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Peroxynitrite-mediated attenuation of α- and β-adrenoceptor agonist-induced vascular responses in vivo

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Abstract

Peroxynitrite is produced by vascular endothelial and smooth muscle cells in response to inflammation, induces vascular relaxation, and alters vascular responses to endothelial-derived relaxing factors. The present study examined the changes in mean arterial pressure and hindquarter, renal, and mesenteric vascular resistances produced by the systemic administration of (i) the catecholamines epinephrine or norepinephrine, (ii) the α_1-adrenoceptor agonist phenylephrine, (iii) the β-adrenoceptor agonist isoproterenol or (iv) [Argδ] vasopressin in pentobarbital-anesthetized rats prior to and following the systemic administration of peroxynitrite. The systemic administration of peroxynitrite significantly inhibited (i) epinephrine-induced pressor and renal and mesenteric vasoconstrictor responses, (ii) norepinephrine-induced pressor and hindquarter, renal, and mesenteric vasoconstrictor responses, (iii) phenylephrine-induced hindquarter and mesenteric vasoconstrictor responses, and (iv) isoproterenol-induced depressor and hindquarter and renal vasodilator responses. In comparison, the systemic administration of peroxynitrite had no effect on arginine vasopressin-induced pressor or vasoconstrictor responses. These results demonstrate selective and consequential attenuation of the hemodynamic effects produced by α- and β-adrenoceptor agonists, suggesting that selective impairment of adrenoceptors by peroxynitrite may play a critical role in the hemodynamic dysfunction associated with inflammatory conditions.

Keywords: Peroxynitrite; Nitric oxide (NO); Superoxide; Catecholamines; Hemodynamics

1. Introduction

Nitric oxide, released by the vascular endothelium in response to shear stress and pulsatile flow, is a potent vasodilator that performs an important physiological role in the normal regulation of vascular tone (Palmer et al., 1987). In response to bacterial endotoxin or inflammatory cytokines the vascular endothelium and smooth muscle also express the inducible isoform of nitric oxide synthase leading to the generation of nitric oxide in high concentration (Radomski et al., 1991; Rees et al., 1990). Vascular endothelial- and smooth muscle-derived nitric oxide production from the cytokine-mediated activation of the inducible isoform of nitric oxide synthase may be responsible for the hypotension and vasodilatation characteristic of early septic shock (Moncada et al., 1991). Concomitant with enhanced nitric oxide production, however, bacterial endotoxin and inflammatory cytokines also increase cellular superoxide anion production from xanthine oxidase, NAD(P)H oxidase, mitochondria, arachidonic acid metabolism, and nitric oxide synthase (Pou et al., 1992; Wolin, 1996).

Nitric oxide reacts at a near diffusion-limited rate (6.7×10^9 M^{-1} s^{-1}) with superoxide anion to form the potent oxidant peroxynitrite (Beckman et al., 1990; Huie and Padmaja, 1993). The second order rate constant for the formation of peroxynitrite is approximately three times greater than the rate of superoxide dismutase-catalyzed dismutation of superoxide anion. Therefore, peroxynitrite formation is a favored reaction under conditions such as atherosclerosis, ischemia-reperfusion, and sepsis, where cellular production of nitric oxide and superoxide anion is increased. Peroxynitrite formation has been demonstrated from isolated cells including vascular endothelial (Kooy and Royall, 1994) and smooth muscle cells (Boota et al., 1996). Moreover, peroxynitrite formation has been demon-

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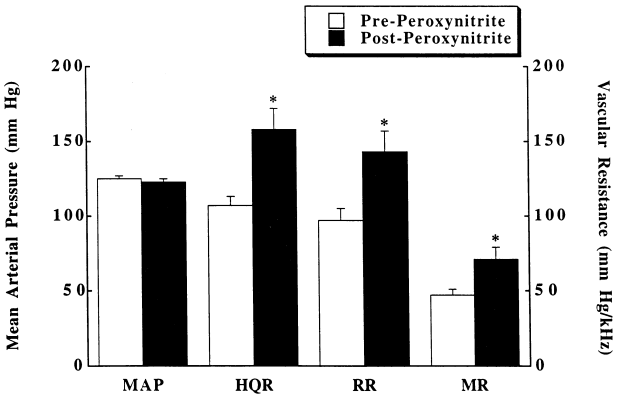

Fig. 1. Summary of the resting hemodynamic values prior to and following the systemic administration of peroxynitrite to pentobarbital-anesthetized rats. The values are the average of the mean arterial pressure (MAP, mm Hg), and hindquarter (HQR), renal (RR), and mesenteric (MR) vascular resistances (mm Hg/kHz) at the time of the dose–responses to epinephrine, norepinephrine, phenylephrine, isoproterenol, and arginine vasopressin prior to (Pre-Peroxynitrite) and 30 min following (Post-Peroxynitrite) the administration of 100 μmol/kg peroxynitrite ($n = 37$). The data are presented as mean ± S.E. $^{*}P < 0.05$ post-peroxynitrite vs. pre-peroxynitrite.

strated within the vasculature in animal models of endotoxemia (Szabo et al., 1995) and in human inflammatory diseases including coronary atherosclerosis (Beckman et al., 1994), myocarditis (Kooy et al., 1997), acute lung injury (Kooy et al., 1995), and sepsis (Kooy et al., 1995, 1997).

Peroxynitrite is an important mediator of free radical toxicity with strong oxidizing properties towards biological molecules including protein and non-protein sulfhydryls (Radi et al., 1991a), deoxyribonucleic acid (King et al., 1992), and membrane phospholipids (Radi et al., 1991b). In addition to its role in oxidative reactions, peroxynitrite nitrates free or protein-associated tyrosines and other phenolics either spontaneously, or via the low molecular mass transition metal-, superoxide dismutase-, or carbon dioxide-catalyzed formation of a nitronium ion-like intermediate (Beckman et al., 1992; Ischiropoulos et al., 1992; Gow et al., 1996). Nitrotyrosine and the metabolites 3-nitro-4-hydroxyphenylacetic acid and 3-nitro-4-hydroxyphenylpropionic acid have been detected in human urine (Ohshima et al., 1990). Moreover, nitrotyrosine has been detected in

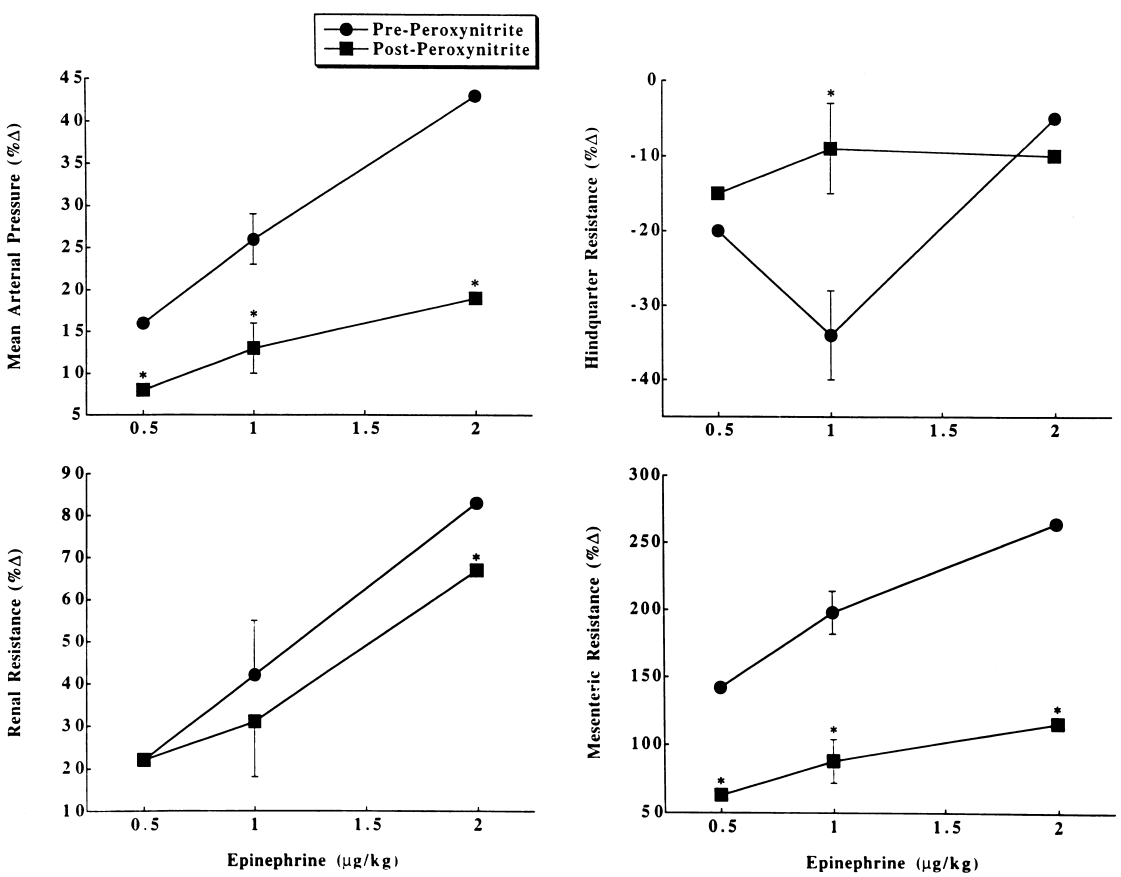

Fig. 2. Effects of peroxynitrite administration on the hemodynamic changes produced by the systemic administration of epinephrine. Epinephrine (0.5–2.0 μg/kg) was systemically administered to pentobarbital-anesthetized rats prior to (Pre-Peroxynitrite) and following (post-peroxynitrite) the systemic administration of peroxynitrite (100 μmol/kg). The changes in mean arterial pressure and hindquarter, renal, and mesenteric vascular resistances produced by the systemic administration of epinephrine are expressed as mean ± S.E. ($n = 5$) of the percentage changes from baseline. $^{*}P < 0.05$ post-peroxynitrite vs. pre-peroxynitrite.

the serum of humans with rheumatoid arthritis (Kaur and Halliwell, 1994) or sepsis (Fukuyama et al., 1997). Furthermore, nitrotyrosine, when systemically administered to pentobarbital-anesthetized rats, significantly attenuates the hemodynamic responses produced by α- and β-adrenoceptor agonists, suggesting that nitrotyrosine or its metabolites may be specific α- and β-adrenoceptor antagonists (Kooy and Lewis, 1996a).

Whereas peroxynitrite has been demonstrated within the vasculature in animal models of endotoxemia (Szabo et al., 1995) and human inflammatory diseases (Beckman et al., 1994; Kooy et al., 1995, 1997), characterization of the peroxynitrite-mediated alterations in vascular reactivity in vivo may be relevant for understanding the pathophysiology of altered vascular function in inflammation-mediated disease states. Herein, we demonstrate that the systemic administration of peroxynitrite significantly attenuates the hemodynamic responses produced by epinephrine, norepinephrine, the specific α_1-adrenoceptor agonist phenylephrine, and the β-adrenoceptor agonist isoproterenol, but has no effect on the hemodynamic responses to arginine vasopressin in pentobarbital-anesthetized rats.

2. Methods

2.1. Rats and surgical procedures

The experimental protocols described in this manuscript were approved by the Institutional Animal Care and Use Committee of The University of Iowa. Sprague–Dawley rats (Madison, WI) weighing between 250 and 350 g were anesthetized with pentobarbital (50 mg/kg, i.p.) and were surgically implanted with femoral arterial and venous catheters (PE-50, Becton Dickinson, 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 (Crystal Biotech, Northborough, MA) were placed around the lower abdominal aorta, renal, and superior 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 (Lacolley et al., 1991). To maintain anesthesia, supplemental doses of pentobarbital (5 mg, i.v.) were given as necessary throughout the experiments.

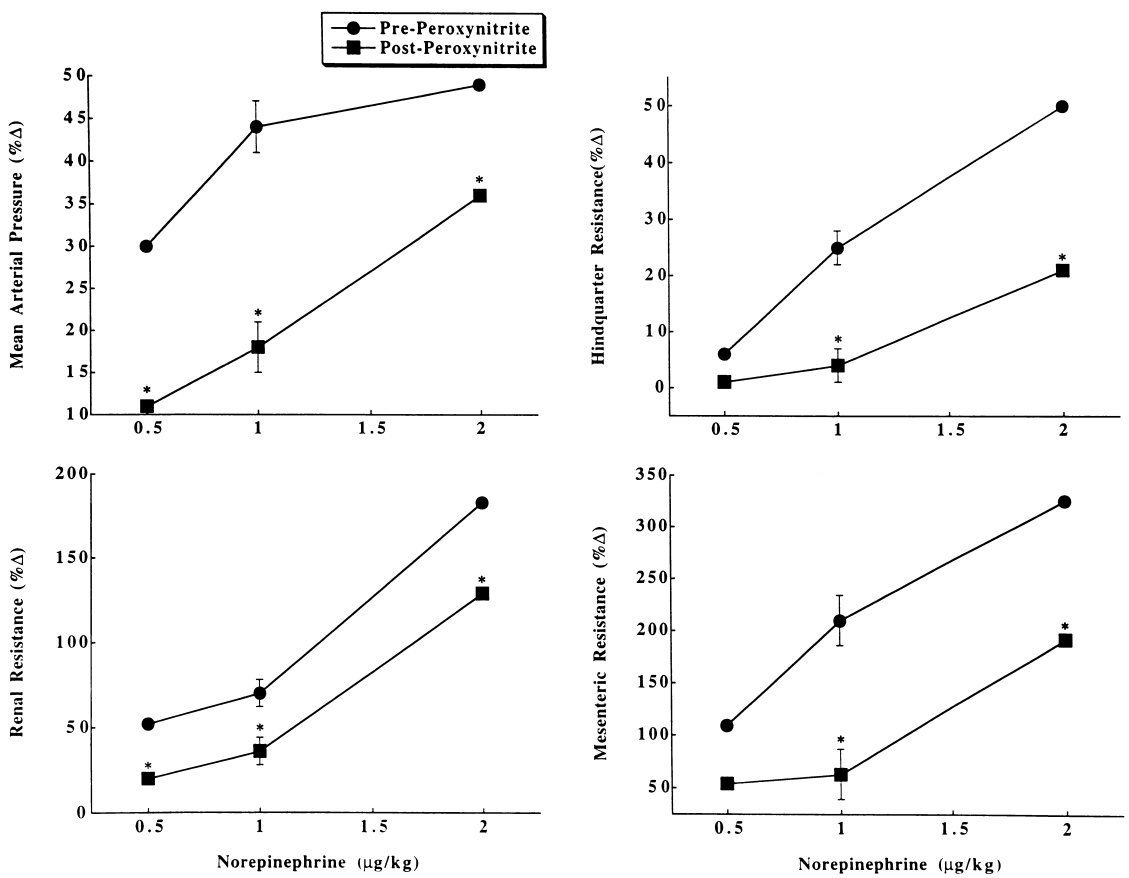

Fig. 3. Effects of peroxynitrite administration on the hemodynamic changes produced by the systemic administration of norepinephrine. Norepinephrine (0.5–2.0 μg/kg) was systemically administered to pentobarbital-anesthetized rats prior to (Pre-Peroxynitrite) and following (Post-Peroxynitrite) the systemic administration of peroxynitrite (100 μmol/kg). The changes in mean arterial pressure and hindquarter, renal, and mesenteric vascular resistances produced by the systemic administration of norepinephrine are expressed as mean ± S.E. ($n = 6$) of the percentage changes from baseline. $* P < 0.05$ Post-Peroxynitrite vs. Pre-Peroxynitrite.

2.2. Experimental protocols

The hemodynamic responses produced by the systemic administration of (i) the catecholamines epinephrine (0.5–2.0 μg/kg) or norepinephrine (0.5–2.0 μg/kg), (ii) the α_1-adrenoceptor agonist phenylephrine (1–4 μg/kg), (iii) the β-adrenoceptor agonist isoproterenol (100–800 ng/kg) or (iv) the non-catecholamine vasoconstrictor arginine vasopressin (50–250 ng/kg) were examined prior to and following the administration of ten consecutive bolus doses of peroxynitrite (10 μmol/kg, i.v.; total peroxynitrite dose = 100 μmol/kg). The second dose response for each of the adrenoceptor agonists was performed approximately 30 min following the final dose of peroxynitrite. The calculated biological half-life of peroxynitrite is approximately 0.6 sec (Ramezanian et al., 1996), eliminating a direct reaction of peroxynitrite with the administered vasoactive compounds as a mechanism for altered vascular responses. For all studies, decomposed peroxynitrite was used as a control.

2.3. Materials

Peroxynitrite was synthesized in a quench flow reactor as previously described (Beckman et al., 1990). Briefly, solutions of 0.6 M $NaNO_2$ and 0.6 M HCl/0.7 M H_2O_2 were vacuum suctioned into a tee-junction and mixed in glass tubing. The acid catalyzed reaction of nitrous acid with hydrogen peroxide to form peroxynitrous acid was quenched by adding 1.5 M NaOH into a second tee-junction at the end of the glass tubing. Excess hydrogen peroxide was removed by addition of hydrated manganese dioxide, which was subsequently removed by filtration. The peroxynitrite solution was stored at −70°C. Prior to each study, the concentration of peroxynitrite was determined spectrophotometrically ($\varepsilon_{302} = 1670\ M^{-1}\ cm^{-1}$). To decompose peroxynitrite, a sample of peroxynitrite was left at 20°C for 2–3 weeks after which no further absorbance was noted at 302 nm. Epinephrine, norepinephrine, phenylephrine, isoproterenol, and arginine vasopressin were from Sigma, St. Louis, MO. Pentobarbital

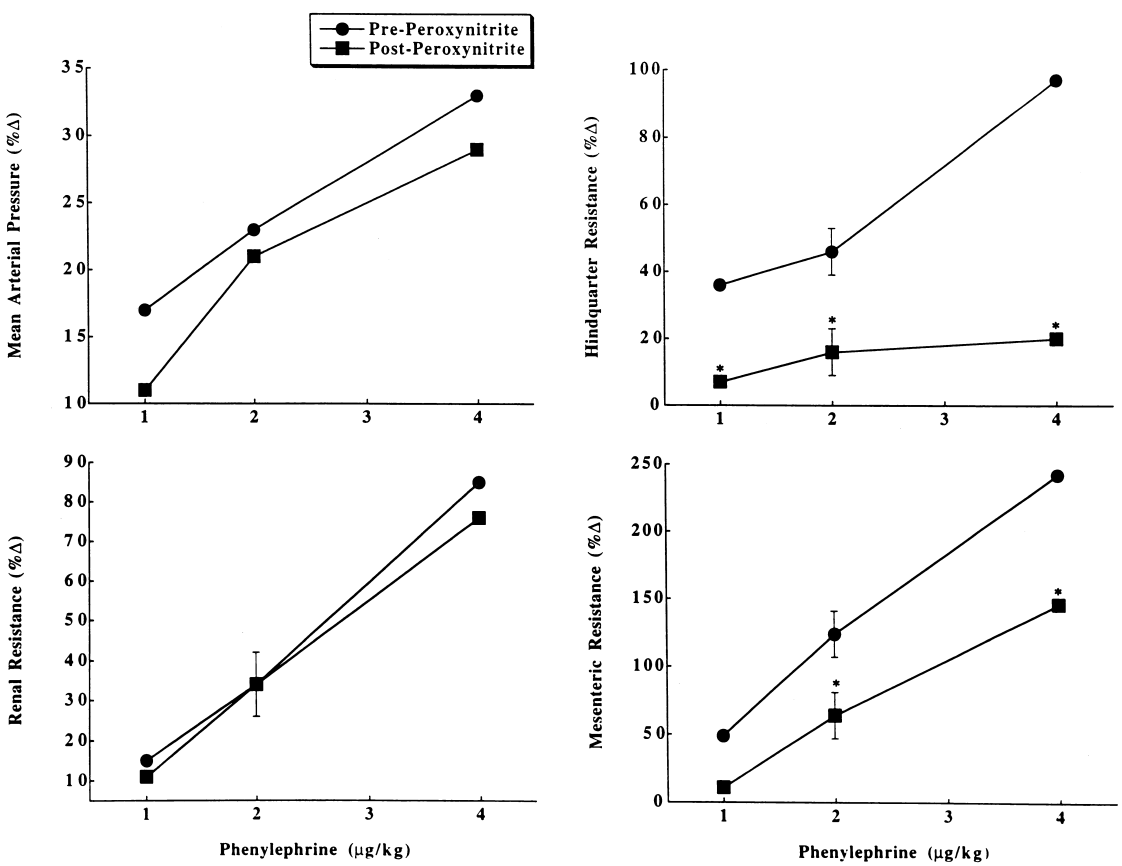

Fig. 4. Effects of peroxynitrite administration on the hemodynamic changes produced by the systemic administration of phenylephrine. Phenylephrine (1–4 μg/kg) was systemically administered to pentobarbital-anesthetized rats prior to (Pre-Peroxynitrite) and following (Post-Peroxynitrite) the systemic administration of peroxynitrite (100 μmol/kg). The changes in mean arterial pressure and hindquarter, renal, and mesenteric vascular resistances produced by the systemic administration of phenylephrine are expressed as mean ± S.E. ($n = 6$) of the percentage changes from baseline. $* P < 0.05$ Post-Peroxynitrite vs. Pre-Peroxynitrite.

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

2.4. Statistics

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. The SE 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. Effect of peroxynitrite on baseline hemodynamic variables

A summary of the baseline hemodynamic variables recorded during the dose responses to epinephrine, norepinephrine, phenylephrine, and isoproterenol before and after the administration of peroxynitrite are shown in Fig. 1. Consistent with our previous report (Kooy and Lewis, 1996b), 30 min following the administration of peroxynitrite significant increases in hindquarter, renal, and mesenteric vascular resistances were evident without changes in mean arterial pressure. The changes in vascular resistance were not significantly different between groups of animals receiving epinephrine, norepinephrine, phenylephrine, isoproterenol, or arginine vasopressin.

The systemic administration of decomposed peroxynitrite had no effect on the baseline hemodynamic variables (data not shown).

3.2. Effects of peroxynitrite on the hemodynamic responses produced by epinephrine, norepinephrine, phenylephrine, isoproterenol, and arginine vasopressin

The changes in mean arterial pressure and vascular resistances produced by the systemic administration of epinephrine prior to and following the administration of peroxynitrite are summarized in Fig. 2. Epinephrine produced dose-dependent increases in mean arterial pressure

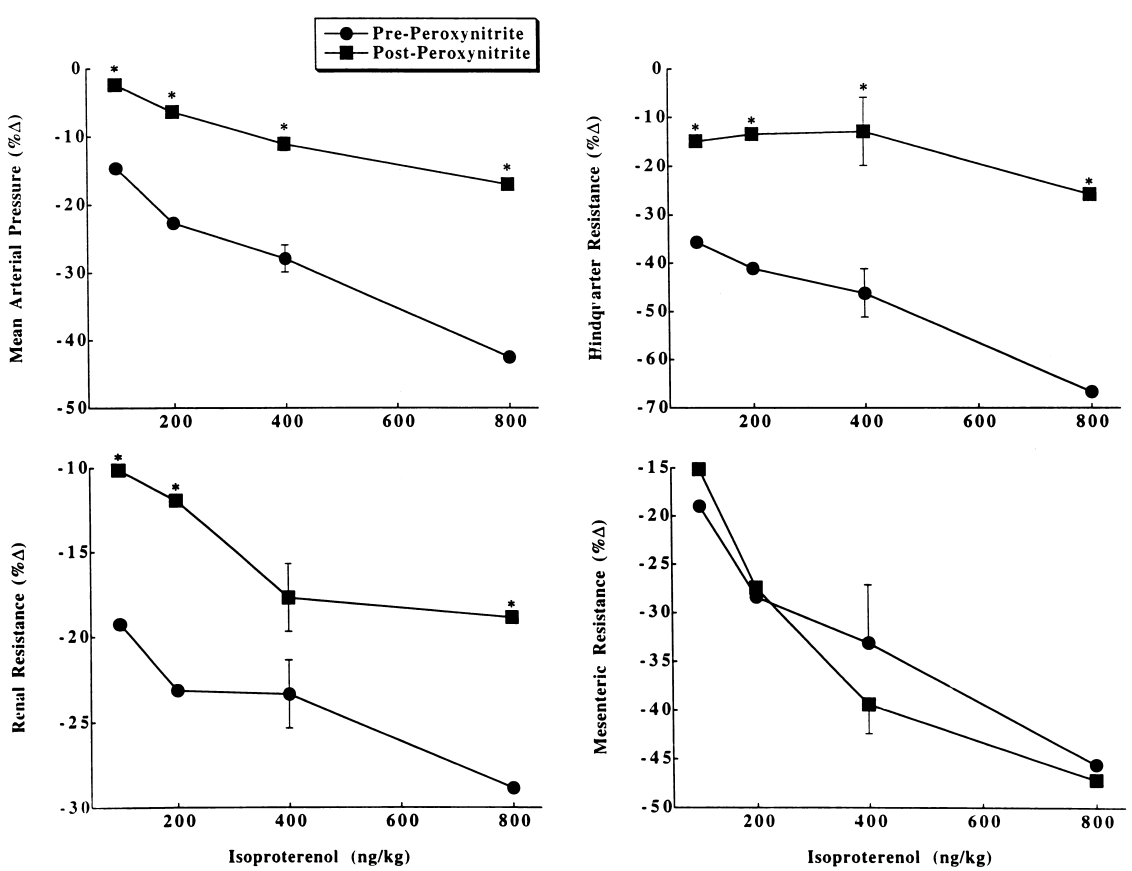

Fig. 5. Effects of peroxynitrite administration on the hemodynamic changes produced by the systemic administration of isoproterenol. Isoproterenol (100–800 ng/kg) was systemically administered to pentobarbital-anesthetized rats prior to (Pre-Peroxynitrite) and following (Post-Peroxynitrite) the systemic administration of peroxynitrite (100 μmol/kg). The changes in mean arterial pressure and hindquarter, renal, and mesenteric vascular resistances produced by the systemic administration of isoproterenol are expressed as mean ± S.E. ($n = 5$) of the percentage changes from baseline. * $P < 0.05$ Post-Peroxynitrite vs. Pre-Peroxynitrite.

accompanied by dose-dependent increases in renal and mesenteric vascular resistances and dose-independent decreases in hindquarter vascular resistance. The epinephrine-induced pressor and renal and mesenteric vasoconstrictor responses were significantly attenuated following the administration of peroxynitrite. The epinephrine-induced hindquarter vasodilatory responses were also significantly attenuated at the 1 μg/kg dose.

The changes in mean arterial pressure and vascular resistances produced by the systemic administration of norepinephrine prior to and following the administration of peroxynitrite are summarized in Fig. 3. Norepinephrine produced dose-dependent increases in mean arterial pressure accompanied by dose-dependent increases in hindquarter, renal, and mesenteric vascular resistances. The norepinephrine-induced pressor and vasoconstrictor responses were significantly attenuated following the administration of peroxynitrite.

The changes in mean arterial pressure and vascular resistances produced by the systemic administration of phenylephrine prior to and following the administration of peroxynitrite are summarized in Fig. 4. Phenylephrine produced dose-dependent increases in mean arterial pressure accompanied by dose-dependent increases in hindquarter, renal, and mesenteric vascular resistances. The phenylephrine-induced hindquarter and mesenteric vasoconstrictor responses were significantly attenuated following the administration of peroxynitrite.

The changes in mean arterial pressure and vascular resistances produced by the systemic administration of isoproterenol prior to and following the administration of peroxynitrite are summarized in Fig. 5. Isoproterenol produced dose-dependent decreases in mean arterial pressure accompanied by dose-dependent decreases in hindquarter, renal, and mesenteric vascular resistances. The isoproterenol-induced depressor and hindquarter and renal vasodilator responses were significantly attenuated following the administration of peroxynitrite.

The changes in mean arterial pressure and vascular resistances produced by the systemic administration of arginine vasopressin prior to and following the administration of peroxynitrite are summarized in Fig. 6. Arginine vasopressin produced dose-dependent increases in mean arterial pressure accompanied by dose-dependent increases

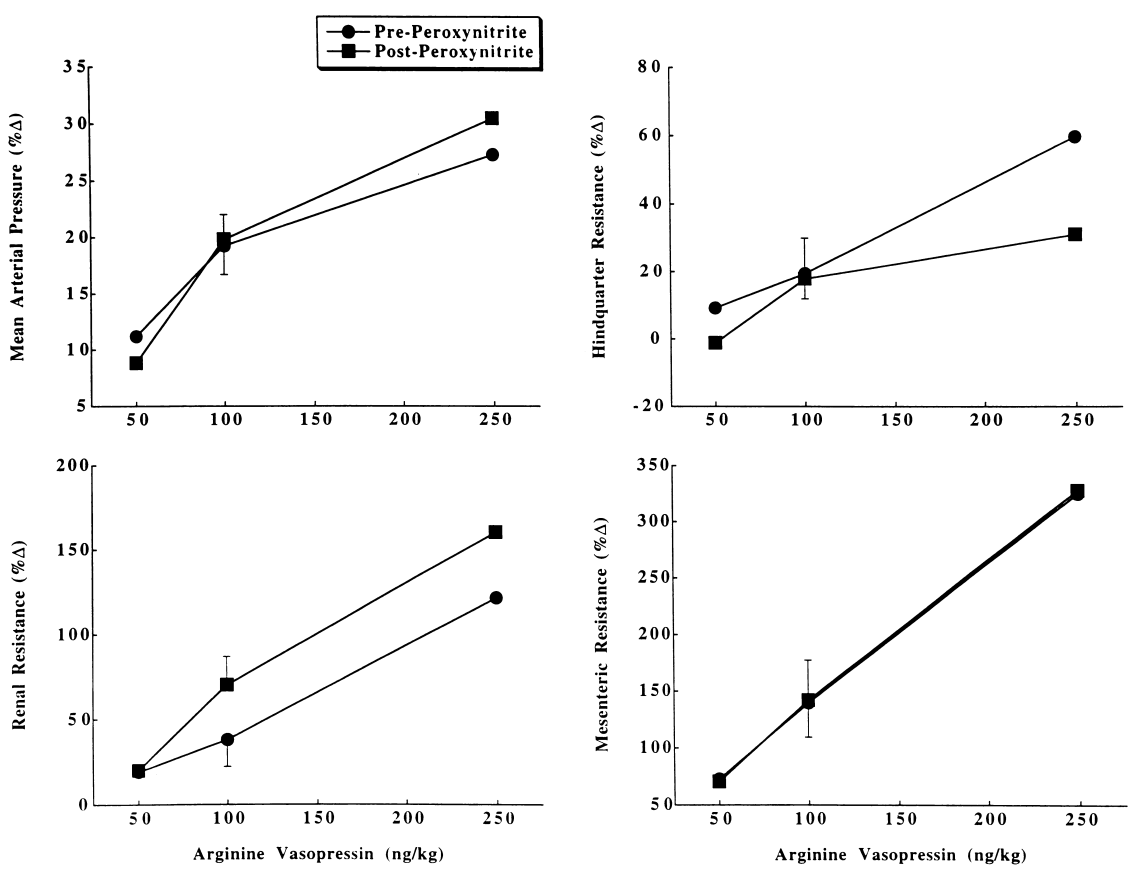

Fig. 6. Effects of peroxynitrite administration on the hemodynamic changes produced by the systemic administration of arginine vasopressin. Arginine vasopressin (50–250 ng/kg) was systemically administered to pentobarbital-anesthetized rats prior to (Pre-Peroxynitrite) and following (Post-Peroxynitrite) the systemic administration of peroxynitrite (100 μmol/kg). The changes in mean arterial pressure and hindquarter, renal, and mesenteric vascular resistances produced by the systemic administration of arginine vasopressin are expressed as mean ± S.E. ($n = 9$) of the percentage changes from baseline. Note there are no statistically significant differences at $P < 0.05$.

in hindquarter, renal, and mesenteric vascular resistances, which remained unchanged following the administration of peroxynitrite.

The hemodynamic responses produced by epinephrine, norepinephrine, phenylephrine, isoproterenol, and arginine vasopressin were similar prior to and following the administration of decomposed peroxynitrite (data not shown).

4. Discussion

The systemic administration of peroxynitrite significantly attenuated the hemodynamic effects produced by the catecholamines epinephrine and norepinephrine in pentobarbital-anesthetized rats. Moreover, peroxynitrite also attenuated the vasoconstrictor responses produced by the α_1-adrenoceptor agonist phenylephrine and the depressor and vasodilator responses produced by the β-adrenoceptor agonist isoproterenol. As previously demonstrated, the hemodynamic effects produced by the systemic administration of bradykinin remained unchanged following the systemic administration of peroxynitrite (Benkusky et al., 1998). These findings demonstrate that peroxynitrite, at the concentration used in the present study, does not inhibit endothelial or vascular smooth muscle function via nonspecific cellular injury. Moreover, the hemodynamic responses produced by arginine vasopressin and nitric oxide (Benkusky et al., 1998) were not altered by the prior administration of peroxynitrite demonstrating the capacity for the vascular smooth muscle to respond to noncatecholamine modulators of vascular tone. Taken together, these results suggest that endogenous vascular endothelial cell- and/or vascular smooth muscle cell-derived peroxynitrite may selectively interfere with adrenergic vascular responses leading to significant alteration in vascular function during states of inflammation, such as atherosclerosis, ischemia-reperfusion, or sepsis, where the formation of peroxynitrite is favored.

Given the diversity of the cellular transduction mechanisms responsible for α-adrenoceptor-mediated vasoconstriction and β-adrenoceptor-mediated vasorelaxation, the most likely site for peroxynitrite-mediated inhibition of these responses is at the receptor level. Peroxynitrite is a potent oxidant, with strong oxidizing properties towards biological molecules including protein and non-protein sulfhydryls (Radi et al., 1991a), deoxyribonucleic acid (King et al., 1992), and membrane phospholipids (Radi et al., 1991b). In addition to its role in oxidative reactions, peroxynitrite nitrates free or protein-associated tyrosines and other phenolics via the low molecular mass metal-, superoxide dismutase-, or carbon dioxide-catalyzed formation of a nitronium ion-like intermediate (Beckman et al., 1992; Ischiropoulos et al., 1992; Gow et al., 1996). As such, peroxynitrite may directly alter the tertiary protein structure of α- and β-adrenoceptors, leading to decreased receptor affinity and decreased catecholaminergic responses.

Alternatively, peroxynitrite-mediated nitration of free tyrosine within the blood may serve as a mechanism whereby peroxynitrite inhibits adrenoceptor activity. The chemical structure of nitrotyrosine is similar to that of the endogenous catecholamines and other adrenoceptor agonists/antagonists, raising the possibility that nitrotyrosine or its metabolites may be direct adrenoceptor antagonists. Moreover, the systemic administration of 2.5 μmol/kg nitrotyrosine significantly attenuates the hemodynamic responses to norepinephrine, epinephrine, phenylephrine, and isoproterenol in pentobarbital-anesthetized rats (Kooy and Lewis, 1996a). Whether the systemic administration of peroxynitrite in the present studies results in significant serum nitrotyrosine concentrations is unknown, since nitrotyrosine concentrations were not measured. However, a comparable serum concentration of nitrotyrosine to that of the aforementioned study (Kooy and Lewis, 1996a) would require only a 2–3% yield from the administration of 100 μmol/kg peroxynitrite. Therefore, the presence of significant serum nitrotyrosine concentrations in the current studies is highly probable.

Despite attenuation of α_1-adrenoceptor-mediated vasoconstriction, the systemic administration of peroxynitrite produces time-dependent increases in hindquarter, renal, and mesenteric vascular resistance. These peroxynitrite-mediated increases in vascular tone may result from (i) attenuation of endothelium-dependent vasorelaxation (Benkusky et al., 1998), (ii) incomplete attenuation of neurogenic vasoconstriction, (iii) concomitant loss of β-adrenoceptor-mediated vasodilatation, or (iv) increased vascular smooth muscle intracellular calcium due to peroxynitrite-mediated alteration in voltage-sensitive calcium channel, sodium–calcium exchanger, or calcium ATPase function (Ishida et al., 1996). Although the vascular resistances are increased following the administration of peroxynitrite, the mean arterial pressure remains unchanged, suggesting that the systemic administration of peroxynitrite leads to a decrease in cardiac output. This decrease in cardiac output may occur through the attenuation of myocardial β_1-adrenoceptor function, as suggested by the attenuation in heart rate response to isoproterenol (data not shown). Moreover, epinephrine and norepinephrine are α- and β-adrenoceptor agonists which increase mean arterial pressure via vascular smooth muscle α-adrenoceptor-mediated vasoconstriction and myocardial β_1-adrenoceptor-mediated increases in cardiac output. Therefore, attenuation of β_1-adrenoceptor activity would also explain why peroxynitrite administration inhibits the pressor responses to epinephrine and norepinephrine but not to the α-adrenoceptor agonist phenylephrine.

In conclusion, peroxynitrite (i) is produced by vascular endothelial (Kooy and Royall, 1994) and smooth muscle cells (Boota et al., 1996), (ii) is present within the vasculature in animal models of inflammation (Szabo et al., 1995)

and in human inflammatory diseases (Beckman et al., 1994; Kooy et al., 1995, 1997), and (iii) selectively attenuates the hemodynamic actions of the endogenous catecholamines epinephrine and norepinephrine. Therefore, peroxynitrite-mediated impairment of adrenoceptors may play a critical role in the hemodynamic dysfunction associated with inflammatory conditions.

Acknowledgements

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