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

P. Holzer and P.H. Guth

Submucosal blood vessels of the mammalian stomach are densely innervated by neurons containing calcitonin gene-related peptide (CGRP), substance P, neurokinin A, and vasoactive intestinal polypeptide (VIP). Because all these peptides are vasodilators in certain vascular beds, we tested the hypothesis that rat α-CGRP, rat VIP, substance P, and neurokinin A are candidate mediators of noncholinergic vasodilator neurons in the gastric mucosa and submucosa. The experiments were performed on urethane-anesthetized Sprague-Dawley rats. Gastric mucosal blood flow (GMBF) was measured by the hydrogen gas clearance technique, and the peptides were infused close arterially to the stomach via a catheter inserted retrogradely in the splenic artery. Basal GMBF was in the range of 35-50 ml/min/100 g. Infusion of rat α-CGRP (15 and 75 pmol/min) significantly increased GMBF in a dose-dependent manner, whereas mean arterial blood pressure was significantly lowered only by the higher dose of CGRP. Substance P (125 and 625 pmol/min) and neurokinin A (50 and 250 pmol/min) failed to alter GMBF, although the higher dose of each peptide led to a significant decrease in mean arterial blood pressure. Infusion of rat VIP (25 pmol/min) failed to affect GMBF and mean arterial blood pressure, whereas a fivefold higher dose of VIP (125 pmol/min) led to a significant rise of GMBF and to significant hypotension. These findings indicate that substance P and neurokinin A are unlikely to be of physiological significance for the regulation of GMBF. CGRP and VIP, however, can be considered as candidate mediators of submucosal nerve endings involved in the neural control of GMBF. By taking account of the origins of these nerve endings, CGRP would transmit sensory nerve-induced vasodilation, while VIP would mediate vasodilation induced by the enteric nervous system. (Circulation Research 1991;68:100–105)

Electrical stimulation of the vagus nerve increases gastric mucosal blood flow (GMBF). In rats, part of this rise of GMBF is mediated by cholinergic neurons, whereas the noncholinergic component is due to activation of capsaicin-sensitive sensory neurons.1,2 In line with this finding, stimulation of sensory neurons by intragastric administration of capsaicin also induces a nonadrenergic noncholinergic vasodilation.3,4 However, the nonadrenergic noncholinergic vasodilator transmitters have not yet been identified. The organization of the gastric circulation requires submucosal arterioles to be dilated to increase GMBF.5,6 Consistent with a regulatory role in mucosal circulation, capsaicin-sensitive sensory neurons7–12 as well as enteric neurons13–18 form a dense plexus of fibers around submucosal arteries and arterioles of the gastrointestinal tract. These nerve fibers contain a variety of peptides, including calcitonin gene-related peptide (CGRP), substance P, neurokinin A, and vasoactive intestinal polypeptide (VIP).7–18 Most if not all CGRP in the rat stomach originates from extrinsic sensory neurons,9–12 whereas VIP appears to be of intrinsic enteric origin only.15,16 Gastric substance P and neurokinin A arise from both extrinsic sensory and intrinsic enteric neurons.7,8,11,15,18

All these peptides are vasodilators in certain vascular beds,19–21 but it is not yet known whether they have a similar effect on blood vessels in the gastric mucosa and submucosa. Their localization in nerve endings sur-
robbing submucosal blood vessels suggests that they play a physiological role in the nonadrenergic noncholinergic neural regulation of GMBF. This proposal is further supported by the presence of receptors for CGRP,22,23 substance P and neurokinin A,24 and VIP25,26 on mesenteric arteries and submucosal arteries and arterioles. The present study was undertaken, therefore, to explore a potential role of these peptides in the nonadrenergic noncholinergic control of mucosal blood flow in the rat stomach. To this end, GMBF was measured by the hydrogen gas clearance technique, and the peptides were infused close arterially into the stomach. This route of administration was considered important for the purpose of the present study because intravenous administration of the peptides might give rise to complex reactions of the cardiovascular system,27 which could mask, at least in part, the local effects of the peptides in the vascular bed under study.

**Materials and Methods**

**Animal Preparation**

Male Sprague-Dawley rats, weighing 270–320 g, were fasted for 20 hours but were allowed free access to water. After the induction of anesthesia with urethane (1.5 g/kg s.c.), the rats were fitted with a tracheal cannula to facilitate spontaneous respiration and allow for the administration of hydrogen. Blood pressure was recorded from a cannula in the right carotid artery, and saline (0.9% NaCl, wt/wt) was continuously infused through a jugular vein at a rate of 1 ml/hr to avoid dehydration. The body temperature of the animals was kept at 36–37°C by means of a heating lamp. After exposure of the stomach by a midline laparotomy, a catheter (PE-10) was inserted retrogradely in the splenic artery close to the celiac artery to enable the close arterial administration of substances to the stomach. This operation was carried out with the aid of a binocular microscope (magnification, ×10). GMBF was determined by the clearance of hydrogen gas. To this end, a needle-type electrode was inserted from the serosa into the basal portion of the gastric mucosa and positioned at the submucosal border of the muscularis mucosae.4 The electrode was made of a platinum wire (125 μm in diameter) that was fixed in a PE-50 catheter and held in place with cyanoacrylate glue (Krazy Glue, Itasca, Ill.). Only the tip of the wire protruding 4 mm from the catheter was inserted in the tissue.4 A reference electrode (Ag-AgCl) was placed inside the peritoneal cavity. Measurement and computerized analysis of the mucosal hydrogen gas clearance were performed as described previously.4,28,29 GMBF was expressed as milliliters per minute per 100 g.

**Experimental Protocol**

The experimental protocol involved alternating 15-minute periods of saturation and desaturation of the tissue with hydrogen gas. Each peptide was tested on six different rats in random order. The peptides or their vehicle (Krebs buffer, pH 7.4) were infused after an equilibration period of 1 hour. Each animal received only two doses of a peptide in an ascending order (or one peptide dose and one infusion of the vehicle). The two infusions were spaced apart by at least 1 hour. Each infusion was started 5 minutes before, and stopped 15 minutes after, the beginning of a hydrogen desaturation period. All peptide solutions were prepared in such a way that each dose was infused at a rate of 25 μl/min.

Preliminary experiments were carried out to establish the threshold dose range for each peptide in influencing GMBF or mean arterial blood pressure (MAP). In the proper experiments, two doses of each peptide were tested for an effect on GMBF: one dose that was too low to affect MAP and a fivefold higher dose that caused significant hypotension. The results are expressed as changes in GMBF and MAP. These parameters refer to the difference between the GMBF and MAP values measured during the infusion of the peptides or their vehicle and those measured in the hydrogen desaturation period immediately before, during which no infusion was run.

**Substances**

Substance P, neurokinin A, and rat VIP were obtained from Sigma, St. Louis. Rat α-CGRP was kindly donated by Dr. J. Rivier, Salk Institute, La Jolla, Calif. The peptides were dissolved in 0.1 M acetic acid to give peptide stock solutions of 0.1 mM. For infusion, the stock solutions were diluted with Krebs buffer, pH 7.4.

**Statistics**

All data are presented as mean±SEM. Statistical evaluation of the results was performed with the Kruskal-Wallis test. Values of p<0.05 are regarded as significant.

**Results**

**General**

Basal GMBF in the present experiments was 41±2 ml/min/100 g (n=35), as measured at the end of the 1-hour equilibration period. MAP at this time point was 96±3 mm Hg (n=35). Basal GMBF and MAP did not change significantly during the experimental period, which lasted another 1.5 hours. In none of the experiments (Figures 1–4) did infusion of the vehicle (Krebs buffer, pH 7.4) at the rate of 25 μl/min influence GMBF and MAP.

**Substance P**

Infusion of 125 and 625 pmol/min substance P was without effect on GMBF, although the higher dose led to a significant fall in MAP (Figure 1). Despite the continuous infusion of substance P, hypotension induced by this peptide was not sustained. The fall in MAP peaked 3–5 minutes after the peptide infusion had begun and waned thereafter so that prepeptide values of MAP were reached after another 5 minutes.
Neurokinin A

The results obtained with neurokinin A were similar to those obtained with substance P. Neurokinin A (50 and 250 pmol/min) did not affect GMBF, but the higher dose lowered MAP significantly (Figure 2). Also, the time course of the hypotensive effect of neurokinin A was comparable to that seen with substance P. Although the peptide was infused continuously, hypertension peaked 3–5 minutes after the infusion of neurokinin A had begun and thereafter waned slowly so that prepeptide values of MAP were reached after another 10 minutes.

Vasoactive Intestinal Polypeptide

Infusion of VIP at the dose of 25 pmol/min was without effect on MAP and GMBF. However, a fivefold higher dose (125 pmol/min) caused a marked decrease in MAP and significantly enhanced GMBF (Figure 3). The hypotensive effect of VIP was sustained as long as the peptide was infused.

Calcitonin Gene-Related Peptide

CGRP was very potent in increasing GMBF. At a dose of 15 pmol/min, which did not affect MAP, this peptide was able to significantly facilitate GMBF. A fivefold higher dose (75 pmol/min) led to a marked increase in GMBF and to significant hypotension (Figure 4). The hypotensive effect of CGRP was sustained as long as the peptide was infused.

**Figure 1.** Effect of close arterial administration of substance P to the rat stomach on gastric mucosal blood flow (MBF, top panel) and mean arterial blood pressure (MAP, bottom panel). Abscissa, dose of peptide infused. 0 referring to vehicle infused at a rate of 25 μl/min; ordinate, changes in MBF and MAP and those measured during a control period before. Data are mean ± SEM; n=6. *p<0.05 vs. vehicle.

**Figure 2.** Effect of close arterial administration of neurokinin A to the rat stomach on gastric mucosal blood flow (MBF, top panel) and mean arterial blood pressure (MAP, bottom panel). Abscissa, dose of peptide infused. 0 referring to vehicle infused at a rate of 25 μl/min; ordinate, changes in MBF and MAP that represent the difference between the values measured during the infusion of peptide or vehicle and those measured during a control period before. Data are mean ± SEM; n=6. *p<0.05 vs. vehicle.

**Discussion**

The present data show that CGRP and VIP, but not substance P and neurokinin A, facilitate GMBF when given close arterially to the stomach of the rat. Because the purpose of the study was to explore a potential role of these neuropeptides in the physiological control of GMBF, two doses of each peptide were chosen such that both a nonhypotensive and a hypotensive dose were tested for a possible effect on GMBF. It was reasoned that this experimental design should allow us to distinguish whether the peptide under study has a local effect on the gastric circulation or whether changes in GMBF might be secondary to changes in MAP. Although no pharmacological characterization of the peptide actions was attempted, it should be recalled that diffusion barriers, enzymatic degradation, and other factors of elimination will have an impact on the potency and efficacy of peptides in vivo. Hence, differences in the activity of various peptides may in part be accounted for by differences in their susceptibility to these factors. Attention also should be drawn to the fact that intravascular administration of substances does not mimic precisely the situation of transmitter release from nerve endings within the vessel wall.
Given all these experimental shortcomings, it was thought that close arterial administration of the peptides to the stomach is the approach of first choice in the exploration of a potential role of substances in the neural control of GMBF.

The ineffectiveness of the tachykinins substance P and neurokinin A in changing GMBF, even at doses that reduced MAP, is consistent with a report that intravenous substance P fails to alter the gastric clearance of aminopyrine, an indirect measure of GMBF. Likewise, substance P and neurokinin A are unable to dilate mesenteric arteries of the rat in vitro, although substance P is a vasodilator in certain other vascular beds. Although receptors for substance P and neurokinin A have been demonstrated on blood vessels of the gastrointestinal tract, it is thought that these receptors are present in the inflamed tissue only but are absent from normal gut. This view is in keeping with the lack of effect of substance P and neurokinin A on GMBF seen in the present study. It follows that substance P- and neurokinin A-containing nerve endings around mucosal and submucosal blood vessels are unlikely to control GMBF.

VIP increased GMBF at doses that also lowered MAP, and it has to be inferred that a rise in GMBF in the face of a reduced perfusion pressure reflects effective dilatation of gastric mucosal blood vessels. This inference is in line with a report that close arterial injection of VIP to the dog stomach induces vasodilatation. Accordingly, VIP receptors have been found in mesenteric arteries and submucosal arterioles of the gastrointestinal tract. Because VIP-containing nerve endings surrounding submucosal blood vessels are of enteric origin, we propose that VIP is a mediator of gastric mucosal vasodilatation induced by the enteric nervous system. In addition, there is evidence that VIP is instrumental in mediating the gastrointestinal nonadrenergic noncholinergic vasodilatation that is induced by stimulation of the parasympathetic nervous system.

The most active peptide seen in the present study was CGRP, which increased GMBF even at subhypotensive doses. This result indicates that, like in other vascular beds, CGRP is a very potent dilator of gastric submucosal and mucosal blood vessels. The present findings obtained with close arterial administration of CGRP to the rat stomach confirm similar findings obtained with intravenous CGRP and are consistent with the in vitro dilator effect of CGRP on mesenteric and gastric arteries. This site of action of CGRP is corroborated by the presence of autoradiographically demonstrable CGRP receptors on mesenteric arteries and gastrointestinal arteries. Rat α-CGRP was used in the present experiments because this is the only form of...
CGRP found in the rat stomach.\textsuperscript{12} Because practically all CGRP in the rat stomach arises from capsaicin-sensitive sensory neurons,\textsuperscript{9,11,12} it would follow that CGRP is the principal mediator of gastric mucosal vasodilatation induced by sensory nerve stimulation, all the more since substance P and neurokinin A, which also occur in these neurons, were found to be inactive on GMBF. There is increasing evidence that CGRP does play a physiological role in the regulation of GMBF since stimulation of sensory neurons in the rat stomach causes the release of CGRP into the circulation\textsuperscript{36} and facilitates GMBF.\textsuperscript{3,4}

This sensory nerve-mediated vasodilatation in the gastric mucosa is thought to be instrumental in maintaining the integrity of the gastric mucosa in the face of injurious factors.\textsuperscript{3,4,34,36}

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