JOURNAL OF PHYSIOLOGY AND PHARMACOLOGY 1993, 44, 2, 139—146

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MICROCIRCULATORY AND MOTOR EFFECTS OF ENDOGENOUS NITRIC OXIDE IN THE RAT GUT

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The aim of the study was to determine the role of endogenous nitric oxide (NO) as the mediator of intestinal blood flow and motility. Experiments were performed on anesthetized rats. Blood flow in the jejunum was determined by Laser-Doppler flowmeter. Motility was monitored on the basis of changes in intrajejunal pressure. Systemic arterial pressure was also recorded. To investigate the potential role of nitric oxide in the regulation of basal intestinal blood flow and motility the NO synthase inhibitor N^G-nitro-L-arginine (L-NNA) was given systemically. Intravenous bolus of L-NNA (15 mg/kg) reduced basal intestinal blood flow and increased both intestinal motility and arterial pressure in the dose-dependent manner. To test the specificity of the NO synthase blockade we administered L-arginine alone or in combination with L-NNA. Pretreatment with L-arginine (100.0 mg/kg i.v.) alone had no major influence but when combined with L-NNA it reversed the intestinal circulatory and motor effects of L-NNA. The resuts of these studies suggest that endogenous NO exerts a tonic relaxatory influence on the smooth muscle of the intestinal vessels and intestinal wall.

Key words: *nitric oxide, intestinal circulation, intestinal motility*

INTRODUCTION

The endogenous nitric oxide (NO) constitutes an important biological control system orginating from L-arginine via the specific synthase activity. This compound is synthetized from the terminal guanidyne nitrogen atom of L-arginine in vascular endothelial and smooth muscle cells (1, 2) and in neurones (3). Its synthesis is affected by various neural and humoral factors. Recent work indicates that purinergic and peptidergic system involving NO may mediate nonadrenergic and noncholinergic (NANC) neural inhibition of smooth muscle cels in the gut (4-6).

NO is potent vasodilator in general circulation and in gastrointestinal vasculature (6-9). This molecule was also shown to have marked inhibitory

effects on the gastrointestinal smooth muscle (6, 10), and on gastric and pancreatic secretion (11-13). The major physiological concerns in the intestinal circulation include microcirculatory events, regulatory processes and the relationship between intestinal blood flow, motility and absorption. Several factors such as neurogenic, metabolic, myogenic, gastrointestinal peptides, tachykinins and neuropeptides have been considered to play an important role in these processes (14-16). Recent experimental evidence suggests that vascular and motor changes induced in the gastrointestinal tract under the influence of some of the above mentioned factors may be be mediated by NO (6). We have previously reported that in anesthetized animals endogenous NO is potent mediator of basal and stimulated gastrointestinal and pancreatic blood flow and secretory activity in these organs (11-13). On the basis of the findings that NO is potent relaxant of the gastrointestinal vasculature (6-9) and musculature (6, 15, 17) of the intestinal wall, we undertook the present study to evaluate the role of endogenous NO in the control of intestinal microcirculatory blood flow and motility in the rat gut.

MATERIAL AND METHODS

Experiments were performed on 35 male Wistar rats weighting 230-420 g. Animals were fasted, but were allowed access to water for 24 h before the experiments. Animals were anesthetized with intraperitoneal injections of 50 mg/kg pentobarbital sodium, intubated and ventilated with room air using a positive pressure respirator. Body temperature was maintained at 37°C by warming each animal with a heating pad monitored by a rectal thermistor and regulator. Mean systemic arterial pressure (AP) was monitored via saline filled catheter inserted into the right carotid artery and connected to a strain-gauge transducer (Statham, P231 D, Iowa, USA). The right jugular vein was cannulated for incjection of drugs and supplemental anesthetic as needed. A midline laparatomy was performed to expose the jejunum. A fiberoptic probe of a laser-Doppler flowmeter (Laser Flo BPM 403 Prefusion Monitor, TSI) was positioned againts the surface of the jejunal serosa along the antimesenteric border. The flow probe was secured outside the animal to prevent any movement of the tip of the probe and ensure continuous optical coupling between the tip of the probe and the jejunal wall. Microcirculatory intestinal blood flow (LDF) measurement were recorded in voltages. The change in LDF was calculated in terms of percentage of control. The intraluminal pressure (IP) of a jejunal segment was measured with a saline-filled open tip polyvinyl catheter inserted into the lumen and connected to pressure transducer. Continuous recordings of AP, LDF and IP were made on the polygraph (Sensor Medics Dynograph, Model R 611). From the tracing of intraluminal pressure, mean motility index (MMI) was calculated (18). Both ends of the jejunum were surgically isolated from the remainder of the small intestine. After the surgical preparation was completed hemodynamic and motility parameters were allowed to stabilize for 30 min before initiating one of the five experimental protocols. In each protocol, a group of at least seven rats was studied. All data are presented as means $\pm$ 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 rats animals were anesthetized and studied, as described above, to quantify the LDF, AP and MMI responses during basal conditions without or with NO synthase blockade

using N^G-nitro-L-arginine (L-NNA) (Sigma Chemical Co., St. Louis, MO). The drug was dissolved freshly in isotonic saline and given intravenously as bolus in a dose of 15 mg/kg.

In group II rats the LDF, AP and MMI were studied before and after pretreatment with L-arginine (Sigma Chemical Co.) plus L-NNA. L-arginine was injected i.v. in a dose of 100 mg/kg and 15 minutes later L-NNA was administered as in group I.

In group III rats the LDF, MMI and AP responses were studied under basal conditions and after stimulation of jejunal motility with erythromycin (Polfa, Poland), which was injected i.v. at a dose of 30 mg/kg.

In group IV rats, intestinal circulatory and motor responses due to i.v. erythromycin were studied after pretreatment of the animals with L-NNA alone as in group I.

In the last group V the animals were first pretreated with combination of L-arginine plus L-NNA as in group II, then 5 min. later received erythromycin as in group and III.

RESULTS

In the five experimental groups, the mean basal LDF was 3.0 ± 0.2 V under control conditions. The mean basal AP range was 115-122 mmHg. In group I intravenous injection of L-NNA decreased LDF by $27.0 \pm 5.0\%$ and increased AP by $36.0 \pm 9.0\%$, and MMI by $62.0 \pm 8.0\%$, respectively (*Figs. 1, 2, 3*).

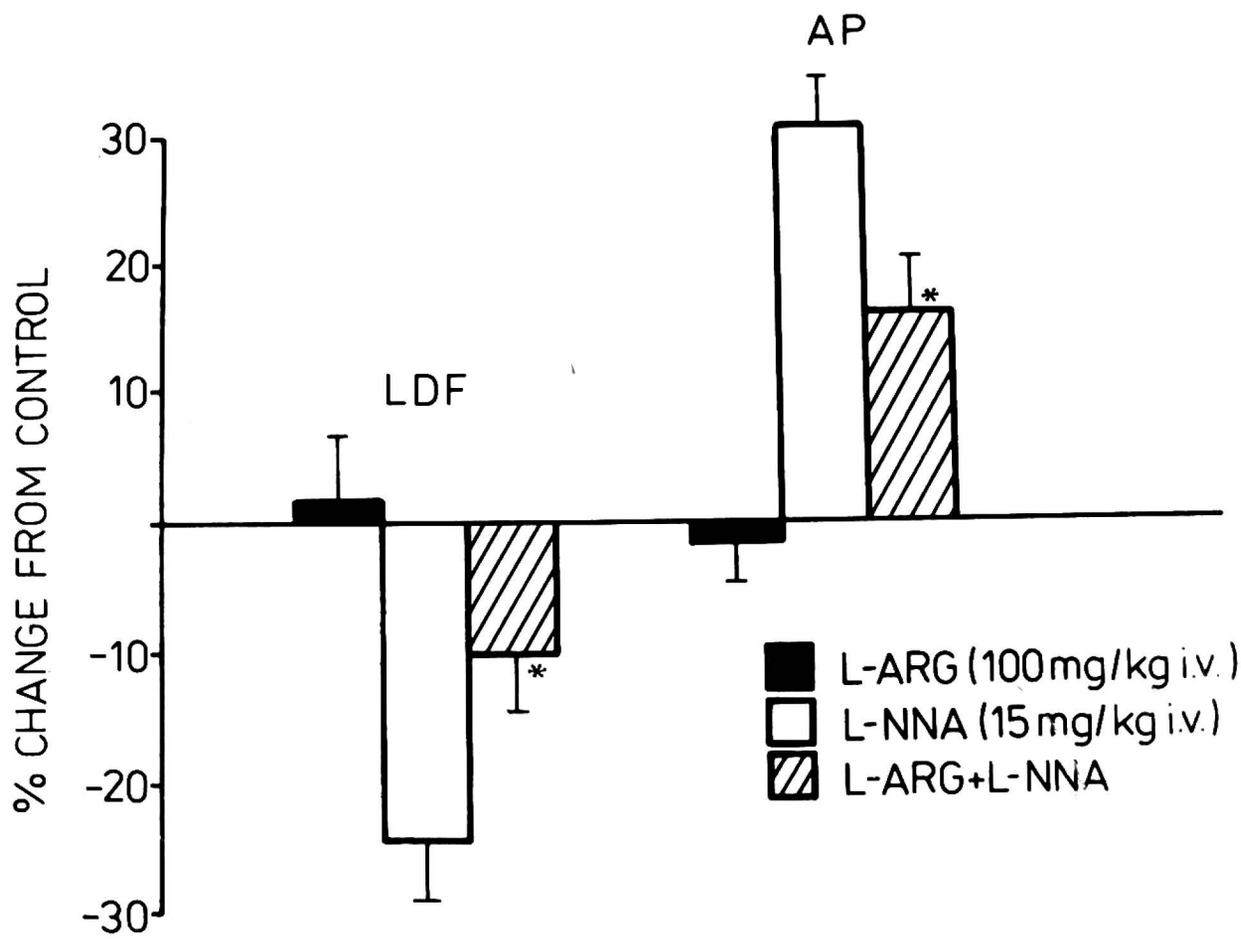

Fig. 1. Effects of L-arginine, L-NNA, and L-arginine + L-NNA on microcirculatory intestinal blood flow (LDF) and systemic arterial pressure (AP). Single asterisk indicate significant ($p < 0.05$) change in comparison with L-NNA alone.

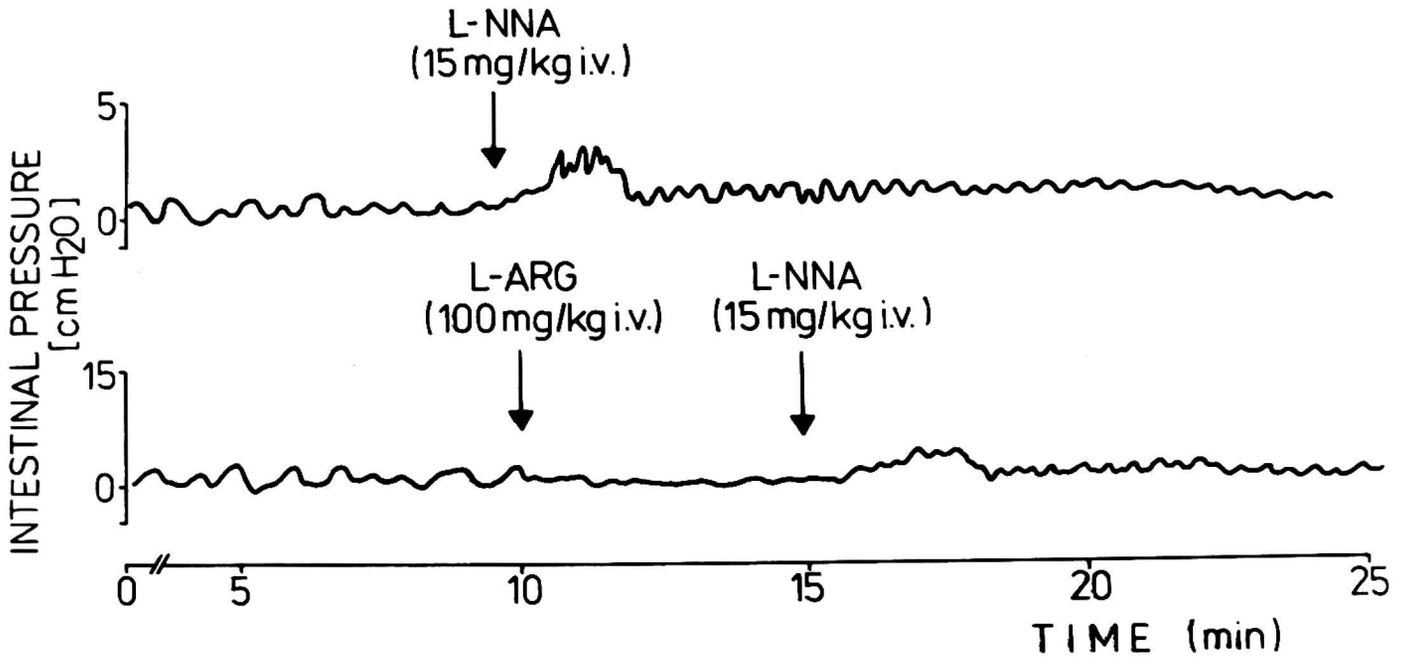

Fig. 2. Effects of L-NNA alone, L-arginine alone and L-arginine + L-NNA on intestinal pressure in two separate experiments.

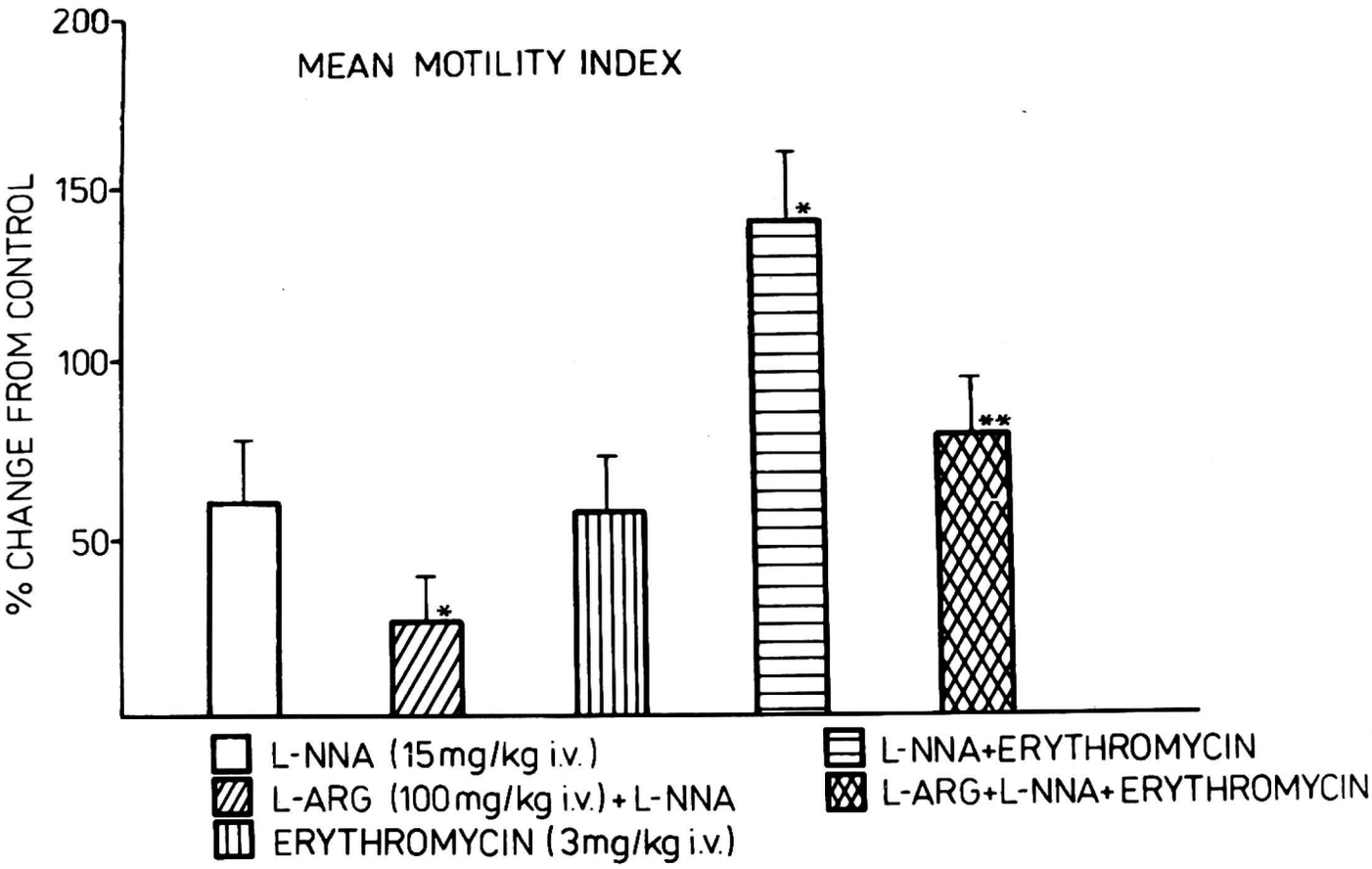

Fig. 3. Effects of L-NNA alone, L-arginine + L-NNA, erytromycin, L-NNA + erytromycin and combination of L-arginine + L-NNA + erytromycin on intestinal mean motility index (MMI). Single asterisk indicates significant ($p<0.05$) decrease below of L-NNA alone and significant increase above erytromycin alone. Double asterisks indicate significant decrease below L-NNA + erytromycin.

In group II pretreatment of animals with L-arginine alone was without any affect on the resting values of LDF and AP but tended to decrease basal jejunal IP. However the last parameter did not change significantly (*Fig. 2*).

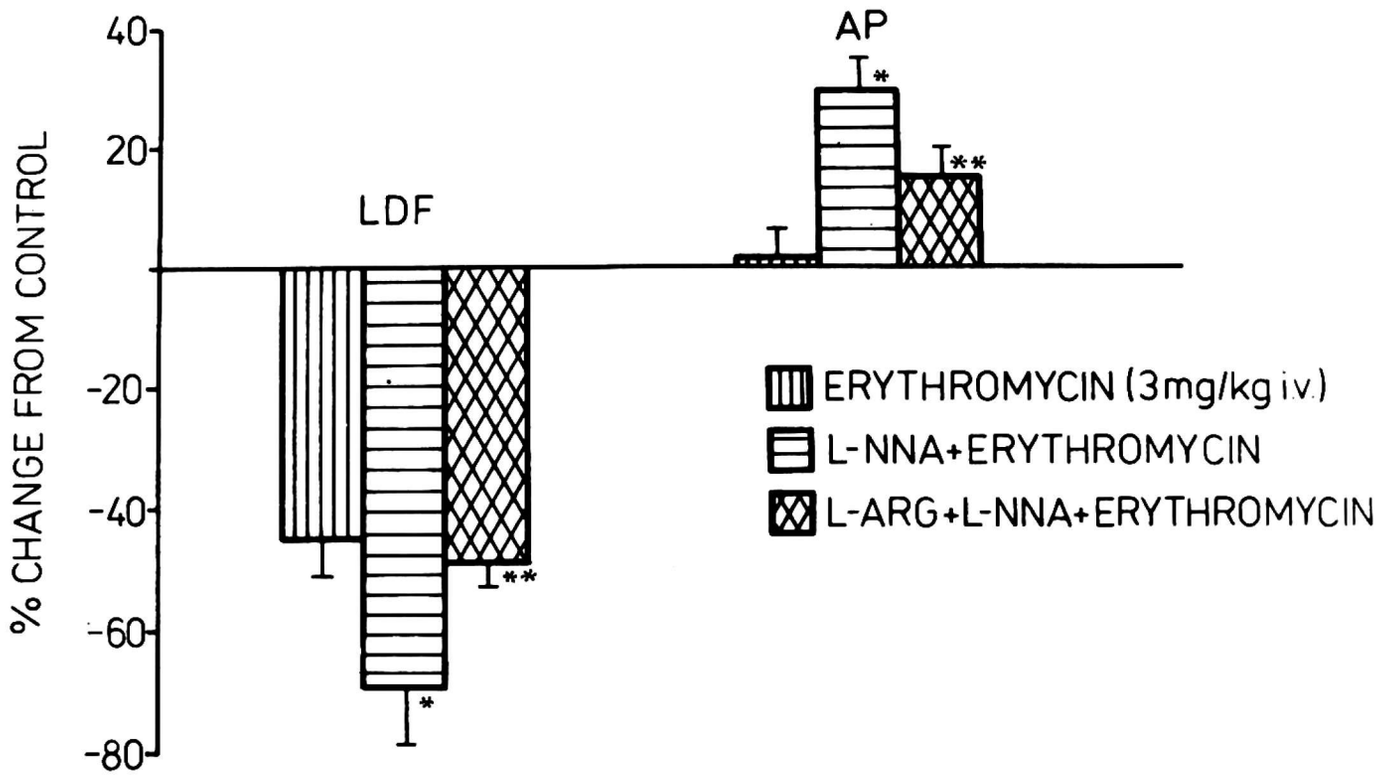

Fig. 4. Effects of erythromycin alone, L-NNA + erythromycin and combination of L-arginine + L-NNA + erythromycin on LDF and AP. Single asterisk indicates significant ($p < 0.05$) change in comparison with erythromycin alone. Double asterisks indicate significant change in comparison with L-NNA + erythromycin.

Combined pretreatment with L-arginine + L-NNA did decrease basal LDF by $10.0 \pm 4.0\%$ ($p < 0.05$) and increased AP and MMI by 16.0 ± 5.0 and $28.0 \pm 11\%$, respectively. However observed increase of LDF, AP and MMI in this experimental group was significantly lower than that observed after L-NNA alone (*Figs 1, 2, 3*).

In group III basal LDF, MMI and AP values were similar to these observed in previous experimental groups. In this group the effects of erythromycin included significant increase of the MMI by $58.0 \pm 16.0\%$ with no change in AP. LDF was decreased by $45.0 \pm 6.0\%$ during peak increase in MMI (*Figs 3, 4*).

In group IV, the effects of erythromycin on MMI and LDF were significantly potentiated by pretreatment with L-NNA. MMI increased by 140.0 ± 18.0 and LDF decreased by $68.0 \pm 9.0\%$ (*Figs 3, 4*).

In group V, the effect of combined pretreatment with L-arginine and L-NNA on erythromycin-iduced changes in LDF and MMI was examined. LDF and MMI values obtained after erytromycin were statistically compared with corresponding control values in group IV. The analysis of this comparison showed that the MMI after erythromycin was $60.0 \pm 15.0\%$ below that seen in the group IV, whereas LDF was $20.0 \pm 4\%$ less decreased then in group IV. AP in group IV and V demonstrated the same changes like in groups I and II (*Figs 3, 4*).

DISCUSSION

In the present study we have assessed the involvement of NO in the control of intestinal microcirculation and motility. In order to determine the role of NO in the maintenance of intestinal microcirculation and motor activity the endogenous generation of NO was impaired using the selective inhibitor of NO synthesis L-NNA (19). The characteristics of the changes in the intestinal microcirculation and arterial pressure, which appeared after acute L-NNA administration in fasted rats are consistent with previous reports that the inhibition of NO formation prompted a marked gastrointestinal ischemia and hypoxia (11). Presumably inhibition of NO syntesis exerts its effects on intestinal circulation by increasing the vasoconstrictor tone of the arteriolar smooth muscle, which regulates resistance to the blood flow through the gut, and the smooth muscle of precapillary sphincters, which regulate the blood flow through the nutrient portion of the intestinal microcirculation. Since systemic arterial pressure was increased by administration of L-NNA, NO appears to be a general tonic vasodilator in the rat circulation.

The inhibition of NO synthesis by L-NNA also increased intestinal motility and induced strong and prolonged contractions similar to phase III of Migrating Motor Complex (MMC). Similar effects were observed in conscious dogs with implanted electrodes along the small bovel, which responded to administration of L-NNA by an immediate apperance of phase III of MMC activity (20). This findings strongly support the hypothesis that NO is mediator of tonic inhibition observed in basal intestinal motor activity (phase I of MMC). Similar effects of NO on circular muscles of colon has been previously described (3), NO mimics the descending relaxation observed in response to intraluminal baloon distention by evoking membrane hyperpolarization that is similar to Inhibitory Junction Potential (IJP) resulted by NANC nerve stimulation (4, 5). It is also possible that the local splanchnic circulatory changes could affect intestinal motility secondary to the decrease of intestinal tissue oxygenation. Therefore, the observed activation of intestinal motor activity could have been the consequence of decreased NO generation and local intestinal hypoxia. The ability of exogenous L-arginine (but not D-arginine) to reverse the vasoconstrictive and motor activity induced by L-NNA 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 (21).

Our observations that L-arginine alone was not able to induce any changes in LDF, AP and MMI indicate that at rest there is not any lack of NO synthesis locally in the gut and in systemic circulation.

It has been found that the well-known antibiotic erythromycin affects gastrointestinal motility in the fasted state and mimics the effects of motilin

(22, 23). Erythromycin-induced prokinetic effect results from the activation of motilin receptors which are mostly involved in phase III of MMC. The finding that erytromycin-induced increase in intestinal motility was potentiated by pretreatment with L-NNA and this response was partially reversed by addition of L-arginine support the notion that NO may be responsible for the maintenance of interdigestive motility pattern.

In the present study the erythromycin-induced increase in IP was accompanied by a decrease in LDF. Since erythromycin strongly influences intestinal motility, the observed microcirculatory effects might be secondary to the contractions of the circular muscle layer of the intestinal wall and mechanical compresion of the microvasculature as suggested previously (24).

In summary, results of the present investigation support the concept that endothelium-derived NO plays an important role in the regulation of intestinal microcirculation under basal conditions. However, the physiological importance of NO in local modulation of intestinal nutrient circulation during various type of autoregulatory hyperemia remains to be clarified. Since NO was found to be generated also in the muscle layers of the intestine and to have potent relaxing action on the intestinal smooth muscle (6) it may also play an important role in local modulation of intestinal motility patterns.

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Received: February 2, 1993
Accepted: February 26, 1993

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