# Selective iNOS Inhibition Is Superior to Norepinephrine in the Treatment of Rat Endotoxic Shock

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**S-methyl-isothiourea (SMT) is a potent inhibitor of NO synthase (NOS) with relative selectivity towards the inducible isoform (iNOS). We compared SMT and norepinephrine for the treatment of experimental endotoxic shock. Anesthetized rats challenged intravenously with lipopolysaccharide (LPS), 10 mg/kg, were treated after 1 h with a 4-h infusion of norepinephrine (titrated to maintain blood pressure within baseline values), SMT at low dose (0.1 mg · $kg^{-1}$ · $h^{-1}$), or at high dose (1 mg · $kg^{-1}$ · $h^{-1}$), or an equivalent volume of saline (2 ml · $kg^{-1}$ · $h^{-1}$). In saline-treated animals, LPS increased plasma nitrate and produced hypotension, low cardiac output (CO), lactic acidosis, and signs of liver and kidney dysfunction. Norepinephrine maintained blood pressure (BP) and reduced the fall in CO, without affecting lactic acidosis, organ dysfunction, and nitrate accumulation. The latter was dose-dependently blunted by SMT. Treatment with this agent prevented hypotension, through systemic vasoconstriction with the high dose and a maintained CO with the low dose. Low, but not high, dose SMT blunted lactic acidosis. Both doses reduced the signs of renal, but not liver, dysfunction. In additional studies, we obtained evidence that, in contrast with the high dose, SMT at low dose did not interfere with the function of constitutive NOS. These findings suggest a potential advantage of selective iNOS inhibition over standard adrenergic support in the therapy of septic shock.** Rosselet A, Feihl F, Markert M, Gnaegi A, Perret C, Liaudet L. Selective iNOS inhibition is superior to norepinephrine in the treatment of rat endotoxic shock. AM J RESPIR CRIT CARE MED 1998;157:162–170.

Nitric oxide (NO) is a short-lived effector molecule derived from the enzymatic oxidation of L-arginine. Small amounts of NO are normally produced by the vascular endothelium under the control of a constitutively expressed NO synthase (ecNOS), a process involved in the physiologic regulation of vascular tone and blood flow distribution. Upon stimulation by lipopolysacharride (LPS) and various cytokines, an inducible NO synthase (iNOS) becomes diffusely expressed, leading to the production of large amounts of NO, which have been implicated in the cardiovascular failure of septic shock (1, 2). Therefore, the pharmacologic inhibition of NO production has been proposed as an adjunct to septic shock therapy. Indeed, L-arginine analogues such as monomethyl-L-arginine (L-NMMA) or L-arginine-methylester (L-NAME), which acts as competitive inhibitors of NO synthase, have been shown to increase blood pressure and partially restore adrenergic vascular reactivity in experimental and human septic shock (reviewed in 1 and 2). Unfortunately, these effects were generally associated with extensive vasoconstriction, depression of cardiac output, and regional hypoperfusion, leading to increased organ damage and mortality (3–7). This deleterious potential may be partially related to the blockade of ecNOS by the aforementioned arginine analogues, which act nonselectively on all isoforms of NO synthase. Therefore, interest is now focusing on the identification of compounds that would reduce iNOS-dependent NO production while preserving ecNOS activity (1, 8, 9). S-substituted thiourea derivatives have been recently identified as highly potent NO synthase inhibitors. With some of these compounds, such as S-methyl-isothiourea (SMT), preferential inhibition of iNOS was demonstrated *in vitro* (10, 11), and studies *in vivo* reported reduced organ damage and mortality during rodent endotoxic shock (11–13).

There is currently no information concerning the effects of such compounds on cardiac output and tissue oxygenation in endotoxic shock. In addition, it is not known whether their administration would have any advantage over standard adrenergic support. Finally, their degree of selectivity towards iNOS has not been established *in vivo*. These clinically relevant questions were addressed in the present study.

## METHODS

### Surgical Preparation and Measurements

All procedures were approved by the Swiss laws on animal experimentation. Forty-five adult male Wistar rats weighing 250 to 300 g were anesthetized intraperitoneally with sodium pentobarbital, 50 mg/kg for induction and 10 mg/kg for maintenance, given as needed (interdigital reflex). Temperature was maintained at 37° C. The rats breathed spontaneously through a tracheal canula, with a $F_{I_{O_2}}$ of 0.5. The right femoral artery was cannulated with PE 50 tubing and connected to a pressure transducer for the measurements of arterial blood pressure (BP) and heart rate (HR) (HP 78342A BP monitor; Hewlett-Packard, Avondale, PA), which were displayed on paper (WR 3310 polygraph recorder; Graphtec), PE 50 catheters were placed into the right femoral vein for the infusion of various agents and into the right atrium via the right internal jugular vein. In a subset of rats (Ex-

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periment 1, *see below*), a thermistor (dismounted from a 7F Swan-Ganz catheter; Baxter Edwards, Irvine, CA), was placed into the ascending thoracic aorta via the right carotid artery for the measurement of transpulmonary thermodilution cardiac output (HP 78231C CO monitor; Hewlett Packard) (14). A 0.15-ml bolus of room-temperature 2.5% glucose solution was injected into the right atrium as the indicator, as previously described (9). CO was averaged from three consecutive determinations and indexed to the weight of the animal to obtain cardiac index (CI). Systemic vascular resistance was calculated with standard formulas and indexed to the weight of the animal (SVRI).

### Experimental Protocols

*Experiment 1.* The purpose of this study was to describe the hemodynamic and metabolic response of endotoxemic rats to the continuous infusion of either norepinephrine (NE) or two different doses of SMT. Thirty-two animals were instrumented as described. Immediately after baseline measurements (T0), endotoxemia was induced intravenously by a bolus of endotoxin (*Escherichia coli* LPS, 10 mg/kg, dissolved in 1 ml of isotonic saline) administered over 15 min. One hour after baseline (T1), rats randomly received an infusion of NE (0.01 to 10 $\mu g \cdot kg^{-1} \cdot min^{-1}$; n = 8), SMT at low (0.1 $mg \cdot kg^{-1} \cdot h^{-1}$; n = 8) or high (1 $mg \cdot kg^{-1} \cdot h^{-1}$; n = 7) dose, or an equivalent volume of isotonic saline (2 $ml \cdot kg^{-1} \cdot h^{-1}$; n = 7). This infusion was continuously given over 4 h (i.e., from T1 to T5). The concentration of NE was titrated, at constant fluid infusion rate, to avoid both progressive hypotension and overcorrection of mean BP. The doses of SMT were chosen on the basis of pilot experiments and previously published data (11).

Arterial blood samples were taken hourly for the determination of blood gases, lactate, and hematocrit (Hct). Additional samples were obtained at T0 and T5 to measure the plasma concentrations of transaminases (ASAT, ALAT), creatinine, and nitrate (the latter being also measured at T3). Plasma nitrate was determined by a spectrophotometric assay, according to a previously published procedure (9, 15). Each sample of blood was replaced with the same volume of isotonic saline. The total volume of sampled blood amounted to 2.8 ml.

*Experiment 2.* This additional study evaluated the influence of SMT, at either the high or the low dose used in Experiment 1, on ecNOS-dependent NO production *in vivo*. The latter is normally involved (*1*) in the physiologic regulation of arterial BP, (*2*) in the depressor response produced by the intravenous administration of acetylcholine (Ach) (16), and (*3*) in the acute hypotension (i.e., within the first hour) after an intravenous LPS challenge in the rat (1). Thirteen rats instrumented for BP measurements were randomly assigned to receive a continuous intravenous infusion over 4 h of either the low dose (0.1 $mg \cdot kg^{-1} \cdot h^{-1}$; n = 5) or the high dose of (1 $mg \cdot kg^{-1} \cdot h^{-1}$; n = 4) SMT, or an equivalent volume of isotonic saline (2 $ml \cdot kg^{-1} \cdot h^{-1}$; n = 4). Under baseline conditions and at the end of the 4-h infusion, the animals were administered sequential boluses of 1, 5, 10, and 25 $\mu g$/kg Ach dissolved in saline (1 ml/kg) and injected into the jugular vein catheter over 15 s with a precision syringe infusor (SP 100i; World Precision Instruments, Sarasota, FL). A transient depressor response was observed and was quantified as the absolute decline in mean BP recorded 30 s after the end of bolus administration. Dose-response curves (drop in mean BP versus Ach concentration) were constructed that, in the range used, were linear. Ach boluses were given at 5-min intervals in order to allow full recovery of mean BP between sequential doses. The mean relative decrease in HR induced by Ach was 7%, except at the higher dose (13%). After the second dose-response curve, rats were allowed to recover for 30 min and then challenged intravenously with a bolus of 10 mg/kg LPS. The observed acute drop in mean BP was recorded after 15 and 30 min. An intravenously administered bolus of L-arginine (L-arg), 100 mg/kg, was finally given at 30 s, and mean BP was recorded 2 and 15 min later (i.e., 32 and 45 min after the onset of LPS administration).

### Data Analysis

Results are expressed as means ± SD. In Experiment 1, effects of time and treatment were statistically analyzed with repeated measures ANOVA. When appropriate, multiple comparisons were made with Bonferroni adjustments for the effects of treatment at specific times, and with Dunnett's test for the effect of time in specific groups (with T0 as a control). In Experiment 2, the effects of LPS and L-arg were analyzed separately in each group, using ANOVA with time as a single, repeated factor. In view of the linear shape, each dose-response curve to Ach was reduced to the slope computed with linear regression. The time course of these slopes and of mean BP over the first 4 h of Experiment 2 were compared between groups, using the same statistical analysis as in Experiment 1.

## RESULTS

### Experiment 1

*Hemodynamic data.* Two rats receiving saline treatment died during the experimental period and were excluded from further analyses. All the other rats survived until the end of the study.

The time-course of hemodynamic variables is presented in Table 1 and Figure 1. Before initiation of treatment (i.e., at T1), a comparable fall in mean BP and CI was noted in all groups, with no change in SVRI. The subsequent hemodynamic profiles were markedly different between groups. In rats treated with saline, mean BP remained stable until T3 and then progressively decreased until T5 because of a severe de-

**TABLE 1**

**SUMMARIZED TIME-COURSE OF HEMODYNAMIC DATA, BLOOD GASES, AND PLASMA NITRATE IN RATS CHALLENGED WITH ENDOTOXIN***

| | Saline | | Norepinephrine | | SMT 0.1 ($mg \cdot kg^{-1} \cdot h^{-1}$) | | SMT 1 ($mg \cdot kg^{-1} \cdot h^{-1}$) | |
|---|---|---|---|---|---|---|---|---|
| | T0 | T5 | T0 | T5 | T0 | T5 | T0 | T5 |
| Mean BP, mm Hg | 112 ± 6 | 78 ± 24$^{\ddagger\parallel}$ | 112 ± 7 | 102 ± 4$^{\dagger}$ | 108 ± 5 | 102 ± 11$^{\dagger}$ | 111 ± 7 | 102 ± 26$^{\dagger}$ |
| CI, ml/min/kg | 265 ± 34 | 137 ± 38$^{\ddagger\parallel}$ | 265 ± 45 | 185 ± 48$^{\dagger\parallel}$ | 264 ± 35 | 217 ± 29$^{\dagger\S\parallel}$ | 263 ± 20 | 135 ± 31$^{\ddagger\S\parallel}$ |
| Heart rate, beats/min | 410 ± 29 | 370 ± 75$^{\ddagger\parallel}$ | 431 ± 22 | 444 ± 27$^{\dagger}$ | 440 ± 32 | 434 ± 28$^{\dagger\S}$ | 411 ± 22 | 366 ± 54$^{\ddagger\S\parallel}$ |
| SVRI, mm Hg/ml/min/kg | 0.43 ± 0.06 | 0.57 ± 0.07$^{\parallel}$ | 0.43 ± 0.08 | 0.59 ± 0.16$^{\parallel}$ | 0.42 ± 0.06 | 0.48 ± 0.06$^{\S}$ | 0.42 ± 0.05 | 0.77 ± 0.17$^{\dagger\ddagger\S\parallel}$ |
| pHa | 7.36 ± 0.03 | 7.18 ± 0.14$^{\parallel}$ | 7.39 ± 0.03 | 7.20 ± 14$^{\parallel}$ | 7.35 ± 0.01 | 7.32 ± 0.05$^{\dagger\ddagger\S}$ | 7.35 ± 0.01 | 7.20 ± 0.13$^{\S\parallel}$ |
| $P_{O_2}$ | 190 ± 53 | 206 ± 36 | 132 ± 38 | 142 ± 19 | 162 ± 47 | 172 ± 58 | 147 ± 58 | 197 ± 44 |
| $Pa_{CO_2}$, mm Hg | 42 ± 4 | 35 ± 5 | 41 ± 5 | 42 ± 13 | 47 ± 3 | 34 ± 4 | 45 ± 2 | 41 ± 13 |
| Base excess, mM | −1.3 ± 2.0 | −13.0 ± 5.5$^{\parallel}$ | −0.1 ± 1.6 | −11.3 ± 4.2$^{\parallel}$ | −0.3 ± 1.0 | −7.8 ± 2.1$^{\dagger\ddagger\S\parallel}$ | −1.1 ± 1.1 | −11.5 ± 4.3$^{\S\parallel}$ |
| Lactate, mM | 0.9 ± 0.1 | 5.4 ± 2$^{\parallel}$ | 1 ± 0.2 | 4.7 ± 1.2$^{\parallel}$ | 0.9 ± 0.1 | 3.4 ± 0.7$^{\dagger\ddagger\S\parallel}$ | 0.9 ± 0.2 | 4.7 ± 1.5$^{\S\parallel}$ |
| Nitrates, $\mu$M | 7 ± 3 | 234 ± 37$^{\parallel}$ | 4 ± 2 | 226 ± 14$^{\parallel}$ | 5 ± 3 | 165 ± 12$^{\dagger\ddagger\S\parallel}$ | 12 ± 4 | 60 ± 6$^{\dagger\ddagger\S\parallel}$ |
| Hematocrit | 0.48 ± 0.02 | 0.40 ± 0.03 | 0.46 ± 0.02 | 0.39 ± 0.03 | 0.46 ± 0.02 | 0.40 ± 0.02 | 0.47 ± 0.03 | 0.41 ± 0.03 |

*Definition of abbreviations*: BP = blood pressure; CI = cardiac index; SVRI = systemic vascular resistance index.

* Four different groups of endotoxemic rats were treated with a continuous infusion of normal saline (NaCl, n = 8) or norepinephrine (NE, n = 8), titrated to maintain blood pressure, or S-methyl-isothiourea (SMT) at low dose (0.1 $mg \cdot kg^{-1} \cdot min^{-1}$, n = 7) or high dose (1 $mg \cdot kg^{-1} \cdot min^{-1}$, n = 7). A bolus of endotoxin was administered intravenously at the onset of experiment (T0). Treatment was started 1 h later (i.e., at T1) and pursued for 4 h (i.e., until T5). The total intravenous fluid load was identical in all groups (2 ml/kg). Data are expressed as the mean ± SD. Statistical symbols: $^{\dagger}p < 0.05$ versus NaCl; $^{\ddagger}p < 0.05$ versus NE; $^{\S}p < 0.05$, SMT 0.1 versus SMT 1; $^{\parallel}p < 0.05$ versus time 0.

pression in CI, associated with a slight rise in SVRI. As per protocol design, treatment with NE allowed maintenance of mean BP within 10 mm Hg of baseline; to achieve this goal, the initial dose of NE (0.01 $\mu g \cdot kg^{-1} \cdot min^{-1}$) had to be progressively increased from T3 to T5 to an average of $3.9 \pm 5$ $\mu g \cdot kg^{-1} \cdot min^{-1}$ ($p < 0.01$). NE attenuated the depression of CI induced by LPS, but it failed to raise SVRI above values in the saline group. SMT at either 0.1 or 1 $mg \cdot kg^{-1} \cdot min^{-1}$ prevented hypotension in the last hour of experiment, but this effect was related to clearly distinct mechanisms: the low dose blunted the depression of CI without ever influencing SVRI (thereby essentially mimicking the effects of NE); in contrast, the high dose caused a rapid, marked increase in SVRI, associated with an accelerated fall in CI (i.e., the CI measured at T2 and T3 was significantly lower than in saline-treated animals).

*Metabolic data.* The results of arterial blood gas determinations, acid-base status, and lactate concentration are shown in Table 1 and Figure 2. $Pa_{O_2}$ remained stable at values largely above 100 mm Hg in all groups for the whole experiment (Table 1). The progressive hyperlacratemia and metabolic acidosis induced by LPS were not significantly influenced by NE or the high dose of SMT. In contrast, these abnormalities were significantly blunted in rats treated with the low dose of SMT. Although there was some variation between groups in the degree of respiratory compensation to metabolic acidosis, the differences in $Pa_{CO_2}$ were not significant at any time point (Table 1).

Hct progressively decreased without significant differences between groups (Table 1). The massive increase in plasma nitrate induced by LPS was dose-dependently blunted by SMT, but it was not influenced by NE treatment (Figure 3). The time-course of plasma transaminases and creatinine are shown in Figure 4. LPS induced a marked augmentation in both ALAT and ASAT levels, without any significant difference between groups, although the change tended to be less pronounced in rats receiving the low dose of SMT and more marked in the NE group. In addition, LPS was responsible for

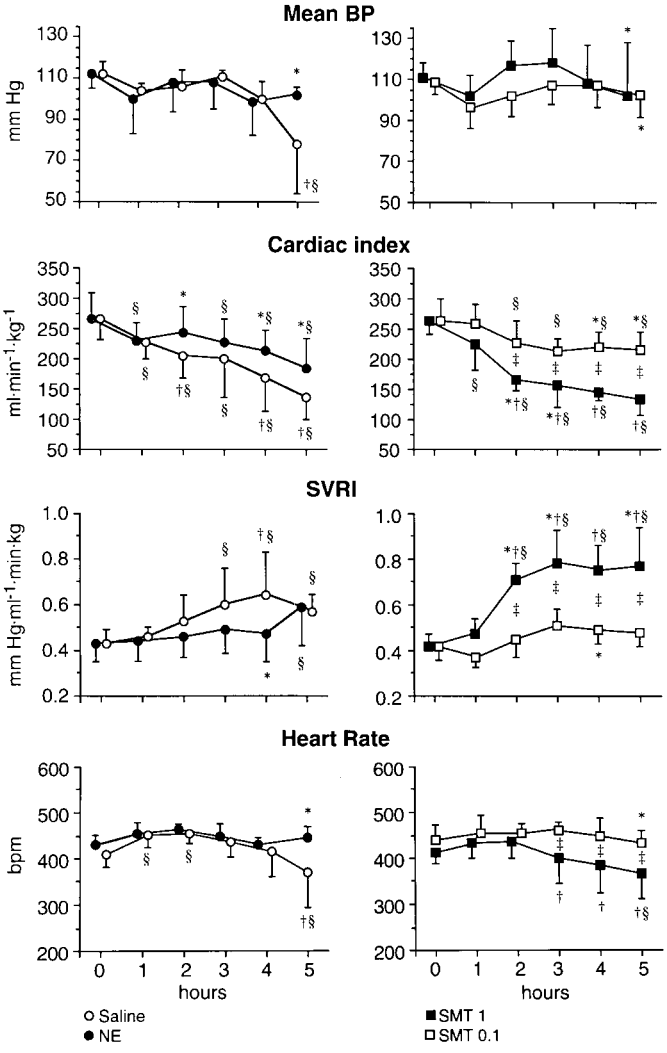


***Figure 1.*** Hemodynamic changes in rats challenged at baseline (time 0) with an intravenous bolus (given over 15 min) of 10 mg/kg LPS. One hour later, rats received a 4-h infusion of norepinephrine (NE, titrated to maintain blood pressure; n = 8), S-methyl-isothiourea (SMT) at low ($0.1 mg \cdot kg^{-1} \cdot h^{-1}$; n = 7) or at high ($1 mg \cdot kg^{-1} \cdot h^{-1}$; n = 7) dose, or an equivalent volume of normal saline ($2 ml \cdot kg^{-1} \cdot h^{-1}$; n = 8). BP = blood pressure; SVRI = systemic vascular resistance indexed to animal weight. Values are mean ± SD. *$p < 0.05$ versus saline; †$p < 0.05$ versus NE; ‡$p < 0.05$, SMT 0.1 versus SMT 1; §$p < 0.05$ versus time 0.

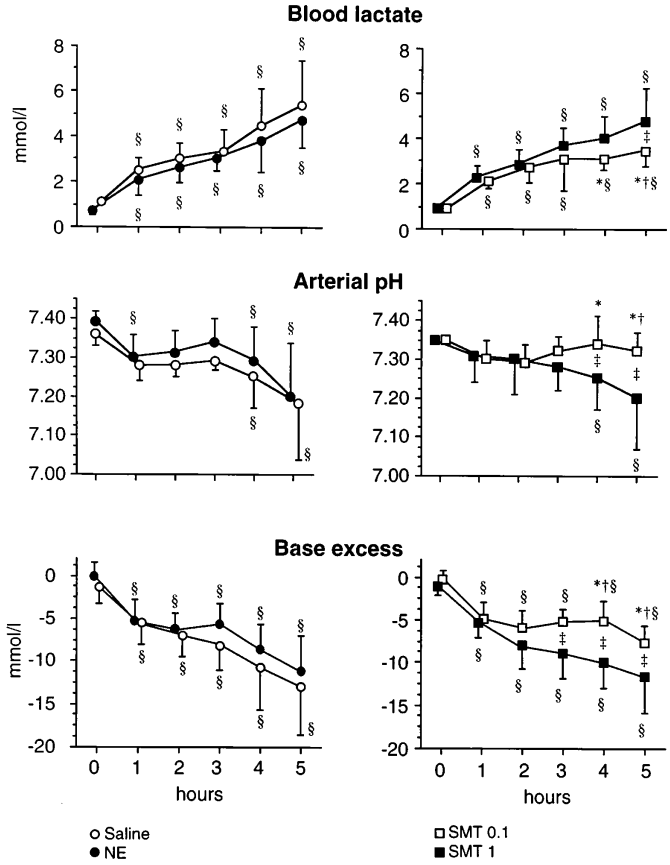


***Figure 2.*** Time course of blood lactate, arterial pH, and base excess in rats challenged at baseline (time 0) with an intravenous bolus (given over 15 min) of 10 mg/kg LPS. One hour later, rats received a 4-h infusion of norepinephrine (NE, titrated to maintain blood pressure; n = 8), S-methyl-isothiourea (SMT) at low ($0.1 mg \cdot kg^{-1} \cdot h^{-1}$; n = 7) or high ($1 mg \cdot kg^{-1} \cdot h^{-1}$; n = 7) dose, or an equivalent volume of normal saline ($2 ml \cdot kg^{-1} \cdot h^{-1}$; n = 8). Values are mean ± SD. *$p < 0.05$ versus saline; †$p < 0.05$ versus NE; ‡$p < 0.05$, SMT 0.1 versus SMT 1; §$p < 0.05$ versus time 0.

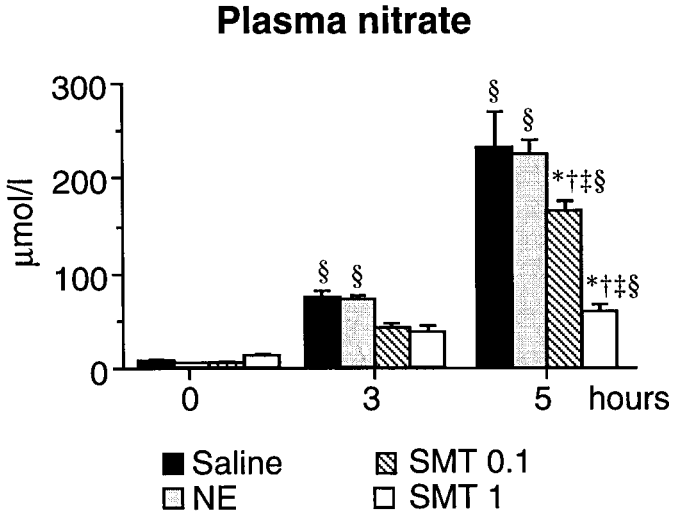


***Figure 3.*** Plasma nitrate concentrations in rats challenged at baseline (time 0) with an intravenous bolus (given over 15 min) of 10 mg/kg LPS. One hour later, rats received a 4-h infusion of norepinephrine (NE, titrated to maintain blood pressure; n = 8), S-methyl-isothiourea (SMT) at low (0.1 mg · $kg^{-1}$ · $h^{-1}$; n = 7) or high (1 mg · $kg^{-1}$ · $h^{-1}$; n = 7) dose, or an equivalent volume of normal saline (2 ml · $kg^{-1}$ · $h^{-1}$; n = 8). Values are mean ± SD. *p < 0.05 versus saline; †p < 0.05 versus NE; ‡p < 0.05, SMT 0.1 versus SMT 1; §p < 0.05 versus time 0.

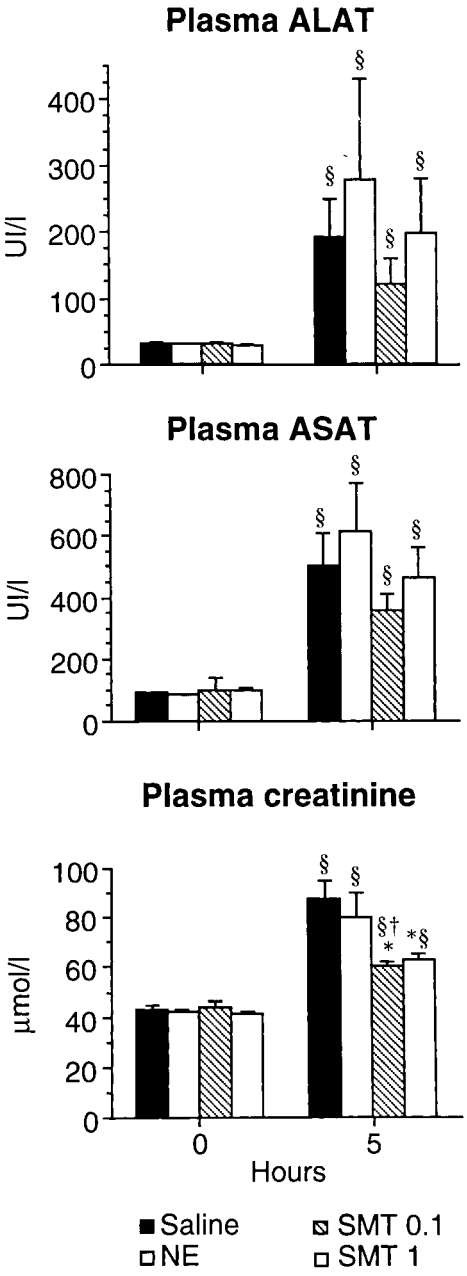


***Figure 4.*** Time course of plasma transaminases (ALAT, ASAT) and creatinine in rats challenged at baseline (time 0) with an intravenous bolus (given over 15 min) of 10 mg/kg LPS. One hour later, rats received a 4-h infusion of norepinephrine (NE, titrated to maintain blood pressure, n = 8), S-methyl-isothiourea (SMT) at low (0.1 mg · $kg^{-1}$ · $h^{-1}$; n = 7) or high (1 mg · $kg^{-1}$ · $h^{-1}$; n = 7) dose, or an equivalent volume of normal saline (2 ml · $kg^{-1}$ · $h^{-1}$; n = 8). Values are mean ± SD; *p < 0.05 versus saline; †p < 0.05 versus NE; ‡p < 0.05, SMT 0.1 versus SMT 1; §p < 0.05 versus time 0.

a large increase in plasma creatinine, which was significantly attenuated by SMT at either dose, but not influenced by NE.

#### Experiment 2

During the 4-h infusion prior to LPS challenge, mean BP remained stable in rats receiving either saline or low dose SMT, but it significantly increased in animals treated with high dose SMT (Table 2). At baseline, the transient, dose-dependent fall in mean BP induced by Ach was the same in all groups. This depressor response was significantly blunted after 4 h of treatment with high dose, but not with low dose, SMT (Figure 5). An acute drop in BP occurred after the LPS challenge, of the same magnitude in animals receiving saline or low dose SMT (Figure 6). In these rats, the subsequent administration of L-arg did not affect mean BP. By contrast, in animals treated with high dose SMT, LPS caused significantly less hypotension, and L-arg conspicuously decreased mean BP.

## DISCUSSION

#### Selectivity of iNOS Inhibition by SMT *In Vivo*

Various thiourea derivatives have been recently reported to act as extremely potent NO synthase inhibitors, both in human and in animal cell lines (8, 17). Among these compounds, SMT appears relatively selective for iNOS. When compared *in vitro* with L-NMMA, SMT was 8-fold more active on the iNOS expressed by stimulated macrophages or vascular smooth muscle cells; on the other hand, it was equipotent with L-NMMA as an inhibitor of ecNOS in unstimulated endothelial cells (10, 11). Little information is presently available on the selectivity of SMT towards iNOS *in vivo*. Very low doses (0.01 mg/kg as an intravenous bolus) increase BP in endotoxemic, but not in control rats (11), an observation consistent with, although not proof of, selectivity. Our results give further insights into the pharmacologic profile of SMT *in vivo*, by demonstrating that selective iNOS inhibition by this compound is dose-dependent.

We used three distinct procedures to evaluate the potential influence of SMT on ecNOS activity, as described in Experiment 2. ecNOS is involved in the physiologic regulation of arterial BP (18, 19) as well as in the endothelial-dependent vasodilation produced by acetylcholine (16, 20). Activation of

**TABLE 2**

**EFFECTS OF SMT ON BASAL SYSTEMIC BLOOD PRESSURE IN NONENDOTOXEMIC RATS***

| Groups | Mean BP (*mm Hg*) | |
|---|---|---|
| | Baseline | After Four Hours of Treatment |
| NaCl | 111 ± 5 | 108 ± 10 |
| SMT 0.1 mg · kg$^{-1}$ · h$^{-1}$ | 110 ± 10 | 108 ± 8 |
| SMT 1 mg · kg$^{-1}$ · h$^{-1}$ | 103 ± 4 | 118 ± 1$^{\dagger\ddagger\S}$ |

* Mean blood pressure (BP) measured at baseline and after a 4-h continuous infusion of saline (NaCl; n = 4) or S-methyl-isothiourea (SMT) (0.1 mg · kg$^{-1}$ · h$^{-1}$; n = 5) or SMT (1 mg · kg$^{-1}$ · h$^{-1}$; n = 4). Data present as the mean ± SD.

Statistical symbols: $^{\dagger}p < 0.05$ versus NaCl; $^{\ddagger}p < 0.05$ versus SMT 0.1; $^{\S}p < 0.05$ versus baseline.

ecNOS also occurs in some pathologic states such as the acute phase of endotoxemia (first hour), bearing a large responsibility in the hypotension observed at this stage of endotoxin shock (1). In absence of endotoxemia, the high (1 mg · kg$^{-1}$ · h$^{-1}$), but not the low (0.1 mg · kg$^{-1}$ · h$^{-1}$), dose of SMT increased BP and largely attenuated the hypotensive response to Ach (Figure 5). Furthermore, in animals treated with the high dose, the acute hypotension induced by LPS did not occur, unless supplemental L-arg was given (Figure 6). These results demonstrate that ecNOS was inhibited by high dose, but not by low dose, SMT. In the endotoxemic rats of Experiment 1, the accumulation of plasma nitrate, a sensitive marker of the enhanced NO release secondary to iNOS expression in this setting (21), was dose-dependently blunted by SMT, indicating that even the low dose was able to downregulate iNOS activity. We cannot rule out that part of this effect was related to some inhibition of iNOS protein expression by SMT since such an effect has been recently demonstrated with the parent compound aminoethyl-isothiourea (22), and further studies should be performed to address this particular issue. From these data, we conclude that SMT may act *in vivo* either as a selective or as a nonselective iNOS inhibitor, depending on the dose used.

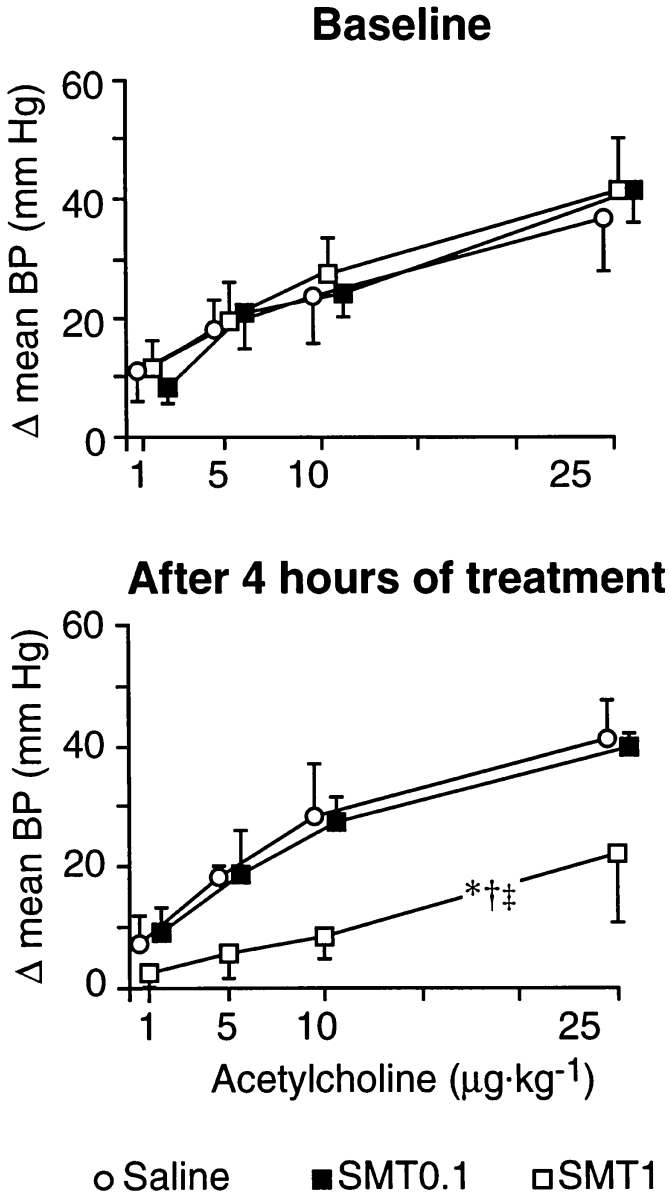


***Figure 5.*** Effects of S-methyl-isothiourea (SMT) on the depressor response to acetylcholine (Ach) *in vivo*. Sequential intravenous boluses of 1, 5, 10, and 25 μg/kg Ach were administered to 13 rats before (baseline) and after a 4-h infusion of 0.1 mg · kg$^{-1}$ · h$^{-1}$ SMT (SMT 0.1, n = 5), 1 mg · kg$^{-1}$ · h$^{-1}$ SMT (SMT 1; n = 4), or an equivalent volume of saline (2 ml · kg$^{-1}$ · h$^{-1}$; n = 4). The depressor response to each bolus of Ach (Δ mean BP) is expressed as the absolute fall in mean blood pressure measured 30 s after administration. Values are mean ± SD. *p < 0.05 versus saline; $^{\ddagger}p <$ 0.05, SMT 0.1 versus SMT 1; $^{\S}p < 0.05$ versus baseline.

### Hemodynamic Effects of SMT during Endotoxic Shock

LPS administration was followed by a progressive fall in BP and CI, accompanied by a slight increase in SVRI. Treatment with SMT significantly prevented hypotension (Figure 1), by mechanisms that clearly depended on whether selective or nonselective iNOS inhibition was achieved. At the high dose, SMT increased SVRI at the expense of further reducing CI, as previously reported with various nonselective NO synthase inhibitors, both in septic and nonseptic conditions (3, 7, 9). The mechanisms proposed to explain the depression of cardiac output caused by NO synthase inhibition include decreased venous return (23), right ventricular failure secondary to pulmonary hypertension (24), coronary vasospasm (25), and increased left ventricular afterload (3).

Contrary to high dose, the low dose of SMT prevented hypotension by maintaining CI, without increasing SVRI. We may confidently exclude an artifact related to differences in volume expansion or blood losses during the protocol: intravenous fluid load and amount of sampled blood were identical in all animals; this is also attested by the similar time-course of Hct in the four groups (Table 1). Although the mechanisms underlying the effects of low dose SMT on CI cannot be inferred from our data, selective iNOS inhibition may have either blunted a negative inotropic effect related to excess NO in the myocardium (26) or improved venous return by enhancing the tone of capacitance vessels. Indeed, we recently showed that the latter mechanism largely contributed to the beneficial hemodynamic effects of the selective iNOS inhibitor, L-canavanine in identical conditions (27).

### Further Effects of SMT during Endotoxic Shock

*Tissue hypoxia.* The cardiovascular failure induced by LPS was accompanied by significant tissue hypoxia, as attested by the simultaneous development of metabolic acidosis and hyperlactatemia (28), indicating a shift of energy metabolism

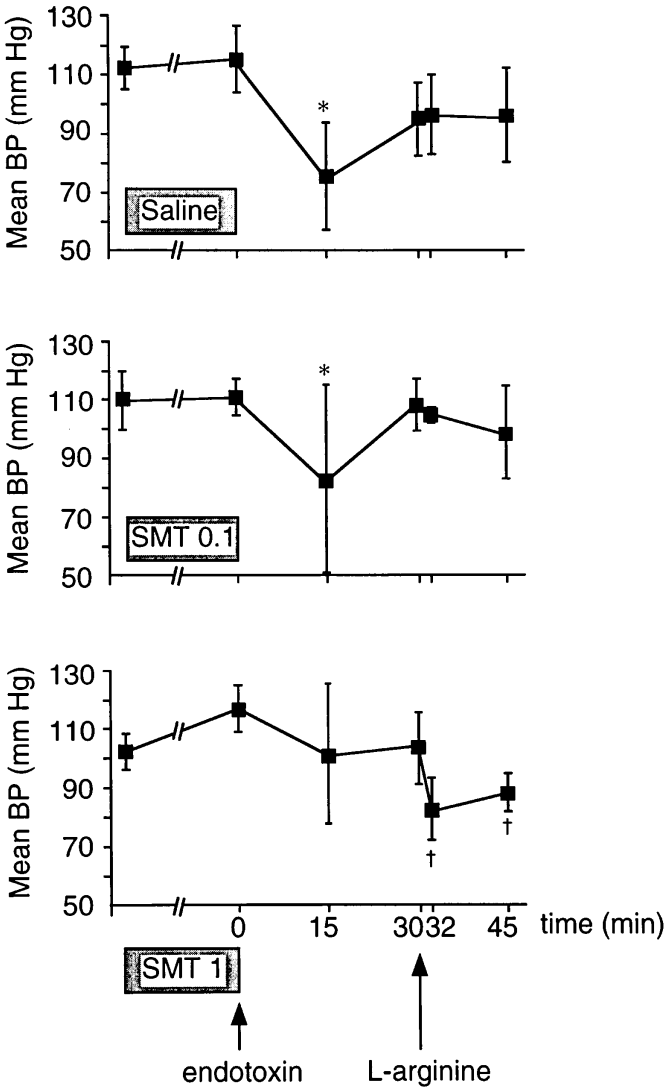


***Figure 6.*** Effects of S-methyl-isothiourea (SMT) on the acute hypotension induced by LPS. Three groups of rats were pretreated with a 4-h intravenous infusion of 0.1 mg · $kg^{-1}$ · $h^{-1}$ SMT (SMT 0.1; n = 5), 1 mg · $kg^{-1}$ · $h^{-1}$ SMT (SMT 1; n = 4), or an equivalent volume of saline (2 ml · $kg^{-1}$ · $h^{-1}$; n = 4). An intravenous bolus of 10 mg/kg LPS was administered and followed 30 min later by an intravenous bolus of 100 mg/kg L-arginine. Values are mean ± SD. *$p < 0.05$, mean BP at 15 min versus time 0; †$p < 0.05$ mean BP at 32 or 45 min versus mean BP at 30 min.

from aerobiosis to anaerobiosis, which might have resulted from two distinct, not mutually exclusive, mechanisms. The first one is that of a perfusion abnormality related to both macrocirculatory and microcirculatory derangements, leading to tissue ischemia and cellular anoxia, whereas the second one involves a primary impairment in the mechanisms of cellular oxygen utilization, independent of tissue blood flow (29–34).

These alterations were significantly reduced in rats receiving the low dose, but they were unaffected by the high dose of SMT (Figure 2). This observation might simply reflect the improved CI produced by the low dose; alternatively, it could also be related to a favorable influence of selective iNOS inhibition on $O_2$ extraction and utilization, in view of the well-characterized depression of cell respiration produced by excess NO (35). Indeed, NO interferes with cellular oxygen utilization and bioenergetic pathways via different mechanisms involving the inhibition of key enzyme in the Kreb's cycle, the mitochondrial electron transport chain, and the glycolytic pathway, as well as the activation of the nuclear enzyme poly-ADP-ribosyl-synthetase after NO-mediated DNA strand breakage (36, 37). Although high dose SMT could exert similar effects at the cellular level, it is likely that this potential benefit would be offset by a decrease in tissue perfusion, a phenomenon repeatedly observed with nonselective NO synthase inhibitors (6, 7). It is noteworthy that two other chemically unrelated selective iNOS inhibitors, namely, 1-amino-2-hydroxy-guanidine and L-canavanine, also reduce metabolic acidosis and hyperlactatemia in endotoxin shock (9, 38). Our results provide further evidence that selective iNOS inhibition improves tissue oxygenation in endotoxemia.

*Organ dysfunction.* Significant liver dysfunction developed in endotoxemic rats, as shown by a marked rise in plasma transaminases. Whether NO contributes to these alterations is currently debated since it appears to have both protective and deleterious effects on the liver in this setting (4). On one hand, NO allows liver perfusion to be maintained (39) through its vasodilatory and antithrombotic properties, and it may scavenge superoxide (40). On the other hand, excess NO may enhance the formation of other free radicals (40, 41) and inhibit several enzymatic pathways indispensable for cell viability (1). In such conditions, the effects of reducing NO production are difficult to predict. Indeed, after administration of NO synthase inhibitors to endotoxemic animals, beneficial, no, or detrimental influences on hepatic damage have all been reported (1), although the beneficial ones were more frequently observed with selective agents (9, 11, 13, 38). The results of the present study are consistent with these considerations; the rise in plasma transaminases induced by LPS was not significantly influenced by treatment with SMT, although it may have been blunted by the low dose (Figure 4).

The progressive development of renal failure, attested by an increase in plasma creatinine, was an additional consequence of LPS administration, and it was significantly attenuated by the two doses of SMT (Figure 4). This effect was probably not solely due to an improved perfusion pressure since the same restoration of BP with NE did not influence plasma creatinine; these data suggest some specific influence of SMT on intrarenal hemodynamics. The improved renal function noted with low dose SMT confirms previous results obtained with selective iNOS inhibitors (9, 11). The same favorable effect was obtained with the high, nonselective dose, at variance with other studies reporting an exacerbation of renal failure after nonselective NO synthase inhibition in endotoxic or bacteremic shock (6, 42). This discrepancy might be related to different degrees of ecNOS blockade or to other, yet unidentified, mechanisms.

The hemodynamic and metabolic effects of SMT at low dose noted in the present study largely confirm previous data obtained with other selective iNOS inhibitors in similar experimental conditions. For example, we recently found that L-canavanine, a selective iNOS inhibitor, prevented hypotension and improved cardiac output while significantly reducing the biologic signs of organ injury and tissue hypoxia when administered to endotoxemic rats (9, 27, 43). Other investigators have also reported effects comparable to those of SMT when using selective iNOS inhibitors such as aminoguanidine (44), aminoethylisothiourea (13), 1-amino-2-hydroxyguanidine (38), and guanidinoethyldisulphide (45) in rodent endotoxic shock. Therefore, it appears that the selective inhibition of iNOS produces very reproducible effects in endotoxemic rodents independently

from the agent used. This convergent information obtained with various chemically unrelated compounds strongly suggests that this approach might be of potential therapeutic benefit in septic conditions.

It is noteworthy that the contrasted effects of SMT observed in our study were obtained at doses that differently reduced plasma nitrate accumulation. Thus, one could argue that the beneficial influence of the low dose of SMT on CI and lactic acidosis was related to the partial, rather than selective, inhibition of iNOS and that comparable effects might be produced with any NOS inhibitor, selective or not, when given at low concentrations. Although we cannot formally rule out this hypothesis since we did not compare different doses of a nonselective NOS inhibitor in our study, there is currently a large body of experimental evidence pointing against such possibility. L-NMMA, the most common nonselective NOS inhibitor, has been used in a number of studies performed in different species and using different models of septic shock, i.e., endotoxic and bacteremic shock. In none of these studies could any beneficial influence of L-NMMA be demonstrated: whatever the dose used, this agent never enhanced CI or tissue oxygenation or reduced organ damage, whereas opposite effects were repeatedly reported (3, 4, 46–49). Even at very low dose (1 mg · $kg^{-1}$ · $h^{-1}$), L-NMMA was shown to detrimentally affect systemic blood flow and to promote the development of lactic acidosis and hepatic damage in hypodynamic endotoxic shock (3). Regarding outcome, L-NMMA increased mortality at high dose and had no effect at low dose (3, 50).

There are two apparent exceptions to the statements made above. The first one is the study by Nava and colleagues (51) who showed that L-NMMA at low dose (10 mg/kg) slightly reduced hepatic damage in endotoxemic rats. The second one is that of Teale and Atkinson (52) who reported an improved survival of septic mice treated with L-NMMA (300 mg/kg) in association with the antibiotics imipenem. However, these results could not be reproduced in the study performed by Evans and colleagues (50) in a similar model.

In humans, preliminary data from the multicenter placebo-controlled, double-blind trial of the NOS inhibitor 546C88 (L-NMMA) in patients with septic shock have been provided recently (53). In this study, infusion of L-NMMA at 5 mg · $kg^{-1}$ · $h^{-1}$ increased blood pressure, allowing adrenergic support to be progressively reduced. However, this effect was associated with a significant reduction in CI. In a previously published study by Petros and colleagues (54), it is worth noting that similar effects on blood pressure and cardiac output were achieved with a dose of L-NMMA of only 1 mg · kg · $h^{-1}$. Thus, when confronting both experimental and clinical data, it appears highly unlikely that any dose of L-NMMA could reproduce the beneficial effects of SMT found in our study.

### Selective iNOS Inhibition versus Adrenergic Support in Endotoxic Shock

In patients with septic shock, the mainstay of cardiovascular support presently remains the administration of adrenergic agents, in particular NE. It is of obvious clinical relevance to compare this standard approach with that based on iNOS inhibition. In the present study, NE was administered as a continuous infusion from T1 to T5 in order to reproduce the same schedule of administration used with SMT, starting with an extremely low dose (0.01 μg/kg/min) subsequently titrated to maintain BP. In fact, titration to an average of 4 μg/kg/min was essentially required during the last hour of study, when severe hypotension occurred in untreated animals. With low dose NE (i.e, from T1 to T4), SURI was unaffected, but CI was consistently higher than in rats treated with saline, reflecting a pharmacologic action on either cardiac pump function or venous return. From T4 to T5, the relatively high dose of NE required to maintain BP caused only a modest increase in SURI (Figure 1), an observation consistent with the known depression of arteriolar sensitivity to α-adrenergic stimulation in endotoxin shock (55, 56).

Although the hemodynamic effects of NE and low dose SMT were essentially the same (Figure 1), only low dose SMT limited the development of lactic acidosis (Figure 2). Two lines of hypotheses may be advanced to explain this difference. First, the changes in blood flow distribution induced by the adrenergic stimulation and selective iNOS inhibition may not be the same. Second, these interventions may differentially affect oxygen extraction and utilization at the cellular level; in particular, the balance between $O_2$ supply and demand may be less favorable with NE because of the thermogenic effect of β-adrenergic stimulation. Whatever the responsible mechanism, our findings indicate a possible advantage of selective iNOS inhibition over standard adrenergic support for the therapy of septic shock, as also suggested by the reduction in plasma creatinine obtained with low dose SMT but not with NE.

### Critiques of the Methods

*Experimental model*. We used an acute endotoxic shock model in the present study, which differs in several ways from human septic shock, so that extrapolations of our results to clinical sepsis may be questionable (33). The acute administration of large doses of endotoxin certainly does not reflect the clinical situations in which a focus of infection may be present for days, causing a more sustained and subacute release of LPS or bacteria (57). In addition, acute endotoxemia is associated with a marked myocardial depression (58) and induces a pattern of hypodynamic shock, characterized by hypotension, low cardiac output, and normal or increased systemic vascular resistance, which clearly differs from the hyperdynamic pattern of clinical septic shock (59). Therefore, further studies are needed to assess the effects of selective iNOS inhibition in experimental models of septic rather than endotoxic shock.

*Choice of the NOS inhibitor*. Because we used only SMT in this study, a potential criticism might be the lack of comparative studies using other NOS inhibitors such as L-NMMA or L-NAME. However, such experiments were not performed in our study since our primary objective was to compare the effects of selective inhibition of iNOS with those of standard adrenergic support in endotoxemic conditions. For this purpose, our choice to use SMT was based on several reports which clearly demonstrated that this compound acts as a selective iNOS inhibitor at relatively low concentration (8, 10). Because such a pharmacologic profile has not been demonstrated with the L-arginine analogs L-NMMA or L-NAME, which definitely act as nonselective NOS inhibitors (60), the issue to compare selective iNOS inhibition and adrenergic support is not feasible with the aforementioned agents.

In addition, it is worth mentioning that the particular profile of action of SMT, acting as a selective iNOS inhibitor at low concentration and as a nonselective NOS inhibitor at high concentration, makes it possible to compare both kinds of inhibition with a single molecule, thus eliminating potentially confounding consequences related to nonspecific effects when comparing the influence of different agents (61). In the case of L-NMMA, such nonspecific effects would make any interpretation extremely difficult since, beyond inhibiting NOS, this compound acts as an inhibitor of system $y^+$-mediated cellular L-arginine transport (62), and it may be used by NOS itself as a substrate for NO production (63). In addition, L-NMMA has

been shown to attenuate the rise in the plasma levels of TNFα caused by LPS in the rat (64). In such conditions, it seems likely that any differences between the effects of SMT and L-NMMA would be extremely difficult to interpret.

In summary, beneficial effects of selective iNOS inhibition were observed on blood flow and tissue oxygenation in rat endotoxemia. When cardiac output was similarly augmented with adrenergic support, tissue oxygenation was not improved. In view of these findings, the selective inhibition of iNOS is worth further consideration as a possible useful intervention in the therapy of septic shock.

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