JOURNAL OF PHYSIOLOGY AND PHARMACOLOGY 1993, 44, 1, 31—42

M. MĄCZKA, P. THOR, K. LORENS, S. J. KONTUREK

NITRIC OXIDE INHIBITS THE MYOELECTRIC ACTIVITY OF THE SMALL INTESTINE IN DOGS

Institute of Physiology, University Medical School, Krakow, Poland

Intestinal motility in fasted animals shows cylic changes (MMC) that are interrupted by feeding. The aim of this study was to determine the possible implication of nitric oxide (NO) (that was proposed as nonadrenergic noncholinergic neurotransmitter) in the motor components of MMC in 5 conscious dogs equipped with monopolar electrodes implanted along the small bowel. In fasted dogs with typical MMCs, L-NNA (an inhibitor of NO synthase) (5 mg/kg-h i. v.) decreased the MMC interval from control 80 $\pm$ 7 to 60 $\pm$ 4 min and increased significantly the spike activity. Infusion of L-arginine (L-Arg) (a substrate of NO synthase) (10 mg/kg-h i. v.) increased the MMC interval from control 79 $\pm$ 7 to 96 $\pm$ 8 min and reduced the slow waves with spikes by about 25%. Similar but transient effects were observed when glycerin trinitrate (GTN) (a donor of NO) (1 mg/kg-h) was administered. After ingestion of meal, the MMC cycles were replaced by irregular spike activity with an average of about 35% slow waves with spikes. Infusion of L-Arg (10 mg/kg-h) reduced by about 90% the postprandial spike activity. Also, infusion of GTN (1 mg/kg-h) strongly reduced the postprandial spike activity. L-NNA in fed dogs caused an initial increase in spike activity followed by phase III-like activity. Similar effects were obtained when L-NNA was infused in dogs with fed-like motility patterns induced by i. v. infusion of caerulein (75 pmol/kg-h). L-NNA added to L-Arg infusion reversed in part the changes of intestinal motility patterns induced by L-Arg. We conclude that NO system exerts a tonic inhibitory influence on intestinal myoelectric activity by reducing the frequency of MMC pacesetter and by suppressing the postprandial spike activity.

Key words: *intestinal motility, nitric oxide, MMC, L-arginine.*

INTRODUCTION

It is well established that the fasting digestive tract in dogs and other species is governed by a regular rhythm (migrating motor complex, MMC) that modulates its motor pattern and secretory activity (1). Boldyreff (2) was first who recognized the interdigestive cycles of synchronous intestinal motor and secretory activities but the mechanisms of these cyclic changes were not fully elucidated. Motilin given in physiological dose induced motility patterns similar to those observed during phase III (3, 4) but it is not clear whether the

increment in plasma motilin is the cause or just a result of phase III contractile activity of the duodenum (5).

Ordinary feeding interrupts both the motor complex and secretory MMC cycle for several hours (6). These changes are accompanied by significant increments in plasma gastrin, cholecystokinin (CCK), secretin and PP but none of these hormones alone in physiological doses is capable to reproduce the postprandial inhibition of MMC cycles (4).

Recently, an evidence was provided that nitric oxide (NO) acts as an inhibitory noncholinergic nonadrenergic (NANC) neurotransmitter in the esophagus (7), the stomach (8), the gut (9). Furthermore, the release of NO-like factor in response to stimulation of NANC nerves was demonstrated (10). Since NANC nerves have been implicated in the intestinal motor activity, we decided to investigate the possible implication of NO in the control of myoelectric activity of the small bowel in fasted and fed conscious dogs with monopolar elctrodes placed along the small intestine.

MATERIAL AND METHODS

The studies were carried out on five mongrel dogs that weighted 15—18 kg and which were prepared surgically with intestinal electrodes and gastric fistula (GF) as described previously (4). Briefly, eight monopolar silver electrodes were implanted at 30 cm intervals along the entire small intestine to record the myoelectric activity of the gut as described previously (6). The recorded electrical changes represented potential differences between individual electrodes and the reference electrode, a coil of silver wire placed subcutaneously. Recordings were made with a type R-611 Beckman recorder starting about 1 mo after surgery. The animals remained in good health throughout the period of study. In all tests, except these with feeding the GF was opened and gastric juice was discarded.

All experiments were carried out in the morning, about 18 h after the dogs last ate; the interval between tests was at least 4 days.

In testes conducted under basal conditions at least two control MMC cycles were first recorded. Intravenous (i. v.) infusion of saline was given at a rate of 40 m/h from the start of all experiments and during recording of the control MMC cycles. After about 2 h of examination under basal conditions, N^G-nitro-L-arginine (L-NNA), an arginine analogue that antagonizes NO synthase (11), L-arginine, a substrate for NO synthase (12, 13), or their combination was infused i. v. for about two hours. In separate experiments glycerin trinitrate (GTN) was injected i. v. or infused in a dose range of 0.5—1.0 mg/kg-h in fasted or fed dogs and the myoelectric activity was recorded for about 2 h.

In tests with feeding, each dog was offered about 500 g of cooked homogenized ground beef that was usually completely consumed. Fed-like motility patterns were also induced by i. v. infusion of caerulein (Farmitalia, Milan, Italy) in a dose of 75 pmol/kg-h. When the myoelectric activity and secretory responses to a meal reached a well sustained plateau, L-NNA, L-arginine or their combination, or GTN was added in a constant dose to i. v. infusion for about 1 h.

In some experiments carried out under basal conditions and after meat feeding, blood samples were taken from the peripheral vein at 15—30 min intervals for the determination of plasma gastrin, CCK, PP, insulin and glucagon as presented previously (4).

Results are expressed as means ± SEM. In tests that compared various stimulants with and without L-NNA, L-arginine, L-NNA plus L-arginine or GTN, the changes in myoelectric activity or plasma hormone concentrations were calculated and averaged to provide myoelectric and hormonal changes for the experimental period. The significance of these differences between means was evaluated by analysis of variance followed by Student's t test. Differences were considered significant if $P<0.05$.

RESULTS

Effect of L-NNA, L-Arg and GTN on fasted pattern of myoelectric activity

In fasted dogs, four typical phases of the MMC were defined in the small intestine. L-NNA infused i. v. (5 mg/kg-h) did not affect the priodic alterations in myoelectric activity of the intestine but caused significant shortening of the MMC interval from control value of about 80 ± 7 min to about 60 ± 4 min mainly due to the reduction in timing of phase I and II (*Fig. 1*).

Table 1. Effects of glycerin trinitrate (GTN) infused i. v. at a dose of 0.5 or 1 mg/kg-h on myoelectric activity of the small intestine as determined in duodenum, midjejunum and ileum and on pancreatic protein secretion in fasted and fed dogs. Means ± SEM of 5 tests on 5 dogs. Asterisk indicates significant difference ($P<0.05$) as compared to saline control.

Type of test	MMC interval (min)	% slow waves with spikes		
		duodenum	jejunum	ileum
Fasted dogs				
CONTROL (SALINE)	82±8	14±3	14±4	13±4
GTN 0.5 mg/kg-h	112±15*	9±4	11±3	9±3
GTN 1.0 „ „ „	132±24*	7±2*	8±2*	8±3*
CAERULEIN (CONTROL)	Interrupted	30±4	33±5	32±8
CAERULEIN+GTN 0.5 mg/kg-h	Interrupted	15±5*	17±7*	21±4*
CAERULEIN+GTN 1.0 „ „ „	Interrupted	12±4*	14±4*	18±4*
Fed dogs				
CONTROL (SALINE)	Interrupted	35±8	36±9	32±8
GTN 0.5 mg/kg-h	Interrupted	17±5*	19±4*	18±6*
GTN 1.0 „ „ „	Interrupted	13±5*	15±4*	16±4*

In contrast, infusion of L-Arg (10 mg/kg-h) in fasted dogs caused a significant prolongation of the MMC interval (to about 96 ± 8 min) (*Fig. 2*). When the infusion of L-Arg was combined with L-NNA, the first phase III

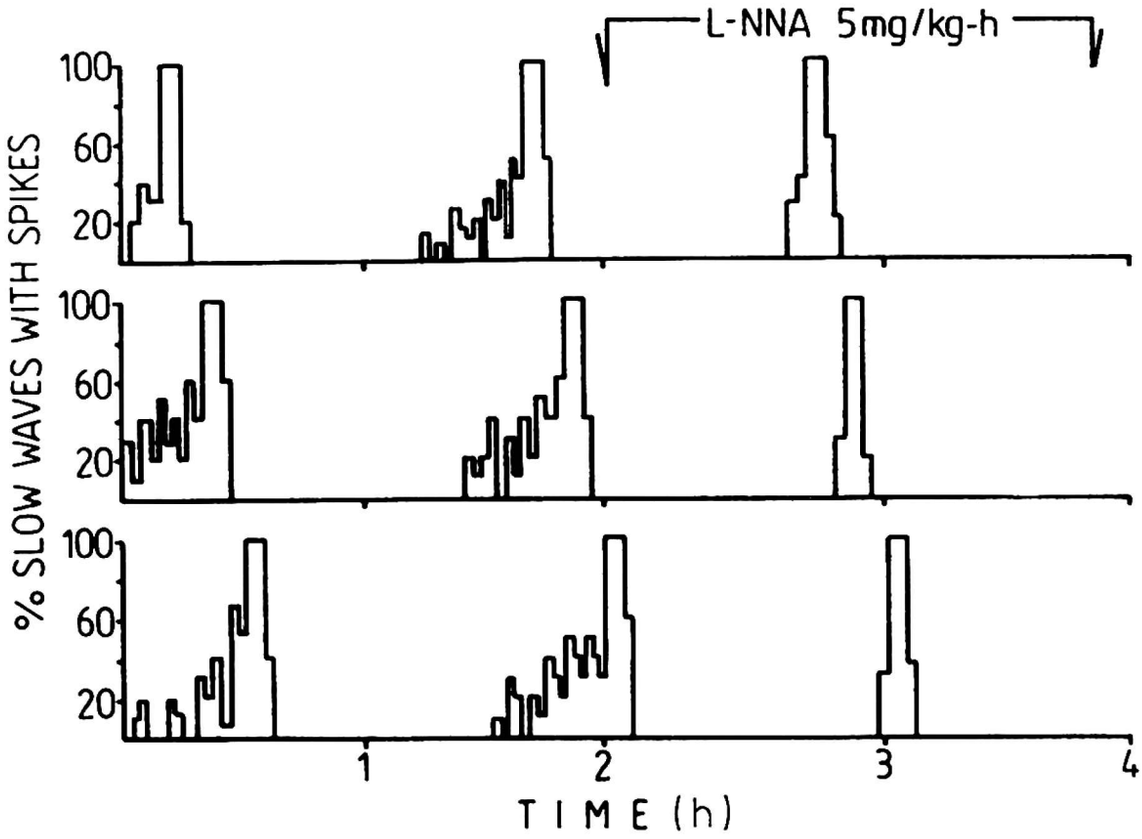

Fig. 1. Effect of i. v. infusion of L-NNA (5 mg/kg-h) on myoelectric activity of small intestine in fasted dogs. Similar results were obtained in other dogs.

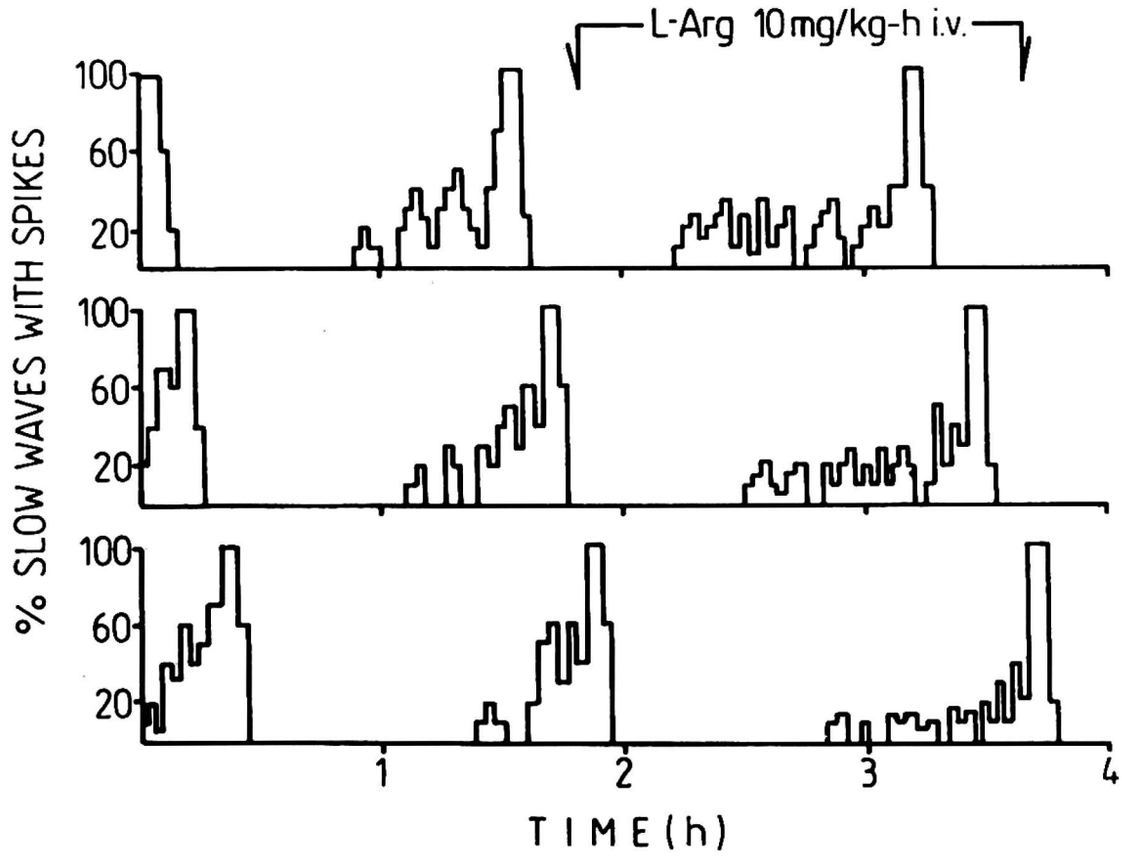

Fig. 2. MMC rate and percent of slow waves spikes in fasted and fed dogs without and with i. v. infusion of L-arginine, L-NNA or their combination. Means ± SEM of 5 tests on 5 dogs.

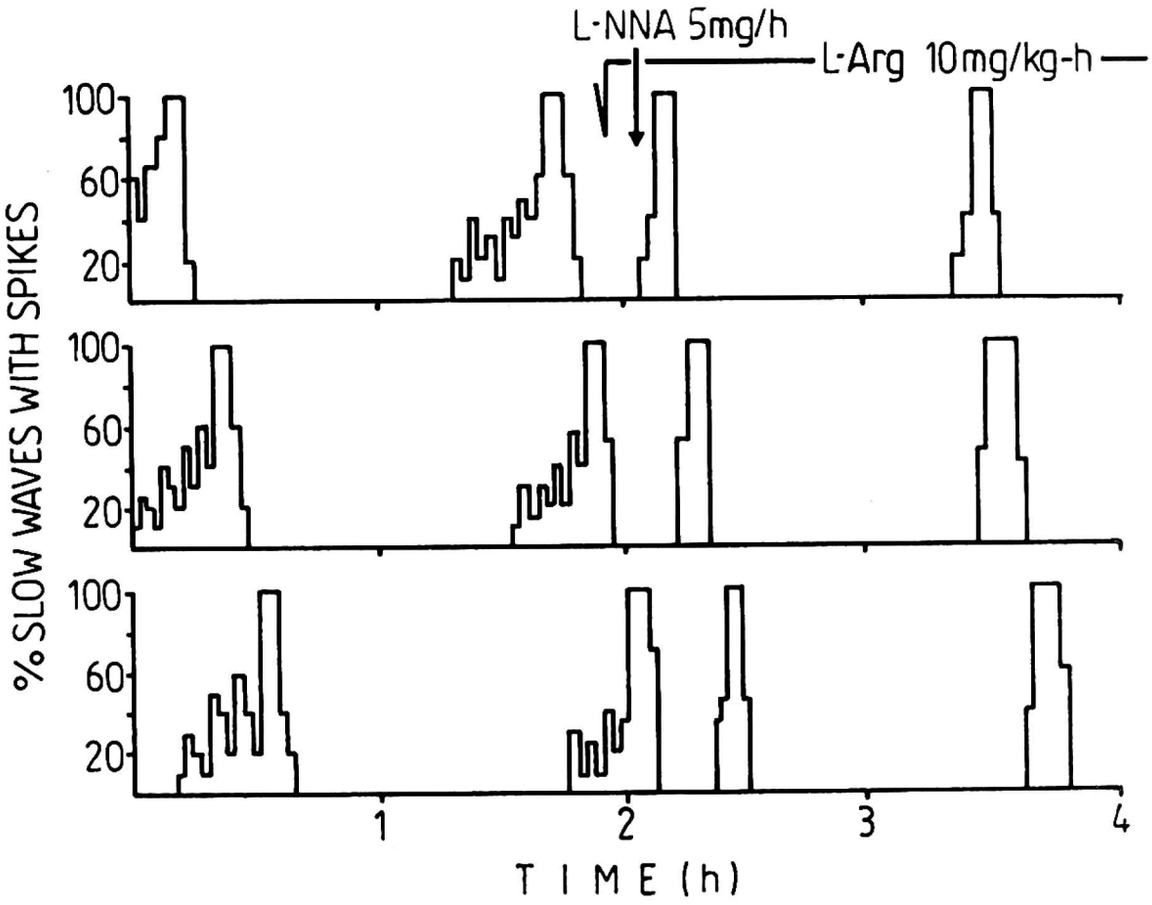

Fig. 3. Effect of i. v. infusion of L-arginine (10 mg/kg-h) on the myoelectric activity of the small bowel. Similar results were obtained in other dogs.

appeared in duodenum almost immediately after injection of L-NNA and the second phase III occurred after about 60 min later (*Fig. 3*). Neither of these phases III were preceded by an increased myoelectric activity (phase II) of the small bowel (*Fig. 4*).

GTN infused i. v. (0.5—1 mg/kg-h) caused a marked delay in the occurrence of MMC and subsequently reduced the spike activity in fasted dogs. The inhibition of spike activity was dose-dependent and appeared shortly after commencing the infusion of GTN.

Effects of L-NNA, L-arginine, L-NNA + L-arginine and GTN on fed patterns of myoelectric activity.

Feeding of a meat meal interrupted the MMC cycle and resulted in a prolong period of low level of irregularly occurring spike potentials (about 35% of slow waves with spikes) over the entire small intestine. Infusion of L-NNA (5 mg/kg-h) in fed dogs resulted in an initial increase of slow waves with spikes reaching 80—100% followed by phase III-like activity that occurred in duodenum and proceeded along the jejunum and ileum (*Fig. 5*). L-Arg

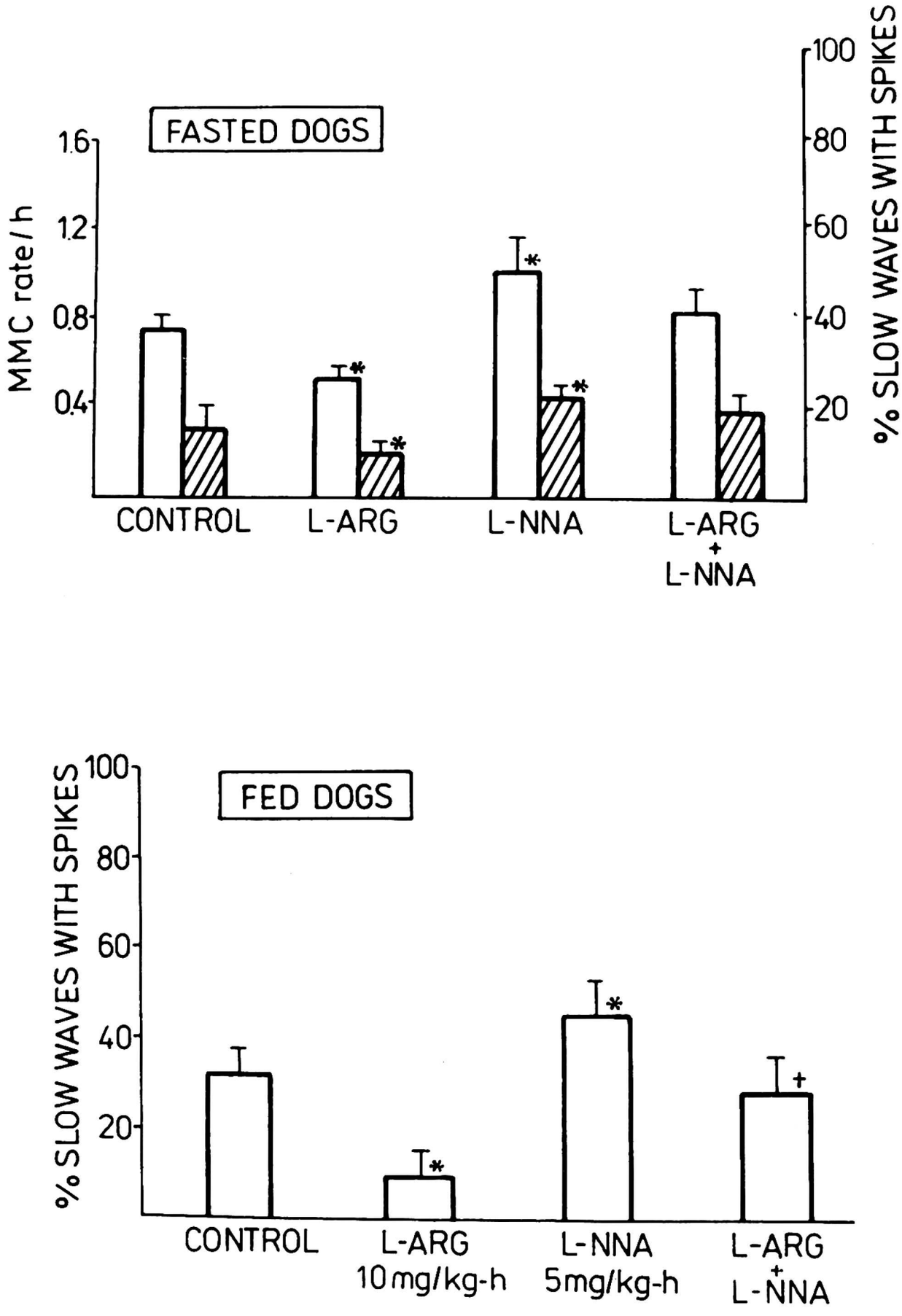

Fig. 4. Effect of i.v. infusion of L-arginine (10 mg/kg-h) combined with injection of L-NNA (5 mg/kg) on myoelectric activity of the small intestine in fasted dogs. Similar results were obtained in other dogs. Asterisk indicates significant ($P<0.05$) change as compared to the values obtained in control tests. Cross indicates significant change as compared to the value obtained with L-NNA alone.

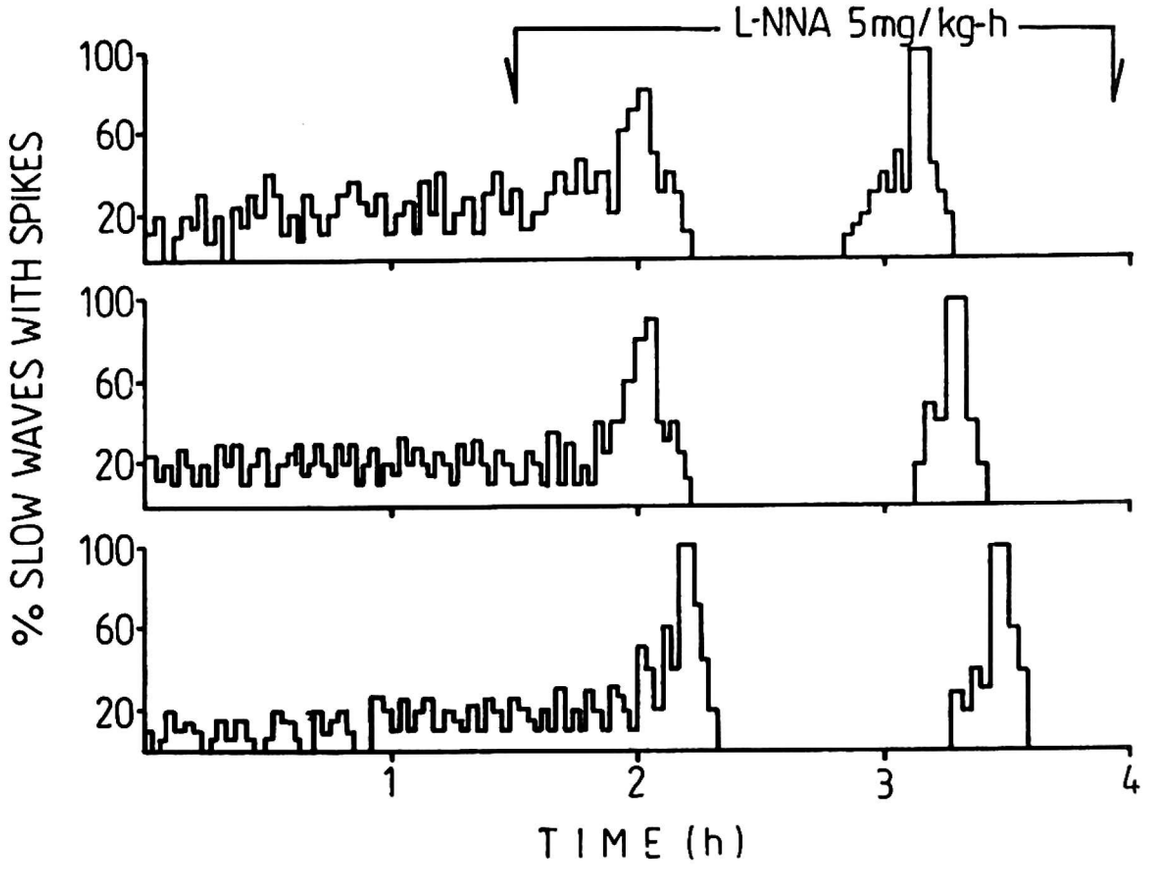

Fig. 5. The myoelectric activity of the small intestine in fed dogs before and during i. v. infusion of L-NNA (5 mg/kg-h). Similar results were obtained in other dogs.

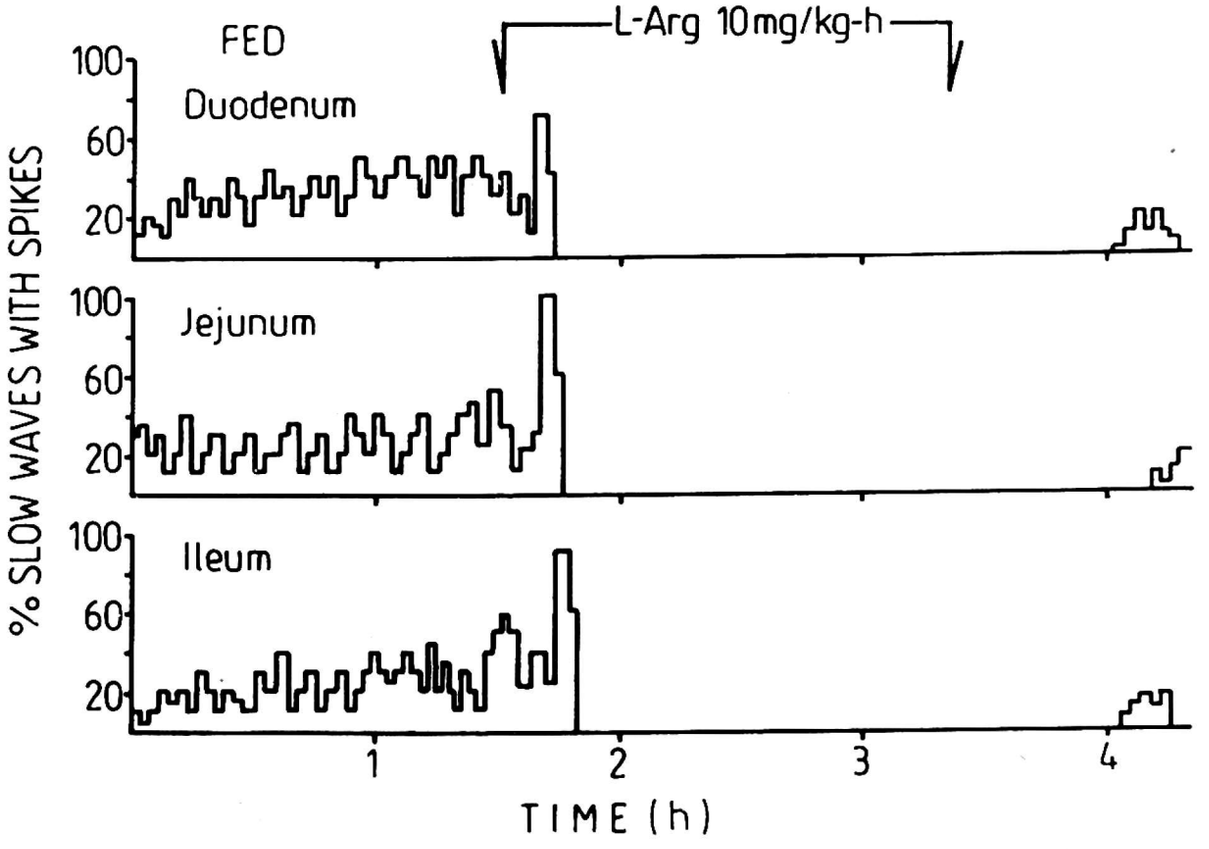

Fig. 6. Effect of i. v. infusion of L-arginine (10 mg/kg-h) on the postprandial myoelectric activity in fed dogs. Similar results were obtained in other dogs.

(10 mg/kg-h) infused i. v. almost completely abolished the postprandial spike activity for the period of infusion (*Fig. 6*). The addition of L-NNA to L-Arg infusion resulted in a significant increase in the intestinal spike activity with the occurrence of phase III migrating along the small bowel (*Fig. 7*).

Administration of GTN (0.5—1 mg/kg-h i. v.) caused almost complete suppression of the fed-like activity for about 30 min but then typical low rate postprandial spike activity was observed despite the administration of GTN.

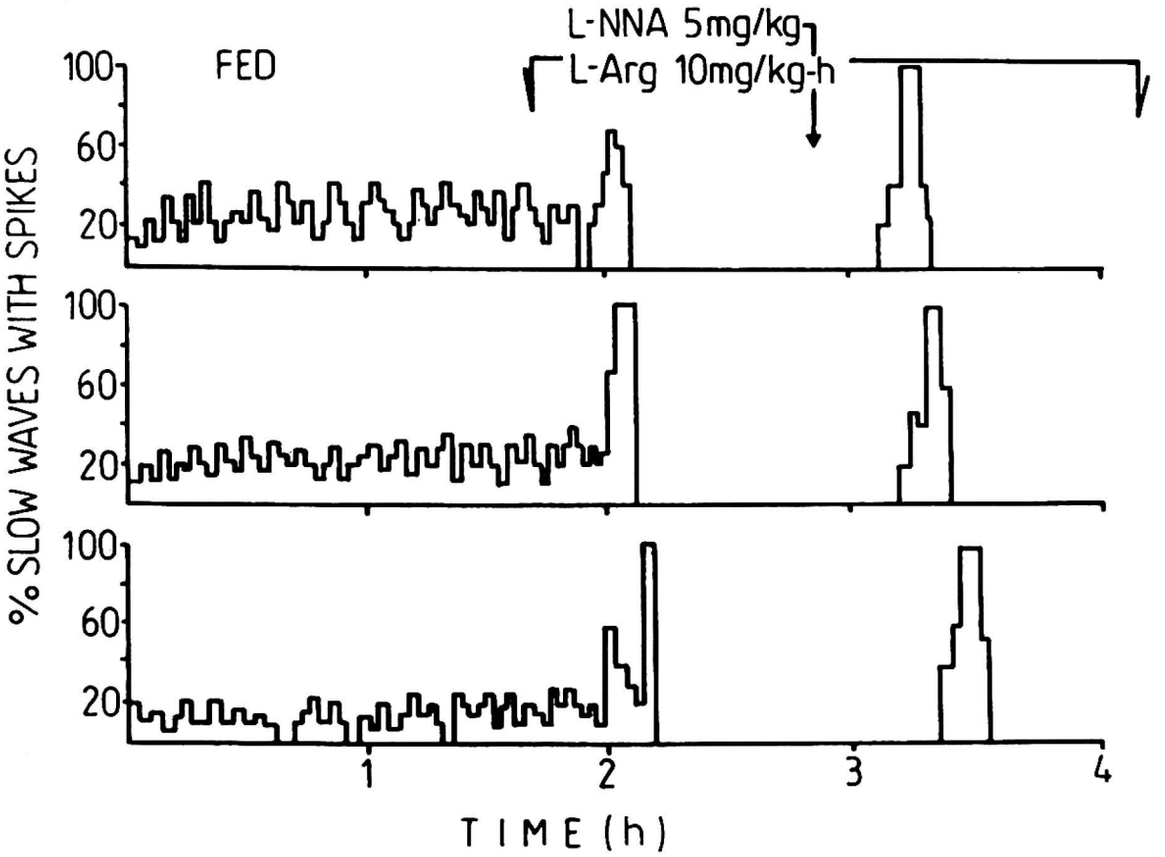

Fig. 7. Effect of i. v. infusion of L-arginine (10 mg/kg-h) plus L-NNA (5 mg/kg) on myoelectric activity of the small intestine in fed dogs. Similar results were obtained in other dogs.

Effects of L-NNA, L-arginine and L-arginine plus L-NNA on plasma gastrin, CCK, PP, insulin and glucagon levels.

The mean values of basal plasma concentrations of gastrin, CCK, PP, insulin and glucagon are shown on *Table 2*. Meat feeding resulted in significant increments in plasma gastrin, CCK, PP, insulin and glucagon. These increments were significantly reduced by the administration of L-NNA alone but not when L-NNA was combined with L-Arg.

Table 2. Effects of L-NNA (5 mg/kg), L-arginine (10 mg/kg-h) or their combination on the postprandial plasma gastrin, CCK, PP, insulin and glucagon levels in tests as on Figs 1 and 2. Mean ± SEM of 5 tests on 5 dogs. Asterisk indicates significant ($P<0.05$) change as compared to the control values obtained in fasted or fed dogs. Cross indicates significant increase as compared to the values obtained in these animals with L-NNA alone.

Type of test	GASTRIN (pM/L)	CCK (pM/L)	PP (pM/L)	INSULIN (uU/ml)	GLU-CAGON (pM/L)
BASAL	26±4	1.2±0.4	28±6	6.4±1.2	34±5
L-NNA	20±3	1.0±0.3	19±3*	3.5±0.7*	24±5*
L-ARG	24±6	1.5±0.4	21±3	11.2±1.3^{+}	42±6^{+}
L-ARG+L-NNA	26±5	1.6±0.4	27±4	7.2±1.8	28±4
FEEDING (CONTROL)	61±9	4.7±1.3	84±9	28.7±3.6	77±10
FEEDING+L-NNA	38±6*	2.6±0.7*	42±6*	21.1±2.0*	59±8*
FEEDING+L-ARG	58±8	5.1±0.5	95±12	34.6±4.2	94±5
FEEDING+L-ARG+ +L-NNA	52±6^{+}	3.6±0.5^{+}	72±10^{+}	23.1±4.4	62±8

DISCUSSION

The results of this study demonstrate that endogenous production of NO is an important pathway that exerts a potent inhibitory effect on intestinal motility patterns both in the interdigestive and postprandial periods. Our dogs equipped with monopolar electrodes along the duodenum, jejunum and ileum showed typical priodicity in myoelectric activity of the small bowel that were first described over 80 years ago by Boldyreff (2) and reconfirmed more recently by numerous investigators (1). The mechanism underlying this interesting phenomenon remains obscure. Both autonomic neural system and gut hormones, especially motilin, have been implicated in the mechanism responsible for generation and progression of MMC and accompanying secretory changes.

NO has been originally considered as an unstable vasodilator secreted from the endothelial cells to act as a local hormone in the immediate vicinity of these cells (12—14). Then, Bult et al. (10) using a superfusion bioassay cascade demonstrated that NO-like factor may be released upon stimulation of the NANC nerves. A better understanding of the physiological action of NO derives from the studies on the effect of substances which suppress the NO system and prevent the release of NO such as L-arginine analogues, N^{G}-monomethyl-L-arginine (L-NMMA) or N^{G}-nitro-L-arginine (L-NNA) (11). Studies using these inhibitors of NO system showed that NO plays an important role in the control of systemic arterial blood pressure by actively dilating the blood vessels (14). The effects of endogenously generated NO have

been mimicked by the use of agents such as GTN which spontaneously release NO and which serve as „donors" of NO (14). Studies in animals revealed that the inhibition of NO synthase by L-NNA antagonizes NANC nerve-mediated relaxation of lower esophageal sphincter and reduces the latency of the contraction in the caudad esophagus (7). These data provided a support for the hypothesis that NO is a transmitter of NANC nerve-mediated responses of circular muscle of esophagus and its sphincter. Similar studies using the rat gastric fundus showed that NO is formed and released upon NANC nerve stimulation to induce relaxation mimicked by exogenously applied NO (GTN) (8). NO has been also implicated in the reflex relaxation of the stomach to accommodate food and fluid (15). The presence of an inhibitory NANC innervation that involves the release of NO was also found in canine ileum and ileo-colonic junction (9). No study so far has been published, however, supporting the role of NO in the control of intestinal motility pattern in conscious animals.

As the major part of the enteric innervation of gut is provided by NANC nerves (16), we assumed that also MMC cycles may involve the release and action of NO. Indeed, the suppression of NO synthase by L-NNA in fasted dogs accelerated the occurrence of successive MMC cycles. This indicates that NO affects the frequency of pacesetter MMC and is required for the progression of phase III along the small bowel. The implication of endogenous NO in the transit of phase III MMC along the gut has also been confirmed using both L-Arg, an endogenous substrate for NO synthesis (12), and GTN, an exogenous donor of NO (14). Infusion of L-Arg in fasted dogs significantly delayed the occurrence of consecutive phase III MMC and reduced significantly the percent of slow waves with spikes. Following administration of GTN, similar prolongation of the MMC interval and the decrease in spike activity were observed to those obtained with L-Arg infusion.

Feeding immediately interrupted the cyclic motor patterns of the gut. Infusion of L-Arg caused a remarkable reduction in the postprandial spike activity in the small bowel similar to that observed with GTN, a donor of NO. Moreover, L-NNA injected during the infusion of L-Arg enhanced the spike activity and initiated phase III in the dog duodenum. Thus, the described changes of the intestinal myoelectric activity induced by activation or suppression of NO system suggest that the action of NO on the intestinal smooth muscle is inhibitory in nature and it could account for the reduction in the frequency of MMC pacesetter potential and for the suppression of the postprandial spike activity. This is in keeping with the previous proposal that NO plays a role as inhibitory neurotransmitter released by NANC nerves in the muscle layer of the gut (8, 9).

Administration of L-NNA caused a significant reduction in the plasma levels of the several hormones such as gastrin, CCK, insulin, glucagon and PP.

Since L-NNA-induced reduction in plasma hormones in fed dogs was reversed by the addition of L-Arg, it is likely that NO exerts its action on intestinal motility through its action on the release of gut hormones. It is not clear, however, whether the changes in plasma hormones caused by L-NNA and/or L-Arg could contribute to the observed alteration in the postprandial spike activity induced by these agents. The fact that L-NNA and L-Arg infused during the fed-like activity patterns induced by caerulein showed similar directional changes to those observed postprandially militates against the major role of endogenously released hormones in the action of endogenous NO on the intestinal motility. It is likely that changes in plasma hormone levels after the administration of L-NNA are secondary to the reduction in the intestinal blood flow and ischemia of the intestinal mucosa due to elimination of the tonic vasorelaxing action of endogenous NO (14). Further studies are needed to elucidate whether or not the changes in plasma gut hormones caused by L-NNA and/or L-Arg contribute to the action on NO on the intestinal motility. In the stomach, NO synthesized from L-arginine, was also found to enhance mucosal vasodilation associated with the stimulation of gastric acid secretory but failed to affect the secretory activity of oxyntic glands (19).

REFERENCES

1. Weisbrodt NW. Motility of the small intestine. In Physiology of the Gastrointestinal Tract (2nd ed), LR Johnson (ed). New York, Raven, 1987, p. 631—663.
2. Boldyreff W. Einige neue Seiten der Tatigkeit des Pankreas. *Ergebn Physiol* 1911; 11: 121—217.
3. Lee KY, Chang TM, Chey WY. Effect of rabbit antimotilin serum on myoelectric activity and plasma motilin concentration in fasting dog. *Am J Physiol* 1983; 245: G547—G553.
4. Konturek SJ, Thor P, Bilski J, Bielanski W, Laskiewicz J. Relationship between duodenal motility and pancreatic secretion in fasted and fed dogs. *Am J Physiol* 1986; 250: G570—574.
5. Sarna S, Chey WY, Chey Y et al. Cause and effect relationship between motilin and migrating myoelectric complexes. *Am J Physiol* 1983; 245: G277—G285.
6. Weisbrodt NW, Copeland EM, Thor PJ. The myoelectric activity of the small intestine of the dog during total parenteral nutrition. *Proc Exp Biol Med* 1976; 153: 121—124.
7. Murray JC, Du A, Ledlow JN, Bates J, Conklin L. Nitric oxide: mediator of nonadrenergic noncholinergic responses of opossum esophageal muscle. *Am J Physiol* 1991; 261: G401—406.
8. Boeckxstaens GE, Pelckmans PA, Bogers JJ et al. Release in nitric oxide upon stimulation of nonadrenergic noncholinergic nerves in the rat gastric fundus. *J Pharmacol Exp Therap* 1991; 256: 441—447.
9. Boeckxstaens GE, Pelckmans PA, Bult H, Man JDG, Herman AG, Maercke YMV. Evidence for nitric oxide as mediator of non-adrenergic non-cholinergic relaxations induced by ATP and GABA in the canine gut. *Br J Pharmacol* 1991; 102: 434—438.
10. Bult H, Boekxstaens GE, Pelckmans PA, Jordaens FH, Maercke YMV, Herman AG. Nitric oxide as an inhibitory non-adrenergic non-cholinergic neurotransmitter. *Nature (Lond)* 1990; 345: 346—347.

11. Mulsch A, Busse R. N^G-nitro-L-arginine (N^5-[imino(nitroamino)methyl]-L-ornithine) impairs endothelium-dependent dilations by inhibiting cytosolic nitric oxide synthesis from L-arginine. *Naunyn-Schmiedeberg's Arch Pharmacol* 1990; 341: 143—147.
12. Palmer RMJ, Ashton DS, Moncada S. Vascular endothelial cells synthesize nitric oxide from L-arginine. *Nature (Lond)* 1988; 333: 664—666.
13. Palmer RMJ, Rees DD, Ashton DS, Moncada S. L-arginine as the physiological precursor from the formation of nitric oxide in endothelium-dependent relaxation. *Biochem Biophys Res Commun* 1988; 153: 1251—1256.
14. Vane JR, Botting RM. Secretory functions of the vascular endothelium. *J Physiol Pharmacol* 1992; 43: 195—207.
15. Desai KM, Sessa WC, Vane JR. Involvement of nitric oxide in the reflex relaxation of the stomach to accommodate food and fluid. *Nature (Lond)* 1991; 351: 477—479.
16. Abrahamsson H. Non-adrenergic non-cholinergic nervous control of gastrointestinal motility patterns. *Arch Int Pharmacodyn Ther* 1986; 28: (Suppl) 50—61.
16. Pique JM, Esplugues JV, Whittle BJR. Endogenous nitric oxide as a mediator of gastric mucosal vasodilation during acid secretion. *Gastroenterology* 1992; 102: 168—174.
17. Konturek SJ, Bilski J, Pawlik WW, Czarnobilski K, Gustaw P, Sendur R. Release of nitric oxide upon vagal and meal-induced stimulation of exocrine pancreatic secretion in dogs. *Digestion* 1992; 52: 97.

R e c e i v e d : November 7, 1992
A c c e p t e d : December 22, 1992
Author's address: Prof. Dr S. J. Konturek Institute of Physiology, University School of Medicine, 31-531 Krakow, ul. Grzegorzecka 16, Poland