Pulmonary vasodilator responses to sodium nitrite are mediated by an allopurinol-sensitive mechanism in the rat

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Am J Physiol Heart Circ Physiol 296: H524–H533, 2009. First published December 12, 2008; doi:10.1152/ajpheart.00543.2008.—Recent studies show that pulmonary vasodilator responses to nitrite are enhanced by hypoxia. However, the mechanism by which nitrite is converted to vasoactive nitric oxide (NO) is uncertain. In the present study, intravenous injections of sodium nitrite decreased pulmonary and systemic arterial pressures and increased cardiac output. The decreases in pulmonary arterial pressure were enhanced when tone in the pulmonary vascular bed was increased with U-46619. Under elevated tone conditions, decreases in pulmonary and systemic arterial pressures in response to nitrite were attenuated by allopurinol in a dose that did not alter responses to the NO donors, sodium nitroprusside and diethylamine/NO, suggesting that xanthine oxidoreductase is the major enzyme-reducing nitrite to NO. Ventilation with a 10% O2 gas mixture increased pulmonary arterial pressure, and the response to hypoxia was enhanced by N\textsuperscript{G}-nitro-L-arginine methyl ester and not altered by allopurinol. This suggests that NO formed by the endothelium and not from the reduction of plasma nitrite modulates the hypoxic pulmonary vasoconstrictor response. Although intravenous injections of sodium nitrite reversed pulmonary hypertensive responses to U-46619, hypoxia, and N\textsuperscript{G}-nitro-L-arginine methyl ester, the pulmonary vasodilator response to nitrite was not altered by ventilation with 10% O2 when baseline pulmonary arterial pressure was increased to similar values in animals breathing room air or the hypoxic gas. These data provide evidence that xanthine oxidoreductase is the major enzyme-reducing nitrite to vasoactive NO, and that this mechanism is not modified by hypoxia.

nitric oxide; xanthine oxidoreductase; pulmonary hypertension; nitric oxide synthase

ENDOTHelial NITRIC OXIDE (NO) formation plays an important role in the regulation of the pulmonary and systemic vascular beds (15, 28). The importance of NO has been demonstrated in experimental animals and in human subjects by the use of NO synthase (NOS) inhibitors (2, 15, 28). Although NO synthesis is important in the regulation of baseline tone in most vascular beds, the role of NO in the regulation of tone in the pulmonary vascular bed of the rat has been questioned (15). NO once released from the endothelium into the blood reacts rapidly with red cell hemoglobin (14, 21a, 26). Furthermore, NO that escapes hemoglobin scavenging can be oxidized to nitrite (21a). NO formed from nitrite that escapes inactivation relaxes vascular smooth muscle by a cGMP-dependent mechanism (7, 26). It has been reported that plasma nitrite concentrations reflect constitutive NOS activity and correlate with endothelial function (21a). In addition, recent research speculates that nitrite anion represents a storage form of NO that can have important pharmacological actions (13, 22). This is a new concept, since nitrite was previously believed to be an inactive metabolite of NO, although the effects of amyl nitrite were first recognized in 1871 (4, 21a). The ability of sodium nitrite to relax vascular smooth muscle was reported in 1953; however, high concentrations were required (12). Nevertheless, it is now widely accepted that, even at micromoles per kilogram doses, nitrite has vasodilator activity in experimental animals and human subjects (8, 17, 29).

Nitrite can be reduced to NO by enzymatic mechanisms and nonenzymatic disproportionation under severe conditions (5, 6, 24). It has been reported that sodium nitrite and acidified sodium nitrate have organ-protective effects in several ischemia-reperfusion injury models (10, 20, 21). In addition to nonenzymatic mechanisms, it has been reported that the reduction of nitrite to NO can be catalyzed by xanthine oxidoreductase (XOR), deoxyhemoglobin, and other heme-containing proteins, and the kinetics of XOR in the reduction of nitrite to NO has been reported (5, 6, 24). Although XOR can theoretically reduce nitrite to NO, the effects of XOR inhibitors on cardiovascular responses to sodium nitrate are uncertain, and studies in the literature report no effect of XOR inhibitors (6, 8).

In contrast, there is increasing evidence that nitrite reduction to NO is catalyzed by deoxyhemoglobin, and it has been reported that pulmonary vasodilator responses to inhaled and injected sodium nitrite are enhanced by hypoxia, which increases deoxyhemoglobin levels (3, 16, 17). In addition, it has been reported that, in the presence of red blood cells, nitrite had no inhibitory effect on hypoxic pulmonary vasoconstriction in the isolated rat lung (7). This suggests that NO must escape red cell scavenging to cause pulmonary vasodilation. Moreover, inhaled nitrite has been reported to produce vasodilation in the pulmonary circulation of the newborn lamb and in the isolated, perfused rat lung (17). However, responses to sodium nitrite have not been determined in the pulmonary vascular bed of the intact rat under normal and elevated tone conditions. In addition, the mechanism of nitrite activation and the role of XOR in the reduction of nitrite to NO are uncertain.

The present study was, therefore, undertaken to investigate responses to sodium nitrite in the intact rat under normal and...
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... elevated tone conditions and to determine the effect of inhibitors of NOS [N\textsuperscript{G}-nitro-L-arginine methyl ester (L-NAME)], XOR (allopurinol and oxypurinol), and of ventilatory hypoxia on responses to nitrite under elevated tone conditions in the pulmonary vascular bed. These results show that sodium nitrite has significant pulmonary and systemic vasodilator activity. Pulmonary vasodilator responses are enhanced when baseline tone is increased by the thromboxane mimic U-46619, ventilatory hypoxia, or when NOS is inhibited. Vasodilator responses to sodium nitrite are attenuated by allopurinol and oxypurinol, indicating that nitrite bioactivation is mediated by XOR. In addition, responses to nitrite are similar with left ventricular [intra-arterial, (ia)] and intravenous (iv) injections, and responses to nitrite are not increased by ventilatory hypoxia under elevated tone conditions. These data suggest that deoxyhemoglobin does not play a major role in nitrite bioactivation. Although allopurinol inhibited vasodilator responses to sodium nitrite, pulmonary and systemic vascular responses to hypoxia were not altered by the XOR inhibitor, suggesting that reduction of plasma nitrite to NO does not modulate the response to hypoxia. These results show that sodium nitrite has significant pulmonary vasodilator activity that is enhanced by U-46619 and NOS inhibition, but is not modulated by hypoxia. These data suggest that XOR plays a major role in reducing nitrite to vasoactive NO.

METHODS

The Institutional Animal Care and Use Committee of Tulane University School of Medicine approved the experimental protocol used in these experiments, and all procedures were conducted in accordance with institutional guidelines. For these experiments, adult male Sprague-Dawley rats (Harlan Sprague-Dawley), weighing 290–450 g, were anesthetized with 100 mg/kg ip Inactin. Supplemental doses of Inactin were given iv as needed to maintain a uniform level of anesthesia. Body temperature was maintained with a heating lamp. The trachea was cannulated with a short segment of polyethylene (PE) tubing to maintain a patent airway, and the animals spontaneously breathed room air or a 10% O\textsubscript{2}-90% N\textsubscript{2} gas mixture. A femoral artery was catheterized with PE50 tubing for measurement of systemic arterial pressure. The left jugular and femoral veins were catheterized with PE50 tubing for iv administration and infusion of drugs and fluids. For measurement of pulmonary arterial pressure, a 3F catheter with a radiopaque marker and curved tip was passed from the right jugular vein into the main pulmonary artery under fluoroscopic guidance (Picker C-arm fluoroscope), as described previously (9, 18). Left ventricular end-diastolic pressure was measured in some experiments by placement of a 3F radiopaque catheter in the chamber under fluoroscopic guidance. Pulmonary and systemic pressures were measured by Nemic Perceptor DT transducers (Boston Scientific), and the data were digitized by a Biopac MP100 data-acquisition system. The pressures were displayed and stored on a Dell PC. Cardiac output was measured with a Cardiomax II (Columbus Instruments) cardiac output computer by the thermal dilution technique. There were 0.2 ml of 0.9% NaCl solution at room temperature injected into the jugular vein catheter with its tip near the right atrium, and changes in blood temperature were measured with a 1.5-F microprobe catheter (Columbus Instruments) positioned in the aortic arch from the left carotid artery. Cardiac output curve data were stored on a PC.

The experiments in this study were designed to do the following: 1) characterize hemodynamic responses to iv injections of sodium nitrite under baseline and elevated tone conditions with U-46619; 2) determine the role of NOS by using the inhibitor L-NAME in mediating the response to sodium nitrite; 3) investigate the role of XOR using allopurinol and oxypurinol in mediating the response to sodium nitrite; 4) investigate the effect of hypoxia to increase deoxyhemoglobin levels (ventilation with 10% O\textsubscript{2}-90% N\textsubscript{2} gas mixture) in modulating the pulmonary vasodilator response to sodium nitrite; and 5) investigate the role of NOS using L-NAME and endogenous nitrite in blood using the XOR inhibitor allopurinol in modulating the pulmonary vasoconstrictor and peripheral vasodilator responses to ventilatory hypoxia.

In the first set of experiments, responses to iv injections of 10–100 μmol/kg sodium nitrite were investigated under baseline conditions. In the second set of experiments, responses to iv injections of sodium nitrite were investigated when pulmonary arterial pressure was increased to an approximate value of 30 mmHg by U-46619, which was infused iv at 80–240 ng/min after an initial priming dose. In the third set of experiments, the role of NOS was investigated, and responses to sodium nitrite were investigated after NOS was inhibited with L-NAME in doses of 10–50 mg/kg iv. In the next set of experiments, the role of XOR was investigated using allopurinol and oxypurinol. Responses to sodium nitrite and the NO donors sodium nitroprusside and diethylamine (DEA)/NO were compared before and after injection of allopurinol (25 mg/kg iv), and the dose of allopurinol was determined from the literature and pilot experiments (29). The effects of L-NAME (5 mg/kg iv) and allopurinol (25 mg/kg iv) on the increase in pulmonary arterial pressure in response to ventilation with the 10% O\textsubscript{2}-90% N\textsubscript{2} gas mixture were investigated to ascertain if NOS and XOR modulate the response to hypoxia. Decreases in systemic arterial pressure in response to left ventricular (ia) and iv injections of sodium nitrite were compared under baseline conditions to ascertain if nitrite reduction to vasoactive NO is similar in arterial (left ventricular) and venous (iv) blood.

In experiments in which the effects of hypoxia on responses to sodium nitrite were investigated, the rats breathed hypoxic gas (10% O\textsubscript{2}-90% N\textsubscript{2}) from a plastic hood over the inlet of the endotracheal tube. In these experiments, sodium nitrite was injected iv after pulmonary arterial pressure reached a steady level after the onset of ventilation with the hypoxic gas mixture. Responses to sodium nitrite were investigated in animals that breathed hypoxic gas and were infused with U-46619 to increase pulmonary arterial pressure to ~30 mmHg. Since hypoxia increased respiratory rate and volume and decreased arterial PCO\textsubscript{2}, responses were investigated in animals in which respiratory movements were blocked with pancuronium (5 mg/kg iv), and the animals were ventilated with a Harvard small-animal respirator. Stroke volume and ventilatory rate were adjusted to maintain PO\textsubscript{2}, PCO\textsubscript{2}, and pH at physiological values. In these experiments, the 10% O\textsubscript{2} gas mixture was delivered to the inlet port on the respirator.

Arterial blood gases were measured in 200-μl arterial blood samples removed from the femoral artery catheter and were analyzed with an iSTAT 1 Analyzer (Abbott Laboratories) or a Radiometer NPT7 series analyzer (Copenhagen). Methemoglobin levels increased from a control value of 1.3 ± 2 to 10.0 ± 1% in arterial blood after three to six injections of 10–100 μmol/kg iv sodium nitrite (n = 11–19).

The thromboxane (TP receptor) agonist, U-46619 (Cayman), was dissolved in 100% ethanol and diluted in 0.9% NaCl solution; sodium nitrite, sodium nitrate, sodium nitroprusside, DEA/NO, pancuronium, allopurinol and oxypurinol (Sigma Aldrich) were dissolved in 0.9% NaCl solution. The agonists were injected iv in small volumes in a random sequence. U-46619 was continuously infused into a separate vein with a Harvard infusion pump.

The data are presented as mean ± SE. Pulmonary vascular resistance was calculated by dividing the mean pulmonary arterial pressure by cardiac output, and left ventricular end-diastolic pressure as an index of left arterial pressure was measured in some experiments and was unchanged. Systemic vascular resistance was calculated by dividing mean systemic arterial pressure by the cardiac output. Area under the curve for decreases in pulmonary and systemic arterial pressure were determined with the Biopac system. The data were analyzed using paired or group t-tests or an analysis of variance...
with a post hoc test. The criterion for statistical significance was a $P < 0.05$.

RESULTS

Response to sodium nitrite. Under baseline conditions in animals breathing room air, iv injections of sodium nitrite, in doses of $10–100 \mu\text{mol/kg}$, produced small decreases in pulmonary arterial pressure, larger decreases in systemic arterial pressure, and small increases in cardiac output (Fig. 1A). Pulmonary and systemic vascular resistances were decreased, and, since maximal decreases in pulmonary arterial pressure were determined in some experiments by injecting larger doses (300 and 1,000 $\mu\text{mol/kg}$ iv) of sodium nitrite, the half-maximal effective dose (ED$_{50}$) for nitrite could be calculated and was $\sim 30 \mu\text{mol/kg}$ iv (Fig. 1A). These data show that sodium nitrite has significant vasodilator activity in the pulmonary and systemic vascular beds.

The effect of injection of sodium nitrite iv and into the left ventricle, which is an ia injection, was compared, and injections at both sites produced similar decreases in systemic arterial pressure (Fig. 1B). These data suggest that nitrite reduction to vasoactive NO occurs in arterial and venous blood.

The iv injections of sodium nitrate, which is not reduced to NO in doses up to 100 $\mu$mol/kg, had no significant effect on pulmonary and systemic arterial pressures (Fig. 1C).

To investigate pulmonary responses under elevated tone conditions and investigate the mechanism of nitrite activation, an iv infusion of thromboxane mimic U-46619 was used to increase pulmonary arterial pressure to an approximate value of 30 mmHg. When pulmonary arterial pressure was increased by U-46619, iv injections of sodium nitrite produced larger dose-related decreases in pulmonary arterial pressure, similar decreases in systemic arterial pressure, and small increases in systemic arterial pressure and cardiac output were 15 and 99 mmHg and 113 ml/min, respectively. Values are means $\pm$ SE; $n$, no. of experiments.

*Significantly different from control, $P < 0.05$.

![Fig. 1. A: bar graphs showing decreases in pulmonary and systemic arterial pressures, percent maximal decrease in pulmonary arterial pressure and half-maximal effective dose (ED$_{50}$), and changes in cardiac output and pulmonary and systemic vascular resistance in response to intravenous (iv) injections of 10–100 $\mu$mol/kg sodium nitrite under baseline conditions in animals breathing room air. B: effect of iv and left ventricular (intra-arterial (ia)) injection of sodium nitrite on systemic arterial pressure. C: effect of iv injections of 10–100 $\mu$mol/kg sodium nitrate on pulmonary and systemic arterial pressure under baseline conditions. Baseline pulmonary and systemic arterial pressure and cardiac output were 15 and 99 mmHg and 113 ml/min, respectively. Values are means $\pm$ SE; $n$, no. of experiments. *Significantly different from control, $P < 0.05$.](http://ajpheart.physiology.org/)
cardiac output (Figs. 1A and 2A). The decreases in pulmonary arterial pressure were significantly greater at 30 and 100 μmol/kg during U-46619 infusion (P < 0.05, group comparison). The ED50 for the decrease in pulmonary arterial pressure in response to sodium nitrite during U-46619 infusion was ~30 μmol/kg iv (Fig. 2A). The iv injections of the 100 μmol/kg dose of sodium nitrite completely reversed the pulmonary hypertensive response to the U-46619 infusion (Fig. 2B).

Effects of XOR inhibitors and time of response onset. To provide information on the kinetics of nitrite activation, the time-to-peak decrease in pressure under elevated tone conditions in response to iv injections of sodium nitrite and sodium nitroprusside was compared. The time-to-peak decrease in pulmonary and systemic arterial pressure in response to sodium nitrite was significantly longer than for sodium nitroprusside when responses to doses that produced similar decreases in pressure were analyzed (Fig. 2C).

To investigate the role of XOR in mediating responses to sodium nitrite, the effects of the XOR inhibitors allopurinol and oxypurinol were investigated under elevated tone conditions. Following injection of allopurinol (25 mg/kg iv), the decreases in pulmonary and systemic arterial pressure in response to iv injection of sodium nitrite were reduced significantly (Fig. 3A). The decreases in pulmonary and systemic arterial pressure in response to iv injections of the NO donors sodium nitroprusside and DEA/NO were not altered by treatment with allopurinol (Fig. 3A). The area under the curve for the decreases in pulmonary and systemic arterial pressure in response to sodium nitrite was reduced significantly after treatment with allopurinol (Fig. 3B). The decreases in pulmonary and systemic arterial pressure in response to iv injections of sodium nitrite were also significantly decreased by oxypurinol (25–50 mg/kg iv), whereas responses to sodium nitroprusside were not altered by this XOR inhibitor (data not shown).

Fig. 2. Bar graphs showing decreases in pulmonary arterial pressure and percent maximal decrease in pulmonary arterial pressure and ED50, decreases in systemic arterial pressure, and changes in cardiac output in response to iv injections of 10–100 μmol/kg sodium nitrite during infusion of U-46619 (A) and reversal of U-46619-induced pulmonary pressure increase by nitrite (B). C: comparison of the time-to-peak decrease in pulmonary and systemic arterial pressure in response to iv injections of sodium nitrite and sodium nitroprusside (SNP) under high-tone conditions in doses that produced similar decreases in pressure. During U-46619 infusion, pulmonary and systemic arterial pressure and cardiac output averaged 29 and 99 mmHg and 99 ml/min, respectively. Values are means ± SE; n, no. of animals. *Significantly different than control, P < 0.05.
**Effect of L-NAME.** Inasmuch as NO in the vascular bed is generated by endothelial NOS, and, under some conditions, endothelial NOS can reduce nitrite to NO, the effect of NOS inhibition on responses to sodium nitrite was investigated, and treatment with L-NAME in doses of 10 or 50 mg/kg iv significantly increased pulmonary and systemic arterial pressures. The iv injection of sodium nitrite produced larger decreases in pulmonary and systemic arterial pressures in L-NAME-treated animals than in control animals when responses are measured at similar levels of pulmonary arterial pressure (Fig. 4A). The percent decreases in pulmonary arterial pressure were significantly greater in U-46619- and L-NAME-treated animals than in control animals (Fig. 4C). When responses are expressed as percent decrease to normalize values in the pulmonary and systemic vascular beds, the percent decreases in pulmonary arterial pressure were significantly greater in U-46619- and L-NAME-treated animals than in control animals (Fig. 4C). The percent decreases in systemic arterial pressure were significantly greater in L-NAME-treated animals, and there were no significant differences (or selectivity) in responses to sodium nitrite in the pulmonary and systemic vascular beds of L-NAME- and U-46619-treated animals (Fig. 4C).

**Effect of L-NAME and allopurinol of the hypoxic pulmonary vasoconstrictor response.** Ventilation with 10% O₂-90% N₂ increased pulmonary arterial pressure, decreased systemic arterial pressure, and decreased arterial PO₂ (Fig. 5A). The hypoxic pulmonary vasoconstrictor response was reversed by iv injection of 100 μmol/kg sodium nitrite (Fig. 5A). The iv injection of L-NAME (5 mg/kg) increased the pulmonary vasoconstrictor response to hypoxia, and the pulmonary pressor response was reversed by iv injection of sodium nitrite (100 μmol/kg iv) (Fig. 5B).
To ascertain if endogenous nitrite in blood is modulating the response to hypoxia by its reduction to vasoactive NO, the effect of the XOR inhibitor allopurinol on the hypoxic pulmonary vasoconstrictor response was investigated, and these data are summarized in Fig. 5C. The increase in pulmonary arterial pressure and the decrease in systemic arterial pressure in response to ventilation with the 10% O₂-90% N₂ gas mixture were not altered after administration of 25 mg/kg iv allopurinol (Fig. 5C). These data suggest that endogenous nitrite in the plasma is not modulating the pulmonary hypertensive response and systemic hypotensive response to hypoxia by its reduction to vasoactive NO by XOR.

Effect of hypoxia on the response to sodium nitrite. It has been reported that the pulmonary vasodilator response to inhaled sodium nitrite is enhanced by hypoxia. However, the effect of hypoxia on the response to injection of sodium nitrite has not been determined in the intact-chest rat. The effect of ventilation with the 10% O₂-90% N₂ gas mixture on the decrease in pulmonary arterial pressure in response to sodium nitrite was investigated in animals in which baseline pulmonary arterial pressure was increased to a high steady level with U-46619 in two sets of experiments.

In the first set of experiments in which the animals spontaneously breathed the hypoxic gas mixture, the decreases in pulmonary arterial pressure in response to iv injections of sodium nitrite were not altered by ventilatory hypoxia when pulmonary arterial pressure was increased to ~30 mmHg with U-46619 when the animals were mechanically ventilated with room air or the 10% O₂ gas mixture and arterial PCO₂ and pH were unchanged (Fig. 6A).

Since ventilation with the 10% O₂ gas mixture increased respiratory rate and volume, resulting in a decrease in arterial Pco₂ and an increase in arterial pH, the effect of hypoxia on the response to nitrite was investigated in a second set of animals treated with pancuronium and ventilated with a small-animal respirator. The iv injection of sodium nitrite in doses of 30 and 100 μmol/kg produced similar decreases in pulmonary arterial pressure when baseline pulmonary arterial pressure was increased to ~30 mmHg with U-46619 in animals that breathed the hypoxic gas mixture (Fig. 6B).
These data indicate that the pulmonary vasodilator response to sodium nitrite is not modulated by hypoxia in the rat when responses are measured at the same level of pulmonary arterial pressure.

**DISCUSSION**

It has been reported that nitrite-dependent vasodilator responses are enhanced by hypoxia and are independent of known NO-generating nitrite reductase activities (6). The results of the present study show that vasodilator responses to sodium nitrite are mediated by an allopurinol-sensitive mechanism, suggesting that XOR is an important nitrite reductase-generating vasoactive NO in the rat and that the pulmonary vasodilator response to nitrite is not modified by hypoxia.

The present results show that iv injections of sodium nitrite decrease pulmonary and systemic arterial pressures. Inasmuch as cardiac output was increased and left ventricular end diastolic pressure was unchanged, the decreases in pressure reflect decreases in pulmonary and systemic vascular resistances. The decreases in pulmonary arterial pressure were modest under baseline conditions when tone was low and were enhanced when pulmonary arterial pressure was increased by U-46619. When pulmonary vascular resistance was increased with the thromboxane mimic, iv injections of sodium nitrite produced dose-related decreases in pulmonary arterial pressure. Inasmuch as the level of vasoconstrictor tone in the pulmonary vascular bed could be estimated by giving larger doses of sodium nitrite until a nadir in pulmonary arterial pressure was achieved, an ED50 could be calculated and was estimated to be 30 μmol/kg iv. When tone was increased with U-46619, the pharmacological properties of the response could be evaluated, and sodium nitrite was found to be 100-fold less potent than sodium nitroprusside, but had full vasodilator efficacy or activity in the pulmonary vascular bed. Vasodilator responses to sodium nitrite were slower in onset than responses to nitroprusside and sodium nitrate, which is not reduced to NO and had no significant vasodilator activity. Although it has been reported that responses to nitrite are independent of known NO-generating reductases, including XOR, the results of the present study show that decreases in pulmonary and systemic arterial pressure in response to iv injections of sodium nitrite are attenuated by allopurinol in a dose that did not alter responses to the NO donors sodium nitroprusside or DEA/NO (6, 8). These data and experiments with oxypurinol provide support for the hypothesis that XOR is a major NO-generating reductase mediating the response to sodium nitrite.

![Fig. 5](http://ajpheart.physiology.org/)

A: bar graphs showing effect of hypoxia on pulmonary arterial pressure and reversal of the hypoxic pulmonary vasoconstrictor response by iv injection of sodium nitrite. B: bar graphs showing effect of L-NAME (5–50 mg/kg iv) on pulmonary arterial pressure and the response to hypoxia and reversal of the hypoxic pulmonary vasoconstrictor response by iv injection of sodium nitrite in the L-NAME-treated animal. Bar graph shows that the response to hypoxia is enhanced by L-NAME. C: bar graphs showing the effect of allopurinol on the increase in pulmonary arterial pressure and decrease in systemic arterial pressure in response to ventilatory hypoxia. Values are means ± SE; n, no. of animals. *Significantly different than control, P < 0.05.
nitrite in the rat. The shift to the right of nitrite dose-response
curve and the decrease in area under the pressure response
curves after treatment with allopurinol suggest that XOR me-
diates >50% of the vasodilator response to injected nitrite.

Although the present data indicate that XOR mediates a
substantial part of the response to nitrite in the rat, several
studies report no effect of XOR inhibitors on vasodilator
responses (6, 8). Moreover it has been reported that NO
production from nitrite occurs primarily in tissue and not in
blood (25). In these studies, the addition of nitrite induced the
formation of large amounts of NO in the heart and liver, and
this was enhanced by decreases in pH and hypoxia (25). The
formation of NO from nitrite in the heart and lung was
inhibited by oxypurinol (24, 25). These data suggest that XOR
is a major nitrite reductase in the rat heart and liver. The results
of the present study are in agreement with these studies in
regard to the role of XOR; however, the present data suggest
that nitrite bioactivation occurs within the vascular system and
can occur in arterial and venous blood, and the process is not
hypoxia sensitive. The reason for the difference in results is
uncertain; however, the results of a recent study show that
allopurinol attenuates decreases in systemic arterial pressure in
response to iv injections of sodium nitrite in l-NAME-treated
rats (29). The results in the rat are consistent with the hypoth-
thesis that XOR plays an important role in mediating responses
to injected nitrite in the rat.

Inasmuch as the present data suggest that XOR is an impor-
tant nitrite reductase in the rat, the effect of the XOR inhibitor
allopurinol on the pulmonary vasoconstrictor response to hyp-
oxia was investigated to determine whether the reduction of
endogenous nitrite in plasma to NO by XOR is modulating the
response. The increase in pulmonary arterial pressure and the
decrease in systemic arterial pressure in response to ventilatory
hypoxia were not altered by allopurinol. These data indicate
that responses to hypoxia are not modulated by the formation
of NO from endogenous nitrite in plasma. These data are not in
agreement with results suggesting that nitrite may play an
important role in modulating the response to hypoxia by acting

\[ \text{PO}_2 = 81 \pm 3 \quad 40 \pm 3 \]  
\[ \text{PCO}_2 = 46 \pm 1 \quad 44 \pm 2 \]  
\[ \text{pH} = 7.36 \pm 0.01 \quad 7.38 \pm 0.02 \]
as a recyclable source of vasoactive NO (5, 13). In contrast to results indicating that endogenous nitrite does not modulate the response to hypoxia, the pressor response to hypoxia was enhanced in the pulmonary vascular bed after administration of l-NAME. These data indicate that endogenous NO produced by NOS in the endothelium has a significant modulatory role in the pulmonary vascular bed of the rat.

Studies in the literature show that nitrite reacts with deoxyhemoglobin to form methemoglobin and NO (3, 26). In the present study, plasma methemoglobin levels increased following repeated injections of sodium nitrite, suggesting a role for deoxymethemoglobin. However, the observation that similar decreases in systemic arterial pressure were observed when sodium nitrite is injected into the left ventricle (an ia injection) or iv suggests that nitrite reduction occurs both in arterial and venous blood and is consistent with the hypothesis that XOR, and not deoxyhemoglobin, is the major nitrite reductase-generating vasoactive NO.

Ventilatory hypoxia increased pulmonary arterial pressure, and the hypoxic vasoconstrictor response was reversed by sodium nitrite. These data are consistent in some respects with results showing that sodium nitrite has marked pulmonary vasodilator activity when pulmonary vascular resistance is increased by ventilatory hypoxia in the neonatal lamb (17). The influence of ventilation with 10% O2 was also investigated and when pulmonary arterial pressure was increased to similar high values with U-46619, and in these studies hypoxia did not modify the pulmonary vasodilator response to iv injection of sodium nitrite. However, when respiration is not controlled, ventilation with hypoxic gas increases respiratory rate and volume and arterial PCO2 is decreased. The resulting respiratory alkalosis can impair nitrite reduction to NO. To prevent hypoxia-induced changes in PCO2 and pH, respiration was paralyzed with pancuronium, and the animals were mechanically ventilated. When the effects of hypoxia on the decrease in pulmonary arterial pressure in response to sodium nitrite were evaluated at similar high levels of pulmonary arterial pressure, the decreases in pulmonary arterial pressure were not modified by hypoxia when arterial PCO2 and pH were constant. These data indicate that, although nitrite is capable of reversing the hypoxic pulmonary vasoconstrictor response, ventilatory hypoxia does not enhance the response to iv injection of sodium nitrite when tone is increased with U-46619 in the anesthetized rat. The present data are different from results in the newborn lamb (17). The reasons for the differences are uncertain; however, it is possible that differences in species, route of nitrite administration, and levels of tone in the pulmonary vascular bed could play a role (17).

In the present study, decreases in systemic arterial pressure in response to iv and left ventricular (ia) injections of sodium nitrite were similar, and the short latency of the response (15–30 s) with ia injection suggests that nitrite reduction to vasoactive NO can occur in arterial and in venous blood. The observation that nitrite activation occurs in arterial blood is consistent with data in the human forearm vascular bed (8).

Although nitrite was believed to be an end-product of NO oxidation and a measure of endothelial NO formation, it has been reported to have significant biological activity in a variety of species, including humans (17, 21a, 29). The cardiovascular effects of inhaled amyl nitrite were first described in 1871, and this agent has been used in the diagnosis and treatment of angina (4, 27). Inhaled nitrite was reported to decrease pulmonary arterial pressure in the newborn lamb when pulmonary vascular resistance was increased by ventilatory hypoxia or U-46619 (17). In another study in the newborn lamb, iv injection of sodium nitrite decreased pulmonary arterial pressure during hypoxia, but had no effect in the normoxic animal (3). In the isolated, perfused rat lung, the inhibition of hypoxic pulmonary vasoconstriction by sodium nitrite was prevented by excess free hemoglobin or the presence of red blood cells (7). These studies suggest that, although deoxymethemoglobin generates NO from nitrite, the NO that escapes scavenging is not sufficient to cause vasodilation (7). The results of the present study in the rat show that iv injections of sodium nitrite decrease pulmonary and systemic arterial pressure, and that pulmonary vasodilator responses are dependent on tone when vascular resistance is increased by U-46619, l-NAME, or ventilatory hypoxia. These data suggest that, when nitrite is administered iv in pharmacological doses, the derived NO can, in part, escape inactivation by hemoglobin and produce a marked pulmonary and systemic vasodilator response that is not selective for the pulmonary vascular bed or hypoxia sensitive and is mediated in large part by XOR. These data indicate, from a pharmacological perspective, that sodium nitrite has full pulmonary vasodilator activity or efficacy but is not 100-fold less potent than sodium nitroprusside on a mole-to-mole basis.

In summary, the results of the present study show that sodium nitrite has significant pulmonary vasodilator activity that is dependent on baseline tone and enhanced by NOS inhibition. Vasodilator responses to nitrite were similar when nitrite was injected ia or iv, and responses to nitrite were attenuated by allopurinol in a dose that did not alter responses to nitroprusside or DEA/NO. These data suggest that, although methemoglobin levels increase, XOR is the major reductase generating vasoactive NO. Although sodium nitrite reversed the pulmonary hypertensive response to hypoxia, decreases in pulmonary arterial pressure in response to nitrite were not enhanced by acute ventilatory hypoxia when pulmonary vascular resistance was increased to similar levels with U-46619. The observation that the hypoxic pulmonary vasoconstrictor response is enhanced by l-NAME but not altered by allopurinol suggests that the response is modulated by the formation of vasoactive NO by NOS, but not by XOR-mediated reduction of endogenous nitrite in blood to NO. These results are consistent with the hypothesis that the pulmonary vasodilator response to nitrite is mediated by XOR and is not hypoxia sensitive, and that nitrite activation can occur in arterial and venous blood. These data suggest that XOR in the cardiovascular system is an important NO-generating nitrite reductase in the rat.

GRANTS

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REFERENCES


