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The peroxynitrite product 3-nitro-L-tyrosine attenuates the hemodynamic responses to angiotensin II in vivo

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Abstract

Peroxynitrite is a potent oxidant formed endogenously by the near diffusion-limited reaction of nitric oxide with superoxide anion. Peroxynitrite specifically adds a nitro group to the *ortho* position of the phenolic ring of free and protein-associated tyrosines to form the stable product 3-nitro-L-tyrosine. Systemic administration of 3-nitro-L-tyrosine markedly inhibits the subsequent hemodynamic responses to α_1- and β-adrenoceptor agonists in anesthetized rats. Angiotensin II is an important modulator of vascular tone. The vasoconstrictor effects of this hormone are known to involve the release of catecholamines from sympathetic tissues. In the present study, we examined whether 3-nitro-L-tyrosine (2.5 μmol/kg i.v.) would attenuate the hemodynamic responses produced by angiotensin II (0.1–1.0 μg/kg i.v.). Angiotensin II produced increases in mean arterial pressure, and renal and mesenteric vascular resistances, but no changes in hindquarter vascular resistance. The pressor and renal and mesenteric vasoconstrictor responses produced by angiotensin II were significantly attenuated 30–60 min following the administration of 3-nitro-L-tyrosine. Further attenuation of these responses was evident 120–180 min following the administration of 3-nitro-L-tyrosine. The α_1-adrenoceptor antagonist prazosin also diminished the pressor and renal and mesenteric vasoconstrictor responses produced by angiotensin II. These results demonstrate that 3-nitro-L-tyrosine inhibits the hemodynamic responses to angiotensin II, possibly through the inhibition of α_1-adrenoceptor-mediated events. The effect of 3-nitro-L-tyrosine on the hemodynamic action of angiotensin II raises the possibility that 3-nitro-L-tyrosine may be involved in the pathogenesis of the hemodynamic disturbances associated with inflammatory conditions, such as atherosclerosis, ischemia-reperfusion, and sepsis, where formation of peroxynitrite is favored.

Keywords: Peroxynitrite; Nitrotyrosine; Nitric oxide; Superoxide; Angiotensin II; Hemodynamics, in vivo

1. Introduction

Peroxynitrite is a potent oxidant formed endogenously by the reaction of nitric oxide with superoxide anion radical (Beckman et al., 1990). The second order rate constant for this reaction is 6.7×10^9 M^{-1} s^{-1} (Huie and Padmaja, 1993), which is three times faster than the reaction of superoxide anion with superoxide dismutase. Therefore, nitric oxide is the one biological molecule which is capable of outcompeting superoxide dismutase for superoxide anion, making peroxynitrite formation the favored reaction under conditions, such as atherosclerosis, ischemia-reperfusion, and sepsis, where cellular production of nitric oxide and superoxide are increased. Rat alveolar macrophages (Ischiropoulos et al., 1992a) and Kuppfer cells (Wang et al., 1991), bovine endothelial cells (Kooy and Royall, 1994), and human neutrophils (Carreras et al., 1994) are capable of generating peroxynitrite in vitro. Moreover, oxidation of dihydrorhodamine 123 in rats with endotoxic and hemorrhagic shock is dependent on peroxynitrite formation in vivo (Kooy et al., 1994a; Szabo et al., 1995a).

Peroxynitrite nitrates free and protein-associated tyrosines and other phenolics either spontaneously, or through the superoxide dismutase- or iron-catalyzed formation of a nitronium ion-like species (Ischiropoulos et al., 1992b; Beckman et al., 1992). Peroxynitrite specifically adds a nitro group to the *ortho* position of the phenolic ring of tyrosine resulting in the formation of the stable product 3-nitro-L-tyrosine (NT) (Ischiropoulos et al., 1992b; Beck-

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man et al., 1992). 3-Nitro-L-tyrosine and the secondary metabolites 3-nitro-4-hydroxyphenylacetic acid and 3-nitro-4-hydroxyphenylpropionic acid have been detected in human urine (Oshima et al., 1990) and 3-nitro-L-tyrosine has been detected in the serum and synovial fluid of humans with rheumatoid arthritis (Kaur and Halliwell, 1994). Moreover, 3-nitro-L-tyrosine residues have been demonstrated in the aortae of endotoxemic rats (Szabo et al., 1995b), and in human coronary atherosclerotic lesions (Beckman et al., 1994), acute lung injury (Kooy et al., 1995) and myocardial inflammation (Kooy et al., 1994b).

The systemic administration of 3-nitro-L-tyrosine to anesthetized rats markedly inhibits the hemodynamic responses produced by the catecholamines norepinephrine and epinephrine, as well as the specific α_1-adrenoceptor agonist phenylephrine and the specific β-adrenoceptor agonist isoproterenol (Kooy and Lewis, 1996). This inhibition by 3-nitro-L-tyrosine may be specific for catecholamine-mediated responses since the hemodynamic effects of the non-catecholamine vasoconstrictor arginine vasopressin were not attenuated by the prior administration of 3-nitro-L-tyrosine (Kooy and Lewis, 1996).

Angiotensin II is an important modulator of cardiovascular function. Systemically administered angiotensin II is a direct vasoconstrictor which also promotes vasoconstriction by facilitating the release of norepinephrine from sympathetic nerve terminals (Chen and Zimmerman, 1995). In order to further evaluate the pharmacological profile of 3-nitro-L-tyrosine, the present study examined the effects of this product of peroxynitrite on the hemodynamic responses produced by angiotensin II in pentobarbital-anesthetized rats.

2. Materials and methods

2.1. Surgery

The experimental protocols described in this manuscript were approved by the Institutional Animal Care and Use Committee of The University of Iowa. Male Sprague-Dawley rats weighing 250–350 g were anesthetized with pentobarbital (50 mg/kg i.p.) and then implanted with femoral arterial and venous catheters (PE-50; Becton Dickinson, Sparks, MD, USA) for the measurement of pulsatile and mean arterial blood pressure mean arterial pressure and the administration of drugs respectively. Immediately following catheterization, a midline laparotomy was performed and miniature pulse Doppler flow probes were placed around the abdominal aorta, renal and mesenteric arteries for the measurement of hindquarter, renal and mesenteric blood flow velocities respectively, and for the determination of hindquarter, renal and mesenteric vascular resistances, as described previously (Lacolley et al., 1991). To maintain anesthesia, supplemental doses of pentobarbital (5 mg/kg i.v.) were given as necessary throughout the experiments.

2.2. Protocols

Once the hemodynamic parameters had stabilized, the effects of bolus i.v. administration of angiotensin II (0.1, 0.25, 0.5, and 1.0 ng/kg) on the above hemodynamic parameters were examined prior to and 30–60 min and 120–180 min following the administration of either saline (0.9% (w/v) NaCl i.v., $n = 5$), 3-nitro-L-tyrosine (2.5 μmol/kg i.v., $n = 5$), or the specific α_1-adrenoceptor antogonist prazosin (100 μg/kg i.v., $n = 5$).

2.3. Statistics

The effects of angiotensin II are expressed as mean ± S.E.M. of the percentage changes from baseline. The data were analyzed by repeated measures analysis of variance (ANOVA) followed by Student's modified t-test with the Bonferroni correction for multiple comparisons (Wallenstein et al., 1980). The standard error terms were derived from the formula $(EMS/n)^{1/2}$ where EMS is the error mean square term from the ANOVA and n is the number of rats (Wallenstein et al., 1980). A value of $P < 0.05$ was taken to denote statistical significance.

2.4. Drugs

Prazosin, angiotensin II, and 3-nitro-L-tyrosine were obtained from Sigma (St. Louis, MO, USA). Pentobarbital

Table 1
A summary of the effects of saline or NT on resting hemodynamic variables in pentobarbital-anesthetized rats

Parameter	Treatment	Pre	Post-NT			
			30–60 min	Δ (%)	120–180 min	Δ (%)
MAP (mmHg)	Saline ($n = 8$)	102 ± 3	101 ± 3	−1 ± 1	100 ± 4	−2 ± 2
	NT ($n = 6$)	106 ± 8	112 ± 8	+6 ± 5	117 ± 7	+12 ± 9
HQR (mmHg/kHz)	Saline ($n = 8$)	48 ± 10	46 ± 9	−4 ± 3	42 ± 11	−11 ± 7
	NT ($n = 6$)	78 ± 10	88 ± 9	+17 ± 10	110 ± 16	+36 ± 10 *
RR (mmHg/kHz)	Saline ($n = 8$)	72 ± 9	74 ± 8	−2 ± 2	68 ± 10	−6 ± 4
	NT ($n = 6$)	94 ± 7	109 ± 8	+20 ± 11	115 ± 12	+23 ± 12
MR (mmHg/kHz)	Saline ($n = 8$)	71 ± 11	67 ± 10	−7 ± 5	63 ± 12	−12 ± 7
	NT ($n = 6$)	28 ± 4	34 ± 3	+22 ± 12	40 ± 4	+28 ± 15

NT, 3-nitro-L-tyrosine (2.5 μmol/kg i.v.); MAP, mean arterial pressure; HQR, hindquarter resistance; RR, renal resistance; MR, mesenteric resistance. The values represent the mean ± S.E.M. of the raw data and the percentage changes from Pre values. * $P > 0.05$, 120–180 min vs. Pre.

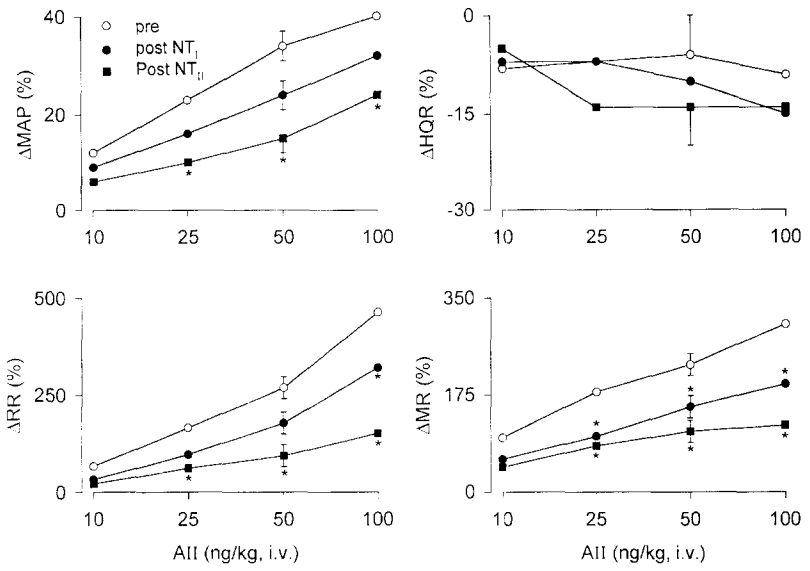

Fig. 1. A summary of the effects of i.v. angiotensin II (AII) on mean arterial pressure (MAP), and renal (RR), hindquarter (HQR), and mesenteric (MR) vascular resistances in pentobarbital-anesthetized rats ($n = 6$) prior to (pre), 30–60 min (post-NT$_I$), and 120–180 min (post-NT$_{II}$) following the administration of 3 nitro-L-tyrosine (NT; 2.5 μmol/kg i.v.). The effects of angiotensin II are expressed as mean ± S.E.M. of the percentage changes from baseline. * $P < 0.05$, post-NT$_I$ or post-NT$_{II}$ vs. pre.

and sterile saline for administration and dilution of chemicals were from Abbott Laboratories (North Chicago, IL, USA).

3. Results

3.1. Effect of 3-nitro-L-tyrosine, saline or prazosin on baseline hemodynamic variables

The values for the baseline hemodynamic variables shown in Table 1 were measured prior to and 30–60 min and 120–180 min following the administration of either 3-nitro-L-tyrosine (2.5 μmol/kg i.v.) or saline (0.9% w/v NaCl i.v.). The hemodynamic values were not different from pre-administration values except for a significant increase in the hindquarter resistance 120–180 min after the administration of 3-nitro-L-tyrosine. The values for the baseline hemodynamic variables prior to and following the administration of the α_1-adrenoceptor antagonist prazosin (100 μg/kg i.v.) are summarized in Table 2. Prazosin produced immediate and sustained decreases in mean arterial pressure and hindquarter and renal but not mesenteric vascular resistances.

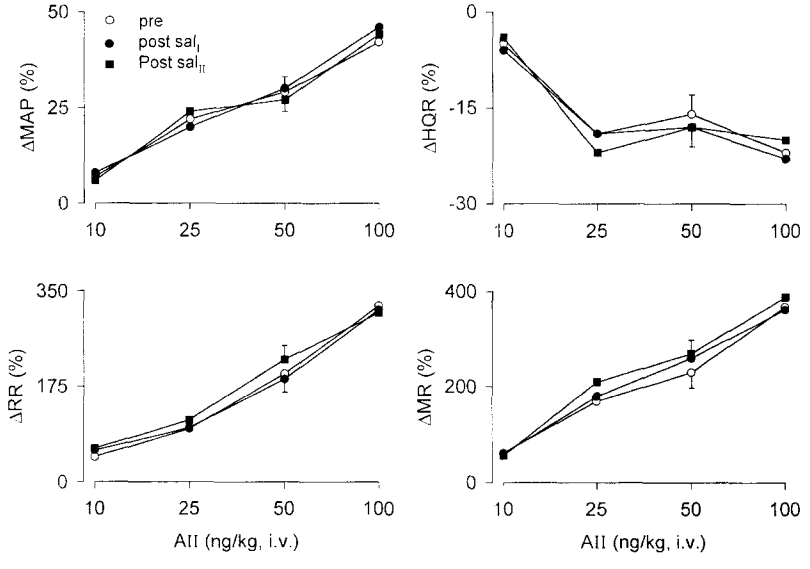

Fig. 2. A summary of the effects of i.v. angiotensin II (AII) on mean arterial pressure (MAP), and renal (RR), hindquarter (HQR), and mesenteric (MR) vascular resistances in pentobarbital-anesthetized rats ($n = 8$) prior to (pre), 30–60 min (post-Sal$_I$), and 120–180 min (post-Sal$_{II}$) following the administration of saline (0.9% NaCl w/v i.v.). The effects of angiotensin II are expressed as mean ± S.E.M. of the percentage changes from baseline. * $P < 0.05$, post-Sal$_I$ or post-Sal$_{II}$ vs. pre.

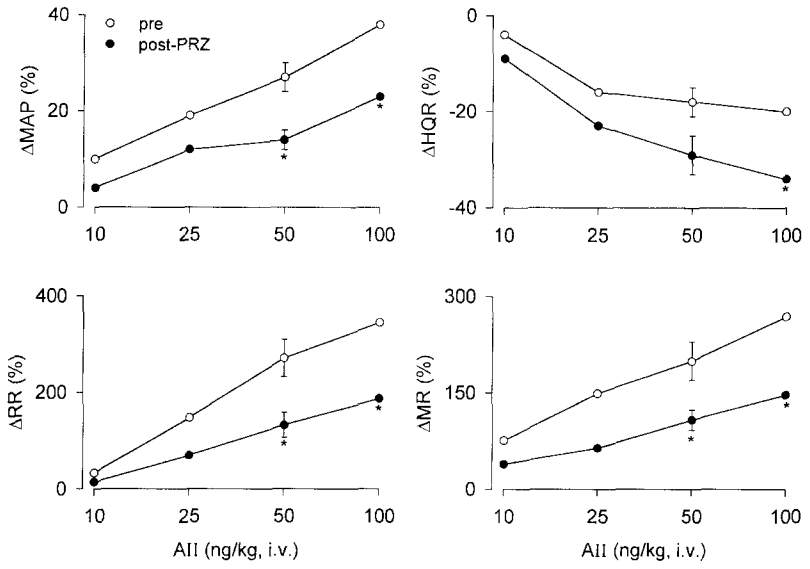

Fig. 3. A summary of the effects of i.v. angiotensin II (AII) on mean arterial pressure (MAP), and renal (RR), hindquarter (HQR), and mesenteric (MR) vascular resistances in pentobarbital-anesthetized rats ($n = 6$) prior to (pre) and following (post-PRZ) the administration of prazosin (100 μg/kg i.v.). The effects of angiotensin II are expressed as mean ± S.E.M. of the percentage changes from baseline. * $P < 0.05$, post-PRZ vs. pre.

3.2. Effects of 3-nitro-L-tyrosine, saline and prazosin on the hemodynamic responses produced by angiotensin II

The dose-dependent effects of angiotensin II (0.1–1.0 μg/kg i.v.) on mean arterial pressure and vascular resistances prior to and 30–60 min and 120–180 min after the administration of 3-nitro-L-tyrosine are summarized in Fig. 1. angiotensin II produced dose-dependent increases in mean anterial pressure and renal and mesenteric vascular resistances but no changes in hindquarter resistance. The vasoconstrictor effects of angiotensin II in the renal and mesenteric beds were reduced 30–60 min and more evidently 120–180 min after the administration of 3-nitro-L-tyrosine. The pressor responses produced by angiotensin II were also substantially diminished 120–180 min after the administration of 3-nitro-L-tyrosine. 3-Nitro-L-tyrosine did not alter the minor effects of angiotensin II in the hindquarter bed. The dose-dependent effects of angiotensin II (0.1–1.0 μg/kg i.v.) on mean arterial pressure and vascular resistances prior to and 30–60 min and 120–180 min after the administration of saline are summarized in Fig. 2. The hemodynamic effects of angiotensin II were similar prior to and following the administration of saline. The dose-dependent effects of angiotensin II prior to and following the administration of prazosin are summarized in Fig. 3. The pressor and renal and mesenteric vasoconstrictor responses produced by angiotensin II were significantly diminished by prazosin. The angiotensin II-induced vasodilation in the hindquarter bed was not markedly affected by prazosin although the highest dose of angiotensin II produced an exaggerated vasodilation in the presence of the α_1-adrenoceptor antagonist.

Table 2
A summary of the effects of prazosin on restiong hemodynamic variables in pentobarbital-anesthetized rats

Parameter	Pre	Post	%Δ
MAP (mmHg)	112±4	84±5	−24±6 *
HQR (mmHg)	62±12	41±7	−34±8 *
RR (mmHg/kHz)	78±10	63±9	−19±4 *
MR (mmHg/kHz)	34±7	28±8	−18±9

MAP, mean arterial pressure; HQR, hindquarter vascular resistance; RR, renal vascular resistance; MR, mesenteric vascular resistance. Values represent mean ± S.E.M. of the raw data and the percentage changes from Pre-values following the administration of prazosin (100 ≅ g/kg, i.v.) to pentobarbital-anesthetized rats. * $P < 0.05$, Post vs. Pre.

4. Discussion

The present study demonstrates that the systemic administration of 3-nitro-L-tyrosine attenuates the vasoconstrictor effects of angiotensin II in pentobarbital-anesthetized rats. angiotensin II is known to exert its hemodynamic effects via direct interaction with angiotensin receptors on the vascular smooth muscle. Moreover, angiotensin II also promotes vasoconstriction through the facilitated release of catecholamines from sympathetic nerve terminals (Chen and Zimmerman, 1995). The systemic administration of 3-nitro-L-tyrosine substantially attenuates the hemodynamic responses produced by α_1-adrenoceptor agonist but does not affect the vasoconstrictor responses produced by arginine vasopressin (Kooy and Lewis, 1996). This suggests that 3-nitro-L-tyrosine or a metabolite (see below) may be a selective adrenoceptor antagonist. Therefore, the 3-nitro-L-tyrosine-mediated attenuation of the vasoconstrictor effects of angiotensin II probably occurs via the inhibition of the α-adrenoceptor-

mediated vasoconstriction related to the angiotensin II-facilitated release of catecholamines. Furthermore, the attenuation of angiotensin II-mediated vasoconstriction by the specific α_1-adrenoceptor antagonist prazosin supports adrenoceptor blockade as the mechanism by which 3-nitro-L-tyrosine inhibits the hemodynamic effects of angiotensin II.

The inhibitory effects of 3-nitro-L-tyrosine on angiotensin II-mediated vasoconstriction increase with time, similar to the attenuation of catecholamine-induced hemodynamic responses following the administration of 3-nitro-L-tyrosine (Kooy and Lewis, 1996). Therefore, 3-nitro-L-tyrosine may not be a direct adrenoceptor antagonist, but may be metabolized to form compounds which are adrenoceptor antagonists. In humans, 3-nitro-L-tyrosine is metabolized to 3-nitro-4-hydroxyphenylacetic acid and 3-nitro-4-hydroxyphenylpropionic acid (Oshima et al., 1990). These compounds share structural similarity with the endogenous catecholamines and may, therefore, serve as adrenoceptor antagonists. Moreover, 3-nitro-L-tyrosine may enter sympathetic nerve terminals or the adrenal glands to be enzymatically converted to nitrated compounds which differ from catecholamines only in the substitution of a nitro (NO_2) group for a hydroxyl (OH) group on the *ortho* position of the phenolic ring. Tyrosine is converted to dopa by tyrosine hydroxylase in sympathetic tissues. Dopa is then converted to dopamine by dopa decarboxylase, a non-specific aromatic decarboxylase, which also produces serotonin, tyramine, and histamine from their respective amino acid precursors (Koelle, 1975). The non-specificity of dopa decarboxylase is also demonstrated by the conversion of α-methyldopa to α-methyldopamine (Hess et al., 1961). 3-Nitro-L-tyrosine is not a substrate for tyrosine hydroxylase (Ischiropoulos et al., 1995), however, 3-nitro-L-tyrosine is structurally similar to dopa and may be converted to 3-nitro-4-hydroxyphenylethylamine by the non-specific actions of dopa decarboxylase. 3-Nitro-4-hydroxyphenylethylamine is a dopamine analogue, which may undergo further conversion to nitrated compounds sharing structural similarity with norepinephrine and epinephrine through the activity of dopamine-β-hydroxylase in the nerves or adrenals and/or phenylethanolamine *N*-methyltransferase in the adrenals. Upon their release, these nitrated catecholamine analogues may act as adrenoceptor antagonists.

Despite the pronounced attenuation of adrenoceptor-mediated vasoconstriction (Kooy and Lewis, 1996) and angiotensin II-mediated vasoconstriction, the systemic administration of 3-nitro-L-tyrosine surprisingly does not affect resting mean anterial pressure or vascular resistances. Conversely, the systemic administration of the specific α_1-adrenoceptor antagonist prazosin had profound effects on baseline hemodynamic variables. To account for these differences, the lack of effect of 3-nitro-L-tyrosine on resting hemodynamic variables may be due to: (1) incomplete blockade of neurogenic vasoconstriction; (2) concomitant loss of β-adrenergic vasodilation (Kooy and Lewis, 1996); or (3) the compensatory activity of circulating hormones, such as AVP (Kooy and Lewis, 1996).

The observation that 3-nitro-L-tyrosine attenuates the hemodynamic effects of adrenoceptor agonists and angiotensin II suggests that this product of peroxynitrite may be involved in the pathophysiological disturbances in hemodynamic function in conditions, such as atherosclerosis, ischemia-reperfusion, and sepsis, where the formation of peroxynitrite is favored.

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