Characterization of the effects of two new arginine/citrulline analogues on constitutive and inducible nitric oxide synthases in rat aorta

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1 New potent inhibitors of nitric oxide synthase (NOS) may be useful in the treatment of septic shock, a disorder characterized by a vascular hyporeactivity to catecholamines caused by an overproduction of nitric oxide (NO^-). We examined the effects of L-thiocitrulline (L-TC) and S-methyl-L-thiocitrulline (L-SMTC), novel NOS inhibitors, on the constitutive and inducible NOS in rat aorta and compared those effects with inhibition due to N^G -methyl-L-arginine (L-NMA).

2 Phenylephrine evoked similar concentration-contraction curves in the control rings and in the rings treated with these different NOS inhibitors ($10\text{ }\mu\text{M}$), whereas $100\text{ }\mu\text{M}$ of L-NMA, L-TC or L-SMTC increased significantly, and to a similar extent, contractions evoked by phenylephrine in aortic rings with endothelium without significantly affecting the maximal responses.

3 Relaxations evoked by acetylcholine, adenosine triphosphate, or calcium ionophore were significantly inhibited in a dose-dependent manner by L-NMA, L-SMTC, or L-TC (10 – $100\text{ }\mu\text{M}$). The potencies of these inhibitors in reducing the relaxations of these vasodilators were not significantly different.

4 In endotoxin-treated preparations with endothelium, the three L-arginine analogues ($10\text{ }\mu\text{M}$) significantly potentiated contractile responses to phenylephrine (pEC_{50} : 6.73 ± 0.12 and 7.3 ± 0.12 , 7.34 ± 0.13 , or 7.22 ± 0.14 ; in the absence and the presence of L-NMA, L-TC, or L-SMTC respectively) and increased maximal contractions from $1.53 \pm 0.15\text{ g}$ to $1.95 \pm 0.13\text{ g}$, $2.08 \pm 0.12\text{ g}$, and $2.03 \pm 0.13\text{ g}$ with L-NMA, L-TC, and L-SMTC respectively. A higher concentration of these NOS inhibitors ($100\text{ }\mu\text{M}$) further increased contractions evoked by this α_1 -agonist without further enhancing the maximal contractions; however, contractions evoked by 10 nM phenylephrine were significantly greater in the presence of L-SMTC or L-TC than in the presence of L-NMA ($100\text{ }\mu\text{M}$) (L-NMA: $0.4 \pm 0.11\text{ g}$; L-TC: $0.78 \pm 0.14\text{ g}$ and L-SMTC: $0.82 \pm 0.17\text{ g}$). The effects of these inhibitors on NO^- synthesis induced by endotoxin were significantly reversed by addition of L-arginine (1 mM) but not by L-citrulline (1 mM). In LPS-treated rings with endothelium, all three NOS inhibitors ($100\text{ }\mu\text{M}$) shifted the concentration-contraction curves evoked by phenylephrine significantly to the left (pEC_{50} : 7.19 ± 0.03 and 7.79 ± 0.08 , 8.01 ± 0.07 , or 8.02 ± 0.07 , in the absence and the presence of L-NMA, L-TC, or L-SMTC, respectively) and increased significantly maximal contractions from $2.05 \pm 0.05\text{ g}$ to $2.38 \pm 0.14\text{ g}$, $2.5 \pm 0.12\text{ g}$, and $2.4 \pm 0.21\text{ g}$ with L-NMA, L-TC, and L-SMTC, respectively. L-TC and L-SMTC were significantly more potent than L-NMA in potentiating contractions evoked by 10 nM and 30 nM phenylephrine.

5 L-TC and L-SMTC produced dose-dependent increases in tone in LPS-treated aortic rings with and without endothelium. In LPS-treated rings with endothelium, L-NMA induced contractions but in preparations without endothelium low concentrations of L-NMA induced small contractions while high concentrations of this inhibitor evoked relaxations. In both preparations L-TC and L-SMTC were significantly more potent than L-NMA in increasing vascular tone.

6 These results suggest that L-SMTC, L-TC and L-NMA were equipotent on basal and agonist-stimulated NO^- synthesis produced by the constitutive isoform of NOS, whereas the two new L-arginine analogues were more potent than L-NMA in inhibiting the production of NO^- induced by endotoxin in rat aorta.

Keywords: Nitric oxide synthase; N^G -methyl-L-arginine; L-thiocitrulline; S-methyl-L-thiocitrulline; rat aorta

Introduction

The nitric oxide synthases (NOS) constitute a family of closely related isoenzymes (Stuehr & Griffith, 1992). In endothelial cells (Palmer *et al.*, 1988; Forsterman *et al.*, 1991) and in neurones (Bredt *et al.*, 1990; Bohme *et al.*, 1991; Snyder, 1992) they are constitutively present and activated by calcium/calmodulin. In contrast, a calcium-calmodulin independent NOS is expressed in macrophages (Marletta *et al.*, 1988) and in vascular smooth muscle (Busse & Mulsch, 1990; Fleming *et al.*, 1990; Beasley *et al.*, 1991; Schini *et al.*, 1991) and endothelial cells (Gross *et al.*, 1990; Kilbourn & Belloni, 1990) of vascular wall cells only after stimulation by endotoxin or cytokines.

Constitutive production of nanomolar amounts of nitric oxide (NO^-) by endothelial cells appears vital to blood pressure homeostasis, whereas the inducible isoform of NOS (iNOS) can generate micromolar quantities of NO^- that probably contribute to both the local tissue damage and the systemic hypotension that characterize septic shock (Julou-Schaeffer *et al.*, 1990; Kilbourn *et al.*, 1990).

Structural modification of one of the two equivalent guanidino nitrogens of L-arginine results in compounds that inhibit NOS. Of these compounds, N^G -methyl-L-arginine (L-NMA), N^G -nitro-L-arginine (L-NOARG), and N^G -nitro-L-arginine methyl ester (L-NAME) have been found to be effective inhibitors (Moore *et al.*, 1990; Rees *et al.*, 1989; 1990), but have been observed to have different potencies against the various NOS isoenzymes. For example, L-NNA preferentially

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blocks the calcium/calmodulin-dependent NOS found in brain (Lambert *et al.*, 1991) and endothelial cells (Gross *et al.*, 1990), whereas L-NMA is more potent against the calcium/calmodulin-independent NOS (Gross *et al.*, 1990). Since both L-NNA and L-NMA inhibit the constitutive isoform of NOS present in endothelial cells (Moore *et al.*, 1990; Rees *et al.*, 1990), these agents can have hypertensive effects even in normotensive animals (Moncada *et al.*, 1991). Newer NOS inhibitors that are isoform-specific or which show an increased potency against NO⁻ production by the inducible isoform of NOS may be useful in treating patients with septic shock.

In the present study we examined the effects of L-NMA, the prototypic inhibitor of NOS, on constitutive NOS (basal and agonist-stimulated production of NO⁻) and on inducible NOS (endotoxin-induced production of NO⁻). In addition, we have characterized the effects of two new arginine/citrulline analogues (Figure 1): L-thiocitrulline (L-TC) and S-methyl-L-thiocitrulline (L-SMTC) (Narayanan & Griffith, 1994), on both isoenzymes in rat aorta.

Methods

Male Wistar rats (300–400 g) were anaesthetized by intraperitoneal injection of sodium pentobarbitone (50 mg kg⁻¹). The thoracic aortae were excised and stored in cold modified Krebs-Ringer solution containing (composition in mM): NaCl 118.3, KCl 4.7, MgSO₄ 1.2, KH₂PO₄ 1.2, CaCl₂ 2.5, NaHCO₃ 25.0; CaEDTA 0.016 and glucose 11.1 (control solution). Arteries were cleaned of fat and connective tissue and cut into rings. In some rings the endothelium was removed mechanically by inserting a small metal wire into the lumen and rolling the tissue back and forth several times on a paper towel wetted with control solution. The preparations were suspended in organ chambers containing 10 ml of control solution at 37°C and aerated with 95% O₂ and 5% CO₂. The aortic rings were stretched progressively to an optimal tension of 2.5 g–3 g. Changes in isometric tension were recorded with an isometric force transducer connected to an analog-to-digital input board in an IBM 386/30 MHz personal computer. The presence or absence of the endothelium was verified by addition of acetylcholine (1 µM) to arteries contracted with phenylephrine (1 µM). The preparations were then rinsed three times with warm control solution, rested for 30 min, and incubated with either solvent, L-NMA, L-TC, or L-SMTC (10 or 100 µM for 30 min, with or without 1 mM L-arginine or L-

citrulline) before a concentration-contraction curve to phenylephrine (1 nM–10 µM) was obtained. In another set of experiments, after incubation with solvent or one of the NOS inhibitors, the preparations were contracted with phenylephrine (0.3 µM–1 µM) and a cumulative dose-response curve was obtained for acetylcholine (ACh), adenosine triphosphate (ATP), or calcium ionophore (A23187). In a separate series of experiments cumulative contraction curves to these NOS inhibitors were obtained in LPS-treated rings with and without endothelium. The preparations were contracted submaximally by addition of phenylephrine (10–50 nM) before the dose-response curves of these NOS inhibitors were obtained. After the maximal response was evoked by these compounds, L-arginine or L-citrulline (1 mM) was added to rings with endothelium.

To induce NOS activity, aortic rings were placed in 24-well multiwell plates with Dulbecco's modified Eagle's medium and Ham's F12 medium (DMEM/F12) (1 ml) containing 100 iu ml⁻¹ streptomycin and 100 iu ml⁻¹ penicillin. The preparations were incubated in culture medium containing 200 ng ml⁻¹ endotoxin (LPS) for 5 h at 37°C in a cell culture incubator before being suspended in the organ chambers.

Drugs

Phenylephrine hydrochloride, acetylcholine chloride, adenosine triphosphate, calcium ionophore (A23187), endotoxin (lipopolysaccharide, *Escherichia coli*, 0128:B12), L-citrulline and L-arginine were purchased from Sigma Chemical Co. (St. Louis, MO, U.S.A.); Dulbecco's Modified Eagle's/Ham's F12 medium (DMEM/F12) was purchased from GIBCO BRL (Grand Island, NY, U.S.A.). N^G-methyl-L-arginine monoacetate was synthesized by the general procedure of Corbin & Reporter (1974). L-Thiocitrulline hydrochloride and S-methyl-L-thiocitrulline hydrochloride were prepared as previously described (Narayanan & Griffith, 1994). All drugs were prepared in distilled water.

Statistical analysis

Results are expressed as the mean ± s.e.mean. Statistical evaluation of the data was performed by Student's *t* test for paired or unpaired observations. When data of more than two groups were compared, an analysis of variance (ANOVA) was used, and individual means were compared with Scheffe's *F* test. *P* values less than 0.05 were considered significant.

Figure 1 Structure of N^G-methyl-L-arginine, L-thiocitrulline and S-methyl-L-thiocitrulline.

Results

Basal release of NO⁻

The addition of L-NMA, L-TC, or L-SMTC (10 μ M or 100 μ M) to the preparations for 30 min had no effect on the basal tension. Phenylephrine (1 nM–1 μ M) evoked concentration-dependent contractions in aortic rings with endothelium. The contractile responses to phenylephrine were not affected in the presence of a 10 μ M concentration of any of these inhibitors and the pEC₅₀ and E_{max} were similar in control and treated rings with endothelium (Table 1), whereas 100 μ M of L-NMA, L-TC, or L-SMTC shifted the dose-response curves to this α_1 -agonist significantly to the left without significantly affecting the maximal responses (E_{max}: 1.95 $\pm$ 0.08 g to 2.13 $\pm$ 0.1, 2.12 $\pm$ 0.19, or 2.2 $\pm$ 0.14 g, respectively) (Table 1). Enhancement of the contractions to phenylephrine was similar for all three inhibitors studied (pEC₅₀: 7.5 $\pm$ 0.14 to 7.84 $\pm$ 0.08, 7.82 $\pm$ 0.12, or 7.78 $\pm$ 0.08, with L-NMA, L-TC, or L-SMTC respectively) (Table 1).

Agonist-induced NO⁻ synthase activity

Following induction of a submaximal contraction with phenylephrine (0.3 μ M–1 μ M), acetylcholine (ACh) (1 nM–10 μ M), adenosine triphosphate (ATP) (0.1 μ M–100 μ M), or calcium ionophore (A23187) (10 nM–1 μ M) induced concentration-dependent relaxations in aortic rings with endothelium. The maximal responses to phenylephrine were not significantly different for the three vasodilators in the presence or the absence of inhibitors (data not shown).

Treatment of aortic rings with endothelium with either L-NMA, L-TC, or L-SMTC (10 μ M) for 30 min resulted in a small but significant inhibition of the relaxations seen at higher concentrations of acetylcholine (Figure 2a), whereas treatment with 100 μ M of these inhibitors produced a significant inhibition of relaxations induced by either low or high concentrations of this vasodilator (Figure 2b). In addition, relaxations evoked by ATP or A23187 were significantly reduced in the presence of 100 μ M L-NMA, L-TC, or L-SMTC (Figure 3a and b). The potency of these three NOS inhibitors in inhibiting relaxations evoked by ACh, ATP, and A23187 were similar.

LPS-induced NOS activity

L-NMA, L-TC, or L-SMTC did not significantly affect the basal tension in LPS-treated rings with and without endothelium (data not shown). The incubation of aortic rings with and without endothelium for 5 h in culture medium

Table 1 Effects of N^G-methyl-L-arginine (L-NMA), L-thiocitrulline (L-TC) and S-methyl-L-thiocitrulline (L-SMTC) 10 μ M and 100 μ M (30 min) on concentration-contraction curves evoked by phenylephrine in aortic rings with endothelium

	pEC ₅₀	E _{max} (g)
Untreated	7.34 $\pm$ 0.16	2.08 $\pm$ 0.05
L-NMA (10)	7.49 $\pm$ 0.21	2.07 $\pm$ 0.1
L-TC (10)	7.54 $\pm$ 0.1	2.12 $\pm$ 0.12
L-SMTC (10)	7.47 $\pm$ 0.14	2.03 $\pm$ 0.05
Untreated	7.5 $\pm$ 0.14	1.95 $\pm$ 0.08
L-NMA (100)	7.84 $\pm$ 0.08*	2.13 $\pm$ 0.1
L-TC (100)	7.82 $\pm$ 0.12*	2.12 $\pm$ 0.19
L-SMTC (100)	7.78 $\pm$ 0.08*	2.2 $\pm$ 0.14

pEC₅₀: negative logarithm of the effective molar concentration of phenylephrine causing 50% of the maximal contraction. E_{max}: maximal contraction evoked by phenylephrine. Statistically significant differences between the untreated and NOS inhibitors-treated rings *P < 0.05.

containing LPS 200 ng ml⁻¹, shifted the concentration-contraction curves to phenylephrine significantly to the right (pEC₅₀: 7.5 $\pm$ 0.14 to 6.73 $\pm$ 0.12 and 7.95 $\pm$ 0.16 to 7.19 $\pm$ 0.03, with and without endothelium respectively) and significantly decreased the maximal contractions from 1.95 $\pm$ 0.08 g to 1.53 $\pm$ 0.15 g and from 2.34 $\pm$ 0.12 g to 2.05 $\pm$ 0.12 g, with and without endothelium respectively. Treatment of LPS-treated rings with endothelium with NOS inhibitors (10 μ M) for 30 min shifted the concentration-contraction curves to phenylephrine significantly to the left and significantly increased the maximal response from 1.53 $\pm$ 0.15 g to 1.95 $\pm$ 0.13,

Figure 2 Effects of N^G-methyl-L-arginine (L-NMA, □), L-thiocitrulline (L-TC, △), or S-methyl-L-thiocitrulline (L-SMTC, ○) (a: 10 μ M and b: 100 μ M; 30 min) on the concentration-relaxation curves evoked by acetylcholine in rat aortic rings with endothelium (●). Results are presented as means $\pm$ s.e. of 5–6 different experiments and are expressed as the percentage of the contraction evoked by phenylephrine (0.3 μ M–1 μ M). Statistically significant differences between the control and NOS inhibitors-treated rings, *P < 0.05.

Figure 3 Effects of L-NMA (□), L-TC (△), or L-SMTC (○) (100 μ M; 30 min) on the concentration-relaxation curves evoked by adenosine triphosphate (a) and calcium ionophore (b) in rat aortic rings with endothelium (●). Results are presented as means $\pm$ s.e. mean of 4 different experiments and are expressed as the percentage of the contraction evoked by phenylephrine (0.3 μ M–1 μ M). Statistically significant differences between the control and NOS inhibitors-treated rings, * P < 0.05.

2.08 $\pm$ 0.12, and 2.03 $\pm$ 0.13 g with L-NMA, L-TC, and L-SMTC, respectively (Figure 4a) (pEC_{50} : 6.73 $\pm$ 0.12 and 7.3 $\pm$ 0.12, 7.34 $\pm$ 0.13, or 7.22 $\pm$ 0.14; in the absence and the presence of L-NMA, L-TC, or L-SMTC). Contractions to this α_1 -agonist obtained in the presence of NOS inhibitors (10 μ M) were not significantly different in LPS-treated rings. The hyporeactivity induced by LPS in aortic rings was reversed in a concentration-dependent manner by L-NMA, L-TC, and L-SMTC, since a higher concentration of these compounds (100 μ M) further potentiated contractions evoked by phenylephrine (Figure 4b) without further increasing maximal responses (pEC_{50} : 6.78 $\pm$ 0.08 and 7.63 $\pm$ 0.09, 7.80 $\pm$ 0.12, or

7.82 $\pm$ 0.11 in the absence and the presence of L-NMA, L-TC, or L-SMTC). In addition, at 10 nM phenylephrine, contractile responses were significantly greater in the presence of L-SMTC or L-TC than in the presence of L-NMA (100 μ M) (L-NMA: 0.4 $\pm$ 0.11 g; L-TC: 0.78 $\pm$ 0.14 g and L-SMTC: 0.81 $\pm$ 0.17 g). The incubation of L-arginine (1 mM) with L-NMA (100 μ M) for 30 min abolished the effect of this inhibitor on the vascular hyporeactivity to phenylephrine induced by LPS (Figure 5), but only partially inhibited the increased contractions to the α_1 -agonist induced by L-TC or L-SMTC (100 μ M) in LPS-treated rings with endothelium (Figure 5). In contrast the incubation of L-citrulline (1 mM) for 30 or 60 min did not alter the effects of the three inhibitors on the production of NO⁻ induced by LPS in rat aortic rings (data not shown). In a similar manner, in LPS-treated rings without endothelium, NOS inhibitors (100 μ M) shifted the concentration-contraction curves evoked by phenylephrine significantly to the left (pEC_{50} : 7.19 $\pm$ 0.03 to 7.79 $\pm$ 0.08, 8.01 $\pm$ 0.07, and 8.02 $\pm$ 0.07 with L-NMA, L-TC, and L-SMTC respectively) and increased significantly the maximal responses from 2.05 $\pm$ 0.05 g to 2.38 $\pm$ 0.14 g, 2.5 $\pm$ 0.12 g, and 2.4 $\pm$ 0.21 g with L-NMA, L-TC, and L-SMTC, respectively (Figure 4c). In addition, at 10 nM and 30 nM phenylephrine contractions were significantly greater in the presence of L-TC or L-SMTC than in the presence of L-NMA (Figure 4c).

Following the induction of a submaximal contraction with phenylephrine (10 nM to 50 nM), L-NMA, L-TC, and L-SMTC (1 μ M–100 μ M) induced concentration-dependent contractions in aortic rings with endothelium incubated for 5 h with LPS (200 ng ml⁻¹) (Figure 6a). Contractions induced by L-SMTC were significantly greater than those induced by L-TC or L-NMA (L-SMTC > L-TC > L-NMA) (Figure 6a). In LPS-treated aortic rings without endothelium, L-TC and L-SMTC evoked concentration-dependent contractions while L-NMA exhibited small contractions at low concentrations (1 μ M–30 μ M) and relaxations at high concentrations (100 μ M–300 μ M). The submaximal contractions induced by phenylephrine were similar for the three compounds in LPS-treated rings with (L-NMA: 0.67 $\pm$ 0.1 g; L-TC: 0.62 $\pm$ 0.07 g; L-SMTC: 0.62 $\pm$ 0.07 g) and without endothelium (L-NMA: 0.86 $\pm$ 0.02 g; L-TC: 0.92 $\pm$ 0.04 g; 0.88 $\pm$ 0.03 g). The ability of these NOS inhibitors to increase phenylephrine-induced tone in LPS-treated rings with endothelium was significantly reversed by L-arginine (1 mM) but not by L-citrulline added at the maximum contractile response to L-NMA, L-TC, or L-SMTC (100 μ M) (data not shown).

Discussion

The present results demonstrate that L-NMA, L-TC, and L-SMTC exhibit similar potencies as inhibitors of the basal and agonist-stimulated NO⁻ production in control rat aortic rings with and without endothelium. In contrast, the two new arginine/citrulline analogues L-TC and L-SMTC, are more potent than L-NMA in reversing the vascular hyporeactivity to phenylephrine and increasing tone in LPS-treated rings with endothelium. This suggests that L-SMTC and L-TC have greater potency than L-NMA against the inducible isoform of NOS. Recent studies with isolated constitutive brain and inducible smooth muscle cells NOS support this finding (Narayanan & Griffith, 1994).

Endothelium containing rings of rat aorta are less sensitive to a wide range of vasoconstrictor stimuli than endothelium-denuded rings (Allan *et al.*, 1983; Frew *et al.*, 1993; Martin *et al.*, 1986). This difference is explained by the high basal production of NO⁻ by endothelial cells in this tissue. In our study, L-NMA, L-TC, and L-SMTC increased, to a similar extent, contractions evoked by phenylephrine in a dose-dependent manner in rat aortic rings with endothelium, suggesting that the three compounds are equipotent in inhibiting the basal release of NO⁻ in rat aorta. In vascular endothelial cells, the production of NO⁻ is subject to complex control and is sti-

Figure 5 Effects of L-NMA (□), L-TC (Δ), or L-SMTC (○) (100 μ M; 30 min), in the presence of L-arginine (1 mM; 30 min) on the concentration-contraction curves evoked by phenylephrine in rat aortic rings with endothelium (■) which were preincubated for 5 h in culture medium containing lipopolysaccharide (200 ng ml⁻¹). Results are presented as means $\pm$ s.e. mean of 4 different experiments and are shown in absolute values. Statistically significant differences between the untreated and NOS inhibitors-treated rings, * P < 0.05.

mulated by a large number of biological mediators such as acetylcholine, peptides, and purine nucleotides (Furchgott, 1984; Furchgott & Zawadzki, 1984). The effects of L-NMA differ according to the species studied or the source of the isolated vessels used. For example, while L-NMA inhibits basal as well as acetylcholine-stimulated production of NO⁻ in a wide range of tissues (Amezcuca *et al.*, 1989; Moore *et al.*, 1990; Rees *et al.*, 1989; 1990; Joly *et al.*, 1994), it inhibits the basal production of NO⁻ in female rat aorta but not that stimulated by vasorelaxant agents (Frew *et al.*, 1990). In our experiments in aortic rings with endothelium, L-NMA induced a dose-dependent inhibition of relaxations evoked by acetylcholine. These inhibitory effects are not specific to acetylcholine, since L-NMA also blocked relaxations evoked by adenosine triphosphate, a receptor-dependent vasodilator, as well as those evoked by a receptor-independent vasodilator, A23187 calcium ionophore. Furthermore, L-TC and L-SMTC produced equipotent effects on relaxations evoked by these three vasodilators. Taken together, these results indicate that the three compounds are comparable in their abilities to inhibit the basal and the agonist-stimulated production of NO⁻.

Treatment of aortic rings with and without endothelium with endotoxin results in a decreased reactivity to phenylephrine. That L-NMA restores contractions to this α_1 -agonist which are impaired by LPS suggests that NO⁻ production via the L-arginine pathway is involved in the alteration of vascular hyporeactivity to phenylephrine. These data confirm previous

Figure 4 Effects of L-NMA (□), L-TC (Δ), or L-SMTC (○) on the concentration-contraction curves evoked by phenylephrine in rat aortic rings (●) with (a: 10 μ M and b: 100 μ M NOS inhibitors; 30 min) and without (c: 100 μ M NOS inhibitors; 30 min) endothelium which were preincubated for 5 h in culture medium containing lipopolysaccharide (200 ng ml⁻¹). Results are presented as means $\pm$ s.e. mean of 6 different experiments and are shown in absolute values. Statistically significant differences between the untreated and NOS inhibitors-treated rings, * P < 0.05.

Figure 6 Contractions induced by L-NMA (□), L-TC (△), or L-SMTC (○) following a submaximal contraction evoked by phenylephrine (10–50 nM) in aortic rings with (a) and without (b) endothelium which were preincubated for 5 h in culture medium containing lipopolysaccharide (200 ng ml⁻¹). Results are presented as means ± s.e. mean of 4 different experiments and shown in absolute values. Statistically significant differences between the contractions evoked by L-NMA and those evoked by L-TC or L-SMTC, **P* < 0.05.

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results showing that endotoxin and cytokines induce the production of NO⁻ in cell culture (Gross *et al.*, 1991; Schini *et al.*, 1991) and in isolated blood vessels (Julou-Schaeffer *et al.*, 1990; Joly *et al.*, 1994). The fact that L-SMTC and L-TC as well as L-NMA potentiate the contractions to phenylephrine suggests that these two new L-arginine analogues inhibit the production of NO⁻ induced by LPS in rat aorta. Comparison of the vascular effects of the three compounds in this study demonstrates that L-NMA is less potent than the other two analogues, which are relatively equipotent in augmenting the contractions to phenylephrine in LPS-treated preparations. The observation that L-SMTC and L-TC produce greater dose-dependent increases in tone in LPS-treated rings with and without endothelium than L-NMA, confirms the enhanced potency of these two new compounds in inhibiting the production of NO⁻ induced by the inducible isoform of NOS, and these results are consistent with studies using isolated inducible NOS enzyme (Narayanan & Griffith, 1994). In addition, the fact that L-TC and L-SMTC have demonstrated similar potency in inducing contractions in LPS-treated rings with and without endothelium suggests that these compounds exert potent inhibitory effects on iNOS in both endothelial and smooth muscle cells of vascular wall. The observations that high concentrations of L-NMA induced relaxations in LPS-treated preparations without endothelium are consistent with previous results obtained in rat aorta (Thomas & Ramwell, 1991) or in canine coronary arteries (Winn *et al.*, 1993). Different hypotheses have been put forward to explain the relaxant effects of L-NMA: one interesting explanation is that L-NMA may have undergone metabolism to L-arginine (Hecker *et al.*, 1990); this could, in turn, lead to increased substrate availability (Hecker *et al.*, 1990).

L-Arginine is a substrate for NOS, and its availability can be rate limiting for induced NO⁻ synthesis (Julou-Schaeffer *et al.*, 1990; Schini & Vanhoutte, 1991). The fact that in LPS-treated rings the increased contractions to phenylephrine induced by L-NMA was fully abolished and those evoked by L-SMTC and L-TC were partially inhibited by L-arginine is consistent with a mechanism involving substrate-inhibitor competition. Furthermore, the observation that L-arginine reverses the increases in tone induced by these inhibitors indicates that these L-arginine analogues exert their actions by a competitive and reversible mechanism of inhibition. A difference in affinity for the inducible NOS among these inhibitors may explain the limited effect of L-arginine against L-SMTC and L-TC. However, since L-SMTC and L-TC are more potent than L-NMA in restoring the contractile response to phenylephrine in LPS-treated rings, a greater molar excess of L-arginine may be required to reverse their effects fully.

This study demonstrates that the two new arginine/citrulline analogues are as potent as L-NMA in inhibiting the NO⁻ synthesis produced by the constitutive isoform of NOS, but L-SMTC and L-TC have greater potency than L-NMA for restoring the contractile response to phenylephrine and producing increases in tone in endotoxin-treated preparations. Further experiments are required to determine whether these new compounds act only through direct inhibition of NOS or also affect L-arginine transport or utilization, resulting in changes in the intracellular L-arginine concentration.

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