ELSEVIER

European Journal of Pharmacology 407 (2000) 309–312

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Short communication

Apparent association of MK-801-sensitive ion channels with L-*S*-nitrosocysteine recognition sites in the hindlimb vasculature of the rat

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Received 24 August 2000; received in revised form 8 September 2000; accepted 12 September 2000

Abstract

This study demonstrates that the decreases in hindquarter vascular resistance produced by the putative endothelium-derived *S*-nitrosothiol, L-*S*-nitrosocysteine, in urethane-anesthetized rats, were attenuated by a lower dose of the *N*-methyl-D-aspartate (NMDA) receptor ion-channel blocker, dizocilpine (MK-801, 200 μg/kg, i.v.), whereas they were augmented by a higher dose of dizocilpine (500 μg/kg, i.v.). In contrast, L-*S*-nitrosocysteine-induced decreases in mesenteric vascular resistance were not affected by either dose of dizocilpine. The vasodilator actions of L-*S*-nitrosocysteine in these beds were not affected by the competitive NMDA receptor antagonist, 2-amino-5-phosphonovaleric acid (2-AP5). The vasodilator actions of the nitric oxide (NO) donor, (*Z*)-1-[*N*-methyl-*N*-[6(*N*-methylammoniohexyl)amino]]diazen-1-ium-1,2-diolate (MAHMA NONOate), in these beds were not affected by dizocilpine or by 2-AP5. These findings suggest that L-*S*-nitrosocysteine recognition sites in hindquarter but not mesenteric beds may be associated with dizocilpine-sensitive ion-channels similar to those in NMDA receptors.

Keywords: *S*-Nitrosothiol; *N*-Methyl-D-aspartate receptor; Nitric oxide

1. Introduction

Systemic injections of the putative endothelium-derived *S*-nitrosothiol, L-*S*-nitrosocysteine, produce vasodilator responses by activation of stereoselective *S*-nitrosothiol recognition sites in vascular smooth muscle of resistance arteries (see Davisson et al., 1996). It is unlikely that these recognition sites are *N*-methyl-D-aspartate (NMDA) receptors since systemic injections of NMDA produce vasoconstrictor responses due to entirely to centrally-mediated increases in sympathetic nerve activity and release of arginine vasopressin (Ohta et al., 1991; Whalen et al., 1999). Although this suggests that NMDA receptors are not present on endothelium or vascular smooth muscle of resistance arteries, this does not discount the possibility that NMDA receptor ion-channels, which are sensitive to blockade by dizocilpine (MK-801, (+)-5-methyl-10,11-dihydro-5*H*-dibenzo[*a*,*d*]cyclohepten-5,10-imine) (Cotman and Iverson, 1987), are present in resistance vessels.

The NMDA ion-channel shares structural homology with ion channels in γ-aminobutyric acid (GABA), glycine and nicotinic acetylcholine receptors (see Ramoa et al., 1990). Moreover, dizocilpine non-competitively blocks nicotinic receptors in the periphery and central nervous system (Ramoa et al., 1990). These findings raise the possibility that a variety of receptors may contain dizocilpine-sensitive ion channels. Accordingly, the aim of this study was to determine whether L-*S*-nitrosocysteine recognition sites in resistance vessels may contain dizocilpine-sensitive ion channels and whether nitric oxide (NO) interacts with these channels. In this study, we determined the effects of dizocilpine and the competitive NMDA receptor antagonist, DL-2-amino-5-phosphonovaleric acid (2-AP5) (Cotman and Iverson, 1987), on the hemodynamic responses produced by systemic injections of L-*S*-nitrosocysteine, the NO-donor, (*Z*)-1-[*N*-methyl-*N*-[6(*N*-methylammoniohexyl)amino]]diazen-1-ium-1,2-diolate (MAHMA NONOate) (Benkusky et al., 1998), and NMDA in urethane-anesthetized rats.

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0014-2999/00/$ - see front matter
PII: S0014-2999(00)00710-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.v.). A catheter was placed in a femoral vein to give drugs and a catheter was placed in a femoral artery to measure mean arterial blood pressure (Lewis et al., 1990). A pulsed Doppler flow probe was placed on the descending aorta (below the level of the kidneys) to measure hindquarter vascular resistance. A pulsed Doppler flow probe was also placed on the superior mesenteric artery to measure mesenteric vascular resistance. Vascular resistances were determined by dividing mean arterial blood pressures by blood flow velocities. The procedures and suitability of measuring percent changes in vascular resistances have been described previously (Davisson et al., 1996; Possas and Lewis, 1997; Travis et al., 1997).

2.2. Experimental procedures and protocols

Protocol 1: Injections of L-*S*-nitrosocysteine (200 nmol/kg, i.v.) and MAHMA NONOate (25 nmol/kg, i.v.) were given before and between 15 and 30 min after injection of (i) saline, (ii) 2-AP5 (50 mg/kg, i.v.), or (iii) dizocilpine (200 or 500 μg/kg, i.v.). Protocol 2: Injections of L-*S*-nitrosocysteine (200 nmol/kg, i.v.) and MAHMA NONOate (25 nmol/kg, i.v.) were given before and between 15 and 30 min after injection of indomethacin (10 mg/kg, i.v.) plus (i) saline, or (ii) dizocilpine (200 or 500 μg/kg, i.v.). Protocol 3: One injection of NMDA (5 μmol/kg, i.v.) was given before and between 15 and 30 min after injection of saline, or 2-AP5 (50 mg/kg, i.v.). Protocol 4: Three injections of NMDA (5 μmol/kg, i.v.) were given before and between 15 and 30 min after injection of saline or dizocilpine (200 or 500 μg/kg, i.v.). Three injections of NMDA were given before and after injection of dizocilpine to test the possibility that the NMDA responses may diminish upon repeated injection. This would be consistent with evidence that dizocilpine is a use dependent inhibitor of NMDA receptors (see Introduction). The responses produced by injections of L-*S*-nitrosocysteine, MAHMA NONOate and NMDA were allowed to subside completely before another injection was given. There were six rats in each group. All drugs were from Sigma (St. Louis, MO, USA) except for dizocilpine, which was a gift from Merck, Sharpe and Dohme (West Point, PA, 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 expressed as mean ± S.E.M. and were analyzed by repeated measures analysis of variance and Student's 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 significance.

3. Results

3.1. Effects of 2-AP5 and dizocilpine on resting hemodynamic parameters

Resting parameters recorded before and between 15 and 30 min after injection of 2-AP5 (50 mg/kg, i.v.) or dizocilpine (200 or 500 μg/kg, i.v.) are summarized in Table 1. Injection of 2-AP5 or dizocilpine did not elicit immediate responses. Likewise, the values recorded 15–30 min after the injection of these compounds were not different from pre-injection values. The administration of saline did not affect the resting hemodynamic parameters ($P > 0.05$ for all comparisons, data not shown).

3.2. Effects of 2-AP5 or dizocilpine on NMDA responses

The effects of NMDA (5 μmol/kg, i.v.) on hindquarter vascular resistance before and after injection of 2-AP5 (50 mg/kg, i.v.) or dizocilpine (200 or 500 μg/kg, i.v.) are summarized in Table 2. NMDA produced increases in hindquarter resistances ($P < 0.05$, for all responses), which were similar in each group ($P > 0.05$, for all comparisons). The NMDA-induced vasoconstrictor response was abolished by 2-AP5. Three injections of NMDA produced similar vasoconstrictor responses before and after injection of saline ($P > 0.05$, for all comparisons). The responses elicited by the first injections of NMDA were similar before and after injection of either dose of dizocilpine

Table 1
Resting hemodynamic parameters recorded before and between 15 and 30 min after the administration of 2-AP5 or dizocilpine

Compound	Parameter	Pre	Post	% Change
2-AP5	MAP (mm Hg)	112 ± 3	110 ± 3	−1 ± 4
	HQR (mm Hg/kHz)	58 ± 6	62 ± 7	+5 ± 4
	MR (mm Hg/kHz)	33 ± 4	32 ± 4	−2 ± 4
$Dizocilpine_{200}$	MAP (mm Hg)	106 ± 3	107 ± 3	+1 ± 2
	HQR (mm Hg/kHz)	64 ± 8	69 ± 10	+9 ± 6
	MR (mm Hg/kHz)	37 ± 4	39 ± 5	+4 ± 4
$Dizocilpine_{500}$	MAP (mm Hg)	110 ± 3	109 ± 3	+1 ± 4
	HQR (mm Hg/kHz)	53 ± 6	61 ± 8	+12 ± 8
	MR (mm Hg/kHz)	34 ± 3	37 ± 4	+8 ± 6

The data are presented as mean ± S.E.M. MAP = mean arterial blood pressure. HQR = hindquarter vascular resistance. MR = mesenteric vascular resistance. 2-AP5 = DL-2-amino-5-phosphonovaleric acid (50 mg/kg, i.v.). Dizocilpine = (MK-801, (+)-5-methyl-10,11-dihydro-5*H*-dibenzo[*a*,*d*]cyclohepten-5,10-imine). $Dizocilpine_{200}$ = dizocilpine (200 μg/kg, i.v.). $Dizocilpine_{500}$ = dizocilpine (500 μg/kg, i.v.). There were six rats in each group. Note that these injections of 2-AP5 and dizocilpine did not affect the resting hemodynamic values ($P > 0.05$, for all responses).

Table 2
Changes in hindquarter vascular resistance produced by L-*S*-nitrosocysteine, MAHMA NONOate and NMDA before and after administration of 2 AP5 or MK 801

Treatment	Compound	ΔHQR (%)	
		Pre	Post
2-AP5	L-SNC	−24±3	−26±3
	MAHMA NONOate	−28±3	−29±4
	NMDA[b]	+21±4	+2±3[a]
$Dizocilpine_{200}$	L-SNC	−26±3	−14±2[a]
	MAHMA NONOate	−29±3	−28±3
	NMDA[b]	+26±3	+14±2[a]
$Dizocilpine_{500}$	L-SNC	−23±3	−41±4[a]
	MAHMA NONOate	−27±3	−32±4
	NMDA[b]	+22±3	+6±4[a]

The data are presented as mean±S.E.M. HQR = hindquarter vascular resistance. 2-AP5 = DL-2 amino-5-phosphonovaleric acid (50 mg/kg, i.v.). Dizocilpine = (MK 801, (+)-5-methyl-10,11-dihydro-5*H*-dibenzo[*a*,*d*]cyclohepten-5,10-imine). $Dizocilpine_{200}$ = dizocilpine (200 μg/kg, i.v.). $Dizocilpine_{500}$ = dizocilpine (500 μg/kg, i.v.). L-SNC = L-*S*-nitrosocysteine (200 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.). NMDA = *N*-methyl-D-aspartate (5 μmol/kg, i.v.). There were six rats in each group.

[a] $P < 0.05$, post-treatment vs. pre-treatment.

[b] These values were produced by a third injection of NMDA before and after administration of MK-801.

($P < 0.05$, for both comparisons, data not shown). However, subsequent injections of NMDA elicited progressively smaller responses. The increases in hindquarter resistance produced by the third injection of NMDA (see Table 2) were substantially diminished after injection of 200 μg/kg (−48±8%, $P < 0.05$) or 500 μg/kg (−67±9%, $P < 0.05$) doses of dizocilpine.

NMDA also elicited pressor responses (e.g., +13±3%, $P < 0.05$) and increases in mesenteric resistance (e.g., +27±3%, $P < 0.05$). These responses were similar before and after injection of saline ($P > 0.05$ for all responses, data not shown) whereas they were abolished by 2-AP5 ($P < 0.05$, for both comparisons, data not shown). The three injections of NMDA elicited similar pressor responses and increases in mesenteric resistance before and after injection of saline ($P > 0.05$ for all responses, data not shown). These responses gradually and markedly diminished after injection of the above doses of dizocilpine such that the third dose of NMDA elicited minimal responses ($P < 0.05$, for all comparisons to first-injection responses, data not shown).

3.3. Effects of 2-AP5 or dizocilpine on L-S-nitrosocysteine and MAHMA NONOate responses

The L-*S*-nitrosocysteine- and MAHMA NONOate-induced decreases in hindquarter resistance before and after injection of 2-AP5 (50 mg/kg, i.v.) or dizocilpine (200 or 500 μg/kg, i.v.) are summarized in Table 2. The L-*S*-nitrosocysteine- and MAHMA NONOate-induced responses were equal to one another ($P > 0.05$, for all comparisons). L-*S*-nitrosocysteine-induced vasodilation was not affected by 2-AP5 (+12±9%, $P > 0.05$). In contrast, L-*S*-nitrosocysteine-induced vasodilation was diminished by the lower dose of dizocilpine (−42±9%, $P < 0.05$) but augmented by the higher dose of dizocilpine (+93±12%, $P < 0.05$). The MAHMA NONOate-induced responses were not affected by 2-AP5 or by either dose of dizocilpine. The decreases in hindquarter resistance produced by L-*S*-nitrosocysteine and MAHMA NONOate were similar before and after injection of saline ($P > 0.05$, for all comparisons). The decreases in mean arterial blood pressure and mesenteric resistances produced by L-*S*-nitrosocysteine and MAHMA NONOate were similar before and after injection of saline, 2-AP5 and either dose of dizocilpine ($P > 0.05$, for all comparisons, data not shown).

3.4. Effects of indomethacin

Injection of indomethacin (10 mg/kg, i.v.) did not affect resting parameters or the responses elicited by L-*S*-nitrosocysteine, MAHMA NONOate or NMDA. Moreover, indomethacin did not alter the effects of 2-AP5 or either dose of dizocilpine on the hemodynamic actions of L-*S*-nitrosocysteine, MAHMA NONOate or NMDA ($P > 0.05$, for all comparisons, data not shown).

4. Discussion

NMDA produced pressor and vasoconstrictor responses, which are due to centrally-mediated increases in sympathetic drive and the release of arginine vasopressin (Ohta et al., 1991; Whalen et al., 1999). These findings suggest that NMDA receptors do not exist on vascular smooth muscle of resistance arteries. The NMDA-induced responses were immediately abolished by 2-AP5 whereas they progressively diminished after injection of dizocilpine. This is consistent with the use-dependent manner by which dizocilpine blocks NMDA receptors (Cotman and Iverson, 1987). Systemic injections of dizocilpine produce a sustained hypertension in conscious rats by centrally-mediated increases in sympathetic drive (Lewis et al., 1989) whereas they did not affect hemodynamic parameters in the urethane-anesthetized rats. It therefore appears that urethane prevents the expression of dizocilpine-induced increases in sympathetic drive.

L-*S*-Nitrosocysteine-mediated decreases in hindquarter resistance were unaffected by 2-AP5. In contrast, the lower dose of dizocilpine reduced whereas the higher dose of dizocilpine augmented L-*S*-nitrosocysteine-induced vasodilation. However, MAHMA NONOate-induced vasodilation was not affected by 2-AP5 or dizocilpine. This suggests that dizocilpine modulates the actions of L-*S*-nitrosocysteine itself and that L-*S*-nitrosocysteine dilates resistance

vessels by activation of recognition sites linked to dizocilpine-sensitive ion channels rather than by interactions with NMDA receptors. Activation of dizocilpine-sensitive ion channels promotes Ca^{2+} influx (Cotman and Iverson, 1987). L-*S*-nitrosocysteine may exert its vasodilator actions in part by activation of recognition sites on vascular endothelial cells. The influx of Ca^{2+} due to subsequent activation of dizocilpine-sensitive ion-channels would have many actions including activation of NO synthase (see Rosenblum, 1992). Accordingly, the lower dose of dizocilpine may attenuate L-*S*-nitrosocysteine-mediated release of endothelium-derived relaxing factors. The increase in Ca^{2+} in vascular smooth muscle produced by activation of dizocilpine-sensitive ion channels would limit L-*S*-nitrosocysteine-mediated vasodilation. Accordingly, the higher dose of dizocilpine may augment L-*S*-nitrosocysteine-mediated vasodilation by blockade of Ca^{2+} influx into vascular smooth muscle. However, the above possibilities are speculative and alternative explanations are conceivable.

L-*S*-Nitrosocysteine-induced vasodilation in the hindquarter beds was immediately affected by dizocilpine. This is consistent with L-*S*-nitrosocysteine being tonically released from vascular endothelial cells (see Travis et al., 1997). Tonic activation of L-*S*-nitrosocysteine recognition sites and the opening of dizocilpine-sensitive ion channels would allow immediate access of dizocilpine to these channels. The NMDA ion-channel shares structural homology with ion channels in a variety of receptors (Ramoa et al., 1990). It is possible that L-*S*-nitrosocysteine recognition sites in the hindquarter bed of the rat contain these dizocilpine-sensitive ion channels. However, the lack of effect of dizocilpine on L-*S*-nitrosocysteine vasodilation in the mesenteric bed suggests that L-*S*-nitrosocysteine recognition sites in this bed are not associated with dizocilpine-sensitive ion channels. The hindlimb beds of the rat are innervated by post-ganglionic sympathetic neurons that may release newly synthesized and preformed stores of *S*-nitrosothiols (see Possas and Lewis, 1997). The dizocilpine-sensitive *S*-nitrosothiol recognition sites in the hindlimb bed may be present to modulate the vasodilator responses to neurogenically-derived *S*-nitrosothiols. The lack of effect of indomethacin on the actions of L-*S*-nitrosocysteine, MAHMA NONOate or NMDA or on the effects of dizocilpine on these agents suggests that cyclooxygenase factors are not involved in the phenomena reported here.

S-Nitrosothiols modulate NMDA receptors by interaction with a redox modulatory site (Lipton et al., 1993). Circulating *S*-nitrosothiols (Stamler et al., 1992, 1997) may regulate NMDA receptors on afferents (Lewis et al., 1987, 1990) or brain regions devoid of a blood–brain barrier (Whalen et al., 1999). L-*S*-nitrosocysteine recognition sites (Davisson et al., 1996, 1997) may contain dizocilpine-sensitive ion channels. Accordingly, dizocilpine may exert its effects (Lewis et al., 1989) by altering responses to endogenous *S*-nitrosothiols (see Travis et al., 1996, 1997).

Acknowledgements

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

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