NITRIC OXIDE IS INVOLVED IN THE MEDIATION OF GASTRIC BLOOD FLOW AND TISSUE OXYGENATION

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Endogenous nitric oxide which is enzymatically formed by endothelial cells from L-arginine has been implicated in the control of gastrointestinal circulation. Its role in the mediation of gastric tissue oxygenation has not been studied. We investigated the role of NO in the control of gastric blood flow and oxygen uptake. In anesthetized dogs, total gastric blood flow, gastric mucosal blood flow, systemic arterial and portal venous pressures and the arteriovenous oxygen content difference were studied. From these measurements gastric vascular resistance and oxygen consumption were calculated. Administration of N\textsuperscript{G}-nitro-L-arginine (L-NNA) induced gastric tissue ischemia and hypoxia. Both, systemic arterial pressure and gastric vascular resistance were increased. Above hemodynamic and metabolic effects of L-NNA were significantly attenuated when administration of L-NNA was combined with L-arginine. Our findings suggest that endogenous NO is a tonic vasodilator modulating gastric blood flow and oxygen uptake through influence on the gastric microcirculatory structures responsible for vascular resistance and the nutrient circulation.

Key words: gastric circulation, nitric oxide, oxygen uptake

INTRODUCTION

Endothelium-derived relaxing factor (EDRF) is though to be a potent vasodilator and inhibitor of platelet aggregation and adhesion (1—3). EDRF is identified as nitric oxide which is synthesized from L-arginine (4). NO appears to be continuously released from endothelial cells stimulated by flowing blood, it is also liberated by many neurohumoral factors. Different stereospecific of NO biosynthesis antagonists such as N-nitro-L-arginine (L-NNA), and N-monomethyl-L-arginine (L-NMMA) have been developed (5, 6). Studies using these inhibitors of NO synthase demonstrated that NO plays an important role as a mediator in the tonic vasodilation observed in the systemic vascular bed.

This local hormonal vasodilator has been implicated in the regulation of gastric macro- and microcirculation at rest and during activation of gastric
secretion (7—12). On the basic of the findings that NO is potent relaxant of the gastric vasculature in the rat, we undertook the present study to evaluate the role of endogenous NO in the control of gastric blood flow and oxygenation of gastric tissues in the dog.

MATERIAL AND METHODS

Experiments were performed on 27 dogs of either sex, weighing between 19 and 23 kg. The animals were fasted for 24 hr before the onset of experiments. Anesthesia was induced by a slow intravenous (i.v.) injection of sodium pentobarbital (25 mg/kg). The animals were ventilated with room air using a positive pressure respirator (Medipan Poland) and a cuffed endotracheal tube. A femoral artery was cannulated and connected to a strain-gauge transducer (Hewlett-Packard Co., Waltham, Mass.) for continuous monitoring of systemic arterial pressure on a recorder (Sensor Medics Dynograph, model 611), and a femoral vein was cannulated for periodic injection of anesthetic as needed.

A midline laparotomy was performed, following which the stomach and spleen were exposed and all nongastric branches of the splenic artery and vein were ligated. One polyethylene catheter was introduced retrogradely into a branch of the splenic artery for intra-arterial (i.a.) infusion of drugs or saline directly into the gastric vascular bed. Two splenic vein branches were also cannulated. One saline-filled catheter was inserted into the portal vein and connected to a pressure transducer for continuous measurement of pressure (PVP). Another catheter was introduced into one of the splenic veins draining the area supplied by the left gastroepiploic artery. This catheter was used to obtain venous blood from the stomach for perfusion through an arteriovenous oxygen content difference analyzer (AVOX System, San Antonio, Tex.). A part of the fundic portion of the stomach supplied by the left gastroepiploic artery was then isolated by clamping with a forceps, thereby forming a gastric pouch. We ligated the larger connections of the left gastroepiploic artery with other gastric arteries as well as all branches supplying the omentum in order to interrupt collateral vessels. Next, the stomach was covered with a saline-soaked gauze and plastic wrap.

Gastric blood flow was determined by means of electromagnetic blood flow probe (2.0—2.5 mm i.d.) placed around the splenic artery proximal to the infusion site. The probe was connected to a blood flow amplifier (T-206 Flowmeter Transonic System Inc, Ithaca). The electromagnetic flow probe was calibrated in vitro and a zero flow reference was established during the experiment by brief occlusion of the splenic artery distal to the flow probe. Gastric oxygen consumption was calculated as the product of simultaneously measured blood flow to the gastric pouch and the arteriovenous oxygen content difference (13). Gastric vascular resistance (GVR) was calculated from the quotient of arterial-venous pressure/blood flow.

Gastric mucosal blood flow (MBF) was determined by laser flowmetry. A fiberoptic probe of a laser Dopler flowmeter (Laser Flo BPM 403, TSI) was positioned againsts the surface of the gastric mucosa. The probe was secured outside the animal to prevent any movement of the tip of the probe and ensure constant (continous) optical coupling between the tip of the probe and the gastric mucosa throughout the experiment. The change in MBF was calculated in terms of percentage of control. Continuous recordings of AP, GBF, MBF and AVO₂ were made on the polygraph.

After administration of sodium heparin (6.0 mg/kg i.v.) the gastric venous catheter and a femoral arterial catheter were attached to a constant-flow pump (Raivin Instruments Co., Woburn, Mass.) to permit gastric venous and femoral arterial blood to flow through separate cuvettes of the oxygen content difference analyzer at 7.0 ml/min. The blood was returned to the animal via femoral vein catheter.
After the surgical preparation was completed, hemodynamic parameters were allowed to stabilize for 30 min. Then one of three experimental protocols was initiated. In each protocol, a group of at least six dogs was studied.

All data are presented as means ± SEM. The significance of changes in measured values from control was determined using the two-tailed Student's test for either grouped or paired data with a confidence limit of less than 5%.

In group I dogs, animals were anesthetized and studied, as described above, to quantify the GBF, MBF, GVO₂, AP, PVP and GVR response during basal conditions without or with NO synthase blockade using L-NNA (Sigma Chemical Co., St. Louis, MO). The drug was dissolved freshly in isotonic saline and given i.v. as infusion in a dose of 2.5 mg/kg/h.

In group II dogs the hemodynamic and metabolic parameters were studied before and after pretreatment with L-arginine (Sigma Chemical Co) 100 mg/kg/h i.v. alone and L-arginine plus L-NNA.

In groups III dogs all measurements were performed before and after i.v. infusion of glyceryl trinitrate (GTN) (Polfa, Poland) in a dose of 0.5 mg/kg/h. GTN was administered alone or in combination with L-NNA.

RESULTS

The control values in these three groups of experiments were GBF 63.6 ± 7.0 ml/min per 100 g tissue, AVO₂ 3.6 ± 0.4 ml O₂/100 ml blood, GVO₂ 2.6 ± 0.5 ml O₂/min per 100 g of tissue. AP 125 ± 8.0 mmHg, PVP 5.6 ± 0.7 mmHg, and GVR 2.8 ± 0.3 mmHg/min blood flow per 100 g tissue. Control value of MBF was 4.6 ± 0.8 Volts.

In group I intravenous infusion of L-NNA decreased GBF by 31.0 ± 4.0%, MBF by 46.0 ± 8.0%, GVO₂ by 38.8 ± 5.0% and increased, AP by 32.6 ± 7.0% and GVR by 55.6 ± 9.0%, respectively. PVP was not change significantly (Figs. 1—4).

In group II pretreatment of animals with L-arginine alone was without any effect on the resting values of GBF, MBF, GVO₂ and PVP but tended to decrease AP and GVR. However the last two parameters did not change significantly. Combined pretreatment with L-NNA + L-arginine did decrease basal GBF by 15.2 ± 3.0%, MBF by 22.6 ± 7.3%, GVO₂ by 23.6 ± 4.0% and increased AP by 10.2 ± 2.0% and GVR by 26.3 ± 8.0% respectively and did not change PVP (Figs. 1—4). However observed changes in gastric blood flow, pressures and GVO₂ after combined treatment with L-NNA + L-arginine were significantly different than that observed after L-NNA alone.

In group III, the effect of GTN alone and of combined pretreatment with L-NNA plus GTN was studied. GTN when infused alone decreased GBF by 18.3 ± 3.0%, MBF by 22.6 ± 5.0%, AP by 25.8 ± 6.0%, PVP by 15.2 ± 3.0% and GVR by 56.7 ± 6.0, respectively. GVO₂ was not affected by administration of GTN. L-NNA when added to GTN did not change significantly any of the examined parameters as compared with corresponding control values (Figs. 1—4).
Fig. 1. Effects of L-NNA alone, L-arginine + L-NNA, GTN alone and GTN + L-NNA on gastric blood flow. Results are shown as a mean values in percentage of control ± SEM. Single asterik indicates significant (p < 0.05) change in comparison with L-NNA alone. Double asteriks indicate significant (p < 0.05) difference in comparison with L-NNA and GTN alone.

Fig. 2. Effects of L-NNA alone, L-arginine + L-NNA, GTN alone and GTN + L-NNA on gastric mucosal blood flow. Single asterik indicates significant (p < 0.05) increase above of L-NNA alone. Double asteriks indicate significant (p < 0.05) increase in comparison with L-NNA and GTN alone.
Fig. 3. Effects of L-NNA alone, L-arginine + L-NNA, GTN alone and GTN + L-NNA on gastric vascular resistance. Single asterik indicates significant (p < 0.05) decrease below L-NNA alone. Double asteriks indicate significant (p < 0.01) decrease in comparison with L-NNA alone and significant increase as compared to GTN alone.

Fig. 4. Changes observed in gastric oxygen uptake after L-NNA alone, L-arginine + L-NNA, GTN alone and GTN + L-NNA. Single asterik indicates significant (p < 0.05) increase above L-NNA alone.
DISCUSSION

The two most important structures of the gastric microcirculation which regulate total blood flow to the stomach and capillary blood flow, respectively, are the arteriolar smooth muscle and the muscle of the precapillary sphincters. More than half of the resistance to the total flow of the blood through the stomach occurs during passage of the blood through the arterioles (14, 15). Small changes in the diameter of these vessels are translated into large changes in blood flow through the gastric arteries. However, it is also true under resting conditions that only one-third to one-fourth of the capillaries are open to the flow of blood at any moment in time (16). Thus, by identifying the diffusion parameters, it becomes apparent that the density of the perfused capillary bed is a critical determinant of tissue oxygen extraction. Furthermore, our calculations indicate that changes in the density of the perfused capillary bed are as significant to oxygen delivery as are the changes in blood flow (17—19).

In the present study we have assessed the role of endogenous NO in the control of gastric circulation and oxygenation of gastric tissues. The characteristics of the changes in the gastric and systemic circulation, which appeared after acute L-NNA administration are consistent with previous reports that the inhibition of NO synthesis prompted a marked gastric ischemia (7, 11). The L-NNA — induced reduction in blood flow through gastric vasculature observed in the current study corresponds with our previous report showing that this agent, when infused i.v. consistently decreases intestinal and pancreatic blood flow (20, 21). The inhibition of NO synthesis evoked a significant decrease in gastric oxygen consumption. This, combined with decreasing gastric blood flow, could be due either to a direct metabolic effect, or to a decrease in blood flow through the nutrient portion of the gastric microcirculation. We have previously shown that many vasoconstrictors suppress gastrointestinal oxygen consumption (16, 22).

We also found that the inhibition of NO synthesis evoked a significant decrease in gastric uptake of oxygen. The declines of GVO₂ paralleled the decreases in GBF and GMBF in response to L-NNA. Hence, inhibition of NO synthesis induced both ischemia and hipoxia in the stomach. In this respect, inhibition of NO synthesis acts upon the gastric circulation in a manner similar to most constrictor agents we have studied.

The microcirculatory sites at which NO acts the gastric vasculature, are the arteriolar smooth muscle and the smooth muscle of the precapillary sphincter. Since significant oxygen exchanges occur only across the capillary endothelium, a reduced uptake of oxygen ensues when blood flow through the capillaries is reduced (16—18). Our data suggests that inhibition of NO synthesis induced indiscriminate contraction of arteriolar and precapillary
sphincteric smooth muscle, thereby reducing total blood flow by constricting arterioles and reducing the nutrient circulation by closing capillaries. Besides decreasing total gastric blood flow the inhibition of NO synthesis induced a redistribution of blood flow into the muscular and/or submucosal compartment of the gastric circulation as evidenced by higher percent of decrease in MBF than percent of decrease in GBF. This may be due to the stimulation of gastric smooth muscle (23, 24) or a greater generation of NO in the mucosal vasculature.

The ability of exogenous L-arginine to reverse the ischemic and hypoxic effects of L-NNA in the stomach gives further support to the specificity and mechanism of action of this agent which is considered to act by competing for the uptake or utilization of L-arginine the substrate for NO synthesis (4). The local and systemic hemodynamic and metabolic effect of L-NNA were also prevented by exogenous donor of NO such as GTN. Moreover, observation that L-arginine alone was not able to induce any changes in the examined parameters indicate that during basal conditions there is not any lack of NO synthesis locally in the stomach and in systemic vasculature.

In summary the findings of the present investigation support the concept that endogenous NO is implicated as tonic vasorelaxing factor in the gastric macro- and microcirculation and regulator of the gastric tissues oxygenation under basal conditions.

REFERENCES


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