Nitroxyl donors retain their depressor effects in hypertension

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Irvine JC, Ravi RM, Kemp-Harper BK, Widdop RE. Nitroxyl donors retain their depressor effects in hypertension. Am J Physiol Heart Circ Physiol 305: H939–H945, 2013. First published July 12, 2013; doi:10.1152/ajpheart.00630.2012.—Nitroxyl (HNO), the redox congener of nitric oxide, has numerous vasoprotective actions including an ability to induce vasodilatation and inhibit platelet aggregation. Given HNO is resistant to scavenging by superoxide and does not develop tolerance, we hypothesised that HNO would retain its in vivo vasodilatory action in the setting of hypertension. The in vitro and in vivo vasodilator properties of the HNO donors Angeli’s salt (AS) and isopropylamine/NONOate (IPA/NO) were compared with the NO’ donor diethylamine/NONOate (DEA/NO) in spontaneously hypertensive rats (SHR) and normotensive [Wistar-Kyoto (WKY) rats]. AS (10, 50, and 200 μg/kg), IPA/NO (10, 50, and 200 μg/kg), and DEA/NO (1, 5, and 20 μg/kg) caused dose-dependent depressor responses in conscious WKY rats of similar magnitude. Depressor responses to AS and IPA/NO were significantly attenuated (P < 0.01) after infusion of the HNO scavenger N-acetyl-l-cysteine (NAC), confirming that AS and IPA/NO function as HNO donors in vivo. In contrast, responses to DEA/NO were unchanged following NAC infusion. Depressor responses to AS and IPA/NO in conscious SHR retained their sensitivity to the inhibitory effects of NAC (P < 0.01), yet those to DEA/NO in SHR were significantly (P < 0.05) enhanced following NAC infusion. Importantly, depressor responses to AS, IPA/NO, and DEA/NO were preserved in hypertension and vasorelaxation to AS and DEA/NO, in isolated aorta, unchanged in SHR as compared with WKY rats. This study has shown for the first time that HNO donors exert antihypertensive effects in vivo and may, therefore, offer a therapeutic alternative to traditional nitrovasodilators in the treatment of cardiovascular disorders such as hypertension.

THE VASOACTIVE PROPERTIES of nitric oxide (NO) donors such as glyceryl trinitrate (GTN) are well recognized, and nitrovasodilators have been used clinically to treat disorders such as angina, heart failure, and hypertension for more than 100 years (26). Yet NO can exist in different redox states, and although the physiological activity of NO has been traditionally attributed to its uncharged form (NO’), it is becoming increasingly apparent that nitroxyl (HNO), the reduced and protonated congener of NO’, exhibits distinct pharmacology from NO’ (13) and may itself have significant therapeutic potential.

The unique actions of HNO, as compared with its redox sibling, are readily apparent in the cardiovascular system. Thus, unlike NO’, HNO increases myocardial contractility (via direct thiol interaction) (28, 29) and is protective in the setting of acute experimental heart failure (23, 28). The positive cardiac inotropic actions of HNO are complemented by its ability to cause vasodilatation and unload the heart (19, 24, 28).

Indeed HNO serves as a potent vasodilator both in vitro (1, 5, 7–11, 34) and in vivo (12, 19, 23, 24). HNO donors such as Angeli’s salt (AS; sodium trioxodinitrate) and isopropylamine NONOate (IPA/NO) exhibit vasorelaxant activity in both large isolated conduit (5, 8, 11) and small resistance (1, 10) arteries. Such effects, like NO’, are mediated predominantly via activation of soluble guanylyl cyclase (sGC) (10, 11) and the resulting accumulation of cGMP (8, 11). However, in contrast with NO’, HNO can also target distinct vascular signaling pathways including voltage-gated K+ (Kv) channels (1, 7, 10, 34), ATP-sensitive K+ channels (KATP) (7), and calcitonin gene-related peptide (CGRP) release (7).

Moreover, HNO has been shown to elicit a vasodepressor effect in vivo, lowering blood pressure in anesthetized rabbits (18) and cats (6), and conscious dogs (23, 24) and rats (12). In dogs, at least, HNO appears to serve as a preferential venuodilator in vivo and does not elevate plasma cGMP (24). However, given circulating levels of cGMP may not reflect changes at the level of the vascular smooth muscle, a clear role for cGMP in the in vivo vasodilatory actions of HNO remains to be determined. Importantly, we have shown that, unlike clinically used nitrovasodilators such as GTN, vascular tolerance to HNO donors does not develop either in vivo (5, 11) or in vivo (12) with continued use. In addition, the vasodilatory capacity of HNO is accompanied by an ability of this nitrogen oxide to inhibit platelet aggregation (5, 21) and limit vascular smooth muscle proliferation (30).

Collectively, the cardioprotective and vasoprotective actions of HNO indicate that HNO donors may serve as an alternative to traditional nitrovasodilators (i.e., GTN) in the treatment of heart failure and vascular disorders such as angina and hypertension. However, before the therapeutic potential of HNO donors can be fully realized, it is important to determine whether their hemodynamic actions are sustained in the setting of disease. Given HNO, unlike NO’, is resistant to scavenging by superoxide (O2·-) (20), does not develop tolerance (11, 12), and can target distinct signaling pathways in the myocardium (28) and vasculature (4), and its bioavailability may be augmented in the face of disease-associated thiol depletion, it may be predicted that the efficacy of HNO would be preserved under pathophysiological conditions. Although not studied directly, the vasodepressor actions of HNO (24) appear to be sustained in the setting of acute experimental heart failure (23).

In addition, recent studies in isolated arteries from hypercholesterolemic (5) and ANG II-induced hypertensive mice (33) have shown that the vasorelaxant actions of HNO are preserved in these diseases, which are associated with elevated vascular O2·- generation and reduced endogenous NO’ bioavailability. Whether such observations translate to the in vivo situation is unclear.

The present study aimed to determine, for the first time, whether the in vivo vasodepressor actions of HNO are maintained in hypertension, a disease state associated with oxidative...
stress, depletion of cellular thiols, and an attenuation of the NO-sGC-cGMP signaling pathway (16). We predicted that the vasodilatory response to HNO donors may be retained, if not potentiated, in an animal model of hypertension. Accordingly, the vasodepressor and vasorelaxant effects of the HNO donors AS and IPA/NO (19) and the NO\(^{-}\) donor diethylamine/NONOate (DEA/NO) were compared in conscious spontaneously hypertensive rats (SHR) and age-matched normotensive Wistar-Kyoto (WKY) rats and in isolated aorta from these animals, respectively.

MATERIALS AND METHODS

Animals. This study was approved by the Monash University Animal Ethics Committee, Monash University, Australia, and conforms to the Guide for the Care and Use of Laboratory Animals published by the National Institutes of Health (NIH Publication No. 85–23, revised 1996). Male WKY rats (15–17 wk of age, 300–350 g, \(n = 17\)) and male SHR (15–17 wk of age, 300–350 g, \(n = 17\)) were housed in standard rat cages at 20 ± 3 °C, with a 12-h:12-h day/night cycle. Food and water were available ad libitum.

In vitro studies. Animals were euthanized by means of stunning and cervical dislocation. The thoracic aorta was isolated, cleaned of fat and connective tissue, and cut into 5-mm ring preparations, leaving the endothelium intact. The vessels were then mounted in 20-ml organ baths, and isometric tension was measured. Data were captured using the CVMS data acquisition system (World Precision Instruments). Vessels were maintained in physiological Krebs’ solution (composition in mmol/l) of 119 NaCl, 4.7 KCl, 1.17 MgSO\(_4\), 25 NaHCO\(_3\), 1.18 KH\(_2\)PO\(_4\), 2.5 CaCl\(_2\), 11.1 glucose, and 0.026 EDTA at 37°C and bubbled continuously with carbogen (95% O\(_2\)-5% CO\(_2\)). After a 30-min equilibration period, vessels were stretched to an optimal passive tension of 2 g.

Vessels were maximally contracted with a K\(^{+}\)-depolarizing solution [composition of potassium physiological salt solution (in mmol/l): 123 KCl, 1.17 MgSO\(_4\), 2.37 KH\(_2\)PO\(_4\), 2.5 CaCl\(_2\), 11.1 glucose, and 0.026 EDTA]. Subsequently, responses to vasorelaxants were examined in vessels precontracted to ~50% potassium physiological salt solution with U46619 (0.3 nmol/l) and titrated concentrations of 0.026 EDTA. After a 30-min equilibration period, vessels were stretched to an optimal passive tension of 2 g.

In vivo studies. Rats were anesthetized with 60 mg/kg ip pentobarbitone, supplemented as required, and catheters were inserted into the right carotid artery and right jugular vein for direct blood pressure measurement and drug administration, respectively. Each catheter was externalized through the back in the neck region and secured by a custom-made harness, and rats were then housed individually. Approximately 24 h after surgery, the carotid artery was connected to a pressure transducer (Gould) attached to a MacLab-8 data acquisition system (ADInstruments). Mean arterial pressure (MAP) and heart rate (HR) were derived from the phasic blood pressure signal. All experiments were performed in conscious unrestrained rats.

Infusion with HNO scavenger N-acetyl-l-cysteine. Rats were randomly assigned to test the depressor effects of bolus intravenous administration of each of the HNO donors, AS (10, 50, and 200 \(\mu\)g/kg) and IPA/NO (10, 50, and 200 \(\mu\)g/kg), and the NO\(^{-}\) donor DEA/NO (1, 5, and 20 \(\mu\)g/kg) over the following three experimental days. On any given day, a three-point dose-response curve was constructed using one of these nitrovasodilators. Thereafter, a 60-min infusion of the HNO scavenger N-acetyl-l-cysteine (NAC; 6.7 \(\mu\)mol/kg \(\cdot\)min \(^{-}\) (12, 23) commenced, and the three-point dose-response curve was repeated in the presence of this infusion (each animal served as its own control). Each animal received only one nitrovasodilator per day.

Sympathetic autonomic blockade. On day 4, submaximal bolus doses of AS (50 \(\mu\)g/kg), IPA/NO (50 \(\mu\)g/kg), and DEA/NO (5 \(\mu\)g/kg) were administered following an intravenous infusion of the \(\beta\)-adrenoceptor antagonist propranolol (1 mg/kg) to determine whether any of the nitrovasodilators were having a direct chronotropic effect.

Data and statistical analysis. Relaxation responses are expressed as a percent reversal of cirazoline precontraction. Individual relaxation curves were fitted to a sigmoidal logistic equation (Graphpad Prism 5.0), and pEC\(_{50}\) values (concentration of agonist giving a 50% relaxation) were calculated and expressed as -logmol/l. Differences between mean pEC\(_{50}\) and maximum relaxation (\(R_{max}\)) values were tested using either a Student’s paired t-test or one-way ANOVA, followed, where appropriate, with a Dunnett’s modified t-test.

In vivo depressor and HR responses are expressed as the maximum change from baseline, and statistical analysis was performed using Student’s paired or unpaired t-test, or two-way ANOVA with repeated measures as appropriate (GraphPad Prism 5.0).

All data are expressed as means ± SE. \(P < 0.05\) was accepted as statistically significant.

Drugs. Drugs and their sources were AS (sodium trioxodinitrate), diethylamine NONOate [Diethylaminommonium (Z)-1-((N,N-diethylamino)diazen-1-ium-1,2-diolate)], U46619 [9,11-dideoxy-9a,11a-methanoepoxy-prosta-5Z,13E-dien-1-oic acid] (Sapphire Bioscience, Crows Nest, NSW, Australia); cirazoline hydrochloride [2-[2-(Cyclopentyloxy)methyl]-4,5-dihydro-H-imidazole], NAC (Sigma-Aldrich, St. Louis, MO); isopropylamine NONOate [sodium (N-isopropylamino)diazen-1-ium-1,2-diolate] (Toronto Research Chemicals, Ontario, Canada). Stock solutions of AS, IPA/NO, and DEA/NO (10 mmol/l) were constituted in 0.01 mol/l NaOH, as were all subsequent dilutions. Stock solutions of U46619 (10 mmol/l) were made up in absolute ethanol (EtOH). All subsequent dilutions of stock solutions were in distilled water. All other drugs were made up in distilled water, and all dilutions were prepared fresh daily.

RESULTS

 Vasorelaxant responses to HNO preserved in hypertension. The HNO donor AS (pEC\(_{50}\) = 6.77 ± 0.07; \(R_{max}\) = 92.1 ± 1.1%) and NO\(^{-}\) donor DEA/NO (pEC\(_{50}\) = 7.42 ± 0.09; \(R_{max}\) = 95.5 ± 1.0%) caused concentration-dependent vasorelaxation of isolated aorta from WKY rats (Fig. 1). We have previously confirmed the contribution of HNO and NO\(^{-}\) to these responses via the sensitivity of AS-mediated vasorelaxation to the HNO scavenger L-cysteine and DEA/NO-mediated vasorelaxation to the NO\(^{-}\) scavenger carboxy-PTIO (11). Vasorelaxation to AS and DEA/NO were unchanged in aorta from SHR as compared with WKY rats (Fig. 1, A and B).

Depressor responses to AS, IPA/NO, and DEA/NO preserved in hypertension. Basal MAP in conscious SHR (172 ± 4 mmHg; \(n = 7\)) was significantly higher than basal MAP in WKY rats (120 ± 1 mmHg; \(n = 7\); \(P < 0.001\)), whereas there was no significant difference in basal HR between SHR (347 ± 9 beats/min; \(n = 7\)) and WKY rats (350 ± 11 beats/min; \(n = 7\)). Moreover, none of the daily treatments affected basal MAP or HR on subsequent days (Table 1).

Bolus intravenous administration of either the HNO donors AS (10, 50, and 200 \(\mu\)g/kg) and IPA/NO (10, 50, and 200 \(\mu\)g/kg) or the NO\(^{-}\) donor DEA/NO (1, 5, and 20 \(\mu\)g/kg) caused dose-dependent transient decreases in MAP in conscious WKY rats and SHR (Fig. 2) that were accompanied by increases in HR (Table 2). In both strains, DEA/NO was the most potent depressor agent with a rank order of potency of DEA/NO >
AS = IPA/NO. Vasodepressor responses to AS, IPA/NO, and DEA/NO were unchanged in conscious SHR as compared with WKY rats (Fig. 2).

In WKY rats and SHR, depressor responses to AS, IPA/NO, and DEA/NO were unaffected by the \(-\text{adrenoceptor antagonist propranolol (Table 2), which itself had no effect on basal MAP (data not shown). However, infusion with propranolol significantly (} P < 0.05, both \( n = 4 \) lowered HR in WKY rats and SHR by 33 \( \pm 7 \) and 45 \( \pm 12 \) beats/min, respectively, and attenuated the nitrovasodilator-induced tachycardia (Table 2), indicative of a baroreflex-mediated increase in HR.

**NAC discriminates between NO˙ and HNO in vivo.** In WKY rats and SHR, dose-dependent depressor responses to AS (\( P < 0.01 \); Fig. 3, A and B) and IPA/NO (\( P < 0.001 \); Fig. 3, C and D) were markedly attenuated following the infusion of the HNO scavenger NAC. NAC did not abolish the response to either AS or IPA/NO with small, but significant (\( P < 0.05 \)), depressor responses (\( \Delta \text{MAP,} \sim 10-15 \text{mmHg} \) observed at

### Table 1. Basal MAP and HR in SHR and WKY rats

<table>
<thead>
<tr>
<th>Experimental Day</th>
<th>1</th>
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<tr>
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<tr>
<td>WKY rats</td>
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<tr>
<td>MAP, mmHg</td>
<td>123 ± 2</td>
<td>121 ± 3</td>
<td>116 ± 2</td>
<td>121 ± 6</td>
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<tr>
<td>HR, beats/min</td>
<td>344 ± 14</td>
<td>352 ± 13</td>
<td>351 ± 19</td>
<td>346 ± 16</td>
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<tr>
<td>SHR</td>
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<tr>
<td>MAP, mmHg</td>
<td>182 ± 5***</td>
<td>166 ± 7***</td>
<td>170 ± 8***</td>
<td>166 ± 4**</td>
</tr>
<tr>
<td>HR, beats/min</td>
<td>336 ± 10</td>
<td>347 ± 10</td>
<td>354 ± 20</td>
<td>348 ± 12</td>
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</table>

Values are means ± SE; \( n = 7 \) to 8 per group. MAP, mean arterial pressure; HR, heart rate. **P < 0.01, ***P < 0.001 spontaneously hypertensive rats (SHR) vs. Wistar-Kyoto (WKY) rats (Student’s unpaired t-test).
Table 2. Effect of the \( \beta \)-blocker propranolol on changes in MAP and HR in response to AS, IPA/NO, and DEA/NO in conscious SHR and WKY rats

<table>
<thead>
<tr>
<th>Nitrovasodilator</th>
<th>AS, 50 ( \mu )g/kg</th>
<th>IPA/NO, 50 ( \mu )g/kg</th>
<th>DEA/NO, 5 ( \mu )g/kg</th>
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<tbody>
<tr>
<td></td>
<td>Control</td>
<td>Propranolol, 1 mg/kg</td>
<td>Control</td>
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</table>
| WKY rats
| \( \Delta \)MAP, mmHg | -17 ± 1 | -24 ± 4 | -26 ± 3 | -30 ± 3 | -26 ± 5 | -19 ± 3 |
| \( \Delta \)HR, beats/min | 60 ± 10 | 19 ± 1* | 78 ± 12 | 19 ± 5* | 79 ± 8 | 19 ± 3** |
| SHR
| \( \Delta \)MAP, mmHg | -19 ± 2 | -25 ± 3 | -23 ± 1 | -26 ± 1 | -20 ± 6 | -23 ± 3 |
| \( \Delta \)HR, beats/min | 52 ± 15 | 8 ± 5* | 67 ± 6 | 13 ± 4** | 64 ± 12 | 11 ± 4* |

Values are means ± SE; \( n = 4 \) per group. AS, Angeli’s salt; IPA/NO, isopropylamine/NONOate; DEA/NO, diethylamine/NONOate. *\( P < 0.05 \), **\( P < 0.01 \) vs. untreated control (Student’s paired \( t \)-test).

doses \( \geq 50 \mu \)g/kg. In contrast, responses to DEA/NO remained unchanged in WKY rats (Fig. 3E) and potentiated in SHR (\( P < 0.05 \); Fig. 3F) in the presence of NAC. NAC itself did not change either basal MAP (\( \Delta = 5 \pm 4 \) mmHg, \( n = 7 \)) or HR (\( \Delta = 1 \pm 16 \) beats/min) in WKY rats but significantly increased basal MAP in SHR (\( \Delta = 20 \pm 9 \) mmHg, \( P < 0.001 \), \( n = 8 \)) with no change in HR (\( \Delta = 16 \pm 26 \) beats/min).

**DISCUSSION**

This study has demonstrated for the first time that the HNO donors AS and IPA/NO retain their in vivo vasodepressor effects in the setting of hypertension. Furthermore, use of the HNO scavenger NAC has confirmed that both AS and IPA/NO retain their identity as HNO donors in vivo under physiological and pathophysiological conditions. With a concomitant ability to lower blood pressure, inhibit platelet aggregation, and increase myocardial contractility, HNO donors may represent a new class of nitrovasodilator for the treatment of vascular disorders.

The study used two HNO donor compounds, AS (Na\(_2\)N\(_2\)O\(_3\)) and IPA/NO (Na[(CH\(_2\)]\(_2\)CHNHN(ONO)]) to establish a class effect of HNO donors upon blood pressure regulation in the

![Fig. 3. Dose-dependent depressor responses induced in conscious WKY rats (left) and SHR (right) by the bolus (intravenous) administration of Angeli’s salt (HNO; \( n = 7 \); A and B), IPA/NO (HNO; \( n = 6 \) to 7; C and D), and DEA/NO (NO\(_\cdot\); \( n = 7 \) to 8; E and F) before (\( \square \), \( \circ \), and \( \triangledown \)) or after (\( \blacksquare \), \( \bullet \), and \( \blacktriangle \)) a 60-min infusion of N-acetyl-l-cysteine (NAC; 6.7 \( \mu \)mol·kg\(^{-1}\)·min\(^{-1}\)). Values are expressed as the maximum change in MAP from baseline and are given as means ± SE. *\( P < 0.05 \), **\( P < 0.01 \), ***\( P < 0.001 \) for response in the presence of NAC vs. untreated control (2-way repeated-measures ANOVA).
setting of normotension and hypertension. Consistent with our previous findings in conscious normotensive rats (12) and those of other investigators in conscious normotensive dogs (19, 24), we found that both AS and IPA/NO caused dose-dependent decreases in blood pressure. To compare the vasoactivity of HNO and NO in vivo, the NO donor DEA/NO was used, which has a similar rate of decomposition as AS and IPA/NO (t1/2, ~2.5 min). Although DEA/NO was found to be a more potent depressor agent in vivo (DEA/NO > IPA/NO = AS), such an observation was indicative of the release of two equivalents of NO' from DEA/NO versus one equivalent of HNO from AS and IPA/NO.

To confirm that HNO mediated the in vivo actions of AS and IPA/NO, the thiol NAC was used. Dose-dependent depressor responses to both AS and IPA/NO were found to be significantly attenuated following infusion of NAC in the conscious WKY rat, yet responses to the NO' donor DEA/NO were unchanged. Thus, our findings, in agreement with previous studies (12, 24, 29), clearly demonstrate that thiols such as NAC can discriminate between the effects of HNO and NO'.

It was next necessary to confirm that IPA/NO and AS retained their ability to donate HNO in the hypertensive setting, given the different oxidative environment existent in this disease state (15) and potential for extracellular oxidation of HNO to NO'. Indeed, dose-dependent depressor responses to both AS and IPA/NO in conscious SHR were significantly attenuated following the infusion with NAC, suggesting that these nitrovasodilators retained their ability to release HNO and there was no significant level of oxidation of HNO to NO' in vivo. As such, this study has, for the first time, established a class effect of HNO donors upon blood pressure regulation under physiological and pathophysiological conditions.

Of note, vasodepressor responses to DEA/NO in SHR were significantly potentiated following NAC infusion, although no change had been observed in the WKY rat (12). These findings are in agreement with previous studies in which NAC infusion has been shown to potentiate vasodilator responses to GTN in the rat (2) and human (22) under pathophysiological conditions. Thus, in disease states such as hypertension, NAC, a thiol donor and antioxidant, may enhance and prolong the activity of NO' via the formation of S-nitrosothiols and/or limiting the scavenging of NO' by reactive oxygen species such as O2'-.

Moreover, these findings have noteworthy clinical implications, suggesting that the efficacy of known NO' donors may be enhanced through the concomitant use of thiol supplemen-
tation in disease states such as hypertension, atherosclerosis, and diabetes, which are characterized by oxidative stress, subsequent thiol depletion, and sGC dysfunction (15, 16).

Interestingly, we also observed that NAC infusion increased basal MAP in conscious SHR, but not WKY rats. Although NAC can interact with a number of signaling molecules to influence the cellular redox milieu, its ability to increase MAP may also suggest that putative endogenous HNO is generated in pathophysiological conditions. Given HNO has recently been shown to serve as an endothelium-derived relaxing factor in the resistance vasculature (1, 34) and biochemical studies have suggested that HNO can be derived from uncoupled NO synthase (NOS) and following oxidation of the NOS intermediates N-hydroxy-L-arginine and hydroxylamine (13), then an augmented endogenous production in the setting of disease could be anticipated. However, it is important to note that although NAC did not alter basal MAP in normotensive WKY rats, we have previously reported a significant increase in MAP in response to NAC in a different cohort of WKY rats (12). As such, NAC appears to have variable effects on basal MAP, and further investigation is required to fully elucidate the role of putative endogenous HNO in the regulation of blood pressure.

Previous studies have shown AS and IPA/NO to have direct positive inotropic effects on the heart (19, 23, 24). To determine whether we could detect a direct chronotropic effect of AS and IPA/NO in our instrumented rats, we tested each nitrovasodilator in the presence of propranolol. However, the concomitant tachycardia to each of the nitrovasodilators was markedly attenuated in the presence of propranolol, indicating that the increased HR responses were baroreceptor reflexly mediated, rather than via any direct cardiac stimulation. By contrast, the depressor responses to AS, IPA/NO, and DEA/NO, in both WKY rats and SHR, were unchanged in the presence of propranolol and thus independent of β-adrenergic stimulation.

With regard to the principle aim of this study, hypertension has been associated with an increase in oxidative stress, thiol depletion, and attenuation of the NO-sGC-cGMP signal transduction pathway (16). This attenuation may stem from a decrease in NO' bioavailability due to scavenging by O2'-- to form peroxynitrite (ONOO') and sGC dysfunction itself (27). Importantly, in contrast with NO', HNO has been shown to be resistant to scavenging by O2'-- (20), can target distinct vascular signaling pathways (Kv channels, KATP channels, and CGRP) (4), does not develop vascular tolerance (5, 11, 12,) and is scavenged by thiols (32), leading us to hypothesize that the HNO signaling pathway may remain effective in the setting of hypertension.

Thus the main finding of this study revealed no change in the depressor activity elicited by the HNO donors AS and IPA/NO in conscious SHR versus WKY rats, confirming that HNO can function as an effective antihypertensive agent. The retained activity of the HNO donors in vivo correlates with our demonstration that the sensitivity of vasorelaxant responses to AS remains unchanged in the isolated aorta of SHR versus WKY rats. Similarly, a recent study by Wyne and colleagues (33) demonstrated preserved HNO-mediated relaxation in isolated aorta from ANG II-treated hypertensive mice. Based upon studies in isolated arteries, we assume that in WKY rats and SHR, the depressor effects of HNO are mediated predominantly via the activation of sGC and the subsequent increase in...
cGMP accumulation (11). Although previous studies have reported that intravenous administration of either IPA/NO or AS does not increase plasma cGMP (19), this may not be reflective of changes at the level of the vasculature.

Interestingly, like the HNO donors, the NO• donor DEA/NO also retained both its in vivo depressor activity and its in vitro relaxant potency in hypertension. Indeed, previous studies in SHR have reported an enhancement of in vivo depressor responses to the NO• donors SNP and MAHMA/ NONOate (17) and a greater increase in cGMP accumulation in response to SNP and GTN in aortic smooth muscle cells (25), compared with age-matched WKY rats. Upon consideration it appears that oxidative stress and dysfunction of the NO•-sGC-cGMP signaling pathway is more prevalent in aged versus adult SHR (14, 27, 35). Thus future studies in aged hypertensive animals may unmask differential effects of HNO versus NO• donors in blood pressure regulation.

In conclusion, this study has clearly demonstrated that the HNO donors AS and IPA/NO retain their vasodepressor activity in the setting of hypertension. Moreover, not only do thiols discriminate between the different redox forms of NO but thioul supplementation may augment the blood pressure lowering action of NO• donors. Furthermore, to our previous demonstration that HNO donors are potent vasorelaxants (7, 10, 11, 34) and resistant to the development of tolerance (11, 12), this study highlights the potential for HNO donors as effective antihypertensive agents. These findings support the growing body of evidence that HNO donors may offer a therapeutic alternative to traditional nitrovasodilators (4, 19, 23, 24) in the treatment of cardiovascular disorders.

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DISCLOSURES

No conflicts of interest, financial or otherwise, are declared by the author(s).

AUTHOR CONTRIBUTIONS


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