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Nitrite potentiates the vasodilatory signaling of S-nitrosothiols

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ABSTRACT

Nitrite and S-nitrosothiols (SNOs) are both byproducts of nitric oxide (NO) metabolism and are proposed to cause vasodilation via activation of soluble guanylate cyclase (sGC). We have previously reported that while SNOs are potent vasodilators at physiological concentrations, nitrite itself only produces vasodilation at supraphysiological concentrations. Here, we tested the hypothesis that sub-vasoactive concentrations of nitrite potentiate the vasodilatory effects of SNOs. Multiple exposures of isolated sheep arteries to S-nitroso-glutathione (GSNO) resulted in a tachyphylactic decreased vasodilatory response to GSNO but not to NO, suggesting attenuation of signaling steps upstream from sGC. Exposure of arteries to 1 μM nitrite potentiated the vasodilatory effects of GSNO in naive arteries and abrogated the tachyphylactic response to GSNO in pre-exposed arteries, suggesting that nitrite facilitates GSNO-mediated activation of sGC. In intact anesthetized sheep and rats, inhibition of NO synthases to decrease plasma nitrite levels attenuated vasodilatory responses to exogenous infusions of GSNO, an effect that was reversed by exogenous infusion of nitrite at sub-vasodilating levels. This study suggests nitrite potentiates SNO-mediated vasodilation via a mechanism that lies upstream from activation of sGC.

1. Introduction

Due to its rapid reaction with hemoglobin, nitric oxide (NO) has a very short circulation half-life (merely 2 ms in blood), thus limiting the range of NO itself to paracrine tissues [1,2]. Nonetheless, endocrine effects of NO have been widely reported [3,4], implicating the existence of byproducts of NO that retain its bioactivity and are stable enough to circulate systemically [5–7]. Over the past two decades much research has been focused on determining the chemical identity of these bioactive NO metabolites, with nitrite and S-nitrosothiols (SNOs) receiving the most attention [5–13].

Nitrite, an anion ubiquitous in both blood and vessel walls, can serve as a source of NO bioactivity with vasodilatory effects [14]. Early work put forth the idea that nitrite's vasoactivity was due to the ability of deoxyhemoglobin to reduce nitrite to NO [5]. Based on the relative abundance of deoxyhemoglobin in hypoxic tissues, this reaction was proposed to promote vasodilation that would serve to restore adequate O_2 delivery [15]. However, more recent work with nitrite suggests that nitrite's vasodilating properties do not depend solely on the reaction with hemoglobin [16–18]. The vasodilating effects of nitrite may also be derived from its direct action on vascular smooth muscle cells [17,19,20]. The mechanism for these effects remains unclear, although it may involve the conversion of nitrite to NO or some other active NO-adduct within the vascular smooth muscle cell by a number of proposed pathways [9,17,19–21].

S-nitrosothiols are a class of molecules containing NO that has bound to sulfur by replacing the hydrogen of a thiol group. These compounds circulate in blood [22] and cause vasodilation by both NO/cGMP dependent and independent pathways [23]. The NO/cGMP dependent pathway, which appears to be the predominant one [24], involves the activation of soluble guanylate cyclase (sGC) by NO within vascular smooth muscle cells [24–26]. However, the mechanism by which extracellular SNOs, most of which are membrane-impermeable, produce activation of intracellular sGC is still not clear. The following possible mechanisms have been proposed for the cross-membrane vasodilatory signaling of SNOs (Fig. S1): 1) SNOs decompose to release free NO either spontaneously or via catalysis by the cell surface protein disulfide isomerase (csPDI) [27,28], followed by diffusion of the NO

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into the cell to activate sGC; 2) SNOs S-transnitrosylate thiol groups on the surface of the cell membrane [29,30] to initiate cross-membrane signaling events that result in transport of the NO moiety into the cell; or SNOs cross the cell membrane after conversion into either 3) membrane-permeable thionitrous acid (HSNO) formed by S-transnitrosation of H_2S [31], 4) S-nitroso-L-cysteine (L-cysNO) which can be taken into the cell via the LAT [32,33], or 5) L-cysNO-glycine formed by hydrolyzation of GSNO via ϒ-glutamyl transpeptidase (ϒ-GT) [34] before being taken into the cell through the dipeptide transporters (PEPT2) [35]. Despite decades of interest, no consensus of support has emerged for any one of these pathways.

The membrane permeability of, and the rate of release of NO from SNOs varies depending on the chemical nature of the molecule. For instance, L-cysNO and its stereoisomer D-cysNO decompose to release NO more rapidly than S-nitroso-glutathione (GSNO) [36]. In contrast, L-cysNO can move across plasma membranes via the L-type amino acid transporter (LAT), whereas D-cysNO and especially GSNO are relatively membrane-impermeable [32]. In this study, we tested the above previously-proposed mechanisms for L-cysNO, D-cysNO, and GSNO mediated vasodilation, with results failing to support any of these pathways. However, we find that SNO-mediated vasodilation is potentiated by the presence of nitrite, an interaction that gives rise to novel hypotheses for the vasoactivity of both SNOs and nitrite.

2. Materials and methods

2.1. Preparation of SNOs and pharmacological compounds

GSNO, L-cysNO, D-cysNO, and their corresponding blank controls were prepared as reported before [37,38] and described in the Online Supplementary Information (SI). Test compounds were purchased from Sigma Aldrich (St. Louis, MO), and dose information and justification for pharmacological selectivity at those doses are provided in the references shown in Table 1.

2.2. Experimental animals

All animal procedures were conducted in accordance with procedures that were pre-approved by the LLU Institutional Animal Care and Use Committee. In both sheep and rats, blood flow and arterial driving pressure to a hind limb were recorded in response to test compounds so that conductance could be calculated as an index of vasodilation.

2.3. Surgical procedures and intra-arterial infusion protocol in sheep

The surgical procedures have been reported before [37,39] and are described in the SI. The conductance and blood flow of the infused femoral artery, mean arterial blood pressure, and heart rate were recorded. Sheep were randomly divided into three groups: a L-NAME group received L-NAME and SNO; a L-NAME + nitrite group received L-NAME, nitrite, and SNO; a control group received SNO but no L-NAME or nitrite. Hexamethonium was given to block neural influences. L-NAME was administrated intravenously (45 mg/kg bolus) to lower the endogenous nitrite level. Sodium nitrite (1 mM) was infused for 15 min at a rate (1 ml/min) calculated to result in femoral arterial blood nitrite concentrations of ~12.4 μM based on dilution of the infusate in the measured femoral flow [17]. GSNO, D-cysNO, or L-cysNO (5 mM; measured value) was infused at rates of 0.2, 0.4, 0.6, and 0.8 ml/min, increasing every 3 min. A baseline period of 30 min was allowed before the nitrite and SNO infusions. Baseline hemodynamic values before infusion of SNOs in sheep that received L-NAME with or without prior nitrite infusion are shown in Table S1.

2.4. Surgical procedures and intra-arterial infusion protocol in rats

The surgical procedures in rats were similar to the sheep experiments mentioned above and described in the SI. Briefly, rats were randomly divided into three groups: a control group received GSNO; a L-NAME group received L-NAME and GSNO; a L-NAME + nitrite group received L-NAME, nitrite, and GSNO. L-NAME (60 mg $kg\cdot^{-1}day^{-1}$, $T_{1/2}$ = 23 h [40]) or saline (1 ml; for the control group) was injected intraperitoneally 4 days (including the day of experiment) before the surgery. After a stable baseline period of 30 min, saline or nitrite (50 μM in saline) was infused. After another 30 min, GSNO (50 μM) was infused into the lower abdominal aorta at rates of 0.05, 0.1, 0.2, and 0.4 ml/min, increasing every 3 min. Arterial pressure and femoral blood flow were recorded continuously and used for calculation of femoral artery conductance while GSNO was infused.

2.5. Wire myography

Arterial rings (2 mm diameter and 5 mm long) were dissected from a portion of the mesenteric artery supplying the duodenum or from the lateral circumflex branch of the femoral artery of adult ewes. Arterial rings were denuded of endothelium and mounted in organ bath chambers as described previously [37,38] and described in the SI. Initial wire myography studies of L- and D-cysNO were performed by measuring SNO dose response curves in femoral arteries after constriction with 10 μM phenylephrine. Subsequently, because mesenteric arteries proved to be more sensitive to vasodilation by SNOs and because the dilation of both vessel types was NO/cGMP dependent [38], experiments of GSNO were performed in mesenteric arteries after constriction with 125 mM KCl [38]. Test compounds were added to the baths 15 min before the contraction and maintained until the end of the experiments, except for HMBA, a thiol modifier (10 μM) [30], which was incubated with vessels for 30 min and then extensively washed out before contraction. To control for spontaneous changes in tension, changes in vessels treated with inert SNO controls (see SI) in parallel vessel baths were subtracted from individual experiments before calculation.

2.6. Analytical methods

Details of analytical methods including tri-iodide chemiluminescence

Table 1
Compounds tested to influence vasodilatory responses.

Name	Chemical description	Concentration	Action
ODQ	1H-[1,2,4] oxadiazolo [4,3-a] quinoxaline-1-one	10 μM	competitive sGC inhibitor [68]
CPTIO	2-(4-carboxyphenyl)-4,4,5-tetramethyl imidazoline-1-oxyl-3-oxide	200 μM	membrane-impermeable NO scavenger [43,69]
BCH	2-Aminobicyclo [2.2.1]heptane-2-carboxylic acid	10 mM	competitive LAT inhibitor [70]
Thr	Threonine	10 mM	competitive LAT inhibitor [70]
Na_2S	Na_2S	1 μM	H_2S donor [71]
PAG	DL-propargyl glycine	1 mM	H_2S synthetase inhibitor [71]
GSM	S-methyl-glutathione	1 mM	ϒ-glutamyl transpeptidase (ϒ-GT) inhibitor [72]
L-NAME	$L\text{-}N^G$-nitro arginine methyl ester	100 μM	nonspecific NOSs inhibitor [73]
HMBA	p-hydroxymercurobenzoic acid	10 μM	membrane -impermeable thiol modifier [30]