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Dietary nitrate increases gastric mucosal blood flow and mucosal defense

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Petersson J, Phillipson M, Jansson EÅ, Patzak A, Lundberg JO, Holm L. Dietary nitrate increases gastric mucosal blood flow and mucosal defense. *Am J Physiol Gastrointest Liver Physiol* 292: G718–G724, 2007. First published November 2, 2006; doi:10.1152/ajpgi.00435.2006.—Salivary nitrate from dietary or endogenous sources is reduced to nitrite by oral bacteria. In the acidic stomach, nitrite is further reduced to bioactive nitrogen oxides, including nitric oxide (NO). In this study, we investigated the gastroprotective role of nitrate intake and of luminally applied nitrite against provocation with diclofenac and taurocholate. Mucosal permeability (^{51}Cr-EDTA clearance) and gastric mucosal blood flow (laser-Doppler flowmetry) were measured in anesthetized rats, either pretreated with nitrate in the drinking water or given acidified nitrite luminally. Diclofenac was given intravenously and taurocholate luminally to challenge the gastric mucosa. Luminal NO content and nitrite content in the gastric mucus were determined by chemiluminescence. The effect of luminal administration of acidified nitrite on the mucosal blood flow was also investigated in endothelial nitric oxide synthase-deficient mice. Rats pretreated with nitrate or given nitrite luminally had higher gastric mucosal blood flow than controls. Permeability increased more during the provocation in the controls than in the nitrate- and nitrite-treated animals. Dietary nitrate increased luminal NO levels 50 times compared with controls. Nitrate intake also resulted in nitrite accumulation in the loosely adherent mucous layer; after removal of this mucous layer, blood flow was reduced. Nitrite administrated luminally in endothelial nitric oxide synthase-deficient mice increased mucosal blood flow. We conclude that dietary nitrate and direct luminal application of acidified nitrite decrease diclofenac- and taurocholate-induced mucosal damage. The gastroprotective effect likely involves a higher mucosal blood flow caused by nonenzymatic NO production. These data suggest an important physiological role of nitrate in the diet.

laser-Doppler flowmetry; ^{51}chromium-labeled EDTA clearance; nitrite; rats; endothelial nitric oxide synthase deficient; gastric mucus

THE ROLE OF NITRATE IN OUR diet has been a subject of great controversy over the years. The anxiety concerning nitrate consumption is related to the presumed association with gastrointestinal cancer. Today, however, there are more studies contradicting than supporting such a correlation (36). Several recent studies have in fact suggested that dietary nitrate has a gastroprotective role (6, 16, 31, 32, 35, 38). Most of the nitrate in our diet originates from green leafy vegetables, a natural source of food for humans. Bacteria in the oral cavity reduce parts of the nitrate to nitrite, and human saliva therefore contains both of these anions (49). A large amount of nitric oxide (NO) is produced in the upper gut when swallowed nitrite reacts with hydrogen ions (6, 35, 37).

The gastric mucosa is covered with a continuous viscous mucous layer, which acts as an important physical preepithelial level of the gastric mucosal defense (4, 48). The mucus also provides a chemical barrier, where the epithelium secretes bicarbonate into the mucus and neutralizes back-diffused acid (3, 43). Earlier studies have shown that acidified nitrite and NO donors increase mucous thickness in the rat (9–11). The second layer of defense, the gastric epithelial barrier, consists of tight junctions. Noxious agents have been shown to disrupt this barrier and increase the clearance of large molecules from blood to lumen (14, 21). Different research groups have shown that inhibition of endogenous NO production leads to an increase in mucosal permeability (22, 28). Several studies have also shown that nonsteroidal anti-inflammatory drugs (NSAIDs) increase the mucosal permeability (15).

There is a current opinion that an adequate mucosal blood flow plays an important role in maintaining the mucosal integrity. Numerous experimental studies have demonstrated the importance of mucosal blood flow in the defense of the gastric mucosa against injury (1, 12, 20, 27, 33, 42, 47, 52). The blood flow carries bicarbonate to neutralize acid and removes cellular waste products. Our group (9) has recently shown that acidified nitrite and acidified nitrite-rich saliva trigger this defense mechanism and increase the gastric mucosal blood flow.

In this study, we addressed the question of whether dietary nitrate and locally administered nitrite might upregulate the mucosal blood flow to such an extent as to protect the gastric mucosa from injury. We wanted to investigate the role of ingested nitrate and its subsequent products nitrite and NO in protecting the stomach from injury produced by NSAIDs and bile salts. For this purpose, we measured the mucosal blood flow and epithelial permeability in vivo in rats and mice and challenged the gastric mucosa by giving the rats the NSAID diclofenac intravenously in combination with luminal administration of the bile salt taurocholate.

METHODS

Animal Preparation

All experiments were approved by the Uppsala University or Karolinska Institute Ethical Committee for Animal Experiments.

Rats. Male Sprague-Dawley rats (B&K Universal, Stockholm, Sweden), weighing 170–280 g, were kept under standardized conditions of temperature (21–22°C) and illumination (12:12-h light-dark cycle). Rats were allowed to adjust to this environment in cages with mesh bottoms with free access to tap water and pelleted food for at least 7 days before the experiment began. The rats were anesthetized

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with 120 mg/kg body wt of 5-ethyl-5- (1-methylpropyl)-2-thiobutabarbital sodium (Inactin; Sigma, St. Louis, MO) injected intraperitoneally. The rats were tracheotomized with a short PE-200 cannula to facilitate spontaneous breathing. Body temperature was maintained at 37–38°C with a heating pad regulated by a rectal thermistor probe. A PE-50 cannula containing Heparin (12.5 IU/ml; Leo Pharma) dissolved in isotonic saline was placed in the right femoral artery to monitor blood pressure, and the left common carotid artery was cannulated for blood sampling. The right femoral vein was cannulated for continuous infusion of a modified Ringer solution (25 mM $NaHCO_3$, 120 mM NaCl, 2.5 mM KCl, and 0.75 mM $CaCl_2$) at a rate of 1.0 ml/h, and the left femoral vein was cannulated for drug infusions.

Mice. Male C57BL/6 mice (B&K Universal) and male mice deficient in endothelial nitric oxide synthase ($eNOS^{-/-}$; The Jackson Laboratory) weighing 24–37 g were used for studies of mucosal blood flow in some experiments. The $eNOS^{-/-}$ mice (background C57/BL/6J-$Nos3^{tm1Unc}$) were generated by gene targeting in embryonic stem cells as previously described (44). The mice were kept under the same standardized conditions as the rats, but they were not fasted before the experiments. The mice were anesthetized by inhalation of ~2.2% isoflurane (Forene; Abbott Scandinavia, Kista, Sweden) via an anesthesia unit (Univentor 400 anesthesia unit; AgnTho's, Lidingö, Sweden) through a breathing mask. Body temperature was maintained at 37–38°C with a heating pad regulated by a rectal thermistor probe. A pulled PE-200 catheter containing heparin (12.5 IU/ml) dissolved in isotonic saline was placed in the left carotid artery and connected to a pressure transducer to monitor blood pressure. The jugular vein was cannulated (pulled PE-200) for continuous infusion of a modified Ringer solution at a rate of 0.35 ml/h.

Tissue Preparation

The abdomen was opened through a midline incision. The gastrohepatic ligaments were cut, and the stomach was gently exteriorized and kept moistened and warm with 37°C saline during the preparation procedure. The short gastric artery and vein were ligated and cut. An incision was made along the greater curvature in the forestomach. The animal was placed on its left side on a Lucite microscope stage on a heating pad, and the stomach was everted through the incision and loosely draped over a truncated cone with the luminal side up. A double-bottom mucosal chamber with a hole in the bottom was fitted over the mucosa, exposing the mucosa through the hole (exposed area: rats 0.8 cm^2, mouse 0.2 cm^2). The junction was sealed with silicon grease (Dow Corning high vacuum grease; Dow Corning, Weisbaden, Germany). The chamber was filled with warm (37°C) unbuffered 0.9% saline solution to keep the tissue moist and prevent the mucous gel from dehydration. The technique has been described in detail previously (23, 26).

Mucosal Permeability

The permeability studies were performed in rats. After completion of surgery and 60 min before the start of the experiment, 50–75 μCi of ^{51}chromium-labeled EDTA (^{51}Cr-EDTA; DuPont-NEN, Boston, MA) was injected as a bolus dose intravenously. This dose was followed by a continuous infusion of ^{51}Cr-EDTA (in the Ringer solution) at a rate of 1.0 ml/h (10–30 μCi/h) throughout the experiment. Three 0.2-ml blood samples were drawn during the experiment. The first was taken 60 min after the bolus injection of ^{51}Cr-EDTA. After each blood sample, the volume loss was compensated for by an injection of an equal volume of 7% BSA (Sigma-Aldrich Chemie, Steinheim, Germany). The blood sample was centrifuged, and 50 μl of the plasma were removed for measurements of radioactivity in counts per minute (cpm). The gastric mucosa was covered with 5 ml of isotonic saline or 5 ml of 10 mM HCl during the experiments. The 10 mM HCl solution was made from a stock solution of 1 M HCl (Titrisol; Merck, Darmstadt, Germany) and adjusted to isotonicity by addition of sodium chloride. The luminal solution and the blood plasma were analyzed for activity in a gamma counter (1282 Compugamma Cs; Pharmacia, Uppsala, Sweden). Each clearance value was calculated by dividing each individual cpm value by a corresponding plasma cpm value. If there was a difference of <10% between the consecutive blood plasma counts, a mean plasma cpm per milliliter value was calculated and used for all clearance samples. If there was a difference of >10% deviation between the blood plasma counts, the activity was plotted against time, and a straight line was drawn between the values. The part of the stomach that had been exposed in the chamber was cut out and weighed after the experiment. Clearance is expressed as milliliters per minute per 100 g wet tissue weight.

Mucosal Blood Flow

Laser-Doppler flowmetry (LDF) (PeriFlux 4001 Master and PeriFlux Pf 3; Perimed, Stockholm, Sweden) was used for blood flow measurements in all experiments. The nature of the Doppler shift from an illuminated tissue depends on the velocity and number of moving red blood cells (40). The laser light (wavelength 635 nm, helium neon laser) is guided to the tissue by an optic fiber. The backscattered light is picked up by a pair of fibers separated by a distance of 0.25–0.5 mm. With this technique, blood flow is determined as a voltage output and expressed as perfusion units (PU). The blood flow was recorded continuously from the mucosal side of the gastric mucosa, with a probe fixed to a micromanipulator and kept at a distance of 0.5–1 mm above the surface of the mucosa, in the solution. This technique for measuring the gastric mucosal blood flow with laser-Doppler flowmetry has been well evaluated previously (25, 29).

Experimental Protocol

Diclofenac and taurocholate: effects on mucosal permeability and blood flow. Blood-to-lumen clearance of ^{51}Cr-EDTA and mucosal blood flow were studied before, during, and after a challenge to the gastric mucosa in rats. The animals were allowed at least 1 h to stabilize after the operation, so that the systemic blood pressure and luminal blood flow were at steady state for at least 20 min before the experiment began. The experiments lasted for 170 min, and the solutions to which the gastric mucosa was exposed to were changed every 10 min. The pH in the removed solutions was measured with a pH electrode (Autobürette ABUp1; Radiometer, Copenhagen, Denmark), and the applied solution was saved for measurements of ^{51}Cr-EDTA activity. The mucosa was exposed to saline for the first 20 min, then to 10 mM isotonic HCl for 130 min, and finally to 20 min of saline. Forty minutes after the start diclofenac (Voltaren; Novartis, Täby, Sweden) was given intravenously at a dose of 5 mg/kg, and 30 min later 20 mM taurocholate (Sigma-Aldrich Chemie) was added to the acidic luminal solution for 40 min (Fig. 1). Mucosal blood flow and mean arterial blood pressure (MAP) were recorded continuously during the experiments, and vascular resistance was calculated as the ratio of MAP to mucosal blood flow ($n = 7$).

Nitrate pretreatment. To investigate whether a high nitrate diet had gastroprotective effects, one group of rats was pretreated with 0.8 mmol/kg sodium nitrate daily in the drinking water for 7 days before the experiments ($n = 5$). The experimental protocol described above was then followed (Fig. 1).

Nitrite administered luminally. To mimic the effect of swallowing nitrite-rich saliva, another group of rats ($n = 8$) was submitted to continuous luminal administration of sodium nitrite (Sigma-Aldrich Chemie) to the mucosa 30 min after the start of the experiment. Fifty microliters of a 100 mM sodium nitrite solution was first applied to the mucosal chamber, containing 5 ml of HCl or saline, resulting in a concentration of 1.0 mM sodium nitrite. Because the system is open to air and the NO that forms from nitrite in the acidic environment declines rapidly, sodium nitrite was thereafter slowly and continuously applied to the mucosa over a period of 10 min (0.5 ml of a 10

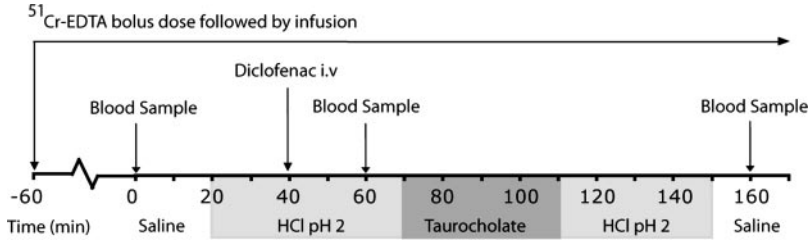

Fig. 1. Experimental protocol.

mM sodium nitrite solution was infused to the mucosal chamber filled with 5 ml HCl or saline, which results in 1.0 mM nitrite in the chamber over the 10 min). This procedure was repeated every 10 min, since the luminal solution was removed for analyses, throughout the experiment, as described (Fig. 1).

Effects of nitrate pretreatment on intragastric NO generation. In 12 additional rats, intragastric NO levels were measured. The rats were given sodium nitrate (0.8 mmol/kg daily, $n = 5$) in the drinking water for 7 days before the experiment. The control animals received ordinary drinking water ($n = 7$). The rats were anesthetized, and a laparotomy was performed after they had been fasted overnight. A thin needle was inserted intragastrically via the stomach wall, and the stomach was inflated with 4 ml of NO-free air. Passage of air into the esophagus or duodenum was prevented by external clamps. After 15 s, the air was aspirated and immediately injected into a chemiluminescence NO analyzer (Aerocrine, Stockholm, Sweden).

Effects of nitrate pretreatment on mucous nitrite content. In another 22 rats (nitrate pretreated: $n = 10$; controls: $n = 12$), nitrite levels in the gastric mucus were measured. Before the removal of the mucous layers, gastric mucosal blood flow was measured for 30 min. The gastric mucosa was covered with warm (37°C) saline throughout the experiments. The loosely adherent mucous layer was removed under a microscope by suction with a catheter connected to a syringe and saved for analyses. The remaining, firmly adherent mucous layer was scraped off the mucosa with a scalpel. The total volume of the different mucous layers was estimated, from measurements of mucous thickness (5) and exposed area, and the samples were stored at −70°C. For measurements of the nitrite content in the mucus, we used a chemiluminescence method described in detail by Feelisch et al. (17).

Effects of removal of loosely adherent mucus on mucosal blood flow. In nitrate-pretreated rats ($n = 7$) and controls ($n = 7$), gastric mucosal blood flow was measured for 30 min, after which the loosely adherent mucous layer was removed as described above. The blood flow was then followed for another 20 min. The gastric mucosa was covered with warm (37°C) saline throughout the experiments.

Effects of nitrite on mucosal blood flow in eNOS-deficient mice. The effects of luminal administration of acidified nitrite on the mucosal blood flow in C57BL/6 mice ($n = 4$) and in eNOS-deficient mice ($n = 4$) were investigated. The gastric mucosa was first covered with 3 ml of warm saline for 20 min, followed by a 10-min period of 1.0 mM sodium nitrite in 3 ml HCl (pH 3), and then another 20 min with saline. Mucosal blood flow and MAP were continuously recorded during the experiments, and mucosal vascular resistance was calculated as the ratio of MAP to mucosal blood flow.

Statistics

The results are expressed as means ± SE. Differences between groups of animals were evaluated by one-way ANOVA and within groups by ANOVA for repeated measurements, followed by Fisher's protected least significant difference test. Differences were regarded as significant if $P < 0.05$. All statistical calculations were performed with Statview II SE graphics software (Abacus Concepts, Berkeley, CA).

RESULTS

Effects of Diclofenac and Taurocholate on Mucosal Permeability

The mucosal permeability, expressed as ^{51}Cr-EDTA clearance (ml·min^{-1}·100 g tissue^{-1}), did not differ between the groups (control, nitrate-pretreated, and nitrite luminally) during the first saline period and increased during the diclofenac and taurocholate provocation in all three groups (Fig. 2). In the control group, however, clearance increased to 0.92 ± 0.12 ml·min^{-1}·100 g^{-1}, which was a significantly greater increase than in the nitrate- and nitrite-treated rats, where clearance only rose to 0.56 ± 0.14 and 0.42 ± 0.07 ml·min^{-1}·100 g^{-1}, respectively.

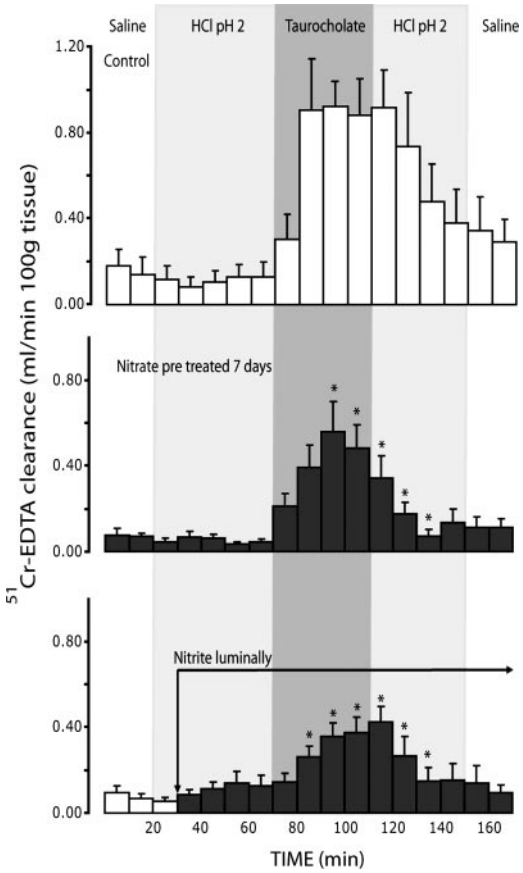

Fig. 2. Mucosal permeability, expressed as blood-to-lumen clearance of ^{51}Cr-EDTA, in control rats ($n = 7$; *top*), nitrate-pretreated rats ($n = 5$; *middle*), and rats given nitrite luminally ($n = 8$; *bottom*) before, during, and after a diclofenac and taurocholate provocation. Values are means ± SE. *$P < 0.05$ compared with control animals.

Effects of Diclofenac and Taurocholate on Blood Flow

In the control group, the blood flow was stable during the saline and HCl periods but increased significantly by 41 ± 12% when taurocholate was applied luminally (Fig. 3). In the nitrite group, blood flow increased after luminal application of nitrite by 47 ± 12% and increased even further when taurocholate was applied (Fig. 3).

Diclofenac resulted in a significant reduction in MAP 40 min after the bolus dose in all groups. In the nitrate-pretreated animals, the blood flow decreased slowly over time in parallel with MAP (from 181 ± 32 to 93 ± 5 PU), resulting in a constantly low vascular resistance, unaffected by taurocholate. There was no difference in MAP between the groups at the beginning of the experiments (control: 101 ± 4 mmHg, nitrate pretreated: 103 ± 6 mmHg, and nitrite treated: 95 ± 2 mmHg) or at the end of the experiments (control: 80 ± 6 mmHg, nitrate pretreated: 73 ± 3 mmHg, and nitrite treated: 77 ± 4 mmHg).

Generation of NO

Animals pretreated with nitrate for 7 days showed greatly increased luminal NO levels (6,280 ± 1,995 parts/billion), compared with control animals (127 ± 40 parts/billion) (Fig. 4).

Effects of Nitrate Pretreatment on Mucous Nitrite Content

Pretreatment with nitrate resulted in an increased concentration of nitrite in the loosely adherent mucous gel layer (8.0 ± 2.0 μM) compared with that in control animals (1.4 ± 0.6 μM) (Fig. 5). There was no difference in nitrite concentration in the firmly adherent mucous layer between the groups (control: 6.0 ± 1.3 μM, nitrate pretreated: 6.1 ± 2.8 μM).

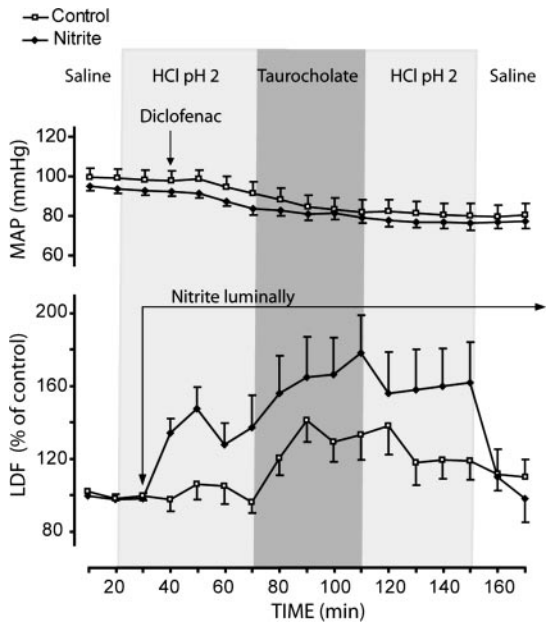

Fig. 3. Mean arterial blood pressure (MAP) and mucosal blood flow [via laser-Doppler flowmetry (LDF), presented as % of values in the first saline period] before, during and after diclofenac and taurocholate provocation in control rats (□; $n = 7$) and rats treated with nitrite luminally (◆; $n = 8$) Values are means ± SE and represents the mean value of a 10-min period.

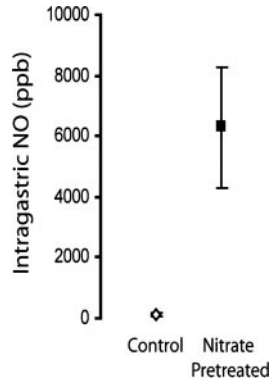

Fig. 4. Intragastric nitric oxide (NO) generation in control animals (◇; $n = 7$) and nitrate-pretreated animals (■; $n = 5$). Values are means ± SE. ppb, Parts per billion.

Effects of Removal of Loosely Adherent Mucus on Mucosal Blood Flow

The nitrate-pretreated animals had significantly higher basal mucosal blood flow than the control animals. When the loosely adherent mucous layer was removed from the nitrate-pretreated animals, the mucosal blood flow decreased significantly from 214 ± 11 to 156 ± 12 PU. In the control animals, removal of the mucus resulted in a slight, nonsignificant reduction from 155 ± 27 to 127 ± 22 PU (Fig. 6).

Effect of Nitrate in the Drinking Water on Mucosal Blood Flow

Figure 7 presents results of baseline mucosal blood flow measurements from control and nitrate-pretreated groups. The results (in PU) show that nitrate-pretreated rats had a significantly higher mucosal blood flow than the controls.

Effects of Nitrite on Mucosal Blood Flow in eNOS-Deficient Mice

The eNOS$^{-/-}$ mice had a significantly higher initial MAP than the C57BL/6 mice (100 ± 12 vs. 74 ± 2 mmHg). The blood flow increased in both eNOS$^{-/-}$ and control mice after luminal application of acidified nitrite (by 17 ± 6 and 28 ± 7%, respectively). There was no difference in the decrease in

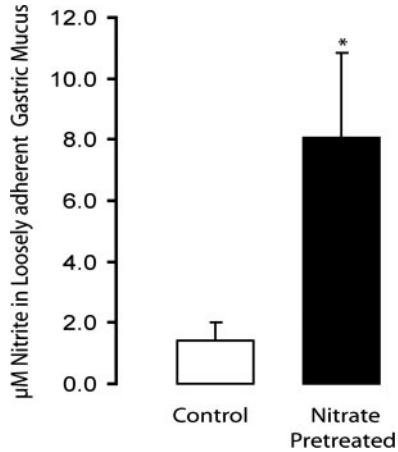

Fig. 5. Nitrite content in loosely adherent mucus in control animals ($n = 12$) and nitrate-pretreated animals ($n = 10$). Values are means ± SE. *$P < 0.05$.

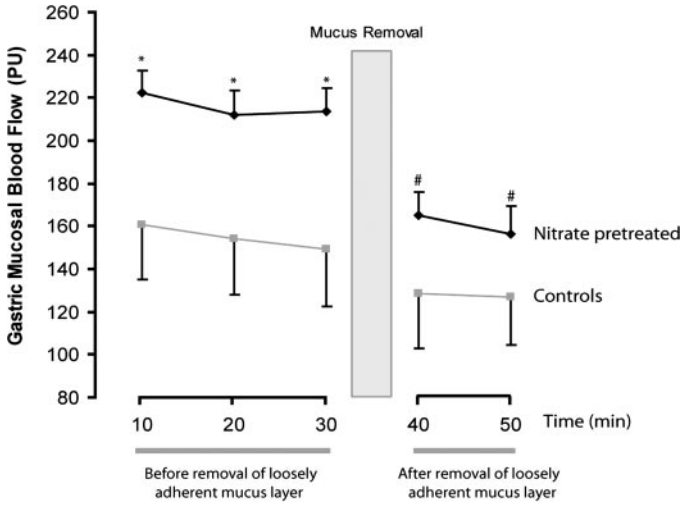

Fig. 6. Gastric mucosal blood flow [in perfusion units (PU)] in nitrate-pretreated animals (◆; $n = 7$) and control animals (gray squares; $n = 7$) before and after removal of the loosely adherent mucus. Values are means ± SE. *$P < 0.05$ compared with control animals. #$P < 0.01$ compared with before mucus removal.

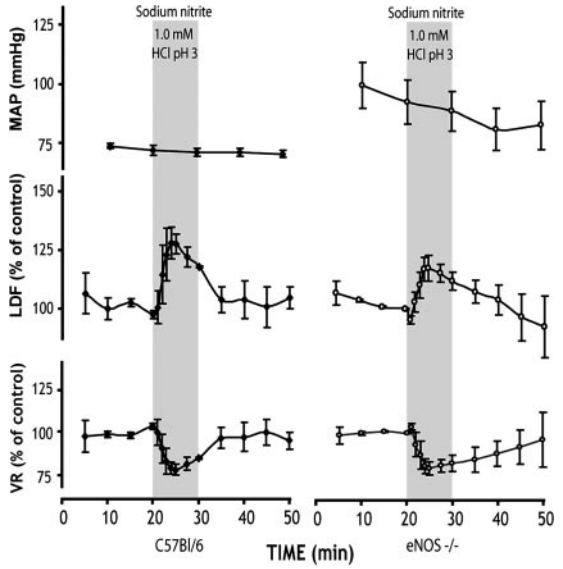

Fig. 8. MAP, gastric mucosal blood flow (LDF), and vascular resistance (VR) after topical application of sodium nitrite (1.0 mM) at pH 3 to the gastric mucosa of C57BL/6 mice ($n = 4$) and endothelial nitric oxide synthase-deficient ($eNOS^{-/-}$) mice ($n = 4$). Values are means ± SE.

vascular resistance between the groups during the acidified nitrite period ($eNOS^{-/-}$: −22 ± 3%; C57BL/6: −21 ± 4%) (Fig. 8).

DISCUSSION

Because, during evolution, vegetables have been a natural source of food for humans, it is an appealing hypothesis that a nitrate-rich diet participates in the physiology of the gastric mucosa.

We show here that high nitrate intake for 1 wk clearly reduced the increase in permeability of the gastric mucosa that is normally induced by diclofenac and taurocholate. The protective effect is similar if nitrite is added continuously to the mucosa. Both nitrite administered luminally and dietary nitrate pretreatment result in an increased mucosal blood flow.

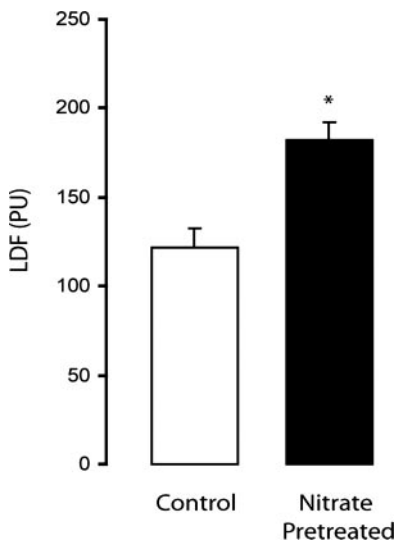

Fig. 7. Gastric mucosal blood flow (in PU via LDF) at the start of the experiments. All control animals ($n = 26$) and nitrate-treated animals ($n = 22$) in the blood flow experiments are included. Values are means ± SE. *$P <$ 0.001 compared with control animals.

The nitrate dose used (0.8 $mmol \cdot kg^{-1} \cdot day^{-1}$ or 68 $mg \cdot kg^{-1} \cdot day^{-1}$) was chosen to compensate for the differences in nitrate metabolism between rats and humans (19). In humans, active transport generates a 10-fold higher concentration of nitrate in saliva than in blood (49). This gradient is not observed in rats. This difference is also clear when one compares the resulting intragastric levels of NO in rats and humans after intake of nitrate. In rats, NO levels can reach up to ~10 ppm as shown here and earlier (9, 45), whereas, in humans, the levels are at least 10-fold higher after ingesting the same amount of nitrate (8). Together, it is very likely that the daily dose of nitrate used here compares to what is readily achievable in humans through a high intake of nitrate-rich vegetables such as spinach, lettuce, or beetroot, which typically contain between 1,800 and 4,200 mg/kg (13).

The high NO levels that we have found in the stomach as a consequence of the dietary nitrate intake confirm earlier reports (8, 9, 35, 37). The ingested nitrate is absorbed in the proximal small intestine and then concentrated in the salivary glands (49). Salivary nitrate is then reduced to nitrite by oral bacteria (16) and is further reduced to NO in the acidic stomach (6, 35). The nitrate-reducing bacteria on rat tongues, for example *Staphylococcus sciuri* and *Staphylococcus intermedius* (34), have been shown to be essential in the generation of gastric NO in nitrate-pretreated animals (45). The gastric pH in fasted rats is as low as 1.4 (39), and under these conditions nitrite is rapidly reduced to NO via formation of nitrous acid (pK_a = 3.4) (51). The NO in the stomach lumen is not produced enzymatically because the high NO level in the stomach after nitrate intake is unaltered by NOS inhibition (46).

When nitrite was applied directly to the acidic gastric mucosa, the blood flow increased immediately. This higher blood flow level was maintained as long as the nitrite was continuously added to the acidic environment. When the luminal HCl

was switched to neutral saline at the end of the experiment, the reduction of nitrite to NO ceased and the blood flow decreased to the control level. Under similar conditions, we have previously shown a clear correlation between the increase in gastric mucosal blood flow and the generation of NO after applying acidified nitrite (9). The high blood flow in this case is probably a direct effect of the generated NO. NO can easily diffuse through the mucus and mucosa to the submucosal arterioles where it causes vasodilatation via guanylyl cyclase activation (9). To investigate the possibility that endogenous NO production via eNOS could be involved in this blood flow increase, we administered acidified nitrite to the gastric lumen in wild-type and eNOS-deficient mice. The results showed that acidified nitrite caused exactly the same effects, vasodilatation and a reduced vascular resistance, in wild-type and eNOS-deficient mice. This clearly demonstrates that eNOS is not involved in the vasodilator effect of luminally applied nitrite.

The nitrate-pretreated animals had higher mucosal blood flow than the control groups. The blood flow level was comparable to that attained on luminal application of acidified nitrite. These animals had high intragastric NO levels as a result of the nitrate intake in the drinking water. The sustained increased mucosal blood flow could not, however, have been due to a large amount of intragastric free NO gas, since the stomach was opened during the animal preparation, resulting in a quick reduction of the NO level. Instead, the increased blood flow might be a consequence of the increased concentration of nitrite that was seen in the outer, loosely adherent mucous gel. The combination of accumulated nitrite in the outer mucous layer and an acidic lumen would result in a slow, prolonged NO formation close to the gastric mucosa. Interestingly, when the loosely adherent mucous layer was removed in the nitrate-treated rats, the blood flow decreased substantially down to a level not significantly different from that in the control animals.

It has been shown previously that the firmly adherent mucous layer is important for gastric protection against luminal acid (3, 5, 41). Within this mucous layer, a pH gradient is formed, with a neutral pH at the epithelial surface (juxtamucosal pH) despite an acidic lumen (41). In this present study, we find for the first time an important protective function of the loosely adherent mucous gel layer. The accumulation of nitrite here will be especially important under conditions when the acid secretion is stopped but the stomach still contains acid. This is true for times in between meals and especially during sleep. We have shown earlier that the juxtamucosal pH is much better maintained in the acid-secreting stomach than in the nonsecreting (41, 43, 48). This is due to delivery of bicarbonate from the acid-secreting parietal cells via the blood to the epithelial cells for transport to the mucus. When the stomach is not secreting but the lumen is still acidic, the accumulated nitrite in the loosely adherent mucous gel might compensate for the reduced protection provided by bicarbonate delivery. NO will be continuously produced and lead to increased blood flow levels.

We have shown in previous studies that the firmly adherent mucous layer increases in thickness after application of acidified nitrite or human nitrite-rich saliva to the mucosa, and under those circumstances our group (9) found a large NO production. Other studies have shown that NO donors increase gastric mucous secretion (10, 11). Preliminary results from our laboratory indicate that rats treated with NOS inhibitors and inducible NOS-deficient mice have a thinner firmly adherent mucous layer, also suggesting that NO is regulating mucous thickness.

To investigate whether dietary nitrate might protect the gastric mucosa against injury, a combination of intravenous diclofenac and luminal acidified taurocholate was given to challenge the gastric mucosa. Diclofenac has been shown to reduce gastric PGE_2 levels and blood flow and increase gastric damage (50), and the bile salt taurocholate has been shown to increase gastric mucosal permeability (7). This combination is clinically relevant, since diclofenac is a widely used NSAID and bile reflux into the stomach is common. In our study, this combination caused a great increase in mucosal permeability accompanied by an increase in mucosal blood flow. Both nitrate intake for 1 wk and luminal administration of acidified nitrite significantly reduced the permeability increase during the challenge. Furthermore, in the nitrate- and nitrite-treated animals, luminal clearance returned to control levels directly after the challenge, which was not seen in the control animals. These results suggest that the gastric mucosa increases its ability to resist gastric irritants when treated with high luminal NO. Further studies are needed to be able to answer the question of whether dietary nitrate can actually reduce ulcer formation after chronic treatment with diclofenac. So far, we have found that nitrate in the drinking water reduces ulcers induced by a bolus dose of diclofenac given by gavage 4 h before the experiment (unpublished observations).

Because NSAIDs are the most commonly prescribed drugs in the United States and the gastrointestinal complications are an enormous clinical and economic problem (2), considerable efforts have been made to try to reduce the side effects. The gastroprotective effects of different NO-donating NSAIDs have been attributed to release of NO and its effects in increasing gastric mucosal blood flow and protecting gastric epithelial cells from necrosis (18, 24). Organic nitrates and nitroglycerin have also been shown to decrease the gastrointestinal side effects of NSAIDs (30). Our results suggest that a nitrate-rich diet, resulting in accumulation of nitrite in the loosely adherent gastric mucus and substantial nonenzymatic NO formation, with increase in gastric mucosal blood flow, protects the gastric mucosa.

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