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Aminoguanidine inhibits both constitutive and inducible nitric oxide synthase isoforms in rat intestinal microvasculature in vivo

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Abstract

The effects of aminoguanidine on the intestinal vascular permeability following endotoxin administration in vivo has been compared to those of the nitric oxide (NO) synthase inhibitor N^G-monomethyl-L-arginine (L-NMMA) in the rat. Concurrent administration of aminoguanidine. (12.5–50 mg/kg, s.c.) with endotoxin (*E. coli* lipopolysaccharide, 3 mg/kg, i.v.), dose dependently increased vascular leakage of radiolabelled albumin in the ileum and colon after 1 h, an effect reversed by the pretreatment with L-arginine (300 mg/kg, s.c.). Aminoguanidine (50 mg/kg, s.c.) also elevated arterial blood pressure over the 1 h investigation period. Similar acute potentiation of endotoxin-provoked vascular injury was observed 1 h following L-NMMA (50 mg/kg s.c.) which also increased blood pressure, indicating the inhibition of constitutive NO synthase. By contrast, administration of aminoguanidine (12.5–50 mg/kg, s.c.) 3 h after endotoxin, at the time of the expression of the inducible NO synthase, reduced the subsequent endotoxin-induced vascular leakage, as did L-NMMA (50 mg/kg). In homogenates of rat ileal or colonic tissue, aminoguanidine inhibited both the constitutive and inducible NO synthase activity showing only 2-fold selectivity for the inducible isoform. Thus, although aminoguanidine inhibits these isoforms of NO synthase, it is not a selective inhibitor of the inducible isoform in the intestinal microvasculature in vivo.

Keywords: Nitric oxide (NO); Aminoguanidine; Nitric oxide (NO) synthase; Microcirculation; Blood pressure; Endotoxin

1. Introduction

Nitric oxide (NO), synthesized from L-arginine by a calcium-dependent NO synthase plays a key role in modulating cardiovascular tone and integrity (Moncada et al., 1991). Inhibition of constitutive NO synthase by N^G-monomethyl-L-arginine(L-NMMA) reduces gastro-intestinal blood flow (Whittle, 1994) and augments the acute intestinal vascular injury induced by low and high doses of endotoxin in the rat (Hutcheson et al., 1990; Laszlo et al., 1994a). In addition, N^G-nitro-L-arginine methyl ester promotes neutrophil adherence (Kubes et al., 1991) and microvascular permeability in the intestinal microcirculation (Kubes and Granger, 1992; Filep and Földes-Filep, 1993). By contrast, expression of a calcium-independent inducible NO synthase provoked by endotoxin and cytokines can bring about cellular injury (Palmer et al., 1992; O'Connor and Moncada, 1991) and is associated with microvascular leakage in the small and large intestine (Boughton-Smith et al., 1993a). Induction of NO synthase is also implicated in the cardiovascular dysfunction seen in septic shock (Kilbourn et al., 1990; Nava et al., 1991; Wright et al., 1992) and in the tissue damage of inflammatory bowel diseases (Boughton-Smith et al., 1993b; Middleton et al., 1993; Miller et al., 1993a,b).

Whereas the currently available L-arginine-based inhibitors of NO synthase can attenuate the cardiovascular and intestinal microvascular consequences of NO synthase induction (Kilbourn et al., 1990; Nava et al., 1991; Boughton-Smith et al., 1993a) identification of a selective inhibitor of the inducible isoform, active both in vitro and in vivo would be of significant pharmacological and therapeutic interest. Aminoguanidine has recently been described as such a selective inhibitor of the inducible NO synthase, based primarily on its activity in a number of cell systems and vascular tissue in vitro, as well as on its relative lack of effects on blood pressure in vivo (Corbett et al., 1992; Misko et al.,

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1993; Hasan et al., 1993; Griffiths et al., 1993; Joly et al., 1994).

In the present study, the acute actions of aminoguanidine in vivo on intestinal microvascular leakage of radiolabelled albumin following endotoxin administration, as well as on blood pressure has been evaluated as an indication of effects on the constitutive NO synthase (Laszlo et al., 1994a), and compared to those of L-NMMA. Furthermore, the actions of aminoguanidine on the microvascular leakage associated with NO induction in the ileum and colon in vivo has also been compared to those of L-NMMA. In addition, the activity in vitro of aminoguanidine and L-NMMA on both constitutive and inducible NO synthase activity in homogenates of rat ileum and colon has been evaluated.

2. Materials and methods

2.1. Experimental protocol

Male Wistar rats (225–275 g) were fasted overnight, but received water ad libitum. Under transient halothane anaesthesia (less than 4 min) endotoxin (*E. coli* lipopolysaccharide 0111:B4; Sigma Chemical Co., Poole, Dorset; 3 mg/kg, i.v.) and [^{125}I]human serum albumin (Amersham, U.K.; 2 μCi/kg, i.v.) were injected and the animals were killed 1 or 4 h later. Aminoguanidine hemisulphate (Sigma Chemical Co.; 12.5–50 mg/kg, s.c.) or N^G-monomethyl-L-arginine hydrochloride (L-NMMA; 546C88, Wellcome Research Laboratories; 50 mg/kg, s.c.), both dissolved in isotonic saline were administered concurrently with endotoxin or 3 h following endotoxin administration. The dose of L-NMMA was selected as near-maximal on the basis of previous studies (Laszlo et al., 1994a). L-Arginine hydrochloride (Sigma Chemical Co.; 300 mg/kg, s.c. in isotonic saline) was injected 15 min before aminoguanidine administration.

2.2. Plasma leakage

As an index of vascular endothelial damage, the plasma leakage of [^{125}I]human serum albumin was determined in the intestinal tissues. Under halothane anaesthesia, blood was collected from the rat abdominal aorta into syringes containing trisodium citrate (final concentration 0.318%) and centrifuged (10 000 × *g*, 10 min, 4°C). The [^{125}I]human serum albumin content of the ileum, colon and plasma was determined in a gamma-spectrometer (Nuclear Enterprises NE 1600) and the albumin content in segments of intestinal tissues was calculated as described previously (Boughton-Smith et al., 1993a). Control values (from rats that had received saline) were subtracted from the treated values for each time-point and the data were expressed as Δ plasma leakage, μl plasma/g tissue.

2.3. Blood pressure

Under pentobarbitone anaesthesia (60 mg/kg, i.p.) systemic arterial blood pressure was measured from the right carotid artery of rats using a pressure transducer (Elcomatic) connected to a Grass Polygraph. The blood pressure was monitored over a 1 h period following administration of aminoguanidine (12.5 and 50 mg/kg, s.c.) or L-NMMA (50 mg/kg, s.c.).

2.4. NO synthase

NO synthase activity was determined as the conversion of radiolabelled L-arginine to L-citrulline by the method described previously (Salter et al., 1991) with minor modifications. Tissues were homogenised (15 s) in ice-cold buffer (250 mg tissue/ml, 10 mM Hepes, 32 mM sucrose, 1 mM dithiothreitol, 0.1 mM EDTA, 10 μg/ml soybean trypsin inhibitor, 10 μg/ml of leupeptin, and 2 μg/ml of aprotonin) adjusted to pH 7.4 (8 M NaOH) followed by centrifugation for 20 min on 10 000 × *g* at 4°C. A 40 μl sample of supernatant was incubated for 10 min at 37°C in 110 μl of reaction buffer comprising of (final concentrations) 50 mM KH_2PO_4, 1 mM $MgCl_2$, 0.2 mM $CaCl_2$, 50 mM valine, 1 mM dithiothreitol, 15 nM L-arginine, 1 mM citrulline, 0.3 mM NADPH, 3 μM FAD, 3 μM FMN, and 157 pM of [^{14}C]L-arginine (110 000 dpm/ml). [^{14}C]L-Arginine monohydrochloride was obtained from Amersham (UK), while the other compounds were from Sigma Chemical Co. (Poole, Dorset, U.K.).

The reaction was arrested via the removal of the substrate L-arginine by the addition (0.5 ml) of a 1:1 suspension of Dowex (AG 50W-8) in water. The mixture was dispersed and diluted via the addition of 0.85 ml distilled water (total volume 1.5 ml). After allowing the resin to settle, the supernatant was removed (0.97 ml) for the estimation of the radiolabelled products by scintillation counting (2 ml Pico-Fluor). Sample protein content was estimated via spectrophotometric assay (Bio-Rad Protein Assay), allowing expression of nitric oxide synthase activity as pmol/min per mg protein.

NO synthase activity determined in ileal and colonic tissue obtained from control rats and following endotoxin administration (3 mg/kg, i.v., for 4 h), was defined as citrulline formation that was abolished by incubation in vitro with L-NMMA (700 μM), and was further characterized by the effects of incubation in vitro with EGTA (1 mM). The effects of incubation of L-NMMA (5–200 μM) or aminoguanidine (15–2000 μM) on such tissue activity was determined, and expressed as % inhibition of control levels.

The concentration of either agent causing 50% inhi-

bition of control activity (IC_{50}) were obtained by computer-based extrapolation of curves fitted from data obtained from each individual preparation, and the mean value ± S.E.M. calculated.

2.5. Statistics

Values are expressed as means ± S.E.M. For statistical comparisons, Students *t*-test for unpaired data or analysis of variance with the Bonferroni test were utilised, where $P < 0.05$ was taken as significant.

3. Results

3.1. Plasma leakage

Administration of endotoxin (3 mg/kg, i.v.) alone did not cause acute plasma leakage in the ileum or colon, determined after 1 h (Fig. 1). Likewise, administration of L-NMMA (50 mg/kg, s.c.) or aminoguanidine (50 mg/kg, s.c.) alone did not significantly provoke acute vascular leakage in the ileum (Δ 13 ± 20 and 6 ± 15 μl plasma/g tissue, $n = 4$ and 6, respectively) or colon (Δ 5 ± 6 and 4 ± 5 μl/g tissue, respectively), determined after 1 h.

Concurrent administration of L-NMMA (50 mg/kg, s.c.) with endotoxin (3 mg/kg, i.v.) increased vascular leakage in the ileal and colonic tissue, determined after 1 h (Figs. 1 and 2). Likewise, concurrent administration of aminoguanidine (12.5–50 mg/kg, s.c.) with endotoxin (3 mg/kg, i.v.) provoked a dose-dependent elevation of vascular leakage in the ileum and colon 1 h later ($P < 0.05$; $n = 5$), which was abolished by pretreatment (15 min) with L-arginine (300 mg/kg, s.c.) as shown in Figs. 1 and 2.

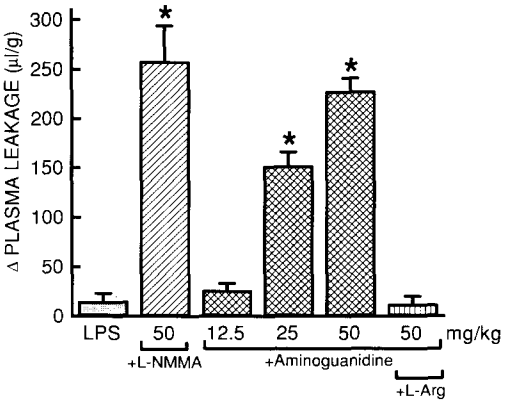

Fig. 2. Provocation of acute plasma leakage in the rat colon by the concurrent administration of N^G-monomethyl-L-arginine (L-NMMA; 50 mg/kg, s.c.) or aminoguanidine (12.5–50 mg/kg, s.c.) with endotoxin (LPS; 3 mg/kg, i.v.), and its reversal by the pretreatment with L-arginine (Arg; 300 mg/kg, s.c.) administered 15 min before aminoguanidine (50 mg/kg, s.c.). Plasma leakage (Δ μl/g tissue) was determined 1 h after endotoxin administration and shown as mean ± S.E.M., where $n = 5$–8 rats in a group. * $P < 0.05$ compared to the LPS-alone group.

Previous studies have demonstrated an increase in intestinal plasma leakage that commenced 3 h after endotoxin administration (Boughton-Smith et al., 1993a; Laszlo et al., 1994a), as also observed in the present study when determined 4 h after challenge (Fig. 3). Administration of L-NMMA (50 mg/kg, s.c.) 3 h following endotoxin challenge, significantly reduced this plasma leakage in ileal and colonic tissues, when

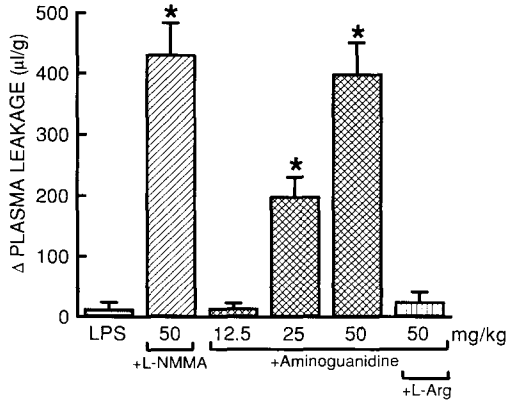

Fig. 1. Provocation of acute plasma leakage in the rat ileum by the concurrent administration of N^G-monomethyl-L-arginine (L-NMMA; 50 mg/kg, s.c.) or aminoguanidine (12.5–50 mg/kg, s.c.) with endotoxin (LPS; 3 mg/kg, i.v.), and its reversal by the pretreatment with L-arginine (Arg; 300 mg/kg, s.c.) administered 15 min before aminoguanidine (50 mg/kg, s.c.). Plasma leakage (Δ μl/g tissue) was determined 1 h after endotoxin administration and shown as mean ± S.E.M., where $n = 5$–8 rats in a group. * $P < 0.05$ compared to the LPS-alone group.

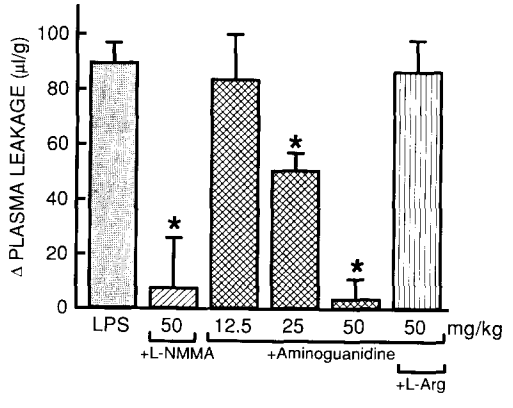

Fig. 3. Inhibition of endotoxin (3 mg/kg, i.v.)-induced plasma leakage in the rat ileum by N^G-monomethyl-L-arginine (L-NMMA; 50 mg/kg, s.c.) or aminoguanidine (12.5–50mg/kg, s.c.) when administered 3 h after challenge with endotoxin, and its reversal by the pretreatment with L-arginine (Arg; 300 mg/kg, s.c.) 15 min before aminoguanidine (50 mg/kg, s.c.). Plasma leakage (Δ μl/g tissue) was determined 4 h after endotoxin administration and shown as mean ± S.E.M., where $n = 5$–8 rats in a group. * $P < 0.05$ compared to the LPS-alone group.

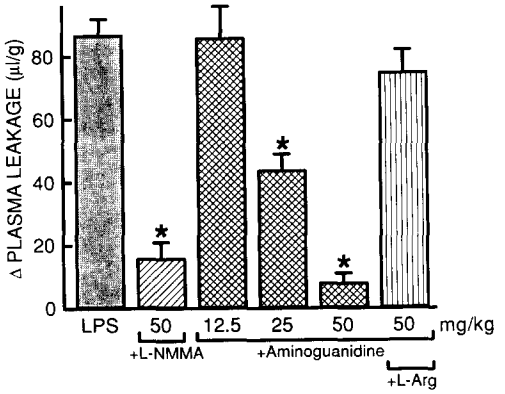

Fig. 4. Inhibition of endotoxin (3 mg/kg, i.v.)-induced plasma leakage in the rat colon by N^G-monomethyl-L-arginine (L-NMMA; 50 mg/kg, s.c.) or aminoguanidine (12.5–50 mg/kg, s.c.) when administered 3 h after challenge with endotoxin, and its reversal by the pretreatment with L-arginine (Arg; 300 mg/kg, s.c.) 15 min before aminoguanidine (50 mg/kg, s.c.). Plasma leakage (Δ μl/g tissue) was determined 4 h after endotoxin administration and shown as mean ± S.E.M., where $n = 5–8$ rats in a group. * $P < 0.05$ compared to the LPS-alone group.

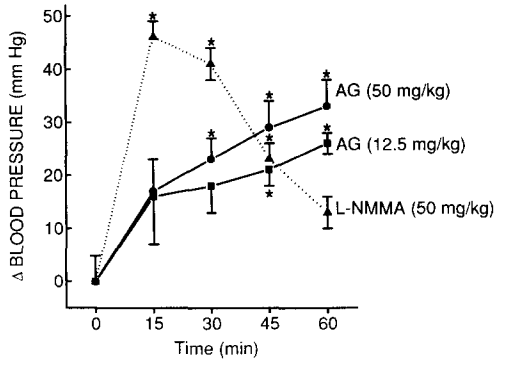

Fig. 5. Elevation of systemic arterial blood pressure by N^G-monomethyl-L-arginine (L-NMMA; 50 mg/kg, s.c.) or aminoguanidine (AG; 12.5–50 mg/kg, s.c.) in the anaesthetised rat. Arterial blood pressure (expressed as Δ mm Hg) was measured over a 1 h period. Data are shown as the means ± S.E.M., where $n = 3–5$ rats in a group. * $P < 0.05$ compared to the initial control value.

determined 1 h later (Figs. 3 and 4). Likewise administration of aminoguanidine (12.5–50 mg/kg, s.c.), 3 h after endotoxin challenge reduced such plasma leakage in the ileum and colon determined 1 h later, an effect which was reversed by pretreatment with L-arginine (300 mg/kg, s.c., 15 min before aminoguanidine) as shown in Figs. 3 and 4.

3.2. Blood pressure

Systemic arterial blood pressure in pentobarbitone-anaesthetised rats was elevated after administration of L-NMMA (50 mg/kg, s.c.) by a maximum of Δ 49 ± 3 mm Hg after 15 min ($P < 0.001$; $n = 5$). Likewise, blood pressure was elevated by administration of aminoguanidine (12.5 and 50 mg/kg, s.c.) by a maximum of Δ 33 ± 5 mm Hg ($P < 0.01$, $n = 5$) treatment, but as shown in Fig. 5, it only reached its peak value after 1 h. Pretreatment with L-arginine (300 mg/kg s.c.) 15 min prior to administration of aminoguanidine (50 mg/kg s.c.) abolished the increase in blood pressure seen after 1 h (Δ 1 ± 3 mm Hg, $n = 4$).

3.3. NO synthase

Basal NO synthase activity was detected in the supernatants of homogenates of segments of ileum and colon, being 57 ± 2 and 389 ± 2 pmol/min per mg protein, respectively ($n = 6$). This basal activity was abolished (< 5 pmol/min per mg protein) by incubation with EGTA (1 mM) in both ileal and colonic tissue samples and therefore reflects a calcium-dependent constitutive isoform. NO synthase activity was elevated in ileal and colonic tissue, 4 h after endotoxin administration, being Δ 263 ± 11 and Δ 409 ± 63 pmol/min per mg protein above basal values, respectively ($n = 5$, $P < 0.001$). This increased activity was not significantly inhibited by incubation with 1 mM EGTA, (Δ 237 ± 4 pmol/min per mg protein above basal for ileum, $n = 5$; $P < 0.001$) and therefore reflects the calcium-independent inducible isoform.

Incubation of the ileal and colonic tissue homogenates with L-NMMA (5–200 μM) or aminoguanidine (15–2000 μM) inhibited both the constitutive and induced NO synthase activity in a concentration-dependent manner (Table 1). The calculated IC_{50} values (concentration inhibiting NO synthase activity by 50%) for L-NMMA against the constitutive activity was 23 ± 4.8 and 4.3 ± 1.8 μM for the ileum and colon respec-

Table 1
Inhibition in vitro by N^G-monomethyl-L-arginine (L-NMMA) or by aminoguanidine of basal (constitutive) and endotoxin-induced (inducible) NO synthase activities obtained from rat ileal and colonic homogenate preparations

	% inhibition			
	Ileum		Colon	
	Constitutive	Induced	Constitutive	Induced
L-NMMA (μM)				
5	14 ± 2	20 ± 1	51 ± 3	39 ± 2
15	29 ± 7	41 ± 2	76 ± 1	61 ± 1
50	56 ± 8	70 ± 1	90 ± 1	87 ± 5
200	89 ± 4	91 ± 1	98 ± 1	95 ± 1
Aminoguaidine (μM)				
15	0 ± 0	6 ± 2	4 ± 1	167 ± 10
50	0 ± 0	10 ± 4	7 ± 3	13 ± 4
200	13 ± 1	46 ± 1	27 ± 4	37 ± 3
700	70 ± 11	85 ± 1	66 ± 3	78 ± 3
2000	97 ± 4	100 ± 1	91 ± 2	95 ± 1

Results are shown as % inhibition of basal activity following 10 min incubation at 37°C, and are the means ± S.E.M. of five experiments.

Table 2
Inhibition in vitro by N^G-monomethyl-L-arginine (L-NMMA) or aminoguanidine of basal (constitutive) and endotoxin-induced (inducible) NO synthase activities obtained from rat ileal and colonic homogenate preparations incubated for 10 minutes and 30 minutes at 37°C

	% inhibition			
	Ileum		Colon	
	Constitutive	Induced	Constitutive	Induced
L-NMMA (15 μM)				
10 min	36±7	46±2	71±3	54±6
30 min	35±9	38±1	77±1	65±2
Aminoguanidine (200 μM)				
10 min	18±1	39±2	24±6	53±8
30 min	20±4	39±3	26±2	45±2

Results are shown as % inhibition of control activity and are the means ± S.E.M. of four experiments.

tively, and 26 ± 4.8 and 10 ± 1.9 μM in these tissues against the inducible activity. Thus, L-NMMA exhibited a 2-fold selectivity against the inducible enzyme, but only in the colonic tissue. The IC_{50} values for aminoguandine against the constitutive activity were 526 ± 163 and 493 ± 125 μM for ileum and colon and 250 ± 141 and 361 ± 116 μM against the inducible activity in these tissues. Thus, aminoguanidine exhibited only a 2- and 1.4-fold selectivity against the inducible NO synthase in the ileum and colon respectively.

In a separate series of studies, the effect of the duration of incubation with L-NMMA or aminoguanidine on NO synthase activities in the broken cell preparation of rat ileum and colon was investigated. As shown in Table 2, a comparable degree of inhibition of either the constitutive or induced NO synthase activity was observed following 10 or 30 min incubation with L-NMMA (15 μM) in the tissue from ileal or colon, as found with aminoguanidine (200 μM).

4. Discussion

In the current study, aminoguanidine provoked acute vascular injury in the small and large intestine when administered concurrently with low doses of endotoxin. Likewise, the NO synthase inhibitor L-NMMA caused acute intestinal vascular injury following concurrent endotoxin challenge, confirming previous studies using both L-NMMA and N^G-nitro-L-arginine methyl ester (Laszlo et al., 1994a). These latter acute effects of these NO synthase inhibitors following endotoxin, were reversed by L-arginine (Laszlo et al., 1994a). Further, the effects of L-NAME were also attenuated by administration of specific receptor antagonists of platelet activating factor or thromboxane, as well as by anti-neutrophil antiserum, suggesting that inhibition of constitutively formed NO allows neutrophil-dependent vascular injury involving these acutely released mediators provoked by endotoxin (Laszlo et al., 1994b,c). Since the effects of aminoguanidine in the ileum and colon were likewise observed within 1 h of administration of endotoxin, at a time when the inducible isoform is not expressed (Salter et al., 1991; Boughton-Smith et al., 1993a), and were reversed by pretreatment with L-arginine, it is likely that such actions also reflect inhibition of the constitutive NO synthase.

The increase in systemic arterial blood pressure following administration of L-NMMA is considered to reflect inhibition of constitutive NO synthase in the vascular endothelium (Rees et al., 1989, 1990; Whittle et al., 1989; Aisaka et al., 1989). Hence, the apparent low hypertensive potency of aminoguanidine following intravenous administration in previous studies, when compared with L-NMMA, has been taken as evidence for minimal action of the constitutive enzyme (Corbett et al., 1992). However, the unusually high activity of L-NMMA in the investigation of Corbett et al. (1992) compared with other studies (Whittle et al., 1989; Rees et al., 1990) will have contributed significantly to the 40-fold potency difference with aminoguanidine (Corbett et al., 1992; Hasan et al., 1993). In the current study, subcutaneous administration of aminoguandine in doses similar to L-NMMA, substantially increased blood pressure, although the response reached its maximal effect at a much slower rate than did L-NMMA. This indicates a distinct pharmacokinetic profile of absorption or distribution of aminoguanidine. Studies in vitro have also suggested a slow tissue penetration of aminoguanidine (Misko et al., 1993; Joly et al., 1994). However, the doses of aminoguanidine that produced this increase in blood pressure are comparable to those previously found to increase blood pressure after intravenous injection (Corbett et al., 1992; Hasan et al., 1993) and these hypertensive effects were abolished by pretreatment with L-arginine in the current study, supporting an action in constitutive NO synthase. It is not yet known how changes in blood pressure correlate with the duration and extent of inhibition of constitutive NO synthase in the intestinal microcirculation by these agents.

Induction of NO synthase in the intestine is associated with an increase in vascular leakage, both events being suppressed by pretreatment with dexamethasone (Boughton-Smith et al., 1993a). Administration of NO synthase inhibitors following NO synthase induction can also ameliorate these vascular changes (Boughton-Smith et al., 1993a; Laszlo et al., 1994a), as confirmed in the present study with L-NMMA. Likewise, administration of aminoguanidine at a time of NO synthase induction, 3 h after endotoxin challenge, dose dependently reduced the vascular leakage in the ileum and

colon. The reversal of these effects of aminoguanidine by L-arginine makes it likely that they reflect inhibition of the inducible NO synthase in vivo. However, the dose-range for aminoguanidine exhibiting such actions was comparable to those eliciting its acute effects of promoting endotoxin-provoked vascular leakage, as found previously with L-NMMA (Laszlo et al., 1994a), suggesting little selectivity in vivo between its actions on the inducible or constitutive isoforms.

Both aminoguanidine and L-NMMA inhibited the calcium-dependent constitutive NO synthase activity following incubation in vitro with ileal and colonic tissue obtained from control rats as well as the calcium-independent activity in tissue obtained from rats pretreated with endotoxin for 4 h to induce NO synthase. The constitutive NO synthase activity in these crude mixed cell homogenates is derived from vascular or neuronal tissue, while the inducible enzyme may originate from the vasculature or inflammatory cells, with both isoforms being also detected in the epithelial cell layer (Tepperman et al., 1993). In previous studies, aminoguanidine inhibited cytokine-stimulated nitrite production from a rat insulinoma cell line and from an endotoxin-induced macrophages cell line (Corbett et al., 1993; Hasan et al., 1993; Misko et al., 1993). Aminoguanidine also attenuated the relaxation of isolated vascular segments associated with NO synthase induction while having no effect on resting tone (Griffiths et al., 1993; Hasan et al., 1993; Misko et al., 1993; Joly et al., 1994). In addition, aminoguanidine inhibited a preparation of inducible NO synthase (Misko et al., 1993) and as with the cell studies, was equipotent with L-NMMA. In the present study on intestinal tissue, L-NMMA was 10 or 30 times more active than aminoguanidine as an inhibitor of the inducible enzyme in vitro from the rat ileum and colon respectively. Moreover, aminoguanidine showed only a 1–2-fold selectivity between its inhibitory potency against the constitutive or inducible isoenzymes from rat ileum and colon, which contrasts with the reported 10–100-fold selectivity of action described in other tissues (Corbett et al., 1992; Hasan et al., 1993; Griffiths et al., 1993; Misko et al., 1993). Such differences may reflect the source or nature of the inducible isoenzyme.

The present findings demonstrate that aminoguanidine can inhibit both constitutive and inducible NO synthase in the rat ileum and colon in vitro and in vivo. Thus, aminoguanidine can augment the acute vascular leakage in the intestine following concurrent administration with endotoxin, or inhibit of the later phase of vascular leakage when its administration is delayed to the time of NO synthase induction. Along with its broad profile of biochemical actions including effects on diamine oxidase and catalase (Ohrui et al., 1992; Ou and Wolf, 1993), these observations in the rat ileum and colon indicate that the use of aminoguanidine as a selective inhibitor of inducible NO synthase, especially for studies in vivo, warrants caution.

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