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Potential role of nitration and oxidation reactions in the effects of peroxynitrite on the function of β-adrenoceptor sub-types in the rat

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Abstract

This study examined the hemodynamic responses elicited by the β-adrenoceptor agonist, isoproterenol (1 and 10 μg/kg, i.v.) before and after administration of (i) peroxynitrite (10 × 10 μmol/kg, i.v.), (ii) the thiol chelator, *para*-hydroxymercurobenzoic acid (pHMBA, 75 μmol/kg, i.v.), and (iii) the electron acceptor, nitroblue tetrazolium (NBT, 10 μmol/kg, i.v.) in pentobarbital-anesthetized rats. The tachycardia elicited by the lower dose of isoproterenol was diminished whereas the tachycardia elicited by the higher dose was not attenuated after administration of peroxynitrite. The falls in hindquarter and renal vascular resistances elicited by both doses of isoproterenol were substantially diminished whereas the isoproterenol-induced falls in mesenteric vascular resistance were not changed after administration of peroxynitrite. All of the isoproterenol-induced responses were markedly attenuated after administration of pHMBA or NBT. These findings suggest that the oxidation and/or nitration of β-adrenoceptors impair the ability of isoproterenol to bind to and/or activate these G protein-coupled receptors. β_1-, β_2- and β_3-adrenoceptors contain extracellular cysteine residues susceptible to oxidation (i.e., disulfide-bridge formation) whereas only the β_1- and β_2-adrenoceptors contain extracellular tyrosine residues susceptible to nitration. These findings also suggest that sustained impairment of β_1- and β_2-adrenoceptor function by peroxynitrite is due to nitration of extracellular tyrosine residues in these receptors. By analogy, β_3-adrenoceptors may not be permanently affected by peroxynitrite because these receptors are devoid of extracellular tyrosine residues.

Keywords: β-adrenoceptor; Peroxynitrite; Redox; Nitration; Vasodilation; Heart rate; (Rat)

1. Introduction

The vasodilator responses elicited by systemic injections of the β-adrenoceptor agonist, isoproterenol, in the hindquarter and renal beds of pentobarbital-anesthetized rats are markedly impaired after administration of peroxynitrite (Benkusky et al., 1999). Peroxynitrite is a powerful oxidant and promotes cysteine–cysteine (i.e., disulfide bond) formation in proteins (Radi et al., 1991a,b). The peroxynitrite-induced oxidation of cysteine residues would be readily subject to reduction by electron donors such as free thiols, catecholamines and superoxide anion (see Altman, 1976). These findings suggest that peroxynitrite may transiently impair the function of β-adrenoceptors by oxidation of cysteine residues in these receptors and that the rate of recovery of function of β-adrenoceptors will depend upon the presence and activity of endogenous electron donors. In addition, peroxynitrite readily nitrates free and protein-associated tyrosine residues (Beckman et al., 1994; Kooy et al., 1997). The covalent attachment of $-NO_2$ to tyrosine residues is essentially irreversible (see Kooy et al., 1997). As such, the loss of isoproterenol-induced vasodilation in the

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hindquarter and renal beds may be due to the "permanent" nitration of tyrosine residues in β-adrenoceptors.

In contrast, the vasodilator responses produced by isoproterenol in the mesenteric bed were not diminished after administration of peroxynitrite (Benkusky et al., 1999). This suggests that the vasodilator actions of isoproterenol in the mesenteric bed are mediated by β-adrenoceptors, which are not susceptible to peroxynitrite-induced oxidation and/or nitration. The extracellular domains of β_1-, β_2- and β_3-adrenoceptors contain thiol residues capable of forming disulfide bridges (Probst et al., 1992; Emorine et al., 1994). It would appear that peroxynitrite-mediated oxidation of cysteine residues would equally diminish the function of these β-adrenoceptor subtypes. However, the oxidation of β-adrenoceptors may not be responsible for the sustained loss of function after administration of peroxynitrite. Specifically, although peroxynitrite probably oxidizes these β-adrenoceptors, it is likely that these receptors are readily reduced by electron donors in the extracellular fluid. In addition, β_1- and β_2-adrenoceptors contain extracellular tyrosine residues, which are susceptible to nitration whereas β_3-adrenoceptors do not (Probst et al., 1992; Emorine et al., 1994). It could be hypothesized that (i) the vasodilator actions of isoproterenol in hindquarter and renal beds are mediated by β_1- and β_2-adrenoceptors susceptible to peroxynitrite-mediated nitration, and (ii) the vasodilator actions of isoproterenol in the mesenteric bed involves the activation of β_3-adrenoceptors, which are not susceptible to nitration.

The hypothesis that peroxynitrite produces a sustained impairment of G_s protein-coupled receptor function by nitration of the receptors is supported by evidence that peroxynitrite attenuates the vasoconstrictor effects of the α_1-adrenoceptor agonist, phenylephrine whereas it does not impair the vasoconstrictor actions of arginine vasopressin (AVP) (Benkusky et al., 1999). The vasoconstrictor actions of phenylephrine are mediated primarily by activation of α_{1A}-adrenoceptors (Blue et al., 1995; Zhu et al., 1997), which contain extracellular cysteine residues capable of disulfide-bond formation and tyrosine residues susceptible to peroxynitrite-induced nitration (Probst et al., 1992; Ford et al., 1994). In contrast, the vasoconstrictor effects of AVP are mediated by AVP V_{1A} receptors, which contain extracellular cysteine residues capable of disulfide-bond formation whereas they do not contain tyrosine residues (Probst et al., 1992; Morel et al., 1992). The aim of this study was to provide functional evidence that the prolonged effects of peroxynitrite on G_s protein-coupled β-adrenoceptor function is due to nitration of tyrosine residues rather than oxidation of cysteine residues in these receptors.

2. Materials and methods

2.1. Rats and experimental procedures

The protocols described below were approved by the University of Iowa Animal Care and Use Committee. Male Sprague–Dawley rats weighing 250–350 g were obtained from Harlan (Madison, WI, USA). All rats were anesthetized with pentobarbital (50 mg/kg, i.p.). A catheter was placed in a femoral artery to measure pulsatile and mean arterial blood pressure and to determine heart rate. A catheter was placed in femoral vein to give drugs. A midline laparotomy was performed and miniature pulsed Doppler flow probes were placed on the lower abdominal aorta, a renal artery and the superior mesenteric artery to measure hindquarter, renal and mesenteric blood flow velocities, and to determine hindquarter, renal and mesenteric vascular resistances, respectively (Benkusky et al., 1998, 1999). Supplemental doses of pentobarbital (3–5 mg, i.v.) were given to maintain anesthesia as necessary throughout surgery and experimentation. The body temperature of each rat was kept at 37 °C with the aid of a heating pad. The rats breathed room air supplemented with 95% O_2–5% CO_2.

The arterial catheter was connected to a Beckman-Dynograph-coupled pressure transducer for the measurement of pulsatile and mean arterial blood pressure. Heart rate was determined from the pulsatile arterial pressure by a Beckman-Dynograph-coupled cardiotachometer. The Doppler leads were connected to a Beckman-Dynograph-coupled Doppler Flowmeter (Department of Bioengineering, University of Iowa) to record blood flow velocities. Vascular resistances were determined by dividing mean arterial blood pressure values by blood flow velocities. The details about the construction reliability of the probes for measuring blood flow velocities, and the determination of percent changes in vascular resistances have been described previously (see Haywood et al., 1981). The rats were allowed 30 min to stabilize before any drug was given.

2.2. Experimental protocols

2.2.1. Effects of isoproterenol before and after administration of saline

A group of rats ($n=7$) received bolus injections of isoproterenol (0.1 and 1.0 μg/kg, i.v.) before and between 20–30 min and again between 90–105 min after an injection of saline. The injections of isoproterenol at 20–30 min post-saline served as a control for the propranolol studies in which the injections of isoproterenol were given 20–30 min after administration of the $\beta_{1,2}$-adrenoceptor antagonist. The injections of isoproterenol at 90–105 min post-saline served as a control for the peroxynitrite, pHMBA and NBT studies. The ten injections of peroxynitrite took 70 min to administer. The injections of isoproterenol were given between 20–30 min after the last injection of peroxynitrite. Accordingly, the injections of isoproterenol were given 90–105 min after the first injection of peroxynitrite. The doses of isoproterenol were given between 60–75 min after the second of two injections of NBT or pHMBA. The two injections of NBT and pHMBA were given 30 min apart. Accordingly, the injections of isoproterenol were given between 90–105 min after the first injection of NBT or pHMBA. The hemodynamic actions produced by the lower dose of isoproterenol were allowed to subside fully before the higher dose was given.

2.2.2. Effects of isoproterenol before and after administration of propranolol

Another group of rats ($n=6$) received bolus injections of isoproterenol (0.1 and 1.0 μg/kg, i.v.) before and 20–30 min

Table 1
Effects of saline or propranolol on resting hemodynamic parameters

Treatment	N	Parameter	Pre	Post	%Change
Saline	7	HR (bpm)	366±11	369±12	+1±7
		MAP (mm Hg)	104±3	103±3	−1±4
		HQR (mm Hg/kHz)	42±5	41±4	−3±5
		RR (mm Hg/kHz)	69±6	72±8	+2±4
		MR (mm Hg/kHz)	31±4	33±4	+5±4
Propranolol	6	HR (bpm)	352±14	301±12	−14±2[a]
		MAP (mm Hg)	106±3	105±3	−1±2
		HQR (mm Hg/kHz)	54±7	55±6	+1±4
		RR (mm Hg/kHz)	66±10	69±10	+2±4
		MR (mm Hg/kHz)	35±4	36±3	+2±3

Each value is the mean±S.E.M. HR=heart rate. MAP=mean arterial blood pressure. HQR=hindquarter vascular resistance. MR=mesenteric vascular resistance. RR=renal vascular resistance. *N*=number of rats. The dose of propranolol was 1 mg/kg, i.v. [a]$P<0.05$, significant change from pre-injection values.

after administration of the $\beta_{1,2}$-adrenoceptor antagonist, propranolol (1 mg/kg, i.v.). The hemodynamic affects of propranolol, and especially the bradycardia had reached their plateau levels by 20 min and stayed at these levels for the duration of the experiments.

2.2.3. Effects of isoproterenol before and after administration of peroxynitrite

A group of rats ($n=5$) received injections of isoproterenol (0.1 and 1.0 μg/kg, i.v.) before and after ten injections of peroxynitrite (10 μmol/kg, i.v.) given 5–10 min apart. The responses elicited by each peroxynitrite injection were allowed to subside fully before another injection was given. The injections of isoproterenol were given 20–30 min after the last injection of peroxynitrite at which time resting parameters had recovered from the effects of the last peroxynitrite.

2.2.4. Effects of isoproterenol before and after administration of pHMBA

Another group of rats ($n=8$) received bolus injections of isoproterenol (0.1 and 1.0 μg/kg, i.v.) before and after the administration of two injections of the lipophobic high molecular weight thiol chelator, *para*-hydroxymercurobenzoic acid (pHMBA, 25 and 50 μmol/kg, i.v.) (see Hoque et al., 1999), given 30 min apart. The hemodynamic responses produced by the lower dose of pHMBA were allowed to subside completely before the higher dose was given. The injections of isoproterenol were given 60–75 min after the last injection of pHMBA by which time the long-lasting affects of pHMBA on hemodynamic parameters had reached plateau levels.

2.2.5. Effects of isoproterenol before and after administration of NBT

Another group of rats ($n=8$) received bolus injections of isoproterenol (0.1 and 1.0 μg/kg, i.v.) before and after administration of two injections of the long-acting electron acceptor, nitroblue tetrazolium (NBT, 5 μmol/kg, i.v.) (Davisson et al., 1993; Hoque et al., 2000), given 30 min apart. The responses elicited by the lower dose of NBT were allowed to subside completely before the higher dose was given. The injections of isoproterenol were given 60–75 min after the last injection of NBT by which time the long-lasting affects of NBT had reached plateau levels.

2.3. Drugs

All drugs were from Sigma (St. Louis, MO, USA). Peroxynitrite was synthesized as described previously (Benkusky et al., 1998, 1999). All drugs were dissolved and/or diluted for injection in saline.

2.4. Statistical analyses

The data are presented as the mean±S.E.M. The data were analyzed by repeated measures analysis of variance followed by Students modified *t*-test with the Bonferroni correction for multiple comparisons between means (see Benkusky et al., 1998, 1999).

3. Results

3.1. Effects of treatments on resting hemodynamic parameters

Resting hemodynamic parameters recorded before and 60–90 min after administration of saline or 20–30 min after administration of propranolol (1 mg/kg, i.v.) are summarized in Table 1. Resting parameters recorded between 60–90 min after injection of saline were not different from pre-injection values ($P>0.05$, for all responses). The administration of propranolol elicited an immediate fall in heart rate (−18±3%, $P<0.05$) but no changes in mean arterial blood pressure or vascular resistances ($P>0.05$, for all responses, data not shown). Resting heart rate recorded 20–30 min after administration of propranolol was lower than pre-injection levels ($P<0.05$) whereas mean arterial blood pressure and vascular resistances were similar to pre-injection values ($P>0.05$, for all responses). The initial hemodynamic

Table 2
Effects of peroxynitrite, pHMBA or NBT on resting hemodynamic parameters

Treatment	N	Parameter	Pre	Post	%Change
Peroxynitrite	5	HR (bpm)	378±13	370±14	−1±6
		MAP (mm Hg)	107±3	119±3	+10±2[a]
		HQR (mm Hg/kHz)	69±7	89±5	+25±6[a]
		RR (mm Hg/kHz)	97±6	123±9	+23±4[a]
		MR (mm Hg/kHz)	29±3	38±3	+28±4[a]
pHMBA	8	HR (bpm)	346±12	300±10	−12±2[a]
		MAP (mm Hg)	104±3	82±3	−20±5[a]
		HQR (mm Hg/kHz)	49±4	65±5	+31±5[a]
		RR (mm Hg/kHz)	86±9	117±8	+36±6[a]
		MR (mm Hg/kHz)	37±4	52±5	+39±6[a]
NBT	8	HR (bpm)	349±10	317±11	−8±2[a]
		MAP (mm Hg)	107±3	94±3	−12±3[a]
		HQR (mm Hg/kHz)	44±5	73±6	+59±8[a]
		RR (mm Hg/kHz)	77±8	105±9	+34±7[a]
		MR (mm Hg/kHz)	39±5	66±7	+63±10[a]

Each value is the mean±S.E.M. Ten injections of peroxynitrite (10 μmol/kg, i.v.) were given. pHMBA=*para*-hydroxymercurobenzoic acid (75 μmol/kg, i.v., in total). NBT=nitroblue tetrazolium (10 μmol/kg, i.v., in total). HR=heart rate. MAP=mean arterial blood pressure. HQR=hindquarter vascular resistance. MR=mesenteric vascular resistance. RR=renal vascular resistance. *N*=number of rats. [a]$P<0.05$, significant change from pre-injection values.

Table 3
Hemodynamic effects of isoproterenol before and after administration of saline

Isoproterenol	Parameter	Pre	Post	%Change
0.1 μg/kg	ΔHR (%)	+16±2[a]	+17±2[a]	+4±5
	ΔMAP (%)	−17±3[a]	−18±3[a]	+5±4
	ΔHQR (%)	−34±4[a]	−35±4[a]	+4±3
	ΔRR (%)	−19±3[a]	−19±3[a]	0±4
	ΔMR (%)	−25±3[a]	−26±3[a]	+3±3
1.0 μg/kg	ΔHR (%)	+33±3[a]	+32±4[a]	−3±4
	ΔMAP (%)	−42±4[a]	−44±3[a]	+5±4
	ΔHQR (%)	−58±6[a]	−61±7[a]	+5±4
	ΔRR (%)	−34±3[a]	−38±4[a]	+9±6
	ΔMR (%)	−49±5[a]	−53±6[a]	+7±5

Each value is the mean±S.E.M. HR=heart rate. MAP=mean arterial blood pressure. HQR=hindquarter vascular resistance. MR=mesenteric vascular resistance. RR=renal vascular resistance. There were seven rats in the group. [a]$P<0.05$, significant response. Note, the hemodynamic responses produced by both doses of isoproterenol were similar before and after administration of saline ($P>0.05$, for all comparisons).

responses produced by systemic injections of peroxynitrite (Benkusky et al., 1998, 1999), pHMBA (Hoque et al., 1999) and NBT (Davisson et al., 1993; Hoque et al., 2000) have been described in detail previously. Resting hemodynamic values recorded before and 60–75 min after administration of peroxynitrite (10×10 μmol/kg, i.v.), pHMBA (25 and 50 μmol/kg, i.v.), or NBT (2×5 μmol/kg, i.v.), are summarized in Table 2. Resting mean arterial blood pressure, hindquarter, renal and mesenteric vascular resistances were elevated 60–75 min after the last injection of peroxynitrite whereas heart rate was not different to pre-injection values ($P<0.05$ for all responses). Resting mean arterial blood pressure and heart rate was reduced whereas hindquarter, renal and mesenteric vascular resistances were elevated 60–75 min after the last injection of pHMBA ($P<0.05$ for all responses). Resting mean arterial blood pressure and heart rate was reduced whereas hindquarter, renal and mesenteric vascular resistances were elevated 60–90 min after the last injection of NBT ($P<0.05$ for all comparisons).

Table 4
Hemodynamic effects of isoproterenol before and after administration of propranolol

Isoproterenol	Parameter	Pre	Post	%Change
0.1 μg/kg	ΔHR (%)	+17±3[a]	+2±2	−86±5[b]
	ΔMAP (%)	−19±2[a]	−4±2	−77±5[b]
	ΔHQR (%)	−31±3[a]	−2±2	−91±6[b]
	ΔRR (%)	−18±3[a]	−1±2	−90±5[b]
	ΔMR (%)	−26±3[a]	−12±3[a]	−51±7[b]
1.0 μg/kg	ΔHR (%)	+35±4[a]	+17±3[a]	−49±5[b]
	ΔMAP (%)	−44±4[a]	−12±3[a]	−71±6[b]
	ΔHQR (%)	−60±7[a]	−10±3[a]	−79±8[b]
	ΔRR (%)	−32±3[a]	−4±4	−87±6[b]
	ΔMR (%)	−51±5[a]	−34±3[a]	−31±4[b]

Each value is the mean±S.E.M. The dose of propranolol was 1 mg/kg, i.v. HR=heart rate. MAP=mean arterial blood pressure. HQR=hindquarter vascular resistance. MR=mesenteric vascular resistance. RR=renal vascular resistance. There were six rats in the group. [a]$P<0.05$, significant response. [b]$P<0.05$, post-propranolol versus pre.

Table 5
Hemodynamic effects of isoproterenol before and after administration of peroxynitrite

Isoproterenol	Parameter	Pre	Post	%Change
0.1 μg/kg	ΔHR (%)	+14±2[a]	+7±2[a]	−52±6[b]
	ΔMAP (%)	−18±3[a]	−3±3	−81±7[b]
	ΔHQR (%)	−30±3[a]	−6±4	−77±10[b]
	ΔRR (%)	−16±3[a]	−4±3	−73±9[b]
	ΔMR (%)	−28±2[a]	−25±3[a]	−7±6
1.0 μg/kg	ΔHR (%)	+37±4[a]	+33±4[a]	−8±5
	ΔMAP (%)	−46±3[a]	−10±3[a]	−76±7[b]
	ΔHQR (%)	−53±5[a]	−14±3[a]	−71±8[b]
	ΔRR (%)	−30±3[a]	−12±3[a]	−62±9[b]
	ΔMR (%)	−54±5[a]	−59±6[a]	+7±5

Each value is the mean±S.E.M. Ten injections of peroxynitrite (10 μmol/kg, i.v.) were given. HR=heart rate. MAP=mean arterial blood pressure. HQR=hindquarter vascular resistance. MR=mesenteric vascular resistance. RR=renal vascular resistance. There were five rats in the group. [a]$P<0.05$, significant response. [b]$P<0.05$, post-peroxynitrite versus pre.

3.2. Effects of saline and propranolol on the hemodynamic effects of isoproterenol

The hemodynamic responses elicited by the 0.1 and 1.0 μg/kg doses of isoproterenol before and after administration of saline are summarized in Table 3. These doses of isoproterenol elicited depressor responses and falls in vascular resistances, and an increase in heart rate. The responses produced by the 1.0 μg/kg dose of isoproterenol were greater than those produced by the 0.1 μg/kg dose ($P<0.05$ for all responses). The responses produced by these doses of isoproterenol were similar before and after administration of saline ($P>0.05$, for all comparisons). The responses elicited by the 0.1 and 1.0 μg/kg doses of isoproterenol before and after administration of propranolol (1 mg/kg, i.v.), are summarized in Table 4. The increase in heart rate elicited by the 0.1 μg/kg dose of isoproterenol was abolished by propranolol. The depressor response and falls in hindquarter and renal resistances elicited by the 0.1 μg/kg dose of isoproterenol were abolished whereas the fall in mesenteric vascular resistance was only partially attenuated by propranolol. The increase in heart rate elicited by the 1.0 μg/kg dose of isoproterenol was only partially attenuated by propranolol. The

Table 6
Hemodynamic effects of isoproterenol before and after administration of pHMBA

Isoproterenol	Parameter	Pre	Post	%Change
0.1 μg/kg	ΔHR (%)	+14±2[a]	+2±2	−78±6[b]
	ΔMAP (%)	−16±2[a]	−3±2	−80±8[b]
	ΔHQR (%)	−30±3[a]	−8±3[a]	−71±9[b]
	ΔRR (%)	−15±2[a]	−2±2	−84±7[b]
	ΔMR (%)	−25±3[a]	−6±4	−81±8[b]
1.0 μg/kg	ΔHR (%)	+39±3[a]	+7±1[a]	−79±10[b]
	ΔMAP (%)	−45±4[a]	−8±2[a]	−78±8[b]
	ΔHQR (%)	−54±5[a]	−12±3[a]	−76±11[b]
	ΔRR (%)	−28±3[a]	−3±3	−86±7[b]
	ΔMR (%)	−50±6[a]	−10±4[a]	−76±9[b]

Each value is the mean±S.E.M. pHMBA=*para*-hydroxymercurobenzoic acid (75 μmol/kg, i.v., in total). HR=heart rate. MAP=mean arterial blood pressure. HQR=hindquarter vascular resistance. MR=mesenteric vascular resistance. RR=renal vascular resistance. There were eight rats in the group. [a]$P<0.05$, significant response. [b]$P<0.05$, post-pHMBA versus pre.

falls in mean arterial blood pressure and hindquarter and renal vascular resistances elicited by the 1.0 μg/kg dose of isoproterenol were markedly attenuated whereas the fall in mesenteric vascular resistance was only partially attenuated by propranolol.

3.3. Effects of peroxynitrite on the hemodynamic actions of isoproterenol

The responses elicited by the 0.1 and 1.0 μg/kg doses of isoproterenol before and after administration of peroxynitrite (10 × 10 μmol/kg, i.v.) are summarized in Table 5. The increase in heart rate elicited by the 0.1 μg/kg dose of isoproterenol was abolished after administration of peroxynitrite. The depressor response and falls in hindquarter and renal vascular resistances elicited by the 0.1 μg/kg dose of isoproterenol were abolished whereas the fall in mesenteric vascular resistance was not diminished after administration of peroxynitrite. The increase in heart rate elicited by the 1.0 μg/kg dose of isoproterenol was not diminished after administration of peroxynitrite. The falls in mean arterial blood pressure and hindquarter and renal vascular resistances elicited by the 1.0 μg/kg dose of isoproterenol were markedly attenuated whereas the fall in mesenteric vascular resistance was not attenuated after administration of peroxynitrite.

3.4. Effects of pHMBA on the hemodynamic actions of isoproterenol

The responses elicited by the 0.1 and 1.0 μg/kg doses of isoproterenol before and after administration of pHMBA (75 μmol/kg, i.v., in total) are summarized in Table 6. The responses produced by these doses of isoproterenol were either abolished or markedly attenuated after administration of pHMBA.

3.5. Effects of pHMBA on the hemodynamic actions of isoproterenol

The responses elicited by the 0.1 and 1.0 μg/kg doses of isoproterenol before and after administration of NBT (2 × 5 μmol/kg, i.v.) are summarized in Table 7. The responses produced by these doses of isoproterenol were either abolished or substantially attenuated after administration of NBT.

Table 7
Hemodynamic effects of isoproterenol before and after administration of NBT

Isoproterenol	Parameter	Pre	Post	%Change
0.1 μg/kg	ΔHR (%)	+16±2[a]	+8±2[a]	−49±8[b]
	ΔMAP (%)	−17±3[a]	−5±1[a]	−68±7[b]
	ΔHQR (%)	−24±3[a]	−7±2[a]	−26±6[b]
	ΔRR (%)	−13±2[a]	−6±2[a]	−46±5[b]
	ΔMR (%)	−29±3[a]	−12±2[a]	−61±8[b]
1.0 μg/kg	ΔHR (%)	+35±3[a]	+16±3[a]	−51±7[b]
	ΔMAP (%)	−42±3[a]	−20±3[a]	−51±5[b]
	ΔHQR (%)	−49±5[a]	−28±4[a]	−39±6[b]
	ΔRR (%)	−27±3[a]	−14±3[a]	−43±8[b]
	ΔMR (%)	−55±7[a]	−32±6[a]	−37±6[b]

Each value is the mean±S.E.M. HR=heart rate. NBT=nitroblue tetrazolium (10 μmol/kg, i.v., in total). MAP=mean arterial blood pressure. HQR=hindquarter vascular resistance. MR=mesenteric vascular resistance. RR=renal vascular resistance. There were eight rats in the group. [a]$P<0.05$, significant response. [b]$P<0.05$, post-NBT versus pre.

4. Discussion

4.1. Role of β-adrenoceptors in the hemodynamic actions of isoproterenol

G_s protein-coupled β-adrenoceptors consist of β_1-, β_2- and β_3-adrenoceptors and an atypical β-adrenoceptor (Harden, 1983; Blue et al., 1989; Cohen et al., 1995a,b, 1999; Emorine et al., 1994; Kaumann, 1996; Kaumann and Molenaar, 1996; Malinkowski and Schlicker, 1996). β-adrenoceptor agonists elicit vasodilation primarily by activation of adenylate cyclase in vascular smooth muscle (Harden, 1983), although these agonists may also release endothelium-derived relaxing factors in some beds (see Whalen et al., 2000). β-adrenoceptor agonists increase pacemaker rate by alteration of ion-channel activity including the cAMP-mediated activation of L-type voltage-sensitive Ca^{2+}-channels (DiFransesco, 1993; Hartzell, 1993; Giles and Shibata, 1981; Irisawa et al., 1993). Catecholamines are potent agonists of β_1- and β_2-adrenoceptors but poor agonists at β_3-adrenoceptors whereas isoproterenol is a potent agonist of all three β-adrenoceptors (Emorine et al., 1994). Moreover, β_3-adrenoceptors are not blocked by doses of propranolol that block β_1-and β_2-adrenoceptors (Cohen et al., 1999; Whalen and Lewis, 1999).

Propranolol eliminated the vasodilator responses elicited by the 0.1 and 1.0 μg/kg doses of isoproterenol in the renal and hindquarter beds, suggesting that isoproterenol acted primarily via $\beta_{1,2}$-adrenoceptors. In contrast, propranolol only partially attenuated the isoproterenol-induced vasodilation in the mesenteric bed. Moreover, although propranolol abolished the increase in heart rate elicited by the 0.1 μg/kg dose of isoproterenol it only partially attenuated the tachycardia elicited by the 1.0 μg/kg dose. Taken together, these findings suggest that isoproterenol can increase heart rate and lower mesenteric resistance by activation of propranolol-insensitive β_3-adrenoceptors and/or atypical β-adrenoceptors (see Whalen and Lewis, 1999).

As mentioned, the ability of propranolol to attenuate the hemodynamic action of isoproterenol was not uniform. The tachycardia and vasodilator responses elicited by the 0.1 μg/kg dose of isoproterenol in the hindquarter and renal beds were abolished by propranolol. In contrast the vasodilator actions of this dose of isoproterenol in the mesenteric vascular bed were attenuated by about 50%. The tachycardia elicited by the 1.0 μg/kg dose of isoproterenol was attenuated by about 50%. In the addition, the vasodilator actions of this dose of isoproterenol in the renal bed was abolished by propranolol whereas the vasodilation in the hindquarter bed was reduced by approximately 80% by the $\beta_{1,2}$-adrenoceptor antagonist. In contrast, the vasodilator actions elicited by the 1.0 μg/kg dose of isoproterenol in the mesenteric bed were reduced by approximately 30% only by propranolol. Taken together, it appears that pacemaker cells in the heart and

vascular smooth muscle cells in the mesenteric circulation contain a substantial population of propranolol-insensitive β-adrenoceptors. In addition, the vascular smooth muscle in the hindquarter circulation appears to contain fewer propranolol-insensitive β-adrenoceptors whereas vascular smooth muscle in the renal circulation may not contain these receptors.

4.2. Effects of pHMBA on the hemodynamic actions of isoproterenol

pHMBA is a lipophobic thiol chelator that readily forms stable covalent bonds with plasma membrane thiol residues at physiological pH (Goodman and Hiatt, 1964). The vasodilator responses and tachycardia elicited by isoproterenol were markedly attenuated by pHMBA. $\beta_{1,2,3}$-adrenoceptors contain cysteine residues in their extracellular domains (Probst et al., 1992; Emorine et al., 1994). As such, pHMBA may reduce the actions of isoproterenol by chelating functional thiol residues in $\beta_{1,2,3}$-adrenoceptors although pHMBA may also chelate thiol residues in ion-channels or other proteins involved in β-adrenoceptor signal transduction (Harden, 1983). However, although pHMBA markedly attenuates α_1-adrenoceptor-mediated vasoconstriction, the thiol chelator augments 5-HT_2-receptor-mediated vasoconstriction (Hoque and Lewis, 1997). To our knowledge, α_1-adrenoceptors and 5-HT_2-receptors recruit similar signal transduction processes. Specifically, activation of these G protein-coupled receptors contracts vascular smooth muscle by enhancing Ca^{2+} release from intracellular storage pools and by opening voltage-sensitive Ca^{2+}-channels (Han et al., 1987; Mylechrane and Phillips, 1989; Fenuik and Humphrey, 1989; Saxena et al., 1989; Satake et al., 1992; Williams and Clarke, 1995).

The finding that phenylephrine-mediated vasoconstriction was attenuated whereas α-methyl-5-HT-mediated vasoconstriction was exaggerated after administration of NBT suggests that pHMBA does not impair voltage-sensitive Ca^{2+}-channels, G proteins or the intracellular processes by which α_1-adrenoceptors and 5-HT_2-receptors contract vascular smooth muscle. Rather, this finding suggests that pHMBA chelates functional thiol residues in the extracellular domains of α-adrenoceptors (see Probst et al., 1992; Ford et al., 1994) and 5-HT_2-receptors (Sanders-Bush, 1988; Zifa and Fillion, 1992) and that the chelation of these thiols has markedly different affects on the activity of these receptors. Moreover, although pHMBA markedly diminishes the vasodilator actions of L-*S*-nitrosocysteine, it does not impair those of the nitric oxide-donor, (*Z*)-1-{*N*-methyl-*N*-[6(*N*-methylammoniohexyl)amino]}diazen-1-ium-1,2-diolate (MAHMA NONOate) (Hoque et al., 1999). The later finding suggests that pHMBA does not interfere with nitric oxide-mediated activation of intracellular soluble guanylate cyclase or cGMP-dependent signaling (see Ignarro, 1990). As such, pHMBA may diminish the actions of isoproterenol by directly affecting β-adrenoceptors rather than G_s proteins, adenylate cyclase, or cAMP-mediated signal transduction process.

4.3. Effects of NBT on the hemodynamic actions of isoproterenol

NBT is a lipophobic electron acceptor that readily oxidizes thiol residues in biological membranes (Altman, 1976; Seidler, 1991; Seidler and van Noorden, 1994). The vasodilator responses and tachycardia produced by isoproterenol were attenuated by NBT although the degree of inhibition was less than that of pHMBA. The cysteine residues in $\beta_{1,2,3}$-adrenoceptors are capable of disulfide-bridge formation (Probst et al., 1992; Emorine et al., 1994). Accordingly, NBT may reduce the actions of isoproterenol by oxidation of functional thiol residues in $\beta_{1,2,3}$-adrenoceptors. Again, NBT may reduce the isoproterenol responses by oxidation of thiol residues in ion-channels or other functional proteins involved in the expression of these responses (Harden, 1983). However, similar to pHMBA, NBT markedly attenuates α_1-adrenoceptor-mediated vasoconstriction whereas it augments 5-HT_2-receptor-mediated vasoconstriction (Hoque and Lewis, unpublished observations). Moreover, although NBT diminishes the vasodilator actions of L-*S*-nitrosocysteine, it does not impair those of MAHMA NONOate (Hoque et al., 2000). Taken together, these findings suggest that NBT may diminish the actions of isoproterenol by directly affecting cardiac β-adrenoceptors rather than their signal transduction processes.

4.4. Effects of peroxynitrite on the hemodynamic actions of isoproterenol

As reported previously (Benkusky et al., 1999), the depressor responses and falls in hindquarter and renal resistances elicited by isoproterenol were substantially attenuated whereas the falls in mesenteric vascular resistance were not attenuated by peroxynitrite. The novel findings were that the increases in heart rate elicited by the 0.1 μg/kg dose of isoproterenol were partially attenuated whereas the increase in heart rate elicited by the 1.0 μg/kg dose isoproterenol were not attenuated by peroxynitrite. By analogy to the findings with propranolol, it is possible that (i) peroxynitrite attenuates isoproterenol-induced vasodilation in the hindquarter and renal beds by impairment of $\beta_{1,2}$-adrenoceptors, and (ii) peroxynitrite does not markedly impair isoproterenol-induced falls in mesenteric vascular resistance or the isoproterenol-induced increases in heart rate because it does not impair the function of β_3-adrenoceptors, which may exist in substantial numbers in cardiac pacemaker cells and the mesenteric bed. Moreover, since $\beta_{1,2,3}$-adrenoceptors are coupled to G_s proteins and linked to similar signal transduction pathways (Probst et al., 1992; Emorine et al., 1994), it appears that peroxynitrite does not interfere with the signal transduction systems recruited by these receptors.

β_1- and β_2-adrenoceptors contain extracellular tyrosine residues, which would be susceptible to nitration whereas β_3-adrenoceptors do not (Probst et al., 1992; Emorine et al., 1994). It could be hypothesized that (i) the vasodilator actions of isoproterenol in the hindquarter and renal beds are mediated by β_1- and β_2-adrenoceptors susceptible to peroxynitrite-mediated nitration, and (ii) the vasodilator actions of isoproterenol in the mesenteric bed involves the activation of β_3-adrenoceptors which are not susceptible to nitration by peroxynitrite. This possibility is supported by the finding that peroxynitrite produces a sustained impairment of the vasoconstrictor effects of the α_1-adrenoceptor agonist, phenylephrine whereas it does not impair the vasoconstrictor actions of AVP (Benkusky et al., 1999). This is because (i) the vasoconstrictor actions of phenylephrine are mediated primarily by activation of α_{1A}-adrenoceptors, which contain extracellular cysteine residues capable of disulfide-bond formation and tyrosine residues susceptible to peroxynitrite-induced nitration (Probst et al., 1992; Emorine et al., 1994), and (ii) the vasoconstrictor effects of AVP are mediated by AVP V_{1A} receptors which contain extracellular cysteine residues capable of disulfide-bond formation whereas they do not contain tyrosine residues in their ligand-binding domains (Probst et al., 1992; Morel et al., 1992).

4.5. Summary

In summary, this study provides evidence that (i) chelation or oxidation of functional thiol residues in vascular and cardiac β_1-, β_2- and β_3-adrenoceptors markedly diminish the ability of isoproterenol to bind or to activate these receptors, (ii) peroxynitrite produces sustained affects on β_1- and β_2-adrenoceptors but not on β_3-adrenoceptors. The sustained effects of peroxynitrite on β_1- and β_2-adrenoceptors may be due to the nitration of tyrosine residues in the extracellular domains of these receptors (Probst et al., 1992; Emorine et al., 1994). By analogy, the lack of sustained effects of peroxynitrite on β_3-adrenoceptors may be due to the lack of tyrosine residues in extracellular binding domains of these receptors (Probst et al., 1992; Emorine et al., 1994). The results with pHMBA and NBT certainly suggest that a burst of peroxynitrite will affect $\beta_{1,2,3}$-adrenoceptors by oxidation of cysteine residues in the extracellular domains of these receptors (Probst et al., 1992; Emorine et al., 1994). However, this effect may be transient since the oxidized cysteine-residues may be rapidly reduced once they are no longer exposed to peroxynitrite.

The present findings provide indirect support for the concept that the β_3-adrenoceptor is resistant to the actions of peroxynitrite because of the paucity of amino acid residues susceptible to oxidation and/or nitration by peroxynitrite. However, definitive studies with selective β_3-adrenoceptor agonists, such as BRL 37344 and CL 316243 (see Cohen et al., 1995b), will be needed to directly address this possibility. Moreover, it is now recognized that the putative β_4-adrenoceptor is the propranolol-*insensitive* state of the β_1-adrenoceptor (see Kaumann et al., 2001; Bundkirchen et al., 2002). This raises the possibility that the propranolol-*insensitive* state of the β_1-adrenoceptor may also be resistant to the actions of peroxynitrite. Inflammatory states are known to be associated with the generation of peroxynitrite (see Beckman et al., 1994; Kooy et al., 1997; Benkusky et al., 1998, 1999). Accordingly, the propensity of peroxynitrite to down-regulate β-adrenoceptor function would have important implications for hemodynamic regulation in inflammatory states. Moreover, the ability of β-adrenoceptor antagonists to regulate hemodynamic status and particular heart rate would be compromised by peroxynitrite-induced down-regulation of β-adrenoceptors.

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