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Nitrotyrosine attenuates the hemodynamic effects of adrenoceptor agonists in vivo: relevance to the pathophysiology of peroxynitrite

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Abstract

Peroxynitrite, which attenuates catecholamine-mediated hemodynamic responses in vivo, nitrates free tyrosine residues to form the specific product, 3-nitro-L-tyrosine. The chemical structure of 3-nitro-L-tyrosine is similar to that of the endogenous catecholamines. Therefore, 3-nitro-L-tyrosine may interfere with catecholamine hemodynamic function in vivo. The hemodynamic responses produced by norepinephrine (1–4 μg/kg, i.v., $n = 6$), epinephrine (0.5–4 μg/kg, i.v., $n = 7$), phenylephrine (1–8 μg/kg, i.v., $n = 5$), and isoproterenol (100–400 ng/kg, i.v., $n = 5$) were attenuated, while the hemodynamic responses produced by arginine vasopressin (50–250 ng/kg; i.v., $n = 5$) were unaffected following the administration of 3-nitro-L-tyrosine (2.5 μmol/kg, i.v.) in pentobarbital-anesthetized rats. These results demonstrate substantial and selective attenuation of the hemodynamic effects produced by α- and β-adrenoceptor agonists, raising the possibility that 3-nitro-L-tyrosine may play a role in the hemodynamic dysfunction associated with inflammatory conditions in which the formation of peroxynitrite is favored.

Keywords: Peroxynitrite; Nitrotyrosine; Nitric oxide (NO); Superoxide; Hemodynamics, in vivo

1. Introduction

Vascular hyporeactivity to catecholamine vasoconstrictors is a characteristic feature of sepsis and the systemic inflammatory response syndrome. Endothelium-derived nitric oxide is a potent vasodilator that plays an important role in the physiological regulation of vascular tone and blood flow (Palmer et al., 1987). In response to bacterial endotoxins and inflammatory cytokines, the vascular endothelium and smooth muscle generate high concentrations of nitric oxide through the expression of the inducible form of nitric oxide synthase (Rees et al., 1990; Radomski et al., 1991). Nitric oxide generated from the inducible nitric oxide synthase has been implicated in the pathophysiology of vascular dysfunction (Moncada et al., 1991). However, there is considerable controversy as to whether these pathological decreases in vascular tone are mediated directly by nitric oxide or through the formation of secondary reaction products.

Inflammatory mediators which enhance cellular production of nitric oxide also increase cellular superoxide anion production from nitric oxide synthase and other cellular sources (Brigham, 1991; Pou et al., 1992). Nitric oxide reacts at a near diffusion-limited rate with superoxide anion ($k = 6.7 \times 10^9$ $M^{-1} \cdot s^{-1}$) to form the potent oxidant, peroxynitrite (Huie and Padmaja, 1993). Peroxynitrite production has been demonstrated from macrophages (Ischiropoulos et al., 1992a), neutrophils (Carreras et al., 1994), and endothelial cells (Kooy and Royall, 1994). The systemic administration of peroxynitrite produces marked reductions in mean arterial pressure and vascular resistances which are subject to rapid tachyphylaxis (Kooy et al., 1996). Moreover, following the development of tachyphylaxis to peroxynitrite, the hemodynamic responses to the catecholamines norepinephrine and epinephrine are substantially attenuated (Kooy et al., 1996).

Peroxynitrite nitrates free or protein-associated tyrosines and other phenolics (Beckman et al., 1992; Ischiropoulos et al., 1992b). The addition of a nitro group to the 3-position of tyrosine results in the formation of the stable product, 3-nitro-L-tyrosine (see Fig. 1). Peroxynitrite-medi-

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ated nitration occurs spontaneously but is also catalyzed by low-molecular-mass transition metals and superoxide dismutase via the formation of a nitronium-ion-like intermediate (Beckman et al., 1992; Ischiropoulos et al., 1992b). 3-Nitro-L-tyrosine and its metabolites (3-nitro-4-hydroxyphenylacetic acid and 3-nitro-4-hydroxyphenylpropionic acid) have been detected in human urine (Ohshima et al., 1990) and 3-nitro-L-tyrosine has been detected in the synovial fluid and serum of human patients with rheumatoid arthritis (Kaur and Halliwell, 1994). 3-nitro-L-tyrosine residues have also been demonstrated in human coronary atherosclerotic lesions (Beckman et al., 1994), acute lung injury (Kooy et al., 1995), and myocardial inflammation (Kooy et al., submitted).

Although 3-nitro-L-tyrosine is used as a marker for the presence and activity of peroxynitrite within tissues during acute and chronic inflammatory disease states, the biological significance of free 3-nitro-L-tyrosine formation has not been previously investigated. The structural similarity of 3-nitro-L-tyrosine to the precursors of endogenous catecholamines (Fig. 1) prompted us to examine whether 3-nitro-L-tyrosine modulates catecholamine function in vivo. We now report that the systemic administration of 3-nitro-L-tyrosine markedly reduces the hemodynamic responses produced by norepinephrine, epinephrine, the specific α_1-adrenoceptor agonist phenylephrine and the β-adrenoceptor agonist isoproterenol in pentobarbital-anesthetized rats. In contrast, the hemodynamic effects produced by arginine-vasopressin were unaffected by 3-nitro-L-tyrosine. These findings are the first evidence that free 3-nitro-L-tyrosine has biological activity and may be involved in the pathophysiological effects of peroxynitrite on hemodynamic function.

2. Methods and materials

2.1. Rats and surgical procedures

The experimental protocols described in this paper were approved by the Institutional Animal Care and Use Committee of The University of Iowa. Male Sprague-Dawley rats ($n = 61$) weighing between 280 and 340 g were anesthetized with pentobarbital (50 mg/kg, i.p.) and were implanted with femoral arterial and venous catheters (PE-50, Becton Dickinson and Company, Sparks, MD) for the measurement of pulsatile and mean arterial blood 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. Experimental protocols

Ten groups of rats were used in these studies. Five groups received 3-nitro-L-tyrosine (2.5 μmol/kg; i.v.) and 5 groups received saline (0.9% w/v NaCl, i.v.). The hemodynamic effects produced by the bolus administration of norepinephrine (1–4 μg/kg, i.v.), epinephrine (0.5–4 μg/kg, i.v.), the α_1-adrenoceptor agonist phenylephrine (1–4 μg/kg, i.v.), the β-adrenoceptor agonist isoproterenol (100–400 ng/kg, i.v.) or arginine vasopressin (100–500 ng/kg; i.v.) were examined prior to, 30–60 and 120–180 min following the administration of either saline or 3-nitro-L-tyrosine. Each saline- and 3-nitro-L-tyrosine-treated group received one of the adrenoceptor agonists or arginine-vasopressin.

2.3. Materials

Sterile saline (0.9% NaCl w/v) and pentobarbital sodium were from Abbott Laboratories (North Chicago, IL). Norepinephrine, epinephrine, phenylephrine, isoproterenol, arginine vasopressin, and 3-nitro-L-tyrosine were from Sigma Chemical Company (St. Louis, MO).

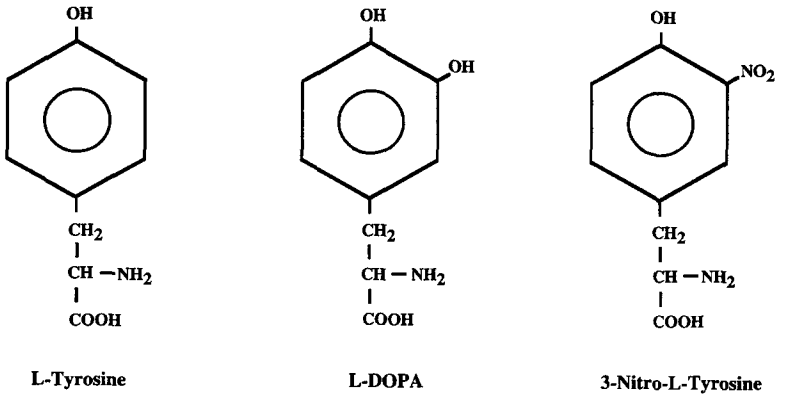

Fig. 1. Chemical structures of L-tyrosine, L-DOPA, and 3-nitro-L-tyrosine.

2.4. Statistics

The data are expressed as mean ± S.E.M. 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.

3. Results

3.1. Effects of 3-nitro-L-tyrosine on baseline hemodynamic variables

Five separate groups of rats received saline and 5 other groups received 3-nitro-L-tyrosine (2.5 μmol/kg, i.v.). Each of the saline-treated or 3-nitro-L-tyrosine-treated groups received either norepinephrine, epinephrine, phenylephrine, isoproterenol, or arginine-vasopressin. The baseline hemodynamic variables in the saline-treated and 3-nitro-L-tyrosine-treated groups used for the arginine vasopressin studies are summarized in Table 1. These values were measured prior to and between 30–60 and 120–180 min following the administration of saline or 3-nitro-L-tyrosine. These times represent those over which the dose–response effects of arginine-vasopressin (and the adrenoceptor agonists in the other groups) were examined. The hemodynamic values recorded 30–60 and 120–180 min after the administration of saline or 3-nitro-L-tyrosine were not different from pre-administration values except for an increase in the mesenteric resistance 120–180 min after the administration of 3-nitro-L-tyrosine. However, the administration of 3-nitro-L-tyrosine did not alter the resting hemodynamic variables in the 4 other groups used in these studies (data not shown).

3.2. Effects of 3-nitro-L-tyrosine on the hemodynamic responses produced by the adrenoceptor agonists and arginine-vasopressin

The effects of norepinephrine on mean arterial pressure and vascular resistances prior to and 30–60 and 120–180 min following the administration of 3-nitro-L-tyrosine are summarized in Fig. 2. Norepinephrine produced dose-dependent increases in mean arterial pressure and hindquarter, renal, and mesenteric vascular resistances. The norepinephrine-induced vasoconstriction in the renal and mesenteric beds was diminished 30–60 min after the administration of 3-nitro-L-tyrosine. The norepinephrine-induced increases in mean arterial pressure and vascular resistances were substantially reduced 120–180 min following the administration of 3-nitro-L-tyrosine. In contrast, the norepinephrine-induced hemodynamic responses were similar prior to and 30–60 and 120–180 min following the administration of saline ($n = 6$, $P > 0.05$ for all comparisons, data not shown).

The effects of epinephrine on mean arterial pressure and vascular resistances prior to and 30–60 min and 120–180 min following the administration of 3-nitro-L-tyrosine are summarized in Fig. 3. Epinephrine produced dose-dependent pressor responses accompanied by increases in renal and mesenteric vascular resistances and a decrease in hindquarter vascular resistance. The vasoconstrictor and vasodilator effects of epinephrine were substantially reduced 30–60 and 120–180 min following the administration of 3-nitro-L-tyrosine. The epinephrine-induced changes in mean arterial pressure and vasucular resistances were similar prior to and 30–60 and 120–180 min following the administration of saline ($n = 8$, $P > 0.05$ for all comparisons, data not shown).

The effects of the selective α_1-adrenoceptor agonist phenylephrine on mean arterial pressure and vascular resistances prior to and 30–60 and 120–180 min following the administration of 3-nitro-L-tyrosine are summarized in Fig. 4. Phenylephrine produced dose-dependent increases in

Table 1
A summary of the effects of saline or NT on resting hemodynamic variables

Parameter	Treatment	Pre-NT	Post-NT			
			30–60 min	Δ (%)	120–180 min	Δ (%)
MAP (mmHg)	Saline	108 ± 4	109 ± 5	+1 ± 1	106 ± 3	−2 ± 1
	NT	113 ± 6	113 ± 7	+0 ± 2	119 ± 9	+5 ± 8
HQR (mmHg/kHz)	Saline	62 ± 11	68 ± 7	+9 ± 6	54 ± 13	−13 ± 7
	NT	78 ± 7	85 ± 11	+10 ± 12	113 ± 25	+39 ± 21
RR (mmHg/kHz)	Saline	104 ± 17	108 ± 16	+5 ± 9	100 ± 10	+4 ± 7
	NT	144 ± 22	135 ± 27	−9 ± 7	162 ± 36	+18 ± 10
MR (mmHg/kHz)	Saline	59 ± 10	68 ± 12	+8 ± 12	60 ± 8	+3 ± 5
	NT	40 ± 6	42 ± 3	+13 ± 16	54 ± 7	+48 ± 16 *

NT = 3-nitro-L-tyrsoine (2.5 μmol/kg, i.v.); MAP = mean arterial pressure; HQR = hindquarter resistance; RR = renal resistance; MR = mesenteric resistance. The saline- and NT-treated groups consisted of 6 pentobarbital-anesthetized rats each. 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-.

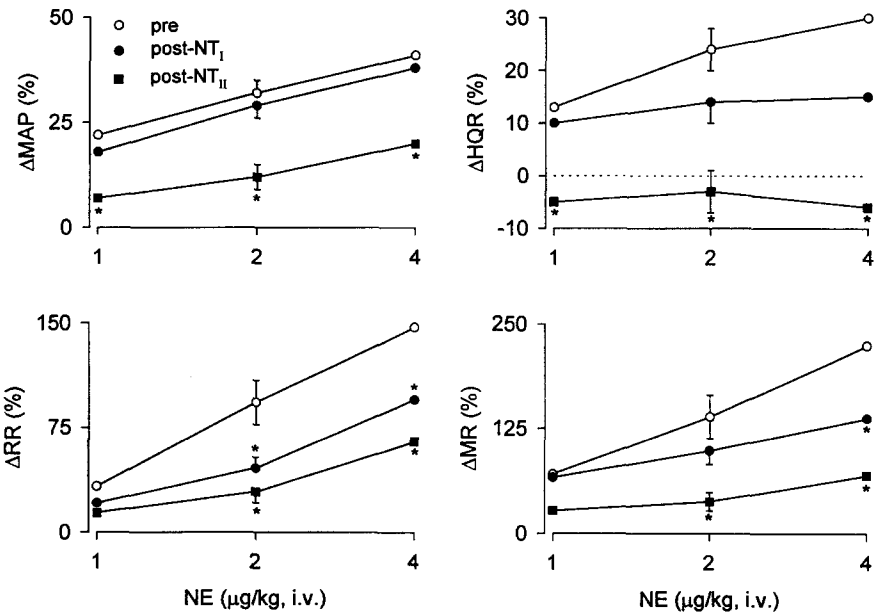

Fig. 2. A summary of the effects of i.v. norepinephrine (NE) on mean arterial pressure (MAP), and renal (RR), hindquarter (HQR), and mesenteric (MR) vascular resistances in pentobarbital-anesthetized rats ($n = 6$) prior to (pre-), 30–60 min (post-NT$_I$), and 120–180 min (post-NT$_{II}$) following the administration of 3 nitro-L-tyrosine (NT; 2.5 μmol/kg, i.v.). The effects of NE 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-.

mean arterial pressure and vascular resistances which were substantially diminished 30–60 and 120–180 min following the administration of 3-nitro-L-tyrosine. The hemodynamic responses produced by phenylephrine were similar prior to and following the administration of saline ($n = 5$, $P > 0.05$ for all comparisons, data not shown).

The effects of the β-adrenoceptor agonist isoproterenol on mean arterial pressure and vascular resistances are summarized in Fig. 5. Isoproterenol produced dose-dependent decreases in mean arterial pressure and vascular resistances. The hypotensive and hindquarter and mesenteric vasodilator effects produced by isoproterenol were

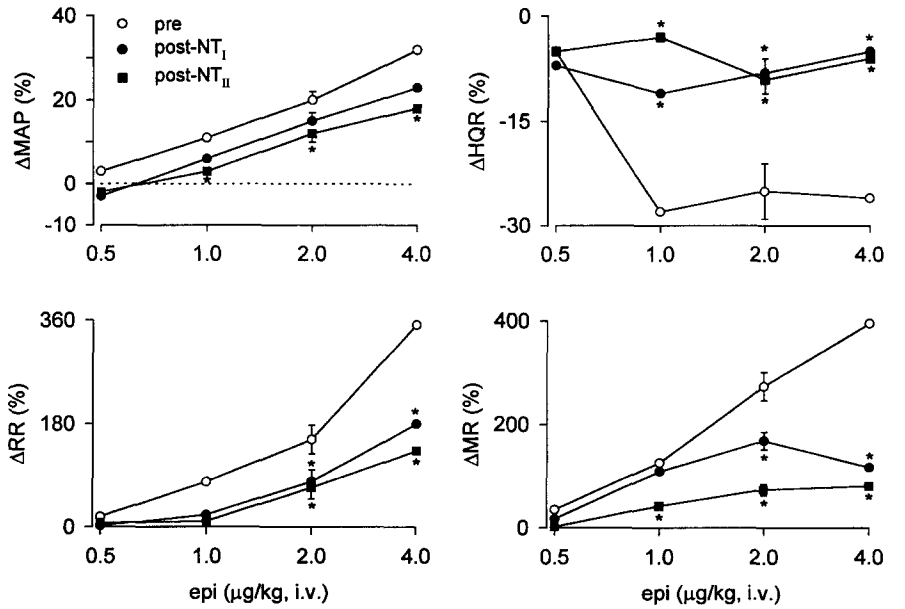

Fig. 3. A summary of the effects of i.v. epinephrine (EPI) on mean arterial pressure (MAP), and renal (RR), hindquarter (HQR), and mesenteric (MR) vascular resistances in pentobarbital-anesthetized rats ($n = 7$) prior to (pre-), 30–60 min (post-NT$_I$), and 120–180 min (post-NT$_{II}$) following the administration of 3 nitro-L-tyrosine (NT; 2.5 μmol/kg, i.v.). The effects of EPI 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-.

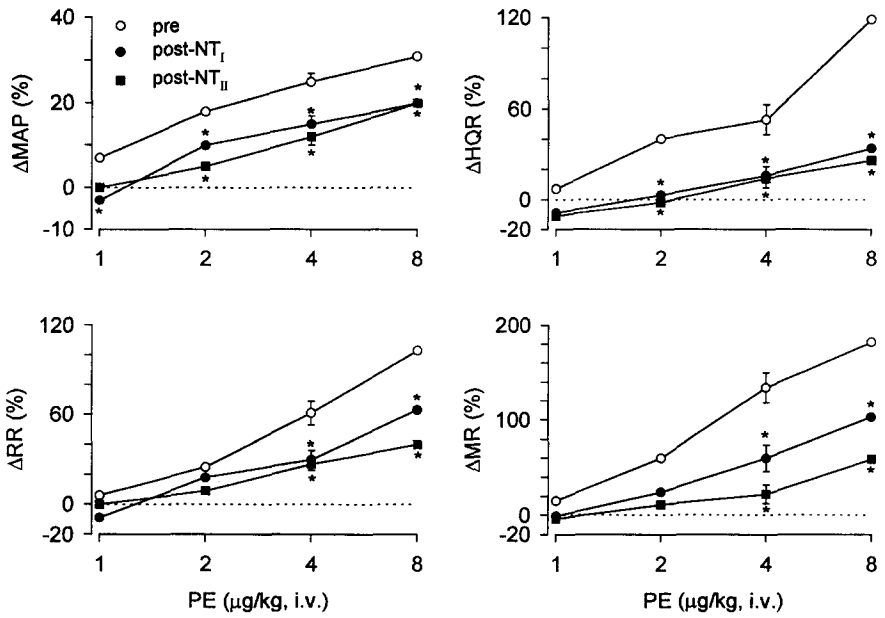

Fig. 4. A summary of the effects of i.v. phenylephrine (PE) on mean arterial pressure (MAP), and renal (RR), hindquarter (HQR), and mesenteric (MR) vascular resistances in pentobarbital-anesthetized rats ($n = 5$) prior to (pre-), 30–60 min (post-NT$_I$), and 120–180 min (post-NT$_{II}$) following the administration of 3 nitro-L-tyrosine (NT; 2.5 μmol/kg, i.v.). The effects of PE 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-.

substantially diminished 30–60 and 120–180 min following the administration of 3-nitro-L-tyrosine. The hemodynamic responses produced by isoproterenol were similar prior to and following the administration of saline ($n = 6$, $P > 0.05$ for all comparisons, data not shown).

The effects of arginine-vasopressin on mean arterial pressure and vascular resistances are summarized in Fig. 6. Arginine vasopressin produced dose-dependent increases in mean arterial pressure and vascular resistances which were not altered 30–60 or 120–180 min following the administration of 3-nitro-L-tyrosine. The hemodynamic responses produced by AVP were similar prior to and 30–60

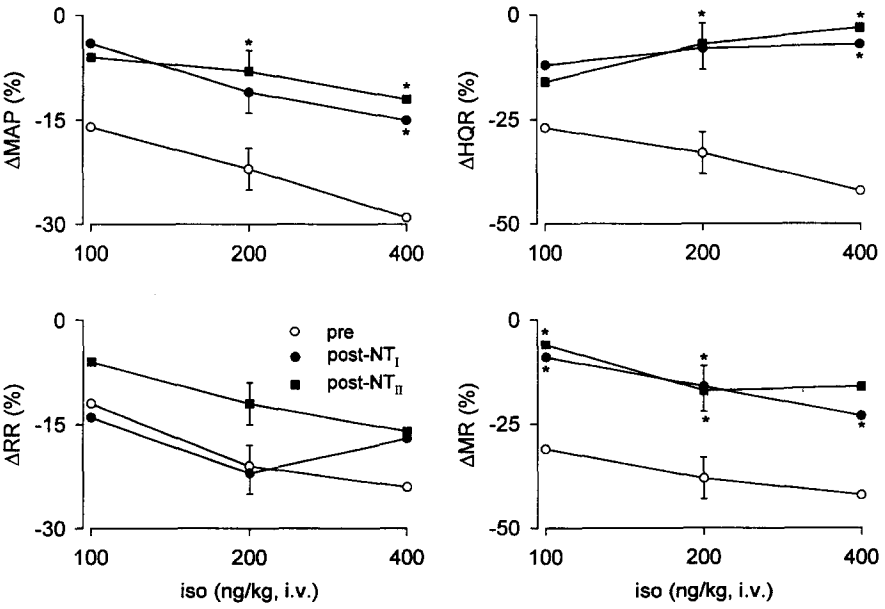

Fig. 5. A summary of the effects of i.v. isoproterenol (ISO) on mean arterial pressure (MAP), and renal (RR), hindquarter (HQR), and mesenteric (MR) vascular resistances in pentobarbital-anesthetized rats ($n = 5$) prior to (pre-), 30–60 min (post-NT$_I$), and 120–180 min (post-NT$_{II}$) following the administration of 3 nitro-L-tyrosine (NT; 2.5 μmol/kg, i.v.). The effects of ISO 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-.

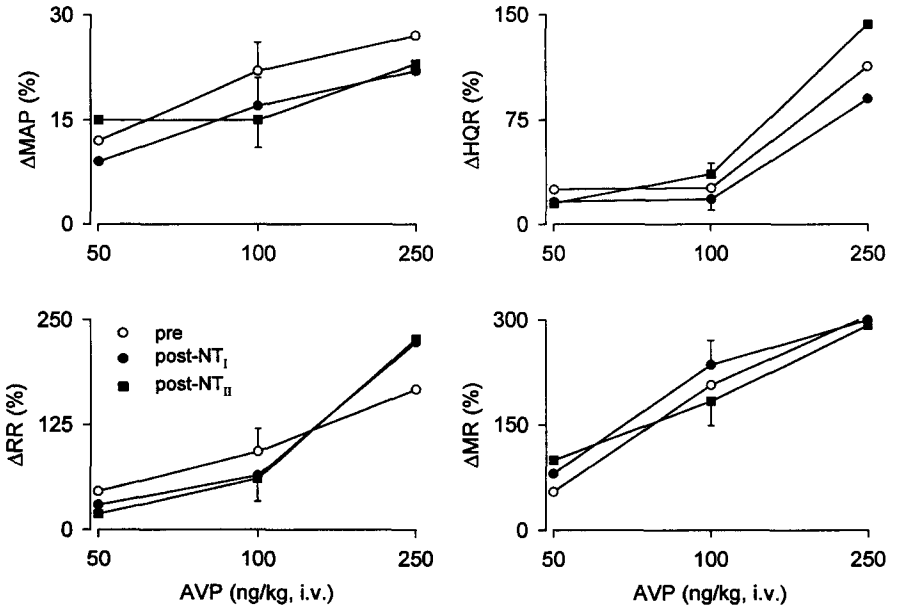

Fig. 6. A summary of the effects of i.v. arginine-vasopressin (AVP) on mean arterial pressure (MAP), and renal (RR), hindquarter (HQR), and mesenteric (MR) vascular resistances in pentobarbital-anesthetized rats ($n = 5$) prior to (pre-), 30–60 min (post-NT$_I$), and 120–180 min (post-NT$_{II}$) following the administration of 3 nitro-L-tyrosine (NT; 2.5 μmol/kg, i.v.). The effects of AVP are expressed as mean ± S.E.M. of the percentage changes from baseline.

and 120–180 min following the administration of saline ($n = 6$, $P > 0.05$ for all comparisons, data not shown).

4. Discussion

Nitrotyrosine is formed endogenously via the peroxynitrite-mediated nitration of tyrosine (Beckman et al., 1992; Ischiropoulos et al., 1992b). Free 3-nitro-L-tyrosine has been detected in the serum of patients with rheumatoid arthritis (Kaur and Halliwell, 1994), which is a chronic, localized inflammatory process. Circulating levels of 3-nitro-L-tyrosine in patients with rheumatoid arthritis reach values of 0.5 μmol/l (Kaur and Halliwell, 1994). Although 3-nitro-L-tyrosine levels have not previously been measured in systemic inflammatory diseases such as sepsis, considerably higher serum levels of 3-nitro-L-tyrosine might be expected. The concentration of tyrosine in human serum is approximately 100 μmol/l providing the potential for peroxynitrite to generate relatively high concentrations of free 3-nitro-L-tyrosine. The doses of systemically administered 3-nitro-L-tyrosine in the present study (2.5 μmol/kg) would produce peak serum concentrations of approximately 40 μmol/l; however, redistribution to the extravascular space might decrease the serum concentration to as low as 4 μmol/l.

The systemic administration of 3-nitro-L-tyrosine markedly diminished the hemodynamic effects of the catecholamines norepinephrine and epinephrine in pentobarbital-anesthetized rats. Moreover, 3-nitro-L-tyrosine also diminished the pressor and vasoconstrictor responses produced by the selective α_1-adrenoceptor agonist phenylephrine and the depressor and vasodilator responses produced by the β-adrenoceptor agonist isoproterenol. These findings suggest that 3-nitro-L-tyrosine or its derivatives (see below) may be α- and β-adrenoceptor antagonists. Furthermore, the observation that 3-nitro-L-tyrosine did not affect the pressor or vasoconstrictor responses produced by the non-catecholamine vasoconstrictor arginine-vasopressin suggests that 3-nitro-L-tyrosine or its derivatives may selectively interact with adrenoceptors.

While the chemical structure of 3-nitro-L-tyrosine is similar to that of the endogenous catecholamines and other adrenoceptor agonists, the inhibitory effects of 3-nitro-L-tyrosine on the catecholamine-induced hemodynamic responses increased with time, raising the possibility that a derivative of 3-nitro-L-tyrosine may be responsible for these effects. 3-Nitro-L-tyrosine is metabolized to 3-nitro-4-hydroxyphenylacetic acid and 3-nitro-4-hydroxyphenylpropionic acid (Ohshima et al., 1990). These compounds have been detected in human urine (Ohshima et al., 1990), suggesting their presence in the circulation. Although their biological activity is unknown, these compounds also share structural similarity with the catecholamines and may serve as adrenoceptor antagonists. Alternatively, 3-nitro-L-tyrosine may enter sympathetic nerve terminals or the adrenal glands to be enzymatically converted to nitrated compounds sharing structural similarity with the endogenous catecholamines. In sympathetic tissues, tyrosine is converted to DOPA by tyrosine hydroxylase. 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). Moreover, DOPA decar-

boxylase also converts 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 similar in chemical structure to DOPA (Fig. 1) and may be converted to 3-nitro-4-hydroxyphenylethylamine by the non-specific actions of DOPA decarboxylase. Except for a substitution of a nitro (NO_2) group for the hydroxyl (OH) group on the *ortho*-position of the phenolic ring, 3-nitro-4-hydroxyphenylethylamine is similar in chemical structure to dopamine. This nitrated dopamine equivalent may then 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 compounds may act as adrenoceptor antagonists.

The systemic administration of 3-nitro-L-tyrosine did not affect resting mean arterial pressure or vascular resistances despite pronounced inhibition of the vasoconstrictor responses produced by the systemic administration of adrenoceptor agonists. The lack of effects of 3-nitro-L-tyrosine on resting hemodynamic variables may be due to (i) incomplete blockade of sympathetic neurogenic vasoconstriction, (ii) concomitant loss of β-adrenergic vasodilation, or (iii) the compensatory activity of circulating hormones such as AVP.

In conclusion, the effect of 3-nitro-L-tyrosine on the hemodynamic action of norepinephrine and epinephrine raises the possibility that 3-nitro-L-tyrosine may be involved in the physiological regulation of vasomotor tone. Moreover, 3-nitro-L-tyrosine may be involved in the pathogenesis of the hemodynamic disturbances in inflammatory conditions such as sepsis, atherosclerosis, or ischemia-reperfusion where the production of peroxynitrite is favored (Huie and Padmaja, 1993).

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