J. BILSKI, S. J. KONTUREK

ROLE OF NITRIC OXIDE IN GASTRODUODENAL ALKALINE SECRETION

Institute of Physiology, Jagiellonian University Medical School, Kraków, Poland

This study was designed to determine the involvement of nitric oxide (NO) in gastric and duodenal alkaline under basal conditions and in response to exogenous and endogenous stimulants in conscious dogs with Heidenhain pouches and duodenal loops. A topical application of HCl or capsaicin increased both gastric and duodenal alkaline secretion. A meat meal stimulated only duodenal alkaline secretion while gastric secretion was not significantly changed. The NO synthase inhibitor, N^G-nitro-L-arginine (L-NNA), significantly inhibited basal gastroduodenal alkaline secretion and almost completely suppressed the alkaline responses to food, acid or capsaicin. L-arginine given alone did not affect significantly basal or stimulated gastroduodenal alkaline secretion but when given together with L-NNA partially reversed the inhibitory effects of L-NNA on this secretion. For the comparison, the administration of indomethacin to suppress the generation of prostaglandin biosynthesis, also reduced basal and stimulated alkaline secretion but this reduction was relatively smaller than that attained by the inhibition of NO synthase with L-NNA. Luminal application of nocloprost, a stable prostaglandin E_2 analog, and glycerin trinitrate caused significant increase in both gastric and duodenal alkaline secretion but these responses were not affected by the administration of L-NNA or indomethacin. We conclude that endogenous NO together with prostaglandins plays a significant role in secretory alkaline response of gastroduodenal mucosa to acid, food and capsaicin.

K e y   w o r d s: *stomach, duodenum, nitric oxide*

INTRODUCTION

Endothelium-derived relaxing factor (EDRF) (1) characterized as nitric oxide (NO) (2, 3) is an unstable vasodilator secreted from the endothelial cells to act as a local hormone in the immediate vicinity of the cells which release it (4). NO is formed from L-arginine by NO synthase in endothelial cells (5).

A better understanding of the physiological action of NO comes from studies on the effects of agents which suppress the NO synthase and which prevent NO release such as L-arginine analogs, N-mono-methyl-L-arginine (L-NMMA) or N^G-nitro-L-arginine (L-NNA) (6, 7). Studies using these inhibitors of NO synthase have shown that NO plays an important role in the

control of systemic arterial blood pressure (8) by actively dilating the blood vessels. NO synthase has been described in a number of tissues including those of upper gastrointestinal tract (9, 10). In the stomach NO has been linked with the control of motility (11) and mucosal integrity and microcirculation (12, 13).

Bicarbonate secretion by the gastroduodenal mucosa has been considered to play an important role in the mucosal defense against acid (14, 15). Although recent studies have suggested that this secretion may be regulated by neural (16, 17) and hormonal factors (18) as well as endogenous prostaglandins (14, 19), the detailed mechanism has not been fully defined. It has been shown that the stimulation of sensory nerve by intragastric capsaicin prevents ethanol injury to gastric mucosa and augments mucosal blood flow, at least in part, by activation of NO-system (20, 21). Endogenous NO has been also implicated in the regulation of gastric mucosal microcirculation under secretory stimulation with pentagastrin (22). Since capsaicin was reported to stimulate mucosal bicarbonate secretion (23) and since alkaline secretion depends on the mucosal blood flow (24, 25), we investigated the possible role of NO in the control of gastric and duodenal bicarbonate secretion under basal conditions and following feeding, mucosal acidification, and administration of capsaicin, prostaglandin (PG) and glycerin trinitrate (GTN) as exogenous donor of NO in conscious dogs without or with pretreatment with NO synthase inhibitor (L-NNA).

MATERIAL AND METHODS

Six mongrel dogs, weighing 18—22 kg, were prepared surgically with a Gregory-type cannula in the vagally denervated Heidenhain pouches (HP) or with isolated duodenal loops (19). The isolated duodenal loops were 10 cm long, and were fashioned from the distal part of the duodenum (starting just below the entrance of the main pancreatic duct). Both ends of each loop were connected to the Herrera type cannula with the straight limb of the cannula inserted into proximal end (for the instillation of saline or test solutions) and the lateral limb placed in the distal end (for the collection of the effluent). Gastrointestinal continuity was restored by end-to-end duodeno-jejunal anastomosis. The operation was performed under aseptic conditions and anaesthesia induced by intravenous (i.v.) injection of 50 mg/kg pentobarbitone sodium (Polfa, Poland). A tracheal tube was inserted in each animal and a Harvard respirator was used to maintain intermittent positive pressure respiration when necessary. During recovery, animals hydration was ensured in first 2 days after surgery by the s.c. administration of 1 l of 0.15 M NaCl per day. Then, liquid meal was given for 1 week and then normal diet. Postoperatively, the dogs lost 2—4 kg of the body weight during the first week but then regained their normal weight during the subsequent 2—3 weeks. They were allowed to recover fully over a period of about 3 months and remained in excellent health throughout the examination period. Food, but not water, were withheld for at least 18 h before each test. In tests involving dogs with the HP, omeprazole was given i.v. in a dose of 10 mg/kg to completely suppress acid secretion. The cannula of each pouch was connected by rubber tube to the barostat to keep a constant pressure of about 5—10 cm H_2O in the pouch lumen as described previously (19). The pouch was bathed every 15 min with 20 ml of saline adjusted to pH 6.0 and applied alone or with test solutions. Duodenal loops were perfused,

at a rate of 20 ml/15 min with saline that was adjusted to pH 6.0 and given alone or with test substances. The volume of the fluid recovered every 15 min from the HP or duodenal loop was recorded to the nearest 0.1 ml and bicarbonate concentrations and outputs were determined by backtitrating the effluent samples to the initial pH level (6.0) and expressed in micromoles per 15 min as described previously (19).

Several tests were performed on each animal including; 1. Control tests with irrigation by saline at pH 6.0 throughout the 4 h study period; 2. Instillation of 100 mM HCl; 3. Feeding with a meat meal (ground beef, 500 g); 4. Capsaicin (topically in increasing concentrations 30—240 μg/ml) 5. GTN (topically in increasing concentrations (0.25—2 mg/ml), each concentration of capsaicin or GTN was applied at same constant volume into the HP or duodenal loops (20 ml/15 min); 6. Nocloprost (topically, 10 μg/ml). In all these tests, basal secretion was first collected for 60 min and then the administration of test substances or feeding was started. Intravenous infusion of saline (40 ml/h) was maintained throughout the study period. In control experiments, the animals received the test substances alone. The control experiments were randomized with those using L-NNA (5.0 mg/kg bolus i.v. injection followed by infusion of 1.0 mg/kg-h), L-arginine (50 mg/kg bolus i.v. injection followed by infusion of 5 mg/kg-h), or L-NNA plus L-arginine added in the same doses to i.v. infusion for 30 min before and during the application of the test substances or feeding. For the comparison, the experiments with saline perfusion, luminal perfusion wth HCl, meat feeding, capsaicin or GTN were repeated after pretreatment with indomethacin (5 mg/kg i.v.) administered 60 min before the experiment.

Results are expressed as means ± SEM. The statistical analysis was done using the non-parametric Mann-Whitney and Friedman two-way analysis of variance. Differences with P value of less than 0.05 were considered significant.

RESULTS

In control tests with saline alone, the HCO_3^- secretion from HP the or duodenal loops showed small fluctuations around the average values of about 9.1 ± 1.4 and 48.8 ± 5.3 μmol/15 min, respectively. Effects of L-NNA, L-arginine or their combination on basal gastric and duodenal HCO_3^- secretion are shown on *Fig. 1*. L-NNA decreased significantly basal HCO_3^- secretion both from gastric and duodenal mucosa. The addition of L-arginine alone to i.v. infusion tended to increase basal duodenal (but not gastric) HCO_3^- secretion but this did not reach statistical significance. The HCO_3^- response to the combination of L-NNA plus L-arginine was significantly higher than that to L-NNA alone.

Topical application of HCl directly to the mucosa of the HP duodenal loops for 15 min was followed by an immediate rise in HCO_3^- outputs from both gastric pouches and duodenal loops reaching peak in the first 30 min and then returning to basal value during subsequent 30 min period. Pretreatment of L-arginine alone tended to increase the HCO_3^- secretion induced by luminal HCl from the duodenal loops but not from gastric pouches but this increase was not statistically significant. The HCO_3^- secretory response to luminal acid was almost completely abolished when animals were given earlier L-NNA but when this pretreatment with L-NNA was combined with L-arginine, the
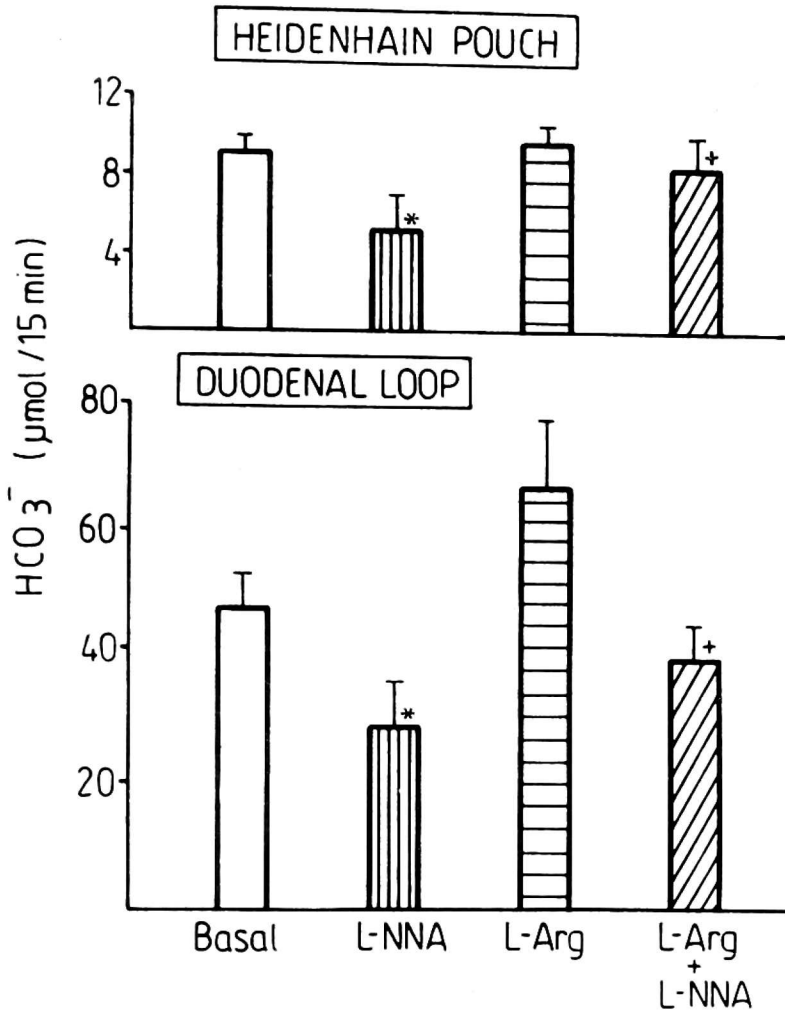
Fig. 1. Basal HCO_3^- outputs from the HP and duodenal loops in tests without and with administration of L-NNA, L-arginine or their combination. In this and subsequent figures, each column or line represents means $\pm$ SEM of 6 tests on 6 dogs. Asterisk indicates significant decrease below the value obtained in control tests without L-NNA and/or L-arginine administration. Cross indicates significant increase above the value obtained in tests with L-NNA administration.
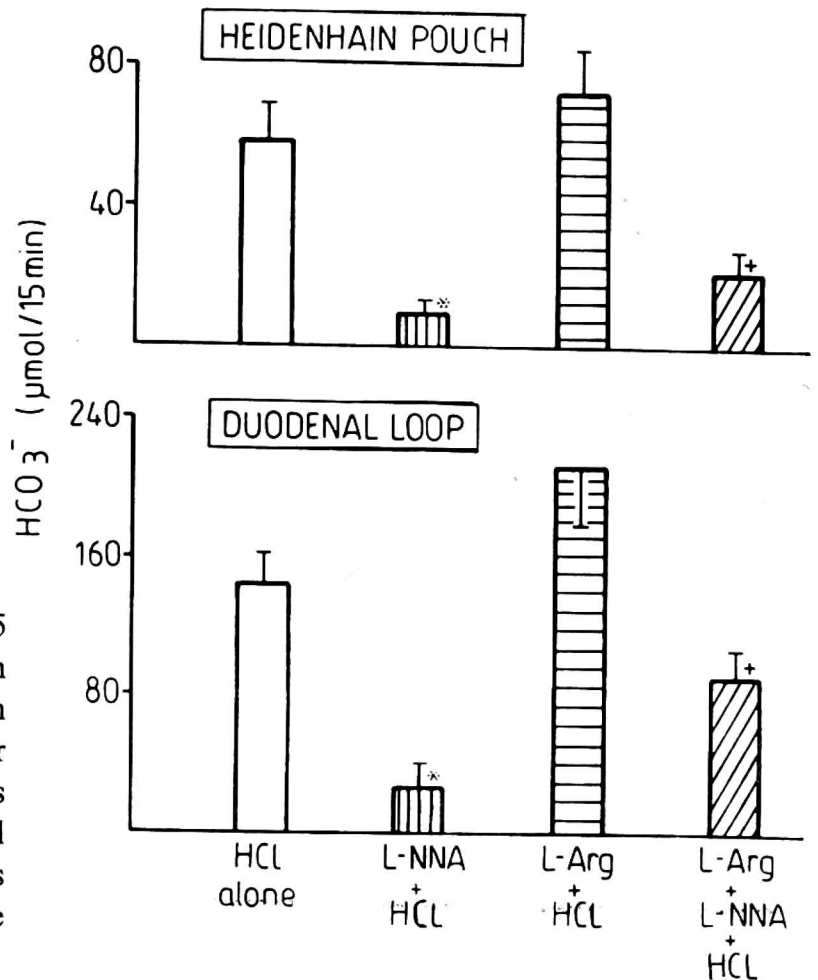
Fig. 2. Peak HCO_3^- outputs during 15 min period after mucosal perfusion with 100 mM HCl in tests without or with administration of L-NNA, L-arginine or their combination. Asterisk indicates significant decrease below the control value with HCl alone. Cross indicates significant increase above the value obtained in tests with L-NNA.

alkaline secretion was significantly higher than that obtained in experiments with L-NNA alone but lower than in tests with instillation of HCl alone (*Figs 2 & 3*).
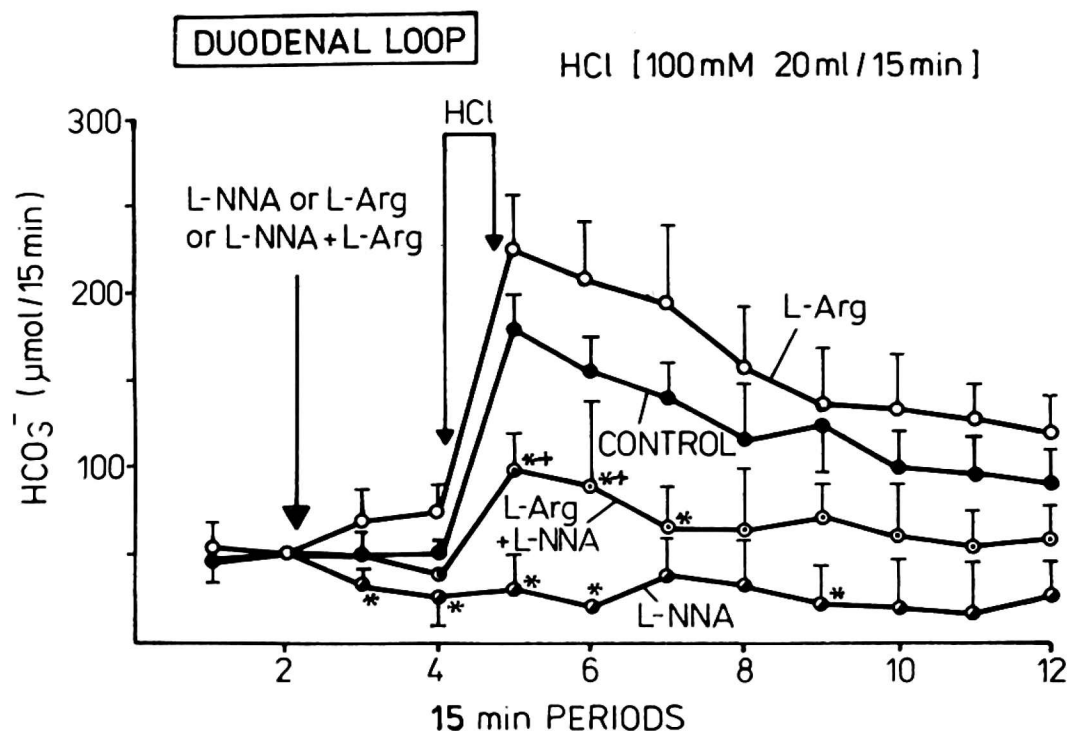

Fig. 3. The HCO_3^- outputs from the duodenal loop in response to the instillation of HCl alone (control) or HCl in combination with the administration of L-NNA, L-arginine or their combination. Asterisk indicates significant change as compared to the control value. Cross indicates significant increase above the value obtained with L-NNA.

Feeding caused only a small and not significant changes in alkaline secretion from the HP and these results are omitted for the sake of clarity. Feeding resulted in a marked rise in HCO_3^- secretion from the duodenal loops. The addition of L-arginine did not result in any significant change in HCO_3^- response as compared to feeding alone. Pretreatment with L-NNA prevented almost completely the HCO_3^- secretion for the entire period after feeding. Combination of L-arginine and L-NNA caused greater increase in HCO_3^- output after food than that observed in experiments with L-NNA alone but still much lower than that obtained with feeding alone (*Fig. 4*).

The effects of various concentrations of capsaicin on HCO_3^- outputs are shown on *Fig. 5*. The exposure of the gastric or duodenal mucosa to capsaicin for 15 min significantly and dose-dependently increased alkaline secretion. The lowest effective concentration was 60 μg/ml in the HP and 30 μg/ml in duodenal loop. Higher concentrations produced greater responses but at concentration 240 μg/ml the animals showed restlessness and some of them retched. Pretreatment with L-NNA almost completely abolished the alkaline response to capsaicin at all concentrations used both in the HP and duodenal loops. Addition of L-arginine to L-NNA partially reduced this inhibition while L-arginine alone was without any significant effect (*Fig. 6*).
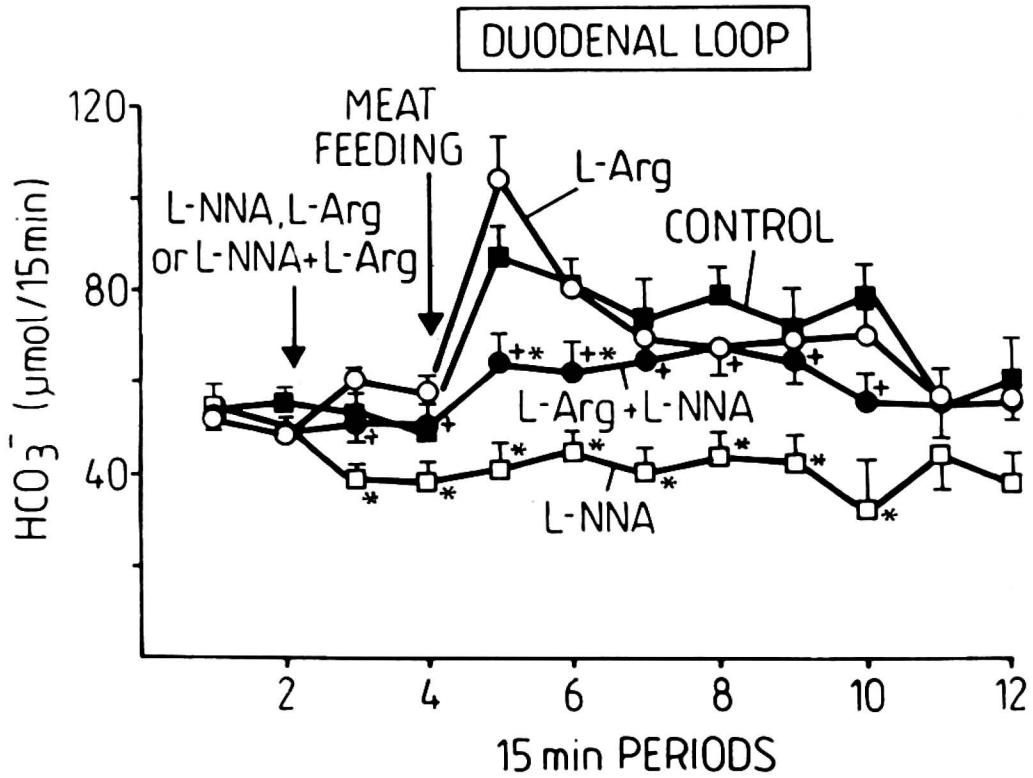
Fig. 4. The HCO_3^- outputs from the duodenal loop before and after meat feeding in tests without or with the administration of L-NNA, L-arginine or their combination. Asterisk indicates significamt decrease below the control value with feeding alone. Cross indicates significant increase above the values obtained in tests with L-NNA.
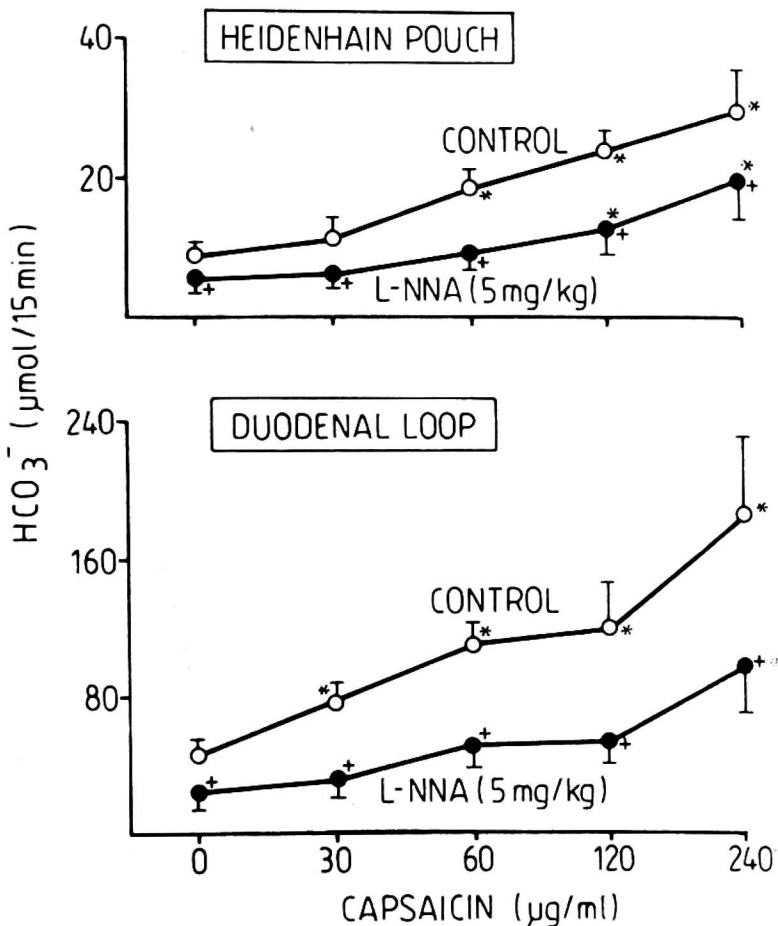

Fig. 5. The HCO_3^- outputs from the HPand duodenal loops in response to luminal application of capsaicin given in graded concentrations alone or in combination with i.v. administration of L-NNA. Asterisk indicates significant increase above the basal value. Cross indicates significant decrease below the value obtained in tests with capsaicin alone.

Fig. 6. The peak HCO_3^- outputs from the HP and duodenal loops in response to luminal perfusion with capsaicin alone or capsaicin combined the administration of L-NNA, L-arginine and L-NNA plus L-arginine. Asterisk indicates significant decrease below the control value the capsaicin alone. Cross indicates significant increase above the values obtained in tests with capsaicin plus L-NNA.
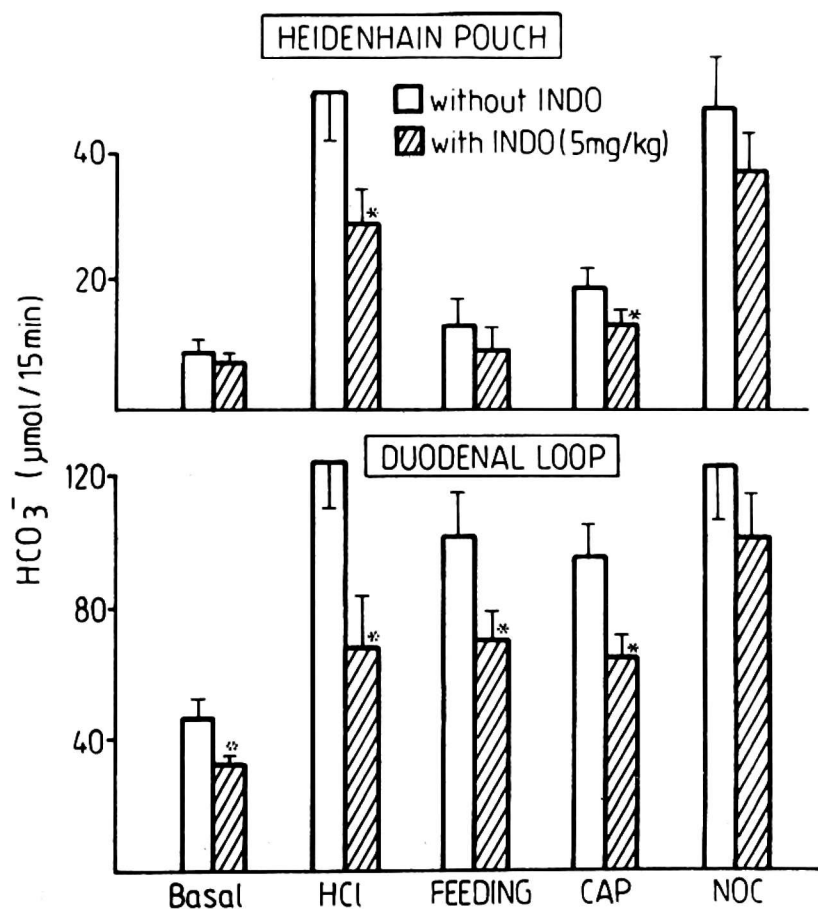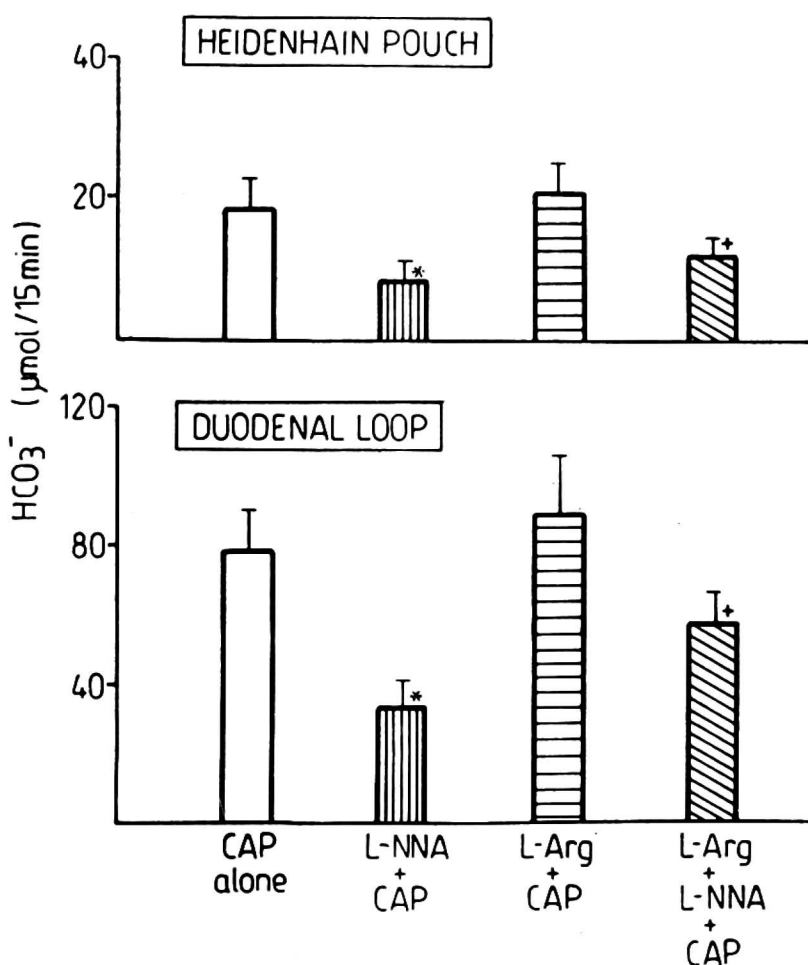

Fig. 7. The HCO_3^- outputs from the HP and duodenal loops in tests under basal conditions and in response to luminal perfusion of HCl, capsaicin and nocloprost or feeding in tests without or with the administration of indomethacin. Asterisk indicates significant decrease below the values obtained in tests without indomethacin.

Pretreatment with indomethacin (5 mg/kg i.v.) caused a significant reduction in basal and stimulated (by HCl instillation, feeding and capsaicin) alkaline secretion mainly from the duodenal loops (Fig. 7).

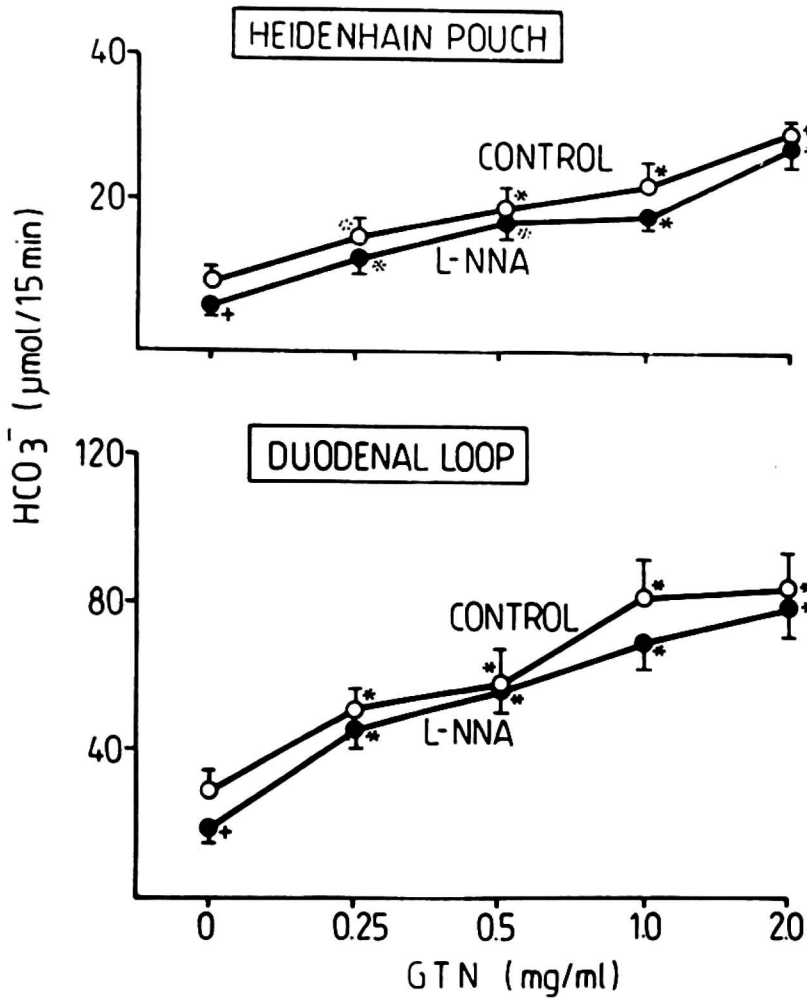Fig. 8. The HCO_3^- secretion from the HP and duodenal loop in response to luminal perfusion with GTN alone or GTN plus L-NNA. Asterisk indicates significant increase above the values. Cross indicates significant decrease below the value obtined in tests without L-NNA.
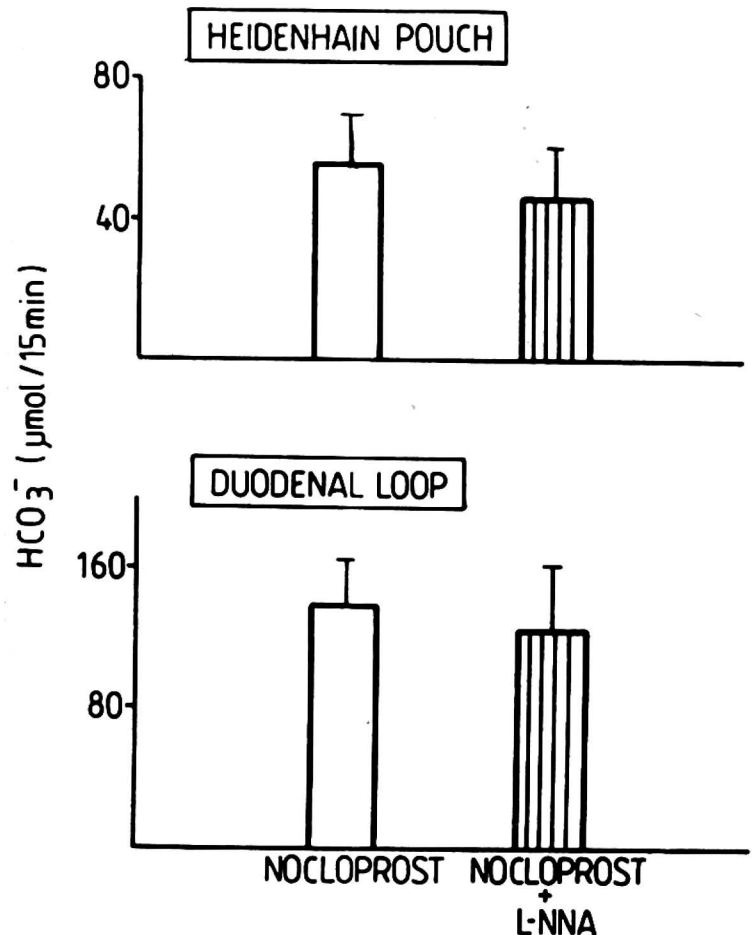

Fig. 9. The HCO_3^- secretion from the HP nd duodenal loop in response to topical application of nocloprost alone or nocloprost plus L-NNA.

GTN given topically in gradually increasing concentrations also caused a significant increase in gastric and duodenal HCO_3^- secretion, but the addition of L-NNA to i.v. infusion did not affect significantly these secretory responses (*Fig. 8*). Also the pretreatment with indomethacin (5 mg/kg i.v.) failed to affect the alkaline response to graded concentrations of GTN and these results have not been included.

Nocloprost applied topically at a concentration of 10 µg/kg increased the alkaline secretion to the value similar to that obtained with the irrigation by 100 mM HCl but the administration of L-NNA (*Fig. 9*) or indomethacin (see *Fig. 7*) failed to affect this secretion from the HP or duodenal loops.

DISCUSSION

This study provides an evidence that endogenous NO plays a significant role in alkaline secretory response of gastroduodenal mucosa to acid and food as well as to capsaicin. This alkaline secretion was most pronounced in the duodenum, and this secretory effect was markedly suppressed when NO sythase was inhibited by L-NNA.

Alkaline secretion by the gastroduodenal mucosa has been demonstrated to be an active process contributing to the mucosal defense against luminal acid. A variety of agents, particularly prostaglandins (PG) and cholinergic agonists (14) were shown to stimulate this secretion. The HCO_3^- response to acid was accompanied by a significant increase in the biosynthesis of mucosal PG and the pretreatment with indomethacin attenuated both alkaline secretion and PG release (15). This report confirms that the suppression of endogenous PG biosynthesis by indomethacin significantly reduces the alkaline response to mucosal acidification and feeding but also demonstrates that the alkaline response to capsaicin can be significantly diminished by the inhibition of mucosal PG. Other studies found that the alkaline response to acid can be partially suppressed by atropine and hexamethonium (16, 17). This indicates that a variety of neuro-hormonal factors may be implicated in the control of alkaline secretion (14).

Recent studies showed that the increase in mucosal HCO_3^- output induced by acid was also reduced by functional ablation of afferent neurons by chronic administration of capsaicin, suggesting that luminal acid alters HCO_3^- secretion through the excitation of the sensory nerves in the mucosa. This is supported by the observation that capsaicin applied to intact duodenal mucosa stimulated alkaline secretion in anesthetized rats (23). Our results confirm that also in conscious dogs capsaicin applied topically stimulates dose-dependently gastroduodenal alkaline secretion but also show for the first time that this stimulation can be completely eliminated by the inhibition of NO biosynthesis

suggesting the implication of NO in the mechanism of alkaline secretion. As the pretreatment with indomethacin, a potent inhibitor of PG biosynthesis (15) also reduced, in part, the alkaline response to capsaicin, it is obvious that NO and PG interact in the stimulation of alkaline secretion by this neurotoxin.

It is likely that topical application of HCl solutions acts on HCO_3^- producing mucosal cells through the stimulation of capsaicin-sensitive afferent neurons. This is supported by previous finding that alkaline response to duodenal mucosa to luminal acidification is attenuated by functional ablation of capsaicin-sensitive afferent nerves in rats (23). Also the increase in gastric blood flow upon the exposure of the mucosa to acid appears to be mediated by capsaicin-sensitive sensory nerves (20) because this vasodilation can be reduced by the pretreatment with the inhibitor of NO synthesis. In the gastrointestinal tract, the NO synthase has been detected in myenteric and submucosal plexus as well as in the gastric mucosa (26, 27). NO could be implicated in the vasodilatory action of several neuropeptides released from the nerve terminals by the action of capsaicin such as calcitonin gene-related peptide (CGRP) or vasoactive intestinal peptide (VIP) (28, 29). The presence of these neuropeptides was detected in the gastric (29) and duodenal mucosa (30). VIP has been shown to stimulate duodenal alkaline secretion (14), but CGRP was reported to exhibit rather an inhibitory effect in rats (31).

Further studies are needed to elucidate whether capsaicin stimulates the HCO_3^--producing cells in gastroduodenal mucosa directly by releasing the neuropeptides from nerve terminals which in turn release NO or whether it directly activates the NO synthase in directly activates the NO synthase in nerve terminals or endothelial cells. The fact that GTN, an exogenous donor of NO, is a potent stimulant of alkaline secretion and that this secretion is not influenced by NO synthase inhibitor, L-NNA, suggests that NO acts directly on HCO_3^--producing cells. Unlike capsaicin, whose HCO_3^--stimulatory activity can be reduced by indomethacin, the action of GTN on alkaline secretion remains unaffected by indomethacin indicating that NO released from GTN does not interact with endogenous prostaglandins in the control of alkaline secretion.

Our results are in contrast with observations reported by Takeuchi et al. (32). In their experiments in rats, NOS inhibitors have stimulated the duodenal alkaline secretion. These results suggested that it is neurally mediated action, dependent on intact vagus. In our experiments, animals have vagally denervated gastric pouches and duodenal loops. In later paper (33) the same authors have shown that NOS inhibitor decreases the alkaline gastric response to hypertonic NaCl solution.

It is of interest, that endogenous NO generated in the oxynic mucosa by secretory stimulants such as pentagastrin did not directly affect the acid secretion but that it made a substantial contribution to the mucosal

vasodilation that is associated with this secretion (22). This conclusion is supported by the finding that the inhibition of NO synthase by N-monomethyl-L-arginine significantly reduced pentagastrin-stimulated gastric mucosal blood flow but failed to affect basal or pentragastrin-induced gastric acid secretion. Thus, in the stomach NO has been implicated in the control of the mucosal blood flow associated with gastric acid secretion but not directly with gastric acid secretory process though a significant correlation was found between mucosal blood flow and gastric acid secretion in the rat stomach (34).

There is an evidence indicating that mucosal blood flow plays an important role in the mucosal alkaline/mucus secretion and mucosal defense. This is supported by the increased susceptibility of the mucosa to damaging agents following the reduction in gastric blood flow (35—37), and by increased mucosal protection against the damage when the blood flow in the mucosa was enhanced (38). Reduction in the mucosal blood flow was reported to play a more important role in duodenum than in the stomach as assessed by the increased susceptibility of the mucosa to acid-induced injury (35). Studies with anesthetized rabbits provided an evidence that mucosal vascular supply of HCO_3^- in the duodenum is a rate limiting factor for the mucosal alkaline secretion (24). Duodenal mucosal resistance to luminal acid can be enhanced by raising the blood flow o the mucosa or by elevating the blood concentration of HCO_3^-. The decrease in the duodenal mucosal blood flow by vasopressin or hemorrhagic shock caused a parallel decrease in alkaline secretion and mucosal blood flow (24, 24, 39). Further studies are needed to determine whether NO acts directly on the secretory cells or *via* increasing the blood flow. Our findings that GTN, which is a potent vasorelaxing agent, stimulates dose-dependently the gastroduodenal alkaline secretion and that capsaicin, which is also known to increase the mucosal blood flow is an effective stimulant of this secretion suggest that the vasodilation is a prerequisite of the enhancement of alkaline secretion by NO.

REFERENCES

1. Furchgott RF, Zawadzki JV. The obligatory role of endothelial cells in relaxating of arterial smooth muscle by acetylcholine. *Nature* 1980; 288: 373—376.
2. Palmer RMJ, Ferridge AG, Moncada S. Nitric oxide release accounts for the biological activity of endothelim-derived relaxing factor. *Nature* 1988; 327: 524—526.
3. Ingarro LJ, Buga GM, Wood KS, Byrns RE, Chaudhuri G. Endothelium-derived relaxing factor produced and released from artery and vein in nitric oxide. *Proc Natl Acad Aci USA* 1987; 84: 9265—9269.
4. Vane JR, Botting RM. Secretory functions of vascular endothelium. *J Physiol Pharmacol* 1992; 43: 195—208.

5. Palmer RMJ, Ashton DS, Moncada S. Vascular endothelial cells synthesize nitric oxide from L-arginine. *Nature* 1988; 333: 664—666.

6. Rees DD, Palmer RMJ, Hodson HF, Moncada S. A specific inhibitor of nitric oxide formation from L-arginine attenuates endothelium-dependent relaxation. *Br J Pharmacol* 1989; 96: 418—424.

7. Moore PK, al-Swayeh OA, Chong NWS, Evans RA, Gibson A. L-Ng/nitro-arginine (L-NOARG), a novel, L-arginine-reversible inhibitor of endothelium-dependent vasodilator in vitro. *Br J Pharmacol* 1990; 99: 408—412.

8. Moncada S, Palmer RMJ, Higgs EA. Biosynthesis of nitric oxide from L-arginine. A pathway for the regulation of cell function and communication. *Biochem Pharmacol* 1989; 38: 1709—1715.

9. Salter M, Knowles RG, Moncada S. Widespread tissue distribution and changes in activity of Ca-dependent and Ca-independent nitric oxide syntheses. *FEBS Letters* 1991; 291 (1): 145—190.

10. Whittle BJR. Nitric oxide and the gastrointestinal tract. In *Immunopharmacology of the Gastrointestinal System*, Wallace JL. (ed), Academic Press, London 1993, pp. 154—167.

11. Desai KM, Zembowicz A, Sessa WC. Vane JR. Nitroxergic nerves mediate vagally induced relaxation in the isolated stomach of the guinea pig. *Proc Natl Acad Sci USA* 1991; 88 (24): 1490—11494.

12. Whittle BJR, Lopez-Belmonte J, Moncada S. Regulation of gastric mucosal integrity by endogenous nitric oxide: interactions with prostanoids and sensory neuropeptides in the rat. *Br J Pharmacol* 1990; 99: 607—611.

13. Lopez-Belmonte J, Whittle BJR, Moncada S. The action of nitric oxide donors in the prevention or induction of injury to the rat gastric mucosa. *Br J Pharmaol* 1993; 108: 73—78.

14. Flemstrom G. Gastric and duodenal mucosal bicarbonte secretion. In *Physiology of Gastrointestinal Tract*, Johnson LR, Christensen J, Jackson MJ, Walsh JR (eds), Raven Press, New York 1987 pp. 1011—1029.

15. Konturek SJ, Bilski J, Tasler J, Konturek J, Bielanski W, Kaminska A. Role of endogenous prostaglandins in duodenal alkaline response to luminal hydrochloric acid or arachidonic acid in conscious dogs. *Digestion* 1986; 34: 268—274.

16. Konturek SJ, Bilski T, Tasler J, Thor P, Cieszkowski M. Cephalic phase of gastroduodenal alkaline secretion. *Am J Physiol* 1987; 252: G742—G747.

17. Smedfors B, Johansson C. Cholinergic influence on duodenal bicarbonate secretion to hydrochloric acid perfusion in the conscious rats. *Scand J Gastroenterol* 1986; 21: 809—815.

18. Konturek SJ, Bilski J, Tasler, Laskiewicz J. Gut hormones in the stimulation of gastroduodenal alkaline secretion in conscious dogs. *Am J Physiol* 1985; 248: G687—G691.

19. Konturek SJ, Tasler J, Bilski J, Kania J. Prostaglandins and alkaline secretion from oxyntic antral and duodenal mucosa of the dog. *Am J Physiol* 1983; 245: G539—G546.

20. Holzer P, Livingston EH, Guth PH. Secretory neurons signal from an increase in rat gastric mucosal blood flow in the face of pending acid injury. *Gastroenterology* 1991; 101: 416—423.

21. Pique JM, Whittle BJR, Esplugues JV. The vasodilatator role of endogenous nitric oxide in the rat microcirculation. *Eur J Pharmacol* 1989; 174: 293—296.

22. Pique JM, Esplugues JV, Whittle BJR. Endogenous nitric oxide as a mediator of gastric mucosa vasodilation during acid secretion. *Gastroenterology* 1992; 102: 168—174.

23. Takeuchi K, Matsumoto J, Ueshima K, Okabe S. Role of capsaicin-sensitive afferent neurons in alkaline secretory response to luminal acid in the rat duodenum. *Gastroenterology* 1991; 101: 954—961.

24. Schiessel R, Starlinger M, Kovats E, Appel W, Feil W, Simon A. Alkaline secretion of rabbit duodenum in vivo: its dependance on acid base balance and mucosal blood flow. In

Mechanisms of Mucosal Protection in the Upper Gastrointestinal Tract, Allen A, Flemstrom G, Garner A, Silsen W, Turnberg LA (eds) Raven Press, New York 1983, pp. 267—272.

25. Schiessel R, Wenzl E, Feil W, Starlinger M. Role of alkaline secretion for protection of duodenal mucosa against luminal acid. *Digestion* 1985; 31: 163—164.

26. Forster ER, Southam E. Location of nitric oxide synthase in rat gastric corpus. *Regul Pept* 1992; 40: 146.

27. Furness JB, Young HM, Li ZS, McConalogue K. Nitric oxide synthase in the enteric nervous system. *Regul Pept* 1992; 40: 151.

28. Holzer P. Local effector functions of capsaicin-sensitive sensory nerve endings: involvement of tachykinins, calcitonin gene-related peptide and other neuropeptides. *Neuroscience* 1988; 24: 739—769.

29. Holzer P, Guth PH. Neuropeptide control of rat gastric mucosal blood flow. Increase by calcitonin gene-related peptide and vasoactive intestinal polypeptide, but nor substance P and neurokinin A. *Circ Res* 1991; 68: 100—105.

30. Feher E, Fodor M. Immunocystochemical demonstration of different neuropeptide-containing nerve elements in the duodenum of cat. *Dig Dis Sci* 1990; 35: 1017.

31. Lenz HJ, Forquignon I, Druge G, Greten H. Effects of neuropeptides on gastric acid and duodenal bicarbonate secretions in freely moving rats. *Reg Pept* 1989; 24: 293—300.

32. Takeuchi K, Ohuchi T, Miyake H, Sugawara H, Okabe S. Effects of nitric oxide synthase inhibitors on gastric alkaline secretion in rats. *Jpn J Pharmacol* 1991; 60: 303—305.

33. Takeuchi K, Ohuchi T, Okabe S. Endogenous nitric oxide in gastric alkaline response in the rat stomach after damage. *Gastroenterology* 1994; 106: 367—374.

34. Pique JM, Leung FW, Tau HW, Livingston E, Scremin OV, Guth PH. Gastric acid blood flow response to stimulation and inhibition of gastric acid secretion. *Gastroenterology* 1988; 95: 642—650.

35. Mersereau WA, Hinchey EJ. Effect of gastric acidity on ulceration induced by hemorrhage in the rat, utilizing a gastric chamber technique. *Gastroenterology* 1973; 64: 1130—1135.

36. Leung FW, Itoh M, Hirabayashi K, Guth PH. Role of blood flow in gastric and duodenal mucosal injury in the rat. *Gastroenterology* 1985; 88: 281—289.

37. Cheung LY, Chang N. The role of gastric mucosal blood flow and H^+ back diffusion in the pathogenesis of acute gastric erosions. *J Surg Res* 1977; 28: 357—359.

38. McGreevy JM, Moody FG. Protection of gastric mucosa against aspirin-induced erosions by enhanced blood flow. *Surgical Forum* 1977; 28: 357—359.

39. Jonsson C, Fandriks L. Bleeding decreases duodenal HCO_3^- secretion by a nervous mechanisms. *Acta Physiol Scand* 1986; 127: 273—274.

Author's address: S. J. Konturek, Institute of Physiology Jagiellonian University, Medical School, ul. Grzegórzecka 16, 31-531 Krakow, Poland