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Blockade of voltage-sensitive Ca^{2+}-channels markedly diminishes nitric oxide- but not L-*S*-nitrosocysteine- or endothelium-dependent vasodilation in vivo

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Abstract

The aim of this study was to determine the hemodynamic responses elicited by systemic injections of (i) the nitric oxide (NO)-donors, sodium nitroprusside (10 nmol/kg, i.v.) and (*Z*)-1-[*N*-methyl-*N*-[6(*N*-methylammoniohexyl)amino]diazen-1-ium-1,2-diolate (MAHMA NONOate, 25 nmol/kg, i.v.), (ii) the endothelium-derived *S*-nitrosothiol, L-*S*-nitrosocysteine (100 nmol/kg, i.v.), and (iii) the endothelium-dependent agonist, acetylcholine (1.0 μg/kg, i.v.), in anesthetized rats, before and after injection of the voltage-sensitive Ca^{2+}-channel (Ca^{2+}_{VS}-channel) blocker, nifedipine (500 nmol/kg, i.v.). Before injection of nifedipine, the agents produced similar falls in mean arterial blood pressure, and in hindquarter and mesenteric vascular resistances. The depressor and vasodilator responses elicited by sodium nitroprusside and MAHMA NONOate were markedly attenuated by nifedipine. The falls in mean arterial blood pressure and mesenteric resistance elicited by L-*S*-nitrosocysteine and acetylcholine were not attenuated but the falls in hindquarter resistance were slightly attenuated by nifedipine. The cyclooxygenase inhibitor, indomethacin (10 mg/kg, i.v.), did not affect the actions of sodium nitroprusside, MAHMA NONOate, L-*S*-nitrosocysteine or acetylcholine or the effects of nifedipine on the hemodynamic actions of these compounds. The decomposition of sodium nitroprusside (0.2 nmol/ml), MAHMA NONOate (0.5 nmol/ml) and L-*S*-nitrosocysteine (2 nmol/ml) to NO upon addition to rat blood was not affected by nifedipine (10 μM). These findings suggest that (i) exogenously applied NO relaxes resistance arteries in vivo by inhibition of Ca^{2+}_{VS}-channels whereas L-*S*-nitrosocysteine and the non-prostanoid endothelium-derived relaxing factor (EDRF) released by acetylcholine acts by additional mechanisms, and (ii) this EDRF may be an *S*-nitrosothiol which acts independently of its decomposition to NO.

Keywords: *S*-Nitrosothiol; Nitric oxide (NO); EDRF (endothelium-derived relaxing factor); Ca^{2+}-channel, voltage-sensitive; Vasodilation

1. Introduction

Systemic injections of endothelium-dependent agonists such as acetylcholine elicit vasodilator responses in resistance arteries in mesenteric and hindlimb vascular beds of conscious rats (Gardiner et al., 1990; Davisson et al., 1996a). Moreover, systemic injections of nitric oxide (NO) synthesis inhibitors elevate mean arterial blood in these rats via increases in peripheral vascular resistances (Gardiner et al., 1990; Davisson et al., 1996a,b; Colombari et al., 1998). These findings suggest that NO is tonically released from endothelial cells in resistance arteries of the rat and that NO plays an important role in regulating the tone of these vessels (see Moncada et al., 1991). On the basis of studies in conduit vessels, it is likely that NO relaxes resistance arteries by activation of soluble guanylate cyclase which generates cGMP in vascular smooth muscle of these arteries (Ignarro, 1990). The vasodilator actions of NO/cGMP in resistance arteries may involve inhibition of (i) Ca^{2+}-dependent contractile processes, (ii) G protein-coupled receptor-induced activation of phospholipase C-mediated mobilization of intracellular stores of Ca^{2+}, (iii) Ca^{2+}-activated Cl^- channels, and (iv) voltage-

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sensitive Ca^{2+}-channels (Ca^{2+}_{VS}-channels) (Abdel-Latif, 1986; Lamb et al., 2000). The latter possibility is supported by evidence that Ca^{2+}_{VS}-channels in ventricular myocytes are inhibited by NO and cGMP (Campbell et al., 1996).

Despite these findings, there is no definitive evidence that NO is the primary endothelium-derived relaxing factor (EDRF) in resistance arteries in vivo. Such evidence would include that systemic injections of selective inhibitors of soluble guanylate cyclase elevate mean arterial blood pressure via inhibition of the vasodilator actions of EDRF. Moreover, although injections of NO-donors elicit pronounced vasodilator responses in mesenteric beds of conscious rats, they elicit minimal responses in the hindlimb beds of these rats (see Gardiner et al., 1990; Davisson et al., 1996a). These findings suggest that NO may not necessarily be the primary EDRF in every vascular bed of the rat. On the basis of findings in conduit arteries, the primary EDRF released within resistance arteries may be the *S*-nitrosothiol, L-*S*-nitrosocysteine (Myers et al., 1990; Rubanyi et al., 1991; Rosenblum, 1992). Systemic injections of L-*S*-nitrosocysteine elicit pronounced vasodilator responses in peripheral vascular beds of conscious rats including the hindlimb beds (Davisson et al., 1996d). The ability of L-*S*-nitrosocysteine to exert vasodilator responses in the hindlimb bed suggests that this *S*-nitrosothiol acts by mechanisms in addition to its decomposition to NO. The NO-independent mechanisms by which L-*S*-nitrosocysteine dilates resistance arteries may involve (i) nitrosation of cysteine residues in functional proteins (Stamler et al., 1992b, 1997) and (ii) activation of stereoselective recognition sites (Davisson et al., 1996d, 1997b; Lewis et al., 1996; Ohta et al., 1997; Hoque et al., 1999), which recognize L-*S*-nitroso-β,β-dimethylcysteine (Travis et al., 1996, 1997) but not L-*S*-nitrosoglutathione (Davisson et al., 1997b; Lewis et al., 1996; Ohta et al., 1997).

The purpose of this study was to provide evidence as to whether NO is the primary non-prostanoid EDRF in resistance vessels of the rat. The findings of Campbell et al. (1996) suggest that NO may act primarily by inhibition of Ca^{2+}_{VS}-channels. Accordingly, prior inhibition of these channels may attenuate the vasodilator actions of endothelium-derived NO. The specific aim of this study was to determine the effects of the Ca^{2+}_{VS}-channel blocker, nifedipine (see Luscher and Vanhoutte, 1990), in the presence or absence of the cyclooxygenase inhibitor, indomethacin (Luscher and Vanhoutte, 1990), on the vasodilator actions of (i) the NO-donors, (*Z*)-1-[*N*-methyl-*N*-[6(*N*-methylammoniohexyl)amino]diazen-1-ium-1,2-diolate (MAHMA NONOate) (Benkusky et al., 1998) and sodium nitroprusside (Feelisch, 1991), (ii) L-*S*-nitrosocysteine, and (iii) acetylcholine, in urethane-anesthetized rats. Urethane-anesthetized rats were used because systemic injections of NO-donors produce pronounced vasodilator responses in hindquarter and mesenteric vascular beds of these rats (Travis et al., 1997; Whalen et al., 1999b,c).

2. Materials and methods

2.1. Rats and surgical procedures

The protocols were approved by the University of Iowa Animal Care and Use Committee. Male Sprague–Dawley rats (275–325 g) were anesthetized with urethane (1 g/kg, i.p.). A catheter was placed in the femoral vein to give drugs. Supplemental doses of urethane (0.1 g/kg, i.v.) were given as necessary during surgery and experimentation. A catheter was also placed in a femoral artery to record mean arterial blood pressure. A midline laparotomy was performed and a pulsed Doppler flow probe was placed around the lower abdominal aorta to measure hindquarter blood flow velocities and to determine hindquarter vascular resistances. A flow probe was placed around the superior mesenteric artery to measure mesenteric blood flow velocities and to determine mesenteric vascular resistances. Vascular resistance at any time point was determined by dividing mean arterial blood pressure by blood flow velocity. Note that a fall in vascular resistance is due to a vasodilation in peripheral resistance arteries. Details of the Doppler technique, including the suitability of determining percent changes in vascular resistance, have been described previously (Haywood et al., 1981; Lacolley et al., 1991a,b). Rat body temperatures were kept at 37°C by a heating pad. The rats breathed room air supplemented with 95% O_2–5% CO_2 via a face mask (Whalen et al., 1999a).

2.2. In vivo protocols

Mean arterial blood pressure was recorded by connecting the arterial catheter to a Beckman Dynograph-coupled pressure transducer. Blood flow velocities were recorded by connecting the flow probe leads to a Beckman Dynograph-coupled Doppler Flowmeter (Department of Bioengineering, University of Iowa) (Davisson et al., 1996a,b,c,d). All drugs were given as bolus intravenous injections. The drug injectates (10–50 μl) were placed into the intravenous catheter and were flushed into the rat by a 150 μl volume of saline. Two groups of rats received an injection of saline, and after 25–30 min, they received injections of the vasodilator agents, sodium nitroprusside (10 nmol/kg, i.v.), MAHMA NONOate (25 nmol/kg, i.v.), acetylcholine (1.0 μg/kg, i.v.) and L-*S*-nitrosocysteine (100 nmol/kg, i.v.). One group ($n = 6$) then received an injection of the vehicle used to dissolve nifedipine, whereas the other group ($n = 8$) received nifedipine (500 nmol/kg, i.v.). After 30–45 min, the vasodilator agents were given again. Two other groups of rats received an injection of indomethacin (10 mg/kg, i.v.) and after 25–30 min, injections of the above doses of the vasodilator

agents. One group ($n = 6$) received vehicle whereas the other group ($n = 8$) received nifedipine (500 nmol/kg, i.v.). After 30–45 min, the vasodilator agents were given again. The doses of the vasodilator agents were chosen because (i) they produce similar depressor and vasodilator responses to one another, and (ii) these responses are about 50% of maximum (see Travis et al., 1997; Whalen et al., 1999a,b,c). Responses elicited by each injection of a vasodilator agent were allowed to subside completely before another injection was given. The order of these injections was changed to properly assess the effects of nifedipine.

2.3. NO measurements

Rats ($n = 20$) were anesthetized with urethane (1 g/kg, i.p.) and blood was drawn from a carotid artery catheter and 800 μl volumes were placed into air-tight wells bubbled with 20% O_2. At this time, 100 μl of nifedipine (10 nmol) or vehicle were added to the wells and the solutions were mixed. The O_2 delivery was discontinued and the air-tight wells were connected to an NO analyzer (see Travis et al., 1996). After 30 s, 100 μl of sodium nitroprusside (0.2 nmol in a final volume of 1 ml), MAHMA NONOate (0.5 nmol) or L-*S*-nitrosocysteine (2 nmol) were added to the wells. The amounts of these compounds were chosen because (i) the blood volume of 275–325g rats is about 20 ml (see Davisson et al., 1996d), (ii) intravenous injections of nifedipine (500 nmol/kg), sodium nitroprusside (10 nmol/kg), MAHMA NONOate (25 nmol/kg) and L-*S*-nitrosocysteine (100 nmol/kg) in these rats would result in peak blood concentrations of about 10, 0.2, 0.5, and 2 nmol/ml, respectively. The NO generated by these compounds was delivered to the NO analyzer by a stream of N_2 gas bubbling over the blood (see Travis et al., 1996). The amounts of NO (pmol) detected between 0–5 and 0–30 s were determined. The 5-s point coincided with the occurrence of the maximal vasodilator effects of these compounds whereas the 30-s time-point coincided to the point at which the responses began to subside.

2.4. Drugs

L-Cysteine, indomethacin, nifedipine, and sodium nitrite were obtained from Sigma (St. Louis, MO, USA). MAHMA NONOate was obtained from Alexis Biochemicals (San Diego, CA, USA). Sodium nitroprusside was obtained from Abbott (Chicago, IL, USA). All drugs were dissolved in saline except nifedipine which was dissolved in 1% dimethylsulfoxide in saline. L-*S*-nitrosocysteine was prepared as described previously (Davisson et al., 1996d, 1997a,b).

2.5. Statistics

The data are presented as mean ± S.E.M. and were analyzed by repeated measures analysis of variance (Winer, 1971) followed by Student's modified *t*-test with Bonferroni corrections for multiple comparisons between means (Wallenstein et al., 1980). A value of $P < 0.05$ was taken to denote statistical significance.

3. Results

3.1. Effects of vehicle on resting parameters in saline- or indomethacin-pretreated rats

Resting parameters before and after administration of vehicle in saline- or in indomethacin-pretreated rats are summarized in Table 1. Post-saline and post-indomethacin values were recorded 20–25 min after injection of these compounds. Post-pretreatment values and the recovery phase values described in Tables 1 and 2 are the mean ± S.E.M. of the averaged pre-injection values recorded when the vasodilator agents were given. The values remained

Table 1
Effects of vehicle on resting hemodynamic parameters in saline- or in indomethacin-pretreated rats

Pretreatment	Treatment	*N*	Parameter	Pretreatment		Treatment	
				Pre	Post	Maximum	Recovery
Saline	Vehicle	6	MAP (mm Hg)	108 ± 3	109 ± 3	106 ± 3	110 ± 3
			HQR (mm Hg/kHz)	59 ± 6	57 ± 7	53 ± 8	59 ± 5
			MR (mm Hg/kHz)	34 ± 3	34 ± 4	38 ± 5	36 ± 4
Indomethacin	Vehicle	6	MAP (mm Hg)	105 ± 3	107 ± 3	108 ± 3	108 ± 3
			HQR (mm Hg/kHz)	52 ± 8	54 ± 7	55 ± 6	57 ± 5
			MR (mm Hg/kHz)	37 ± 4	36 ± 4	37 ± 5	39 ± 3

The data are mean ± S.E.M. MAP = mean arterial blood pressure. HQR = hindquarter vascular resistance. MR = mesenteric vascular resistance. *N* = number of rats in each group. The dose of indomethacin was 10 mg/kg, i.v. The data in the column designated "Maximum" refer to the baseline values at the point of the maximal effects of vehicle. The data in the column designated "Recovery" refer to the values recorded from 35–45 min after administration of the vehicle used to dissolve nifedipine. The volumes of vehicle solutions (1% dimethylsulfoxide in saline) were equivalent to the vehicle solutions containing nifedipine (see Table 2). Note that the injections of saline, indomethacin or vehicle did not affect resting hemodynamic parameters ($P > 0.05$ for all comparisons).

Table 2
Effects of nifedipine on resting hemodynamic parameters in saline- or in indomethacin-pretreated rats

Pretreatment	Treatment	N	Parameter	Pretreatment		Treatment	
				Pre	Post	Maximum	Recovery
Saline	Nifedipine	8	MAP (mm Hg)	107 ± 3	108 ± 3	71 ± 4[a]	106 ± 3
			HQR (mm Hg/kHz)	67 ± 8	65 ± 7	47 ± 5[a]	61 ± 6
			MR (mm Hg/kHz)	39 ± 4	40 ± 4	25 ± 3[a]	38 ± 3
Indomethacin	Nifedipine	8	MAP (mm Hg)	109 ± 3	108 ± 3	72 ± 4[a]	108 ± 3
			HQR (mm Hg/kHz)	62 ± 5	64 ± 5	43 ± 4[a]	65 ± 6
			MR (mm Hg/kHz)	37 ± 4	39 ± 4	23 ± 3[a]	40 ± 4

The data are presented as mean ± S.E.M. MAP = mean arterial blood pressure. HQR = hindquarter vascular resistance. MR = mesenteric vascular resistance. N = number of rats. The dose of indomethacin was 10 mg/kg, i.v. The dose of nifedipine (dissolved in 1% dimethylsulfoxide in saline) was 500 nmol/kg, i.v. The data in the column designated "Maximum" refer to the baseline values at the point of the maximal effects of nifedipine (5–10 min post-injection). The data in the column designated "Recovery" refer to the values recorded from 35–45 min after administration of nifedipine. Note that the administration of saline or indomethacin did not affect resting hemodynamic parameters ($P > 0.05$ for all comparisons).

[a] $P < 0.05$, post-nifedipine vs. post-saline or post-indomethacin.

constant over this time. Specifically, values recovered to pre-injection levels after recovery from each injection. Injections of saline or indomethacin did not elicit immediate responses ($P > 0.05$, for all responses, data not shown). As can be seen in Table 1, post-injection values recorded 25–30 min after injection of saline or indomethacin were similar to pre-injection values. Injection of vehicle did not produce initial responses (see column designated "Maximum"). Post-vehicle injection values recorded during the injection of the vasodilator agents (see column designated "Recovery") were not different to pre-injection values (i.e., post-saline or post-indomethacin values).

3.2. Effects of nifedipine on resting parameters in saline- or indomethacin-pretreated rats

Resting parameters before and after injection of nifedipine in saline- or in indomethacin pretreated rats are summarized in Table 2. Injections of saline or indomethacin did not affect resting parameters ($P > 0.05$ for all comparisons). In saline-pretreated rats, injection of nifedipine elicited immediate falls in mean arterial blood pressure ($-34 \pm 4\%$) and in hindquarter ($-27 \pm 3\%$) and mesenteric ($-36 \pm 5\%$) resistances that reached maximum within 5–10 min ($P < 0.05$ for all responses, column designated "Maximum"). However, mean arterial blood pressure ($-2 \pm 3\%$), hindquarter ($-6 \pm 5\%$) and mesenteric ($-3 \pm 3\%$) resistance returned to pre-injection values by 15–20 min ($P > 0.05$, for all responses, column designated "Recovery") and stayed at these levels for the rest of the experiment. Responses elicited by nifedipine were similar in indomethacin- and in saline-pretreated rats ($P > 0.05$, for all comparisons).

3.3. Effects of vehicle on the hemodynamic actions of the vasodilator agents

Maximal responses elicited by sodium nitroprusside, MAHMA NONOate, L-*S*-nitrosocysteine and acetylcholine before and after injection of vehicle in saline- or indomethacin-pretreated rats are summarized in Table 3. The responses elicited by these agents were about 50% of maximum. Prior to injection of vehicle, each agent elicited similar falls in mean arterial blood pressure, and hindquarter and mesenteric resistances in saline- or indomethacin-pretreated rats ($P > 0.05$, for all comparisons). Responses reached maximum in 5–10 s and began to decline at 30–45 s, excepting the acetylcholine responses which be-

Table 3
Hemodynamic actions of the vasodilator compounds before and after administration of vehicle in saline-pretreated or in indomethacin-pretreated rats

Compound	Parameter	Saline		Indomethacin	
		Pre	Post-VEH	Pre	Post-VEH
Sodium nitroprusside	ΔMAP (%)	−34 ± 3	−35 ± 3	−31 ± 4	−34 ± 3
	ΔHQR (%)	−36 ± 3	−37 ± 3	−37 ± 3	−38 ± 3
	ΔMR (%)	−29 ± 4	−30 ± 3	−27 ± 3	−28 ± 3
MAHMA NONOate	ΔMAP (%)	−36 ± 3	−36 ± 3	−34 ± 4	−36 ± 3
	ΔHQR (%)	−32 ± 4	−34 ± 3	−31 ± 5	−33 ± 3
	ΔMR (%)	−35 ± 4	−38 ± 4	−36 ± 5	−34 ± 4
L-*S*-nitroso-cysteine	ΔMAP (%)	−37 ± 3	−35 ± 4	−37 ± 3	−35 ± 4
	ΔHQR (%)	−34 ± 3	−36 ± 4	−32 ± 3	−31 ± 4
	ΔMR (%)	−32 ± 4	−30 ± 3	−35 ± 4	−37 ± 3
Acetylcholine	ΔMAP (%)	−31 ± 3	−32 ± 3	−33 ± 4	−32 ± 3
	ΔHQR (%)	−32 ± 3	−35 ± 4	−36 ± 3	−34 ± 4
	ΔMR (%)	−27 ± 3	−28 ± 3	−29 ± 3	−30 ± 3

The data are presented as mean ± S.E.M. VEH = vehicle (dimethylsulfoxide, equal volumes to nifedipine). MAP = mean arterial blood pressure. HQR = hindquarter vascular resistance. MR = mesenteric vascular resistance. The dose of sodium nitroprusside was 10 nmol/kg, i.v. The dose of MAHMA NONOate ((*Z*)-1-[*N*-methyl-*N*-[6(*N*-methylammoniohexyl)amino]diazen-1-ium-1,2-diolate) was 25 nmol/kg, i.v. The dose of L-*S*-nitrosocysteine was 100 nmol/kg, i.v. The dose of acetylcholine was 1.0 μg/kg, i.v. There were six rats in each group. Note that the responses produced by the vasodilator agents were similar before and after administration of vehicle in both saline- and in indomethacin-pretreated rats ($P > 0.05$, for all comparisons).

Table 4
Hemodynamic actions of the vasodilator compounds before and after administration of nifedipine in saline-pretreated or in indomethacin-pretreated rats

Compound	Parameter	Saline		Indomethacin	
		Pre	Post-NIF	Pre	Post-NIF
Sodium nitroprusside	ΔMAP (%)	−33±3	−14±2[a]	−37±3	−15±2[a]
	ΔHQR (%)	−32±3	−6±3[a]	−34±3	−7±3[a]
	ΔMR (%)	−34±3	−9±3[a]	−35±3	−11±3[a]
MAHMA NONOate	ΔMAP (%)	−32±4	−12±3[a]	−36±4	−14±3[a]
	ΔHQR (%)	−34±5	−11±3[a]	−37±5	−13±3[a]
	ΔMR (%)	−37±5	−17±4[a]	−32±5	−15±4[a]
L-*S*-nitrosocysteine	ΔMAP (%)	−38±3	−33±4	−38±3	−33±4
	ΔHQR (%)	−37±3	−28±3[a]	−39±3	−29±3[a]
	ΔMR (%)	−31±4	−29±3	−36±4	−32±3
Acetylcholine	ΔMAP (%)	−29±3	−28±3	−29±3	−28±3
	ΔHQR (%)	−31±3	−23±2[a]	−31±3	−23±2[a]
	ΔMR (%)	−33±4	−29±3	−36±4	−34±3

The data are presented as mean±S.E.M. MAP = mean arterial blood pressure. HQR = hindquarter vascular resistance. MR = mesenteric vascular resistance. NIF = nifedipine (500 nmol/kg, i.v.). The dose of sodium nitroprusside was 10 nmol/kg, i.v. The dose of MAHMA NONOate ((*Z*)-1-[*N*-methyl-*N*-[6(*N*-methylammoniohexyl)amino]diazen-1-ium-1,2-diolate) was 25 nmol/kg, i.v. The dose of L-*S*-nitrosocysteine was 100 nmol/kg, i.v. The dose of acetylcholine was 1.0 μg/kg, i.v. There were eight rats in each group.

[a] $P < 0.05$, post-nifedipine vs. pre. Note, that nifedipine exerted similar effects in saline- and in indomethacin-pretreated rats ($P > 0.05$, for all between-group comparisons).

gan to decline at 25–30 s. In both groups, the responses elicited by the vasodilator agents were similar before and after injection of vehicle.

3.4. Effects of nifedipine on the hemodynamic actions of the vasodilator agents

The maximal responses elicited by sodium nitroprusside, MAHMA NONOate, L-*S*-nitrosocysteine and acetylcholine before and after injection of nifedipine in saline- or indomethacin-pretreated rats are summarized in Table 4. Pre-vehicle injection responses elicited by these agents were similar to those in Table 1 ($P > 0.05$, for all comparisons). The falls in mean arterial blood pressure, and hindquarter and mesenteric resistances elicited by sodium nitroprusside and MAHMA NONOate were markedly attenuated after injection of nifedipine in saline- and indomethacin-pretreated rats. Nifedipine-induced attenuation of these responses was similar in both groups ($P > 0.05$, for all comparisons). In contrast, the falls in mean arterial blood pressure and mesenteric resistance elicited by L-*S*-nitrosocysteine and acetylcholine were not attenuated by nifedipine in saline- or indomethacin-pretreated rats. However, the falls in hindquarter resistance elicited by L-*S*-nitrosocysteine and acetylcholine were slightly diminished by nifedipine in saline- or indomethacin-pretreated rats ($P < 0.05$ for both comparisons). The nifedipine-induced attenuation of these responses were similar in both treatment groups ($P > 0.05$, for all comparisons).

3.5. Effects of nifedipine on the decomposition of nitrosyl factors to NO

The total amounts of NO (pmol) obtained after addition of sodium nitroprusside, MAHMA NONOate or L-*S*-nitrosocysteine to rat blood containing nifedipine (10 nmol/ml) or vehicle are summarized in Table 5. In saline-treated samples, 31 ± 6% of the total NO generated by sodium nitroprusside was detected in the first 5 s and 68 ± 7% of the total NO was detected in the first 30 s. The total amount of NO detected was 47 ± 5% of what could be generated from sodium nitroprusside. In saline-treated samples, 22 ± 5% of the total NO generated by MAHMA NONOate was detected in the first 5 s whereas 44 ± 6% was detected in the first 30 s. The total amount of NO was 66 ± 7% of what could be generated from MAHMA NONOate (each molecule of MAHMA NONOate can decompose to two molecules of NO). In saline-treated samples, 20 ± 4% of the total NO generated by L-*S*-nitrosocysteine was detected in the first 5 s and 47 ± 6% of the total NO was detected in the first 30 s. The total amount of NO detected was only 3 ± 1% of what could be generated from L-*S*-nitrosocysteine. More NO was detected after addition of MAHMA NONOate to blood than after addition of sodium nitroprusside or L-*S*-nitrosocysteine.

Table 5
Effects of vehicle and nifedipine on the decomposition of sodium nitroprusside, MAHMA NONOate and L-*S*-nitrosocysteine to nitric oxide

Compound	Pretreatment	Nitric oxide (pmol)		
		0–5 s	0–30 s	Total
Sodium nitroprusside	Saline	32±4	69±7	97±6
	Vehicle	35±4	66±8	94±5
	Nifedipine	30±3	70±9	102±9
MAHMA NONOate	Saline	148±12[a]	315±26[a]	679±31[a]
	Vehicle	152±11[a]	314±21[a]	703±28[a]
	Nifedipine	160±10[a]	300±22[a]	688±25[a]
L-*S*-nitrosocysteine	Saline	14±3[b]	34±4[b]	69±5[b]
	Vehicle	12±2[b]	36±3[b]	68±4[b]
	Nifedipine	15±3[b]	37±3[b]	71±5[b]

The data are presented as mean±S.E.M. of 4 separate determinations. The concentration of nifedipine was 10 nmol/ml. The concentration of sodium nitroprusside was 0.2 nmol/ml. MAHMA NONOate = (*Z*)-1-[*N*-methyl-*N*-[6(*N*-methylammoniohexyl)amino]diazen-1-ium-1,2-diolate (0.5 nmol/ml). The concentration of L-*S*-nitrosocysteine was 2 nmol/ml. Note that the decomposition of these compounds to nitric oxide was not affected by vehicle or nifedipine ($P > 0.05$ for all comparisons to saline-treated samples).

[a] $P < 0.05$, MAHMA NONOate vs. appropriate dose of sodium nitroprusside.

[b] $P < 0.05$, L-*S*-nitrosocysteine vs. appropriate dose of sodium nitroprusside and MAHMA NONOate.

Although more L-*S*-nitrosocysteine was added than either sodium nitroprusside or MAHMA NONOate, less NO was detected after addition of L-*S*-nitrosocysteine. Vehicle or nifedipine did not affect the decomposition of the above compounds to NO ($P < 0.05$, for all comparisons).

4. Discussion

4.1. Effects of indomethacin

Systemic injections of indomethacin did not affect (i) resting parameters, (ii) the vasodilator actions of sodium nitroprusside, MAHMA NONOate, L-*S*-nitrosocysteine, or acetylcholine, or (iii) the inhibitory effects of the Ca^{2+}_{VS}-channel blocker, nifedipine, on the vasodilator actions of sodium nitroprusside and MAHMA NONOate (see below). Accordingly, the effects of nifedipine on NO-mediated vasodilation may not involve prostanoid factors (Luscher and Vanhoutte, 1990).

4.2. Role of NO in the vasodilator actions of nitrosyl factors

L-*S*-nitrosocysteine (2 nmol/ml), sodium nitroprusside (0.2 nmol/ml), MAHMA NONOate (0.5 nmol/ml) generated markedly different amounts of NO upon addition to rat blood. These concentrations would arise upon injection of 100 nmol/kg dose of L-*S*-nitrosocysteine, 10 nmol/kg of sodium nitroprusside, and 25 nmol/kg of MAHMA NONOate. MAHMA NONOate generated more NO than sodium nitroprusside which generated more NO than L-*S*-nitrosocysteine. It appears that the actions of these NO-donors cannot simply be explained by their ability to generate NO since the above doses of these agents elicited similar vasodilator responses.

4.3. Role of NO in the vasodilator actions of MAHMA NONOate

Each molecule of MAHMA NONOate generates two molecules of NO by a simple first-order reaction in biological fluids (see Benkusky et al., 1998). It is probable that the vasodilator actions of this compound are due to the generation of NO in the blood. Although the fate of blood borne NO cannot be readily determined, it is likely that significant amounts of NO enter vascular smooth muscle of resistance arteries. However, substantial amounts of NO are also likely to enter circulating cells such as red blood cells where the NO may be trapped by hemoglobin (see Jia et al., 1996). NO may also be rapidly degraded or converted to nitrosonium (NO^+) or nitroxyl (NO^-) ions that are capable of rapid reactions with blood proteins (Stamler et al., 1992b; Jia et al., 1996). In particular, nitrosonium ions may react with circulating thiols to form *S*-nitrosothiols such as *S*-nitrosoalbumin (Stamler et al., 1992a) and superoxide anion to form peroxynitrite (Benkusky et al., 1998). It is probable that NO was trapped or metabolized before reaching the NO detector since 60% of NO that could be obtained from MAHMA NONOate was detected after addition to blood.

4.4. Role of NO in the vasodilator actions of sodium nitroprusside

Each molecule of sodium nitroprusside generates one molecule of NO in biological fluids (see Feelisch, 1991). About 50% of the amounts of NO that could be obtained from sodium nitroprusside were detected after addition to rat blood. It could be expected that a greater amount of NO in blood would translate into larger vasodilator responses. Although, sodium nitroprusside generated less NO than MAHMA NONOate, sodium nitroprusside and MAHMA NONOate elicited similar vasodilator responses. The NO generated by sodium nitroprusside in the first 10 s were much less than the amounts of NO generated by MAHMA NONOate. This time period coincides with the peak vasodilation produced by these agents (Davisson et al., 1997a; Possas and Lewis, 1997). This suggests that the actions of sodium nitroprusside are not explained by the NO it generates in blood. Sodium nitroprusside may decompose to NO upon contact with blood and vascular smooth muscle cells such that higher amounts of NO enter these cells. As such, there would be less NO in blood to transport to the NO detector. MAHMA NONOate may decompose to NO spontaneously or by contact with proteins in blood such that there would be more NO available to be transported to the NO detector and less NO to enter blood and vascular smooth muscle cells. Accordingly, the total NO which enters red blood cells after addition of the above concentrations of MAHMA NONOate and sodium nitroprusside may be similar. By analogy, MAHMA NONOate and sodium nitroprusside may have elicited similar vasodilator responses because similar amounts of NO entered vascular smooth muscle in resistance vessels. The one electron reduction of sodium nitroprusside also results in five molecules of cyanide (see Feelisch, 1991). Cyanide may not be involved in sodium nitroprusside-induced vasodilation since cyanide elicits minor depressor responses in urethane-anesthetized rats (Hoque et al., 1993). Sodium nitroprusside is an iron–nitrosyl compound capable of nitrosation reactions (Stamler et al., 1992b). The possible role of these reactions in sodium nitroprusside vasodilation will be discussed below.

4.5. Role of NO in the vasodilator actions of L-S-nitrosocysteine

If a covalent bond breaks in an organic compound such that both electrons remain with one fragment, the reaction is called heterolytic. If a bond breaks in such a way that each fragment gets one electron, free radicals are formed,

and the reaction is called homolytic (see March, 1985). If an nitrosothiol (RS–NO) breaks into two radicals (RS• and NO•) it is a homolytic reaction. If it breaks into two ions (RS^- and NO^+) or (RS^+ and NO^-) then it is heterolytic reaction (see Feelisch, 1991). Homolytic decomposition of L-*S*-nitrosocysteine yields NO and cystine (Feelisch, 1991). L-*S*-nitrosocysteine (2 nmol/ml) generated less NO upon addition to blood than either MAHMA NONOate (0.5 mol/ml) or sodium nitroprusside (0.2 nmol/ml). L-*S*-nitrosocysteine may deliver NO more effectively to cells so that less is available for detection by the NO analyzer. However, heterolytic decomposition of L-*S*-nitrosocysteine does not result in NO (Feelisch, 1991). Accordingly, L-*S*-nitrosocysteine may decompose less efficiently to NO in vivo than sodium nitroprusside or MAHMA NONOate. The results suggest that L-*S*-nitrosocysteine acts by mechanisms other than decomposition to NO (Kowaluk and Fung, 1990; Mathews and Kerr, 1993).

4.6. Effects of nifedipine on resting hemodynamic parameters

Nifedipine elicited substantial depressor and vasodilator responses. This indicates that Ca^{2+}_{VS}-channels regulate the tone of resistance vessels in vivo (see Abdel-Latif, 1986; Satake et al., 1992; Lamb et al., 2000). Since the nifedipine responses subsided within 15–20 min, it is possible that blockade of Ca^{2+}_{VS}-channels was relatively transient. However, the finding that the vasodilator actions of sodium nitroprusside and MAHMA NONOate were markedly diminished 15–20 min after injection of nifedipine suggests that blockade of Ca^{2+}_{VS}-channels was still in effect. The findings that the vasodilator actions of the NO-donors, sodium nitroprusside and MAHMA NONOate, were markedly attenuated by nifedipine whereas those of L-*S*-nitrosocysteine were not, suggest that NO acts mainly by inhibition of Ca^{2+}_{VS}-channels whereas L-*S*-nitrosocysteine acts by additional mechanisms. For example, L-*S*-nitrosocysteine may diminish phospholipase C-mediated release of intracellular pools of Ca^{2+} (Abdel-Latif, 1986). The ability of nifedipine to block NO-mediated vasodilation would result in the development of hypertension via increases in peripheral vascular resistances if NO is indeed EDRF in resistance arteries. The lack of vasoconstriction after injection of nifedipine would argue that NO is not EDRF in these arteries.

4.7. Role of Ca^{2+}_{VS}-channels in the hemodynamic effects of NO-donors

The vasodilator responses elicited by sodium nitroprusside and MAHMA NONOate were markedly attenuated by nifedipine. Nifedipine may not have affected decomposition of the NO-donors to NO since it did not affect NO levels obtained by addition of the donors to blood. This suggests that (i) blockade of Ca^{2+}_{VS}-channels precludes exogenous NO from relaxing vascular smooth muscle in resistance arteries, and (ii) endogenous NO relaxes these arteries mainly by blocking Ca^{2+}_{VS}-channel activity. NO may not directly affect Ca^{2+}_{VS}-channels (Campbell et al., 1996). However, nitrosonium (NO^+) or nitroxyl (NO^-) ions may directly react with amino acids in Ca^{2+}_{VS}-channels (Campbell et al., 1996) and other ion-channels (Bolotina et al., 1994; Koh et al., 1995). In addition, sodium nitroprusside may nitrosate Ca^{2+}_{VS}-channels (Stamler et al., 1992b). Nitrosation of Ca^{2+}_{VS}-channels may not be responsible for NO-mediated vasodilation since nitrosation events augment their activity (Campbell et al., 1996). Inhibitory effects of NO on Ca^{2+}_{VS}-channel activity may involve production of cGMP in vascular smooth muscle (see Campbell et al., 1996). The redox forms of NO activate a variety of K^+-channels, which leads to hyperpolarization of vascular smooth muscle (Koh et al., 1995). Accordingly, NO may indirectly reduce Ca^{2+}_{VS}-channel activity in resistance arteries by activation of K^+-channels.

4.8. Role of Ca^{2+}_{VS}-channels in the hemodynamic effects of L-S-nitrosocysteine

The vasodilator actions of the putative endothelium-derived *S*-nitrosothiol, L-*S*-nitrosocysteine (Myers et al., 1990; Rubanyi et al., 1991; Rosenblum, 1992), were minimally affected by nifedipine. These findings do not preclude the possibility that L-*S*-nitrosocysteine inhibits Ca^{2+}_{VS}-channel activity either directly or indirectly (i.e., via changes in membrane potential or the NO-mediated generation of cGMP) in resistance vessels. However, *S*-nitrosothiols increase Ca^{2+}_{VS}-channel activity in cardiac myocytes by redox modulation of cysteine residues in these channels (Campbell et al., 1996). Taken together, it appears that L-*S*-nitrosocysteine dilates resistance arteries by mechanisms in addition to NO-mediated inhibition of Ca^{2+}_{VS}-channels. A likely mechanism by which L-*S*-nitrosocysteine relaxes vascular smooth muscle in the presence of nifedipine is the inhibition of Ca^{2+}-dependent contractile processes (see Abdel-Latif, 1986). L-*S*-nitrosocysteine may (i) diminish the ability of neurogenically derived catecholamines from activating phospholipase C, (2) directly reduce phospholipase C activity, and/or (3) directly interfere with Ca^{2+}-dependent contractility. L-*S*-nitrosocysteine is highly lipophobic (Kowaluk and Fung, 1990) and is unlikely to enter vascular smooth muscle.

S-nitrosothiols modulate the activities of functional proteins by nitrosation of amino acids and nitrosylation of heme-iron moieties in these proteins (Stamler et al., 1992b; Lei et al., 1992; Lander et al., 1993; Lipton et al., 1993; Lipton and Stamler, 1994; Stamler, 1994; George and Shibata, 1995; Koh et al., 1995; Broillet and Firestein, 1996; Campbell et al., 1996; Meffert et al., 1996; Wolosker et al., 1996; Miyamoto et al., 1997). Accordingly, L-*S*-nitrosocysteine may relax vascular smooth muscle by nitrosation of a membrane-bound protein which activates

intracellular signal transduction processes which inhibit phospholipase C activity or reduce Ca^{2+}-mediated contractility. Although the identity of this putative protein is not known, there is evidence that L-*S*-nitrosocysteine activates stereoselective recognition sites (Davisson et al., 1996d, 1997b; Lewis et al., 1996; Ohta et al., 1997) which recognize L-*S*-nitroso-β, β-dimethylcysteine (Travis et al., 1996, 1997) but not larger *S*-nitrosothiols such as *S*-nitrosoglutathione (Davisson et al., 1997b; Lewis et al., 1996; Ohta et al., 1997). These recognition sites, which are susceptible to covalent modification by a lipophobic thiol chelator (Hoque et al., 1999), may be a novel class of receptor which upon activation initiates signal transduction processes which inhibit Ca^{2+}-dependent contractile processes. Moreover, these receptors may contain amino acid sequences which allows for nitrosation and activation of these receptors (see Stamler et al., 1997).

4.9. Role of Ca^{2+}_{VS}-channels in the hemodynamic effects of acetylcholine

The dose of the endothelium-dependent vasodilator, acetylcholine, used in these studies elicited similar responses to MAHMA NONOate, sodium nitroprusside and L-*S*-nitrosocysteine. Any effect of nifedipine on the vasodilator actions of acetylcholine would probably arise from modulation of the actions of EDRF on vascular smooth muscle rather than EDRF release since endothelial cells are devoid of nifedipine-sensitive Ca^{2+}_{VS}-channels (Luscher and Vanhoutte, 1990). The vasodilator responses elicited by acetylcholine were minimally affected by nifedipine. This suggests that the EDRF released by acetylcholine can relax resistance arteries by mechanisms other than blockade of Ca^{2+}_{VS}-channels. The observation that L-*S*-nitrosocysteine and the EDRF released by acetylcholine are similarly affected by nifedipine is consistent with the possibility that the EDRF released by acetylcholine in resistance vessels is an *S*-nitrosothiol (Myers et al., 1990; Rubanyi et al., 1991; Rosenblum, 1992). However, K^+ (Edwards et al., 1998), an epoxyeicosatrienoic acid product of cytochrome P_{450} epoxygenase (Fisslthaler et al., 1999) or a cannabinoid receptor agonist (Edwards and Weston, 1998) may also be EDRFs released by acetylcholine.

There is considerable evidence that NO plays a major role in the expression of acetylcholine-induced relaxation in conduit arteries but not in resistance arteries (Hwa et al., 1994; Woodman et al., 2000). Hwa et al. (1994) demonstrated that the acetylcholine-induced responses in mesenteric resistance arteries were attenuated by blockade of Ca^{2+}-activated K^+-channels but not by agents such as hemoglobin and methylene blue, which markedly attenuated acetylcholine-induced responses in mesenteric conduit arteries (Hwa et al., 1994). Moreover, Woodman et al. (2000) found that the selective inhibitor of soluble guanylate cyclase, 1*H*-[1,2,4]oxadiazolo[4,3,-a]quinoxalin-1-one (ODQ) failed to attenuate acetylcholine-induced vasorelaxation in resistance arteries in the hindlimb but that high K^+ did attenuate this relaxation. Interestingly, the vasorelaxant effects of L-*S*-nitrosocysteine are markedly attenuated after inhibition of Ca^{2+}-activated K^+-channels (George and Shibata, 1995). ODQ diminishes the vasorelaxant potency of acetylcholine in conduit arteries (Moro et al., 1996). However, the finding that the maximal relaxation elicited by the endothelium-dependent agonist was not affected by ODQ (Moro et al., 1996) supports the possibility that acetylcholine releases an EDRF other than NO in these arteries. Our results suggest that this may also be the case for resistance arteries and that the EDRF may be an *S*-nitrosothiol. However, it is certainly feasible that acetylcholine elicits the release of *S*-nitrosothiols in conduit arteries up-stream from resistance vessels.

4.10. Summary

The vasodilator responses elicited by systemic injections of NO-donors were markedly attenuated by nifedipine. Accordingly, the vasodilator actions of NO in resistance vessels may be due primarily to inhibition of Ca^{2+}_{VS}-channels. In contrast, the vasodilator actions of L-*S*-nitrosocysteine and acetylcholine were minimally affected by nifedipine. This suggests that (i) L-*S*-nitrosocysteine and the EDRF released by acetylcholine act by mechanisms in addition to inhibition of Ca^{2+}_{VS}-channels, and (ii) the EDRF released by acetylcholine in resistance arteries has biological activity that is not shared by NO. Whether this EDRF is an *S*-nitrosothiol or other factors entirely remains to be established.

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References

Abdel-Latif, A.A., 1986. Calcium-mobilizing receptors, polyphosphoinositols, and the generation of second messengers. Pharmacol. Rev. 38, 227–272.

Benkusky, N.A., Lewis, S.J., Kooy, N.W., 1998. Attenuation of vascular relaxation after development of tachyphylaxis to peroxynitrite. Am. J. Physiol. 275, H501–H508.

Bolotina, V.M., Najibi, S., Palancino, J.J., Pagano, P.J., Cohen, R.A., 1994. Nitric oxide directly activates calcium-dependent K^+ channels in vascular smooth muscle. Nature 368, 850–853.

Broillet, M.-C., Firestein, S., 1996. Direct activation of the olfactory cyclic nucleotide-gated channel through modulation of sulfhydryl groups by NO compounds. Neuron 16, 377–385.

Campbell, D.L., Stamler, J.S., Strauss, H.C., 1996. Redox modulation of L-type calcium channels in ferret ventricular myocytes: dual actions of nitric oxide and *S*-nitrosothiols. J. Gen. Physiol. 108, 277–293.

Colombari, E., Davisson, R.L., Shaffer, R.A., Talman, W.T., Lewis, S.J., 1998. Hemodynamic effects of L-glutamate in the NTS of conscious rats: possible role of vascular nitrosyl factors. Am. J. Physiol. 274, H1066–H1074.

Davisson, R.L., Bates, J.N., Johnson, A.K., Lewis, S.J., 1996a. Use-dependent loss of acetylcholine- and bradykinin-mediated after nitric oxide synthase inhibition: Evidence for preformed stores of nitric oxide-containing factors in vascular endothelial cells. Hypertension 28, 354–360.

Davisson, R.L., Shaffer, R.A., Johnson, A.K., Lewis, S.J., 1996b. Use-dependent loss of active sympathetic neurogenic vasodilation following nitric oxide synthase inhibition in conscious rats: evidence for the presence of preformed stores of nitric oxide-containing factors. Hypertension 28, 347–353.

Davisson, R.L., Shaffer, R.A., Johnson, A.K., Lewis, S.J., 1996c. Stimulation of lumbar sympathetic nerves may produce hindlimb vasodilation via the release of pre-formed stores of nitrosyl factors. Neuroscience 72, 881–887.

Davisson, R.L., Travis, M.D., Bates, J.N., Lewis, S.J., 1996d. Hemodynamic effects of L- and D-*S*-nitrosocysteine in the rat: stereoselective *S*-nitrosothiol recognition sites. Circ. Res. 79, 256–262.

Davisson, R.L., Possas, O.S., Murphy, S.P., Lewis, S.J., 1997a. Neurogenically derived nitrosyl factors mediate lumbar sympathetic vasodilation in the hindlimb vasculature of the rat. Am. J. Physiol. 272, H2369–H2376.

Davisson, R.L., Travis, M.D., Bates, J.N., Johnson, A.K., Lewis, S.J., 1997b. Stereoselective actions of *S*-nitrosocysteine in the central nervous system of conscious rats. Am. J. Physiol. 272, H2361–H2368.

Edwards, G., Weston, A.H., 1998. Endothelium-derived hyperpolarizing factor—a critical appraisal. Prog. Drug. Res. 50, 107–133.

Edwards, G., Dora, K.A., Gardener, M.J., Garland, C.J., Weston, A.H., 1998. K^+ is an endothelium-derived hyperpolarizing factor in rat arteries. Nature 396, 269–272.

Feelisch, M., 1991. The biochemical pathways of nitric oxide formation from nitrovasodilators: appropriate choice of exogenous NO donors and aspects of preparation and handling of aqueous NO solutions. J. Cardiovasc. Pharmacol. 17 (Suppl. 3), 25–33.

Fisslthaler, B., Popp, R., Kiss, L., Potente, M., Harder, D.R., Fleming, I., Busse, R., 1999. Cytochrome P450 2C is an EDHF synthase in coronary arteries. Nature 401, 493–497.

Gardiner, S.M., Compton, A.M., Kemp, P.A., Bennett, T., 1990. Regional and cardiac hemodynamic responses to glyceryl trinitrate, acetylcholine, bradykinin and endothelin-1 in conscious rats: effects of N^G-nitro-L-arginine methyl ester. Br. J. Pharmacol. 101, 632–639.

George, M.J., Shibata, E.F., 1995. Regulation of calcium activated potassium channels by *S*-nitrosothiols and cyclic guanosine monophosphate in rabbit coronary myocytes. J. Invest. Med. 43, 451–458.

Haywood, J.R., Shaffer, R.A., Fastenow, C., Fink, G.D., Brody, M.J., 1981. Regional blood flow measurement with pulsed Doppler flowmeter in conscious rat. Am. J. Physiol. 241, H273–H278.

Hoque, A.M., Davisson, R.L., Johnson, A.K., Lewis, S.J., 1993. Nitro blue tetrazolium (NBT) in combination with methylene blue prevents the vasodilator actions of sodium nitroprusside in vivo. FASEB J. 7, A432, Abstract.

Hoque, A., Bates, J.N., Lewis, S.J., 1999. In vivo evidence that L-*S*-nitrosocysteine may exert its vasodilator effects by interaction with thiol residues in the vasculature. Eur. J. Pharmacol. 384, 169–172.

Hwa, J.J., Ghibaudi, L., Williams, P., Chatterjee, M., 1994. Comparison of acetylcholine-dependent relaxation in large and small arteries of rat mesenteric vascular bed. Am. J. Physiol. 266, H952–H958.

Ignarro, L.J., 1990. Nitric oxide: a novel signal transduction mechanism for transcellular communication. Hypertension 16, 477–483.

Jia, L., Bonaventura, C., Bonaventura, J., Stamler, J.S., 1996. S-nitrosohemoglobin: a dynamic activity of blood involved in vascular control. Nature 380, 221–226.

Koh, S.D., Campbell, J.D., Carl, A., Sanders, K.M., 1995. Nitric oxide activates multiple potassium channels in canine colonic smooth muscle. J. Physiol. 489, 735–743.

Kowaluk, E.A., Fung, H., 1990. Spontaneous liberation of nitric oxide cannot account for in vitro vascular relaxation by *S*-nitrosothiols. J. Pharmacol. Exp. Ther. 25, 1256–1264.

Lacolley, P.J., Lewis, S.J., Brody, M.J., 1991a. Role of the sympathetic nerve activity in the generation of vascular nitric oxide in urethane anesthetized rats. Hypertension 17, 881–887.

Lacolley, P.J., Lewis, S.J., Brody, M.J., 1991b. L-N^G-nitro arginine produces an exaggerated hypertension in anesthetized SHR. Eur. J. Pharmacol. 197, 239–240.

Lamb, F.S., Kooy, N.W., Lewis, S.J., 2000. Role of Cl^- channels in alpha-adrenoceptor-mediated vasoconstriction in the anesthetized rat. Eur. J. Pharmacol. 401, 403–412.

Lander, H.M., Sehajpal, P.K., Novogrodsky, A., 1993. Nitric oxide signaling: a possible role for G-proteins. J. Immunol. 151, 7182–7187.

Lei, S.Z., Pan, Z.-H., Aggarwal, S.K., Chen, H.-S.V., Hartman, J., Sucher, N.J., Lipton, S.A., 1992. Effects of nitric oxide production on the redox modulatory site of the NMDA receptor-channel complex. Neuron 8, 1087–1099.

Lewis, S.J., Travis, M.D., Bates, J.N., 1996. Stereoselective *S*-nitrosocysteine recognition sites in rat brain. Eur. J. Pharmacol. 312, R3–R5.

Lipton, S.A., Stamler, J.S., 1994. Actions of redox related congeners of nitric oxide at the NMDA receptor. Neuropharmacology 33, 1229–1233.

Lipton, S.A., Choi, Y., Pan, Z., Lei, S.Z., Chen, H.V., Sucher, N.J., Loscalzo, J., Singel, D.J., Stamler, J.S., 1993. A redox-based mechanism for the neuroprotective and neurodestructive effects of nitric oxide and related nitroso-compounds. Nature 364, 626–632.

Luscher, T.F., Vanhoutte, P.M., 1990. The Endothelium: Modulator of Cardiovascular Function. CRC Press, Boca Raton, FL.

March, J., 1985. Advanced Organic Chemistry, Reactions, Mechanisms, and Structure. 3rd edn. Wiley, New York, NY, pp. 179–181.

Mathews, R.W., Kerr, S.W., 1993. Biological activity of *S*-nitrosothiols: the role of nitric oxide. J. Pharmacol. Exp. Ther. 267, 1529–1537.

Meffert, M.K., Calakos, N.C., Scheller, R.H., Schulman, H., 1996. Nitric oxide modulates synaptic vesicle docking/fusion reactions. Neuron 16, 1229–1236.

Miyamoto, A., Laufs, U., Pardo, C., Liao, J.K., 1997. Modulation of bradykinin receptor ligand binding affinity and its coupled G-proteins by nitric oxide. J. Biol. Chem. 272, 19601–19608.

Moncada, S., Palmer, R.M.J., Higgs, E.A., 1991. Nitric oxide; physiology, pathophysiology and pharmacology. Pharmacol. Rev. 43, 109–142.

Moro, M.A., Russel, R.J., Cellek, S., Lizasoain, I., Su, Y., Darley-Usmar, V.M., Radomski, M.W., Moncada, S., 1996. cGMP mediates the vascular and platelet actions of nitric oxide: confirmation using an inhibitor of the soluble guanylate cyclase. Proc. Natl. Acad. Sci. U. S. A. 93, 1480–1485.

Myers, P.R., Minor, R. Jr., Guerra, R. Jr., Bates, J.N., Harrison, D.G., 1990. Vasorelaxant properties of the endothelium-derived relaxing factor more closely resembles *S*-nitrosocysteine than nitric oxide. Nature 345, 161–163.

Ohta, H., Bates, J.N., Lewis, S.J., Talman, W.T., 1997. Actions of *S*-nitrosocysteine in the nucleus tractus solitarii unrelated to the release of nitric oxide. Brain Res. 746, 98–104.

Possas, O.S., Lewis, S.J., 1997. NO-containing factors mediate reflex hindlimb vasodilation produced by superior laryngeal nerve stimulation. Am. J. Physiol. 273, H234–H243.

Rosenblum, W.I., 1992. Endothelium-derived relaxing factor in brain blood vessels is not nitric oxide. Stroke 23, 1527–1535.

Rubanyi, G.M., John, A., Wilcox, D., Bates, J.N., Harrison, D.G., 1991. Evidence that a *S*-nitrosothiol, but not nitric oxide, may be identical with endothelium-derived relaxing factor. J. Cardiovasc. Pharmacol. 17, 541–545.

Satake, N., Kiyoto, S., Shibata, S., Gandhi, V., Jones, D.J., Morikawa,

M., 1992. Possible mechanisms of inhibition with atropine against noradrenaline-induced contraction in the rabbit aorta. Br. J. Pharmacol. 107, 553–558.

Stamler, J.S., 1994. Redox signaling: nitrosylation and related target interactions of nitric oxide. Cell 78, 931–936.

Stamler, J.S., Jaraki, O., Osborne, J., Simon, D.I., Keaney, J.F., Vita, J., Singel, D., Valeri, C.R., Loscalzo, J., 1992a. Nitric oxide circulates in mammalian plasma primarily as an *S*-nitroso adduct of serum albumin. Proc. Natl. Acad. Sci. U. S. A. 89, 7674–7677.

Stamler, J.S., Singel, D.J., Loscalzo, J., 1992b. Biochemistry of nitric oxide and its redox-activated forms. Science 258, 1898–1902.

Stamler, J.S., Toone, E.J., Lipton, S.A., Sucher, N.J., 1997. (S)NO signals: Translocation, regulation, and a consensus motif. Neuron 18, 691–696.

Travis, M.D., Stoll, L.L., Bates, J.N., Lewis, S.J., 1996. L- and D-*S*-nitroso-β,β-dimethylcysteine differentially increase cGMP in cultured vascular smooth muscle. Eur. J. Pharmacol. 318, 47–53.

Travis, M.D., Davisson, R.L., Bates, J.N., Lewis, S.J., 1997. Hemodynamic effects of L- and D-*S*-nitroso-β,β-dimethylcysteine in the rat. Am. J. Physiol. 273, H1493–H1501.

Wallenstein, S., Zucker, C.L., Fleiss, J.C., 1980. Some statistical methods useful in circulation research. Circ. Res. 47, 1–9.

Whalen, E.J., Johnson, A.K., Lewis, S.J., 1999a. Hemodynamic actions of systemically injected pituitary adenylate cyclase activating polypeptide-27 in the rat. Eur. J. Pharmacol. 365, 205–215.

Whalen, E.J., Johnson, A.K., Lewis, S.J., 1999b. Tachyphylaxis to PACAP-27 after inhibition of NO synthesis: a loss of adenylate cyclase activation. Am. J. Physiol. 277, R1453–R1461.

Whalen, E.J., Travis, M.D., Johnson, A.K., Lewis, S.J., 1999c. Rapid tachyphylaxis to the hemodynamic effects of PACAP-27 after inhibition of nitric oxide synthesis. Am. J. Physiol. 276, H2117–H2126.

Winer, B.J., 1971. Statistical Principles of Experimental Design. McGraw-Hill, New York, NY, pp. 752–809.

Wolosker, H., Reis, M., Assreuy, J., Meis, L., 1996. Inhibition of glutamate uptake and proton pumping in synaptic vesicles by *S*-nitrosylation. J. Neurochem. 66, 1943–1948.

Woodman, O.L., Wongsawatkul, O., Sobey, C.G., 2000. Contribution of nitric oxide, cyclic GMP and K^+-channels to acetylcholine-induced dilatation of rat conduit and resistance arteries. Clin. Exp. Pharmacol. Physiol. 27, 34–40.