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Differential effects of lumenal L-arginine and N^G-nitro L-arginine on blood flow and water fluxes in rat ileum

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1 The role of endogenous mucosal nitric oxide (NO) in the local regulation of H_2O absorption and blood flow in rat ileum was studied by perfusing L-arginine (L-Arg) (0.1–1.0 mM) and N^G-nitro L-arginine (L-NOARG) (0.01–1.0 mM) through the lumen. D-Arginine (D-Arg) or L-Arg (1 mM), combined with L-NOARG, were used to determine if any of the measured intestinal effects of L-NOARG were exerted through NO formation.

2 Net and unidirectional H_2O fluxes and effective mucosal blood flow were measured using ^3H_2O and [^{14}C]-inulin in the perfusate. Mucosal NO formation was measured as the appearance of lumenal NO_2^-.

3 L-NOARG, beginning at a concentration of 0.1 mM, decreased net H_2O absorption, but had only minor effects on unidirectional H_2O fluxes or on blood flow. L-NOARG increased blood pressure, beginning at a concentration of 0.5 mM.

4 L-Arg had no significant effects on net H_2O absorption or blood pressure, and only minor effects on unidirectional H_2O fluxes and blood flow.

5 NO appearance in the lumen was marginally decreased by 1.0 mM L-NOARG, but not increased by L-Arg.

6 Mucosal blood flow resistance paralleled systemic blood pressure suggesting that vascular effects on the mucosa were exerted only after L-NOARG had reached the general circulation.

7 Lumenal L-Arg reversed the effects of lumenal L-NOARG on net H_2O absorption and blood pressure, but D-Arg did not.

8 It was concluded that there is tonic NO production by the rat intestinal mucosa that promotes H_2O absorption, but does not affect blood flow resistance. Mucosal NO production was not related to the observed effects on mucosal function.

Keywords: Nitric oxide; intestine; H_2O absorption; blood flow; mucosa; blood pressure; H_2O fluxes

Introduction

Nitric oxide (NO), or a related product, is produced by enterocytes, vascular tissue, smooth muscle, neurones, fibroblasts, mast cells and white blood cells (Blachier *et al.*, 1991; Nathan, 1992), all of which are found in the intestinal mucosa. The role of local NO production on intestinal mucosal function has not been well defined. Under normal conditions, NO is produced by constitutive NO synthases (cNOS) and has signalling functions. An inducible NOS (iNOS) produces larger amounts of NO following insults, and NO under these conditions can protect or damage tissues (Lopez-Belmonte *et al.*, 1993). Both cNOS and iNOS are produced in the intestine (Salter *et al.*, 1991; Boughton-Smith *et al.*, 1992). Most research concerned with the role of NO in the intestine revolves around intestinal motility, blood flow and the role of NO in preventing mucosal damage during shock stimuli. Relatively little work has focused on the role of NO in regulating intestinal absorption.

NO is produced by intestinal non-adrenergic non-cholinergic neurones and inhibits intestinal motility (Boeckxstaens *et al.*, 1991), but there are few of these neurones in the mucosa (Young *et al.*, 1992). NO is also a neural vasodilator (Amerini *et al.*, 1992) and is also produced by endothelial cells in the intestinal vasculature (Andriantsitohaina & Surprenant, 1992). The specific effect of endogenous NO on mucosal vasodilatation, however, has not been determined. NO maintains mucosal and vascular integrity at low and intermediate levels but causes damage at higher concentrations (Lopez-Belmonte *et al.*, 1993). Hence, NO has known functions in the intestine but the role of NO in many aspects of gut function requires further elucidation.

NOS produce NO from L-arginine (L-Arg) (Nathan, 1992), and production is inhibited by compounds such as N^G-nitro-L-arginine (L-NOARG) (Rees *et al.*, 1990). The D-isomers are inactive, and their lack of effect, when the L-isomers are active, has been used as a criterion of effects exerted through NO production. N^G-nitro-L-arginine and L-arginine are absorbed by the intestinal mucosa and, therefore, can have local effects as well as systemic effects, including effects on the intestine, after these compounds have been absorbed into the general circulation (Gardiner *et al.*, 1990b). A criterion for local mucosal effects would be that lumenal agents acted on mucosal functions at lower concentrations than those that exerted systemic effects. NO formed in the mucosa will diffuse into the lumen and interstitial space. NO rapidly and spontaneously forms NO_2^- and NO_3^- in varied proportions depending on the site of formation (Wennmalm *et al.*, 1992). Lumenal NO_2^- appearance, therefore, was used as an estimate of mucosal NO production. Absorptive site blood flow, arterial blood pressure and unidirectional H_2O fluxes were also measured to determine cardiovascular effects, and, because, it is sometimes possible to infer the transport mechanism that is affected by treatments from these parameters (Mailman, 1984).

In these experiments, H_2O absorption and mucosal blood flow were initially considered as representative of local effects, while blood pressure was used as an example of systemic effects. The experiments presented here were designed to determine the role of mucosal NO in regulating the intestinal mucosal functions of H_2O absorption and its supporting blood flow.

Methods

Animal preparation

Female Sprague Dawley rats, 287 ± 7 g, were fasted for 24 h with H_2O allowed *ad libitum*. The animals were anaesthetized

with sodium pentobarbitone (50 mg kg^{-1}, i.p.). The trachea was cannulated. A femoral vein was cannulated for infusion of supplemental anaesthetic, as needed, and for hydration with Krebs-Ringer bicarbonate solution (KRB) at 1.2 ml h^{-1}. A femoral artery was cannulated with plastic tubing containing heparinized (50 u ml^{-1}) KRB for measurement of blood pressure. A laparotomy was performed. A 20 cm segment of ileum, with its nerve and blood supply intact, beginning about 2 cm from the ileocecal junction was cannulated with an inflow and effluent cannula. The segment was flushed with KRB at 37°C. The segment was curled back into the abdominal cavity, and the incision was closed with wound clips and covered with saline-soaked sponges and plastic wrap. Body temperature was monitored with a rectal thermistor and maintained at 38°C with a heat lamp.

The ileal lumen was perfused by a syringe pump (Sage) through a condensor that maintained the lumenal perfusate at 37°C. The perfusate was labelled KRB (composition, mM: NaCl 118, KCl 4.7, $CaCl_2$ 2.5 and $NaHCO_3$ 25) containing ^3H_2O (about 50,000 c.p.m. ml^{-1}) and [^{14}C]-inulin (25 mg l^{-1}; about 5,500 c.p.m. ml^{-1}). A second, continuously variable, syringe pump was connected to the inflow cannula for addition of labelled KRB in control animals or L-Arg, D-Arg and/or L-NOARG, in labelled KRB, in experimental animals. The concentration of these compounds was changed by adjusting the flow rates of one or both pumps.

Protocol

After surgery, the lumenal perfusion was started (0.25 ml min^{-1}). The labelled KRB perfusate either contained no added agent or contained L-Arg or D-Arg (1 mM). After a 50 min equilibration period, the effluent was collected for four 10 min periods that served as initial baseline periods, so that each animal served as its own control. The second syringe pump was then started, beginnning period 1. In control animals, the second perfusate was the same as the baseline perfusate. In experimental animals, the second perfusate contained L-Arg or L-NOARG dissolved in the base perfusate. After four 10 min periods, the flow, and thus the concentration of L-Arg or L-NOARG, was changed two more times for the same periods and then turned off to reestablish control levels of flow and concentration. A final group of four 10 min periods was carried out, ending with period 16. Total perfusion rates varied between 0.25 to 0.28 ml min^{-1}.

L-NOARG concentrations were 0.01–1 mM and L-Arg 0.1–1 mM when added to KRB. When the base perfusion contained L-Arg or D-Arg (1 mM), the second syringe pump delivered only L-Arg or D-Arg (1 mM) in control animals or L-NOARG at 0.1, 0.5 and 1 mM in 1 mM D-Arg or L-Arg in experimental animals. At the end of the experiment, a blood sample was taken for measurement of plasma ^3H_2O and [^{14}C]-inulin. The perfused segment of gut was removed and weighed. Values were expressed per g gut.

Measurements

^3H_2O and [^{14}C]-inulin were measured by liquid scintillation counting in a Triton X-100/toluene/Liquifluor (Beckman) cocktail. Quench corrections were made by the external standard channels ratio method. Net and unidirectional H_2O fluxes were measured as previously described (Mailman, 1984). In brief, net H_2O absorption was determined from the inflow rate and the change in [^{14}C]-inulin concentration. The absorptive and secretory H_2O fluxes were determined from the net H_2O absorption and the change in specific activity of ^3H_2O due to unlabelled H_2O entering the lumen from the plasma. Blood levels of ^3H_2O were estimated from the final plasma concentration, assuming a linear increase over time.

Effective mucosal blood flow at the site of H_2O absorption was measured as absorptive site blood flow (ASBF), as previously described (Mailman, 1981). Briefly, the clearance of ^3H_2O from the lumen was calculated by dividing the total amount of ^3H_2O absorbed by the effluent concentration of ^3H_2O. This technique equates the lumenal ^3H_2O concentration with plasma concentration at the mucosal absorptive site because of the rapid equilibration of H_2O within the mucosa, as shown elsewhere (Mailman, 1981).

NO_2^- in the lumen perfusion solutions and effluent was measured by the Greiss reaction. Preliminary experiments showed that addition of HCl, to initiate the conversion of NO_2^- to NO, caused a small amount of turbidity in otherwise clear effluent. Hence, HCl (final concentration 1.2 M) and sulphanillic acid (final concentration 1.2 mM) were added first, and optical density at 540 nm was measured. Then, N-(1-naphthyl)ethylenediamine HCl (final concentration 0.3 mM) was added, and optical density was reread. The NO_2^- concentrations were determined from the change in optical density in standards and samples. Net NO_2^- appearance was calculated from the difference between the amounts of NO_2^- in the inflow and effluent solutions. The amounts were calculated from the concentrations and flow rates in the two inflow perfusates and the effluent solution.

Blood pressure (BP) was measured by transducer and was continuously recorded on a polygraph (Narco). ASBF resistance per 100 g gut was caculated as BP $ASBF^{-1}$ 100^{-1}.

Drugs

L-Arg HCl, D-Arg HCl and L-NOARG were obtained from Sigma and dissolved in the lumenal perfusion solutions. L-Arg and L-NOARG (1 mM) decreased the pH of the Krebs solution by only 0.12 and 0.09, respectively.

Statistics

The parameter values in periods after the initial control periods are expressed as a change from the mean of the initial period values. Initial values are given in the figure legends. Statistical comparisons were by two – factor ANOVA and Dunnett's (1955) or unpaired *t* test. ANOVA was used to compare all periods and treatments in KRB control animals with those perfused with L-NOARG or L-Arg. If significant differences were present within treatments, then the Dunnett *t* test was used to compare several treatments to a single control (KRB controls vs L-Arg at 0.1, 0.5, 1 mM and L-NOARG at 0.01, 0.05, 0.1 mM and 0.1, 0.5, 1 mM), at each 10 min period so as to account for any changes due to time/and or the small changes in perfusion rates and any effects of the baseline perfusion. Unpaired *t* test, after ANOVA, was used for comparing each period in D-Arg controls to D-Arg plus L-NOARG (0.1, 0.5, 1 mM) and L-Arg controls to L-Arg plus L-NOARG (0.1, 0.5, 1 mM). Values are given as mean ± s.e.mean. Significance was considered at $P<0.05$ by two-tailed comparisons. Calculations were carried out using Statview 512 on a Macintosh computer.

Results

Effects of L-NOARG alone

Net H_2O absorption was not decreased by L-NOARG (0.01 or 0.05 mM) but was decreased after 20 min of 0.1 mM L-NOARG and then returned to control levels 30 min after the L-NOARG was stopped (Figure 1). The maximum decrease was to about 50% of control. In a second series of experiments, net H_2O absorption was decreased by L-NOARG (0.1–1.0 mM) and returned to control levels 30 min after the L-NOARG was stopped (Figure 1). The maximum decrease was to about 50% of control, similar to the effect of 0.1 mM L-NOARG seen in the previous experiments.

Blood pressure was not significantly changed by the concentration of L-NOARG (0.1 mM) that first reduced net H_2O

absorption when L-NOARG was perfused at 0.01–0.1 mM (Figure 2). Blood pressure was increased after 20 min of 0.5 mM and during 1.0 mM L-NOARG, but not at 0.1 mM or the first 20 min of 0.05 mM that decreased net H_2O absorption, and blood pressure remained elevated after L-NOARG was stopped (Figure 2).

L-NOARG had only minor effects on H_2O fluxes or ASBF. L-NOARG caused a significant decrease in the secretory H_2O flux only in one period at 1 mM and decreases in the absorptive H_2O flux and ASBF in the last two periods at 1 mM and in the first period after 1 mM L-NOARG was stopped (not shown).

Lumenal NO_2^- appearance was inconsistently affected by lumenal L-NOARG (not shown). NO_2^- appearance was significantly decreased, relative to controls, in one period during infusion of 0.1 mM L-NOARG and in three periods at 1 mM. The significant changes represented about a 30% decrease from control. There were no significant changes in lumenal NO_2^- appearance in any other experiments even if changes in net H_2O absorption or blood pressure occurred (not shown).

Effects of L-Arg alone

L-Arg had no significant effects on net H_2O absorption or blood pressure (not shown). L-Arg significantly increased ASBF and the absorptive H_2O flux only in one period (not shown). The unidirectional secretory H_2O fluxes were significantly increased by L-Arg in four periods at 0.5–1.0 mM (not shown).

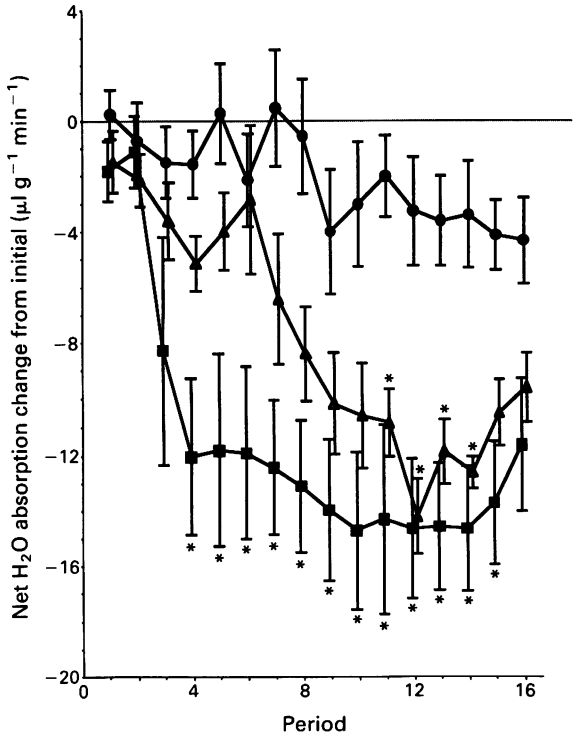

Figure 1 Net H_2O absorption from rat ileum during lumenal perfusion with Krebs solution (●) ($n = 11$) or N^G-nitro L-arginine (L-NOARG) at 0.01, 0.05, 0.1 mM and after stopping L-NOARG during periods 1–4, 5–8, 9–12 and 13–16, respectively (▲) ($n = 8$) or L-NOARG at 0.1, 0.5, 1 mM and after stopping L-NOARG during periods 1–4, 5–8, 9–12 and 13–16, respectively (■) ($n = 7$). Points represent 4–10 min periods at each concentration. Values are expressed as a change from initial control values. Initial values were 21.6 ± 2.2, 21.9 ± 2.3 and 26.6 ± 2.7 µl g^{-1} min^{-1} for the Krebs perfusion, 0.01–0.1 L-NOARG and 0.1–1 L-NOARG experiments, respectively. Mean ± s.e.mean. *represents a significant difference from periods in control animals, $P < 0.05$.

Effects of L-NOARG with D-Arg

Net H_2O absorption was decreased about 75% by L-NOARG (0.1–1.0 mM) in the presence of a background perfusion of 1 mM D-Arg, and it returned to control levels 20 min after the L-NOARG was stopped (Figure 3). The initial net H_2O

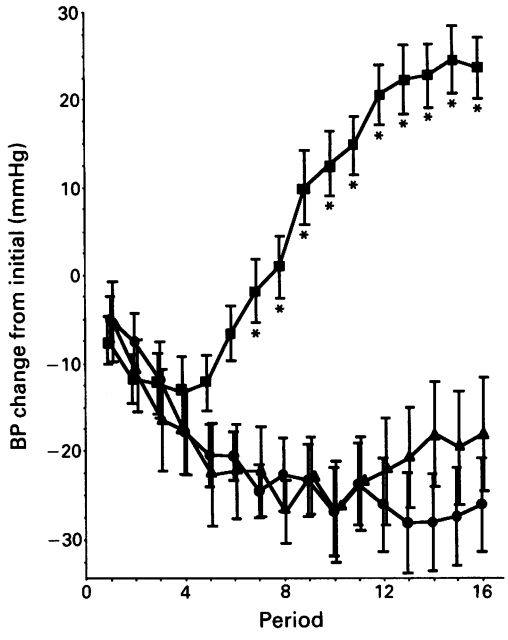

Figure 2 Blood pressure (BP) during lumenal perfusion with Krebs solution (●) ($n = 11$) or N^G-nitro L-arginine (L-NOARG) at 0.01, 0.05, 0.1 mM and after stopping L-NOARG during periods 1–4, 5–8, 9–12 and 13–16, respectively (▲) ($n = 8$) or L-NOARG at 0.1, 0.5, 1 mM and after stopping L-NOARG during periods 1–4, 5–8, 9–12 and 13–16, respectively (■) ($n = 7$). Points represent 4–10 min periods at each concentration. Values are expressed as a change from initial control values. Initial values were 106 ± 5, 118 ± 6 and 110 ± 5 mmHg for the Krebs, 0.01–0.1 L-NOARG and 0.1–1 L-NOARG experiments, respectively. Mean ± s.e.mean. *represents a significant difference from periods in control animals, $P < 0.05$.

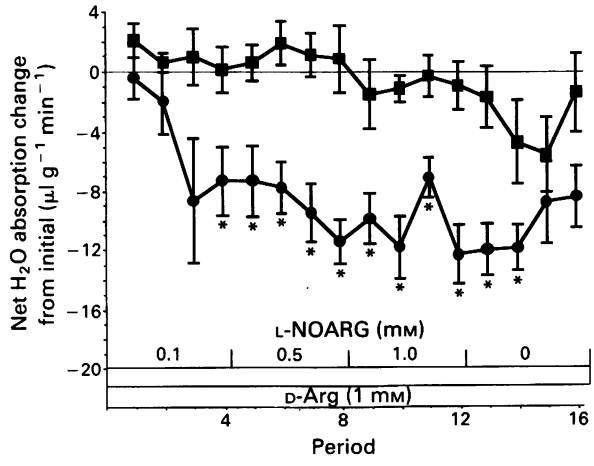

Figure 3 Net H_2O absorption during lumenal perfusion through rat ileum with 1 mM D-arginine (D-Arg) (■) ($n = 6$) or D-Arg + N^G-nitro L-arginine (L-NOARG) (●) ($n = 6$) at 0.1, 0.5, 1 mM and after stopping L-NOARG during periods 1–4, 5–8, 9–12 and 13–16, respectively. D-Arg and L-NOARG concentrations are shown on the X-axis. Points represent 4–10 min periods at each concentration. Values are expressed as a change from initial control values. Initial values were 16.7 ± 2.4 and 13.3 ± 1.5 µl g^{-1} min^{-1} for D-Arg and D-Arg + L-NOARG experiments, respectively. Mean ± s.e.mean. *represents a significant difference from periods in control animals, $P < 0.05$.

absorption during baseline perfusion with 1 mM D-Arg (Figure 3 legend) was significantly ($P<0.01$) lower than during perfusion with Krebs solution (Figure 1 legend).

Blood pressure was increased by L-NOARG (0.1–1.0 mM) in the presence of 1 mM D-Arg and remained elevated after L-NOARG was stopped (Figure 4). The responses of both net H_2O absorption and blood pressure were qualitatively similar to the effects of L-NOARG alone (Figures 1 and 2) but were potentiated, in that they occurred earlier or at a lower concentration, in the presence of D-Arg.

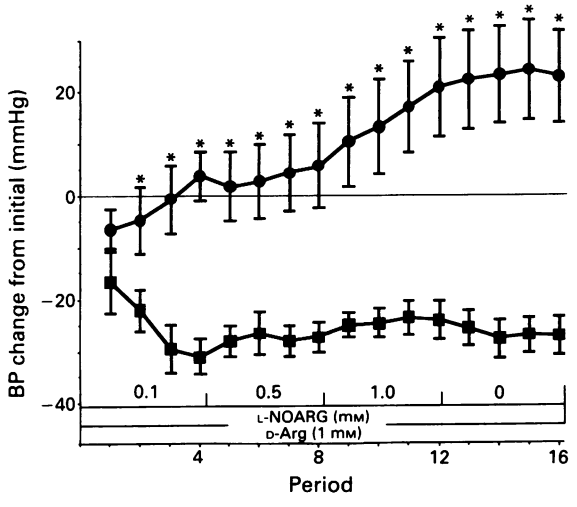

Figure 4 Blood pressure during lumenal perfusion through rat ileum with 1 mM D-arginine (D-Arg) (■) ($n=6$) or D-Arg + N^G-nitro L-arginine (L-NOARG) (●) ($n=6$) at 0.1, 0.5, 1 mM and after stopping L-NOARG during periods 1–4, 5–8, 9–12 and 13–16, respectively. D-Arg and L-NOARG concentrations are shown on the X-axis. Points represent 4–10 min periods at each concentration. Values are expressed as a change from initial control values. Initial values were 117 ± 6 and 101 ± 8 mmHg for the D-Arg and D-Arg + L-NOARG experiments, respectively. Mean ± s.e.mean. *represents a significant difference from periods in control animals, $P<0.05$.

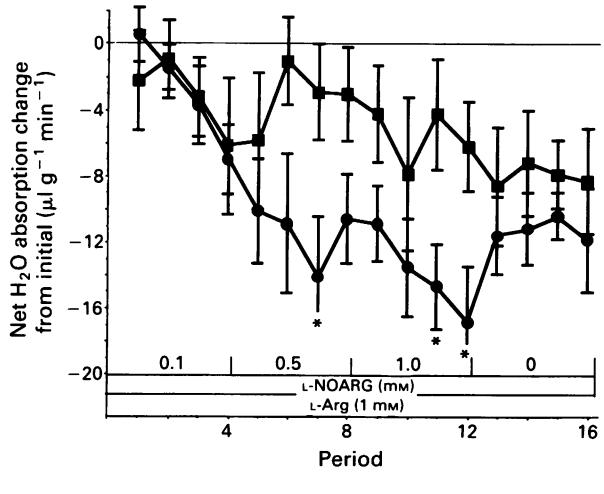

Figure 5 Net H_2O absorption from rat ileum during baseline lumenal perfusion with 1 mM L-arginine (L-Arg) (■) ($n=6$) or L-Arg + N^G-nitro L-arginine (L-NOARG) (●) ($n=6$) at 0.1, 0.5 and 1 and after stopping L-NOARG during periods 1–4, 5–8, 9–12 and 13–16, respectively. L-Arg and L-NOARG concentrations are shown on the X-axis. Points represent 4–10 min periods at each concentration. Values are expressed as a change from initial control values. Initial values were 19.1 ± 1.6 and 20.0 ± 2.4 µl g^{-1} min^{-1} for L-Arg and L-Arg + L-NOARG experiments, respectively. Mean ± s.e.mean. *represents a significant difference from periods in control animals, $P<0.05$.

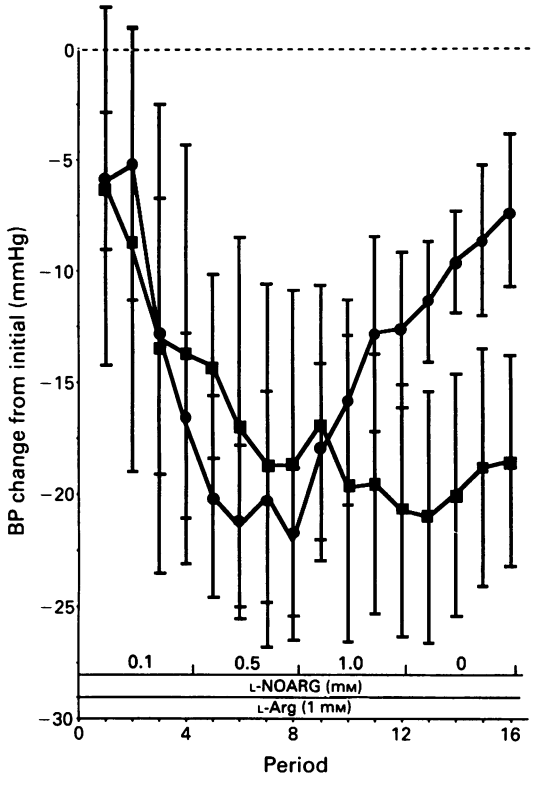

Figure 6 Blood pressure during lumenal perfusion through rat ileum with 1 mM L-arginine (L-Arg) (■) ($n=6$) or L-Arg + N^G-nitro L-arginine (L-NOARG) (●) ($n=6$) at 0.1, 0.5, 1 mM and after stopping L-NOARG during periods 1–4, 5–8, 9–12 and 13–16, respectively. L-Arg and L-NOARG concentrations are shown on the X-axis. Points represent 4–10 min periods at each concentration. Values are expressed as a change from initial control values. Initial values were 108 ± 9 and 119 ± 7 mmHg for L-Arg and L-Arg + L-NOARG experiments, respectively. Mean ± s.e.mean.

There were few significant effects of L-NOARG with D-Arg on unidirectional H_2O fluxes or ASBF, and these were similar to those observed with L-NOARG alone.

Effects of L-NOARG with L-Arg

Net H_2O absorption was inconsistently decreased by L-NOARG (0.5–1.0 mM) in the presence of a background perfusion at 1 mM L-Arg, and it returned to control levels after the L-NOARG was stoppped (Figure 5). The effects of L-NOARG in reducing net H_2O absorption were inhibited by L-Arg compared to L-NOARG alone (Figure 1). The increase in blood pressure produced by L-NOARG alone (Figure 2) was completely blocked by L-NOARG (0.1–1.0 mM) in the presence of 1 mM L-Arg (Figure 6).

L-NOARG, in the presence of L-Arg, had only minor effects on unidirectional H_2O fluxes or ASBF similar to L-NOARG when by itself. The absorptive H_2O flux decreased only in two periods and there were no significant effects on ASBF or the secretory H_2O flux.

Effects on ASBF resistance

ASBF was little changed by any of the treatments. Hence, ASBF resistance paralleled blood pressure in all experiments (not shown).

Discussion

These experiments were designed to determine if endogenous mucosal NO could regulate H_2O absorption and blood flow.

Net H_2O absorption and blood flow were intially considered as local effects, while blood pressure was considered a systemic effect, of agents applied lumenally. There are several cell types in the intestinal mucosa that can produce NO (Blachier *et al.*, 1991; Salter *et al.*, 1991; Nathan, 1992). Lumenal L-NOARG will inhibit tonic NO production (Rees *et al.*, 1990). L-Arg will increase NO production if endogenous L-Arg availability is rate limiting (Creager *et al.*, 1992). Lumenal agents would be presented to the mucosa at higher concentrations than those reached in the general circulation after absorption. Once in the circulation, systemic effects could include effects on the intestine. The data indicate that there is tonic mucosal NO production that increases net H_2O absorption but that blood flow is not locally affected.

Lumenal L-NOARG decreased net H_2O absorption. Decreases were observed beginning at concentrations of 0.1 mM. These responses suggested that tonic NO production increased net H_2O absorption, and blocking NO production removed the stimulation. Lower lumenal concentrations of L-NOARG that decreased net H_2O absorption did not change blood pressure, and, thus, L-NOARG had not been absorbed into the circulation in amounts sufficient to exert systemic effects. Hence, the effect of L-NOARG on net H_2O absorption was local and not part of a systemic response. In experiments by others, i.a. administration of N^G-nitro-L-arginine methyl ester (L-NAME) did not change fluid transport in feline intestine (Kubes, 1992). This difference in results could be related to the route of administration of the NO production inhibitor or species differences.

L-NOARG decreased net H_2O absorption without parallel effects on the unidirectional H_2O fluxes, i.e. changes in unidirectional H_2O fluxes were not significant, but the difference between them was consistently changed to favour reduced net absorption. This suggested that active absorption by enterocytes was affected. The net flux of H_2O is only about 15% of the unidirectional H_2O fluxes, and is due to the bias of active transcellular transport imposed on the unidirectional H_2O fluxes that are affected to a greater extent by local cardiovascular events (Mailman, 1981; 1984). Thus, net H_2O absorption can be significantly changed in the absence of significant changes in unidirectional H_2O fluxes. Previous studies have shown that the unidirectional secretory fluxes are increased when mucosal capillary pressure is increased, thus favouring passive secretion driven by increased interstitial pressure (Mailman, 1984). Capillary pressure would be little changed by L-NOARG, in these experiments, because transmission of the increased arterial pressure to the capillaries was reduced by the increased resistance, that is largely precapillary (Kubes & Granger, 1992). The unidirectional absorptive fluxes are increased when ASBF increases, due to a washout effect of the blood flow on absorbed substances (Mailman, 1984). ASBF was little changed by L-NOARG, in these experiments, and thus, there was little significant effect on the absorptive fluxes. Some of the effect of L-Arg in increasing unidirectional H_2O fluxes may be accounted for by its causing small increases in ASBF and increasing capillary pressure as precapillary resistance decreased in the face of constant arterial pressure.

NO can act through stimulation of soluble guanylate cyclases (Moncada *et al.*, 1991). Increased guanosine 3':5'-cyclic monophosphate (cyclic GMP) in enterocytes, as caused by heat-stable enterotoxin acting on particulate guanylate cylase, for example, causes intestinal secretion (Waldman & Murad, 1987). Reducing mucosal NO should reduce enterocyte cyclic GMP if these are target cells of NO. In the present experiments, reduced NO caused decreased absorption, an effect opposite to that expected from reducing cyclic GMP. However, enterocytes have relatively little soluble guanylate cyclase (Waldman & Murad, 1987), thus making it unlikely that enterocytes were acted on directly by NO. The effects of L-NOARG on transport may be due to effects on regulatory cells in the mucosa that, in turn, affect enterocytes.

Another cell type that may be involved are the fibroblasts that underlie the epithelial layer. Fibroblasts enhance the secretory response of cultured epithelial cells to agents that are known to stimulate NO release, as well as the release of other bioactive substances (Berschneider & Powell, 1992). Therefore, direct fibroblast-enterocyte interactions would cause increased absorption if NO production had been blocked by L-NOARG. Again, this response would be opposite to the observed response.

L-NOARG may act on mucosal neurones to decrease mucosal absorption. NO can interfere with cholinergic transmission (Lefebvre *et al.*, 1992), and cholinergic stimulation increases intestinal secretion or reduces absorption (Cooke, 1984). There is tonic neural inhibition of intestinal absorption (Andres *et al.*, 1985; Cooke, 1989). If NO interferes with cholinergic transmission, then L-NOARG would increase cholinergic stimuli and, thus, decrease absorption, as was observed. However, there are relatively few NO producing neurones in the mucosa (Young *et al.*, 1992), although the NO may be released by other types of cells and then act on mucosal neurones. The cell types involved and the mechanism of the decreased absorption due to lumenal L-NOARG requires further study.

Lumenal L-Arg partially blocked the effect of L-NOARG in reducing net H_2O absorption but completely blocked the increased blood pressure occurring at 0.5 and 1 mM. The relatively greater effect of L-Arg in blocking the effects of L-NOARG on blood pressure, as compared to net H_2O absorption, may be due to the relative concentrations of these two compounds reached in the mucosa, as compared to the circulation and/or to the relative sensitivity of the vasculature, as compared to the cells involved in the response of H_2O absorption. Lumenal D-Arg did not block the effects of L-NOARG. These findings further supported the suggestion that NO was the active agent because effects on NO production are enantiomer specific. However, D-Arg potentiated the effects of L-NOARG in decreasing absorption and increasing blood pressure. D-Arg may decrease the availability of L-Arg and thus, reduce NO production. This possibility is consistent with the effect of D-Arg, by itself, in decreasing the initial baseline net H_2O absorption.

The use of lumenal D-Arg or L-Arg to characterize the responses to L-NOARG as due to inhibition of NO formation assumed that their interaction occurred at NOS. Another possibility is that L-Arg or D-Arg may differentially inhibit the transport of L-NOARG across the mucosa, where local effects could occur, and then into the circulation, where systemic effects could occur. Transport was not measured in these experiments, but neither L-NOARG nor D-Arg greatly inhibited the uptake of L-Arg into endothelial cells (Bogle *et al.*, 1992). The endothelial cell transport system resembles the y^+ system (Mann *et al.*, 1990) that is also the major L-Arg transport pathway in the intestine (Satoh *et al.*, 1989; Cheeseman *et al.*, 1992). Hence, effects of L-Arg or D-Arg on L-NOARG transport across the mucosa are not likely and were not tested.

Although the inhibition of L-NOARG effects on H_2O absorption and blood pressure by L-Arg suggested that NO was a mediator, the small effects of L-NOARG on the entry rate of NO_2^- into the lumen did not support this possibility. L-NOARG caused only inconsistent decreases in lumenal NO_2^- and there was no parallel between lumenal NO_2^- and the effects of any treatment on H_2O absorption or blood pressure. Lumenal NO_2^- may not reliably represent lumenal NO entry, because NO_2^- moves across the gut mucosa (Witter *et al.*, 1979; Witter & Balish, 1979). Hence, lumenal NO_2^- may underestimate NO production and also be subject to variation because of changes in transport rate.

L-Arg had little or no effect on net H_2O absorpton, blood pressure, ASBF or lumenal NO_2^-. This suggested that lumenal L-Arg was not rate limiting for NO production, in agreement with other findings (Griffith *et al.*, 1991; Creager *et al.*, 1992). It is possible that the small effects on the

unidirectional H_2O fluxes were due to L-Arg acting as a transported and metabolized amino acid.

Local ASBF resistance paralleled systemic blood pressure, indicating that L-NOARG did not have a local effect on the mucosal vasculature. This is surprising because L-NOARG would have a locally higher concentration in the mucosa than after dilution in the systemic circulation. An analogous effect was observed when rats were given N^G-monomethyl-L-arginine (L-NMMA) or L-NAME by i.v. or drinking H_2O routes (Gardiner *et al.*, 1990a,b). In these experiments, the mesenteric vasculature was more sensitive than the hindquarters to i.v. NOS antagonists and responded more rapidly. During oral administration, mesenteric blood flow was not greatly changed, relative to controls at the same time, but mesenteric conductance was decreased, and the mesenteric responses were less than those of the hindquarters. A similar effect has been observed in the renal circulation (Granger *et al.*, 1992). L-NAME, at approximately the same dose delivered to the renal circulation, had much greater effects when infused i.v. than when infused i.a. The differences were attributed to systemic effects on the kidneys, and generalized sympathetic stimulation was suggested as a possible mechanism. Local i.a. NOS antagonists increased total intestinal resistance and decreased blood flow in cats, but the blood flow distribution within the gut was not examined (Kubes & Granger, 1992). Possibly, the L-NOARG may act on the intestinal microcirculation at resistance vessels that are sufficiently distant from the mucosa that they are not affected by mucosal levels of L-NOARG but are affected by circulating levels. Other research has shown that the resistance of the mesentery was increased by i.v. NOS antagonists, but the intestinal resistance was not affected even though blood pressure increased (Pizcueta *et al.*, 1991). Alternatively, there could be local and systemic compensations that tend to maintain mucosal blood flow at constant levels and that override local effects of L-NOARG.

In summary, lumenal L-NOARG decreased net H_2O absorption at lower concentrations than those that increased blood pressure, suggesting that tonic NO production by the mucosa increased H_2O absorption. Lumenal L-Arg reduced the effects of L-NOARG, but D-Arg did not, further, supporting a role of NO. However, the rate of entry of NO_2^- into the lumen, used as a measure of NO production, did not support a role of mucosal NO in the observed effects. A local effect of NO on mucosal blood flow resistance was not found.

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