Mitochondrial aldehyde dehydrogenase mediates vasodilator responses of glyceryl trinitrate and sodium nitrite in the pulmonary vascular bed of the rat

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Mitochondrial aldehyde dehydrogenase mediates vasodilator responses of glyceryl trinitrate and sodium nitrite in the pulmonary vascular bed of the rat. Am J Physiol Heart Circ Physiol 299:H819–H826, 2010. First published June 11, 2010; doi:10.1152/ajpheart.00959.2009.—It has been reported that mitochondrial aldehyde dehydrogenase (ALDH2) catalyzes the formation of glyceryl dinitrate and inorganic nitrite from glyceryl trinitrate (GTN), leading to an increase in cGMP and vasodilation in the coronary and systemic vascular beds. However, the role of nitric oxide (NO) formed from nitrite in mediating the response to GTN in the pulmonary vascular bed is uncertain. The purpose of the present study was to determine if nitrite plays a role in mediating vasodilator responses to GTN. In this study, intravenous injections of GTN and sodium nitrite decreased pulmonary and systemic arterial pressures and increased cardiac output. The decreases in pulmonary arterial pressure under baseline and elevated tone conditions and decreases in systemic arterial pressure in response to GTN and sodium nitrite were attenuated by cyanamide, an ALDH2 inhibitor, whereas responses to the NO donor, sodium nitroprusside (SNP), were not altered. The decreases in pulmonary and systemic arterial pressure in response to GTN and SNP were not altered by allopurinol, an inhibitor of xanthine oxidoreductase, whereas responses to sodium nitrite were attenuated. GTN was ~1,000-fold more potent than sodium nitrite in decreasing pulmonary and systemic arterial pressures. These results suggest that ALDH2 plays an important role in the bioactivation of GTN and nitrite in the pulmonary and systemic vascular beds and that the reduction of nitrite to vasoactive NO does not play an important role in mediating vasodilator responses to GTN in the intact chest rat.

Allopurinol; cyanamide; U-46619; NO2-; nitroarginine methyl ester

Glyceryl trinitrate (GTN) and, to a lesser extent, amyl nitrite have been used in the treatment of angina and heart failure for more than a century (4, 5, 38). However, the molecular mechanism by which GTN relaxes vascular smooth muscle is still the subject of current investigation and remains unknown. Although it is well established that nitric oxide (NO) activates soluble guanylyl cyclase, increases cGMP formation, and relaxes vascular smooth muscle, the role of NO release in mediating the vasorelaxant response to GTN is uncertain (11, 17, 21, 26, 30, 37, 39). Although studies in the literature provide evidence that NO is released from GTN, other studies show that NO is not released and suggest that the activation of guanylyl cyclase is mediated by a closely related but not currently identified chemical species with NO-guanylyl cyclase stimulating properties (2, 11, 16, 23, 25, 29–31, 36, 39). There is substantial evidence in the literature that mitochondrial aldehyde dehydrogenase (ALDH2) plays an important role in the bioactivation of GTN (8–10, 24). This enzyme catalyzes the formation of 1,2-glyceryl dinitrate (1,2-GDN) and inorganic nitrite from the metabolism of GTN, leading to the production of cGMP and vasorelaxation (3, 10). Nitrite anion can be reduced to NO by enzymatic and nonenzymatic mechanisms, and it has been reported that vasodilator responses to GTN in the coronary and systemic vascular beds can be attenuated by cyanamide, an inhibitor of ALDH2 (3, 8, 45). However, the effects of cyanamide and allopurinol on responses to GTN have not been determined in the pulmonary vascular bed. The purpose of the present study was to investigate the effects of cyanamide and allopurinol on responses to GTN and sodium nitrite to determine if the formation of vasoactive NO from nitrite plays a role in mediating responses to GTN in the pulmonary and systemic vascular beds in the rat. The results of this study suggest that ALDH2 plays a role in the bioactivation of GTN and nitrite but that the reduction of nitrite to vasoactive NO does not contribute to vasodilator responses to GTN in the pulmonary and systemic vascular beds in the intact rat.

METHODS

The Institutional Animal Care and Use Committee of the Tulane University School of Medicine approved the experimental protocol employed in these studies, and all procedures were conducted in accordance with institutional guidelines. In these experiments, adult male Sprague-Dawley rats (Charles River) weighing 275–450 g were anesthetized with Inactin (100 mg/kg ip) (Sigma-Aldrich) and were placed in the supine position on an operating table. Supplemental doses of Inactin were administered intravenously to maintain a uniform level of anesthesia. Body temperature was maintained with a heating lamp. The trachea was cannulated with a short segment of PE-240 tubing to maintain a patent airway. The animals spontaneously breathed room air. A femoral artery was catheterized with PE-50 tubing for measurement of systemic arterial pressure. The left jugular and femoral veins were catheterized with PE-50 tubing for intravenous injections and infusions of agents. For pulmonary arterial pressure measurements, a specifically designed 3-Fr single-lumen catheter with a curved tip and with radio-opaque markers was passed from the right jugular vein to the main pulmonary artery under fluoroscopic guidance (Picker-Surveyor Fluoroscope), as previously described (7). Pulmonary and systemic arterial pressures were measured with Namic Perceptor DT transducers (Boston Scientific), digitized by a Biopac MP100 data acquisition system (Biopac Systems), and stored on a Dell personal computer (PC). Cardiac output was measured by the thermodilution technique with a Cardiomax II computer (Columbus Instruments). A known volume (0.2 ml) of room
temperature 0.9% NaCl solution was injected in the jugular vein catheter with its tip near the right atrium, and changes in blood temperature were detected by a 1.5-Fr thermistor microprobe (Columbus Instruments) positioned in the aortic arch from the left carotid artery, and the indicator dilution curves were stored on the PC.

In the first set of experiments, the effects of the ALDH2 inhibitor cyanamide, in a dose of 25 mg/kg iv, on responses to intravenous injections of GTN (Lilly), sodium nitrite, and sodium nitroprusside (SNP) (Sigma-Aldrich) were investigated in the pulmonary and systemic vascular beds under baseline conditions. Cyanamide (Sigma-Aldrich) was dissolved in 0.9% NaCl with the addition of small amounts of 0.1 N NaOH.

In the second set of experiments, the effect of cyanamide, 25 mg/kg iv, on responses to intravenous injections of GTN, sodium nitrite, and SNP were investigated when pulmonary arterial pressure was increased to a high steady level by continuous intravenous infusion of the thromboxane (TP) receptor agonist U-46619. After initial loading at a high priming rate, the U-46619 infusion was adjusted (240–400 ng/min) to maintain pulmonary arterial pressure at ~30 mmHg. U-46619 (Cayman Chemical) was dissolved in 95% ethyl alcohol, diluted with 0.9% NaCl solution to working solutions, and infused with a Harvard infusion pump.

In the third set of experiments, the effect of the ALDH2 inhibitor cyanamide, 25 mg/kg iv, on responses to GTN, sodium nitrite, and SNP were also investigated in animals treated with N\(^\text{G}\)-nitro-L-arginine methyl ester (L-NAME), 25–50 mg/kg iv, to inhibit endogenous NO formation. L-NAME (Sigma-Aldrich) was dissolved in a 0.9% NaCl solution.

In the last set of experiments, the effect of the xanthine oxidoreductase (XOR) inhibitor allopurinol on responses to GTN, SNP, and sodium nitrite were investigated and compared in the pulmonary and systemic vascular beds under baseline tone condi-

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Fig. 1. Bar graphs showing changes in pulmonary and systemic arterial pressures and cardiac output in response to iv injections of glyceryl trinitrate, sodium nitrite, and sodium nitroprusside before and after administration of 25 mg/kg cyanamide iv. n = No. of experiments. *P < 0.05 when control responses (filled bars) are compared with responses after cyanamide treatment (open bars).
tions and when pulmonary arterial pressure was increased to a high steady value with the TP agonist U-46619. Allopurinol (Sigma-Aldrich) was dissolved in 0.9% NaCl with the addition of small amounts of 0.1 N NaOH.

The hemodynamic data are expressed as means ± SE, and pulmonary and systemic arterial pressures are presented as mean pressures. The hemodynamic data were analyzed using paired and group t-tests and with an ANOVA with Dunnett’s post hoc test. The criteria used for statistical significance was $P < 0.05$.

Table 1. Effect of cyanamide on systemic and pulmonary arterial pressure and cardiac output under baseline conditions

<table>
<thead>
<tr>
<th>Condition</th>
<th>Pulmonary Arterial Pressure, mmHg</th>
<th>Systemic Arterial Pressure, mmHg</th>
<th>Cardiac Output, ml/min</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>19 ± 1</td>
<td>109 ± 4</td>
<td>121 ± 5</td>
</tr>
<tr>
<td>Cyanamide (25 mg/kg iv)</td>
<td>22 ± 1</td>
<td>125 ± 5*</td>
<td>113 ± 5</td>
</tr>
</tbody>
</table>

Values are means ± SE; $n = 8–10$ rats in each group. *$P < 0.05$ compared with control.

RESULTS

Effects of cyanamide. The effects of the ALDH2 inhibitor cyanamide on responses to GTN, sodium nitrite, and SNP were investigated in the pulmonary and systemic vascular beds of the rat, and the results of these experiments are summarized in Fig. 1. Under baseline conditions, the intravenous injections of GTN and SNP (1–10 µg/kg) and sodium nitrite (10–100 µmol/kg) produced small decreases in pulmonary arterial pressure, larger dose-dependent decreases in systemic arterial pressure, and increases in cardiac output. After the administration of cyanamide (25 mg/kg iv), the decreases in pulmonary and systemic arterial pressure in response to intravenous injections of GTN and sodium nitrite were significantly reduced, whereas decreases in the pressure response to SNP were not significantly changed (Fig. 1). The effect of cyanamide on baseline pulmonary and systemic arterial pressure and cardiac output is shown in Table 1.

The effects of cyanamide (25 mg/kg iv) on decreases in pressure in response to the nitrosovasodilators were investi-
gated under elevated tone conditions in the pulmonary vascular bed, and these data are summarized in Fig. 2. When pulmonary arterial pressure was increased to ~30 mmHg by intravenous infusion of U-46619, the intravenous injections of GTN, sodium nitrite, and SNP produced larger dose-dependent decreases in pulmonary arterial pressure, similar dose-dependent decreases in systemic arterial pressure, and increases in cardiac output. The decreases in pulmonary and systemic arterial pressures in response to GTN and sodium nitrite were significantly reduced after administration of cyanamide, whereas decreases in pulmonary and systemic pressures in response to SNP were not altered (Fig. 2). The effect of U-46619 and cyanamide on mean vascular pressures and cardiac output is shown in Table 2.

The effects of cyanamide (25 mg/kg iv) on responses to the nitrovasodilators were investigated in animals treated with L-NAME to inhibit NO synthase. The administration of L-NAME produced significant increases in pulmonary and systemic arterial pressures and a decrease in cardiac output (7). In animals treated with L-NAME (25–50 mg/kg iv), pulmonary arterial pressure was significantly increased, and decreases in pulmonary and systemic arterial pressures in response to intravenous injections of GTN and sodium nitrite were attenuated by cyanamide treatment. Decreases in pulmonary and systemic arterial pressure in response to SNP were not significantly altered after administration of cyanamide (Fig. 3). The effect of L-NAME and cyanamide on mean vascular pressures and cardiac output is shown in Table 3.

Effect of allopurinol. The effects of the XOR inhibitor allopurinol on responses to GTN, sodium nitrite, and SNP were

### Table 2. Effect of cyanamide on systemic and pulmonary arterial pressure and cardiac output in U-46619-infused animals

<table>
<thead>
<tr>
<th></th>
<th>Pulmonary Arterial Pressure, mmHg</th>
<th>Systemic Arterial Pressure, mmHg</th>
<th>Cardiac Output, ml/min</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>31 ± 1</td>
<td>110 ± 5</td>
<td>122 ± 7</td>
</tr>
<tr>
<td>Cyanamide (25 mg/kg iv)</td>
<td>30 ± 1</td>
<td>134 ± 6*</td>
<td>116 ± 6</td>
</tr>
</tbody>
</table>

Values are means ± SE; n = 8–9 rats/group. *P < 0.05 compared with control.
investigated when pulmonary arterial pressure was increased with U-46619, and these results are summarized in Fig. 4. Following administration of allopurinol (25 mg/kg iv), the decreases in pulmonary and systemic arterial pressures in response to sodium nitrite were significantly reduced, whereas decreases in pulmonary and systemic arterial pressure in response to GTN and SNP were not significantly changed (Fig. 4). The effect of U-46619 and allopurinol on mean vascular pressures and cardiac output in these experiments is shown in Table 4. Under control baseline tone conditions, the three vasodilator agents produced small decreases in pulmonary arterial pressure, larger decreases in systemic arterial pressure, and increases in cardiac output. The decreases in pulmonary and systemic arterial pressure in response to sodium nitrite were significantly decreased after administration of allopurinol, whereas decreases in pressure in response to GTN and SNP were not altered by the XOR inhibitor (data not shown).

### Comparison of responses to GTN and sodium nitrite.

The decreases in systemic and pulmonary arterial pressures in Table 3. **Effect of cyanamide on systemic and pulmonary arterial pressure and on cardiac output in L-NAME-treated animals**

<table>
<thead>
<tr>
<th></th>
<th>Pulmonary Arterial Pressure, mmHg</th>
<th>Systemic Arterial Pressure, mmHg</th>
<th>Cardiac Output, ml/min</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>28 ± 2</td>
<td>138 ± 4</td>
<td>85 ± 11</td>
</tr>
<tr>
<td>Cyanamide (25 mg/kg iv)</td>
<td>29 ± 2</td>
<td>125 ± 4</td>
<td>80 ± 14</td>
</tr>
</tbody>
</table>

Values are means ± SE; n = 6 rats/group. L-NAME, N^3^-nitro-L-arginine methyl ester.

Table 4. **Effect of allopurinol on systemic and pulmonary arterial pressure and cardiac output in U-46619-infused animals**

<table>
<thead>
<tr>
<th></th>
<th>Pulmonary Arterial Pressure, mmHg</th>
<th>Systemic Arterial Pressure, mmHg</th>
<th>Cardiac Output, ml/min</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>30 ± 1</td>
<td>104 ± 5</td>
<td>116 ± 10</td>
</tr>
<tr>
<td>Allopurinol (25 mg/kg iv)</td>
<td>31 ± 1</td>
<td>112 ± 5</td>
<td>111 ± 9</td>
</tr>
</tbody>
</table>

Values are means ± SE; n = 8–9 rats/group.

### Figures

**Fig. 4.** Bar graphs showing changes in pulmonary and systemic arterial pressures and cardiac output in response to iv injections of glyceryl trinitrate, sodium nitrite, and sodium nitroprusside before (filled bars) and after administration of the xanthine oxidoreductase (XOR) inhibitor allopurinol at 25 mg/kg iv in U-46619-infused animals (open bars). n = No. of experiments. *P < 0.05.
U-46619-infused animals in response to intravenous injections of GTN and sodium nitrite were compared, and these data are shown in Fig. 5. GTN was ~1,000-fold more potent compared with sodium nitrite in decreasing pulmonary and systemic arterial pressures when doses are expressed on a micromole per kilogram basis (Fig. 5).

Pathway for GTN bioactivation. A proposed pathway for GTN bioactivation by ALDH2 is shown in Fig. 6.

DISCUSSION

GTN and amyl nitrite have been used in the treatment of angina pectoris and heart failure for 140 years (4, 5, 38), and it is generally believed that the therapeutic effect of these drugs involves the release of NO from nitrite, the activation of soluble guanylyl cyclase, and relaxation of blood vessels (26, 37). It has been reported that ALDH2 plays an important role in the in vivo bioactivation of GTN (8–10, 24, 45). The results of the present study show that decreases in pulmonary and systemic arterial pressures in response to intravenous injections of GTN were attenuated by cyanamide, an inhibitor of ALDH2, and provide support for the hypothesis that ALDH2 plays an important role in the bioactivation of GTN (10). The mechanism by which GTN activates soluble guanylyl cyclase is uncertain (25, 26, 32). The nitrite anion that is formed from the reaction of GTN with ALDH2 can be reduced to vasoactive NO or a NO-like compound that activates soluble guanylyl cyclase (26, 37). The pathway by which ALDH2 is hypothesized to activate GTN is shown in Fig. 6. The cellular metabolism of GTN by ALDH2 yields 1,2-glyceryl dinitrate (1,2-GDN) and nitrite anion. The results of the present study using cyanamide and allopurinol and comparing the biological activity of GTN and nitrite are consistent with the hypothesis that vasodilator responses to GTN in the intact-chest rat are mediated by the formation of a molecule that activates soluble guanylate cyclase and that the reduction of nitrite to nitric oxide (NO) is not involved.

Fig. 5. Dose-response curves comparing decreases in pulmonary and systemic arterial pressures in response to iv injections of glyceryl trinitrate and sodium nitrite in U-46619-infused animals when doses are expressed on a μmol/kg iv basis. n = No. of experiments.
suggest that the conversion of inorganic nitrite to vasoactive NO, even if all three nitrate groups of GTN were reduced to NO, would not be able to account for the vasodilator activity of GTN in the intact-chest rat. These data are consistent with the results of studies showing that vasorelaxant responses to GTN can occur independent of its NO-releasing properties in isolated vessels (2, 11, 16, 23, 29–31, 39). The present results are consistent with this hypothesis; however, the identity of the chemical species formed from GTN by ALDH2 that activates soluble guanylyl cyclase in the intact vascular bed is uncertain (25, 26, 37). The results of the present study showing that the vasodilator responses to sodium nitrite are attenuated by cyanamide in the rat are consistent with the observations of Ohtake and colleagues (40) showing that decreases in systemic arterial pressure in response to nitrite are attenuated by cyanamide in 1-NNAME-treated rats. The observation that responses to sodium nitrite and GTN are attenuated when responses to the NO donor SNP are not altered provide support for the hypothesis that ALDH2 plays a role in the bioactivation of nitrite and GTN in the pulmonary and systemic vascular beds. The effect of acetaldehyde, chloral hydrate, and disulfiram, agents that have been reported to inhibit ALDH2 (10), on decreases in systemic arterial pressure in response to intravenous injections of GTN were investigated, and the results of these experiments show that responses to GTN are attenuated by these agents.

Recent studies provide support for the hypothesis that the nitrite anion represents a storage form of NO that can have therapeutic effects (12, 15, 19, 27). Although nitrite was believed to be an inactive end product of NO metabolism that reflects endothelial function, the vasodilator actions of nitrite were recognized as early as 1867 by Brunton (5) who used amyl nitrite to treat angina. Cardiovascular responses to sodium nitrite have been investigated by Weiss and coworkers (41–43) in the last century and by Gladwin and coworkers in recent times (12, 14, 19). The ability of sodium nitrite to relax isolated arteries was first reported by Furchgott and Bhadra-kom (18) in 1953, and the effect of nitrite on soluble guanylyl cyclase activity and cGMP levels was documented by Ignarro et al. (21, 25, 26) and by Murad and coworkers (1, 36, 37). The mechanism by which nitrite is reduced to NO in the rat is uncertain, but the reduction of inorganic nitrite to NO can be mediated by enzymatic mechanisms and by nonenzymatic disproportionation at low pH (12, 13, 16, 19, 22, 27, 35). Moreover, it has also been reported that vasodilator responses to sodium nitrite can be attenuated by allopurinol, suggesting that XOR can play a role in bioactivation of nitrite (7, 20, 40). The results of the present study show that vasodilator responses to sodium nitrite are inhibited by cyanamide. These data suggest that both XOR and ALDH2 can act to reduce nitrite to vasoactive NO in the rat (7, 20, 40).

GTN has been used in the treatment of heart failure and relieves the symptoms of pulmonary congestion (6, 33, 44). The results of the present study in the rat and previous studies in the dog show that GTN has significant vasodilator activity in the pulmonary vascular bed and that pulmonary vasodilator responses are enhanced when vasoconstrictor tone is increased (28). In addition, studies in the intact-chest dog under constant flow conditions show that decreases in pulmonary (lobar) arterial pressure in response to intravenous injections of GTN are associated with decreases in small intrapulmonary vein pressure (28). These data suggest that GTN would be expected to decrease capillary hydrostatic pressure and reduce fluid leak in the lung. The results of the present study extend results of previous studies by showing that ALDH2 plays an important role in the bioactivation of GTN in the pulmonary vascular bed of the rat (28, 29).

In summary, the results of the present study show that GTN and sodium nitrite decrease pulmonary and systemic arterial pressures and that vasodilatory responses are attenuated by cyanamide, an inhibitor of ALDH2. Responses to sodium nitrite were attenuated by allopurinol, whereas the XOR inhibitor did not alter responses to GTN. In terms of relative vasodilator activity, GTN was ~1,000-fold more potent than sodium nitrite in the intact-chest rat and suggests that, if all three nitrate groups were reduced to NO, this could not account for the vasodilator activity of GTN. These data are consistent with the hypothesis that ALDH2 plays an important role in the bioactivation of GTN and nitrite. These data suggest that the reduction of nitrite to vasoactive NO or any other metabolic transformation of nitrite is not involved in mediating vasodilator responses to GTN in the pulmonary and systemic vascular beds in the intact-chest rat.

GRANTS
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DISCLOSURES
No conflicts of interest are declared by the authors.

REFERENCES
H826  CARDIOVASCULAR RESPONSES TO GLYCERYL TRINITRATE AND SODIUM NITRITE


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