Nitric Oxide Synthase Inhibition Promotes Endothelium-Dependent Vasodilatation and the Antihypertensive Effect of L-Serine

Ramesh C. Mishra, Saswati Tripathy, Kaushik M. Desai, Dale Quest, Yanjie Lu, Jawed Akhtar, Venkat Gopalakrishnan

Abstract—L-serine is a precursor of central neurotransmitters. Its cardiovascular effects are largely unstudied. We compared the in vitro effects of L-serine and acetylcholine in phenylephrine-constricted third-order branches of mesenteric arterioles in the NO synthase inhibitor N^\text{G}-nitro L-arginine methyl ester (L-NAME), pretreated hypertensive rats, and a control group of normotensive male Sprague-Dawley rats. The changes in mean arterial pressure and heart rate evoked by acute intravenous infusion of either L-serine (0.1 to 3.0 mmol/kg) or acetylcholine (0.1 to 10.0 mmol/kg) were determined in anesthetized rats. L-serine evoked concentration-dependent (10 to 200 μmol/L) vasodilation in endothelium-intact but not in endothelium-denuded vessels. It was abolished by the inclusion of a combination of apamin (SKCa channel inhibitor) and TRAM-34 (IKCa channel inhibitor) or ouabain (Na^+ pump inhibitor) and Ba^{2+} (Kp channel inhibitor) or when the vessels were constricted by potassium chloride. The maximal response to L-serine was higher in the L-NAME treatment group (control 20% versus L-NAME 40%) in relation to the maximal response to acetylcholine (control 93% versus L-NAME 79%). L-serine evoked a rapid, reversible, dose-dependent fall in mean arterial pressure without increasing heart rate and was more pronounced in L-NAME-treated rats (maximal response: >60 mm Hg) than in the control rats (maximal response: 25 mm Hg). This was inhibited (P<0.01) by apamin + charybdotoxin pretreatment. The in vitro and in vivo data confirm that L-serine promotes vasodilatation in resistance arterioles and evokes a greater fall in mean arterial pressure in NO synthase–inhibited hypertensive rats via activation of apamin and charybdotoxin/TRAM-34-sensitive KCa channels present on the endothelium. (Hypertension. 2008;51:791-796.)

Key Words: amino acids ■ blood pressure ■ endothelium ■ hypertension ■ vasodilation

The nonessential amino acid L-serine biosynthesized from glycine or threonine and the metabolism of glucose, other than being a neurotransmitter, is a precursor for nucleotides, phospholipids, and central neurotransmitters such as glycine, serotonin, and D-serine.1-5 L-Serine treatment has been attempted in the management of schizophrenia, depression, and chronic fatigue syndrome and for preventing microcephaly, psychomotor retardation, and seizures encountered in rare inborn errors of L-serine biosynthesis.2,3 Recently, N-arachidonoyl L-serine was shown to promote endothelium-dependent vasodilatation isolated in rat arteries.4 This study failed to address whether L-serine itself would promote vasodilatation or regulate blood pressure (BP). Previous studies have shown that the amino acid taurine reduces BP by reducing sympathetic discharge, oxidative stress, and increased salt excretion in hypertensive animals and patients.5-9 There are no reports on the direct cardiovascular effects of L-serine. Therefore, we investigated the in vitro effect of L-serine using third-order branches of rat mesenteric arterioles constricted with phenylephrine (PE), a preparation that represents the resistance function of circulation.10 These observations were supported by in vivo studies that examined the acute effect of L-serine infusion on the regulation of BP and heart rate (HR) in anesthetized rats.

Methods

Animals and Methods

The investigation, approved by our university review committee, conformed to the Guide for the Care and Use of Laboratory Animals stipulated by the Canadian Council on Animal Care and National Institutes of Health publication 85-23. Twelve-week-old male Sprague-Dawley rats (300 to 350 g) were obtained from Charles River (St Constant, Quebec, Canada). A group of rats received treatment with the NO synthase (NOS) inhibitor N^\text{G}-nitro-L-arginine methyl ester (L-NAME; 700 mg/L in drinking water ad libitum) for 5 days to evoke chronic inhibition of NOS in vivo.11,12 Control rats received plain water. The rats from both groups were anesthetized with an intraperitoneal injection of thiopental sodium (100 mg/kg).11,12 The detailed methodology for the measurement of mean arterial pressure (MAP) and HR in vivo and vasodilator responses in...
the third-order branches of mesenteric arterioles in vitro using a wire myograph system are provided in the online supplement (please see http://hyper.ahajournals.org).11–13

**Materials**

Acetylcholine (ACh), barium chloride (Ba2+), indomethacin, L-serine, L-NAME, ouabain, and PE were obtained from Sigma-Aldrich Canada Ltd. Apamin and charybdotoxin (ChTX) were from EMD Biosciences Inc. TRAM-34 was a gift from Dr Heike Wulff, University of California at Davis. Thiopental sodium was obtained from Abbott Laboratories Ltd. U46619 and another lot of L-serine were purchased from Calbiochem.

**Statistical Analysis**

The vasodilator responses were normalized as the percentage of response to a fixed concentration of PE. Concentration-response curves were computer fitted (Prism, GraphPad Software Inc). The change in MAP after infusion of each dose was plotted to generate the dose-response (DR) curves to L-serine and ACh. The data are expressed as means±SEMs (n=9). Differences between the means of 2 groups were tested for significance by a 1-way ANOVA followed by Tukey posthoc test. The differences were considered significant when \( P<0.05 \).

**Results**

**In Vitro Study**

L-serine ≤1 mmol/L failed to alter the basal tone. In PE-constricted arterioles with intact endothelium, L-serine evoked concentration-dependent vasodilation; its effect was higher in the vessels of L-NAME–treated rats. The data from a typical experiment that compares the vasodilator response to L-serine and ACh in endothelium-intact vessels and the lack of response to either agonist in endothelium-denuded preparations from an L-NAME–treated rat is shown (Figure 1). The response to L-serine was of slower onset with lower efficacy (Emax) in relation to the response to ACh. Like ACh, L-serine failed to evoke vasodilatation, even at very high concentrations in endothelium-denuded preparations (Figure 1). The Emax for L-serine (40±3%) in endothelium-intact vessels was shifted significantly to the left (\( P<0.01 \)) in the L-NAME treatment group compared with the Emax (20±3%) in the control group (Figure 2). The concentration-response curve to ACh was shifted to the right in the L-NAME treatment group (Figure 2). The Emax

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**Figure 1.** A representative tracing that demonstrates the pattern of vasodilator responses to the cumulative addition of increasing concentrations of either L-serine (0.1 to 500 \( \mu \text{mol/L} \)) or ACh (0.1 pmol/L to 100 \( \mu \text{mol/L} \)) in endothelium-intact (ENDO [↑] A and B) or endothelium-denuded (ENDO [↓] C and D) third-order branches of mesenteric arterioles constricted with PE (10 \( \mu \text{mol/L} \)) in vitro after isolation from a 12-week-old male Sprague-Dawley rat that received chronic L-NAME treatment (700 mg/L PO in drinking water ad libitum) for 5 days. W denotes when the tissues were washed in normal Krebs buffer to attain recovery. Similar responses were noted in vessels from 8 chronic L-NAME–treated rats.

**Figure 2.** The line graphs compare the concentration-response (CR) curves to L-serine (A) determined in PE-constricted mesenteric arterioles isolated from either normotensive control group (○) or chronic L-NAME–treated hypertensive rats (●) with intact endothelium. The lack of responses to L-serine in endothelium-denuded vessels in both control (○) and chronic L-NAME–treated (●) vessels are also shown. The CR curves to ACh (B) determined in either the control group (○) or in the chronic L-NAME treatment group (●) with intact endothelium or the lack of responses in endothelium-denuded vessels in control (○) and chronic L-NAME–treated (●) vessels are shown. Each data point represents the mean±SEM of >20 arterioles isolated from >8 rats from control and chronic L-NAME treatment groups. *\( P<0.05 \) and **\( P<0.01 \) vs data point in the control group.
values for L-serine and ACh are compared (Figure 3). In endothelium-intact arterioles, addition of the cyclooxygenase (COX) inhibitor indomethacin (10 μmol/L), apamin (SKCa inhibitor, 1 μmol/L), or TRAM-34 (IKCa inhibitor, 1 μmol/L) alone failed to significantly affect the Emax to either L-serine or ACh, whereas incubation with a combination of either apamin+TRAM-34 or ouabain (Na+ pump inhibitor, 20 μmol/L)+Ba2+ (K+ channel inhibitor, 50 μmol/L) abolished the responses to both agonists in the vessels of L-NAME–treated rats (Figure 3). The Emax values for L-serine (40%) and ACh (>90%) were similar when U46619 (1 μmol/L) was used instead of PE to evoke vasoconstriction; in contrast, in vessels preconstricted with a depolarizing concentration of KCl (80 mmol/L), L-serine failed to evoke vasodilatation (Figure S1, see http://hyper.ahajournals.org). Small increases in KCl (between 10 and 20 mmol/L) in PE-constricted vessels evoked a concentration-dependent vasodilatation, and the addition of L-serine failed to enhance the dilator response further; inclusion of Ba2++ouabain but not apamin+TRAM-34 abolished the responses to K+, confirming that efflux of K+ contributes to vasodilatation in mesenteric arterioles (Figures S2 and S3).

In Vivo Study
Basal MAP was significantly higher (P<0.01) in the L-NAME treatment group (135±6 mm Hg; n=12) compared with the control group (93±8 mm Hg; n=9). L-serine evoked a dose-dependent fall in MAP in both groups, but the onset was abrupt and more pronounced in the L-NAME–treated group (Figure 4). Apamin+ChTX infusion inhibited the depressor response to L-serine (Figure 4B). Infusion of saline alone failed to affect BP, and the dose-dependent effect persisted when pH was maintained at 7.3. The entire DR panel was reproducible a second time in either group. In chronic L-NAME–treated rats, the fall in MAP was evident, even at the low concentration of 0.3 mmol/kg, and the maximal fall attained at 3.0 mmol/kg was much higher (from 140 mm Hg to 50 mm Hg) in this group (Figure 4). The responses to relatively lower doses of L-serine were completely abolished after infusion of apamin+ChTX (Figure 4), whereas ACh infusion evoked a dose-dependent fall in MAP in both groups (Figure 5). As expected, the dose-dependent fall in MAP evoked by ACh was completely abolished only in L-NAME–treated rats that received apamin+ChTX pretreatment (Figure 5). The pooled data from several experiments revealed that the fall in MAP evoked by L-serine was much lower in the control group compared with ACh, with a minimal degree of rightward shift in the DR curve after apamin+ChTX pretreatment (Figure 6). In L-NAME–treated rats, apamin+ChTX infusion completely abolished the responses to L-serine at concentrations ranging up to 1 mmol/kg, and the rightward shift in the DR curve to L-serine was much higher in comparison with the ACh response (Figure 6). The fall in MAP evoked by L-serine and ACh was accompanied by nonsignificant increases in HR in control and L-NAME treatment groups (data not shown).

Discussion
L-Serine Activates Endothelial KCa Channels
The data are summarized as follows: (1) L-serine evoked concentration-dependent vasodilatation in endothelium-intact but not in endothelium-denuded arterioles; (2) the Emax values for l-serine were similar irrespective of whether PE or the thromboxane analog, U46619, was used; (3) the responses were unaffected by the inclusion of NOS inhibitor, L-NAME, or the COX inhibitor, indomethacin, but were abolished in the combined presence of apamin (SKCa inhibitor)+TRAM-34 (IKCa inhibitor, 1 μmol/L, 10 μmol/L, or TRAM-34 (1 μmol/L) was used; (3) the responses were completely abolished after infusion of apamin+ChTX (Figure 4), whereas ACh infusion evoked a dose-dependent fall in MAP in both groups (Figure 5). As expected, the dose-dependent fall in MAP evoked by ACh was completely abolished only in L-NAME–treated rats that received apamin+ChTX pretreatment (Figure 5). The pooled data from several experiments revealed that the fall in MAP evoked by L-serine was much lower in the control group compared with ACh, with a minimal degree of rightward shift in the DR curve after apamin+ChTX pretreatment (Figure 6). In L-NAME–treated rats, apamin+ChTX infusion completely abolished the responses to L-serine at concentrations ranging up to 1 mmol/kg, and the rightward shift in the DR curve to L-serine was much higher in comparison with the ACh response (Figure 6). The fall in MAP evoked by L-serine and ACh was accompanied by nonsignificant increases in HR in control and L-NAME treatment groups (data not shown).
inhibitor); (4) inclusion of a combination of ouabain+/Ba²⁺ pretreatment abolished the responses confirming the contribution of vascular Na⁺ pump and Kᵥ channels; (5) the responses were higher after chronic l-NAME treatment; (6) the vasodilator effect of l-serine was absent in high KCl preconstricted/depolarized vessels; and (7) in PE constricted vessels, however, the addition of small increments of KCl evoked vasodilatation, and this was abolished by ouabain+/Ba²⁺ pretreatment but not by apamin+/TRAM-34 combination. The addition of l-serine failed to further augment the dilator responses to KCl. These data suggest that, like ACh, l-serine promotes K⁺ efflux from the endothelium. This mediates vasodilatation via entry of K⁺ through the Na⁺ pump and its subsequent efflux through the Kᵥ channels present on mesenteric vascular smooth muscle cells. In addition, in vivo studies established that l-serine evoked a dose-dependent fall in MAP that was significantly higher in L-NAME–treated hypertensive rats; the antihypertensive effect of l-serine was inhibited by apamin+/ChTX pretreatment; and, whereas these observations indicate that l-serine promotes NO- and COX-independent endothelium-dependent vasodilatation, likely via selective activation of SKCa/IKCa channels present on the endothelium, the profound fall in MAP seen in L-NAME–treated rats (Emax > 70 mm Hg) suggests that l-serine might also recruit other additional mechanisms in reducing BP. The significance of these findings is discussed below.

The mean plasma concentration of serine in human adults is 130 μmol/L.² In the present study, we observed that the vasodilator responses to l-serine occur at concentrations

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**Figure 4.** A typical experiment compares the fall in MAP to acute intravenous infusion of various doses of l-serine (0.1 to 3.0 mmol/kg) in control rat before (A) and 45 minutes after (B) and in a chronic L-NAME (700 mg/L PO for 5 days) –treated rat before (C) and 45 minutes after (D) the infusion of apamin+/ChTX (75 μg/kg each).

**Figure 5.** A typical experiment compares the fall in MAP to acute intravenous infusion of various doses of ACh (0.1 to 10.0 nmol/kg) in a control rat before (A) and 45 minutes after (B) and in a chronic L-NAME–treated rat before (C) and 45 minutes after (D) the infusion of apamin+/ChTX (75 μg/kg each).
between 10 and 200 μmol/L in L-NAME–treated mesenteric arterioles. These data suggest that, under physiological conditions, elevation in plasma L-serine occurring subsequent to food intake and enhanced glucose metabolism could promote endothelium-dependent but NO- and COX-independent vasodilation to ensure increased delivery of nutrients to the tissues. This is consistent with the observation that, other than NO, endothelium-derived hyperpolarization factor (EDHF) contributes to flow-mediated vasodilatation in vivo.14

Antihypertensive Effect of Amino Acids

Central infusion of amino acids can induce hypotension within 3 minutes after administration in anesthetized rats, as was first shown in 1972. Although L-serine was stated as a hypotensive agent in the Abstract, the Results section specifically clarified that the effect of L-serine on BP was not studied. The results compared the hypotensive effect of single-dose intracisternal infusion of amino acids with the following order of efficacy: taurine > GABA > L-alanine > glycine. Taurine evoked central respiratory depression and hypothermia, whereas these effects were least with L-serine. Since then, several studies have substantiated the antihypertensive effect of taurine in hypertensive animals and patients.5–8 The evidence suggests that taurine reduces sympathetic discharge and oxidative stress and enhances salt excretion.5–9 The present study shows that acute intravenous infusion of L-serine evokes a rapid, dose-dependent fall in MAP with recovery to basal MAP after each dose. The responses were elicited for a second time without significant changes in efficacy. The fall in MAP was accompanied by an insignificant increase in HR. The present study is the first evidence that L-serine elicits a substantial antihypertensive effect in the NO-compromised state.

Recently, N-arachidonoyl L-serine, isolated from bovine brain, has been shown to promote NO- and COX-independent but endothelium-dependent vasodilation by binding to a novel receptor that is distinctly different from the classical cannabinoid receptor activated by anandamide.4 N-arachidonoyl L-serine also exerts anti-inflammatory/vascular protective effects. However, this study failed to address whether L-serine, per se, would promote vasodilation.4 L-serine inhibits γ-glutamyl transpeptidase activity and reduces leukocyte-endothelial cell interaction by blocking the bioconversion of leukotriene C4 to leukotriene D4. A dietary increase in L-serine lowers plasma homocysteine concentration.16 Elevations in plasma levels of γ-glutamyl transpeptidase and homocysteine are known risk factors for cardiovascular disease.16,17 Thus, the study of the vascular actions of L-serine is of potential clinical importance.

NOS Inhibition Augments the L-Serine Effect

In both in vitro and in vivo studies, the DR curves to L-serine were shifted to the left, whereas the DR curves to ACh were shifted to the right after chronic L-NAME treatment (Figures 2 and 6). It is known that when NO-dependent vasodilatation is compromised, EDHF compensates for the loss of NO to preserve endothelium-dependent vasodilatation.18 In the rat model, unlike ACh, which recruits both NO and EDHF, L-serine seems to selectively promote endothelial SKCa- and IKCa-mediated EDHF-dependent vasodilatation. These data are consistent with an earlier report that L-serine augmented the efflux of L-arginine, the precursor for NO, and, thus, is not linked to NO generation.19 Because L-serine–evoked vasodilatation was abolished by the apamin + TRAM-34 combination, it is tempting to suggest that L-serine promotes selective activation of EDHF. Because we have not performed in situ recording for alterations in membrane potential, it would not be appropriate to claim that L-serine activates EDHF. However, based on the data that apamin + TRAM-34 combination abolished the vasodilator effect and apamin + ChTX combination significantly reduced the hypotensive responses to L-serine in L-NAME–treated rats, it is reasonable to state that the L-serine effect may be mediated by activation of endothelial SKCa and IKCa channels. Several candidate molecules have been proposed as EDHFs (eicosatetraenoic acids, H2O2, K+, myoendothelial gap junctions, and C-type natriuretic peptide). There is blood vessel heterogeneity; there is also agonist-dependent variation in the recruitment of these messenger molecules.
candidates. Although C-type natriuretic peptide was proposed as the EDHF in mesenteric arteries, others state that the evidence is insufficient to support C-type natriuretic peptide as a bona fide EDHF. Based on our present data on a lack of vasodilator responses to L-serine in the presence of either a ouabain + Ba^2+ combination or an elevated K^+ (15 mM/L) state, it is reasonable to suggest that an increase in K^+ concentration in the myoendothelial region contributes to L-serine–evoked vasodilatation in the rat mesenteric artery. Although the vasodilator E_max for L-serine was modest (40%), the maximal fall in MAP evoked by L-serine was much higher (>70 mm Hg) after chronic NOS inhibition. This is consistent with the report that EDHF-dependent vasodilatation is less important in maintaining basal vascular conductance and that it plays a crucial role in agonist-evoked vasodilatation in mesenteric and hind-limb vascular beds. It could be argued that the greater fall in MAP in L-NAME–treated rats could be because of elevated MAP. This is unrelated to elevated MAP, because the reduction in MAP evoked by L-serine in normal rats subjected to PE infusion or in spontaneously hypertensive rats was only modest, and it was comparable to the level seen in control rats (unpublished observations). These data confirm that the acute dose-dependent response to L-serine is exaggerated when the NO system is blunted. The profound degree of fall in MAP in L-NAME–treated rats suggests that L-serine–evoked vasodilator responses may be much higher in other vascular beds, but this remains to be determined.

**Clinical Perspective**

L-serine–evoked vasodilatation is a compensatory mechanism that could be exploited to reverse elevated total peripheral resistance in the early phases of hypertension when endothelial dysfunction corresponds with impaired EDRF functionality of NO. In humans, oral treatment with L-serine prevents central resistance in the early phases of hypertension when endothelial dysfunction is blunted, and it is an avenue in the treatment of hypertension. In vitro and in vivo studies indicate that L-serine–evoked vasodilator responses are substantially increased in hypertensive vessels.

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We are grateful to Dr Heike Wulff, Department of Pharmacology, University of California at Davis, for the generous gift sample of TRAM-34.

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**Disclosures**

None.

**References**


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Methods

In Vitro Study

The rat mesentery was carefully excised, cleaned of adherent tissues. A section of mesentery around 100 mm distal to the pylorus was rapidly removed and placed in ice-cold Krebs buffer. With the aid of a dissection light microscope, the third order arterioles (inner diameter ~120 µm) were carefully isolated. Four to eight rings (~2 mm length) were cut from the isolated blood vessels to be used for isometric tension measurements. Rings were suspended between a micropositioner and force transducer with stainless steel wire (40 µm diameter) in a myograph chamber (10 ml volume), Model 610M Multi Wire Myograph System (Danish Myotechnology, Denmark). Resting tension (2 mN) was fixed for initial equilibration period of 1 h in Krebs buffer with the following composition (in mmol/L): 120, NaCl; 1.8, CaCl₂; 4.8, KCl; 1.2, MgCl₂; 1.2, KH₂PO₄; 25, NaHCO₃; 10, glucose; pH 7.4 gassed with 95% O₂, 5% CO₂ and maintained at 37°C. The rings were washed every 20 min. in buffer. Isometric tension was recorded using the Powerlab data acquisition system (AD Instruments Pvt. Ltd., Sydney, Australia). In some studies, endothelium was removed mechanically by gently rubbing the intimal surface of the rings with a thin wire before the vessels were mounted in the wire myograph. The endothelium was considered as removed if the vasodilator response to acetylcholine (ACh) was reduced to ≤ 10% of its original level (>92%) with no change in the vasodilator response to sodium nitroprusside (SNP). The concentration-response (CR) relationship for L-serine (1 µmol/L - 500 µmol/L), D-serine (1 µmol/L - 500 µmol/L) and ACh (1 pmol/L – 10 µmol/L) were determined in these vessels after sustained tonic vasoconstrictor response to a fixed concentration (≈EC₈₀) of α₁ selective agonist, phenylephrine (PE, 10 µmol/L) was attained. In some experiments, instead of
PE, vasoconstriction was attained using either the thromboxane analog, U46619 (1 µmol/L) or depolarizing stimulus with potassium chloride (KCl 80 mmol/L). When investigating the effects of various inhibitors such as a non-selective cyclooxygenase inhibitor, indomethacin (10 µmol/L), or apamin (SKCa inhibitor, 1 µmol/L) or TRAM-34 (IKCa inhibitor, 1 µmol/L), or ouabain (Na+ pump inhibitor, 20 µmol/L), or Ba²⁺ (Kir channel inhibitor, 50 µmol/L), they were added either alone or in a combination as indicated to the organ baths 20 min prior to the addition of PE. The concentration of the above agents were chosen based on the data established in an earlier study. These agents were maintained until the responses to increasing concentrations of either L-serine or ACh were determined in vessels isolated from both control and chronic L-NAME treated rats.

**In Vivo Study**

The anaesthetized rats were allowed to breathe spontaneously through a tracheal cannula. In the present study, instead of carotid artery, the femoral artery was cannulated and connected to a pressure transducer to record the changes in mean arterial pressure (MAP) and heart rate (HR) using the PowerLab data acquisition system (AD Instruments Pvt. Ltd. Sydney, Australia). Femoral vein was cannulated to administer drugs as bolus injections in a limited volume of 0.1 ml of various agents prepared in saline and the pH was adjusted to 7.3. After ensuring that the MAP and HR maintained at a stable level, the responses to bolus infusions of either L-serine (between 0.1 and 3.0 mmol/kg) or ACh (0.1 - 10 nmol/kg) were determined. Enough time was allowed between responses for the MAP to recover to the resting level. In an attempt to selectively inhibit the EDHF mediated responses in vivo, the animals were given slow infusions
of apamin (75µg/kg) followed by charybdotoxin (ChTX 75 µg/kg) in a total time period of 15 min. Although TRAM-34 would be a more selective agent for blocking endothelial IKCa channel, to avoid the effect of dimethylsulfoxide (DMSO) in which stock concentration of TRAM-34 was dissolved, we used ChTX that was soluble in saline. The MAP increased dramatically within 5 min following the slow infusion of ChTX and it returned to a level that was slightly above the resting MAP level in about 30 min. time interval. In our previous studies, we used intravenous infusions of apamin and ChTX at dose levels of 50 µg/kg each. However, in the present study, we increased the dose of each toxin to 75 µg/kg with a time interval of 45 min after infusion of the toxins to determine the dose-response (DR) curves to ACh and L-serine. This was found to be an optimal condition since the blockade of hypotensive responses to ACh were highly significant.

Figure Legends

Figure S1.
The maximal vasodilator responses (E_max) to L-serine (L-S, 500 µmol/L) attained in either phenylephrine (PE 10 µmol/L) or thromboxane analog, U46619 (1 µmol/L) or high potassium chloride (KCl, 80 mmol/L) constricted third order branches of mesenteric arterioles maintained in wire myograph after isolation from chronic L-NAME treated rats. Each data point is mean ± s.e.m. of 5 separate experiments using vessels isolated from 4 L-NAME treated rats.

** P<0.01 compared to the responses to L-S data in the presence of either PE or U46619.
Figure S2.

The data of a representative experiment of concentration dependent vasodilator responses to cumulative increases in either L-serine (Panel a: L-serine 50 - 200 µmol/L) or KCl (Panel b: KCl 9.7-18.7 µmol/L followed by L-serine 100-500 µmol/L) in PE (10 µmol/L) constricted endothelium-intact mesenteric arteriole isolated from a L-NAME treated rat. Panel c shows that in the same vessel, after pretreatment with apamin + TRAM-34 (1 µmol/L) addition of the first dose of KCl (9.7 mmol/L) evoked vasodilatation whereas when the tissues were washed and incubated with a combination of Ba\(^{2+}\) (50 µmol/L) and ouabain (20 µmol/L), either the addition of KCl (9.7 - 18.7 mmol/L) or ACh (10 µmol/L) failed to elicit vasodilatation. Note: In this particular experiment, after addition of apamin+TRAM-34, the vessels rapidly relaxed to the 96% and came back to constricted state and oscillated. In several experiments, we have noticed that apamin+TRAM-34 did not abolish the dilator responses to KCl.

Figure S3.

The bar diagram shows the pooled mean ± s.e.m. data from 4 separate experiments comparing the maximal vasodilator responses to KCl (18.7 mmol/L) attained in PE (10 µmol/L) constricted arterioles isolated from L-NAME treated rats either before (control □) or after apamin+TRAM-34 (■) or ouabain+Ba\(^{2+}\) (□) pretreatments \textit{in vitro}. ** \(P<0.01\) compared to the responses to KCL either in the absence or presence of apamin+TRAM-34.
Figure S1  Online Supplement to the Revised MS ID#: Hypertension/2007/099598-R1

% Vasodilatation

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<th>L-S PE</th>
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** LS
Prolonged exposure to potassium chloride (KCl) significantly reduced vasodilatation compared to U46619. (P<0.01, **P<0.001)**