Long-Lasting Changes of Rat Blood Pressure to Vasoconstrictors and Vasodilators Induced by Nitric Oxide Donor Infusion: Involvement of Potassium Channels

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ABSTRACT

We investigated the effects of the exposure of the rat vascular system to nitric oxide (NO), using infusion of either NO donor sodium nitroprusside (SNP) or S-nitroso-acetyl-DL-penicillamine (SNAP) on mean arterial pressure (MAP) responses to vasoconstrictors (phenylephrine, angiotensins I and II) and to vasodilators (bradykinin, acetylcholine, SNP, and iloprost). SNP (250 nmol/kg/min) or SNAP (85 nmol/kg/min) infused for 30 min decreased MAP by 40 to 60 mm Hg. MAP returned to normal levels 5 to 10 min after the end of infusion. After infusion of SNP or SNAP the effects of phenylephrine, angiotensin I, and angiotensin II were reduced by 40 to 80%, whereas the responses to bradykinin or acetylcholine were enhanced by 50 to 80%. These changes in vascular responsiveness persisted for at least 24 h after the SNP infusion. Pretreatment with either tetraethylammonium (360 μmol/kg) or 4-aminopyridine (4-AP; 1 μmol/kg) did not alter the effects of phenylephrine or bradykinin in control animals, but prevented SNP-induced changes in responsiveness to phenylephrine or bradykinin. On the other hand, administration of tetraethylammonium, reversed hyporesponsiveness to phenylephrine, whereas 4-AP was ineffective. Tetraethylammonium and 4-AP did not alter the increased responses to bradykinin. Glibenclamide was without effect in any situation. These results indicate that NO-induced changes on vascular responsiveness to vasoconstrictors and vasodilators are much more profound and long-lasting than described previously and that the effects of NO appear to be, at least in part, mediated by persistent activation of a tetraethylammonium-sensitive population of K+ channels.

The L-arginine/nitric oxide (NO) pathway (Moncada et al., 1991) has been implicated in the control of several biological processes in the cardiovascular and nervous systems. In these systems, NO is released by the action of constitutive Ca2+-dependent NO synthases (NOS; Moncada et al., 1991; Snyder and Bredt, 1992). Another isoform of this enzyme, the inducible Ca2+-independent NOS (iNOS), is expressed in phagocytic and other cell types after activation by endotoxin (bacterial lipopolysaccharide; LPS) and/or cytokines. Much larger amounts of NO are produced by this enzyme, accounting for the cytotoxicity of macrophages toward parasites and tumor cells and for the progressive hypotension present in pathological conditions such as septic shock (Moncada et al., 1991).

Excessive NO production has been shown to play a pivotal role in septic shock. Current knowledge suggests that high and continuous NO production during septic shock is the major cause of the vascular hyporesponsiveness to vasoconstrictors (Gray et al., 1990, 1991; Julou-Shaeffer et al., 1990; Mulder et al., 1994). For instance, inhibition of iNOS attenuates the circulatory changes and multiple organ failure caused by LPS in the rat (Wu et al., 1996). Moreover, guanylate cyclase inhibition reverses the hyporesponsiveness to vasoconstrictors (Fleming et al., 1991).

Besides NO, endothelial cells produce other chemical species able to induce vasodilatation, among them prostacyclin (Moncada et al., 1976) and endothelium-derived hyperpolarizing factor (EDHF; Chen et al., 1988; Taylor and Weston, 1988). Changes in membrane potential leading to repolarization/hyperpolarization have been associated with the relaxation induced by some endothelium-dependent vasodilatory substances. These membrane-potential changes initially were associated with the release of EDHF, whose chemical identity still is unknown (for reviews, see Garland et al., 1995; Mombouli and Vanhoutte, 1997; and Edwards and Weston, 1998). Although it has been shown that NO is also able to induce hyperpolarization in arterial smooth muscle (Tare et al., 1990); there are evidences indicating that relax-
ant response to endothelial-derived NO could be blocked independently of the accompanying smooth muscle hyperpolarization (for a review, see Garland et al., 1995), suggesting that the concomitant release of NO and EDHF may underlie the relaxant effect of some vasodilatory substances. Both NO and EDHF seem to induce K⁺ channel opening, thus explaining the hyperpolarization. For instance, NO activates voltage-dependent K⁺ channels, causing hyperpolarization and relaxation of pulmonary arterial smooth muscle (Yuan, 1996; Zhao et al., 1997). Similarly, EDHF-induced relaxation of vascular smooth muscle seems to involve opening of voltage-dependent K⁺ channels (Pettersson et al., 1997). Finally, the mechanisms of potassium channel activation induced by NO is still a matter of controversy. For instance, the NO-induced opening of calcium-dependent K⁺ channels can be mediated through a cGMP-dependent protein kinase (Archer et al., 1994) or directly by NO itself (Bolotina et al., 1994; Mistry and Garland, 1998). In addition, NO seems to activate potassium channels in pathological conditions such as septic shock (Hall et al., 1996; Price et al., 1997; Wu et al., 1998).

In the current report, we have attempted to study the effects of the exposure of the rat vascular system to NO and its consequences regarding the responses to vasoconstrictors and to vasodilators. The classical approach of increasing NO production by endotoxin (LPS) did not seem adequate for this, because LPS is known to release a vast array of mediators, several of them with important actions in the vascular system (for a review, see Brandtzæg, 1996). Therefore, we have developed a model for exposing the vascular system of the rat to NO, namely the infusion of NO donors. As our results will show, a brief exposure to NO is enough to render the rat vascular system hyporesponsive to vasoconstrictors, which resembles remarkably the hemodynamic changes seen in septic shock. This NO effect is long-lasting, persisting for at least 24 h. In addition, simultaneously to the hyporesponsivity to vasoconstrictors, NO donor infusion potentiated the vascular responses to vasodilators. Finally, opening of K⁺ channels seems to be involved in NO effects.

Materials and Methods

Surgical Procedures. All procedures were approved by our Institutional Ethics Committee and are in accordance with National Institutes of Health Animal Care Guidelines. Both male and female Wistar rats (3–4 months old; weighing 200–300 g) were used in this study. Animals were kept at a 12-h light/12-h dark cycle and had free access to food and water. They were anesthetized with ketamine/xylazine (90/15 mg/kg i.m., supplemented at 45- to 60-min intervals), as suggested by Gratton et al. (1995). The left femoral vein and right carotid artery were isolated, and heparinized polyethylene catheters (PE 20 and PE 50) were inserted for drug administration and recording of MAP and heart rate (HR) and blood withdrawal, respectively. Immediately after artery cannulation, heparin diluted (30 IU/ml) in sterile Dulbecco’s PBS (137 mM NaCl, 2.7 mM KCl, 1.5 mM KH₂PO₄, 8.1 mM NaHPO₄, pH 7.4) was injected to prevent clotting. When necessary, another catheter was inserted into the bladder for urine withdrawal. Animals were allowed to breathe spontaneously via a tracheal cannula. Body temperature was monitored by a rectal thermometer and maintained at 36 ± 1°C. Data were recorded (at a 10-s sampling rate) with a Digi-Med Blood Pressure Analyzer system (model 190) connected to a Digi-Med System Integrator (model 200; Micro-Med, Louisville, KY) software, running under Windows 95 (Microsoft Corporation, Redmond, WA). All MAP and HR values shown are those recorded at the peak of the effect produced by a given compound. Results are expressed as a mean either of changes in MAP (mm Hg) or changes in HR (beats per minute; bpm) in relation to basal values. All animals were sacrificed by an overdose of pentobarbital immediately after ending the experiment.

NO Donor Infusions. Figure 1 shows a typical experiment using phenylephrine and sodium nitroprusside (SNP). The same basic protocol was followed throughout the study. After surgery, a stabilization period of 30 min was allowed for. Then, increasing doses of vasoconstrictors (3, 10, and 30 nmol/kg phenylephrine; 3, 10, and 30 pmol/kg angiotensin I and angiotensin II) or vasodilators (3, 10, and 30 nmol/kg acetylcholine, bradykinin, and SNP; 1, 3, and 10 nmol/kg iloprost) were injected as boluses in a volume of 50 μl followed by a catheter flush with 150 μl of sterile PBS. Control values were obtained by injection of 200 μl of sterile PBS. Changes in MAP began immediately after injection and lasted for 2 to 3 min, the peak being observed in the first minute for all vasoactive compounds tested. The next dose was only injected once the changes in MAP induced by the previous one had subsided fully (usually within 10 min of injection). After obtaining a control dose-response curve to a given agonist and once MAP had fully returned to baseline levels, infusion of SNP (250 nmol/kg/min), S-nitroso-N-acetyl-DL-penicillamine (SNAP; 85 nmol/kg/min), or N-acetyl-DL-penicillamine (NAP; 85 nmol/kg/min) was started and maintained for a 30-min period. MAP fell to about 40 mm Hg for SNP and SNP infusions and remained around this value throughout the infusion period. A recovery period of 30 min, starting with the end of the infusion, then was allowed. MAP returned to baseline levels within 5 to 15 min, and, by the end of the recovery period, it was around preinfusion levels. At given times (30, 60, and 120 min) after the end of recovery period, successive dose-response curves to the same vasoconstrictor or vasodilator compound were obtained. Only one such compound was studied in any given animal. Control groups were subjected to a similar protocol, in which the animals were infused with PBS alone during 30 min (20 μl/min/30 min). Next, we studied the effect of NO donor infusion on vascular responses to selected compounds 24 h after the end of the infusion. Because maintaining animals anesthetized for long time periods is difficult, this part of the study was conducted by using a somewhat different protocol. Groups of naive animals were anesthetized with tri bromoethanol (125 mg/kg, i.p.) to allow insertion and fixation of a butterfly catheter into the caudal vein for the 30-min infusion of SNP, SNAP, or PBS as described above. After the infusion, the
caudal catheter was removed and the animal was accommodated in warmed surroundings until recovery of anesthesia (1 h). Twenty-four hours later, the animals were anesthetized with ketamine/xylazine and dose-response curves to phenylephrine or to bradykinin were performed as described above.

For nitrate/nitrite (NO$_3^-$) assay, blood (300 μl each) and urine samples (collected over a 5-min period) were obtained from the carotid artery and from the cannulated bladder, respectively, before and during infusion of SNP, SNAP, NAP, or PBS. At given periods after the end of the infusion (60, 120, 180, and 240 min), additional blood and urine samples were obtained in the same manner. Samples were centrifuged at 1000g for 10 min, and the plasma or the clear supernatant was saved and stored at −20°C. To avoid MAP changes due to hemorrhage, 300 μl of PBS was injected after withdrawal of each blood sample.

**Treatment with K$^+$ Channel Inhibitors.** For these experiments we used two protocols. In the first, dose-response curves to phenylephrine or to bradykinin were obtained as described above. Then, nonspecific K$^+$ channel blocker tetraethylammonium (TEA; 360 μmol/kg), the ATP-dependent K$^+$ channel blocker glibenclamide (40 μmol/kg), or the voltage-dependent K$^+$ channel blocker 4-amino-pyridine (4-AP; 1 μmol/kg) was slowly (during 3–5 min) injected i.v., and, after stabilization of the MAP (usually 5–10 min), infusion with SNP was initiated as described. Thirty minutes after the end of the infusion, new dose-response curves to phenylephrine and to bradykinin were obtained as described. The effects of K$^+$ channel inhibitors alone were evaluated in animals in which the infusion of SNP was substituted by PBS. The second protocol was similar, except that K$^+$ channel blockers were injected after the infusion of SNP. In this case, dose-response curves to phenylephrine or to bradykinin were obtained before and 30 min after the end of SNP infusion. K$^+$ channel blockers were injected, and new dose-responses curves to phenylephrine or to bradykinin were obtained. Finally, for studying TEA effects on the long-lasting effect of NO donor infusion, it also was injected 24 h after SNP infusion and dose-response curves to phenylephrine were made.

**NO$_x$ Assay.** Briefly, plasma (deproteinized by zinc sulfate and diluted 1:1 with Milli-Q water) and urine (nondiluted) were subjected to nitrate conversion, as described by Granger et al. (1990). Nitrate was converted to nitrite by using Escherichia coli nitrate reductase for 2 h at 37°C. Samples were centrifuged for bacteria removal, and 100 μl of each sample was mixed with Griess reagent (1% sulfanilamide in 10% phosphoric acid/0.1% naphthyl-ethylenediamine in Milli-Q water) in a 96-well plate and read at 540 nm in a plate reader. Standard curves of nitrite and nitrate (0–150 μM) were run simultaneously. Because under these conditions nitrate conversion was always greater than 90%, no corrections were made. Values are expressed as μM NO$_x$ (nitrate + nitrite).

**Drugs.** The following drugs and reagents were used in this study: phenylephrine, angiotensin I, angiotensin II, acetylcholine, bradykinin, SNP, NAP, sulfanilamide, α-naphthyl-ethylenediamine, sodium nitrate, sodium nitrite, and glibenclamide (all purchased from Sigma Chemical Co., St. Louis, MO); ketamine (obtained from Parke-Davis, Sao Paulo, SP, Brazil); xylazine (Rompun; kindly donated by Bayer, Sao Paulo, SP, Brazil); iloprost (a kind gift from Dr. E. Antunes, Universidade de Campinas, SP, Brazil); TEA (Sigma) and 4-AP (Research Biochemicals, Natick, MA), which were kindly donated by Dr. M. C. O. Salgado (Faculty of Medicine, Ribeirao Preto, USP, Brazil); and S-nitroso-N-acetyl-DL- penicillamine (synthesized in house by the method of Field et al., 1978). All compounds were diluted in sterile PBS except glibenclamide, which was prepared as a concentrated stock solution in dimethyl sulfoxide.

**Statistical Analysis.** Results are expressed as the mean ± S.E.M. (n = 4–7 for each group). Statistical significance was analyzed by either Student’s t test for paired or unpaired samples or one-way ANOVA followed by Bonferroni’s post hoc t test, when applicable. A value of $P < .05$ was considered statistically significant.

**Results**

**Effects on MAP.** A typical experiment using phenylephrine before and after SNP infusion is shown in Fig. 1. For the sake of brevity, only the dose-response curve obtained 30 min after the end of NO donor infusion is depicted. All vasoconstrictors increased MAP dose-dependently (Fig. 2, circles). The responses evoked by angiotensin II (data not shown) were identical with those caused by angiotensin I. To allow for a better analysis, we performed time-matched dose-response curves in control animals, which were infused with sterile PBS only. Similarly, the hypotensive effects of bradykinin and acetylcholine also were dose-dependent (Fig. 3, circles).

**Effects of NO Donor Infusion.** Both SNP and SNAP infusions caused reductions in normal MAP (90 ± 1.5 mm Hg after infusion and recovery, MAP values were 95.7 ± 6.9, 94.9 ± 8.6, and 107.6 ± 4.1 mm Hg for animals that received PBS, SNP, and SNAP, respectively.)

![Fig. 2. Effects of SNP and SNAP infusions on vasoconstrictive effects of phenylephrine and angiotensin I on MAP of anesthetized rats.](image)
Hg; \( n = 83 \) of around 40 to 60 mm Hg (for a typical recording, see Fig. 1). On the other hand, HR was not changed by SNP or SNAP infusions. For instance, the average HR of control animals during sterile PBS infusion was \( 226 \pm 8 \) bpm, whereas values observed in SNP and SNAP groups were \( 208 \pm 15 \) bpm and \( 235 \pm 10 \) bpm (\( n = 10 \) each), respectively. None of these parameters was changed by infusion of NAP, the non-nitrosylated parent compound of SNAP. Although we did test the influence of SNAP at higher concentrations (up to 1000 nmol/kg/min) and for longer periods of infusion (up to 120 min; data not shown), we selected the 250-nmol/kg/min regimen because the hypotension was reproducible and fully reversible, indicating that animals were not developing hemodynamic failure. For SNAP we selected the 85-nmol/kg/min regimen because its effects on MAP were identical with those of SNP.

The hypotensive effects of both NO donors were of immediate onset, well maintained throughout the infusion, and reversed to basal MAP levels within 5 to 10 min after infusion cessation. Indeed, 30 min after ending infusion of SNP or SNAP, MAP had returned to \( 102.3 \pm 2.9 \) (\( n = 33 \)) and \( 106.8 \pm 2.7 \) mm Hg (\( n = 23 \)), respectively, as compared with the \( 94.6 \pm 2.9 \) mm Hg measured in control animals after infusion of PBS (\( n = 28 \)). Although we determined both plasma and urine NOx levels during and after the infusion of NO donors, only slightly nonsignificant increases for SNP and no changes for SNAP infusions were found. For example, plasma NOx assayed 60 min after the end of infusion was \( 20.8 \pm 3.4 \) mM, \( 30.8 \pm 3.8 \) mM, and \( 46.7 \pm 7.7 \) mM for PBS, SNP, and SNAP infused animals, respectively (\( n = 3 \)).

Infusion of NO donors for a period of 30 min decreased the hypertensive effects of both phenylephrine (Fig. 2, left; for a typical recording, see Fig. 1) and angiotensins I (Fig. 2, right) and II (data not shown). For instance, 30 min after termination of the infusion period, MAP increases induced by 3, 10, and 30 nmol/kg phenylephrine in either SNP- or SNAP-infused rats were reduced by approximately \( 80\% \), \( 46\% \), and \( 40\% \), respectively (\( P < .05 \), when compared with responses in control animals; Fig. 2A). Similar results were obtained at 60 min after either SNP or SNAP infusion (Fig. 2B). Although a similar pattern can be observed 120 min after SNP infusion, it was not found in SNP-infused animals when compared with control animals (Fig. 2C). Although we do not have a clear explanation for this discrepancy, it may be related to the fact that SNP releases NO much faster than SNAP or to some degree of desensitization to the phenylephrine effect. Interestingly, previous NO donor infusion 24 h prior to experiment elicited a significant reduction (\( P < .05 \)) in phenylephrine responses when compared with the one obtained in animals infused with PBS alone (Fig. 2D).

Next, we examined the influence of SNP and SNAP infusions on the effects of some structurally unrelated vasodilators (an amine, a peptide, a nitrovasodilator, and an eicosanoid) and found that bradykinin and acetylcholine each had their actions increased by more than \( 60\% \) at 30 min postinfusion (\( P < .05 \)) when compared with their respective control groups (Fig. 3A). Similar patterns also were observed at 60 and 120 min after ending infusion (Fig. 3B and C, respectively). The same potentiating effect caused by NO donor infusion was seen with acetylcholine (Fig. 3, right). The potentiation of bradykinin-induced responses elicited by SNP infusion was present even after 24 h, as shown in Fig. 3D. On the other hand, the hypotensive responses to SNP (a direct vasodilator) or iloprost (an endothelium-independent vasodilator) were unchanged by SNP infusion, at all time periods examined. For instance, when injected before SNP infusion, SNP and iloprost (both 10 nmol/kg) decreased MAP of \( 27.3 \pm 4.0 \) and \( 43.7 \pm 3.3 \) mm Hg, respectively, whereas their effects after NO donor infusion were \( 33.4 \pm 3.3 \) and \( 48.1 \pm 3.8 \) mm Hg, respectively. Similar results were obtained in SNAP-infused animals (data not shown). Infusion of \( N \)-acetyl-DL-penicillamine (SNAP non-nitrosylated parent compound) did not influence the effects of any of these vasoconstrictor or vasodilator compounds tested (data not shown).

**Effects of K⁺ Channel Blockers.** Next, we sought to study the involvement of K⁺ channels in responses induced by NO donor infusion. Some control experiments were made initially. Glibenclamide (40 \( \mu \)mol/kg, at dose able to fully
inhibit the vasodilatory effect of cromakalim, an ATP-dependent K⁺ channel opener; data not shown) caused an initial MAP reduction of 23.4 ± 4.0 mm Hg (n = 16) followed by a sustained increase of 45.8 ± 3.4 mm Hg (n = 16), which was accompanied by a heart rate fall of 51 ± 5 bpm. Both TEA (360 μmol/kg; n = 15) and 4-AP (1 μmol/kg; n = 14) increased MAP transiently by 43.4 ± 3.6 and 26.6 ± 6.6 mm Hg, respectively, without altering the heart rate. All of these effects subsided in the next 10 to 20 min. Neither TEA nor 4-AP affected the hypotension caused by SNP infusion, but glibenclamide attenuated it by 30 to 40% (data not shown). At the doses used, none of K⁺ channel blockers induced any changes in the effects of phenylephrine or bradykinin in control animals (data not shown).

When administered before SNP infusion, glibenclamide failed to interfere in the reduced phenylephrine effects (Fig. 4A, left) or in the enhanced bradykinin effects (Fig. 4A, right) induced by NO donor infusion. On the other hand, 4-AP and TEA completely blocked the altered responses to both phenylephrine and bradykinin caused by NO donor infusion (Fig. 4, B and C).

A somewhat different picture emerged when K⁺ channel blockers were tested after the exposure of the rat vascular systems to NO (i.e., after the infusion of NO donor). The effectiveness of SNP infusion on phenylephrine and bradykinin actions was checked before K⁺ channel blocker injection (Fig. 5, squares). In this protocol, we found that only TEA blocked SNP effects on phenylephrine responses, albeit in part (Fig. 5, B and C, left). The same inhibitory effect of TEA on the NO-induced hyporesponsiveness to phenylephrine was seen when the K⁺ channel blocker was administered 24 h after SNP infusion (Fig. 5D). Moreover, both 4-AP and TEA were effective in attenuating the vascular response to lower doses of bradykinin (3 nmol/kg) but not to higher ones (Fig. 5, B and C, right). Similar to the preinfusion treatment, glibenclamide failed to restore responses to both phenylephrine and bradykinin to normal levels when injected after NO donor infusion (Fig. 5A). Dimethyl sulfoxide (the vehicle used...
to dissolve glibenclamide) did not influence any of the evaluated parameters.

**Discussion**

The main findings of the present report can be summarized as follows: 1) infusion of NO donors induced a state of profound hyporesponsiveness to vasoconstrictors (phenylephrine and angiotensins I and II) in the rat vascular system, which resembles the pattern seen in septic shock; 2) exposure to NO donors also potentiated endothelium-dependent vasodilator responses to bradykinin and to acetylcholine; 3) these NO-induced modifications in vascular responsiveness persisted for at least 24 h after NO donor infusion; 4) blockade of K⁺ channels with TEA or, more specifically, of voltage-dependent K⁺ channels with 4-AP before NO donor infusion practically abolished the changes in responses to phenylephrine and bradykinin; and 5) 4-AP, when administered after NO donor infusion, failed to normalize reduced phenylephrine and increased bradykinin responses, whereas TEA reversed the hyporesponsiveness to phenylephrine (even 24 h after NO donor infusion) but was without effect on bradykinin responses.

The effects of NO donor infusion can be ascribed to NO because NAP, the non-nitrosylated parent compound of SNAP, was completely devoid of any effect. In addition, MAP returned rapidly to basal levels once the infusion was terminated. We did not find increased NOx nor changes in nitrosothiol levels in plasma during or after NO donor infusion (data not shown). These findings indicate that the effective amount of NO released during infusions was rather small. Katsuki et al. (1977) showed diminished pressor responses to acetylcholine and angiotensins I and II in the rat vascular system, which resembles the pattern seen in septic shock; 2) exposure to NO donors also potentiated endothelium-dependent vasodilator responses to bradykinin and to acetylcholine; 3) these NO-induced modifications in vascular responsiveness persisted for at least 24 h after NO donor infusion; 4) blockade of K⁺ channels with TEA or, more specifically, of voltage-dependent K⁺ channels with 4-AP before NO donor infusion practically abolished the changes in responses to phenylephrine and bradykinin; and 5) 4-AP, when administered after NO donor infusion, failed to normalize reduced phenylephrine and increased bradykinin responses, whereas TEA reversed the hyporesponsiveness to phenylephrine (even 24 h after NO donor infusion) but was without effect on bradykinin responses.

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The only hypothesis that we can offer at present would be that events causing an increase in bradykinin and acetylcholine responses seem to be occurring at the endothelial level. It may be that, for example, ion channels (or other transduction mechanisms) are affected differentially by NO in endothelial and smooth muscle cells. This possibility warrants further investigation.

Data presented here do not offer explanations on why a short exposure to NO may cause long-term changes in vessel sensitivity. However, considering that the presence of cysteiny1 sulfhydryl groups on regulatory domains of K⁺ channels is critical for their activity (Ruspeberg et al., 1991; Islam et al., 1993; Wang et al., 1997) and that NO is highly reactive toward sulfhydryl groups, it is conceivable that reaction of NO with -SH groups may affect K⁺ channel activity. Indeed, nitrothiolsylation of cytoplasmic domain of potassium channels increases their open probability (Abderrahmane et al., 1998).

Another explanation for the long-term effect of NO infusion in vessel sensitivity is related to the formation of intracellular nitrosothiols (RSNO), formed by the rapid reaction of NO with intracellular thiols (such as glutathione). These compounds can undergo homolytic cleavage of the S–N bond to give NO and thyl radical (for a review, see Stamler, 1994). Recently, NO release from intracellular S-nitrosothiol pools has been shown to play a direct role in the decrease of rat blood pressure induced by acetylcholine and bradykinin (Davison et al., 1996). Therefore, if NO donor infusion replenishes S-nitrosothiol pools in endothelial cells and these pools release NO back it would explain, at least in part, 1) why only endothelium-dependent vasodilators had their effects potentiated by NO donor infusion and 2) the long-term hyposensitivity to vasoconstrictors and hypersensitivity to vasodilators. Thus, an increased, continuous NO release from S-nitrosothiol pools would, in turn, increase the open probability of K⁺ channels, leading to hyperpolarization. Alternatively, our data could be explained by an increase in EDHF release caused by NO donor infusion. This suggestion is based on reports showing that NO inhibits endothelial NO synthase (Buga et al., 1993) and also damps EDHF production (Bauersachs et al., 1996). Therefore, the impaired NO synthesis and the consequent increase in EDHF release would explain the observed changes in vascular response induced by NO donor infusion. Although our data do not allow for exclusion of this possibility, it seems unlikely be-
cause a long-term inhibition on NO synthesis would have some effect on the blood pressure. These possibilities are now being investigated in our laboratory.

We sought to investigate the involvement of soluble guanylate cyclase on NO-induced changes on vascular responsiveness, but guanylate cyclase inhibitors, methylene blue and 1H-[1,2,4]-oxadiazolo-[4,3-a]-quinoxalin-1, failed to change the vascular responses to SNP, SNAP, acetylcholine, bradykinin, and phenylephrine in either PBS- or NO donor-infused animals (data not shown). One possible explanation for these findings would be that NO donor effects are not related to guanylate cyclase activation. For instance, NO donors open calcium-activated K⁺ channels in cell-free membrane patches (Bolotina et al., 1994; Mistry and Garland, 1998). However, several reports show that in other preparations (such as pulmonary artery rings) NO effects on the same type of K⁺ channels seem to be mediated by a GMP-dependent protein kinase (for example, Archer et al., 1994), indicating that this still is an unresolved issue. Alternatively, guanylate cyclase inhibitors may have failed to inhibit the enzyme activity in vivo because of pharmacokinetic aspects or the inherent complexity of blood pressure as the experimental preparation.

In accordance with the present results, i.v. injection of E. coli also induces hyporesponsiveness to vasoconstrictors and hyperresponsiveness to vasodilators, which persist for at least 24 h after septic shock onset (manuscript in preparation). The involvement of potassium channels in this model currently is being evaluated in our laboratory.

In conclusion, our results indicate, for the first time, that NO donor infusion reproduces the changes in vascular responsiveness to vasoconstrictors and vasodilators seen in septic shock. In addition, our results suggest that the role of bradykinin in the septic shock hypotension may be more important than described previously. Another important piece of information provided by our in vivo study is that the onset of these vascular changes induced by NO appear to need activation of K⁺ channels, mainly of the voltage-dependant type, but not of the ATP-sensitive type. The long-lasting effects of NO on phenylephrine responses, however, are likely to depend on TEA-sensitive K⁺ channel population, whereas NO should rely on some other mechanism when responses to bradykinin are considered. Finally, our data demonstrate that the NO-induced changes in vascular responsiveness are much more profound, long-lasting, and important than anticipated previously. If applicable to septic shock, our data may help to understand the hyporesponsiveness to vasoconstrictors and point out the putative importance of the hyperresponsiveness to vasodilators in this condition. Further studies directed to understanding the relationship between NO, K⁺ channel activity, and altered responses to vasoconstrictors and vasodilators ultimately may lead to a better management of septic shock.

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