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Analysis of Responses to Glyceryl Trinitrate and Sodium Nitrite in the Intact Chest Rat

Bobby D. Nossaman, M.D.^{1,2}, Edward A. Pankey, M.D.¹, Adeleke R. Badejo Jr., Ph.D.¹, David B. Casey, M.D.¹, Satvika Uppu, B.S.³, Subramanyam N. Murthy, Ph.D.¹, and Philip J. Kadowitz, Ph.D.¹

¹Department of Pharmacology, Tulane University School of Medicine, 1430 Tulane Avenue, New Orleans, Louisiana 70112-2699 USA

²Department of Anesthesiology, Critical Care Medicine Section, Ochsner Medical Center, 1514 Jefferson Highway, New Orleans, Louisiana 70121 USA

³Department of Chemistry, Southern University, Baton Rouge, Louisiana 70803 USA

Abstract

Responses to glyceryl trinitrate/nitroglycerin (GTN), S-nitrosoglutathione (GSNO), and sodium nitrite were compared in the intact chest rat. The iv injections of GTN, sodium nitrite, and GSNO produced dose-dependent decreases in pulmonary and systemic arterial pressures. In as much as cardiac output was not reduced, the decreases in pulmonary and systemic arterial pressures indicate that GTN, sodium nitrite, and GSNO have significant vasodilator activity in the pulmonary and systemic vascular beds in the rat. Responses to GTN were attenuated by cyanamide, but not allopurinol, whereas responses to nitrite formed by the metabolism of GTN were attenuated by allopurinol and cyanamide. The results with allopurinol and cyanamide suggest that only mitochondrial aldehyde dehydrogenase is involved in the bioactivation of GTN, sodium nitrite, and GSNO, whereas both pathways are involved in the bioactivation of nitrite anion in the intact rat. The comparison of vasodilator activity indicates that GSNO and GTN are more than 1000 fold more potent than sodium nitrite in decreasing pulmonary and systemic arterial pressures in the rat. Following administration of 1H-[1,2,4]-oxadiazolo[4,3-]quinoxaline-1-one (ODQ), responses to GTN were significantly attenuated, indicating that responses are mediated by the activation of soluble guanylyl cyclase. These data suggest that the reduction of nitrite to nitric oxide formed from the metabolism of GTN, cannot account for the vasodilator activity of GTN in the intact rat and that another mechanism; perhaps the formation of an S-NO, may mediate the vasodilator response to GTN in this species.

Keywords

Mitochondrial aldehyde dehydrogenase; Xanthine oxidoreductase; Nitric oxide; S-nitrosothiols; Glyceryl trinitrate/nitroglycerin; Sodium nitrite; Sodium nitroprusside; Allopurinol; Cyanamide; L-NAME

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Corresponding Author: Philip J. Kadowitz, Ph.D., Department of Pharmacology, SL83, Tulane University School of Medicine, 1430 Tulane Avenue, New Orleans, Louisiana 70112-2699 USA, Phone: 504 988-2634, Fax: 504 988-5283, pkadowi@tulane.edu.

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Introduction

Glyceryl trinitrate/nitroglycerin (GTN) and to a lesser extent, nitrite of amyl, have been used in the treatment of angina and heart failure since the latter part of the nineteenth century [1; 2; 3]. The bioactivation of GTN in vascular smooth muscle is hypothesized to involve the formation of nitric oxide (NO) or an S-nitrosothiol (S-NO) from inorganic nitrite that activates soluble guanylyl cyclase (sGC) increasing cGMP levels and promoting vasodilation [4; 5; 6; 7; 8; 9]. In arterial smooth muscle preparations, the activation of sGC by GTN in biochemical studies has been shown to require the addition of thiols and it has been reported that the release of NO is difficult to detect by a number of methods when GTN is added to vascular smooth muscle preparations [10; 11; 12; 13; 14; 15; 16]. There is evidence in the literature showing that the bioactivation of GTN is catalyzed by mitochondrial aldehyde dehydrogenase (ALDH2) [17; 18; 19]. Stamler and coworkers reported that ALDH2 denitrates GTN to 1,2-glyceryl dinitrate (GDN), and inorganic nitrite and that nitrite is converted to a metabolite that activates sGC and promotes vasodilation [19]. Moreover, studies by Furchgott, Ignarro, et. al., and others have shown that large doses of nitrite are needed to relax vascular smooth muscle and increase cGMP levels [5; 6; 20; 21]. In addition, there is little information on the mechanism by which inorganic nitrite is reduced to vasoactive NO in the pulmonary and systemic vascular beds [22; 23; 24]. The mechanism by which GTN decreases pulmonary and systemic arterial pressures was investigated in experiments with cyanamide, allopurinol, and 1H-[1,2,4]-oxadizaolo[4,3-]quinoxaline-1-one (ODQ). ODQ inhibits sGC by oxidizing the heme iron on the enzyme making it insensitive to NO [25; 26; 27; 28]. Results of recent studies show that responses to GTN are attenuated by cyanamide, but that allopurinol had no significant effect in the pulmonary and systemic vascular beds of the intact rat [29]. It has also been reported that ODQ attenuates vasodilator responses to the NO donor, sodium nitroprusside, in the rat [30]. The purpose of the present study was to compare cardiovascular and pulmonary responses to GTN, sodium nitrite, and to the S-nitrosothiol, S-nitrosoglutathione (GSNO), in the intact chest rat and provide information on the mechanism of the bioactivation of these vasodilator agents. These results show that responses to GTN, sodium nitrite, and GSNO are attenuated by ODQ and suggest that the xanthine oxidoreductase (XOR) catalyzed conversion of GTN to vasoactive NO cannot account for the vasodilator activity of GTN in the intact chest rat. These results suggest that an alternate mechanism involving the formation of another chemical species, perhaps an S-NO, may mediate the activation of sGC and the vasodilator response to GTN 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 Rivers) weighting 280–420 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 ip in order 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 iv injections and infusions of agents. For pulmonary arterial pressure measurement, a specially designed 3F single lumen catheter with a curved tip and with radio-opaque marker was passed from the right jugular vein into the main pulmonary artery under fluoroscopic guidance (Picker-Surveyor Fluoroscope) as previously described [31]. 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 PC. Cardiac output was measured by the thermodilution technique with a Cardiamax II computer (Columbus Instruments). A known volume (0.2 ml) of room temperature 0.9% NaCl solution was injected into the jugular vein catheter with its tip near the right atrium and changes in blood temperature were detected by a 1.5F 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 series of experiments, the dose-response curves for the vasodilator agents, GTN, sodium nitrite, and GSNO were compared in the intact chest rat under baseline tone conditions.

In the next series of experiments, the effect of the ALDH2 inhibitor, cyanamide, 25 mg/kg iv, and the XOR inhibitor, allopurinol, 25 mg/kg iv, on responses to iv injections of GTN and sodium nitrite (Sigma-Aldrich) were investigated and compared in the pulmonary and systemic vascular beds. Allopurinol and cyanamide (Sigma-Aldrich) were dissolved in 0.9% NaCl with the addition of small amounts of 0.1N NaOH.

In the final series of experiments, the effects of the sGC inhibitor, 1H-[1,2,4]-oxadiazolo[4,3-*q*]quinoxaline-1-one, ODQ, 5 mg/kg iv, on responses to GTN, sodium nitrite, and GSNO were investigated [32; 33]. ODQ inhibits sGC by oxidizing the heme iron on sGC rendering the enzyme insensitive to NO [25; 26; 27; 28]. The dose of ODQ was determined in previous studies [34; 35].

The hemodynamic data are expressed as mean $\pm$ SE and were analyzed using ANOVA for repeated measures. The criterion used for statistical significance was $P < 0.05$.

Results

GTN has been used in the medical management of angina and heart failure for over 140 years [1; 2; 3]. However, the mechanism by which GTN relaxes vascular smooth muscle remains uncertain [10; 11; 12; 13; 14; 15; 16]. Recently Stamler and coworkers reported that vascular ALDH2 catalyzed the conversion of GTN to 1,2-GDN and nitrite anion that is reduced to NO [19]. The localization of ALDH2 in the liver is mitochondrial [36], however, recent studies have shown that the localization of the enzyme is also cytosolic in vascular smooth muscle [37]. The main products formed from GTN metabolism are 1,2-GDN and inorganic nitrite with the formation of smaller amounts of 1,3-GDN [19]. However, the mechanism by which nitrite is converted to a substance that activates sGC and increases cGMP levels is still uncertain [22; 23; 24]. As previously reported and shown in figure 1, both sodium nitrite and GTN have significant vasodilator activity in the pulmonary and systemic vascular beds, however GTN is more potent than sodium nitrite by more than 3 orders of magnitude (Fig. 1).

Although GTN and nitrite of amyl have been used in the treatment of angina and heart failure for many years [1; 2; 3], the mechanism of action is unknown. The smooth muscle relaxing effects of inorganic nitrite were first demonstrated in 1953 by Furchgott and Bhadrakom and the effects of nitrite and GTN on sGC activity and cGMP levels were reported by Ignarro and coworkers [5; 6; 20; 21]. The studies of Ignarro and colleagues show that added thiols were needed for the activity of GTN in biochemical studies in vascular smooth muscle preparations and the hypothesis that S-nitrosothiols (S-NOs) were formed was suggested [5; 21]. Although GTN was 1000 fold more potent than sodium nitrite in decreasing pulmonary and systemic arterial pressures, the data in figure 1 indicate that the GSNO has very potent vasodilator activity approaching that of GTN in the pulmonary and systemic vascular beds in the intact rat. The results in figure 1 show that the dose-response

curve for GSNO is very close to the curve for GTN on the abscissa and that GSNO is approximately 1000 fold more potent than sodium nitrite in decreasing pulmonary and systemic arterial pressure (Fig. 1).

The time course of decreases in pressure in response to GTN, sodium nitrite, and GSNO is shown in figure 2 where it can be seen that there is more similarity when comparing responses of GSNO to GTN than GSNO to sodium nitrite.

Although it has been reported that ALDH2 is involved in the bioactivation of GTN, less is known of the mechanisms involved in the bioactivation of inorganic nitrite [10; 11; 12; 13; 14; 15; 16]. The observation that responses to nitrite could be attenuated by cyanamide, an ALDH2 inhibitor, and by allopurinol, an XOR inhibitor, in L-NAME treated rats was first reported by Ohtake et al in 2006 [38]. These observations were confirmed in our laboratory and the results in figure 3 are similar to previous data showing that vasodilator responses to sodium nitrite were attenuated by cyanamide and by allopurinol suggesting that both ALDH2 and XOR were involved in the bioactivation of inorganic nitrite in the intact rat [29]. In addition as previously reported, cyanamide attenuated responses to GTN, whereas allopurinol had no significant effect on vasodilator responses to GTN indicating a difference in the bioactivation of GTN and inorganic nitrite [29].

The mechanism by which GTN decreases pulmonary and systemic arterial pressures was investigated in experiments with ODQ. ODQ inhibits sGC by oxidizing the heme iron on the enzyme rendering it insensitive to NO [25; 26; 27; 28]. ODQ has been shown to attenuate vasodilator responses to sodium nitroprusside in the intact rat [30]. The effects of ODQ on responses to GTN, sodium nitrite, and GSNO were investigated and these data are summarized in figure 4. Following administration of ODQ in a dose of 5 mg/kg iv, the decreases in pulmonary and systemic arterial pressures in response to iv injections of GTN, sodium nitrite, and GSNO were significantly attenuated (Fig. 4). These data suggest that decreases in pulmonary and systemic arterial pressures in response to GTN, sodium nitrite, and GSNO are mediated by the activation of sGC in the intact rat. The mechanism by which GTN activates sGC is uncertain [5; 21; 39]. Inorganic nitrite, formed from GTN by ALDH2 [19], can be reduced to vasoactive NO or a NO-like substance or product that activates sGC [21; 29; 40]. However, recently published potency data suggest that nitrite cannot account for the vasodilator activity of GTN in the rat [29]. It is therefore possible that an unknown product or intermediate formed from GTN is more potent than nitrite in mediating the vasodilator response to GTN [29]. The chemical identity of the metabolite that is formed from GTN that activates sGC is unknown at the present time, but the present results suggest that an S-NO-like intermediate may play a role based on the studies of Ignarro and coworkers and the observations that the bioactivation of GTN and nitrite are mediated by different mechanisms and that an S-NO compound has similar activity to GTN in the pulmonary and systemic vascular beds in the intact chest rat [5; 21; 41]. It is therefore possible that a compound with vasodilator activity similar to GSNO may play a role in mediating the vasodilator response to GTN in the rat.

Discussion

GTN has been used in the treatment of angina and heart failure for more than 140 years [1]. However, the mechanism by which GTN produces its beneficial effect is uncertain [10; 11; 12; 42; 43]. It has recently been shown that ALDH2 is involved in the bioactivation of GTN [19]. ALDH2 metabolizes GTN to 1,2-GDN and inorganic nitrite, which is converted to a product or intermediate that activates sGC, increases cGMP levels and promotes vasodilation [4; 5; 6; 7; 8; 9; 19]. In agreement with previous studies, the results of the present study show that GTN and sodium nitrite have significant vasodilator activity in the

pulmonary and systemic vascular beds of the intact chest rat. The present results show that pulmonary and systemic vasodilator responses to GTN and sodium nitrite are attenuated by cyanamide, an inhibitor of ALDH2 [44]. These results are consistent with the results of Chen and Stamler and provide support for the hypothesis that ALDH2 plays an important role in the bioactivation of GTN [19]. However, in potency studies when responses to GTN and sodium nitrite are compared and doses are expressed on an nmol/kg basis, a marked difference in activity is apparent with GTN being approximately 3 orders of magnitude more potent than sodium nitrite. It has been reported that GTN is metabolized to GDN and nitrite [19]. Moreover, if GTN were metabolized to glycerol and all three nitrite groups were reduced to NO, there would still be a potency difference of approximately 1000. Although it has been reported that nitrite can be metabolized to vasoactive NO when GTN is added to vascular smooth muscle preparations, it has proven difficult, if not impossible, to detect the formation of NO [10; 11; 12; 13; 14; 15; 16]. The failure to detect the formation of NO and the low potency of nitrite in producing vasodilation indicates that the simple reduction of nitrite to vasoactive NO cannot account for the vasodilator activity of GTN and another mechanism or intermediate may be involved. The studies of Ignarro and coworkers show that when GTN is added to vascular smooth muscle preparations, added thiols are needed for the activation of sGC and increases in cGMP levels [5; 21; 41; 45]. Ignarro and coworkers hypothesized that the activation of sGC by GTN requires the formation of an S-NO-like compound or intermediates [5; 21; 41s].

In the present study, vasodilator responses to the S-NO, GSNO, was compared with responses to GTN and sodium nitrite. The results of these studies show that all three agents have significant vasodilator activity in the pulmonary and systemic vascular beds. Moreover when dose-response curves for GTN and GSNO were compared, it was observed that these agents had similar potency and that GSNO was approximately 1000 fold more potent than sodium nitrite. In addition, there are some similar properties when comparing responses to GTN and GSNO. These data can be interpreted to provide support for the hypothesis that an S-NO-like intermediate, if formed, would have sufficient vasodilator activity to account for the response to GTN. However, the formation of an S-NO and the chemical nature of the S-NO that could hypothetically be formed in the reaction of ALDH2 and GTN has not been identified. The precise pathway for the bioactivation of GTN is uncertain. It is well established that GTN activates sGC and that ALDH2 has an important role in the bioactivation of GTN [5; 19; 21; 41]. ALDH2 catalyzes the conversion of GTN to GDN and nitrite that is converted to an NO-like intermediate that increases cGMP and promotes vasodilation [5; 19; 21; 41]. It has recently been reported that GTN is bioactivated by vascular ALDH2 that is cytosolic in localization [46]. However the intermediate that activates sGC is still unknown and there is good evidence in the literature and in the present study to indicate that the formation of NO from nitrite does not mediate the response to GTN [11; 47].

In regard to the mechanism of action of nitrite, the results of the present study with ODQ, an agent that oxidizes the heme iron on sGC and decreases the response to NO, show that ODQ attenuates vasodilator responses to GTN, sodium nitrite, and GSNO in the pulmonary and systemic vascular beds of the intact rat suggesting that responses are mediated by the activation of sGC.

In summary, the results of the present study show that GTN and the GTN metabolite, inorganic nitrite, have significant vasodilator activity in the pulmonary and systemic vascular beds and that GTN is approximately 3 orders of magnitude more potent than sodium nitrite. The experiments with ODQ, an agent that oxidizes the heme iron on sGC rendering it insensitive to NO, suggest that vasodilator responses to GTN, sodium nitrite, and GSNO are mediated by the activation of sGC in the intact rat. Responses to GTN are

inhibited by cyanamide, but not by allopurinol, suggesting they are mediated by ALDH2 and that XOR does not have significant role in the bioactivation of GTN. In contrast, responses to nitrite are attenuated by cyanamide and allopurinol suggesting that both ALDH2 and XOR can mediate the bioactivation of inorganic nitrite and that the bioactivation of GTN and nitrite are mediated by different mechanisms in the pulmonary and systemic vascular beds in the rat. The present results indicate that responses to GSNO are similar to GTN in potency and in some respects are similar in time course. The present results are consistent with the hypothesis that inorganic nitrite cannot account for the vasodilator activity of GTN and that another molecule or intermediate, perhaps an S-NO, may be involved.

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Abbreviations

GTN	glyceryl trinitrate/nitroglycerin
GDN	glyceryl dinitrate
NO	nitric oxide
S-NO	S-nitrosothiol
GSNO	S-nitrosoglutathione
sGC	soluble guanylyl cyclase
ALDH2	mitochondrial aldehyde dehydrogenase
XOR	xanthine oxidoreductase
ODQ	1H-[1,2,4]-oxadiazolo[4,3-]quinoxaline-1-one
L-NAME	N(G)-nitro-L-arginine methyl ester hydrochloride

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- Nitroglycerin/glyceryl trinitrate (GTN) has been used for over 140 years
- Mechanism of action remains unknown
- GTN metabolizes to 1,2-GDN and nitrite anion
- Nitrite anion derived from GTN does not produce nitric oxide

Figure 1.

Dose-response curves comparing decreases in pulmonary and systemic arterial pressures in response to iv injections of glyceryl trinitrate (GTN), S-nitrosoglutathione (GSNO), and sodium nitrite under baseline tone conditions. n = number of experiments. * $P < 0.05$.

Figure 2 A **Figure 2 B**

Figure 2 C **Figure 2.**

Records from an experiment showing the time course of decreases in pressure in response to an iv injection of (A) *S*-nitrosoglutathione, (B) glyceryl trinitrate (GTN), and (C) sodium nitrite under baseline tone conditions.

Figure 3.

Bar graphs showing changes in pulmonary and systemic arterial pressures and cardiac output in response to iv injections of sodium nitrite (left panels) and glyceryl trinitrate (GTN) (right panels) before and after administration of cyanamide (25 mg/kg iv) or before and after administration of allopurinol (25 mg/kg iv). n = number of experiments. *P < 0.05.

Figure 4.

Bar graphs showing changes in pulmonary and systemic arterial pressures in response to iv injections of glyceryl trinitrate (GTN), sodium nitrite, and S-nitrosoglutathione (GSNO) before and after administration of ODQ (5 mg/kg iv). n = number of experiments. *P < 0.05.