Decreases in splanchic vascular resistance contribute to hypotensive effects of L-serine in hypertensive rats

Ramesh C. Mishra,1 Saswati Tripathy,1 Jugal D. Gandhi,1 John Balsevich,3 Jawed Akhtar,2 Kaushik M. Desai,1 and Venkat Gopalakrishnan1

1Department of Pharmacology and the Cardiovascular Research Group, 2Department of Medicine and the Royal University Hospital, College of Medicine, University of Saskatchewan, and 3Plant Biotechnology Institute, National Research Council of Canada, Saskatoon, Saskatchewan, Canada

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Mishra RC, Tripathy S, Gandhi JD, Balsevich J, Akhtar J, Desai KM, Gopalakrishnan V. Decreases in splanchic vascular resistance contribute to hypotensive effects of L-serine in hypertensive rats. Am J Physiol Heart Circ Physiol 298: H1789–H1796, 2010. First published March 26, 2010; doi:10.1152/ajpheart.00810.2009.—L-Serine administration reduces mean arterial pressure (MAP) in spontaneously hypertensive rats (SHR) and Wistar-Kyoto (WKY) rats rendered hypertensive by chronic oral treatment with the nitric oxide synthase inhibitor N\textsuperscript{o}-nitro-L-arginine methyl ester (L-NAME). To determine if the fall in MAP was due to decreases in vascular resistance or cardiac output (CO), and to record regional hemodynamic effects, we measured the distribution of fluorescent microspheres to single bolus intravenous injections of L-serine (1 mmol/kg) in 14-wk-old male WKY, SHR, and L-NAME-treated WKY rats. MAP and total peripheral resistance (TPR) were significantly higher (P < 0.01), whereas CO was lower in L-NAME-treated WKY (P < 0.01 and SHR (P < 0.05). L-Serine administration led to a rapid fall in MAP (WKY 22%, L-NAME-WKY 46%, SHR 34%), and TPR (WKY 24%, L-NAME-WKY 68%, SHR 53%), whereas CO was elevated. In WKY rats, L-serine induced an increase in blood flow only in the small intestine (53%) while it was more profound in several vascular beds of hypertensive rats [L-NAME-WKY: small intestine (238%), spleen (184%), diaphragm (85%), and liver (65%); SHR: small intestine (217%), spleen (202%), diaphragm (116%), liver (85%), and large intestine (105%); pancreas (96%), and liver (93%). Pretreatment with a combination of apamin (a small calcium-activated potassium channel inhibitor) and charybdotoxin (an intermediate calcium-activated potassium channel inhibitor) abolished the L-serine-induced changes in blood flow and TPR. L-Serine acts predominantly on apamin- and charybdotoxin-sensitive potassium channels in the splanchic circulation to increase blood flow, thereby contributing to the fall in TPR and the pronounced blood pressure-lowering effect of L-serine in hypertensive rats.

**fluorescent microspheres; N\textsuperscript{o}-nitro-L-arginine methyl ester; L-serine; regional blood flow; spontaneously hypertensive rats**

THE PLASMA CONCENTRATION OF L-SERINE IS 130 µMOL/L IN BOTH HUMANS AND RATS (6, 7, 21). RECENTLY, WE DEMONSTRATED THAT IN VITRO ADDITION OF L-SERINE (10–200 µMOL/L) EVOKED CONCENTRATION-DEPENDENT, ENDOTHELIUM-MEDIATED, BUT NITRIC OXIDE (NO)-AND PROSTANOID-INDEPENDENT VASODILATION IN PHENYLEPHRINE-CONSTRICTED THIRD-ORDER BRANCHES OF RAT MESENTERIC ARTERIOLES SET UP IN A WIRE MYOGRAPH APPARATUS. THE MAXIMAL VASODILATOR RESPONSES EVOKED BY L-SERINE WERE HIGHER IN VESSELS ISOLATED FROM RATS RENDERED HYPERTENSIVE BY 4 DAYS OF CHRONIC ORAL TREATMENT WITH THE NITRIC OXIDE SYNTHASE (NOS) INHIBITOR, N\textsuperscript{o}-NITRO-L-ARGININE METHYL ESTER (L-NAME). SUCH ENDOTHELIOUM-DEPENDENT VASODILATOR RESPONSES TO L-SERINE WERE SUGGESTED TO BE MEDIATED VIA RECRUITMENT OF ENDOTHELIAL CALCIUM-ACTIVATED POTASSIUM (K\textsubscript{Ca}) CHANNELS (25). IN SUPPORT OF THIS IN VITRO OBSERVATION, IN VIVO STUDIES PERFORMED BY ACUTE SINGLE BOLUS INTRAVENOUS ADMINISTRATION OF L-SERINE LED TO A RAPID, REVERSIBLE, AND DOSE-DEPENDENT (0.1–3 MMOL/KG) FALL IN MEAN ARTERIAL PRESSURE (MAP). THE MAXIMAL FALL IN MAP EVOKED BY L-SERINE (3.0 MMOL/KG) WAS SIGNIFICANTLY HIGHER IN BOTH L-NAME-INDUCED HYPERTENSION RATS (93 MMHG) AND SPONTANEOUSLY HYPERTENSIVE RATS (SHR, 81 MMHG) THAN THE FALL RECORDED IN NORMOTENSIVE SPRAGUE-DAWLEY (30 MMHG, I.E., 25%) OR WISTAR-KYOTO (WKY 46 MMHG) RATS (25, 26). THESE DATA LED US TO HYPOTHESIZE THAT L-SERINE COULD TARGET SEVERAL VASCULAR BEDS TO PROMOTE VASODILATION AND PERHAPS RECRUIT MULTIPLE MECHANISMS TO EVOLVE A PRONOUNCED BLOOD PRESSURE (BP)-LOWERING EFFECT IN HYPERTENSIVE RATS.

Importantly, we had not determined if the BP-lowering effect of L-serine in hypertensive animals was due to a decrease in total peripheral resistance (TPR) or a fall in cardiac output (CO). Moreover, the contribution of the various regional vascular beds to the overall systemic hemodynamic effects of L-serine has not been studied. Therefore, the present study used the fluorescent microsphere distribution technique to quantify the changes in regional blood flow, regional vascular resistance in different tissues, and the systemic hemodynamic effects evoked by L-serine. The changes in MAP, heart rate (HR), TPR, and CO following acute intravenous infusion of a sub-maximal dose of L-serine (1 mmol/kg) were determined in anesthetized 14-wk-old male normotensive WKY, SHR, and WKY rats rendered hypertensive by chronic oral treatment with the NOS inhibitor, L-NAME.

**MAterials AND METHODS**

**Animals.** Twelve-week-old male WKY rats (320–350 g) and SHR (250–270 g) obtained from Charles River (St. Constant, Quebec, Canada) were acclimatized for 1 wk and used in the study. This study was approved by our University Committee for Animal Care and Supply, which follows the guidelines established by the Canadian Council on Animal Care and the United States National Institutes of Health (NIH publication No. 85-23, revised 1996). One group of WKY rats received L-NAME (0.7 mg/ml in drinking water given ad libitum) for 4 days (8, 25, 26, 30). All other rats received plain water. The rats were 14 wk old at the time of experiments. SHR and L-NAME-treated rats served as hypertensive models, whereas WKY rats served as the control group.

**Surgical procedure and drug infusions.** All rats employed in the study were fasted for a period of at least 8 h before the microsphere administration was carried out. Rats were anesthetized with thiopental...
sodium (100 mg/kg ip) as described earlier (8, 30). Body temperature was maintained at 37°C with a controlled heating pad. The trachea was cannulated to allow spontaneous breathing. The right femoral artery was cannulated with polyethylene tubing (PE-50) and connected to a pressure transducer to record changes in MAP and HR using a PowerLab data acquisition system (AD Instruments, Sydney, Australia). The left femoral vein was cannulated and connected to a reciprocal syringe pump (Harvard Apparatus, Quebec, Canada) for collecting the reference blood sample (19). The left femoral vein was cannulated to administer saline (vehicle) and l-serine (prepared in saline, pH adjusted to 7.3) given as a bolus injection (1 mmol·0.4 ml⁻¹·kg⁻¹). The right carotid artery was cannulated with PE-50 tubing connected to a pressure transducer. A cannula was guided through the common carotid artery in the left ventricle to administer the fluorescent microsphere, and it was ensured that different colors of microspheres were infused either before (control) or soon after the bolus administration of l-serine. The placement of the cannula tip in the left ventricle was confirmed by pressure waveform as described earlier (12, 14). In some studies, a combination of apamin plus charybdotoxin (75 µg·kg⁻¹ of each) was infused slowly over a 10-min period, and this was administered 45 min before bolus administration of l-serine to ensure that small (SK_Ca)- and intermediate (IK_Ca)-conductance K_Ca channels were blocked in vivo as established earlier (10, 12, 14, 32).

**Hemodynamic measurements using fluorescent microspheres.** Four different colors (green, yellow, red-orange, and carmine) of 15-µm-diameter fluorescent microspheres (FluoSpheres) were purchased and stored at 4°C. It is now feasible to infuse up to four different colors of microspheres in the same rat to determine the changes in organ blood flow both before and after administration of different drugs by carefully pairing microspheres that have different absorption/emission wavelength characteristics (4, 10, 12, 14, 18, 32). Microspheres were sonicated and vigorously vortexed for 2–3 min just before use to avoid sedimentation, then diluted to a final volume of 0.3 ml. Microspheres (80,000–100,000) were suspended in 0.3 ml of 0.9% saline containing 0.01% wt/vol Tween 20. Microspheres were vortexed and immediately injected in the left ventricle through the carotid artery cannula over a period of 20 s and flushed with 0.3 ml saline over a period of 20 s. Concurrently, for the reference blood sample, blood was withdrawn from the femoral artery, downstream from the site of microsphere injection, at a rate of 0.5 ml/min, starting 10 s before simultaneous infusion of test drug and microspheres by injection and continued for a further 70 s. The reference blood sample was transferred to a tube with anticoagulant, mixed well, and kept on ice (19). This procedure was carried out first with vehicle (saline) administration to get the baseline values and then with l-serine (1 mmol/kg) administration after 10 min, by employing a different-colored microsphere (14, 18, 19) to determine l-serine-induced changes in various hemodynamic parameters in the same animal. An overdose of thiopental was administered 10 min after the last microsphere injection to terminate the experiment, and all organs were rapidly removed.

The heart, left and right kidney, liver, lungs, spleen, brain, stomach, small and large intestine, diaphragm, and pancreas were collected in whole or part to quantify the fluorescence intensity of the dye from microspheres captured in each tissue. Briefly, the organs were removed and rinsed with cold saline, patted dry, weighted, and placed in 15-ml glass tubes. Both the reference blood samples and tissues were digested for 24–48 h in 3–4 ml of potassium hydroxide (4.0 M) with 0.01% wt/vol Tween 20. Microspheres were sonicated and vigorously vortexed for 2–3 min just before use to avoid sedimentation, then diluted to a final volume of 0.3 ml. Microspheres (80,000–100,000) were suspended in 0.3 ml of 0.9% saline containing 0.01% wt/vol Tween 20. Microspheres were vortexed and immediately injected in the left ventricle through the carotid artery cannula over a period of 20 s and flushed with 0.3 ml saline over a period of 20 s. Concurrently, for the reference blood sample, blood was withdrawn from the femoral artery, downstream from the site of microsphere injection, at a rate of 0.5 ml/min, starting 10 s before simultaneous infusion of test drug and microspheres by injection and continued for a further 70 s. The reference blood sample was transferred to a tube with anticoagulant, mixed well, and kept on ice (19). This procedure was carried out first with vehicle (saline) administration to get the baseline values and then with l-serine (1 mmol/kg) administration after 10 min, by employing a different-colored microsphere (14, 18, 19) to determine l-serine-induced changes in various hemodynamic parameters in the same animal. An overdose of thiopental was administered 10 min after the last microsphere injection to terminate the experiment, and all organs were rapidly removed.

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**Measurement of hemodynamic.** An F-2500 fluorescence spectrometer (Hitachi, Tokyo, Japan) was used to measure fluorescence intensity at excitation and emission wavelengths between 350 and 750 nm using 5-nm slit width. Each individual sample was read in a 5-ml quartz cuvette in duplicate (4, 15, 19, 32).

**Validation of regional hemodynamics.** Saline administration did not affect the relative distribution of microspheres or evoke changes in MAP. Because regional hemodynamic study is performed for the first time in our laboratory, before investigating the effect of l-serine, it was important to validate our data with responses to an endothelium-dependent vasodilator agonist, acetylcholine (ACh). A single bolus intravenous administration of a submaximal dose of ACh (10 nmol/kg) significantly (\( P < 0.001 \)) increased the blood flow to the heart (47% increase from control value) and both kidneys (32%) of WKY rats compared with saline administration in the same rats. ACh administration failed to affect the blood flow to heart and kidneys in hypertensive rats (l-NAME-treated WKY and SHR). Moreover, both basal and ACh-evoked increases in blood flow values were closely similar for the left and right kidneys with a variation of <5% between them (Fig. 1). The blood flow to the liver was the lowest in all three groups, and it remained unaffected following ACh administration. The sequence of saline (vehicle) and ACh administration did not affect the responses to ACh in all three groups.

**Materials.** ACh, l-serine, and l-NAME were purchased from Sigma-Aldrich Canada (Oakville, Ontario, Canada); thiopental sodium was from Abbott Laboratories (St. Laurent, Quebec, Canada). Apamin and charybdotoxin were purchased from EMD Biosciences (La Jolla, CA). Fluorescent microspheres were purchased from Invitrogen (Carlsbad, CA).
RESULTS

MAP values and the effect of L-serine. MAP was elevated in L-NAME-treated WKY and SHR groups compared with the age-matched normotensive WKY group (Table 1). The data from a representative experiment comparing the effect of a submaximal dose of L-serine on MAP changes in a rat from each group are shown (Fig. 2, A–C). The data show that apamin plus charybdotoxin pretreatment abolished the hypertensive response to L-serine in all three rats (Fig. 2, A–C).

L-Serine (1 mmol/kg) administration led to a much greater fall in MAP in both L-NAME-treated WKY and SHR strains than in the WKY group (Fig. 2 and Table 1).

Comparison of systemic hemodynamic effects of L-serine. MAP and TPR values were higher in both L-NAME-treated WKY and SHR models compared with the WKY rats (Table 1). CO was lower in both L-NAME-treated WKY and SHR models. L-Serine administration significantly reduced MAP and TPR in the control WKY group. The fall in MAP and TPR was significant in L-serine-induced hypertensive rats (P < 0.001) and in the SHR (P < 0.01). The reduced levels of CO seen in both hypertensive rat models were elevated following L-serine administration such that they were higher than the basal values in WKY rats (Table 1).

Comparison of basal blood flow changes. Saline administration did not affect basal blood flow values in all the three groups (WKY, L-NAME-treated WKY, and SHRs). In normotensive WKY rats, among the total of 12 organs studied, the left and right kidneys received the highest blood flow (5.4–6.0 ml·min⁻¹·g⁻¹) followed by heart (3.4–3.7 ml·min⁻¹·g⁻¹). All other organs received blood flows between 1.0 and 1.5 ml·min⁻¹·g⁻¹, with the exception of liver, which received the lowest level (~0.4 ml·min⁻¹·g⁻¹) of blood flow (Fig. 3, A–C).

In chronic L-NAME-treated WKY rats, the basal regional blood flow values were relatively lower in most organs studied with the exception of heart and brain (Fig. 3, A and B). However, the decreases in basal blood flow to the spleen (P < 0.01), small intestine, and both kidneys (P < 0.05) were significant compared with their respective basal blood flow values in the control group of WKY rats. In the SHR, a significant (P < 0.01) reduction in blood flow to several organs was noted compared with WKY rats (Fig. 3). The greatest reduction was in the kidneys and the spleen (P < 0.01) followed by small intestine, pancreas, and large intestine (P < 0.05) (Figs. 3 and 4). Despite the reduction in blood flow to most tissues, the regional blood flow to the heart (P < 0.01) was significantly higher in the SHR group (Fig. 3, A and B).

L-Serine-induced changes in regional blood flow. In the normotensive WKY rats, the L-serine-induced increase in blood flow (given as % increase above the control value) was significant only in the heart (34%, P < 0.05) and small intestine (53%, P < 0.05) compared with saline infusion values in the same group (Fig. 3, A and B). In L-NAME-treated WKY rats, the increases in blood flow were significant in the following order: small intestine (238%, P < 0.01), spleen (184%, P < 0.01), diaphragm (85%, P < 0.01), large intestine (69%, P < 0.05), liver (65%, P < 0.05), heart (59%, P < 0.01), and kidneys (34–36%, P < 0.05) (Figs. 3 and 4). In the SHRs, the increases in blood flow were noted in several tissues in the following rank order: small intestine (217%, P < 0.01) > spleen (202%, P < 0.01) > diaphragm (116%, P < 0.05), large intestine (105%, P < 0.05), pancreas (96%, P < 0.05), and kidney (82%, P < 0.05) (Figs. 3 and 4). In tissues or organs in which L-serine administration elevated blood flow to a significant extent, pretreatment with the apamin plus charybdotoxin combination normalized it to a level equivalent to that seen following saline treatment (Figs. 3 and 4). Blood flows to the stomach and lungs were not significantly affected by L-serine administration in any of the groups (Fig. 3). The increase in blood flow evoked by L-serine was significant in the diaphragm of L-NAME-treated WKY and SHR, and this was also seen in another skeletal muscle, gluteal muscle (Fig. 4). Pretreatment with the apamin plus charybdotoxin combination abolished L-serine-induced elevated blood flow to both diaphragm and gluteal muscle (Fig. 4).

Differences in the basal regional vascular resistance. Chronic L-NAME treatment of WKY rats (Table 2) led to an elevation in vascular resistance. This was significant (P < 0.01) in small intestine (127%), spleen (148%), and large intestine (114%) followed by a significant (P < 0.05) increase in all other tissues [liver (66%), diaphragm (34%), kidney (85%), pancreas (60%), stomach (65%), and lungs (61%)] compared with the vascular resistance in WKY rats receiving

Table 1. Systemic hemodynamic changes evoked by L-serine (1 mmol/kg iv) in 14-wk-old male normotensive WKY rats, WKY (L-NAME), and SHR

<table>
<thead>
<tr>
<th>Parameters</th>
<th>WKY</th>
<th>WKY (L-NAME)</th>
<th>SHR</th>
</tr>
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<tbody>
<tr>
<td>Body wt, g</td>
<td>340 ± 16</td>
<td>322 ± 9</td>
<td>272 ± 15</td>
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<tr>
<td>MAP, mmHg</td>
<td>108 ± 4</td>
<td>84 ± 5⁵</td>
<td>142 ± 7⁶</td>
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<td>HR, beats/min</td>
<td>357 ± 3</td>
<td>381 ± 9</td>
<td>368 ± 11</td>
</tr>
<tr>
<td>CO, ml/min</td>
<td>103 ± 3</td>
<td>114 ± 4</td>
<td>79 ± 5⁵</td>
</tr>
<tr>
<td>TPR, mmHg·ml⁻¹·min⁻¹</td>
<td>1.05 ± 0.04</td>
<td>0.80 ± 0.03³</td>
<td>1.79 ± 0.02⁴</td>
</tr>
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</table>

Each data point is the mean ± SE of 6 rats. WKY, Wistar-Kyoto rats; WKY (L-NAME), WKY rats rendered hypertensive by chronic oral treatment with N⁶-nitro-L-arginine methyl ester (L-NAME); SHR, spontaneously hypertensive rats; MAP, mean arterial pressure; HR, heart rate; CO, cardiac output; TPR, total peripheral resistance. ⁵P < 0.05, ⁶P < 0.01, and ⁷P < 0.001 compared with vehicle treatment in the same group. ³P < 0.05 and ⁴P < 0.01 compared with respective data of WKY group.
saline administration (Table 2). Pretreatment with L-NAME did not elevate resistance in the heart or brain (Table 2). In the SHR group (Table 2), a significant increase \((P < 0.01)\) in vascular resistance was present in all tissues except heart and brain.

**Effects of L-serine on regional vascular resistance.** Only in the small intestine of the control group of WKY rats did L-serine administration produce a significant decrease (42%) in regional vascular resistance (Table 2). However, L-serine lowered vascular resistance in most vascular beds of L-NAME-treated WKY rats (Table 2). This was significant \((P < 0.001)\) in small intestine (80%), spleen (61%), and liver (61%), followed by \((P < 0.01)\) in diaphragm (59%), large intestine (56%), pancreas (54%); and \((P < 0.05)\) in both kidneys (45%) (Table 2).

**DISCUSSION**

**Validation of microsphere distribution.** The following findings validate that the regional hemodynamics data gathered with L-serine are appropriate. First, the basal values for volume of blood flow distribution to different organs following saline administration was very profound, and it was normalized to a level seen in the saline-treated normotensive WKY group (Table 2). With the exception of heart, stomach, brain, and lungs, administration of L-serine lowered vascular resistance in almost all other tissues in the SHR group in the following rank order: \((P < 0.001)\) in small intestine (66%), spleen (64%), liver (61%); \((P < 0.01)\) in diaphragm (59%), large intestine (56%), pancreas (54%); and \((P < 0.05)\) in both kidneys (45%) (Table 2).

**Fig. 2.** A representative experiment that compares the lack of fall in mean arterial pressure (MAP) following iv single bolus administration of vehicle (saline 0.4 ml/kg) and the fall in MAP following L-serine (L-S, 1 mmol/kg) bolus administration in 14-wk-old male WKY rat (A), WKY (l-NA) rat (B), and SHR (C). The horizontal bar with arrows indicates the time point when the microspheres were administered. The microspheres were also administered in all three groups either before or after pretreatment with a slow infusion of a combination of apamin and charybdotoxin (ChTX) (75 \(\mu g/kg\) each) over 15 min, and the responses to L-serine were determined after 45 min. Similar data as shown were reproduced in 7 rats under each group.

**Fig. 3.** Regional blood flow (ml·min\(^{-1}\)·g\(^{-1}\)) in heart, left and right kidneys (A), small intestine, spleen, brain (B), liver, lungs, and stomach (C) of 14-wk-old male WKY, WKY (l-NA), and SHR subsequent to bolus administration of L-serine (1 mmol/kg, filled bars) or the responses to L-serine determined after 45 min in rats subjected to pretreatment with a combination of apamin and charybdotoxin (75 \(\mu g/kg\) of each, hatched bars) infusion. The data are compared with vehicle administration (iv administration of saline 0.4 ml/kg, open bars) performed in the same groups, which did not affect basal blood flow to all organs/tissues studied. Each bar is a mean ± SE value of 6 rats. *\(P < 0.05\) and **\(P < 0.01\) compared with respective saline treatment value in the same group. †\(P < 0.01\) compared with data for L-serine treatment in the same group. a\(P < 0.05\) and b\(P < 0.01\) compared with respective data for the WKY group.
Regional and Systemic Hemodynamic Effects of L-serine

**A**

Fig. 4. Comparison of regional blood flow (ml·min⁻¹·g⁻¹) in large intestine, pancreas (A), diaphragm, and gluteal muscle (B) following iv administration of either saline (open bars) or L-serine (1 mmol/kg, filled bars) or the responses to the same dose of L-serine administration tested after pretreatment with a combination of apamin (Apa) + charybdotoxin (75 μg/kg, hatched bars) infusion in WKY, WKY (L-NAME), and SHR. Each bar is a mean ± SE value of 6 rats. *P < 0.05 and **P < 0.01 compared with respective saline treatment group. †P > 0.05 compared with data for the WKY group. ‡P > 0.05 compared with data for WKY group.

administration reported in the present study is consistent with data reported by other laboratories (4, 5, 10, 1213–19, 29, 32). Second, the variation in blood flow levels between the two kidneys (right vs. left) is much lower (<5%) than the accepted level of a difference of <15% reported by other studies (15, 17). Third, although a high concentration of ACh is known to enhance vasodilatation in all vascular beds under both in vitro and ex vivo conditions, previous studies using the microsphere distribution technique have shown that infusion of ACh at a rate of 2 μmol·kg⁻¹·min⁻¹ promotes a significant increase in blood flow to the heart and kidneys (15, 17). Consistent with the above data, intravenous administration of a single bolus submaximal dose of ACh (10 mmol/kg) for an appropriate comparison with L-serine administration increased the blood flow to heart and kidneys (Fig. 1).

**Mechanism of the hemodynamic effects of L-serine.** It could be argued that a greater fall in MAP evoked by L-serine in hypertensive animals was simply a consequence of the higher baseline values, thereby increasing the potential for a greater hypotensive response. However, when MAP was raised by a constant infusion of phenylephrine, the fall in MAP evoked by L-serine was small and comparable to normotensive rats (25). Thus the exaggerated responses to L-serine in hypertensive rats cannot be attributed simply to a baseline effect. It could also be argued that the greater effect of L-serine to lower MAP in hypertensive animals was nothing more than a reflection of the remodeled arterial walls known to occur in chronic hypertension, which could result in a generalized amplifier effect. However, the fall in MAP evoked by L-serine was more pronounced in male WKY rats subjected to L-NAME treatment for 4 days [MAP before L-serine treatment was 142 ± 7 mmHg; after 1 mmol/kg L-serine treatment it was 77 ± 6 mmHg; fall = 65 mmHg] than in an age-matched 14 wk-old male SHR strain [MAP before L-serine treatment 166 ± 6 mmHg and after treatment 109 ± 7 mmHg; fall = 57 mmHg]. Although vascular remodeling is likely present in the established phase of hypertension in 14 wk-old SHR, it is unlikely that L-NAME-treated rats would have undergone significant vascular modeling following 4 days (96 h) of chronic L-NAME treatment. These data suggest that vascular remodeling and elevated MAP per se do not account for the greater degree of fall in MAP following single bolus administration of L-serine in hypertensive animals.

The data from the present study suggest that the effect of L-serine in the small intestinal and the splanchnic regional circulation contributes largely to the changes in systemic hemodynamic parameters such as the reduction in TPR and the associated fall in MAP in all three groups. In addition, L-serine also enhanced blood flow and reduced vascular resistance in both hypertensive rat models in the abdominal visceral region.

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**Table 2. Regional vascular resistance evoked by bolus iv infusion of vehicle (saline 0.4 ml/kg) or L-serine (1 mmol/kg, 0.4 ml/kg) in 14-wk-old male WKY (320–350 g), WKY (L-NAME) (320–350 g), and SHR (250–270 g)**

<table>
<thead>
<tr>
<th>Tissue</th>
<th>WKY Control (saline)</th>
<th>WKY Control (L-serine)</th>
<th>WKY (L-NAME) (saline)</th>
<th>WKY (L-NAME) (L-serine)</th>
<th>SHR (saline)</th>
<th>SHR (L-serine)</th>
</tr>
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<tbody>
<tr>
<td>Small intestine</td>
<td>81 ± 5.9</td>
<td>47 ± 3.5*</td>
<td>184 ± 10.3*</td>
<td>38 ± 5.4*</td>
<td>199 ± 16.6*</td>
<td>68 ± 17.3*</td>
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<tr>
<td>Spleen</td>
<td>75 ± 7.9</td>
<td>59 ± 8.3</td>
<td>186 ± 7.3*</td>
<td>73 ± 6.7*</td>
<td>245 ± 27.6*</td>
<td>87 ± 17.2*</td>
</tr>
<tr>
<td>Liver</td>
<td>256 ± 16.7</td>
<td>220 ± 15.9</td>
<td>426 ± 16.4*</td>
<td>210 ± 14.8*</td>
<td>563 ± 21.4*</td>
<td>217 ± 18.3*</td>
</tr>
<tr>
<td>Diaphragm</td>
<td>76 ± 8.7</td>
<td>59 ± 8.4</td>
<td>102 ± 7.8*</td>
<td>49 ± 8.1*</td>
<td>148 ± 12.2*</td>
<td>61 ± 16.0*</td>
</tr>
<tr>
<td>Large intestine</td>
<td>77 ± 9.4</td>
<td>72 ± 10.2</td>
<td>165 ± 12.6*</td>
<td>89 ± 14.1*</td>
<td>256 ± 20.1*</td>
<td>114 ± 17.7*</td>
</tr>
<tr>
<td>Left kidney</td>
<td>20 ± 2.5</td>
<td>17 ± 2.4</td>
<td>37 ± 2.6*</td>
<td>28 ± 2.7</td>
<td>58 ± 3.1*</td>
<td>32 ± 3.0*</td>
</tr>
<tr>
<td>Right kidney</td>
<td>19 ± 1.4</td>
<td>16 ± 1.4</td>
<td>35 ± 3.4*</td>
<td>27 ± 3.8</td>
<td>57 ± 3.5*</td>
<td>31 ± 3.5*</td>
</tr>
<tr>
<td>Pancreas</td>
<td>93 ± 8.5</td>
<td>90 ± 12.3</td>
<td>149 ± 14.8*</td>
<td>139 ± 15.2</td>
<td>268 ± 14.1*</td>
<td>122 ± 17.8*</td>
</tr>
<tr>
<td>Heart</td>
<td>30 ± 4.4</td>
<td>22 ± 4.0</td>
<td>35 ± 4.4</td>
<td>21 ± 3.4*</td>
<td>32 ± 4.0</td>
<td>26 ± 3.5*</td>
</tr>
<tr>
<td>Stomach</td>
<td>116 ± 10.2</td>
<td>126 ± 13.3</td>
<td>191 ± 15.3*</td>
<td>171 ± 15.0</td>
<td>272 ± 27.4*</td>
<td>234 ± 23.2</td>
</tr>
<tr>
<td>Brain</td>
<td>112 ± 6.4</td>
<td>117 ± 6.1</td>
<td>134 ± 21.2</td>
<td>130 ± 19.9</td>
<td>143 ± 16.9</td>
<td>97 ± 16.8</td>
</tr>
<tr>
<td>Lungs</td>
<td>125 ± 14.5</td>
<td>115 ± 12.7</td>
<td>201 ± 21.7*</td>
<td>179 ± 22.2</td>
<td>263 ± 24.9*</td>
<td>211 ± 22.1</td>
</tr>
</tbody>
</table>

Each data point is the mean ± SE of 6 rats. *P < 0.05, **P < 0.01, and ***P < 0.001 compared with vehicle treatment in the same group. †P < 0.05 and ‡P < 0.01 compared with respective data of WKY group.
namely the spleen, liver, diaphragm, and, to a minor extent, in
the large intestine. These data confirm that L-serine predomi-
nantly targets the blood vessels in the abdominal region. 
Although basal MAP levels were elevated in both L-NAME-
treated WKY and SHR strains, there were differences in the 
regional vascular resistance and tissue selectivity between the 
L-NAME-treated WKY and SHR. In the case of SHR, elevated 
regional vascular resistance was present in almost all organs/
tissues studied except brain and heart, whereas the increase in 
vascular resistance was pronounced in the abdominal vascular 
beds in the L-NAME-treated WKY rats. Moreover, L-serine 
lowered vascular resistance in the pancreas and both kidneys in 
the SHR group and not in the L-NAME-treated hypertensive 
WKY rats. Thus there are subtle differences in the regional 
vascular bed flow responses to L-serine between L-NAME-
treated hypertensive rats and SHR. Pretreatment with a com-
bination of apamin (a SKCa inhibitor) plus charybdotoxin (an 
IKCa inhibitor) infusion abolished the enhanced regional blood 
flow changes evoked by L-serine. When TRAM-34, a more 
selective IKCa inhibitor than charybdotoxin, was combined 
with apamin (n = 2), it also gave similar results to the apamin 
plus charybdotoxin combination. This confirms that the in vivo 
regional hemodynamic changes evoked by L-serine are linked 
to selective activation of SKCa and IKCa channels in the small 
intestinal and splanchnic vascular circuits as suggested earlier 
(20, 25, 26).

**Amino acids and BP.** It has been known for a long time that 
ingestion of dietary proteins and gluconeogenic amino acids 
like L-serine increase glomerular filtration rate and renal 
plasma flow in both animal models and humans (2, 3). Previous 
studies have shown that plasma and tissue levels of L-serine 
were lower in SHR compared with WKY rats of the same age 
(21). Recent studies have reinforced that amino acids such as 
glutamic acid and taurine have significant BP-lowering effects 
in humans (31, 36). Despite these studies, there are no system-
atic studies that establish the direct vascular effects of amino 
acids.

**L-Serine targets the splanchnic vascular bed in hypertensive 
rats.** Recently, we established that L-serine-evoked concentra-
tion-dependent endothelium-mediated vasodilatation was 
mediated by activation of endothelial KCa channels (20, 25, 26). 
Others have showed that N-octanoyl serine also evoked NO-
independent vasodilatation (24). Acute intravenous adminis-
tration of L-serine evoked a much higher fall in MAP in SHR and 
L-NAME-treated WKY rats (25, 26). However, the degree of 
vasodilatation evoked by L-serine in our in vitro study was 
relatively much lower in the mesenteric arteries isolated from 
control (20%) vs. L-NAME-treated (42%) rats compared with the 
in vivo effect (25). The regional hemodynamic effects of 
L-serine obtained in the present study support our in vitro and 
in vivo findings (25, 26). Moreover, the observation of a 
dose-dependent fall in MAP following acute intravenous bolus 
administration of L-serine to newborn piglets was accompanied 
by a significant reduction in splanchnic vascular resistance and 
enhanced blood flow, particularly to the small intestine (un-
published observation, Dr. Po-Yin Chueng’s laboratory, 
University of Alberta). Taken together, all these data confirm that 
the effect of L-serine predominantly occurs in the small intesti-
tinal and splanchnic vascular beds, and this may contribute to 
the fall in systemic vascular resistance.

L-Serine administration had no effect on blood flow changes 
or vascular resistance in the stomach, brain, or lungs. Although 
the blood flow is higher in the diaphragm, it is not known 
whether that is because of changes in the visceral blood flow 
by collateral circuits. However, examination of blood flow 
changes in another skeletal muscle (gluteus muscle) also gave 
identical results. Previous studies have shown that a splanchnic 
vascular network supplied via the celiac, superior, and inferior 
mesenteric arteries that branch off from the aorta give rise to 
small arteries that branch and anastomose extensively to main-
tain blood flow to the visceral organs (28). Blood flow to this 
region accounts for 25% of the overall CO, and it is enhanced 
in the postprandial state (11, 28). The intrinsic mechanisms and 
the metabolic factors that govern intestinal vasodilatation un-
der such conditions are not fully understood, but adenosine is 
recognized as a potent vasodilator of the splanchnic vascular 
bed (27). It is not known whether L-serine-induced vasodilata-
tion is linked in some ways to vascular adenosine receptor 
activation/purinergic transmission (27). Alternatively, it is also 
possible that L-serine is transported through the neutral amino 
acid transporter, LAT-1, into endothelial cells, and the move-
ment of L-serine through this transporter may be linked to 
avtivation of endothelial KCa channels to promote vasodilata-
tion (20). Under the in vivo setting, the high level of L-serine 
generated following ingestion of carbohydrate and protein-rich 
diet in the gut may be transported through the same transporter 
in the intestinal epithelium and the vascular endothelium dur-
ing the process of absorption. Thus elevated L-serine levels 
could serve as a candidate that may selectively promote splanchnic 
vasodilatation to enhance food absorption (1b). The 
data from the present study provide the impetus to pursue all of 
these possibilities in the future. The L-serine-induced increased 
blood flow and reduced TPR is not linked to activation of the 
endothelial NO system, since the responses to L-serine were 
more much higher in chronic L-NAME-treated hypertensive rats. 
This is consistent with an earlier report that L-serine in fact 
promotes the efflux of arginine, the substrate for NO, and thus 
L-serine responses are not linked to generation of NO (22).

Although L-serine enhanced hepatic blood flow in hypertensive 
rats, it was relatively lower, since liver receives the majority of 
blood through the hepatic portal vein and a minor fraction from 
the hepatic artery (28). Thus most of the microspheres coming 
to this region would be trapped in the splanchnic organs before 
entering the liver through the hepatic portal vein, and our 
microspheres would have measured only the portion of blood 
received by liver through the hepatic artery. Thus limited blood 
flow determination in liver is a physical limitation of this 
technique. L-Serine administration failed to affect the blood 
flow to lungs, brain, and stomach. Theoretically, pulmonary 
blood flow should be equal to CO. Lung blood flow could not 
be correctly determined by this technique because the micro-
spheres were injected directly in the left ventricle. It may be 
possible that our microspheres would have measured only the 
lung nutritive blood flow. To summarize the overall data, based 
on blood flow changes and vascular resistance in different 
vascular beds, it is reasonable to conclude that the major effect 
of L-serine is localized to small intestine and splanchnic vas-
cular beds. Thus the blood flow changes in the visceral region 
following acute L-serine administration could contribute to the 
alterations in systemic hemodynamic changes of fall in TPR 
and MAP.
Physiological significance and clinical relevance. Oral treatment with \( L \)-serine (\( >1.5 \) g/day) in humans has been attempted to treat chronic fatigue syndrome, depression, schizophrenia, and rare in-born errors of metabolism associated with \( L \)-serine deficiency (1, 6, 7). The data from the present study suggest that acute intravenous administration of \( L \)-serine lowers visceral/deficiency (1, 6, 7). The data from the present study suggest that treatment with \( L \)-serine (6, 7, 9, 35).

References

4. Castellino P, Levin R, Shohat J, DeFronzo RA. Free amino acids pools in the spontaneously hypertensive rat: \( L \)-serine, an endocannabinoid-like brain constituent with vasodilatory dynamics. It has been demonstrated that inadequate splanchnic perfusion could lead to increased morbidity and mortality in intensive care patients (23). In vivo, \( L \)-serine is biosynthesized from glucose, threonine, or glycine metabolism (6, 7). Thus postprandial elevation in the \( L \)-serine level following carbohydrate and protein diet could evoke vasodilatation in the small intestinal, splanchnic vasculature to promote absorption of nutrients. On the one hand, although a glucose breakdown product like methylglyoxal promotes oxidative stress and cardiovascular complications, \( L \)-serine may provide cardiovascular protection (6, 7, 9, 35).

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Disclosures

No conflicts of interest are declared by the authors.

References


