β₁ Receptors Protect the Renal Afferent Arteriole of Angiotensin-Infused Rabbits from Norepinephrine-Induced Oxidative Stress

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Renal afferent arterioles (Aff) from angiotensin II (AngII)-infused rabbits have enhanced contractions to AngII that are normalized by tempol (superoxide dismutase mimetic), whereas contractions to norepinephrine (NE) are normal and unaffected by tempol. Tested was the hypothesis that β-receptor stimulation with NE prevents enhanced reactivity and superoxide generation. Preconstricted Aff from AngII- or vehicle-infused rabbits were perfused at physiologic pressure. Aff from vehicle-infused rabbits had strong, endothelium-independent relaxations to dobutamine (β₁-receptor agonist; 14 ± 6%; P < 0.0001; mean ± SD) but only weak relaxations to salbutamol (β₂-receptor agonist; 13 ± 3%; P < 0.05) or BRL-37,344 (β₂-receptor agonist; 14 ± 3%; P < 0.05). Contractions to NE were similar in Aff from vehicle- and AngII-infused rabbits (~36 ± 5 versus ~34 ± 3%; NS) and were unaffected by tempol (~32 ± 4%; NS). In contrast, phenylephrine contractions (α₁ agonist) were enhanced in Aff from AngII-infused rabbits (~59 ± 6 versus ~46 ± 4%; P < 0.05) and normalized by tempol. NE contractions in Aff from AngII-infused rabbits (~34 ± 4%) were enhanced (P < 0.01) by propranolol (nonselective β antagonist; −53 ± 6%), CGP-20,712A (selective β₁-receptor antagonist; −61 ± 9%), or Rp-cAMP (competitive inhibitor of cAMP; −56 ± 4%); were normalized by tempol; but were unaffected by ICI-118,551 (selective β₂-receptor antagonist) or SR-59,230A (selective β₂-receptor antagonist). Superoxide generation in Aff from AngII-infused rabbits that were assessed from ethidium:dihydro-ethidium was enhanced by addition of CGP-20,712A to NE but was normalized by tempol. Aff have robust α₁-receptor contraction and β₁-receptor dilation. NE elicits β₁ signaling via cAMP that moderates oxidative stress and contractions in Aff from AngII-infused rabbits.

Oxidative stress suggests an increased production or a decreased metabolism of reactive oxygen species (ROS). Superoxide (O₂⁻) mediates enhanced contractility in many models of hypertension (1,2). O₂⁻ is metabolized by superoxide dismutase (SOD) to H₂O₂. Harrison and colleagues (1,3) showed that, whereas prolonged infusion of angiotensin II (AngII) generates O₂⁻ in blood vessels, infusions of norepinephrine (NE) fail to induce vascular oxidative stress. Moreover, administration of permanent SOD reverses hypertension in the AngII-induced but not the NE-induced rat model (3). They concluded that AngII selectively induces vascular oxidative stress, which contributes to hypertension.

Relatively low rates of infusion of AngII into mice (4), rats (1,5,6), rabbits (7), or humans (8) increase BP gradually (“slow pressor response”). This is considered a model of human hypertension because the plasma levels of AngII increase only within a physiologic range of 50 to 100% (9). The hypertension entails a renal mechanism because it depends on salt intake (10) and an enhanced vascular reactivity of the renal afferent arteriole (Aff) to AngII (11). Unlike AngII, infusion of NE leads to tachyphylaxis and a decreased BP response (8). The AngII slow pressor response has been related to ROS because it is prevented by co-infusion of the permanent SOD mimetic nitroxide tempol (4,6).

Low rates of infusion of the thromboxane-prostanoid receptor (TP-R) mimetic U-46,619 (12) or endothelin-1 (ET-1) (13) also elicit slow pressor responses that are accompanied by oxidative stress. Moreover isolated, pressurized Aff that are dissected from the kidneys of rabbits that are undergoing an AngII slow pressor response have an enhanced contractility not only to AngII but also to ET-1 and U-46,619 (11) that depend on O₂⁻ because they are moderated or prevented by bath addition of the permanent SOD mimetic nitroxide tempol (11). In contrast, contractions to bath addition of NE or high [K⁺] are not enhanced in Aff from AngII-infused rabbits and are not affected by tempol. This suggests an alternative explanation for the previous studies: Prolonged AngII infusion generates vascular ROS that enhance the responses to AngII and also to a number of other G protein–coupled receptor agonists such as ET-1, U-46,619, and phenylephrine (PE). In contrast, activation of β-adrenergic receptors by NE prevents the generation of ROS and consequently the enhanced contraction. This suggests that the unique response is to NE rather than to AngII. Therefore,
these studies were undertaken to investigate the hypothesis that activation of a specific subtype of β-adrenergic receptors by NE prevents ROS-induced enhanced contractility of Aff from AngII-infused rabbits.

Materials and Methods

Animal Protocols

The protocol was approved by the Georgetown University Animal Care and Use Committee. Male New Zealand white rabbits (1.8 to 2.1 kg) were maintained on tap water and standard diet (Na+ content 0.4 g/100g) and prepared as described previously (11). Under local anesthesia with EMLA cream (2.5% lidocaine and 2.5% prilocaine), sterile osmotic minipumps (Alzet, DURET Corp., Cupertino, CA) were implanted subcutaneously in rabbits (n = 6 per group) to infuse vehicle (0.154 M NaCl) or human AngII (Peninsula Laboratories, San Carlos, CA) at 200 ng/kg per min (AngII) for 12 to 14 d.

Isolation and Microperfusion of Aff

Rabbits were anesthetized with xylazine (10 mg/kg intramuscularly), ketamine (50 mg/kg intramuscularly), and pentobarbital sodium (10 mg/kg intravenously) followed by heparin (1000 USP intravenously) for anticoagulation. Microdissection and microperfusion at 60 mmHg of Aff were performed as described previously (14,15). Each experiment in each series used a separate arteriole.

Experimental Protocol

The first aim was to test the role of the β-adrenoreceptor subtype and the endothelium in the relaxation of Aff from vehicle-infused rabbits (n = 6 per group). Aff were preconstricted by 60 to 70% from control luminal diameter with PE (10−6 M; α1-receptor agonist). Thereafter, dobutamine (10−7 M; β1-receptor agonist) (16), salbutamol (10−6 M; β2-receptor agonist) (17), or BRL-37,344 (BRL; 10−6 M; β2-receptor agonist) (17) was added to the bath. These dosages were selected in pilot studies to show <90% of maximal responses. Vessels were observed for 3 to 5 min, which allowed for maximal responses. Parallel studies were undertaken in vessels (n = 6) that were denuded of endothelium by microperfusion with 0.1% goat anti-human von Willebrand factor antibody plus 2% guinea pig serum complement for 10 min (14,18). This entirely prevents relaxation responses to acetylcholine but leaves intact responses to sodium nitroprusside (14). A full dosageresponse to the most effective agent, dobutamine (10−10 to 10−6 M) was performed with and without endothelium removal.

The second aim was to contrast the role of O2− in contractile responses of Aff from AngII- versus vehicle-infused rabbits (n = 6 per group). Constrictions to bath additions of NE (10−6 M; activating both α1- and β-adrenergic receptors) and PE (10−7 M; activating α1-adrenergic receptors) were related to a standard maximal contraction with NE (10−6 M) plus 40 mM KCl (NAK). These responses were maintained for 3 min. Thereafter, Aff were washed with buffer, recovered for 10 min, and incubated for 20 min with tempol (10−4 M) to assess the effects of metabolism of O2− (19). Tempol at 10−4 M blocks fully the contractile responses of afferent arterioles to O2− that are generated by a quinolone (19) but does not change basal tone or responses of vessels from normal rabbits that are infused with a vehicle to AngII, U-46,619, NE, or ET-1 (7).

The third aim was to contrast the effect of nonselective β-adrenergic receptor blockade alone or with tempol (10−4 M) on contractions to NE or to high [K+] of Aff from AngII- or vehicle-infused rabbits (n = 6 per group). Aff were incubated for 20 min with a vehicle (MEM solution), propranolol (10−6 M; nonselective β-adrenergic receptor antagonist) (20), tempol (10−4 M), or a combination of propranolol and tempol (order randomized). Luminal diameters were measured during basal conditions and after superfusion with NE (10−7 M) or KCl (40 mM).

The fourth aim was to test the role of cAMP in mediating the effects of β-adrenergic receptor stimulation with NE in mitigating the enhanced contractions in Aff from AngII-infused rabbits (n = 6 per group). Constrictions to NE (10−6 M) or KCl (40 mM/L) were obtained before and after 20 min of incubation with vehicle, adenosine-3’,5’-cyclic monophosphorothioate Rp-isomer (Rp-cAMP, 5 × 10−6 M; cAMP antagonist) (21,22) or tempol (10−4 M) or a combination of Rp-cAMP and tempol (order randomized).

The fifth aim was to evaluate the β-adrenergic receptor subtype that modulates NE contractions of Aff from AngII-infused rabbits (n = 6 per group). Aff were incubated for 30 min with CGP-20,712A (CGP; 10−8 M; selective β1-adrenergic receptor agonist), ICI-118,551 (ICI; 10−8 M; selective β2-adrenergic receptor agonist), or SR-59,230A (SR; 10−6 M; selective β3-adrenergic receptor antagonist) (23) alone or in combination with tempol (orders randomized). Luminal diameters were measured during basal conditions and after superfusion with NE (10−7 M).

The sixth aim was to test the specificity of β1-adrenergic receptor blockade by CGP in Aff from vehicle-infused rabbits (n = 6 per group). Aff were incubated for 30 min with vehicle (MEM solution) or the β1-adrenergic receptor agonist CGP (10−8 M). Thereafter, they were preconstricted with PE (10−6 M), after which dobutamine (10−7 M), salbutamol (10−6 M), or BRL (10−6 M) was added to the bath.

The seventh aim was to test the hypothesis that β1-adrenergic receptor activation during NE prevents an increase in O2− generation in Aff. O2− generation was assessed by fluorescence microscopy of perfused Aff (n = 6) with dihydroethidium (DHE). DHE is freely permeable to cells. It is oxidized by O2− to the highly fluorescent compound ethidium, which is trapped intracellularly and intercalated into DNA (24). The conversion of DHE to ethidium was quantified by a dual-wavelength determination using an excitation wavelength of 380 nm and an emission wavelength of 460 nm for DHE and an excitation wavelength of 535 nm and an emission wavelength of 605 nm for ethidium (25). Single-agent signal capture was achieved by cycling at 3-s intervals between a 460- and 605-nm filter. Changes in O2− were expressed as the ratio of ethidium:DHE fluorescence (26). The system used an Olympus IX70 fluorescence microscope equipped with dual photomultipliers (PMT, Photon Technology Int., Lawrenceville, NJ). Excitation was provided by a 75-W xenon arc lamp using a 380/460 nm wavelength combination isolated with a computer-controlled monochromator. Ethidium and DHE emit blue and red light, respectively, that was directed to a dual PMT assembly by a beam splitter that directed light to the two separate PMT using barrier filters centered at 460 and 605 nm, respectively. The ratio of ethidium:DHE was monitored in real time and recorded by software (Felix32; Photon Technology Int.).

For testing of β1-adrenergic receptor activation of ROS generation during NE action, Aff from AngII-infused rabbits were cannulated and load with HBSS that contained DHE (10−5 M) and glutamine (10 mM). After 30 min for equilibration, the ratio of ethidium:DHE was assessed for 10-min periods during vehicle and after 30 min of incubation with NE (10−7 M), 30 min of incubation with NE+CGP (10−8 M; β1-adrenergic receptor blocker), and 30 min of incubation with NE plus a combination of CGP and tempol (10−4 M). A separate set of Aff were incubated throughout with a vehicle as a time control.

Drugs and Solutions

All solutions were prepared fresh daily. Reagents were purchased from Sigma (St. Louis, MO). Rp-cAMP was obtained from Biolog-Life Science Institute (La Jolla, CA). Earle’s Deficient BME Solution was

used for dissection. It contains 8.89 g/L NaCl, 26 mM NaHCO₃, 2 mM l-glutamine, and 5% BSA (pH 7.40 to 7.45). It was filtered (0.8 μm) and prepared fresh daily. MEM solution that contained 26 mM NaHCO₃ and 5% BSA was used for perfusion, and MEM that contained 26 mM NaHCO₃ and 0.15% BSA was used for superfusion. HBSS was purchased from Invitrogen (Carlsbad, CA).

Statistical Analyses
Statistical tests of the percentage of vasoconstriction or vasodilation used 2 × 2 factorial repeated-measures ANOVA (Statistical Software v.5.0; University of Hamburg, Hamburg, Germany). When appropriate, post hoc comparisons between groups were made with t test. Statistical significance was defined as P < 0.05. Data are presented as mean ± SD.

Results
The diameter of Aff from AngII- and vehicle-infused rabbits was similar in the basal state (17.3 ± 1.2 versus 16.9 ± 1.2 μm; NS) and during NAK (6.5 ± 0.5 versus 6.3 ± 0.6 μm; NS)

β-Adrenoceptors Subtype Mediating Relaxation of Aff
Dobutamine (10⁻⁷ M; a selective β₁-adrenergic receptor agonist) potently relaxed PE-constructed Aff (78 ± 6%; P < 0.001), whereas salbutamol (10⁻⁶ M; selective β₂-adrenergic receptor agonist) had a significantly (P < 0.001) weaker effect (13 ± 3%; P < 0.05) as did BRL (10⁻⁶ M; selective β₂-adrenergic receptor agonist, 14 ± 3; P < 0.05). None of these relaxations was affected by endothelial removal (Figure 1A). Dobutamine induced a dosage-dependent, endothelium-independent relaxation, with an IC₅₀ of 4.99 × 10⁻² ± 2.03 × 10⁻⁴ M (Figure 1B). We conclude that β-receptor vasodilation in rabbit Aff is mediated predominantly by β₁-adrenergic receptors via an endothelium-independent mechanism.

Role of O₂⁻ in Contractions of Aff to NE and PE
Compared with Aff from vehicle-infused rabbits, Aff from AngII-infused rabbits had similar contractions to NE (−36 ± 5 versus −34 ± 3%; NS) but enhanced contractions to PE (−59 ± 6 versus −46 ± 4%; P < 0.05; Figure 2). Bath addition of tempol did not modify the diameter in the basal state or during NAK. Bath addition of tempol to Aff from AngII-infused rabbits did not affect their response to NE but reduced their contractions to PE to the level of Aff from vehicle-infused rabbits (49 ± 5 versus 46 ± 4; NS). We conclude that PE generates O₂⁻ in Aff from AngII-infused rabbits, which enhances their responsiveness, whereas NE does not.

Effect of β-Adrenergic Receptor Blockade on Contractions of Aff to NE
Aff from rabbits that were infused with vehicle or AngII had similar contractions to KCl and NE (Figure 3). Bath addition of tempol, propranolol, or tempol plus propranolol did not modify contractions of Aff to KCl in either group and did not modify contractions to NE in vehicle-infused rabbits. However, propranolol enhanced contractions to NE in Aff from AngII-infused rabbits (−34 ± 3 versus −53 ± 6%; P < 0.01; Figure 3B). This effect of propranolol was prevented by tempol (53 ± 6%; NS versus propranolol alone). We conclude that activation of β-adrenergic receptors in Aff from AngII-infused rabbits prevents enhanced O₂⁻−-dependent contractions to NE.

Role of cAMP in Moderating NE Contraction of Aff from AngII-Infused Rabbits
KCl contractions of Aff from AngII-infused rabbits were unaffected by tempol, Rp-cAMP, or tempol plus Rp-cAMP (Figure 4). Whereas tempol did not affect NE contractions of Aff from AngII-infused rabbits, these contractions were enhanced by Rp-cAMP (−38 ± 3 versus −56 ± 4%; P < 0.01). This effect of Rp-cAMP was blocked by bath addition of tempol (−37 ± 6%; NS versus tempol alone). We conclude that cAMP is generated in Aff from AngII-infused rabbits mediates the effects of β-adrenergic receptors to inhibit O₂⁻− generation and enhance NE contractions.

Subtype of β-Adrenergic Receptor Preventing Enhanced Contractions to NE in Aff from AngII-Infused Rabbits
NE contractions of Aff from AngII-infused rabbits were unaffected by tempol alone, ICI (selective β₂-adrenergic receptor...
antagonist), SR (selective β2-adrenergic receptor antagonist), or ICI or SR plus tempol (Figure 5). However, CGP (10⁻⁸ M; selective β1-adrenergic receptor antagonist) enhanced NE contractions (−40 ± 3 versus −61 ± 9%; P < 0.001). This effect was prevented by tempol (−39 ± 5%; NS versus tempol alone). We conclude that the β1-adrenergic receptors that inhibit O₂⁻ generation and enhance contractions to NE in Aff from AngII-infused rabbits are subtype I.

Specificity of CGP as a β1-Adrenergic Receptor Antagonist
The addition of CGP (10⁻⁸ M) to the bath of Aff from vehicle-infused rabbits prevented the relaxation to dobutamine (10⁻⁷ M; selective β1-adrenergic receptor agonist; 78 ± 5 versus 3 ± 5%; P < 0.001) but not to salbutamol (selective β2-adrenergic receptor agonist) or BRL (selective β2-receptor agonist) (Figure 6). We conclude that CGP is a selective β1-adrenergic receptor antagonist in this preparation.
Role of $\beta_1$ Adrenergic Receptors in Preventing $O_2^-$ Generation with NE from AngII-Infused Rabbits

NE at $10^{-7}$ M slightly but significantly increased the ethidium:DHE fluorescence ratio (before 0.01 ± 0.08 versus during NE 0.28 ± 0.10; $P < 0.05$). After Aff were incubated with CGP ($10^{-6}$ M; $\beta_1$-adrenergic receptor blocker), NE increased the ethidium:DHE ratio significantly (1.33 ± 0.5 CGP+NE versus 0.28 ± 0.1 NE alone; $P < 0.001$). This was restored to the level of NE alone by addition of tempol ($10^{-4}$ M) to vessels that were incubated with CGP and NE (0.23 ± 0.11; NS versus NE+vehicle; Figure 7). The ratio of ethidium:DHE did not change in Aff that were incubated with a vehicle (time control).

Discussion

We confirm that prolonged infusion of AngII does not modify NE contractions of Aff, in contrast to the enhanced responses reported with AngII, ET-1, and U-46,619 (7,11). The new findings are that the absence of an enhanced response to NE in these vessels cannot be ascribed to a protective role of $\alpha_1$-adrenergic receptor stimulation because the contractile response to phenylephrine is enhanced similarly to AngII, ET-1, and U-46,619. Blockade of $\beta_1$-adrenergic receptors unmasks an enhanced NE response in Aff from AngII-infused rabbits, similar to PE. This implicates $\beta_1$-adrenergic receptors in moderating the responsiveness. Because this effect is reversed by incubation with tempol, it can be ascribed to $\beta_1$-adrenergic receptor-dependent inhibition of vascular $O_2^-$ generation. The $\beta_1$-adrenergic receptor subtype that is responsible is identified as type I because the $\beta_1$-adrenergic receptor agonist dobutamine causes a much more powerful relaxation of preconstricted Aff than $\beta_2$- or $\beta_3$-adrenergic agonists and the $\beta_1$-adrenergic receptor antagonist CGP enhances NE contractions in Aff from AngII-infused rabbits, whereas $\beta_2$- or $\beta_3$-adrenergic receptor antagonists are ineffective. Fluorescence microscopy demonstrated that blockade of $\beta_1$-adrenergic receptors during stimulation with NE enhanced $O_2^-$ in the perfused Aff, which was restored by incubation with tempol. The effects of $\beta_1$-adrenergic receptors to moderate contractions are independent of the endothelium. Because this effect is blocked by Rp-cAMP, it seems to be mediated by generation of cAMP. Therefore, the absence of an enhanced response to NE in Aff from AngII-infused rabbits is related to $\beta_1$-adrenergic receptors that activate cAMP and counteract $\alpha_1$-adrenergic receptor stimulation of $O_2^-$. Tempol is freely membrane permeable and acts as a catalytic SOD mimic (15). Tempol fully prevents the graded vasoconstriction of isolated, perfused Aff to the $O_2^-$-generating quinolone paraquart (19). Studies with fluorescence indicators of $O_2^-$ have confirmed that tempol, in the dosage used in this study, fully prevents the generation of $O_2^-$ in isolated, perfused vasa recta that are stimulated with AngII (27). This was confirmed in this study, in which enhanced vascular $O_2^-$ generation by NE during $\beta_1$-adrenergic receptor blockade was normalized by bath addition of $10^{-4}$ M tempol.

Contractions to NE in Aff are mediated via $\alpha_1$-adrenergic receptor–dependent transmembrane increases in intracellular [$Ca^{2+}$] (28) and release of $Ca^{2+}$ from intracellular stores (29). The finding that PE seemed to have enhanced ROS generation in Aff from AngII-infused rabbits is consistent with its effect to increase ROS generation in vitro in vascular smooth muscle cells (30) and in vivo in rat aorta, where the effect is accompanied by upregulation of p47phox expression and mediated via NAPDH oxidase (31).

Within the cardiovascular system, $\beta_1$-adrenergic receptors are expressed predominantly in cardiomyocytes, where they mediate chronotropic and inotropic effects (32), whereas $\beta_2$-adrenergic receptors are expressed predominantly in peripheral blood vessels, where they mediate vasorelaxation (32). $\beta_2$-Adrenergic receptors can cause endothelium-dependent relaxation of coronary microvessels but have a less defined role presently in the cardiovascular system (33). Therefore, the finding that vasorelaxation of Aff was much more prominent with a $\beta_2$- than with a $\beta_1$- or $\beta_3$-adrenergic receptor agonist would not be expected from Land’s hypothesis. This may relate to a selective action of $\beta_1$-adrenergic receptors on the terminal renal Aff, where specialized granular smooth muscle cells are stimulated to release renin predominantly by $\beta_1$-adrenergic recep-

Figure 6. Mean ± SD values ($n = 6$ per group) for effect of CGP ($10^{-6}$ M; ■) compared with vehicle (□) on the relaxation responses of Aff from vehicle-infused rabbits that were preconstricted with $10^{-6}$ M PE to dobutamine ($10^{-7}$ M; $\beta_1$-adrenergic receptor agonist), salbutamol ($10^{-6}$ M; $\beta_2$-receptor agonist), or BRL ($10^{-6}$ M; $\beta_3$-adrenergic receptor agonist). ***$P < 0.001$ versus vehicle.

Figure 7. Mean ± SD values ($n = 6$) for reactive oxygen species (ROS) generation from the ratio of fluorescence of ethidium: dihydroethidium (Eth:DHE) during incubation of Aff from AngII-infused rabbits with vehicle, $10^{-7}$ M NE, NE plus CGP ($10^{-6}$ M; $\beta_1$-adrenergic receptor antagonist), or NE plus CGP plus $10^{-4}$ M tempol (TMP). *$P < 0.05$, ***$P < 0.001$ versus vehicle; b$p < 0.01$ versus NE alone.
tors (34,35). It seems that a similar receptor activation also induces vasorelaxation of this blood vessel. Moreover, recent studies have shown predominant β-adrenergic receptor regulation of vascular tone by the β1 subclass in some small vessels, depending on species and site (36). Recent studies in the rat have concluded that β1, not β2-adrenergic receptors predominate on vascular smooth muscle cells of mesenteric resistance vessels (17) and renal microvessels and macula densa epithelium (37). Functionally, the β1-adrenergic receptor is the predominant class mediating vasodilation in the small blood vessels of the kidney and mesentery of rats or mice (17,38). In an in vivo analysis in humans, Wellstein et al. (39) estimated that 77% of β-adrenergic receptor–related hypotension is mediated via β1-adrenergic receptors and 23% via β2-adrenergic receptors. Studies in the rabbit have confirmed that β1-adrenergic receptor blockade with atenolol inhibits the renin release in response to angiotensin receptor blockade, which is considered to be an effect that is mediated at the terminal Aff (40).

The effect of β1-adrenergic receptors to inhibit ROS generation seems to depend on protein kinase A (PKA) because it is blocked by Rp-cAMP. Studies in the isolated mesenteric resistance vessel of the rat have shown that β1-adrenogenic receptors induce vasorelaxation via separate PKA-dependent and -independent pathways (22).

Within the peripheral circulation, β-adrenergic receptor stimulation can lead to endothelium-dependent or -independent vasorelaxation. Removal of the endothelium from the terminal renal Aff did not modify the vasorelaxation to β1- or β2-, or β1-adrenergic receptor stimulation, indicating that relaxation at this site is endothelium independent. This conclusion held for the entire dosage-response range for the β1-adrenergic receptor agonist dobutamine. This is consistent with recent conclusions from studies with fluorescence covalent ligands for β1-adrenergic receptors that locate these receptors on vascular smooth muscle cells of rat mesenteric resistance vessels in the plasma membrane, Golgi, endoplasmic reticulum, and perinuclear spaces (17).

Whereas epinephrine is a potent agonist of β1- and β2-adrenergic receptors, NE activates predominantly β1-adrenergic receptors. This is consistent with the finding that the β1-adrenergic receptor antagonist CGP increased NE vasoconstriction markedly in Aff from AngII-infused rabbits, whereas a β2- or β1-adrenergic receptor antagonist was not effective. CGP was validated as a selective β1-adrenergic receptor antagonist because it prevented the response to the β1-adrenergic receptor agonist dobutamine without affecting the response to a β2- or β1-adrenergic receptor agonist.

Whereas there is much evidence of receptor-dependent stimulation of vascular ROS by agonists such as PE, AngII, ET-1, or U-46, 619, less is known concerning receptor-mediated antioxidant defense, for example by catecholamines. Activation of dopamine D1 and D3 receptors in rat renal vascular smooth muscle cells inhibits ROS generation (41). D3 receptor activation in human embryonic kidney HEK-293 cells inhibits ROS generation by activation of heme oxygenase-1 (42) and inhibition of the assembly and activity of NAPDH oxidase (43). Because the D3 receptor–mediated inhibition of ROS generation is independent of cAMP in HEK-293 cells, whereas D1-like receptor–mediated inhibition of ROS in renal vascular smooth muscle cells is mediated in part by cAMP, the D1 dopamine receptor, like the β1-adrenergic receptor, may exert their antioxidant effects via cAMP.

If our finding that β1 receptors protect the renal Aff of AngII-infused rabbits from NE-induced oxidative stress and associated enhanced contractility is relevant to other microvessels, then this may help to explain why prolonged treatment of hypertension with a β1-adrenergic receptor antagonist, atenolol, in contrast to the antioxidant calcium channel blocker, amlodipine (44), fails to restore endothelial function or reverse remodeling of subcutaneous microvessels (45). Moreover, atenolol fails to provide effective cardiovascular protection in trials of hypertensive patients (46). This has led some authorities to recommend that β1-adrenergic receptor antagonists no longer be prescribed as primary therapy for hypertension (47).

A potential explanation that requires study is that such therapy prevents the counterbalance of α-adrenergic receptor–mediated ROS generation during NE stimulation by activated β1-adrenergic receptors.

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