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Short communication

Redox regulation of *S*-nitrosocysteine-mediated vasodilation in vivo

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Abstract

This study examined the effects of the lipophobic electron acceptor, nitroblue tetrazolium (2×5 μmol/kg, i.v.) on the vasodilation produced by the putative endothelium-derived *S*-nitrosothiol, L-*S*-nitrosocysteine (400 nmol/kg, i.v.), and the nitric oxide (NO) donor, (*Z*)-1-[*N*-methyl-*N*-[6(*N*-methylammoniohexyl)amino]diazen-1-ium-1,2-diolate (MAHMA NONOate, 25 nmol/kg, i.v.), in anesthetized rats. The administration of nitroblue tetrazolium resulted in delayed but long-lasting increases in vascular resistances. The L-*S*-nitrosocysteine-induced vasodilator responses were markedly diminished whereas the MAHMA NONOate-induced responses were not affected by nitroblue tetrazolium. These results support the possibility that L-*S*-nitrosocysteine interacts with membrane thiols that are subject to nitroblue tetrazolium-induced oxidation (i.e., disulfide-bridge formation) and that nitroblue tetrazolium-induced vasoconstriction may involve a loss of potency of endothelium-derived *S*-nitrosothiols.

Keywords: L-*S*-Nitrosocysteine; Nitric oxide (NO); Redox; Vasodilation; (Rat)

1. Introduction

L-*S*-Nitrosocysteine is a putative endothelium-derived *S*-nitrosothiol (Myers et al., 1990; Rubanyi et al., 1991; Rosenblum, 1992). L-*S*-nitrosocysteine may relax vascular smooth muscle by (i) its decomposition to nitric oxide (NO) and the nitrosation of thiol residues in functional proteins (Stamler et al., 1992, 1997), and (ii) activation of stereoselective recognition sites (Davisson et al., 1996a,b,c, 1997; Lewis et al., 1996). These recognition sites may be novel receptors that specifically recognize L-*S*-nitrosocysteine and similar lipophobic *S*-nitrosothiols such as L-*S*-nitroso-β,β-dimethylcysteine (Travis et al., 1996, 1997). However, it is also possible that the stereoisomeric form and size of L-*S*-nitrosocysteine gives this *S*-nitrosothiol access to 'nitrosation motifs' in identified functional proteins including receptors and ligand-gated and voltage-dependent ion channels (Stamler et al., 1992, 1997).

The vasodilator actions of L-*S*-nitrosocysteine are substantially diminished by the lipophobic thiol chelator, *para*-hydroxymercurobenzoic acid (*p*HMBA), whereas the vasodilator actions of the NO donors, (*Z*)-1-[*N*-methyl-*N*-[6(*N*-methylammoniohexyl)amino]diazen-1-ium-1,2-diolate (MAHMA NONOate) and sodium nitroprusside are minimally affected (Hoque et al., 1999). This suggests that L-*S*-nitrosocysteine recognition sites contain reduced thiol residues. In theory, these recognition sites may contain cysteine residues that are subject to oxidation (i.e., disulfide bond formation yielding cystine) by endogenous and exogenously applied electron acceptors. The formation of these disulfide bonds may diminish the ability of L-*S*-nitrosocysteine to bind to and/or to activate these recognition sites. The main aim of the present study was to determine whether the lipophobic electron acceptor, nitroblue tetrazolium (Altman, 1976; Seidler, 1991), modifies the hypotensive and vasodilator actions of L-*S*-nitrosocysteine, MAHMA NONOate or sodium nitroprusside, in urethane-anesthetized rats.

2. 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 (250–350 g) were anesthetized with urethane (1 g/kg, i.p.). Catheters were put into a femoral vein to give drugs

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and into a femoral artery to record mean arterial blood pressure. Pulsed Doppler flow probes were put on a renal artery, the superior mesenteric artery and the descending aorta to determine renal, mesenteric and hindquarter vascular resistances, respectively (Davisson et al., 1993, 1996a,b,c). Rat body temperature was maintained at 37°C via a heating pad. The rats breathed room air supplemented with 95% O_2–5% CO_2.

2.2. Experimental procedures and drugs

The hemodynamic effects of L-*S*-nitrosocysteine (400 nmol/kg, i.v.), MAHMA NONOate (25 nmol/kg, i.v.) and sodium nitroprusside (10 μg/kg, i.v.) were determined before and after two injections of saline ($n = 6$) or nitroblue tetrazolium (5 μmol/kg, i.v., $n = 8$). Injections of saline or nitroblue tetrazolium were given 15 min apart. Nitrosyl factors were injected 75–105 min after the second injection of saline or nitroblue tetrazolium. The vasodilator responses elicited by each injection of the nitrosyl factors were allowed to subside completely before another injection was given. All drugs were from Sigma (St. Louis, MO, USA) except for sodium nitroprusside which was from Abbott (Chicago, IL, USA) and MAHMA NONOate which was from Alexis Biochemicals (San Diego, CA, USA). L-*S*-nitrosocysteine was prepared as described previously (Davisson et al., 1996a,b,c). Data are presented as mean ± S.E.M. and were analyzed by repeated measures analysis of variance and Student's modified *t*-tests with Bonferroni corrections for multiple comparisons (see Davisson et al., 1996a,b,c). A value of $P < 0.05$ denoted statistical significance.

3. Results

3.1. Effects of nitroblue tetrazolium on resting hemodynamic parameters

Resting parameters recorded before and after administration of two injections of saline or nitroblue tetrazolium (2 × 5 μmol/kg, i.v.) are shown in Table 1. Resting parameters recorded 75–105 min after the second injection of saline were not different to those before any injection of saline was given. Resting mean arterial blood pressure was lower whereas hindquarter, renal and mesenteric vascular resistances were higher 75–105 min after the second injection of nitroblue tetrazolium.

Table 1
Resting hemodynamic parameters before and 75–105 min after injection of saline or NBT

Treatment	Parameter	Pre	Post	% Change
Saline	MAP (mm Hg)	105 ± 3	103 ± 3	−1 ± 3
	HQR (mm Hg/kHz)	42 ± 6	41 ± 7	−2 ± 4
	RR (mm Hg/kHz)	83 ± 10	79 ± 12	−3 ± 6
	MR (mm Hg/kHz)	34 ± 4	36 ± 4	+5 ± 6
NBT	MAP (mm Hg)	106 ± 4	91 ± 3	−14 ± 3[a]
	HQR (mm Hg/kHz)	33 ± 5	56 ± 6	+39 ± 7[a]
	RR (mm Hg/kHz)	77 ± 12	114 ± 15	+43 ± 8[a]
	MR (mm Hg/kHz)	39 ± 6	68 ± 8	+71 ± 9[a]

The data are presented as the mean ± S.E.M. NBT = nitroblue tetrazolium (2 × 5 μmol/kg, i.v.). MAP = mean arterial blood pressure. HQR = hindquarter resistance. RR = renal resistance. MR = mesenteric resistance. There were six rats in the saline-treated group and eight rats in the NBT-treated group.

[a] $P < 0.05$, Post-NBT vs pre-NBT.

Table 2
Effects of NBT on the hemodynamic actions of the nitrosyl factors

Compound	Parameter	Pre	Post-NBT
L-SNC	ΔMAP	−36 ± 4	−13 ± 3[a]
	ΔHQR, %	−34 ± 4	−8 ± 3[a]
	ΔRR, %	−21 ± 3	−7 ± 2[a]
	ΔMR, %	−49 ± 5	−19 ± 3[a]
MAHMA NONOate	ΔMAP, %	−29 ± 3	−27 ± 3
	ΔHQR, %	−31 ± 4	−28 ± 5
	ΔRR, %	−25 ± 3	−27 ± 3
	ΔMR, %	−44 ± 6	−39 ± 7
SNP	ΔMAP, %	−36 ± 3	−33 ± 3
	ΔHQR, %	−31 ± 3	−44 ± 3[a]
	ΔRR, %	−23 ± 4	−19 ± 4
	ΔMR, %	−47 ± 5	−28 ± 4[a]

The data are presented as the mean ± S.E.M. L-SNC = L-*S*-nitrosocysteine (400 nmol/kg, i.v.). MAHMA NONOate = (*Z*)-1-[*N*-methyl-*N*-[6(N-methylammoniohexyl)amino]]diazen-1-ium-1,2-diolate (25 nmol/kg, i.v.). SNP = sodium nitroprusside (10 μg/kg, i.v.). NBT = nitroblue tetrazolium (2 × 5 μmol/kg, i.v.). MAP = mean arterial blood pressure. HQR = hindquarter resistance. RR = renal resistance. MR = mesenteric resistance. There were eight rats in the group.

[a] $P < 0.05$, Post-NBT vs pre-NBT.

3.2. Effects of nitroblue tetrazolium on the hemodynamic actions of the nitrosyl factors

The effects of L-*S*-nitrosocysteine (400 nmol/kg, i.v.), MAHMA NONOate (25 nmol/kg, i.v.) and sodium nitroprusside (10 μg/kg, i.v.) before and after administration of nitroblue tetrazolium (2 × 5 μmol/kg, i.v.) are summarized in Table 2. L-*S*-Nitrosocysteine produced a depressor response and falls in vascular resistances. The L-*S*-nitrosocysteine-induced responses were markedly attenuated after administration of nitroblue tetrazolium. The MAHMA NONOate- and sodium nitroprusside-induced responses were equivalent to those of L-*S*-nitrosocysteine ($P > 0.05$ for all comparisons). The MAHMA NONOate-induced responses were not affected by nitroblue tetrazolium. The sodium nitroprusside-induced depressor response and fall in renal resistance were not affected by nitroblue tetrazolium. However, the sodium nitroprusside-induced falls in hindquarter resistance were augmented whereas the sodium nitroprusside-induced falls in mesenteric resistance were attenuated after administration of nitroblue tetrazolium. The effects of L-*S*-nitrosocysteine, MAHMA NONOate and sodium nitroprusside were similar before and after injections of saline ($P > 0.05$, for all comparisons, data not shown).

4. Discussion

Nitroblue tetrazolium is lipophobic electron acceptor with a redox potential of −50 mV (Altman, 1976; Seidler, 1991). Nitroblue tetrazolium binds to biological tissues and readily oxidizes cysteine residues to the disulfide, cystine (see Altman, 1976). Systemic injections of nitroblue tetrazolium produce initial falls in mean arterial blood pressure and hindquarter and mesenteric vascular resistances but a marked increase in renal vascular resistance in pentobarbital-anesthetized rats (Davisson et al., 1993). Although the mechanisms responsible for the immediate effects of nitroblue tetrazolium have not been established, it appears that changes in redox state of proteins and especially cysteine residues have pronounced effects on vascular tone.

The principal findings of this study were that the vasodilator actions of L-*S*-nitrosocysteine were substantially diminished by nitroblue tetrazolium whereas the vasodilator actions of the NO donor, MAHMA NONOate, were not affected. The nitroblue tetrazolium-induced increases in resting resistances (see below) complicates the interpretation of the effects of this electron acceptor on the hemodynamic actions of the nitrosyl factors. However, the differential effects of nitroblue tetrazolium on L-*S*-nitrosocysteine- and MAHMA NONOate-induced vasodilation supports considerable evidence that the biological actions of *S*-nitrosothiols are not only dependent upon their decomposition to NO and the generation of guanosine 3′,5′-cyclic monophosphate (cGMP) in target tissues (see Stamler et al., 1992, 1997; Mathews and Kerr, 1993; Gordge et al., 1998). The vasodilator actions of L-*S*-nitrosocysteine are also attenuated by pHMBA whereas the vasodilator actions of MAHMA NONOate are not affected (Hoque et al., 1999). Taken together, these results suggest that: (i) the vasodilator actions of L-*S*-nitrosocysteine depend upon the oxidation–reduction state of membrane thiol residues, which are susceptible to oxidation by nitroblue tetrazolium; (ii) the formation of disulfide bonds in these proteins may prevent L-*S*-nitrosocysteine from binding to and/or nitrosating these sites; and (iii) the oxidation of membrane thiols does not impair the mechanisms by which NO relaxes vascular smooth muscle. The vasodilator actions of sodium nitroprusside in the hindquarter bed were augmented whereas the vasodilator actions of this NO donor in the mesenteric bed were substantially diminished by nitroblue tetrazolium. At present, we have no explanation for why nitroblue tetrazolium has opposing effects on sodium nitroprusside-induced vasodilation in these beds. However, it should be noted that sodium nitroprusside is an iron-nitrosyl (see Feelisch, 1991) and that its NO moiety is in the form of NO^+ which is capable of nitrosation reactions (see Stamler et al., 1992, 1997). The vasodilator actions of sodium nitroprusside may involve nitrosation of thiol residues in functional proteins in vascular membranes.

The other principal finding of this study is that these urethane-anesthetized rats displayed a hypotension but increases in peripheral vascular resistances 75–105 min after injection of the second dose of nitroblue tetrazolium (5 μmol/kg, i.v.). The fall in mean arterial blood pressure is therefore likely to involve a fall in cardiac output. The mechanisms by which nitroblue tetrazolium produces these delayed vasoconstrictor effects in the peripheral vasculature have not been determined. These actions of nitroblue tetrazolium are probably not due to increases in sympathetic neurogenic vasoconstriction since nitroblue tetrazolium markedly diminishes the vasoconstrictor effects of the α_1-adrenoceptor agonist, phenylephrine (Hoque and Lewis, 1996). Nitroblue tetrazolium non-competitively inhibits NO synthase by providing an alternative substrate for the NADPH diaphorase activity common to all NO synthase isoforms (see Hope et al., 1991; Gabbott and Bacon, 1993). It is certainly possible that the delayed increases in vascular resistances induced by nitroblue tetrazolium are due to its entry into vascular endothelial cells and the subsequent inhibition of NO synthase activity. However, the vasodilator actions of L-*S*-nitrosocysteine and NO donors are substantially augmented after administration of the NO synthesis inhibitor, N^G-nitro-L-arginine methyl ester, which also elicited substantial increases in vascular resistances (see Davisson et al., 1996a,b,c). There is considerable circumstantial evidence that L-*S*-nitrosocysteine is an endothelium-derived nitrosyl factor (Myers et al., 1990; Rubanyi et al., 1991; Rosenblum, 1992). The results of the present study raise the possibility that the nitroblue tetrazolium-induced increases in vascular resistances may also involve the attenuation of the vasodilator actions of endothelium-derived *S*-nitrosothiols. It is possible that the ability of nitroblue tetrazolium to oxidize cysteine residues in membrane proteins including putative stereoselective *S*-nitrosothiol recognition sites (Davisson et al., 1996a,b,c, 1997; Lewis et al., 1996; Travis et al., 1996, 1997) is responsible for the loss of response to L-*S*-nitrosocysteine although other properties of this amphoteric electron acceptor might also be involved (see Altman, 1976; Seidler, 1991).

In summary, the results of this study support the possibility that the vasodilator effects of L-*S*-nitrosocysteine may involve activation (perhaps by *trans*-nitrosation) of functional proteins that contain reduced thiol residues (see Hoque et al., 1999). The covalent modification or oxidation of these thiol residues may represent a mechanism by which endogenous electron acceptors (see Altman, 1976; Seidler, 1991) regulate the activity of endothelium-derived *S*-nitrosothiols (see Myers et al., 1990; Feelisch, 1991; Rosenblum, 1992; Davisson et al., 1996a,b,c).

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