

The effect of nitric oxide inhibitors and s-nitrosothiols on hemodynamic parameters in an animal model

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Background: Nitric oxide (NO) is becoming an increasingly important signaling molecule implicated in a growing number of physiological and pathophysiological processes. We sought to test the hypothesis that co-administration of S-nitro-N-acetylpenicillamine (SNAP) or S-nitrosocaptopril (CapSNO) with N^G-methyl-L-arginine ester (L-NAME) or N^G-methyl-L-arginine acetate (L-NMMA) may reverse the elevated systolic, diastolic, and mean arterial pressures caused by the administration of L-NAME or L-NMMA only.

Materials and methods: Blood pressure was measured using the CODA 6 machine. The hemodynamic parameters systolic blood pressure (SBP), diastolic blood pressure (DBP), mean arterial pressure (MAP) and heart rate (HR) were determined for each rat group. There was intravenous (IV) administration of the control (0.3 mL, saline) or dosage of 12.5 mg/kg body weight of SNAP or CapSNO via IV.

Results: In CapSNO and L-NAME-treated rats, CapSNO significantly decreased SBP from 131.12 ± 4.29 mmHg to 121.42 ± 4.24 mmHg after 5 minutes ($P < 0.0001$), and then L-NAME (administered at t = 5 min) increased SBP to 165.29 ± 6.79 mmHg at 10 minutes ($P < 0.05$). In SNAP and L-NAME-treated rats, SNAP significantly decreased SBP from 135.86 ± 2.84 mmHg to 106.98 ± 1.99 mmHg ($P < 0.0001$) after 5 minutes, and L-NAME increased SBP to 146.18 ± 3.19 mmHg after 25.0 minutes ($P < 0.05$). In rats treated with SNAP and L-NAME, SNAP decreased DBP to 74.98 ± 2.49 mmHg ($P < 0.0001$) after 5 minutes while L-NAME increased DBP to 105.01 ± 2.60 mmHg after 25 minutes ($P < 0.05$). In SNAP and L-NAME treated group there was an increase in HR after the administration of SNAP (486.60 ± 30.82 at 0 minutes to 555.66 ± 32.37 beats min⁻¹ at 5 minutes; $P < 0.0001$), followed by a decrease in HR to 336.90 ± 17.48 beats min⁻¹ at 25 minutes ($P < 0.05$) after the administration of L-NAME (at t = 5 min).

Conclusion: The data suggest that the actions of L-NAME and L-NMMA reversed the effects of NO released from SNAP or CapSNO. These drugs could be beneficial in the control of blood pressure in hypertensive patients.

Keywords: nitric oxide, N^G-methyl-L-arginine ester, N^G-methyl-L-arginine acetate, S-nitro-N-acetylpenicillamine, blood pressure, S-nitrosocaptopril

Introduction

Nitric oxide (NO) is a signaling molecule that is involved in a variety of physiological and pathological cellular processes in various tissues including vasculature, central nervous system, and skeletal muscle.¹ Nitric oxide is synthesized as a product of the conversion of its physiological precursor, L-arginine, to L-citrulline, a reaction which is catalyzed by a family of enzymes known as the NO synthases (NOS). Three isoforms of NOS have been identified in mammalian tissues, namely, NOS 1 (neuronal

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NOS, nNOS), NOS 2 (inducible NOS, iNOS) and NOS 3 (endothelial NOS, eNOS).² In contrast to the activities of nNOS and eNOS, which are tightly regulated by calcium-dependent calmodulin binding, iNOS does not require calcium ion or post-translational modification for its activity. Therefore, iNOS expression is associated with prolonged, exaggerated NO generation up to more than 1000-fold compared with nNOS and eNOS.^{3,4}

The NOS inhibitors are guanidino amino acids which act competitively at the NOS active site. These include N^G-nitro-L-arginine (L-NNA), N^G-methyl-L-arginine ester (L-NAME, a methyl ester pro-drug that is activated to become L-NNA) and N^G-methyl-L-arginine acetate (L-NMMA). L-NMMA, L-NNA, and L-NAME act as competitive, stereospecific inhibitors of NOS synthase and in some cases irreversible inhibitors of both the constitutive and inducible NO synthase. However, L-NMMA and L-NAME are relatively more selective for the constitutive isoforms and compete for the substrate, L-arginine, thereby inhibiting the formation of NO.^{5,6} L-NMMA and L-NAME are important in the regulation of regional vascular conductance in conscious animals.^{7,8} Vallance and colleagues found that arterial infusion of L-NMMA caused a 50% fall in basal blood flow.⁹ Further, Rees et al reported that L-NAME was about 10-fold more potent than L-NMMA in increasing blood pressure.¹⁰

In contrast, NO donors are drugs that generate NO through mechanisms that are independent of NOS. Commonly used agents are the organic nitrates (eg, glyceryl trinitrate [GTN], isosorbide dinitrate [ISDN]), sodium nitroprusside (SNP), sydnonimines (eg, molsidomine [SIN-1]), s-nitrosothiols (eg, S-nitrosoglutathione [GSNO], S-nitroso-N-acetylpenicillamine [SNAP]), NONOates (eg, SPERMINE-NONOate, DETA-NONOate), and hybrid donors (eg, nitroaspirins, nicorandil). S-nitroso-N-acetylpenicillamine and S-nitrosocaptopril (CapSNO) are examples of synthetic s-nitrosothiols which have been used extensively in research. S-nitroso-N-acetylpenicillamine has been shown to have a powerful vasodilatory effect and anti-platelet activity in the cardiovascular system¹¹ and was found to significantly reduce mean arterial pressure in animals.¹² However, its use as an anti-platelet agent is limited by its intense vasodilatory and hence hypotensive effects. CapSNO is a S-nitroso derivative and a hybrid compound of both captopril and NO.¹³ As a unique compound, it has properties of both a direct nitrovasodilator and an angiotensin converting enzyme (ACE) inhibitor and is not cross-tolerant with GTN.¹⁴ CapSNO has been found to dilate coronary arteries by virtue of its NO moiety and is a potential anti-anginal drug.¹⁵

Experimental evidence from animal studies suggested that SNAP had a beneficial effect of reducing blood pressure, although this was associated with decreased glucose tolerance.¹⁶ We sought to test the hypothesis that co-administration of L-NAME or L-NMMA with SNAP or CapSNO may reverse the elevated systolic, diastolic, and mean arterial pressure caused by the administration of L-NAME or L-NMMA only. Therefore, this study examined the effects of L-NMMA and L-NAME on hemodynamic parameters such as systolic, diastolic, mean arterial pressure, and heart rate in normotensive rats. The study also sought to investigate the co-administration of SNAP or CapSNO with L-NAME or L-NMMA on these hemodynamic parameters.

Materials and methods

Animals

Sprague-Dawley rats (250–350 g) were obtained from the Basic Medical Sciences Animal House, The University of the West Indies, Mona and were housed under the supervision of the attendants present. The rats were fed a diet of Purina Laboratory Chow and water administered ad libitum. A total of 44 rats were used for this study, and 6 to 8 rats were used for each set of experiments. All procedures were approved and conducted in accordance with the guidelines of the University Hospital of the West Indies, The University of West Indies and the Faculty of Medical Sciences (UHWI/UWI/FMS) Ethics Committee.

Sample preparation

A dosage of 30 mg kg⁻¹ body weight (BW) of L-NAME and L-NMMA (Sigma-Aldrich, St. Louis, MO, USA) were used for analysis.¹⁷ L-NAME and L-NMMA were dissolved in saline (0.3 mL; 0.9% NaCl) just before the beginning of the analysis. The solution was then administered into the tail vein of the rat immediately after the basal reading was taken for observation of the effect of the NOS inhibitors only on hemodynamic parameters.

Hemodynamic studies

Blood pressure was measured via a non-invasive method, using the CODA 6 machine (Kent Scientific Corporation, Torrington, CT, USA). This machine enabled measurement of systolic blood pressure (SBP), diastolic blood pressure (DBP), mean arterial pressure (MAP) and heart rate (HR) (in beats per minute) of each rat.¹⁸ The CODA system uses volume pressure recording (VRP) to measure blood pressure in mice and rats. The VRP correlates 99% with direct blood pressure and telemetry. Blood pressure was non-invasively measured

by determining the tail blood volume with a volume pressure recording sensor and an occlusion tail-cuff. Acclimatization readings were taken (which were not used in the final data analysis), followed by the acquisition of basal readings ($t = 0$ min). After basal readings were obtained, the machine was set to pause briefly to allow for intravenous administration of the control (0.3 mL, saline) or dosage of 12.5 mg/kg BW of SNAP or CapSNO (Sigma-Aldrich, St. Louis, MO, USA),¹⁷ via IV and then allowed to run for a further 5 minutes. At the 5-minute interval ($t = 5$ min), the machine was then set to pause briefly and immediately after which L-NAME, or L-NMMA were separately administered via IV at a dose of 30 mg kg⁻¹ BW. The machine was then allowed to continue to run uninterrupted for a further 20 minutes with blood pressure readings taken at 5-minute intervals by the machine until 5 cycles had been completed. Twenty-five minutes was done to ensure sufficient time to observe the effects of the drugs administered and to minimize undue stress to the animals in the study.

Statistical analysis

Each data point was expressed as mean \pm standard error of the mean (SEM). The significance between groups and within groups was determined using the *t* test or the 2-way ANOVA with $P \leq 0.05$ considered to be significant.

Results

In L-NAME-treated rats, there was a significant increase in SBP from 142.75 ± 5.75 mmHg to 185.12 ± 2.00 mmHg ($P < 0.0001$) after 5 minutes followed by a decrease to 143.58 ± 3.25 mmHg at the 15-minute interval. In L-NMMA-treated rats there was a significant increase in SBP from 137.23 ± 2.43 mmHg to 146.48 ± 2.37 mmHg after 5 minutes followed by a decrease to 125.32 ± 1.71 mmHg at 25 minutes (Figure 1). The increase in SBP was significantly higher in rats treated with L-NAME (185.12 ± 2.00 mmHg) compared with L-NMMA (146.48 ± 2.37 mmHg; $P < 0.00016$) and saline (113.00 ± 3.13 mmHg; $P < 0.00012$) after 5 minutes.

In CapSNO and L-NAME-treated rats, administration of CapSNO significantly decreased SBP to its lowest value of 121.42 ± 4.24 mmHg after 5 minutes ($P < 0.0001$). The administration of L-NAME (at $t = 5$ min) resulted in fluctuations in the SBP with a sharp increase to 165.29 ± 6.79 mmHg at 10 minutes ($P < 0.05$) and a gradual decrease to 162.50 ± 2.76 mmHg at 25.0 minutes. In SNAP and L-NAME-treated rats, administration of SNAP caused a significant decrease in SBP to its lowest value of 106.98 ± 1.99 mmHg ($P < 0.0001$) after 5 minutes. Administration of L-NAME (at

$t = 0$ min) caused a gradual increase to 146.18 ± 3.19 mmHg after 25-minutes. In SNAP and L-NMMA-treated rats, SNAP decreased SBP to 126.29 ± 2.51 mmHg ($P < 0.0001$) after 5 minutes (Figure 1).

L-NAME significantly increased the DBP to 134.16 ± 2.85 mmHg ($P < 0.0001$) after 5 minutes, followed by a decrease to 110.48 ± 3.15 mmHg after 15 minutes, then an increase to 128.86 ± 1.62 mmHg ($P < 0.05$) after 25 minutes (Table 1). In rats treated with SNAP and L-NAME, SNAP decreased the SBP to 74.98 ± 2.49 mmHg ($P < 0.0001$) 5 minutes after the administration of SNAP. On administration of L-NAME the DBP increased to 105.01 ± 2.60 mmHg at 25 minutes ($P < 0.05$). The DBP in rats treated with SNAP and L-NAME (89.15 ± 4.69 mmHg) was significantly lower than in rats treated with CapSNO and L-NAME (133.26 ± 6.15 mmHg; $P = 0.000011$) after 10 minutes (Table 1). In SNAP and L-NMMA-treated rats, the DBP of 112.84 ± 2.82 mmHg after 20 minutes, was significantly higher than saline control (86.00 ± 1.50 mmHg; $P = 0.0019$).

In examining the MAP, L-NAME (150.82 ± 2.32 mmHg) exhibited a significant hypertensive effect compared with saline (101.75 ± 2.72 mmHg at 5 minutes; $P < 0.0001$). There was a significant reduction at 15 minutes where rats treated with L-NAME had MAP of 121.22 ± 3.05 mmHg which was significantly higher than in those treated with L-NMMA (96.22 ± 1.24 mmHg; $P < 0.0001$; Figure 2). The decrease in MAP in rats treated with SNAP and L-NAME was more significant after 5 minutes (85.29 ± 2.24 mmHg) than in those treated with CapSNO and L-NAME (100.96 ± 3.80 mmHg; $P = 0.00068$). Further, the MAP for rats treated with CapSNO and L-NAME (143.61 ± 6.33 mmHg) was significantly higher than in those treated with SNAP and L-NAME (100.64 ± 4.58 mmHg; $P < 0.0001$) after 10 minutes (Figure 2).

A reduction in HR from 433.70 ± 10.08 beats min⁻¹ at 0 minutes to 362.46 ± 15.81 beats min⁻¹ at 5 minutes ($P < 0.05$) was observed in rats treated with L-NAME. This was followed by an increase to 405.21 ± 22.75 beats min⁻¹ after 10 minutes (Table 2). A similar trend was observed in rats treated with L-NMMA where the HR decreased from 411.68 ± 12.72 to 363.76 ± 11.75 beats min⁻¹ ($P < 0.05$) after 5 minutes followed by an increase to 414.73 ± 24.50 beats min⁻¹ after 15 minutes ($P < 0.05$). In the SNAP and L-NAME treated group there was an increase in HR after the administration of SNAP (486.60 ± 30.82 at 0 minutes to 555.66 ± 32.37 beats min⁻¹ at 5 minutes; $P < 0.0001$). This was followed by a gradual decrease in HR to 336.90 ± 17.48 beats min⁻¹ at 25 min ($P < 0.05$) after the administration of L-NAME (Table 2).

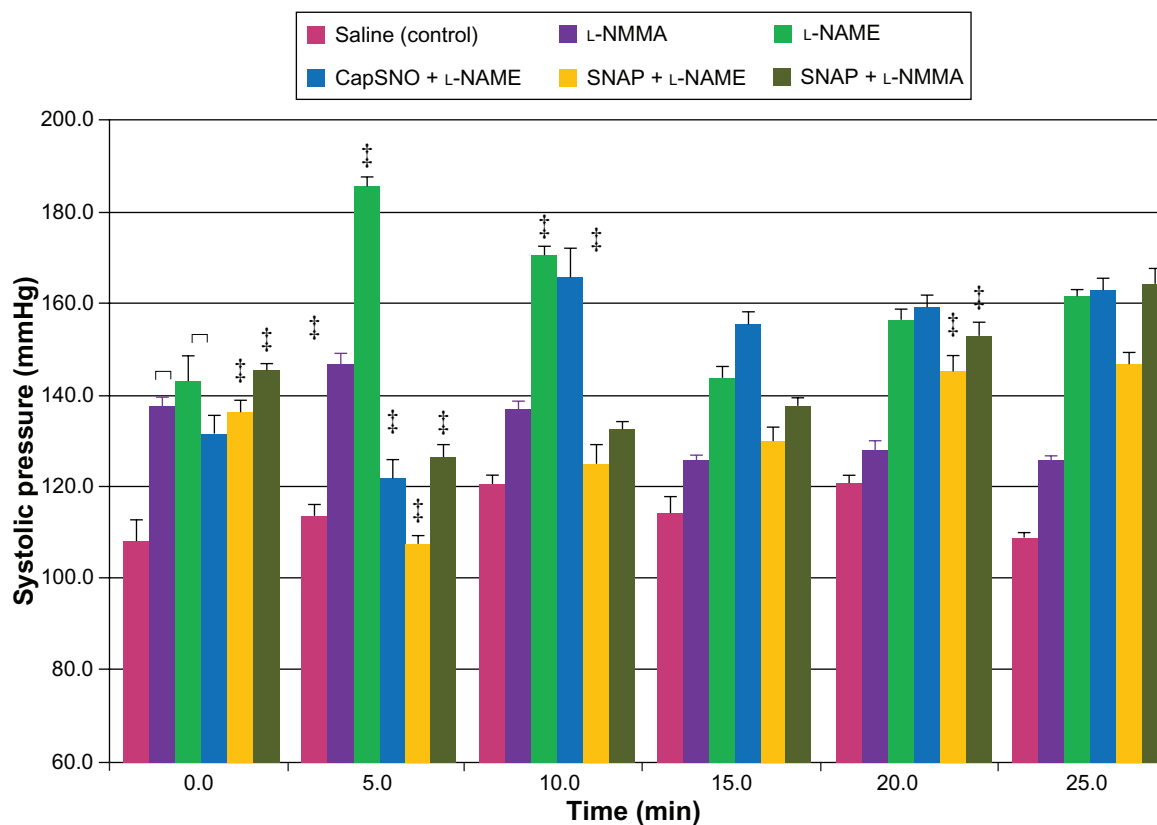


Figure 1 The effect of SNAP, CapSNO, L-NAME, and L-NMMA on systolic blood pressure.

Note: ‡Significant differences between values at time point given and $t = 0$ or $t = 5.0$ min; for full names of chemical compounds see Introduction.

Discussion

Nitric oxide (NO) is an important mediator with a wide variety of biological functions including the control of blood vessel tone and neurotransmission. Analysis of the hemodynamic data revealed that SNAP and CapSNO reduced systolic, diastolic, and mean arterial pressures in normotensive rats. According to the data obtained, SNAP caused the most significant decrease in blood pressure and was shown to be more potent than CapSNO. The blood pressure-lowering effect of SNAP correlates with findings proposed by Shaffer et al who showed that CapSNO administered at

a dosage of 12.5 mg/kg BW could significantly decrease blood pressure in anesthetized and conscious rats when compared with captopril-treated rats.¹³ The findings from this study are in congruence with similar findings in which, as we have indicated, GSNO and SNAP had beneficial effects based on their reduction of blood pressure in normotensive dogs.^{12,16} GSNO and SNAP are potent vasodilators and are able to cause a significant decrease in DBP and SBP.^{19,20} The mechanism involves the release of the NO from the s-nitrosothiols leading to the activation of soluble guanylate cyclase and a decrease in Ca^{2+} concentration.^{21,22}

Table 1 The effect of SNAP, CapSNO, L-NAME, and L-NMMA on diastolic blood pressure

Time (min)	Saline	Diastolic blood pressure (mmHg)				
		L-NAME	L-NMMA	CapSNO + L-NAME	SNAP + L-NAME	SNAP + L-NMMA
0.0	97.25 ± 3.2	92.59 ± 5.49	90.70 ± 2.19	99.36 ± 4.16	91.86 ± 3.43	93.79 ± 1.93
5.0	97.00 ± 2.5	134.16 ± 2.85‡	95.75 ± 1.47	91.28 ± 3.81	74.98 ± 2.49‡	94.23 ± 2.41
10.0	94.50 ± 1.5	123.13 ± 1.79	93.60 ± 1.63	133.25 ± 6.15‡*	89.15 ± 4.69*	97.62 ± 1.88
15.0	89.75 ± 2.3	110.48 ± 3.15	82.14 ± 1.48	121.77 ± 2.83	91.68 ± 2.80	103.55 ± 3.05
20.0	86.00 ± 1.50*	124.95 ± 2.00‡	82.58 ± 2.11	127.59 ± 2.75‡	102.04 ± 2.96‡	112.84 ± 2.82‡
25.0	84.50 ± 1.30	128.86 ± 1.62	84.10 ± 1.78	130.05 ± 3.01	105.01 ± 2.60	121.63 ± 3.21

Notes: ‡Significant differences between values at time point given and $t = 0$ or $t = 5.0$ min; *Significant differences between the groups; for full names of chemical compounds see Introduction.

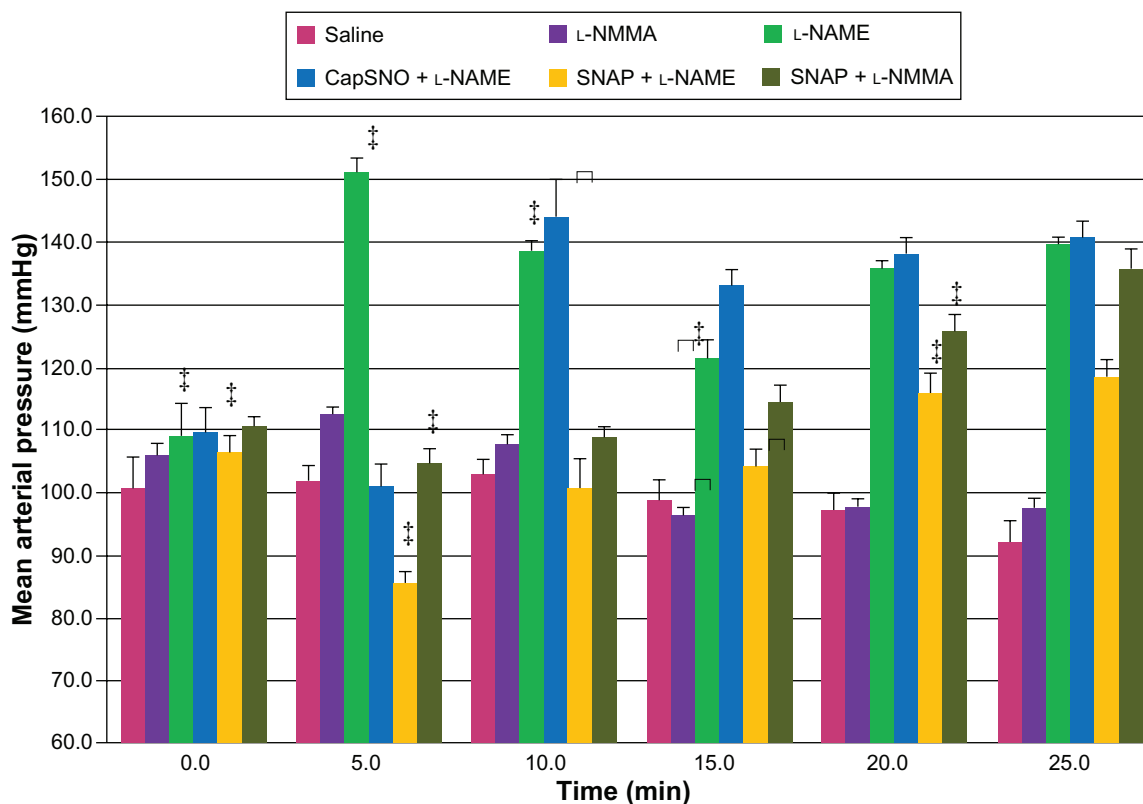


Figure 2 The effect of SNAP, CapSNO, L-NAME, and L-NMMA on mean arterial pressure.

Note: ‡Significant differences between values at time point given and $t = 0$ or $t = 5.0$ min; for full names of chemical compounds see Introduction.

In the present study, L-NMMA and L-NAME reversed the hypotension caused by SNAP and CapSNO. L-NMMA and L-NAME act by competitive inhibition of NO synthase and are firmly established to produce both acute and chronic hypertension in many animal species.²³ Samsell et al found that arterial blood pressure increased 36% in rats on chronic administration of L-NAME and 37% in rats on L-NAME plus prazosin.²⁴ The hypertensive response as observed by the elevated mean arterial pressure by L-NAME or L-NMMA administration could have been caused by a number of factors such as: i) decreasing the vasodilator effects of NO on the vasculature, or other hypertensive factors, normally

attenuated by NO, ii) an enhanced response of the vascular smooth muscle to vasoconstrictors such as phenylephrine during the NO synthesis blockade, iii) a diminished vasodilator response of the vascular smooth muscle to endogenous dilators or exogenous NO donors such as SNAP or CapSNO, and iv) the release of endothelium-derived contracting factors. However, our data do not directly address these possibilities.^{25–28}

A key finding of the study is that while both NO inhibitors fully reversed the hypotensive effect of SNAP and CapSNO, L-NAME was more potent than L-NMMA in increasing blood pressure. Researchers have found that

Table 2 The effect of SNAP, CapSNO, L-NAME and L-NMMA on heart rate

Time (min)	Saline	Heart rate (beats min^{-1})				
		L-NAME	L-NMMA	CapSNO + L-NAME	SNAP + L-NAME	SNAP + L-NMMA
0.0	327.00 \pm 6.62	433.70 \pm 10.08	411.68 \pm 12.72	458.04 \pm 16.32	486.60 \pm 30.82	474.31 \pm 24.84
5.0	351.75 \pm 13.31	362.46 \pm 15.81	363.76 \pm 11.75	466.87 \pm 14.77 [‡]	555.66 \pm 32.37 [‡]	479.53 \pm 5.40 [‡]
10.0	352.25 \pm 15.12	405.21 \pm 22.75	382.22 \pm 17.89	380.71 \pm 12.86	570.84 \pm 40.07	409.22 \pm 7.44
15.0	338.75 \pm 9.38	389.35 \pm 24.08	414.73 \pm 24.50	355.07 \pm 9.52	594.95 \pm 46.04	411.06 \pm 15.24
20.0	344.50 \pm 4.25	385.40 \pm 11.42 [‡]	406.64 \pm 19.26 [‡]	321.95 \pm 5.27	576.26 \pm 50.37 [‡]	397.88 \pm 13.28 [‡]
25.0	339.75 \pm 8.40	391.39 \pm 11.69	395.32 \pm 19.91	336.90 \pm 17.48	590.53 \pm 44.02	363.33 \pm 9.51

Note: ‡Significant differences between values at time point given and $t = 0$ or $t = 5.0$ min; for full names of chemical compounds see Introduction.

the blood pressure-raising effect of L-NAME was 2 to 3 times greater than that previously reported in similar studies using L-NMMA.^{27,29} One possible explanation is that human endothelial cells enzymatically degrade L-NMMA to L-arginine, which would oppose the pressor effect.³⁰ Further, in normotensive human subjects, L-NAME acutely increased blood pressures into the hypertensive range.³¹ The largest effect of L-NAME was on DBP, which exceeded 85 mmHg in 74%, 90 mmHg in 52%, and 100 mmHg in 19% of subjects. DBP did not exceed 109 mmHg in any subject, and in all subjects, blood pressure returned to baseline values by 24 hours without any deleterious side effects.³¹

L-NAME causes acute vasoconstriction in a number of vascular beds and studies by Gardiner et al and Baylis et al showed that administration of L-NAME for several hours to conscious rats resulted in increases in arterial blood pressure.^{32,33} This NO inhibition in rats was associated with a decrease in cardiac output and blood flow to several vascular beds, and the bradycardia that accompanied the NO inhibition was reversed with atropine.^{32,34} These studies as well as results in this study suggest strongly that NO synthesis inhibition in the rat has marked effects on arterial blood pressure regulation for both short-term (minutes and hours) and long-term (days and weeks) periods. NO synthesis inhibitors have also been administered to conscious dogs by Persson et al who found that a single injection of L-NNA caused an increase in arterial blood pressure that lasted for 24 hours.³⁵ The results of these studies differ from ours in the type of NO inhibitor used, and the concentrations of the NO inhibitors used in this study caused an effect which lasted for less than 1 hour.

An increase in sympathetic activity is another possible mediator of the increase in arterial blood pressure observed during the study due to L-NAME or L-NMMA administration. Sakuma et al showed that renal sympathetic nerve activity acutely increased after administration of L-NAME.²⁵ NO may act centrally to exert a tonic inhibitory influence on sympathetic nerve activity (SNA). Reduction in this tonic restraint has been postulated to contribute to the hypertension caused by synthetic NOS inhibitor. In addition, Pucci et al found that the pressor and renal vasoconstrictor effects of L-NAME were not impaired in anesthetized rats with blockade of either ganglionic transmission, α -adrenergic receptors, arginine vasopressin, the renin-angiotensin system, or prostanoids.³⁶ Therefore, whether increased sympathetic activity mediates part of the L-NAME pressor effect in the present study is not still clear. Further, in previous studies, intrarenal infusion of NO synthesis inhibitors

induced potent renal vasoconstriction and anti-natriuresis.³⁷ Liu et al recorded renal SNA in conscious rabbits and found that intravenous L-NAME increased blood pressure and reflexively decreased renal SNA, whereas the return of blood pressure to baseline with hydralazine caused renal SNA to return to, but not exceed, baseline.³⁷ This suggested that L-NAME alone did not exert sympathoexcitatory effects upon renal SNA. On the contrary, McKeogh et al suggested that the predominant effect of systemic NOS inhibition is to decrease SNA to both the heart and kidneys.³⁸

The hypertension induced by L-NMMA and L-NAME was accompanied by bradycardia and the hypotension induced by SNAP or CapSNO was accompanied by tachycardia. A study by Manning and Hu showed that L-NAME caused significant decreases in the heart rate component of baroreceptor reflex sensitivity to nitroprusside.³⁹ Studies have shown that NO donors directly (ie, independent of their effects on the autonomic nervous system) increase HR through a NO-cGMP-mediated stimulation of the pacemaker current I(f) in isolated sinoatrial node cells and spontaneously beating atrial preparations.^{40,41} The direct positive chronotropic effect of exogenous NO is functionally relevant in vivo, both in animals^{42,43} and in humans.⁴¹ For instance, topical administration of SNP to the sinoatrial node in the pig heart in situ increases HR in the absence of changes in arterial blood pressure.⁴² Furthermore, intravenous infusion of SNP or molsidomine increases HR independent of autonomic activation in the rabbit⁴³ and in humans when arterial blood pressure is clamped by simultaneous application of phenylephrine.⁴⁴

In conclusion, the results demonstrate that SNAP and CapSNO reduced systolic, diastolic, and mean arterial pressures in rats. SNAP caused more significant decrease in blood pressure and was shown to be more potent than CapSNO. This hypotension was associated with an increase in heart rate. The data suggest that L-NAME and L-NMMA inhibit the action of NOS and that their action overrides the effect of NO released from SNAP or CapSNO. This may help to control blood pressure in hypertensive patients.

Disclosure

The authors have nothing to declare.

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