ELSEVIER

European Journal of Pharmacology 384 (1999) 169–172

ejp

www.elsevier.nl/locate/ejphar

Short communication

In vivo evidence that L-*S*-nitrosocysteine may exert its vasodilator effects by interaction with thiol residues in the vasculature

Azizul Hoque [a], James N. Bates [b], Stephen J. Lewis [a,*]

[a] *The Department of Pharmacology, University of Iowa, Iowa, IA 52242, USA*
[b] *The Department of Anesthesia, University of Iowa, Iowa, IA 52242, USA*

Received 21 September 1999; accepted 28 September 1999

Abstract

This study examined the effects of the lipophobic thiol chelator, *para*-hydroxymercurobenzoic acid (25 and 50 μmol/kg, i.v.) on the falls in mean arterial blood pressure and regional vascular resistances produced by L-*S*-nitrosocysteine (400 nmol/kg, i.v.) and the nitric oxide (NO)-donors, (*Z*)-1-[*N*-methyl-*N*-[6(*N*-methylammoniohexyl)amino]]diazen-1-ium-1,2-diolate (MAHMA NONOate, 25 nmol/kg, i.v.) and sodium nitroprusside (10 μg/kg, i.v.), in urethane-anesthetized rats. The L-*S*-nitrosocysteine-induced responses were markedly diminished whereas the MAHMA NONOate- and sodium nitroprusside-induced responses were minimally affected by *para*-hydroxymercurobenzoic acid. These results suggest that the vasodilator actions of L-*S*-nitrosocysteine involves the interaction with membrane thiols in vascular smooth muscle of resistance arteries and that *para*-hydroxymercurobenzoic acid does not markedly affect NO-mediated vasodilation.

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

1. Introduction

The putative endothelium-derived *S*-nitrosothiol, L-*S*-nitrosocysteine (Myers et al., 1990; Rubanyi et al., 1991; Rosenblum, 1992), may relax vascular smooth muscle by its decomposition to nitric oxide (NO) (Ignarro, 1990), the nitrosation of thiol residues in functional proteins (Stamler et al., 1992, 1997), and the activation of stereoselective recognition sites (Davisson et al., 1996, 1997; Lewis et al., 1996; Ohta et al., 1997). These recognition sites may be a novel class of receptor which specifically recognizes L-*S*-nitrosocysteine and structurally similar *S*-nitrosothiols such as L-*S*-nitroso-β,β-dimethylcysteine (Travis et al., 1996, 1997) but not larger molecular weight *S*-nitrosothiols such as L-*S*-nitrosoglutathione (Lewis et al., 1996; Davisson et al., 1997). Alternatively, the stereoisomeric configuration of L-*S*-nitrosocysteine may merely confer access to 'nitrosation motifs' in established functional proteins (Stamler et al., 1997).

At present, there is no direct evidence that *S*-nitrosothiols dilate resistance arteries in vivo by nitrosation of thiol residues in functional proteins in plasma membranes of vascular smooth muscle. The chelation of thiol residues in vascular membranes would help to determine the importance of these thiol residues in the vasodilator actions of L-*S*-nitrosocysteine and NO. The lipophobic thiol chelator, *para*-hydroxymercurobenzoic acid, readily forms stable covalent bonds with plasma membrane thiol residues at physiological pH (Goodman and Hiatt, 1964). The main aim of this study was to determine the effects of *para*-hydroxymercurobenzoic acid on the vasodilator actions of L-*S*-nitrosocysteine and the NO-donors, sodium nitroprusside (Feelisch, 1991), and (*Z*)-1-[*N*-methyl-*N*-[6(*N*-methylammoniohexyl)amino]]diazen-1-ium-1,2-diolate (MAHMA NONOate) (Benkusky et al., 1998), in urethane-anesthetized rats. The results of these study suggest that L-*S*-nitrosocysteine exerts its vasodilator actions by interaction with membrane-bound thiol residues whereas NO-mediated vasodilation is not dependent upon these thiol residues.

* Corresponding author. Tel.: +1-319-335-7943; fax: +1-319-335-7903.
E-mail address: stephen-lewis@uiowa.edu (S.J. Lewis)

0014-2999/99/$ - see front matter
PII: S0014-2999(99)00686-X

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 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., 1996). 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 15–20 min after each of two injections of saline ($n = 6$) or two injections of *para*-hydroxymercurobenzoic acid (25 and 50 μmol/kg, i.v., $n = 8$). The second injections of saline or *para*-hydroxymercurobenzoic acid were given 60 min after the first injections. The effects of the nitrosyl factors were allowed to subside completely before another injection was given. In five rats prepared as described above, the hemodynamic actions of MAHMA NONOate (15 nmol/kg, i.v.) were determined before and after administration of *para*-hydroxymercurobenzoic acid (25 and 50 μmol/kg, i.v.). Again, MAHMA NONOate was injected 15–20 min after the last dose of *para*-hydroxymercurobenzoic acid. All drugs were from Sigma (St. Louis, MO, USA) except for sodium nitroprusside which was from Abbott (Chicago, Ill, USA) and MAHMA NONOate which was from Alexis Biochemicals (San Diego, CA, USA). L-*S*-nitrosocysteine was prepared as described previously (Davisson et al., 1996). The data are presented as mean ± S.E.M. and were analyzed by repeated measures analysis of variance followed by Students modified *t*-test with the Bonferroni correction for multiple comparisons between means (see Davisson et al., 1996). A value of $P < 0.05$ was taken to denote statistical significance.

3. Results

3.1. Effects of para-hydroxymercurobenzoic acid on resting hemodynamic parameters

The resting hemodynamic values recorded between 25 and 40 min after injection of two doses of *para*-hydroxymercurobenzoic acid (25 and 50 μmol/kg, i.v.) are summarized in Table 1. Resting mean arterial blood pressures and vascular resistances were not different to pre-injection values 25–40 min after injection of the first dose of *para*-hydroxymercurobenzoic acid ($P > 0.05$, for all comparisons). Resting MAP was reduced 25–40 min after administration of the second dose of *para*-hydroxymercurobenzoic acid ($-22 \pm 4\%$ of pre-injection values, $P < 0.05$). Resting hindquarter and mesenteric resistances were not different whereas renal vascular resistance was slightly higher ($+19 \pm 5\%$, $P < 0.05$) than pre-injection levels. Neither injection of saline affected resting hemodynamic values ($P > 0.05$, for all comparisons, data not shown). Injections of *para*-hydroxymercurobenzoic acid (25 and 50 μmol/kg, i.v.) produced similar responses to those described above in the group of rats which received injections of MAHMA NONOate (15 nmol/kg, i.v.) ($P > 0.05$, for all comparisons, data not shown).

Table 1
Resting hemodynamic values recorded 25–40 min after injections of para-hydroxymercurobenzoic acid
The data are presented as the mean ± S.E.M. pHMBA = *para*-hydroxymercurobenzoic acid. MAP = mean arterial blood pressure. HQR = hindquarter resistance. RR = renal resistance. MR = mesenteric resistance. There were eight rats in the group.

Parameter	Pre	Post-pHMBA (μmol/kg, i.v.)	
		25	50
MAP (mm Hg)	109 ± 4	105 ± 3	83 ± 4[a]
HQR (mm Hg/kHz)	36 ± 4	35 ± 5	32 ± 4
RR (mm Hg/kHz)	66 ± 6	72 ± 7	79 ± 6[a]
MR (mm Hg/kHz)	36 ± 5	38 ± 4	39 ± 6

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

3.2. Effects of para-hydroxymercurobenzoic acid on the hemodynamic actions of the vasodilator agents

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 *para*-hydroxymercurobenzoic acid (25 and 50 μmol/kg, i.v.) are summarized in Table 2. L-*S*-nitrosocysteine produced falls in mean arterial blood pressure and vascular resistances. The L-*S*-nitrosocysteine-induced responses were markedly attenuated by the lower and higher doses of *para*-hydroxymercurobenzoic acid. The MAHMA NONOate-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 *para*-hydroxymercurobenzoic acid. The sodium nitroprusside-induced depressor and vasodilator responses were equivalent to those of L-*S*-nitrosocysteine ($P > 0.05$, for all comparisons). The hemodynamic actions of sodium nitroprusside were not affected by the lower dose of *para*-hydroxymercurobenzoic acid. The sodium nitroprusside-induced falls in mean arterial blood pressure and

Table 2
Effects of *para*-hydroxymercurobenzoic acid on the hemodynamic actions of L-*S*-nitrosocysteine, MAHMA NONOate and sodium nitroprusside
The data are presented as the mean ± S.E.M of the maximal percent changes. 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.). pHMBA = *para*-hydroxymercurobenzoic acid. MAP = mean arterial blood pressure. HQR = hindquarter resistance. RR = renal resistance. MR = mesenteric resistance. There were eight rats in the group.

Compound	Parameter	Pre	Post-pHMBA (μmol/kg, i.v.)	
			25	50
L-SNC	ΔMAP	−38 ± 3	−24 ± 3[a]	−18 ± 3[a]
	ΔHQR, %	−36 ± 4	−21 ± 3[a]	−15 ± 3[a]
	ΔRR, %	−21 ± 3	−9 ± 2[a]	−2 ± 3[a]
	ΔMR, %	−49 ± 5	−34 ± 3[a]	−23 ± 3[a]
MAHMA NONOate	ΔMAP, %	−32 ± 3	−33 ± 3	−36 ± 4
	ΔHQR, %	−34 ± 4	−31 ± 4	−36 ± 4
	ΔRR, %	−22 ± 3	−19 ± 3	−24 ± 5
	ΔMR, %	−46 ± 5	−41 ± 6	−52 ± 7
SNP	ΔMAP, %	−42 ± 3	−41 ± 3	−33 ± 3[a]
	ΔHQR, %	−38 ± 3	−32 ± 3	−29 ± 3[a]
	ΔRR, %	−26 ± 4	−22 ± 3	−24 ± 4
	ΔMR, %	−51 ± 4	−48 ± 4	−39 ± 3[a]

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

hindquarter and mesenteric vascular resistances were slightly diminished by the higher dose of *para*-hydroxymercurobenzoic acid whereas the falls in renal resistance were not affected.

In order to determine whether *para*-hydroxymercurobenzoic acid may affect the hemodynamic responses produced by a lower dose of an NO-donor, we determined the effects of MAHMA NONOate (15 nmol/kg, i.v.) before and after administration of *para*-hydroxymercurobenzoic acid (25 and 50 μmol/kg, i.v., administered as above). This dose of MAHMA NONOate produced falls in mean arterial blood pressure (−17 ± 3%, $P < 0.05$), hindquarter vascular resistance (−19 ± 3%, $P < 0.05$), renal vascular resistance (−9 ± 2%, $P < 0.05$) and mesenteric vascular resistance (−29 ± 4%, $P < 0.05$). These responses were approximately 50% of those produced by the 25-nmol/kg dose of MAHMA NONOate ($P < 0.05$, for all comparisons). After administration of *para*-hydroxymercurobenzoic acid, the lower dose of MAHMA NONOate produced falls in mean arterial blood pressure (−19 ± 2%, $P < 0.05$), hindquarter vascular resistance (−23 ± 3%, $P < 0.05$), renal vascular resistance (−13 ± 3%, $P < 0.05$) and mesenteric vascular resistance (−34 ± 3%, $P < 0.05$). These MAHMA NONOate-induced responses were similar before and after administration of *para*-hydroxymercurobenzoic acid ($P > 0.05$, for all pre- vs. post-*para*-hydroxymercurobenzoic acid comparisons). The effects of L-*S*-nitrosocysteine, MAHMA NONOate (25 nmol/kg, i.v.) and sodium nitroprusside were similar before and after each injection of saline ($P > 0.05$, for all comparisons, data not shown).

4. Discussion

This study demonstrates that the vasodilator effects of L-*S*-nitrosocysteine are markedly reduced by the lipophobic thiol chelator, *para*-hydroxymercurobenzoic acid. L-*S*-nitrosocysteine may exert its effects by nitrosation of thiol residues in functional proteins (see Stamler et al., 1992, 1997) and by activation of stereoselective recognition sites which do not recognize larger *S*-nitrosothiols such as L-*S*-nitrosoglutathione (see Lewis et al., 1996). These recognition sites may be discrete receptors for L-*S*-nitrosocysteine, which are nitrosated upon occupation by this *S*-nitrosothiol. Alternatively, the size and stereoisomeric configuration of L-*S*-nitrosocysteine may confer access to 'nitrosation motifs' in established receptors, ion channels and other functional proteins (Stamler et al., 1997). Nonetheless, it appears that the vasodilator effects of L-*S*-nitrosocysteine are dependent upon thiol residues in biological membranes. However, this study does not provide specific evidence that L-*S*-nitrosocysteine exerts its effects by nitrosation of membrane proteins. It is possible that the covalent attachment of *para*-hydroxymercurobenzoic acid to thiol residues merely prevents access of L-*S*-nitrosocysteine to established functional proteins or to putative stereoselective recognition sites.

The vasodilator actions of the 25-nmol/kg dose of MAHMA NONOate (a dose which produced similar responses to those of L-*S*-nitrosocysteine) were not affected by *para*-hydroxymercurobenzoic acid whereas the vasodilator actions of sodium nitroprusside were slightly reduced by the higher dose of *para*-hydroxymercurobenzoic acid. This suggests that the vasodilator actions of these NO-donors are not markedly dependent upon thiol residues in vascular membranes and that *para*-hydroxymercurobenzoic acid does not affect the intracellular mechanisms by which NO exert its vasodilator effects (see Ignarro, 1990). The finding that *para*-hydroxymercurobenzoic acid did not affect the hemodynamic responses produced by the 15-nmol/kg dose of MAHMA NONOate (which produced substantially smaller responses than the 25-nmol/kg dose of MAHMA NONOate) strengthens the argument that the thiol chelator does not affect NO-mediated vasodilation. It should be noted that sodium nitroprusside is an iron–nitrosyl and that its NO moiety exists as NO^+, which is capable of nitrosation reactions (see Stamler et al., 1992). The ability of *para*-hydroxymercurobenzoic acid to reduce the vasodilator actions of sodium nitroprusside suggests that the nitrosation of thiol residues

may be a mechanism of action of sodium nitroprusside in vivo.

The hypotension produced by the higher dose of *para*-hydroxymercurobenzoic acid was associated with a minor increase in renal vascular resistance but no changes in hindquarter or mesenteric vascular resistances. This suggests that *para*-hydroxymercurobenzoic acid-induced hypotension is due mainly to a fall in cardiac output. *Para*-hydroxymercurobenzoic acid markedly reduces the vasoconstrictor effects of the α_1-adrenoceptor agonist, phenylephrine (Hoque and Lewis, 1996). Accordingly, the minimal effects of *para*-hydroxymercurobenzoic acid on resting resistances may involve the combined blockade of neurogenic vasoconstriction and endothelium-dependent nitrosyl factor-mediated vasodilation.

In summary, the vasodilator effects of the putative endothelium-derived *S*-nitrosothiol (Myers et al., 1990; Rubanyi et al., 1991; Rosenblum, 1992), L-*S*-nitrosocysteine, in resistance vessels appear to require the presence of reduced thiol residues in vascular membranes whereas the vasodilator actions of NO does not. These cysteine residues may be in discrete L-*S*-nitrosocysteine recognition sites (see Davisson et al., 1997) or may be in established functional proteins subject to L-*S*-nitrosocysteine-mediated nitrosation (see Stamler et al., 1992, 1997). Taken together, these findings support considerable evidence that L-*S*-nitrosocysteine exerts its biological actions by mechanisms other than its decomposition to NO (see Stamler et al., 1992, 1997). Finally, these findings raise the possibility that the covalent modification or redox regulation of thiol residues may represent a mechanism by which endogenous factors regulate the activity of endothelium-derived L-*S*-nitrosocysteine (Myers et al., 1990; Rubanyi et al., 1991; Rosenblum, 1992).

Acknowledgements

This study was supported by National Institutes of Health grant HL-14388.

References

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

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

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

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, 25–33, (suppl. 3).

Goodman, I., Hiatt, R.B., 1964. Chemical factors affecting spontaneous motility of the small intestine in the rat: 1. Sulphydryl reactants. Biochem. Pharmacol. 13, 871–879.

Hoque, A., Lewis, S.J., 1996. Stereoselective recognition sites for *S*-nitrosothiols: role of thiol residues. Hypertension 28, 170, Abstract.

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

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

Myers, P.R., Minor, R.L., Guerra, R., Bates, J.N., Harrison, D.G., 1990. Vasorelaxant properties of the endothelium-derived relaxing factor more closely resemble *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.

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.

Stamler, J.S., Singel, D.J., Loscalzo, J., 1992. 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.