British Journal of Pharmacology (2006) 147, 755–764
www.nature.com/bjp

Downregulation of propranolol-sensitive β-adrenoceptor signaling after inhibition of nitric oxide synthesis

[1,2,5]Erin J. Whalen, [3]James N. Bates, [1,3]Alan Kim Johnson & *,[4]Stephen J. Lewis

[1]Department of Pharmacology, University of Iowa, Iowa City, IA, U.S.A.; [2]Department of Psychology, University of Iowa, Iowa City, IA, U.S.A.; [3]Department of Anesthesia, University of Iowa, Iowa City, IA, U.S.A. and [4]Department of Physiology and Pharmacology, College of Veterinary Medicine, University of Georgia, Athens, GA 30602-7389, U.S.A.

1 The β-adrenoceptor agonist, isoprenaline, elicits vasodilation and tachycardia in anesthetized rats *via* activation of propranolol-sensitive β_1- and β_2-adrenoceptors and also by propranolol-insensitive β_1- and β_3-adrenoceptors.

2 The aim of this study was to determine whether the relative contribution of propranolol-sensitive and -insensitive β-adrenoceptors to the changes in heart rate (HR) and vascular resistances elicited by isoprenaline is altered after blockade of nitric oxide (NO) synthase, in pentobarbital-anesthetized rats.

3 The hemodynamic responses elicited by isoprenaline (0.1 and 0.5 $\mu g\,kg^{-1}$, i.v.) were determined before and after injection of saline or the NO synthase inhibitor, N^G-nitro-L-arginine methylester (L-NAME, 50 $\mu mol\,kg^{-1}$, i.v.), and again after injection of the β_1- and β_2-adrenoceptor antagonist, propranolol (1 mg kg^{-1}, i.v.). The responses elicited by the above doses of isoprenaline were also determined before and during infusion of the α_1-adrenoceptor agonist, phenylephrine (3 $\mu g\,kg^{-1}\,min^{-1}$, i.v.), and again 15–20 min after injection of propranolol (1.0 mg kg^{-1}, i.v.).

4 Both doses of isoprenaline elicited tachycardia and reductions in vascular resistances. Propranolol eliminated the responses elicited by the lower dose of isoprenaline and substantially diminished the responses elicited by the higher dose of the β_1-, β_2- and β_3-adrenoceptor agonist. The maximal vasodilator responses elicited by both doses of isoprenaline were not diminished whereas the maximal increases in HR were higher after injection of L-NAME. The ability of propranolol to diminish the hemodynamic actions of isoprenaline was substantially diminished in L-NAME-treated rats, whereas propranolol retained its potency in rats that received an equi-pressor infusion of the α_1-adrenoceptor agonist, phenylephrine.

5 The finding that the maximal vasodilator responses elicited by isoprenaline were not diminished by L-NAME suggests that the vasodilation elicited by this drug was due to direct activation of β-adrenoceptors on vascular smooth muscle and that the full compliment of isoprenaline-sensitive receptors was not changed after inhibition of NO synthesis. However, these results suggest that the activities of propranolol-sensitive β-adrenoceptors are downregulated, whereas propranolol-insensitive β-adrenoceptors are upregulated upon the loss of exposure to endothelial nitrosyl factors.
British Journal of Pharmacology (2006) **147,** 755–764. doi:10.1038/sj.bjp.0706675;
published online 6 February 2006

Keywords: β-Adrenoceptors; isoprenaline; nitric oxide; tachycardia; vasodilation; rats

Abbreviations: D-NAME, N^G-nitro-D-arginine methyl ester; HQR, hindquarter vascular resistance; HR, heart rate; L-NAME, N^G-nitro-L-arginine methyl ester; MAP, mean arterial blood pressure; MR, mesenteric vascular resistance; NO, nitric oxide; PKA, cAMP-dependent protein kinase; RR, renal vascular resistance

Introduction

β-Adrenoceptors including β_1-, β_2- and β_3-adrenoceptors and atypical β-adrenoceptors are expressed in most mammalian cells (Harden, 1983; Probst *et al*., 1992; Emorine *et al*., 1994; Cohen *et al*., 1995; Kaumann, 1996; Lefkowitz, 2004). β_1-, β_2- and β_3-adrenoceptors are Gs protein-coupled receptors that activate adenylate cyclase, which converts ATP to cAMP (Harden, 1983; Seino & Shibasaki, 2005; Young *et al*., 2005). cAMP elicits its effects in cells *via* activation of cAMP-dependent protein kinase (PKA) (Harden, 1983; Seino & Shibasaki, 2005; Young *et al*., 2005). Catecholamines are relatively potent agonists of $\beta_{1,2}$-adrenoceptors but weak agonists at β_3-adrenoceptors, whereas isoprenaline is a potent agonist of β_1-, β_2- and β_3-adrenoceptors (Emorine *et al*., 1994). The putative β_4-adrenoceptor has been redefined as the propranolol-insensitive state of the β_1-adrenoceptor (see Kaumann *et al*., 2001; Bundkirchen *et al*., 2002).

Recent *in vitro* studies have demonstrated the presence of functional atypical (propranolol-insensitive) β_1-adrenoceptors in human myocardium (Bundkirchen *et al*., 2002). There is little information as to whether this rarely described state of β_1-adrenoceptors impacts the cardiovascular actions of endogenous catecholamines or exogenously applied β-adrenoceptor agonists *in vivo* and under what conditions the activity

*Author for correspondence; E-mail: slewis@vet.uga.edu
[5]Current address: Division of Cardiology, Department of Medicine, 452 Clinical Research Laboratory Building, Box 3821 Medical Center, Duke University Medical Center, Durham, NC 27710, U.S.A.

of these receptors is up- or downregulated. However, we have demonstrated that higher doses of isoprenaline elicit pronounced increases in heart rate (HR) and equally pronounced depressor and vasodilator responses in anaesthetized rats, which are only partially affected by propranolol (Whalen & Lewis, 1999). Propranolol is a potent antagonist of β_2-adrenoceptors and appropriately configured β_1-adrenoceptors, but not β_3-adrenoceptors (Cohen *et al*., 1999). These findings raise the possibility that higher doses of isoprenaline elicit their haemodynamic responses *via* the activation of propranolol-insensitive β_1-adrenoceptors and/or β_3-adrenoceptors, as well as propranolol-sensitive β_1- and β_2-adrenoceptors.

The depressor and vasodilator actions of isoprenaline and the membrane-permeable cAMP analog 8-(4-chlorophenylthiol)-cAMP (8-CPT-cAMP) are minimally reduced in pentobarbital-anesthetized rats after the acute injection of the nitric oxide (NO) synthesis inhibitor, N^G-nitro-L-arginine methylester (L-NAME) (Whalen *et al*., 1999a; 2000). This suggests that the vasodilator actions of isoprenaline *in vivo* may be due to activation of β-adrenoceptors on vascular smooth muscle rather than the release of newly synthesized NO or related nitrosyl factors such as s-nitrosothiols (Myers *et al*., 1990; Rosenblum, 1992) from the vascular endothelium.

The aim of this study was to examine the possibility that endothelium-derived nitrosyl factors affect the status of propranolol-insensitive β_1- and β_3-adrenoceptors in cardiac pacemaker cells and in vascular smooth muscle cells in resistance arteries *in vivo*. In these studies, we determined the changes in HR, mean arterial blood pressure (MAP), and hindquarter (HQR), mesenteric (MR) and renal (RR) vascular resistance elicited by 0.1 and 0.5 $\mu g\,kg^{-1}$ doses of isoprenaline in pentobarbital-anesthetized rats before and after injection of saline or L-NAME and again after injection of propranolol. To control for the increases in MAP and vascular resistances elicited by L-NAME, the hemodynamic responses elicited by 0.1 and 0.5 $\mu g\,kg^{-1}$ doses of isoprenaline were determined before and during an equi-pressor infusion of the selective α_1-adrenoceptor agonist, phenylephrine, and again after subsequent injection of propranolol.

We now report that the ability of propranolol to block the hemodynamic responses elicited by isoprenaline was markedly reduced in L-NAME-treated, but not in phenylephrine-treated, rats. This suggests that the relative status of β-adrenoceptor subtypes is not affected by changes in resting parameters *per se*. Rather, these findings suggest that propranolol-sensitive β_1- and β_2-adrenoceptors are downregulated, whereas propranolol-insensitive β_1-adrenoceptors and/or β_3-adrenoceptors are upregulated after inhibition of NO synthesis.

Methods

Surgical and experimental procedures

All studies were carried out in accordance with the National Institutes of Health Guide for the Care and Use of Laboratory Animals (NIH Publication No. 80-23) revised in 1996. The protocols were approved by the Animal Care and Use Committees of the University of Iowa and University of Georgia. Male Sprague–Dawley rats (250–350 g) were anesthetized with pentobarbital (50 mg kg^{-1}, i.p.). To prepare rats for study 1, a catheter was inserted into a femoral vein to give bolus injections of the test agents. The rats received supplemental doses of pentobarbital (5 mg kg^{-1}, i.v.) to maintain anesthesia as necessary during surgery and experimentation. A catheter was placed in a femoral artery to measure pulsatile arterial blood pressures and MAP. A midline laparotomy was performed and pulsed Doppler flow probes were placed around the superior mesenteric artery, a renal artery and lower abdominal aorta (below the level of the kidneys) to continuously record mesenteric, renal and hindquarter blood flow velocities, respectively, and to determine MR, RR and HQR vascular resistances, respectively (Davisson *et al*., 1996; Whalen *et al*., 1999b–d). The body temperature of each rat was maintained at 37°C *via* a rectal thermometer connected to a thermostatically-controlled heating pad. Exactly the same procedures were used to prepare rats for study 2, except that another catheter was placed into the same femoral vein to allow continuous infusion of the selective α_1-adrenoceptor agonist phenylephrine, which will be described below.

Immediately after the above surgeries, the arterial catheter was connected to a Beckman Dynagraph-coupled pressure transducer to record MAP and pulsatile arterial blood pressures. HR was derived from pulsatile arterial blood pressure by a Beckman Dynagraph-coupled cardiotachometer. The leads from the Doppler flow probes were connected to a Beckman Dynagraph-coupled Doppler flowmeter (Department of Bioengineering, University of Iowa) to continuously record blood flow velocities. Vascular resistance at any point in time was determined by dividing MAP by blood flow velocity in the particular bed. The above techniques have been detailed previously (Whalen *et al*., 1999b–d; 2000; Lewis *et al*., 2005a–c). Details of the Doppler technique, including the reliability of the method for the estimation of flow velocity and determination of percent changes in vascular resistances, have also been detailed previously (Haywood *et al*., 1981). In this series of studies, the arithmetic and/or percent changes in all parameters at the point of maximal drug-induced response were determined. All rats were given 25–30 min to stabilize after surgery before beginning the experiments.

General statements about protocols under studies 1 and 2

All rats received bolus intravenous injections of isoprenaline (0.1 and then 0.5 $\mu g\,kg^{-1}$) given 5 min apart before and after various treatments. The 5-min interval between injections allowed the responses elicited by the 0.1 $\mu g\,kg^{-1}$ dose to subside completely before the 0.5 $\mu g\,kg^{-1}$ dose was given. The dose of propranolol was 1 mg kg^{-1} and the dose of L-NAME and D-NAME was 50 $\mu mol\,kg^{-1}$. The responses elicited by L-NAME and propranolol had reached plateau levels at the times the injections of isoprenaline were given (see below).

Study 1

Group 1 rats ($n=6$) received injections of isoprenaline before and beginning 25–30 min after a bolus injection of saline and again 15–20 min after another injection of saline. Group 2 rats ($n=6$) received injections of isoprenaline before and beginning 25–30 min after a bolus injection of saline and again 15–20 min after an injection of propranolol. Group 3 rats ($n=6$) received injections of isoprenaline before and beginning 25–30 min after a bolus injection of L-NAME, and again 15–20 min after an injection of saline. Group 4 rats ($n=6$) received injections

of isoprenaline before and beginning 25–30 min after injection of L-NAME, and again beginning 15–20 min after injection of propranolol. Group 5 rats ($n=5$) received injections of isoprenaline before and beginning 25–30 min after injection the inactive of enantiomer, D-NAME.

Study 2

In group 6 rats ($n=10$), the hemodynamic responses elicited by bolus injections of isoprenaline (0.1 and 0.5 $\mu g\,kg^{-1}$) were determined before, during intravenous infusion (3 $\mu g\,kg^{-1}\,min^{-1}$) of a 30 $\mu g\,\mu l^{-1}$ solution of phenylephrine (Lacolley *et al*., 1991), and again 15–20 min after injection of propranolol (1.0 $mg\,kg^{-1}$). The effects of phenylephrine on HR, MAP, HQR, RR and MR were determined but only the HR, MAP and HQR results will be reported since the changes in these parameters elicited by the infusion of phenylephrine were qualitatively and quantitatively similar to those elicited by L-NAME (see Results) whereas the increases in MR and RR were much greater than those elicited by L-NAME.

Drugs

Sodium pentobarbital and sterile saline were obtained from Abbott (Chicago, IL, U.S.A.). All other drugs were obtained from Sigma (St Louis, MO, U.S.A.). These drugs were dissolved and diluted for injection in sterile saline.

Statistics

The data are presented as mean ± s.e.m. and were analyzed by repeated measures analysis of variance (ANOVA) followed by Students modified *t*-test with Bonferroni corrections for multiple comparisons between means using the error mean square terms from the repeated measures ANOVAs (see Whalen *et al*., 1999b–d). A value of $P<0.05$ denoted statistical difference.

Results

General observations

The hemodynamic responses elicited by isoprenaline in the saline + propranolol and L-NAME + propranolol treatment groups will be described in detail later in this article. With respect to the control studies for the above groups, it should be mentioned that the responses elicited by the 0.1 and 0.5 $\mu g\,kg^{-1}$ doses of isoprenaline were similar before and after each of the two injections of saline (saline + saline group, $P>0.5$, for all comparisons). For example, the arithmetic increases in HR elicited by the 0.1 $\mu g\,kg^{-1}$ dose of isoprenaline before and after the injection of the first and second injections of saline were $+62\pm7$, $+65\pm8$ and $+63\pm7$ beats min^{-1}, respectively ($P>0.5$, for all comparisons).

The injection of L-NAME augmented the increases in HR elicited by the 0.1 and 0.5 $\mu g\,kg^{-1}$ doses of isoprenaline in the L-NAME + saline group. The responses elicited by the 0.1 and 0.5 $\mu g\,kg^{-1}$ doses of isoprenaline were similarly augmented after the injection of L-NAME and the subsequent injection of saline. Importantly, the responses elicited by isoproterenol after injection of saline were equal to those after the preceding injection of L-NAME ($P>0.5$, for all comparisons). For example, the arithmetic increases in HR elicited by the 0.1 $\mu g\,kg^{-1}$ dose of isoprenaline before and after the injection of L-NAME and the subsequent injection of saline were $+58\pm8$, $+87\pm9$ and $+88\pm6$ beats min^{-1}, respectively ($P>0.5$, post-L-NAME and post-saline values *versus* pre; $P>0.5$, post-saline *versus* post-L-NAME values). Finally, the hemodynamic responses elicited by the 0.1 and 0.5 $\mu g\,kg^{-1}$ doses of isoprenaline were similar before and after the injection of D-NAME ($P>0.5$, for all comparisons, data not shown). For example, the arithmetic increases in HR elicited by the 0.1 $\mu g\,kg^{-1}$ dose of isoprenaline before and after the injection of D-NAME were $+54\pm6$ and $+57\pm9$ beats min^{-1}, respectively ($P>0.5$).

L-NAME studies

Isoprenaline-induced changes in HR Resting HR values before the injection of the 0.1 $\mu g\,kg^{-1}$ dose of isoprenaline in the saline + propranolol treatment group (left-hand panels) and the L-NAME + propranolol treatment groups (right-hand panels) are summarized in Figure 1. The injection of saline did not affect HR, whereas the subsequent injection of propranolol elicited a sustained bradycardia (-41 ± 4 beats min^{-1}, $-11\pm1\%$, $P<0.05$). L-NAME elicited a sustained bradycardia (-35 ± 4 beats min^{-1}, $-10\pm1\%$, $P<0.05$) and the subsequent injection of propranolol elicited an additional sustained

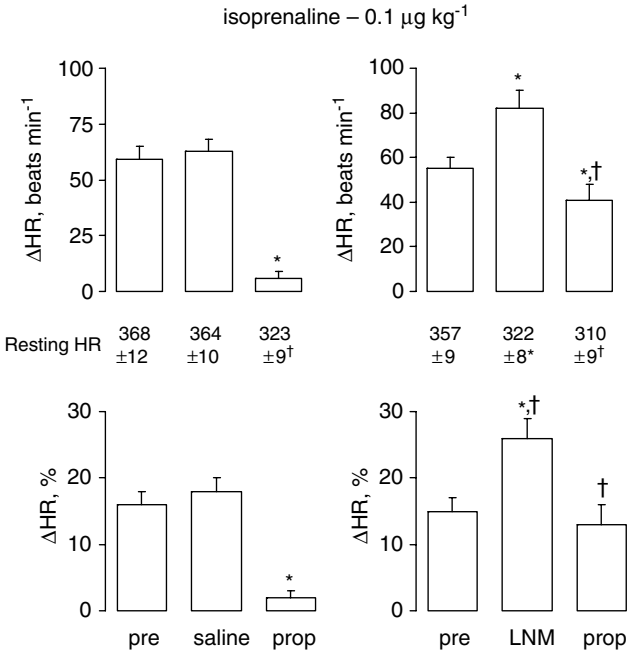

Figure 1 Summary of the maximal changes in HR elicited by isoprenaline (0.1 $\mu g\,kg^{-1}$, i.v.) before and after injection of saline or L-NAME (LNM, 50 $\mu mol\,kg^{-1}$, i.v.) and again after an injection of propranolol (prop, 1 $mg\,kg^{-1}$). There were six rats in each group. All values are mean ± s.e.m. Statistical symbols for resting HR. $^{*}P<0.05$, significant change from Pre. $^{\dagger}P<0.05$, Post-propranolol *versus* post-saline or post-L-NAME. Statistical symbols for isoprenaline-induced changes in HR. $^{*}P<0.05$, significant difference from Pre. $^{\dagger}P<0.05$, Post-L-NAME *versus* post-saline, and post-L-NAME + propranolol *versus* post-saline + propranolol. The numbers of rats and doses of drugs are the same for Figures 2–10.

reduction in HR (-11 ± 2 beats min^{-1}, $-3.5\pm0.5\%$, $P<0.05$). Note that the HR values after injection of propranolol were similar in the saline + propranolol and L-NAME + propranolol treatment groups ($P>0.05$). The arithmetic and percent increases in HR elicited by the 0.1 μg kg^{-1} dose of isoprenaline in the two groups of rats are also summarized in Figure 1. The isoprenaline-induced increases in HR were similar before and after injection of saline, but were abolished after the subsequent injection of propranolol (residual tachycardia $=+6\pm3$ beats min^{-1}, $+2\pm1\%$, $P>0.05$). The isoprenaline-induced increases in HR were augmented by L-NAME ($+28\pm4$ beats min^{-1}, $+11\pm2\%$, $P<0.05$). These responses were diminished but not abolished by propranolol (residual tachycardia $=+41\pm7$ beats min^{-1}, $+14\pm3\%$, $P<0.05$).

The increases in HR elicited by the 0.5 μg kg^{-1} dose of ISO in the saline + propranolol or L-NAME + propranolol groups are summarized in Figure 2. The pre-injection values were similar to those shown in Figure 1. The 0.5 μg kg^{-1} dose of isoprenaline elicited pronounced increases in HR that were greater in magnitude than those of the 0.1 μg kg^{-1} dose ($P<0.05$). The increases in HR elicited by the 0.5 μg kg^{-1} dose of isoprenaline were similar before and after the injection of saline. These responses were reduced, but not abolished, after the subsequent injection of propranolol ($+37\pm6$ beats min^{-1}, $+11\pm2\%$, $P<0.05$). The isoprenaline-induced increases in HR were augmented by L-NAME ($+45\pm5$ beats min^{-1}, $+17\pm2\%$, $P<0.05$). These responses were diminished after the subsequent injection of propranolol. However, the residual tachycardia in the L-NAME + propranolol group ($+111\pm6$ beats min^{-1}, $+36\pm3\%$) was greater ($P<0.05$) than in the saline + propranolol group ($+37\pm6$ beats min^{-1}, $+11\pm2\%$).

Isoprenaline-induced changes in MAP

Resting MAP values before the injections of the 0.1 μg kg^{-1} dose of isoprenaline in the saline + propranolol and L-NAME-propranolol groups are summarized in Figure 3. Resting MAP values were similar before and after injection of saline and the subsequent injection of propranolol. L-NAME elicited a sustained increase in MAP ($+37\pm3$ mmHg, $+35\pm3\%$, $P<0.05$). Subsequent injection of propranolol did not affect resting MAP ($+2\pm2$ mmHg, $1\pm2\%$, $P>0.05$). The changes in MAP elicited by the 0.1 μg kg^{-1} dose of isoprenaline in the saline + propranolol or L-NAME + propranolol groups are summarized in Figure 3. Isoprenaline elicited decreases in MAP that were similar before and after injection of saline. These responses were abolished by propranolol. The arithmetic decreases in MAP elicited by isoprenaline were augmented after injection of L-NAME (11 ± 2 mmHg), whereas the percent decreases in MAP were not. The isoprenaline-induced decreases in MAP were diminished, but not abolished, by propranolol. The decreases in MAP elicited by the 0.5 μg kg^{-1} dose of isoprenaline in the saline + propranolol or L-NAME + propranolol groups are summarized in Figure 4. The decreases in MAP elicited by this dose of isoprenaline were greater than those elicited by the 0.1 μg kg^{-1} dose. The decreases in MAP elicited by the 0.5 μg kg^{-1} dose of isoprenaline were similar before and after injection of saline. These responses were reduced, but not abolished, by propranolol. The arithmetic decreases in MAP elicited by isoprenaline were augmented by L-NAME (10 ± 2 mmHg), whereas the percent reductions were not. The isoprenaline-induced decreases in MAP were diminished by

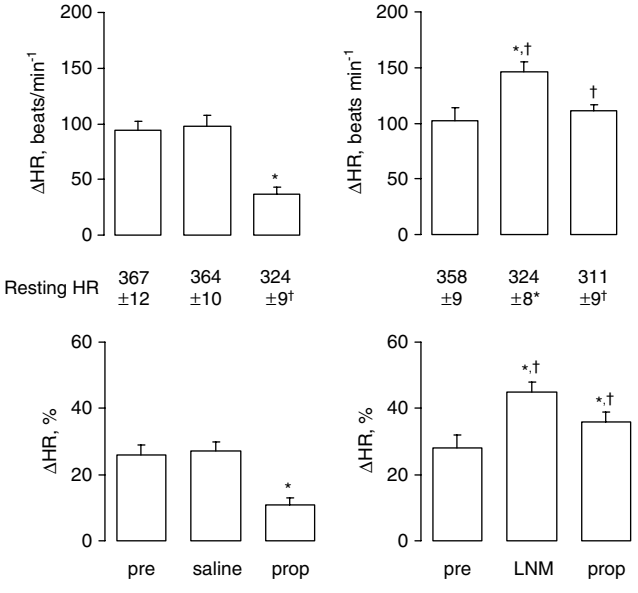

Figure 2 Summary of the maximal changes in HR elicited by isoprenaline (0.5 μg kg^{-1}) before and after injection of saline or LNM and again after injection of propranolol (prop). All values are mean ± s.e.m. Symbols for resting HR. *$P<0.05$, significant difference from Pre. †$P<0.05$, Post-propranolol *versus* post-saline or post-L-NAME. Symbols for isoprenaline-induced changes in HR. *$P<0.05$, significant difference from Pre. †$P<0.05$, post-L-NAME *versus* post-saline and post-L-NAME + propranolol *versus* post-saline + propranolol.

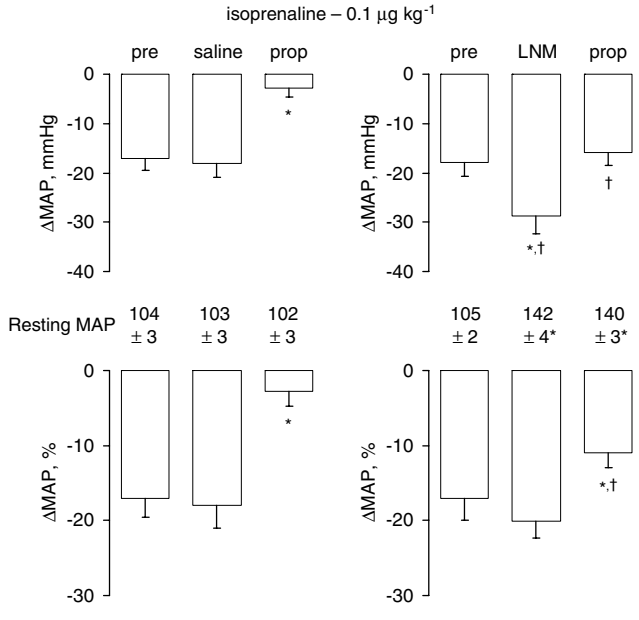

Figure 3 Summary of the maximal changes in MAP elicited by isoprenaline (0.1 μg kg^{-1}) before and after injection of saline or LNM and again after injection of propranolol (prop). All values are mean ± s.e.m. Symbols for resting MAP. *$P<0.05$, significant difference from Pre. †$P<0.05$, post-propranolol *versus* post-saline or post-L-NAME. Symbols for isoprenaline-induced changes in MAP. *$P<0.05$, significant change from Pre. †$P<0.05$, post-L-NAME *versus* post-saline and post-L-NAME + propranolol *versus* post-saline + propranolol.

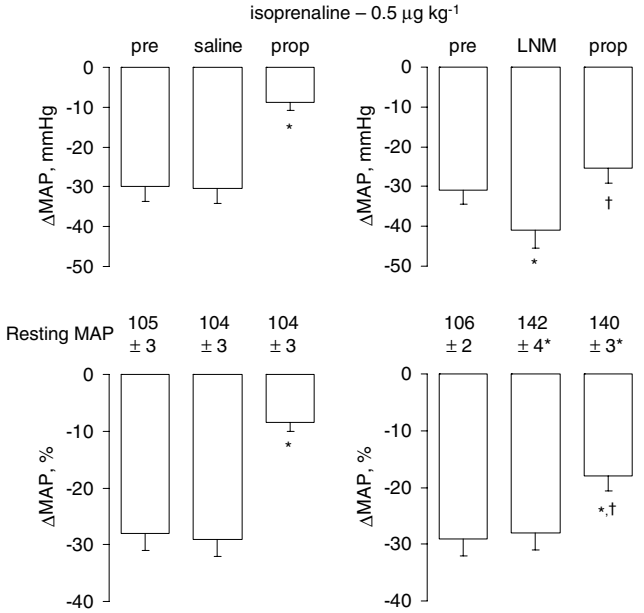

Figure 4 Summary of the maximal changes in MAP elicited by isoprenaline ($0.5\,\mu g\,kg^{-1}$) before and after injection of saline or LNM and again after injection of propranolol (prop). All values are mean ± s.e.m. Symbols for resting MAP. $^*P<0.05$, significant difference from Pre. $^\dagger P<0.05$, post-propranolol *versus* post-saline or post-L-NAME. Symbols for isoprenaline-induced changes in MAP. $^*P<0.05$, significant change from Pre. $^\dagger P<0.05$, post-L-NAME *versus* post-saline and post-L-NAME + propranolol *versus* post-saline + propranolol.

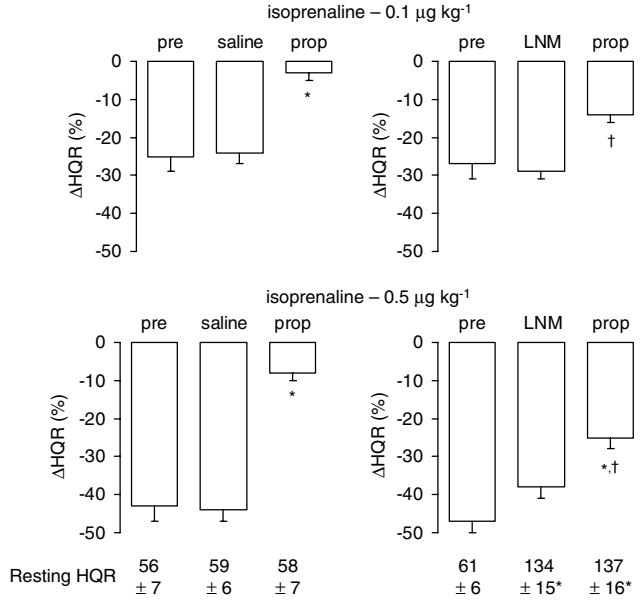

Figure 5 Summary of the maximal changes in HQR elicited by isoprenaline (0.1 or $0.5\,\mu g\,kg^{-1}$) before and after injection of saline or LNM and again after injection of propranolol (prop). All values are mean ± s.e.m. Symbols for resting HQR. $^*P<0.05$, significant difference from Pre. $^\dagger P<0.05$, post-propranolol *versus* post-saline or post-L-NAME. Symbols for isoprenaline-induced changes in HQR. $^*P<0.05$, significant change from Pre. $^\dagger P<0.05$, post-L-NAME *versus* post-saline and post-L-NAME + propranolol *versus* post-saline + propranolol.

propranolol. However, the residual responses were substantially greater than those in the saline + propranolol group.

Isoprenaline-induced changes in hindquarter vascular resistance

Resting HQR values before the injections of the 0.1 and $0.5\,\mu g\,kg^{-1}$ doses of isoprenaline in the saline + propranolol and L-NAME + propranolol groups are summarized at the bottom of Figure 5. Resting HQR values were similar before and after the injection of saline and subsequent injection of propranolol. L-NAME elicited a sustained increase in HQR ($+73\pm8\%$, $P<0.05$). The subsequent injection of propranolol did not affect resting MAP ($+3\pm2$ mmHg, $+2\pm2\%$, $P>0.05$). The percent decreases in HQR elicited by the $0.1\,\mu g\,kg^{-1}$ dose of isoprenaline were similar before and after injection of saline (see the top left panel of Figure 5). These responses were abolished by propranolol. The percent decreases in HQR elicited by the $0.1\,\mu g\,kg^{-1}$ dose of isoprenaline were similar before and after injection of L-NAME (see the top right panel of Figure 5). The isoprenaline-induced decreases in HQR were diminished, but not abolished, by propranolol. The changes in HQR elicited by the $0.5\,\mu g\,kg^{-1}$ dose of isoprenaline in the saline + propranolol or L-NAME-propranolol treatment groups are summarized in the bottom panels of Figure 5. The percent decreases on HQR elicited by the $0.5\,\mu g\,kg^{-1}$ dose of isoprenaline were greater than those elicited by the $0.1\,\mu g\,kg^{-1}$ dose. The percent decreases in HQR elicited by the $0.5\,\mu g\,kg^{-1}$ dose of isoprenaline were similar before and after injection of saline. These responses were reduced but not abolished by propranolol. The percent decreases in HQR elicited by the $0.5\,\mu g\,kg^{-1}$ dose of isoprenaline were similar before and after injection of L-NAME. The isoprenaline-induced decreases in HQR were diminished after subsequent injection of propranolol. However, the residual responses were substantially greater than in the saline + propranolol group ($P<0.05$).

Isoprenaline-induced changes in mesenteric vascular resistances

Resting MR values before the injections of the 0.1 and $0.5\,\mu g\,kg^{-1}$ doses of isoprenaline in the saline + propranolol and L-NAME + propranolol groups are summarized at the bottom of Figure 6. Resting MR values were similar before and after the injection of saline and the subsequent injection of propranolol. L-NAME elicited a sustained increase in MR ($+158\pm16\%$, $P<0.05$). The subsequent injection of propranolol did not affect resting MAP ($+5\pm3\%$, $P>0.05$). The percent decreases in MR elicited by injections of the 0.1 and $0.5\,\mu g\,kg^{-1}$ doses of isoprenaline in the saline + propranolol and L-NAME + propranolol groups are also summarized in Figure 6. The percent decreases in MR elicited by the $0.5\,\mu g\,kg^{-1}$ dose of isoprenaline were greater than those elicited by the $0.1\,\mu g\,kg^{-1}$ dose. The percent decreases in MR elicited by the 0.1 and $0.5\,\mu g\,kg^{-1}$ doses of isoprenaline were similar before and after injection of saline. These responses were reduced, but not abolished, by propranolol. The percent decreases in MR elicited by the 0.1 and $0.5\,\mu g\,kg^{-1}$ dose of isoprenaline were similar before and after injection of

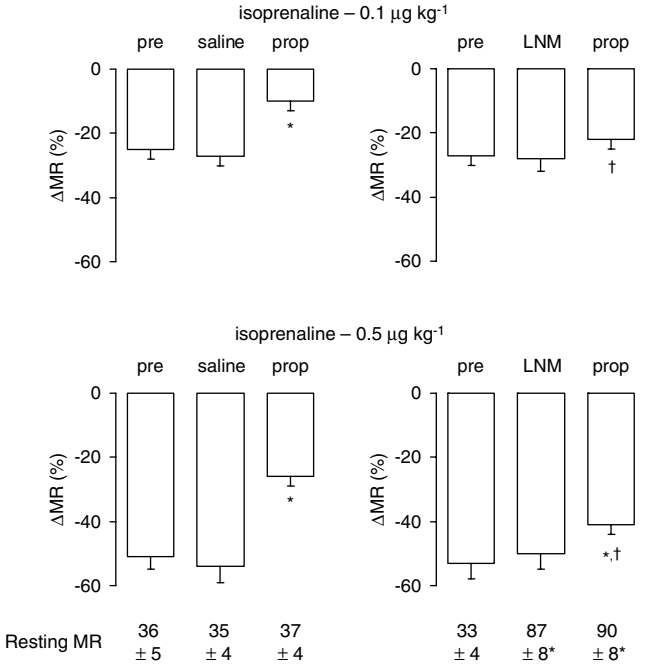

Figure 6 Summary of the maximal changes in MR elicited by isoprenaline (0.1 and 0.5 $\mu g\,kg^{-1}$) before and after injection of saline or LNM and again after injection of propranolol (prop). All values are mean ± s.e.m. Symbols for resting MR. $^{*}P<0.05$, significant difference from Pre. $^{\dagger}P<0.05$, Post-propranolol *versus* post-saline or post-L-NAME. Symbols for isoprenaline-induced changes in MR. $^{*}P<0.05$, significant change from Pre. $^{\dagger}P<0.05$, post-L-NAME *versus* post-saline and post-L-NAME + propranolol *versus* post-saline + propranolol.

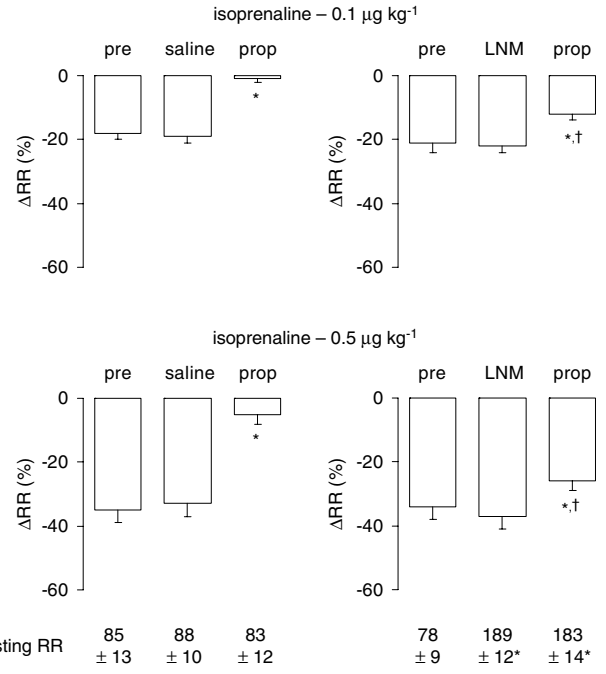

Figure 7 Summary of the maximal changes in RR elicited by isoprenaline (0.1 and 0.5 $\mu g\,kg^{-1}$) before and after injection of saline or LNM and again after injection of propranolol (prop). All values are mean ± s.e.m. Symbols for resting RR. $^{*}P<0.05$, significant difference from Pre. $^{\dagger}P<0.05$, post-propranolol *versus* post-saline or post-L-NAME. Symbols for isoprenaline-induced changes in RR. $^{*}P<0.05$, significant change from Pre. $^{\dagger}P<0.05$, post-L-NAME *versus* post-saline and post-L-NAME + propranolol *versus* post-saline + propranolol.

L-NAME. The isoprenaline-induced decreases in MR were diminished after the subsequent injection of propranolol. However, the residual responses were substantially greater than in the saline + propranolol group ($P<0.05$).

Isoprenaline-induced changes in renal vascular resistances

Resting RR values before the injections of the 0.1 and 0.5 $\mu g\,kg^{-1}$ doses of isoprenaline in the saline + propranolol and L-NAME + propranolol groups are summarized at the bottom of Figure 7. Resting RR values were similar before and after injection of saline and subsequent injection of propranolol. L-NAME elicited a sustained increase in RR ($+136 \pm 14\%$, $P<0.05$). The subsequent injection of propranolol did not affect resting RR ($-3 \pm 5\%$, $P>0.05$). The percent decreases in RR elicited by the 0.1 and 0.5 $\mu g\,kg^{-1}$ doses of isoprenaline in the saline + propranolol and L-NAME + propranolol groups are also summarized in Figure 7. The percent decreases in MR elicited by the 0.5 $\mu g\,kg^{-1}$ dose of isoprenaline were greater than those of the 0.1 $\mu g\,kg^{-1}$ dose. The percent decreases in MR elicited by the 0.1 and 0.5 $\mu g\,kg^{-1}$ doses of isoprenaline were similar before and after injection of saline. These responses were virtually abolished by propranolol. The percent decreases in MR elicited by the 0.1 and 0.5 $\mu g\,kg^{-1}$ doses of isoprenaline were similar before and after injection of L-NAME. The isoprenaline-induced decreases in MR were diminished after the subsequent injection of propranolol. However, the residual responses were substantially greater than in the saline + propranolol group ($P<0.05$).

Phenylephrine studies

Isoprenaline-induced changes in HR Resting HR values before the injections of the 0.1 and 0.5 $\mu g\,kg^{-1}$ doses of isoprenaline, prior to and during the continuous infusion of phenylephrine, and after the subsequent injection of propranolol are summarized in Figure 8. The infusion of phenylephrine elicited a sustained reduction in HR (e.g., -21 ± 4 beats min^{-1}, $-6 \pm 1\%$, $P<0.05$; pre 0.1 $\mu g\,kg^{-1}$ dose values), that was most likely due to baroreceptor-reflex-mediated changes in autonomic outflow in response to the phenylephrine-induced increases in MAP (see below). Resting HR values during the infusion of phenylephrine (e.g., 328 ± 9 beats min^{-1}, pre 0.1 $\mu g\,kg^{-1}$ dose values) were similar to those following the injection of L-NAME (e.g., 322 ± 8 beats min^{-1}, see the right-hand panels of Figure 1). Subsequent injection of propranolol elicited a further reduction in HR in the rats receiving phenylephrine (e.g., -24 ± 5 beats min^{-1}, $-6 \pm 1\%$, $P<0.05$; pre 0.1 $\mu g\,kg^{-1}$ dose values). The arithmetic and percent increases in HR elicited by the 0.1 $\mu g\,kg^{-1}$ dose of isoprenaline were abolished by propranolol. The effects of propranolol on the isoprenaline-induced tachycardia were identical to those in saline-treated rats (see the left panels of Figure 1). The arithmetic and percent increases in HR elicited by the 0.5 $\mu g\,kg^{-1}$ dose of isoprenaline were reduced, but not abolished, by propranolol. The effects of propranolol on the isoprenaline-induced tachycardia were identical to those in saline-treated rats (see the left panels of Figure 1). More specifically, the residual

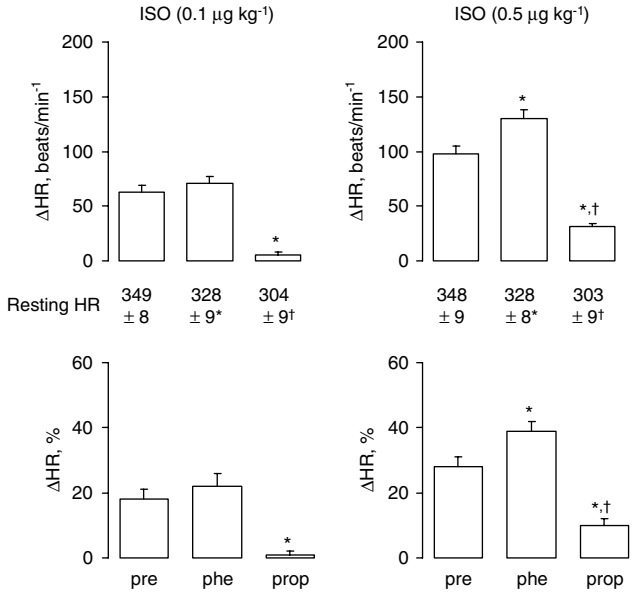

Figure 8 Summary of the maximal changes in HR elicited by isoprenaline (0.1 and 0.5 $\mu g\,kg^{-1}$) before and during infusion of phenylephrine (3 $\mu g\,kg^{-1}\,min^{-1}$) and again upon injection of propranolol (prop). All values are mean ± s.e.m. Symbols for resting HR. $^{*}P<0.05$, significant difference from Pre. $^{\dagger}P<0.05$, Post-propranolol *versus* post-saline or post-L-NAME. Symbols for isoprenaline-induced changes in HR. $^{*}P<0.05$, significant change from Pre. $^{\dagger}P<0.05$, post-L-NAME *versus* post-saline and post-L-NAME + propranolol *versus* post-saline + propranolol.

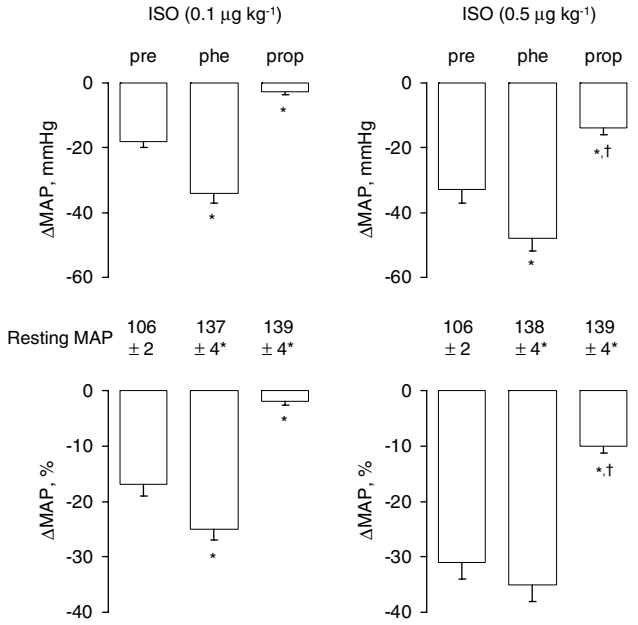

Figure 9 Summary of the maximal changes in MAP elicited by isoprenaline (0.1 and 0.5 $\mu g\,kg^{-1}$) before and during infusion of phenylephrine (3 $\mu g\,kg^{-1}\,min^{-1}$) and again after injection of propranolol (prop). All values are mean ± s.e.m. Symbols for resting MAP. $^{*}P<0.05$, significant difference from Pre. $^{\dagger}P<0.05$, post-propranolol *versus* post-saline or post-L-NAME. Symbols for isoprenaline-induced changes in MAP. $^{*}P<0.05$, significant change from Pre. $^{\dagger}P<0.05$, post-L-NAME *versus* post-saline and post-L-NAME-propranolol *versus* post-saline + propranolol.

tachycardia elicited by isoprenaline after the injection of propranolol in the saline-treated and in the phenylephrine-infused rats were $+37\pm6$ beats min^{-1} ($+11\pm2\%$) and $+31\pm5$ beats min^{-1} ($+10\pm2\%$), respectively ($P>0.05$ for all comparisons).

Isoprenaline-induced changes in MAP

Resting MAP values before the injections of the 0.1 and 0.5 $\mu g\,kg^{-1}$ dose of isoprenaline, prior to and during the continuous infusion of phenylephrine, and after the subsequent injection of propranolol are summarized in Figure 9. The infusion of phenylephrine elicited a sustained increase in MAP (e.g., $+31\pm3$ mmHg, $+28\pm3\pm1\%$, $P<0.05$; pre 0.1 $\mu g\,kg^{-1}$ dose values). Resting MAP values during the infusion of phenylephrine (e.g., 137 ± 4 mmHg, pre 0.1 $\mu g\,kg^{-1}$ dose values) were similar to those following the injection of L-NAME (e.g., 142 ± 4 mmHg, see right-hand panels of Figure 3). Subsequent injection of propranolol did not affect the resting MAP. The arithmetic and percent decreases in MAP elicited by the 0.1 $\mu g\,kg^{-1}$ dose of isoprenaline were abolished by propranolol. The effects of propranolol on the isoprenaline-induced depressor responses were identical to those in saline-treated rats (see the left panels of Figures 3 and 4). The arithmetic and percent decreases in MAP elicited by the 0.5 $\mu g\,kg^{-1}$ dose of isoprenaline were reduced, but not abolished, by propranolol. The effects of propranolol on the isoprenaline-induced depressor responses were identical to those in saline-treated rats (see the left panels of Figures 3 and 4). More specifically, the depressor response elicited by isoprenaline after the injection of propranolol in the saline-treated and in the phenylephrine-infused rats were -9 ± 2 mmHg ($-8\pm2\%$) and -12 ± 2 mmHg ($-10\pm2\%$), respectively ($P>0.05$ for all comparisons).

Isoprenaline-induced changes in hindquarter vascular resistance

Resting HQR values before the injections of the 0.1 and 0.5 $\mu g\,kg^{-1}$ doses of isoprenaline, prior to and during the continuous infusion of phenylephrine, and after subsequent injection of propranolol (while maintaining the infusion of phenylephrine) are summarized in Figure 10. The infusion of phenylephrine elicited a sustained increase in HQR (e.g., $+110\pm13\%$, $P<0.05$; pre 0.1 $\mu g\,kg^{-1}$ dose values). The resting HQR values during infusion of phenylephrine (e.g., 147 ± 15 mmHg kHz^{-1}, pre 0.1 $\mu g\,kg^{-1}$ dose values) were similar ($P>0.05$) to that following the administration of L-NAME in the saline-treated rats (134 ± 15 mmHg kHz^{-1}, see the right-hand panels of Figure 5). The subsequent injection of propranolol did not affect resting HQR. The percent decreases in HQR elicited by the 0.1 $\mu g\,kg^{-1}$ dose of isoprenaline were abolished by propranolol. The effects of propranolol on the isoprenaline-induced depressor responses were identical to those in saline-treated rats (see left panels of Figure 5). The percent decreases in HQR elicited by the 0.5 $\mu g\,kg^{-1}$ dose of isoprenaline were reduced, but not abolished, by propranolol. The effects of propranolol on the isoprenaline-induced depressor responses were identical to those in saline-treated rats (see left panels of Figure 5). More specifically, the reductions in HQR elicited by isoprenaline after the injection

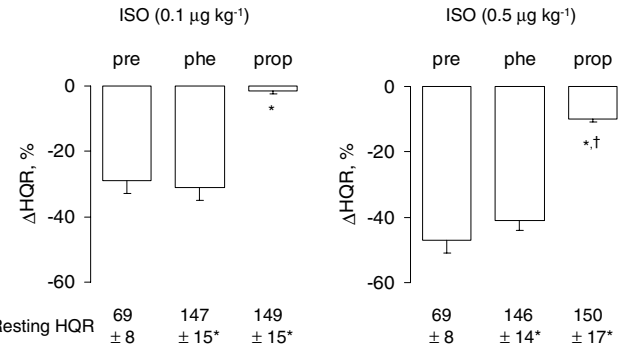

Figure 10 Summary of the maximal changes in HQR elicited by isoprenaline (0.1 and 0.5 $\mu g\,kg^{-1}$) before and during infusion of phenylephrine ($3\,\mu g\,kg^{-1}\,min^{-1}$) and again after injection of propranolol (prop). All values are mean ± s.e.m. Symbols for resting HQR. $^{*}P<0.05$, significant difference from Pre. $^{\dagger}P<0.05$, Post-propranolol *versus* post-saline or post-L-NAME. Symbols for isoprenaline-induced changes in HQR. $^{*}P<0.05$, significant change from Pre. $^{\dagger}P<0.05$, post-L-NAME *versus* post-saline and post-L-NAME + propranolol *versus* post-saline + propranolol.

of propranolol in the saline-treated and in the phenylephrine-infused rats were $-8\pm2\%$ and $-10\pm1\%$, respectively ($P>0.05$).

Discussion

The present study examined the effects of the NO synthesis inhibitor, L-NAME, and the β_1- and β_2-adrenoceptor antagonist, propranolol, on the hemodynamic responses elicited by two doses of isoprenaline, namely 0.1 and 0.5 $\mu g\,kg^{-1}$, in pentobarbital-anesthetized rats. The rationale for the use of the lower dose of isoprenaline was that the increases in HR and the vasodilator responses in the hindquarter and renal beds would be abolished by propranolol, whereas the vasodilator responses in the mesenteric bed would be substantially, but not completely, abolished by the β_1- and β_2-adrenoceptor antagonist (see Whalen & Lewis, 1999). Therefore, any changes in the contribution of propranolol-sensitive and propranolol-insensitive β-adrenoceptors after inhibition of NO synthesis following administration of L-NAME would be readily detectable. The rationale for the use of the higher dose of isoprenaline was that the hemodynamic actions of this agonist would be only partially affected by propranolol (see Whalen & Lewis, 1999), which would therefore allow for the ready evaluation of the activity of propranolol-sensitive and -insensitive β-adrenoceptors after inhibition of NO synthesis.

The increase in HR and the vasodilator responses in the hindquarter and renal beds elicited by the 0.1 $\mu g\,kg^{-1}$ dose of isoprenaline were abolished by propranolol in naïve rats, whereas the vasodilation in the mesenteric bed was substantially attenuated, but not abolished, after injection of the β_1- and β_2-adrenoceptor antagonist. These findings suggest that the majority of responses elicited by the lower dose of isoprenaline were due to activation of propranolol-sensitive β_1- and β_2-adrenoceptors whereas propranolol-insensitive β_1- and β_3-adrenoceptors, may also be present in the mesenteric circulation. The hemodynamic actions of the 0.5 $\mu g\,kg^{-1}$ dose of isoprenaline were substantially attenuated, but not abolished, by propranolol in naïve rats. This suggests that propranolol-insensitive β_1- and/or β_3-adrenoceptors are present in cardiac pacemaker cells and in the vascular smooth muscle of the hindquarter, renal and mesenteric vasculature.

This study confirms that the vasodilator actions of isoprenaline are not substantially diminished in pentobarbital-anesthetized rats treated with the NO synthesis inhibitor L-NAME (Whalen *et al*., 2000). It therefore appears that the vasodilator actions of isoprenaline in these rats were mainly due to activation of Gs protein-coupled β-adrenoceptors on vascular smooth muscle rather than the release of endothelium-derived nitrosyl factors such as NO and *S*-nitrosothiols (see Myers *et al*., 1990; Rosenblum, 1992; Stamler *et al*., 1992; 1997; Danser *et al*., 1998; Batenburg *et al*., 2004a, b). As such, it appears that the maximal vasodilation elicited by the full compliment of β-adrenoceptor subtypes available to isoprenaline is not altered after inhibition of NO synthesis.

One principal finding of this study was that the ability of propranolol to block the vasodilator actions of both doses of isoprenaline was substantially diminished in L-NAME-treated rats. Again, since the maximal responses to isoprenaline were not affected by L-NAME, it appears that the activity of propranolol-sensitive β_1- and β_2-adrenoceptors was reduced, whereas the activity of propranolol-insensitive β-adrenoceptors (e.g., β_3-adrenoceptors and the propranolol-insensitive state of β_1-adrenoceptors) was increased after inhibition of NO synthesis. In contrast, the ability of propranolol to block the hypotensive and vasodilator actions of the 0.1 and 0.5 $\mu g\,kg^{-1}$ doses of isoprenaline was not affected in rats that were receiving an equi-pressor infusion of the α_1-adrenoceptor agonist, phenylephrine. Taken together, it appears that the changes in the relative contribution of propranolol-sensitive and -insensitive β-adrenoceptors to the hemodynamic actions of isoprenaline in L-NAME-treated rats is not simply due to the increases in MAP and vascular resistances elicited by the NO synthesis inhibitor.

It is possible that the affinity of propranolol for β_1- and β_2-adrenoceptors or the signal transduction processes initiated by these receptors were diminished after loss of exposure to endothelial-derived nitrosyl factors (see Myers *et al*., 1990; Rosenblum, 1992; Stamler *et al*., 1992; 1997; Danser *et al*., 1998; Batenburg *et al*., 2004a, b), following administration of L-NAME. These nitrosyl factors may elicit their effects *via* generation of cGMP (Ignarro, 1990) and/or nitrosation of cysteine residues in functional proteins (Stamler *et al*., 1992; 1997). For example, nitrosation of β_1- and β_2-adrenoceptors may confer optimal receptor configuration for the agonist and the antagonist such that the loss of nitrosyl factors would lead to the downregulation of β_1- and β_2-adrenoceptor affinity for isoprenaline and propranolol. Conversely, the loss of nitrosyl factors and/or cGMP signaling mechanisms may upregulate β_3-adrenoceptors and/or induce the conformational change necessary to produce the propranolol-insensitive form of the β_1-adrenoceptor. It should be noted that the vasodilator actions of the membrane-permeable cAMP analog, 8-CPT-cAMP, are also augmented after injection of propranolol in anesthetized rats (Whalen *et al*., 1998). As such, the vasodilator actions of isoprenaline in rats treated with L-NAME and propranolol may involve the upregulation of propranolol-insensitive β_1- and β_3-adrenoceptors and cAMP signal transduction processes, perhaps *via* the loss of activity of cGMP-dependent phosphodiesterases that degrade cAMP (White *et al*., 1993).

This study also confirms that the increases in HR elicited by isoprenaline are augmented after injection of L-NAME (Whalen *et al*., 1999a). These *in vivo* findings complement *in vitro* studies that have demonstrated the existence and functional importance of NO synthase isoforms in cardiac pacemaker and muscle cells (see Balligand *et al*., 1993; Han *et al*., 1994; Gauthier *et al*., 1998). The tachycardia elicited by the membrane-permeable cAMP analog, 8-CPT-cAMP, is also augmented after injection of L-NAME in anesthetized rats (Whalen *et al*., 1999a). As such, the enhanced tachycardia elicited by isoprenaline in L-NAME-treated rats may involve the upregulation of β-adrenoceptors and cAMP signal transduction processes.

The other principal finding of this study was that the ability of propranolol to block the tachycardia elicited by the 0.1 and $0.5\,\mu g\,kg^{-1}$ doses of isoprenaline was markedly diminished in L-NAME-treated rats. In contrast, the ability of propranolol to diminish the increases in HR to isoprenaline were not diminished in rats that were receiving the infusion of phenylephrine. Since resting HRs were similar after the administration of propranolol in the L-NAME- and phenylephrine-treated rats, it appears that the differential effect of propranolol is not a function of the baseline HR. Taken together, it is possible that the loss of nitrosyl factors generated in cardiac pacemaker cells (see Balligand *et al*., 1993; Han *et al*., 1994; Gauthier *et al*., 1998) promotes the downregulation of propranolol-sensitive β_1- and β_2-adrenoceptor functions, whereas it promotes the upregulation of propranolol-insensitive β_1- and β_3-adrenoceptor functions. However, since the increases in HR elicited by 8-CPT-cAMP are also augmented after administration of propranolol (see Whalen *et al*., 1998), it is possible that the tachycardia elicited by isoprenaline in the presence of propranolol is due to the combination of an increased activity of propranolol-insensitive β_1- and β_3-adrenoceptors and enhanced cAMP-dependent signaling.

In summary, this study provides evidence that the activity of propranolol-sensitive β_1- and β_2-adrenoceptors is diminished, whereas the activity of propranolol-insensitive β_1- and β_3-adrenoceptors may be increased after inhibition of NO synthesis. It is possible that the differences in amino-acid sequences of β_1-, β_2- and β_3-adrenoceptor subtypes (see Harden, 1983; Probst *et al*., 1992; Emorine *et al*., 1994; Cohen *et al*., 1995) regulate the differential effects of nitrosyl factors and cGMP-dependent protein kinase on the functional status of these receptors. Moreover, in a recent study, the *S*-nitrosothiol, *S*-nitrosoglutathione, was shown to reversibly inhibit α_1-adrenoceptor-mediated vasoconstriction and ligand binding in the pulmonary artery. These effects of *S*-nitrosoglutathione were independent of cGMP-dependent protein kinase and strongly suggestive of receptor *S*-nitrosylation (Nozik-Grayck *et al*., 2006). The potential influence of nitrosyl factors on more traditional regulators of β-adrenoceptor function, signaling and expression, namely, protein kinase A, G protein-coupled receptor kinases, β-arrestin proteins and phosphodiesterases (Claing *et al*., 2002; Kohout & Lefkowitz, 2003; Lefkowitz & Whalen, 2004), and the consequences of such regulation remain to be determined.

This study was supported in part by grants DK66086, HL-14388 and HL-57472, NASA NAG5-6171, N00014-97-1-0145, and AHA 0350433N.

References

BALLIGAND, J-L., KELLY, R.A., MARSDEN, P.A., SMITH, T.W. & MICHEL, T. (1993). Control of cardiac muscle cell function by an endogenous nitric oxide signaling system. *Proc. Natl. Acad. Sci. U.S.A.,* **90,** 347–351.

BATENBURG, W.W., DE VRIES, R., SAXENA, P.R. & DANSER, A.H. (2004a). L-S-nitrosothiols: endothelium-derived hyperpolarizing factors in porcine coronary arteries? *J. Hypertens.,* **22,** 1927–1936.

BATENBURG, W.W., POPP, R., FLEMING, I., DE VRIES, R., GARRELDS, I.M., SAXENA, P.R. & DANSER, A.H. (2004b). Bradykinin-induced relaxation of coronary microarteries: *S*-nitrosothiols as EDHF? *Br. J. Pharmacol.,* **142,** 125–135.

BUNDKIRCHEN, A., BRIXIUS, K., BOLCK, B. & SCHWINGER, R.H. (2002). Bucindolol exerts agonistic activity on the propranolol-insensitive state of β_1-adrenoceptors in human myocardium. *J. Pharmacol. Exp. Ther.,* **300,** 794–801.

CLAING, A., LAPORTE, S.A., CARON, M.G. & LEFKOWITZ, R.J. (2002). Endocytosis of G protein-coupled receptors: roles of G protein-coupled receptor kinases and beta-arrestin proteins. *Prog. Neurobiol.,* **66,** 61–79.

COHEN, M.L., BLOOMQUIST, W., KRIAUCIUNAS, A., SHUKER, A. & CALLIGARO, D. (1999). Aryl propanolamines: comparison of activity at human β_3-adrenoceptors, rat β_3-adrenoceptors and atrial receptors mediating tachycardia. *Br. J. Pharmacol.,* **126,** 1018–1024.

COHEN, M.L., GRANNEMAN, J.G., CHAUDRY, A., SCHENK, K.W., CUSHING, D.J. & PALKOWITZ, A.D. (1995). Is the 'atypical' β-receptor in the rat stomach fundus the rat β_3 receptor? *J. Pharmacol. Exp. Ther.,* **272,** 446–451.

DANSER, A.H., DE VRIES, R., SCHOEMAKER, R.G. & SAXENA, P.R. (1998). Bradykinin-induced release of nitric oxide by the isolated perfused rat heart: importance of preformed pools of nitric oxide-containing factors. *J. Hypertens.,* **16,** 239–244.

DAVISSON, R.L., TRAVIS, M.D., BATES, J.N. & LEWIS, S.J. (1996). Hemodynamic effects of L- and D-*S*-nitrosocysteine in the rat: stereoselective *S*-nitrosothiol recognition sites. *Circ. Res.,* **79,** 256–262.

EMORINE, L., BLIN, N. & STROSBERG, A.D. (1994). The human β_3-adrenoceptor: the search for a physiological function. *Trends Pharmacol. Sci.,* **15,** 3–7.

GAUTHIER, C.H., LEBLAIS, V., KOBZIK, L., TROCHU, J.N., KHANDOUDI, N., BRIL, A., BALLIGAND, J.L. & LE MAREC, H. (1998). The negative inotropic effect of β_3-adrenoceptor stimulation is mediated by activation of a nitric oxide synthase pathway in human ventricle. *J. Clin. Invest.,* **102,** 1377–1384.

HAN, X., SHIMONI, Y. & GILES, W.R. (1994). An obligatory role for nitric oxide in autonomic control of mammalian heart rate. *J. Physiol.,* **476,** 309–314.

HARDEN, T.K. (1983). Agonist-induced desensitization of the β-adrenergic receptor-linked adenylate cyclase. *Pharmacol. Rev.,* **35,** 5–32.

HAYWOOD, J.R., SHAFFER, R.A., FASTENOW, C., FINK, G.D. & BRODY, M.J. (1981). Regional blood flow measurement with pulsed Doppler flowmeter in conscious rat. *Am. J. Physiol.,* **241,** H273–H278.

IGNARRO, L.J. (1990). Nitric oxide: a novel signal transduction mechanism for transcellular communication. *Hypertension,* **16,** 477–483.

KAUMANN, A.J. (1996). (−)CGP 12177-induced increase of human atrial contraction through a putative third β-adrenoceptor. *Br. J. Pharmacol.,* **117,** 93–98.

KAUMANN, A.J., ENGELHARDT, S., HEIN, L., MOLENAAR, P. & LOHSE, M. (2001). Abolition of (−)-CGP 12177-evoked cardiostimulation in double β_1/β_2-adrenoceptor knockout mice. Obligatory role of β_1-adrenoceptors for putative β_4-adrenoceptor pharmacology. *Naunyn-Schmiedeberg's Arch. Pharmacol.,* **363,** 87–93.

KOHOUT, T.A. & LEFKOWITZ, R.J. (2003). Regulation of G protein-coupled receptor kinases and arrestins during receptor desensitization. *Mol. Pharmacol.,* **63,** 9–18.

LACOLLEY, P.J., LEWIS, S.J. & BRODY, M.J. (1991). Role of the sympathetic nerve activity in the generation of vascular nitric oxide in urethane anesthetized rats. *Hypertension,* **17,** 881–887.

LEFKOWITZ, R.J. & WHALEN, E.J. (2004). Beta-arrestins: traffic cops of cell signaling. *Curr. Opin. Cell Biol.,* **16,** 162–168.

LEFKOWITZ, R.J. (2004). Historical review: a brief history and personal retrospective of seven-transmembrane receptors. *Trends Pharmacol. Sci.,* **25,** 413–422.

LEWIS, S.J., GRAVES, J.E., BATES, J.N. & KOOY, N.W. (2005a). Peroxynitrite elicits dysfunction of stereoselective *S*-nitrosocysteine recognition sites. *J. Cardiovasc. Pharmacol.,* **46,** 637–645.

LEWIS, S.J., HOQUE, A. & BATES, J.N. (2005b). Differentiation of L- and D-*S*-nitrosothiol recognition sites. *J. Cardiovasc. Pharmacol.,* **46,** 660–671.

LEWIS, S.J., HOQUE, A., WALTON, T.M. & KOOY, N.W. (2005c). Potential role of nitration and oxidation reactions in the effects of peroxynitrite on the function of β-adrenoceptor sub-types in the rat. *Eur. J. Pharmacol.,* **518,** 187–194.

MYERS, P.R., MINOR JR, R., GUERRA JR, R., BATES, J.N. & HARRISON, D.G. (1990). Vasorelaxant properties of the endothelium-derived relaxing factor more closely resembles *S*-nitrosocysteine than nitric oxide. *Nature,* **345,** 161–163.

NOZIK-GRAYCK, E., WHALEN, E.J., STAMLER, J.S., MCMAHON, T.J., CHITANO, P. & PIANTADOSI, C.A. (2006). *S*-nitrosoglutathione inhibits α_1-adrenergic receptor-mediated vasoconstriction and ligand binding in pulmonary artery. *Am. J. Physiol.,* **290,** L136–L143.

PROBST, W.C., SNYDER, L.A., SCHUSTER, D.I., BROSIUS, J. & SEALFON, S.C. (1992). Sequence alignment of the G-protein coupled receptor superfamily. *DNA Cell Biol.,* **11,** 1–20.

ROSENBLUM, W.I. (1992). Endothelium-derived relaxing factor in brain blood vessels is not nitric oxide. *Stroke,* **23,** 1527–1535.

SEINO, S. & SHIBASAKI, T. (2005). PKA-dependent and PKA-independent pathways for cAMP-regulated exocytosis. *Physiol. Rev.,* **85,** 1303–1342.

STAMLER, J.S., SINGER, D.J. & LOSCALZO, J. (1992). Biochemistry of nitric oxide and its redox-activated forms. *Science,* **258,** 1898–1902.

STAMLER, J.S., TOONE, E.J., LIPTON, S.A. & SUCHER, N.J. (1997). S)NO signals: Translocation, regulation, and a consensus motif. *Neuron,* **18,** 691–696.

WHALEN, E.J. & LEWIS, S.J. (1999). *In vivo* evidence that isoproterenol may increase heart rate in the rat by mechanisms in addition to activation of cardiac β_1- or β_2-receptors. *Eur. J. Pharmacol.,* **382,** 207–210.

WHALEN, E.J., JOHNSON, A.K. & LEWIS, S.J. (1998). Blockade of β-adrenoceptors up-regulates cAMP signal transduction *in vivo*. *Eur. J. Pharmacol.,* **355,** 189–193.

WHALEN, E.J., JOHNSON, A.K. & LEWIS, S.J. (1999a). Effects of nitric oxide synthase inhibition on sympathetically-mediated tachycardia *in vivo*. *Eur. J. Pharmacol.,* **365,** 217–223.

WHALEN, E.J., JOHNSON, A.K. & LEWIS, S.J. (1999b). Tachyphylaxis to PACAP-27 after inhibition of NO synthesis: a loss of adenylate cyclase activation. *Am. J. Physiol.,* **277,** R1453–R1461.

WHALEN, E.J., JOHNSON, A.K. & LEWIS, S.J. (1999c). Hemodynamic actions of systemically injected pituitary adenylate cyclase activating polypeptide-27 in the rat. *Eur. J. Pharmacol.,* **365,** 205–215.

WHALEN, E.J., JOHNSON, A.K. & LEWIS, S.J. (2000). Adrenoceptor dysfunction after inhibition of NO synthesis. *Hypertension,* **36,** 376–382.

WHALEN, E.J., TRAVIS, M.D., JOHNSON, A.K. & LEWIS, S.J. (1999d). Rapid tachyphylaxis to the hemodynamic effects of PACAP-27 after inhibition of nitric oxide synthesis. *Am. J. Physiol.,* **276,** H2117–H2126.

WHITE, R.E., LEE, A.B., SHCHERBATCO, A.D., LINCOLN, T.M., SCHONBRUNN, A. & ARMSTRONG, D.L. (1993). Potassium channel stimulation by natriuretic peptides through cGMP-dependent dephosphorylation. *Nature,* **361,** 263–266.

YOUNG, L.H., LI, J., BARON, S.J. & RUSSELL, R.R. (2005). AMP-activated protein kinase: a key stress signaling pathway in the heart. *Trends Cardiovasc. Med.,* **15,** 110–118.

(Received November 22, 2005
Accepted January 4, 2006
Published online 6 February 2006)