and heart rate (HR) were calculated on-line. The arterial cannula was also used for continuous infusion of saline (1.5 ml h^{-1}), to avoid dehydration, and for the administration of drugs, either as bolus or infusion (15 μl min^{-1}). The infusion of fluid did not interfere with the measurement of MAP. The stomach was exposed by a midline laparotomy and 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 0.7–0.8 ml min^{-1} throughout the experiment.

Blood flow measurements

Gastric mucosal blood flow was measured by 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). Determination of blood flow was discontinuous, as the experimental protocol involved alternating 15-min periods of saturation, and desaturation, of the tissue with hydrogen. The current representing the actual hydrogen concentration at the site of the electrode was amplified, digitized and recorded on a personal computer. The washout curve was then fitted to a monoexponential curve, the power of which was used to calculate the average gastric mucosal blood flow (ml min^{-1} [100 g]$^{-1}$) during the 15-min periods of desaturation (Livingston *et al.*, 1989).

Blood flow in the left gastric artery (LGA) was determined with the ultrasonic transit time shift technique (Barnes *et al.*, 1983; Burton & Gorewit, 1984) by the use of a small animal flowmeter (model T106; Transonic, Ithaca, New York, U.S.A.) and a 1-mm ultrasonic flow probe (model 1RB; Transonic). The flow probe has been developed for measurements in small vessels and repeatedly used and validated in different arteries of the rat (Myers & Hernandez, 1992; Holzer *et al.*, 1993; 1994; D'Almeida *et al.*, 1995). The LGA was isolated from the adjacent tissue over a segment of 3 mm close to its entry into the stomach. The flow probe was put around the artery and held in place by a micromanipulator. The blood flow values, in ml min^{-1}, were likewise digitized and recorded on the computer.

To account for alterations of MAP which occurred due to the administration of drugs, vascular conductance in the gastric mucosa (GMVC) or LGA (LGVC) was calculated as blood flow divided by MAP. Effects on gastric haemodynamics were expressed as % changes of vascular conductance relative to baseline values (100%).

Macroscopic lesions

At the end of each experiment the stomach was excised and opened along the greater curvature. Macroscopic haemorrhagic lesions were quantified by computerized image analysis (MCID M4; Imaging Research, St. Catharines, Ontario, Canada) and expressed as % of the area of the glandular mucosa.

Study groups

Five studies were performed. *The first study* investigated the effects of NKA and SP, infused intra-aortically at different rates (0.14–3.78 nmol min^{-1} kg^{-1}), on the gastric mucosal hyperaemic response to acid back-diffusion which was induced by disrupting the mucosal barrier with 15% ethanol in the presence of 0.05 M HCl. *The second study* assessed the mechanisms and receptors behind the haemodynamic effects of NKA in the gastric mucosa. For this purpose the effect of NKA (1.26 nmol min^{-1} kg^{-1}) on basal blood flow and the acid-evoked hyperaemia was studied in rats pretreated with either vehicle (2 × 1 ml kg^{-1}), the NK_1 receptor antagonist, SR 140,333 (2 × 150 nmol kg^{-1}), the NK_2 receptor antagonist, MEN 10,627 (2 × 100 nmol kg^{-1}) or the mast-cell stabilizer, ketotifen (2 × 2.3 μmol kg^{-1}). The dose of 1.26 nmol min^{-1} kg^{-1} of NKA was chosen, since it had proved to be submaximally effective in the first study. The same dose of NKA (1.26 nmol min^{-1} kg^{-1}) was used in *study 3* to assess the peptide's effects on basal blood flow and the acid-induced hyperaemia in the LGA. *Study 4* addressed the question whether vasoconstriction induced by arginine vasopressin (AVP; 0.1 nmol min^{-1} kg^{-1}) would also inhibit the acid-evoked gastric mucosal vasodilatation. The dose of AVP was selected in preliminary experiments such that the AVP-induced gastric mucosal vasoconstriction was of similar magnitude to that induced by NKA in study 2. *Study 5* was performed to probe for an implication of endogenous tachykinins and mast cell mediators in the gastric mucosal hyperaemia due to acid back-diffusion. To this end the vasodilator response to intraluminal ethanol/acid was determined in rats that had been pretreated with vehicle (2 × 1 ml kg^{-1}), SR 140,333 (2 × 150 nmol kg^{-1}), MEN 10,627 (2 × 100 nmol kg^{-1}) or ketotifen (2 × 2.3 μmol kg^{-1}).

Experimental protocol

After the preparation had been completed the animals were allowed to equilibrate for 30 min. For haemodynamic measurements in the gastric mucosa two cycles of saturation/desaturation were recorded to determine basal mucosal blood flow while the stomach was luminally perfused with 0.05 M HCl. Previous studies have shown that at moderate concentrations (0.05–0.15 M), HCl alone does not alter gastric blood flow as compared to saline perfusion (Holzer *et al.*, 1991) since the intact mucosal barrier effectively prevents acid back-diffusion in this experimental model. The third cycle was performed to investigate the haemodynamic effects of the peptides NKA, SP or AVP under basal conditions (perfusion of the stomach with 0.05 M HCl) or during acid back-diffusion (gastric perfusion with 0.05 M HCl plus 15% ethanol). The intra-aortic infusion of the peptides was started concurrently with the induction of acid back-diffusion at the beginning of the third saturation/desaturation period. SR 140,333 (2 × 150 nmol kg^{-1}), MEN 10,627 (2 × 100 nmol kg^{-1}) or ketotifen (2 × 2.3 μmol kg^{-1}) was administered intra-aortically at two doses, the first one being injected at the beginning of the second saturation/desaturation period while the second dose

was given at the beginning of the third saturation/desaturation period. According to this protocol the first desaturation period always measured basal gastric mucosal blood flow, the second period accounted for basal gastric mucosal blood flow in the presence of vehicle, tachykinin antagonists or ketotifen whereas the third desaturation period determined the haemodynamic effects of NKA, SP or AVP on basal blood flow or on the hyperaemia caused by acid back-diffusion.

A similar protocol was adhered to when the haemodynamic effects of NKA were evaluated in the LGA. Basal blood flow in the LGA (perfusion of the stomach with 0.05 M HCl) was recorded during the 15 min period immediately before the intra-aortic infusion of NKA was started. The effects of NKA in the LGA on basal blood flow and on the hyperaemic response to acid back-diffusion (gastric perfusion with 15% ethanol in 0.05 M HCl) were determined from the average blood flow measured during the period of 15–30 min after the start of the peptide's infusion and induction of acid back-diffusion, respectively.

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). AVP (Bachem; Bubendorf, Switzerland), SP and NKA (Peptide Institute; Osaka, Japan) were dissolved in 0.1 M acetic acid to give stock solutions of 1 mM. Dilutions ready for infusion were made with saline containing 2% (wt./wt.) bovine serum albumin (Sigma; Vienna, Austria). Stock solutions (10–15 mM) of SR 140,333 ((S))1-{2-[3-(3,4-dichlorophenyl)-1-(3-isopropoxyphenyl-acetyl)piperidin-3-yl]-ethyl}-4-phenyl-1-azoniabicyclo[2.2.2]-octane chloride) (Sanofi; Montpellier, France) and MEN 10,627 (*cyclo*(Met-Asp-Trp-Phe-Dap-Leu)*cyclo*(2β–5β)) (Menarini; Florence, Italy) were made in dimethyl sulphoxide and further diluted with saline. Ketotifen (Sigma) was dissolved in saline.

Statistical analysis

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

Results

Effects of neurokinin A (NKA) and substance P (SP)

Intra-aortic infusion of NKA or SP (0.14–3.78 nmol min^{-1} kg^{-1}) led, in most cases, to a transient fall of MAP, which at the higher doses of the peptides amounted to 20–30 mmHg (Figure 1). This hypotensive effect was not further evaluated, since it was not observed in all study groups and had vanished when blood flow measurements with the hydrogen clearance technique were begun 15 min later. During acid back-diffusion (gastric perfusion with 15% ethanol in 0.05 M HCl) both

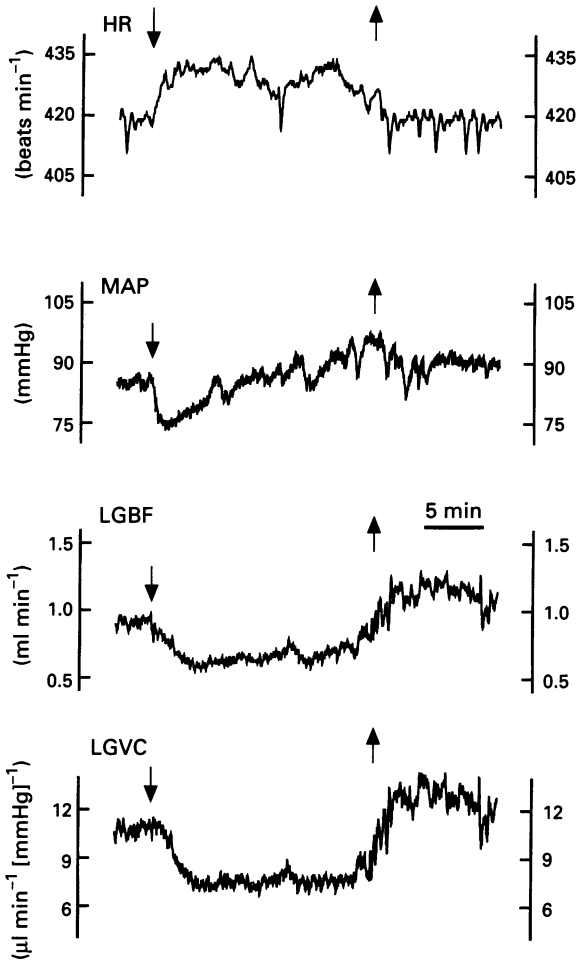

Figure 1 Tracing of the effects of neurokinin A on heart rate (HR), mean arterial pressure (MAP), blood flow (LGBF) and vascular conductance (LGVC) in the left gastric artery under basal conditions (gastric perfusion with 0.05 M HCl). The arrows indicate the start (↓) and the end (↑) of a 20 min intra-aortic infusion of neurokinin A (1.26 nmol min^{-1} kg^{-1}).

Table 1 Haemodynamic effects of substance P (SP) and neurokinin A (NKA) in the gastric mucosa during acid back-diffusion

Infusion rate (nmol min^{-1} kg^{-1})	n	*MAP* (mmHg) *pre*	*post*	*HR* (beat min^{-1}) *pre*	*post*	*GMBF* (ml min^{-1} 100g^{-1}) *pre*	*post*	*GMVC* (μl min^{-1} g^{-1} [mmHg]$^{-1}$) *pre*	*post*
Vehicle	8	93±1	90±2	419±16	420±16	32.9±2.6	60.9±5.1*	3.5±0.3	6.7±0.5*
SP									
0.42	6	83±3	81+3	376±10	375±9	39.0±7.1	51.1±5.6*	4.6±0.7	6.4±0.7*
1.26	6	83±4	80±4	386±19	394±22	42.4±2.3	44.9±3.5	5.1±0.1	5.7±0.5
3.78	7	89±5	85±4	396±19	424±20*	36.0±3.0	35.6±4.5†	4.1±0.4	4.2±0.5†
NKA									
0.14	6	93±5	91±5	392±12	387±11	35.4±3.1	56.4±4.9*	3.9±0.4	6.3±0.7*
0.42	6	85±3	83±4	393±15	400±13	36.6±5.2	41.1±5.7	4.3±0.7	4.9±0.6†
1.26	14	89±2	91±3	382±4	402±7*	40.4±3.3	32.1±2.1*†	4.5±0.3	3.5±0.3*†
3.78	7	84±3	88±3	394±11	457±13*	38.5±3.6	28.4±2.5*†	4.6±0.4	3.3±0.3*†

Mean arterial blood pressure (MAP), heart rate (HR), blood flow (GMBF) and vascular conductance (GMVC) in the gastric mucosa were determined during the 15 min period before and the 15–30 min period after the intra-aortic infusion of substance P (SP), neurokinin A (NKA) or vehicle (15 μl min^{-1}) had been started. Acid back-diffusion was induced by changing the gastric perfusion medium from 0.05 M HCl to 0.05 M HCl plus 15% ethanol simultaneously with the start of the SP/NKA/vehicle infusion. Data shown are mean ± s.e.mean; *n* as indicated. *$P<0.05$ versus pre-infusion. †$P<0.05$ versus vehicle.

peptides were devoid of any significant effect on MAP as measured 15–30 min after the peptide infusion had begun (Tables 1 and 2). Under basal conditions (gastric perfusion with 0.05 M HCl alone), however, NKA (1.26 nmol min^{-1} kg^{-1}) slightly, but significantly ($P<0.005$) increased MAP from 88±1 to 94 ±2 mmHg ($n=41$). Both NKA and SP, when infused at high rates (1.26–3.78 nmol min^{-1} kg^{-1}), increased the HR of the rats irrespectively of whether acid back-diffusion had been induced or not, whereas no change was observed during vehicle infusion (Tables 1–3). Overall analysis of the cardiac effect of NKA (1.26 nmol min^{-1} kg^{-1}) revealed that the peptide increased HR from 397±8 to 436±8 ($P<0.001$, $n=37$) under basal conditions and from 378±9 to 402±11 ($P<0.01$, $n=18$) during acid back-diffusion. The hypertensive and positive chronotropic effects of NKA (1.26 nmol min^{-1} kg^{-1}), however, did not reach the level of statistical significance in all experimental groups (Tables 1–3) and were, therefore, not further analysed.

Disruption of the gastric mucosal barrier with 15% ethanol in the presence of 0.05 M HCl, which is known to cause acid back-diffusion into the mucosa (Holzer *et al.*, 1991), led to a 60–90% increase in gastric mucosal blood flow (Tables 1, 4 and 5) and a 170% enhancement of blood flow in the LGA (Table 2). The changes in GMVC and LGVC were similar as gastric hyperaemia took place in the absence of any alteration of MAP (Tables 2 and 5; Figures 2–4). HR was also unaffected (Table 1, 2, 4 and 5). Intra-aortic infusion of NKA and SP (0.14–3.78 nmol min^{-1} kg^{-1}) dose-dependently reduced the gastric mucosal dilator response to acid back-diffusion, NKA being at least three times more potent than SP (Table 1, Figure 2). At the higher doses of NKA (1.26–3.78 nmol min^{-1} kg^{-1}) the ethanol/acid-induced vasodilatation was even reversed to vasoconstriction. The inhibition of the vasodilator response to acid back-diffusion by NKA (1.26 nmol min^{-1} kg^{-1}) was also seen when blood flow was measured in the LGA (Table 2). Concomitantly with the suppression of the ethanol/acid-evoked hyperaemia the area of the macroscopically damaged mucosa was increased by NKA and SP (Figure 2). These pro-ulcerogenic effects became manifest with the highest tachykinin doses only, NKA being again more potent than SP.

Under basal conditions, with 0.05 M HCl being perfused through the stomach, NKA (1.26 nmol min^{-1} kg^{-1}) reduced both blood flow and vascular conductance in the gastric mucosa (Table 3) and LGA (Table 2, Figure 1) by about 30%. As can be seen in Figure 1, the vasoconstrictor effect of NKA was rapid in onset, sustained, and quickly waned after termination of the peptide's infusion. Under these conditions, no macroscopically visible lesions in the gastric mucosa were observed after intra-aortic infusion of vehicle or NKA (1.26 nmol min^{-1} kg^{-1}).

Effects of arginine vasopressin (AVP)

Intra-aortic infusion of AVP (0.1 nmol min^{-1} kg^{-1}) caused sustained hypertension without significantly altering HR (Table 4). Concomitantly, gastric mucosal blood flow and GMVC

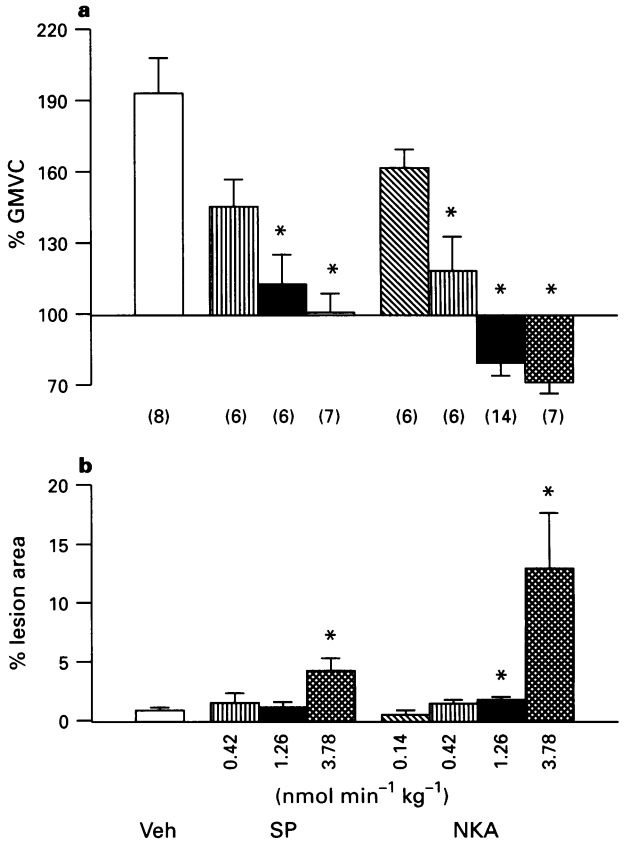

Figure 2 Effects of substance P (SP) and neurokinin A (NKA) on the rise of gastric mucosal vascular conductance (GMVC, a) and the formation of macroscopic mucosal lesions (b) due to acid back-diffusion (gastric perfusion with 15% ethanol in 0.05 M HCl). The effects on GMVC are expressed as percentage of the pre-infusion values (100%). The extent of macroscopically damaged mucosa is given as percentage of the area of the glandular mucosa. The intra-aortic infusion of vehicle (Veh, 15 μl min^{-1}), SP or NKA, at the rate indicated, was started concurrently with the induction of acid back-diffusion (0 min), GMVC being recorded during the period 15–30 min. Data shown are mean±s.e. mean; number of experiments per group as indicated in parentheses. * $P<0.05$ versus Veh.

Table 2 Haemodynamic effects of neurokinin A (NKA) in the left gastric artery under basal conditions (Basal) and during acid back-diffusion (ABD)

		MAP (mmHg)		*HR* (beat min^{-1})		*LGBF* (ml min^{-1})		*LGVC* (μl min^{-1} [mmHg]$^{-1}$)		*LGVC*
Infusion	n	*pre*	*post*	*pre*	*post*	*pre*	*post*	*pre*	*post*	*(%)*
Basal										
Vehicle	6	87±8	87±6	344±35	350±40	0.54±0.12	0.54±0.11	6.3±1.2	6.2±1.2	98±2
NKA	6	92±5	98±7	348±17	403±21*	0.52±0.10	0.43±0.08*	5.5±1.0	4.4±0.8*	79±2†
ABD										
Vehicle	8	87±5	87±4	375±9	381±11	0.51±0.07	1.26±0.12*	6.1±1.0	14.5±1.3*	271±33
NKA	8	90±5	92±6	378±24	404±23*	0.56±0.09	0.93±0.10*†	6.3±1.2	10.4±1.3*†	184±25†

Mean arterial blood pressure (MAP), heart rate (HR), blood flow (LGBF) and vascular conductance (LGVC) in the left gastric artery were determined as average values during the 15 min period before and the 15–30 min period after the intra-aortic infusion of neurokinin A (NKA, 1.26 nmol min^{-1} kg^{-1}) or its vehicle (15 μl min^{-1}) had been started. The effect of drug infusion on LGVC is expressed as percentage of the pre-infusion values (100%). Under basal conditions (Basal) the stomach was perfused with 0.5 M HCl. Acid back-diffusion (ABD) was induced by changing the gastric perfusion medium from 0.05 M HCl to 0.05 M HCl plus 15% ethanol simultaneously with the start of the NKA/vehicle infusion. Data shown are mean±s.e.mean; n as indicated. *$P<0.05$ versus pre-infusion, †$P<0.05$ versus vehicle.

were reduced (Figure 3, Table 4) to an extent comparable with that seen under NKA (1.26 nmol min^{-1} kg^{-1}; Table 3), without generation of macroscopic gastric mucosal lesions. The same dose of AVP (0.1 nmol min^{-1} kg^{-1}), however, was unable to alter the gastric mucosal hyperaemic response to acid back-diffusion (Figure 3, Table 4). The ethanol/acid-induced formation of macroscopic lesions in the gastric mucosa was also unaffected by AVP. The area of macroscopic damage after gastric ethanol/acid perfusion was $0.69 \pm 0.14\%$ and $0.57 \pm 0.09\%$ ($n=6$ per group) in vehicle- and AVP-treated rats, respectively.

Effects of SR 140,333, MEN 10,627 and ketotifen

The NK_1 receptor antagonist, SR 140,333 (2×150 nmol kg^{-1}), the NK_2 receptor antagonist MEN 10,627 (2×100 nmol kg^{-1}), and the mast cell stabilizer, ketotifen (2×2.3 μmol kg^{-1}) had no significant effect on MAP, HR or gastric mucosal blood flow ($n=13-14$, data not shown). Nor was there a significant modification of the hyperaemic response to acid back-diffusion by any of these drugs (Table 5).

The inhibitory effect of NKA (1.26 nmol min^{-1} kg^{-1}) on the gastric mucosal vasodilator response to acid back-diffusion was left unchanged by SR 140,333 (2×150 nmol kg^{-1}) and ketotifen (2×2.3 μmol kg^{-1}), but was prevented by MEN 10,627 (2×100 nmol kg^{-1}). As shown in Figure 4, MEN 10,627 restored the NKA-suppressed hyperaemia to the level that was observed under infusion of the NKA vehicle. Since in this series of experiments NKA (1.26 nmol min^{-1} kg^{-1}) failed to worsen significantly the ethanol/acid-induced gastric mucosal damage ($n=8$, data not shown) it was not possible to

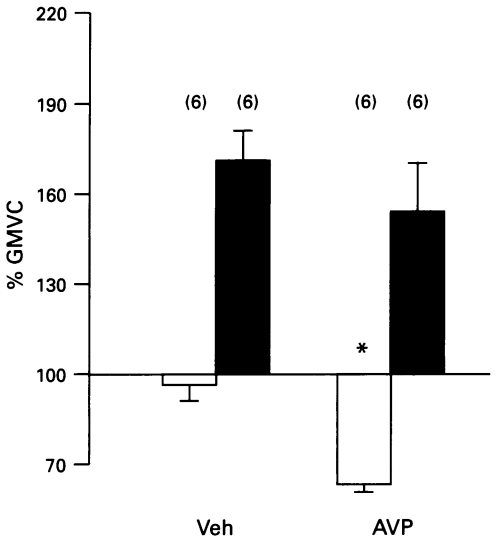

Figure 3 Effects of arginine vasopressin (AVP) on gastric mucosal vascular conductance (GMVC) under basal conditions (open columns) and during acid back-diffusion (solid columns). The effects on GMVC are expressed as a percentage of the pre-infusion values (100%). The intra-aortic infusion of vehicle (Veh, 15 μl min^{-1}) or AVP (0.1 nmol min^{-1} kg^{-1}) was started concurrently with the induction of acid back-diffusion (0 min), GMVC being recorded during the period 15–30 min. Data shown are mean ± s.e. mean; number of experiments per group as indicated in parentheses. *$P<0.05$ versus Veh.

Table 3 Haemodynamic effects of neurokinin A (NKA) in the gastric mucosa under basal conditions

			MAP (mmHg)		*HR* (beat min^{-1})		*GMBF* (ml min^{-1} $100g^{-1}$)		*GMVC* (μl min^{-1} g^{-1} $[mmHg]^{-1}$)	
Pretreatment	*Infusion*	n	*pre*	*post*	*pre*	*post*	*pre*	*post*	*pre*	*post*
None	Vehicle	6	97±4	97±5	425±21	425±13	45.1±3.4	45.2±4.0	4.6±0.3	4.7±0.5
None	NKA	6	97±2	103±3*	434±16	478±11*	42.2±8.0	32.9±4.8*	4.4±0.9	3.2±0.5*
Vehicle	NKA	7	88±2	93±3*	391±17	418±19	43.5±4.8	32.5±3.8*	4.9±0.5	3.5±0.5*
SR 140,333	NKA	7	93±3	98±4*	441±23	467±22*	43.7±4.3	42.6±4.1	4.7±0.5	4.4±0.5
Vehicle	NKA	9	90±2	95±2*	409±19	439±17*	46.2±4.7	36.3±2.8*	5.3±0.5	3.9±0.3*
MEN 10,627	NKA	8	100±4	101±4	427±25	431±26	42.4±3.5	33.7±2.2*	4.2±0.3	3.3±0.2*
Vehicle	NKA	13	86±3	89±3	389±11	435±12*	43.7±2.3	35.6±4.5*	5.1±0.3	3.9±0.4*
Ketotifen	NKA	7	87±6	92±6	409±17	455±23*	43.6±4.7	36.5±3.5*	5.1±0.5	3.9±0.3*

Mean arterial blood pressure (MAP), heart rate (HR), blood flow (GMBF) and vascular conductance (GMVC) in the gastric mucosa were determined during the 15 min period before and from the 15–30 min period after the intra-aortic infusion of neurokinin A (NKA, 1.26 nmol min^{-1} kg^{-1}) or its vehicle (15 μl min^{-1}) had been started. The stomach was perfused with 0.05 M HCl throughout the experiment. The tachykinin NK_1 receptor antagonist SR 140,333 (2×150 nmol kg^{-1}), the NK_2 receptor antagonist MEN 10,627 (2×100 nmol kg^{-1}) or the mast cell stabilizer ketotifen (2×2.3 μmol kg^{-1}) was given in two equal doses, 30 min and immediately before NKA/vehicle infusion. Data shown are mean ± s.e.mean; *n* as indicated. *$P<0.05$ versus pre-infusion.

Table 4 Haemodynamic effects of arginine vasopressin (AVP) in the gastric mucosa under basal conditions (BASAL) and during acid back-diffusion (ABD)

		MAP (mmHg)		*HR* (beat min^{-1})		*GMBF* (ml min^{-1})		*GMVC* (μl min^{-1} g^{-1} $[mmHg]^{-1}$)	
	n	*pre*	*post*	*pre*	*post*	*pre*	*post*	*pre*	*post*
Basal									
Vehicle	6	89±2	88±3	411±18	416±24	39.5±2.3	37.5±2.1	4.4±0.3	4.3±0.2
AVP	6	85±1	106±3*†	431±32	413±30	38.4±1.5	30.0±2.0*†	4.5±0.2	2.8±0.2*
ABD									
Vehicle	6	94±4	89±6	458±21	468±28	37.9±2.9	61.6±6.8*	4.2±0.4	7.1±0.9*
AVP	6	93±5	100±4*†	450±22	444±18	34.8±6.1	53.9±6.5*	4.0±0.9	5.5±0.9*

Mean arterial blood pressure (MAP), heart rate (HR), blood flow (GMBF) and vascular conductance (GMVC) in the gastric mucosa were determined during the 15 min period before and from the 15–30 min period after the intra-aortic infusion of arginine vasopressin (AVP, 0.1 nmol min^{-1} kg^{-1}) or its vehicle (15 μl min^{-1}) had been started. Under basal conditions (Basal) the stomach was perfused with 0.05 M HCl. Acid back-diffusion (ABD) was induced by changing the gastric perfusion medium from 0.05 M HCl to 0.05 M HCl plus 15% ethanol simultaneously with the start of the AVP/vehicle infusion. Data shown are mean ± s.e.mean; *n* as indicated. *$P<0.05$ versus pre-infusion, †$P<0.05$ versus vehicle.

examine whether or not the tachykinin antagonists and ketotifen would prevent NKA from aggravating mucosal injury. Unlike the anti-vasodilator action, the effect of NKA (1.26 nmol min^{-1} kg^{-1}) in reducing basal gastric mucosal blood flow was prevented by SR 140,333 (2×150 nmol kg^{-1}) whereas MEN 10,627 (2×100 nmol kg^{-1}) and ketotifen (2×2.3 μmol kg^{-1}) were ineffective (Table 3).

Discussion

The present data demonstrate that NKA potently modulates the gastric circulation under resting conditions and during acid challenge of the mucosa. Of particular novelty was the finding that the hyperaemic response to acid back-diffusion, induced by perfusing the stomach with 15% ethanol in 0.05 M HCl (Holzer *et al.*, 1991), was dose-dependently depressed by SP and NKA. The observation that NKA inhibited the ethanol/acid-evoked hyperaemia in the LGA to a smaller extent than that in the gastric mucosa could be indicative of a primary action in the mucosal microcirculation while the muscular microcirculation is little affected. The anti-vasodilator effect of NKA and SP was accompanied by aggravation of the ethanol/acid-induced damage to the mucosa, which attests to the essential role of hyperaemia in protecting the gastric mucosa from influxing acid (Holzer *et al.*, 1991; Guttu *et al.*, 1994).

The ability of SP to exacerbate acid back-diffusion injury is consistent with the action of this peptide in worsening ethanol injury (Karmeli *et al.*, 1991). The deleterious effect of NKA seen here, however, is at variance with the reported ability of NKA and NK_2 receptor-selective agonists to protect the gastric mucosa from ethanol damage (Evangelista *et al.*, 1989; Stroff *et al.*, 1996) at doses that are in the range of, or beyond, those used in our experiments. The assumption that the hyperaemic reaction to ethanol/acid perfusion is blunted because tachykinins reduce acid back-diffusion by virtue of their gastroprotective action is invalidated by the pro-ulcerogenic effect of NKA and SP under conditions of acid back-diffusion.

The discrepancy between our data and those of other studies is most probably related to the profoundly different nature of the experimental models and the associated microcirculatory conditions. While ethanol/acid challenge provokes vasidilatation, and the ensuing hyperaemia is an important factor in limiting acid back-diffusion injury, blood flow does not rise in response to challenge with ethanol alone (Holzer *et al.*, 1991) and in fact decreases after exposure to high concentrations of ethanol because of venular constriction (Yonei & Guth, 1988). The protective effect of NKA against ethanol injury is associated with a decrease, not increase, in blood flow (Stroff *et al.*, 1996), which shows that hyperaemia is not a prerequisite for protection from ethanol injury whereas suppression of the acid/ethanol-evoked hyperaemia is invariably followed by aggravation of damage (Holzer *et al.*, 1991; Li *et al.*, 1992; Quintero *et al.*, 1992; Raybould *et al.*, 1992; Pethö *et al.*, 1994). In order to accommodate the divergent data in a single hypothesis we propose that NKA and SP exert two independent and opposing effects on gastric mucosal homeostasis: a blood flow-independent protective action and a pro-ulcerogenic action due to inhibition of the hyperaemic response to acid back-diffusion. Evidence for this conjecture comes from the observation that SP and NKA worsened mu-

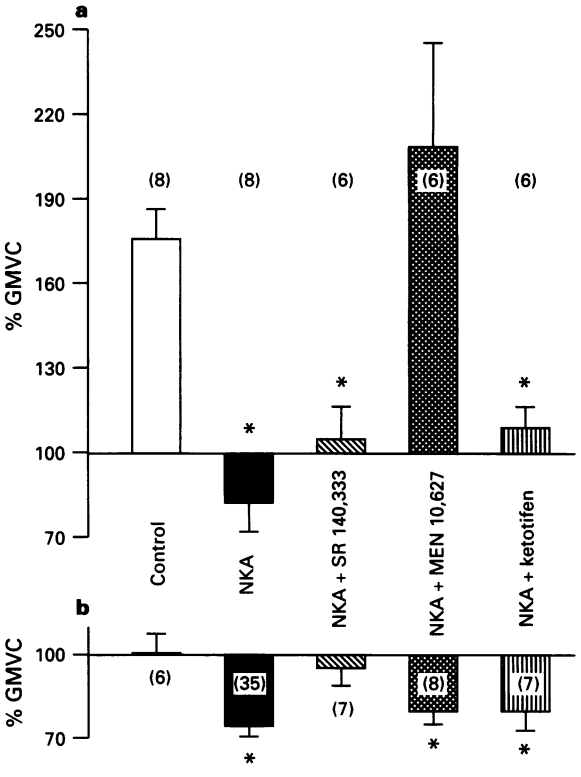

Figure 4 Effects of SR 140,333, MEN 10,627 and ketotifen on the action of neurokinin A (NKA) in suppressing the gastric mucosal vasodilator response to acid back-diffusion (a) and in reducing resting gastric mucosal blood flow (b). The effects on gastric mucosal vascular conductance (GMVC) are expressed as a percentage of the pre-infusion values (100%). SR 140,333 (2×150 nmol kg^{-1}), MEN 10,627 (2×100 nmol kg^{-1}) or ketotifen (2×2.3 μmol kg^{-1}) was administered intra-aortically in two equal doses, 30 min and immediately before the intra-aortic infusion of NKA (1.26 nmol min^{-1} kg^{-1}) or its vehicle (15 μl min^{-1}) was started. Acid back-diffusion was induced concurrently with the NKA/vehicle infusion (0 min), GMVC being recorded during the period 15–30 min. Data shown are mean ± s.e. mean; number of experiments per group as indicated in parentheses. *$P < 0.05$ versus control.

Table 5 Effect of the tachykinin receptor antagonists SR 140,333 and MEN 10,627, and of ketotifen on the haemodynamic responses to acid back-diffusion

		MAP (mmHg)		*HR* (beat min^{-1})		*GMBF* (ml min^{-1} $100g^{-1}$)		*GMVC* (μl min^{-1} g^{-1} $[mmHg]^{-1}$)		*GMVC*
	n	*pre*	*post*	*pre*	*post*	*pre*	*post*	*pre*	*post*	*(%)*
Vehicle	10	97±3	96±4	432±15	435±18	48.7±3.2	89.5±8.1	5.0±0.3	9.4±0.8	187±14
SR 140,333	7	90±3	90±4	445±13	433±14	44.7±4.9	85.4±16.2	4.9±0.4	9.3±1.4	186±21
MEN 10,627	9	94±3	91±3	411±18	415±17	40.1±3.7	86.5±11.8	4.3±0.4	9.4±1.2	223±26
Ketotifen	6	94±5	88±5	426±15	429±12	46.3±4.0	86.1±11.9	5.0±0.5	10.1±2.0	198±17

Mean arterial blood pressure (MAP), heart rate (HR), blood flow (GMBF) and vascular conductance (GMVC) in the gastric mucosa were determined during the 15 min period before and from the 15–30 min period after acid back-diffusion had been induced by changing the gastric perfusion medium from 0.05 M HCl to 0.05 M HCl plus 15% ethanol. The effect of acid back-diffusion on GMVC is expressed as percentage of the pre-infusion values (100%). The tachykinin NK_1 receptor antagonist SR 140,333 (2×150 nmol kg^{-1}), the NK_2 receptor antagonist MEN 10,627 (2×100 nmol kg^{-1}) or the mast cell stabilizer ketotifen (2×2.3 μol kg^{-1}) was given in two equal doses, 30 min and immediately before acid back-diffusion. Data shown are mean ± s.e.mean; *n* as indicated.

cosal injury only when the ethanol/acid-induced vasodilatation was totally suppressed. According to our hypothesis this result means that the injurious action of a partially reduced hyperaemic reaction is balanced by the blood flow-independent protective influence of tachykinins. This beneficial effect, though, is completely overridden when the protective hyperaemia due to acid back-diffusion is abolished by high tachykinin doses.

SP was less potent than NKA in inhibiting the gastric mucosal hyperaemia and in enhancing the formation of mucosal lesions caused by acid back-diffusion, which suggests that these tachykinin effects may not be mediated by NK_1 receptors. Indeed, the NK_1 receptor antagonist, SR 140,333 (2×150 nmol kg^{-1}) failed to alter the ability of NKA to prevent the gastric hyperaemia due to acid back-diffusion whereas pretreatment of the rats with the NK_2 receptor antagonist, MEN 10,627 (2×100 nmol kg^{-1}) restored the vasodilator response to normal. Since the antagonist doses used here have been described as effective and receptor-selective (Emonds-Alt *et al.*, 1993; Maggi *et al.*, 1994) it is concluded that the anti-vasodilator effect of NKA is mediated by NK_2 receptors. Because of the higher potency of NKA in our model compared to that of SP, only the action of NKA was further analysed by pharmacological means.

SP has been reported to inhibit the CGRP-induced vasodilatation in the rat skin and the capsaicin-induced mucosal hyperaemia in the rat stomach by releasing mast cell proteases which readily inactivate CGRP by cleavage (Brain & Williams, 1988; Grønbech & Lacy, 1994). Since the gastric vasodilatation in response to acid back-diffusion is likewise brought about by capsaicin-sensitive afferent neurones (Holzer *et al.*, 1991; Raybould *et al.*, 1992) that utilize CGRP as their major vasodilator mediator (Li *et al.*, 1992; Holzer *et al.*, 1994) it was deemed worth examining whether factors liberated from mast cells are responsible for the inhibitory effect of NKA on the ethanol/acid-induced gastric vasodilatation. However, pretreatment of rats with a dose of ketotifen (2×2.3 μmol kg^{-1}) that effectively protects gastric mast cells from degranulation by SP (Grønbech & Lacy, 1994) did not reverse the inhibition by NKA of the gastric mucosal vasodilator response to acid back-diffusion. Thus, the anti-vasodilator action of NKA is unlikely to involve degranulation of mast cells.

Another finding of the current study was that NKA attenuated basal blood flow through the gastric mucosa and LGA. Previous studies have reported that NKA, at doses lower than those used here, and SP do not alter resting gastric mucosal blood flow (Yokotani & Fujiwara, 1985; Holzer & Guth, 1991; Grønbech & Lacy, 1994). The presently observed vasoconstrictor effect of NKA (1.26 nmol min^{-1} kg^{-1}) is confirmed by the ability of NKA_{4-10} (130 nmol kg^{-1}) to reduce gastric mucosal blood flow (Stroff *et al.*, 1996). Like the suppression of the ethanol/acid-evoked hyperaemia, the NKA-induced attenuation of basal blood flow was not altered by ketotifen, which indicates that mast cell-derived vasoconstrictors such as platelet-activating factor are not involved. The possibility that catecholamines, which mediate tachykinin-evoked tachycardia (Hancock & Lindsay, 1995), play a role in the vasoconstrictor mechanism deserves further study. It is important to realize, though, that the vasoconstrictor action of NKA does not seem to be responsible for inhibition of the vasodilator response to acid back-diffusion. This conclusion is supported by two independent sets of data.

Firstly, the tachykinin receptors underlying the vasoconstrictor and anti-vasodilator actions of NKA are different. Thus, pretreatment of rats with SR 140,333 suppressed the ability of NKA to reduce resting mucosal blood flow whereas MEN 10,627 was ineffective. It follows that, unlike the peptide's anti-vasodilator effect which is mediated by NK_2 receptors, the vasoconstrictor effect is brought about by NK_1 receptors. NK_1 receptor-induced constrictor responses have also been noted in the rat mesenteric veins (Claing *et al.*, 1992) and in the rabbit intrapulmonary arteries (Shirahase *et al.*, 1995).

Secondly, vasoconstriction *per se* was shown to be unable to suppress the hyperaemic response to acid back-diffusion. This inference is based on the observation that AVP, at a dose (0.1 nmol min^{-1} kg^{-1}) that constricted the gastric mucosal vasculature to a similar extent to an effective anti-vasodilator dose of NKA (1.26 nmol min^{-1} kg^{-1}), failed to blunt significantly the ethanol/acid-evoked hyperaemia. To overcome acid-evoked hyperaemia, a much higher degree of AVP-induced vasoconstriction, corresponding to the vasodilator response induced by acid back-diffusion, would be required. These findings corroborate the conjecture that NKA-evoked vasoconstriction and inhibition of the vasodilator reaction to acid back-diffusion take place independently of each other. The reversal of the ethanol/acid-induced vasodilatation to vasoconstriction, which was seen with high doses of NKA (1.26–3.78 nmol min^{-1} kg^{-1}), is likely to reflect a combination of the peptide's two independent haemodynamic actions. The observation that this NK_1/NK_2 receptor-mediated action was completely reversed by a NK_2 receptor antagonist is in keeping with our notion that vasoconstriction *per se*, be it induced by NKA or AVP, does not interfere with the acid-evoked hyperaemia. A comparison with other vasoconstrictor peptides discloses that NKA is roughly as potent as angiotensin II (Wachter *et al.*, 1996) but at least one order of magnitude less active than AVP in constricting the gastric mucosal vasculature.

Despite the demonstration of potent modulator effects of exogenous NKA in the gastric circulation, the present study failed to provide evidence that endogenously released tachykinins have a bearing on gastric blood flow regulation. Neither resting blood flow nor the mucosal vasodilator response to acid back-diffusion was significantly altered by the tachykinin receptor antagonists, SR 140,333 and MEN 10,627. The lack of effect of these antagonists on resting gastric mucosal blood flow might have been foreseen since perivascular afferent neurones, a major source of tachykinins in the stomach (Green & Dockray, 1988), seem to be inactive under basal conditions and antagonism of the co-released vasodilator messenger CGRP does not affect resting gastric blood flow (Li *et al.*, 1992; Holzer *et al.*, 1994). However, NK_1 and NK_2 receptor blockade also failed to affect significantly (enhance) the gastric hyperaemic response to acid back-diffusion, a condition that effectively stimulates gastric afferent neurones. It can hence be inferred that endogenous tachykinins, should they be released, do not appreciably modulate the ethanol/acid-evoked vasodilatation although it should be mentioned in passing that the NK_2 receptor antagonist, MEN 10,627, caused a slight, insignificant enhancement of the hyperaemia due to acid back-diffusion (Table 5). Further pertinent in this respect is that certain forms of chronic inflammation cause upregulation of tachykinins (Koch *et al.*, 1987; Swain *et al.*, 1992; Bost, 1995) and tachykinin receptors (Mantyh *et al.*, 1988; 1995) in the gastrointestinal tract. It is conceivable, therefore, that in the chronically inflamed gastric mucosa, endogenous tachykinins may gain pathogenic significance as inhibitors of protective mucosal hyperaemia.

In conclusion, the current data demonstrate that tachykinins, particularly NKA, inhibit the protective gastric hyperaemia in response to acid back-diffusion through an NK_2 receptor-mediated mechanism. In addition, NKA constricts the gastric vasculature under resting conditions, an effect that involves NK_1 receptors and is unrelated to the peptide's anti-vasodilator activity. The relevance of these tachykinin actions for gastric circulatory control remains to be elucidated.

This work was supported by the Austrian Science Foundation (FWF, grants P9473-MED and P11834-MED). The authors are grateful to Dr C.A. Maggi for providing a sample of MEN 10,627, to Dr X. Emonds-Alt for providing a sample of SR 140,333 and to Mr Wolfgang Schluet for organizational help.

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(Received May 20, 1996
Revised August 2, 1996
Accepted September 13, 1996)