

Chemistry and Antihypertensive Effects of Tempol and Other Nitroxides

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Abstract—Nitroxides can undergo one- or two-electron reduction reactions to hydroxylamines or oxammonium cations, respectively, which themselves are interconvertible, thereby providing redox metabolic actions. 4-Hydroxy-2,2,6,6-tetramethylpiperidine-*N*-oxyl (tempol) is the most extensively studied nitroxide. It is a cell membrane-permeable amphiphile that dismutates superoxide catalytically, facilitates hydrogen peroxide metabolism by catalase-like actions, and limits formation of toxic hydroxyl radicals produced by Fenton reactions. It is broadly effective in detoxifying these reactive oxygen species in cell and animal studies. When administered intravenously to hypertensive rodent models, tempol caused rapid and reversible dose-dependent reductions in blood pressure in 22 of 26 studies. This was accompanied by

vasodilation, increased nitric oxide activity, reduced sympathetic nervous system activity at central and peripheral sites, and enhanced potassium channel conductance in blood vessels and neurons. When administered orally or by infusion over days or weeks to hypertensive rodent models, it reduced blood pressure in 59 of 68 studies. This was accompanied by correction of salt sensitivity and endothelial dysfunction and reduced agonist-evoked oxidative stress and contractility of blood vessels, reduced renal vascular resistance, and increased renal tissue oxygen tension. Thus, tempol is broadly effective in reducing blood pressure, whether given by acute intravenous injection or by prolonged administration, in a wide range of rodent models of hypertension.

I. Introduction

A. Development of Knowledge Concerning Nitroxides

The biological activity of nitroxides was recognized in 1964 by Emmerson and Howard-Flanders who reported that nitroxides sensitized bacteria to the lethal effects of radiation (Emmerson and Howard-Flanders, 1964, 1965). This finding sparked interest in their therapeutic potential. In 1965, McConnell and Griffith demonstrated that nitroxides are “free radicals” and paramagnetic “spin labels.” They showed further that

nitroxides could be linked stably and covalently to proteins and other agents as biomarkers for molecules of interest such as poly-L-lysine, bovine serum albumin, hemoglobin, or catalase (Griffith and McConnell, 1966; Grebenshchikov et al., 1972). Early studies of nitroxide synthesis and action were described by Rozantsev, Swartz, and coworkers (Rozantsev, 1970; Chumakov et al., 1972, 1974; Grebenshchikov et al., 1972; Rozantsev and Sholle, 1979; Rozantsev and Zhdanov, 1987; Kocherginsky and Swartz, 1995).

In the 1990s Schnackenberg, Welch, and Wilcox reported that intravenous, intraperitoneal, or per os administration of 4-hydroxy-2,2,6,6-tetramethylpiperidine-*N*-oxyl (tempol)¹ to hypertensive rat models led to a reduction in blood pressure (BP) and lipid peroxidation (Schnackenberg et al., 1998; Schnackenberg and Wilcox, 1999). They reported that the acute antihypertensive response to nitroxides was related to their in vitro su-

¹ Abbreviations: 1K,1C, one-kidney, one-clip; 20-HETE, 20-hydroxyeicosatetraenoic acid; 2K,1C, two-kidney, one-clip; 3-CP, 3-carbamoyl-PROXYL; 8-iso-PGF_{2α}, 8-isoprostane prostaglandin F_{2α}; A-192621, (±)-*trans,trans*-2-(4-*n*-propoxyphenyl)-4-(1,3-benzodioxol-5-yl)-1-(2,6-dienthylphenyl) aminocarbonylmethylpyrrolidine-3-carboxylic acid; A₁-R, adenosine type 1 receptor; ACEI, angiotensin-converting enzyme inhibitor; ACh, acetylcholine; Ang II, angiotensin II; ARB, angiotensin receptor blocker; AT₁-R, angiotensin type 1 receptor; AT₂-R, angiotensin type 2 receptor; BH₄, tetrahydrobiopterin; BK, large-conductance, Ca²⁺-activated potassium; BP, blood pressure; BSO, buthionine sulfoximine; CAT-1, 4-trimethylammonium-2,2,6,6-tetramethylpiperidine-1-oxyl iodide; CD, collecting duct; CKD, chronic kidney disease; COX, cyclooxygenase; Cu/Zn-SOD, copper-zinc superoxide dismutase; D1, dopamine-1; D1R, dopamine-1 receptor; DEXA, dexamethasone; DHE, dihydroethidium; DM, diabetes mellitus; DOCA, deoxycorticosterone acetate; DR, dopamine receptor; DSS, Dahl salt-sensitive rat; EC, endothelial cell; EDCF, endothelium-dependent contracting factor; EDHF, endothelium dependent hyperpolarizing factor; EDRF, endothelium dependent relaxant factor; ENaC, epithelial sodium channel; eNOS, endothelial nitric-oxide synthase; EPR, electron paramagnetic resonance; ERK, extracellular signal regulated kinase; ET-1, endothelin-1; ET-A-R, endothelin type A receptor; ET-B, endothelin type B; EUK-134, manganese 3-methoxy-*N,N'*-bis(salicylidene)ethylenediamine chloride; GFR, glomerular filtration rate; gp91ds-tat, [H]RKKRRRQRRCSTRIRRL[NH₃]; GRK, G-protein-coupled receptor kinase; H₂O₂, hydrogen peroxide; HIF, hypoxia inducible factor; HR, heart rate; iNOS, inducible nitric oxide synthase; JNK, c-Jun N-terminal kinase; K_{ATP}, ATP-dependent potassium; L-NAME, L-nitroarginine methyl ester; MAP, mean arterial pressure; MAPK, mitogen-activated protein kinase; MDA, malondialdehyde; Mn-SOD, manganese superoxide dismutase; MnTMPyP, Mn(III)tetrakis[1-methyl-4-pyridyl] porphyrin; MRI, magnetic resonance imaging; NE, norepinephrine; NF-κB, nuclear factor κB; nNOS, neuronal nitric-oxide synthase; NO, nitric oxide; NOS, nitric-oxide synthase; Nox-1, neutrophil oxidase-1; O₂⁻, superoxide anion; OH, hydroxyl radical; ONOO⁻, peroxyntrite; paraquat, 1,1'-dimethyl-4,4'-bipyridinium dichloride; PE, phenylephrine; PEG, polyethylene glycol; PG, prostaglandin; PGI₂, prostacyclin; PKC, protein kinase C; pO₂, partial pressure of oxygen; PRA, plasma renin activity; PVN, paraventricular nucleus; RAAS, renin-angiotensin-aldosterone system; RBF, renal blood flow; Ren-2, renin-2; ROS, reactive oxygen species; RRM, reduced renal mass; RSNA, renal sympathetic nerve activity; RVLN, rostroventrolateral medulla; RVR, renal vascular resistance; SD, Sprague-Dawley; SHR, spontaneously hypertensive rat(s); SHR_{SP}, stroke-prone spontaneously hypertensive rat; SNS, sympathetic nervous system; SOD, superoxide dismutase; SQ-29,548, 7-(3-((2-((phenylamino)carbonyl)hydrazino)methyl)-7-oxabicyclo(2.2.1)hept-2-yl)-5-heptenoic acid; STZ, streptozotocin; TAL, thick ascending limb; tempamine, 4-amino-2,2,6,6-tetromethylpiperidine-*N*-oxyl; TEMPO, 2,2,6,6-tetramethylpiperidine-1-oxyl; tempol, 4-hydroxy-2,2,6,6-tetramethylpiperidine-*N*-oxyl; tempol-H, tempol hydroxylamine; tempone, 4-oxo-2,2,6,6-tetramethylpiperidine-*N*-oxyl; TGF, tubuloglomerular feedback; tiron, 4,5-dihydroxy-1,3-benzene disulfonic acid; TP-R, thromboxane-prostanoid receptor; U46,619, 9,11-dideoxy-9,11-methanoepoxy-prostaglandin F₂; VSMC, vascular smooth muscle cell; WKY, Wistar-Kyoto rat(s); Y-27632, (+)-(*R*)-*trans*-4-(1-aminoethyl-*N*-4-pyridyl)cyclohexanecarboxamide dihydrochloride.

peroxide dismutase (SOD)-mimetic activity (Patel et al., 2006) and dependent on potentiating the effects of nitric-oxide synthase (NOS) and on inhibition of the sympathetic nervous system (SNS) by actions that included activation of ATP-dependent potassium (K_{ATP}) channels (Chen et al., 2007a), whereas the long-term response to tempol entailed correction of salt sensitivity (Welch et al., 2005b), renal hypoxia (Welch and Wilcox, 2001; Welch et al., 2003, 2005a), and renal vasoconstriction (Kawada et al., 2002; Wang et al., 2003b, 2004, 2006b). They showed further that local microperfusion of tempol into the interstitium of the kidney of the spontaneously hypertensive rat (SHR) model of oxidative stress restored NO signaling between the macula densa and afferent arteriole (Welch and Wilcox, 2001) and that systemic infusion of tempol improved the efficacy with which the kidney used oxygen for tubular sodium (Na⁺) transport and thereby increased the renal cortical pO₂ (Welch et al., 2005a).

Fink, Xu, and coworkers first demonstrated the NO-independent effects of tempol to reduce SNS activity (Xu et al., 2001, 2002) and related this to the antihypertensive response (Xu et al., 2004) via activation of large-conductance, Ca²⁺-activated potassium (BK) channels (Xu et al., 2005, 2006).

Nishiyama and coworkers reported that O₂⁻ activated renal sympathetic nerves directly whereas local neural application of tempol prevented nerve firing (Shokoji et al., 2003, 2004; Majid et al., 2005). With Majid, he reported that NOS blockade in the dog unexpectedly enhanced the natriuresis and diuresis in response to tempol (Majid and Nishiyama, 2002) and related this result to enhanced generation of ROS in the kidney after NOS blockade (Majid et al., 2004).

These studies laid the foundation for an explosion of scientific interest in nitroxides as agents to reduce ROS and BP. These are the subject of this review. The larger field of the role of ROS in hypertension and aging has been extensively reviewed (Cai and Harrison, 2000; Wilcox and Welch, 2001; Himmelfarb et al., 2002; Wilcox, 2002, 2003, 2005; Cai et al., 2003; Touyz, 2003, 2004; Himmelfarb, 2004; Modlinger et al., 2004; Wilcox and Gutterman, 2005; Harrison et al., 2007; Lambeth, 2007; Lambeth et al., 2007).

In this review we describe the published experience of the BP-lowering actions of nitroxides such as tempol. The emphasis is placed on dose, delivery, responsiveness, and mechanisms of action. We do not consider the larger field of organ protection by tempol. Studies with tempol are of importance both because of the potential role of tempol as a therapeutic agent to reduce ROS and BP and because of the insight these studies yield into the roles of ROS in hypertension.

B. Biochemistry of Nitroxides

Nitroxides share a reducible nitroxide (N-O) group as part of a six- or five-member carbon ring. Some exam-

ples discussed in this review from the very large family of nitroxides are represented in Fig. 1. Tempol is a cell membrane-permeable amphiphilic nitroxide. It is a redox cycling agent that can metabolize superoxide anion (O_2^-) and many other ROS (Krishna et al., 1992, 1996a, 1998; Li et al., 2006). Tempol is among the most potent of the nitroxides in protecting cells and tissues from the damaging effects of ROS (Krishna et al., 1998; Li et al., 2006). The action of nitroxides to metabolize ROS is ascribed primarily to cyclic one- or two-electron transfer among three oxidation states: the oxammonium cation, the nitroxide, and the hydroxylamine (Fig. 2A). Nitroxides undergo a very rapid, one-electron reaction in vivo to the corresponding hydroxylamine (Swartz, 1990; Okajo et al., 2006), which has antioxidant activity (Krishna et al., 1992, 1998; Wu et al., 1997; Hahn et al., 2000). Hydroxylamines can be converted to nitroxides by hydrogen peroxide (H_2O_2) or other oxidants such as transition metals (Dikalov et al., 1998). Indeed, incubation of tempol hydroxylamine (tempol-H) with H_2O_2 in the presence of cytochrome *c* oxidase (Chen et al., 1989) yields radical tempol (Moore et al., 1992). Nitroxides can be converted to the corresponding oxammonium compounds by hypervalent heme (Krishna et al., 1992) and thereafter can undergo fast one-electron reactions to the

nitroxide or by interaction with NADPH can undergo two-electron reactions to the hydroxylamine. These reactions contribute to the pro-oxidant and potentially adverse effects of nitroxides (Israeli et al., 2005). A rapid exchange between the nitroxide, hydroxylamine, and oxammonium cation species confers recycling and catalytic activity on nitroxides (Krishna et al., 1992). This interaction among the nitroxide species has been reviewed recently (Soule et al., 2007). Tempol is rapidly converted to tempol-H in tissues but does not undergo significant further metabolism over several hours (Hyodo et al., 2006).

Saito et al. demonstrated that hydroxyl radical ($\cdot OH$) interacts both with the nitroxide group and with the 4-position of the piperidine ring of tempol to form 4-oxo-2,2,6,6-tetramethylpiperidine-*N*-oxyl (tempone) with the appearance of a new triplet electron paramagnetic resonance (EPR) signal (Saito et al., 2003) (Fig. 2B). However, at physiological levels of pH, this reaction accounts for only approximately 10% of the reduction of $\cdot OH$ by tempol (Deffner and Schimmack, 1976; Saito et al., 2003). This reaction is also rapidly reversible because tempone was metabolized in cells (Kroll et al., 1999) or in mice to tempol over 10 min (Kroll and Borchert, 1999; Kroll et al., 1999).

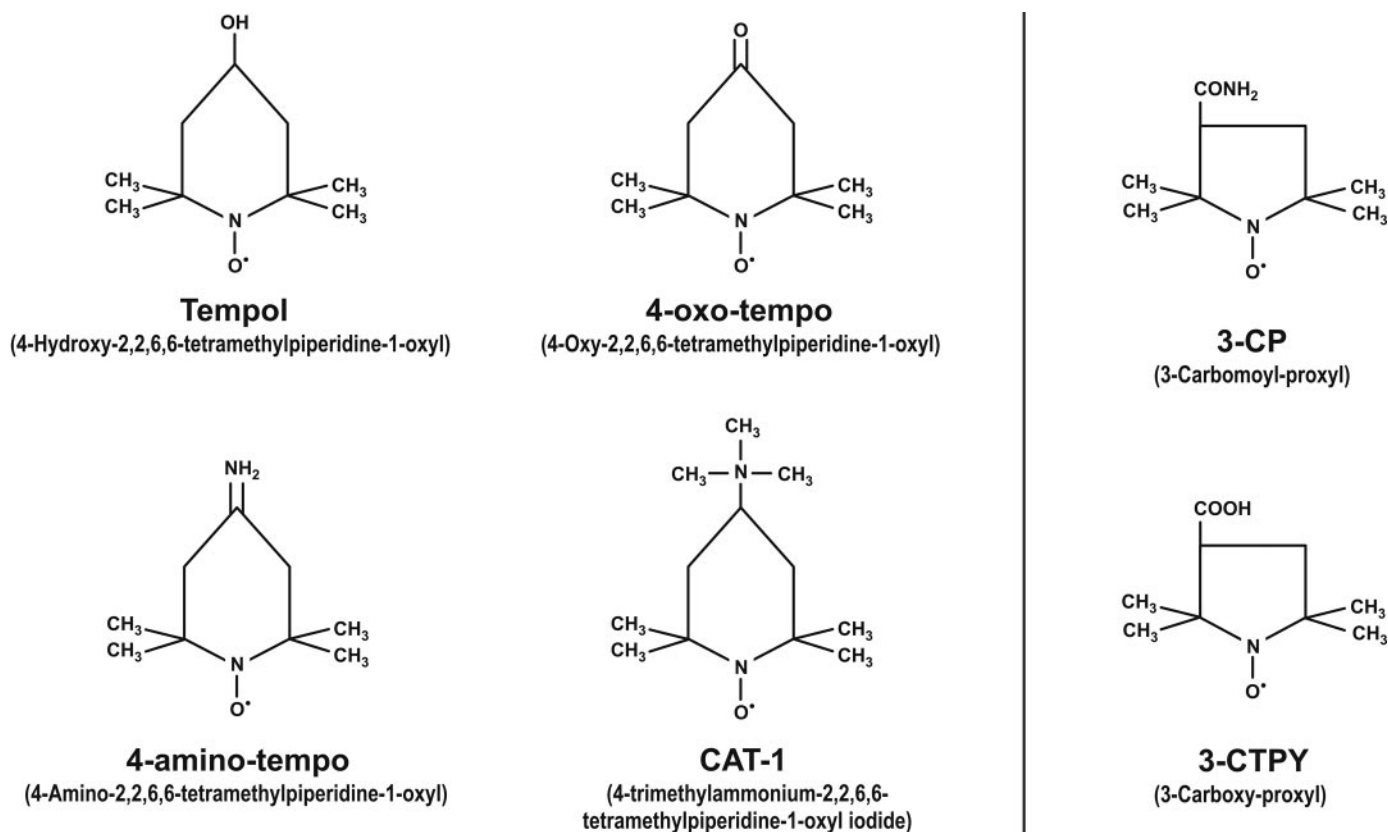
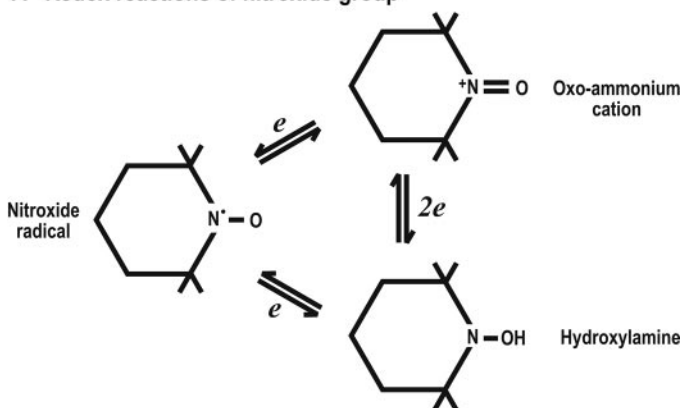


FIG. 1. Some examples of six- and five-member ring nitroxide compounds. [Reprinted from Patel K, Chen Y, Dennehy K, Blau J, Connors S, Mendonca M, Tarpey M, Krishna M, Mitchell JB, Welch WJ, and Wilcox CS (2006) Acute antihypertensive action of nitroxides in the spontaneously hypertensive rat. *Am J Physiol Regul Integr Comp Physiol* 290:R37–R43. Copyright © 2006 American Physiological Society. Used with permission.]

A Redox reactions of nitroxide group



B Redox reaction of the 4-position of piperidine ring

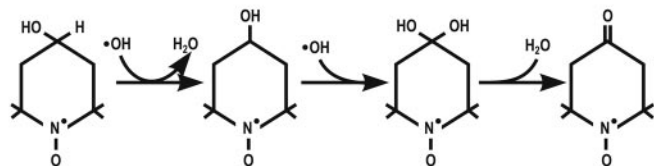


FIG. 2. A, oxidized and reduced nitroxide forms, and their interconversion. [Reprinted from Soule BP, Hyodo F, Matsumoto K, Simone NL, Cook JA, Krishna MC, and Mitchell JB (2007) The chemistry and biology of nitroxide compounds. *Free Radic Biol Med* 42:1632–1650. Copyright © 2007 Elsevier Limited. Used with permission.] B, conversion of tempol to tempone by reaction of the 4-position of the piperidine ring with hydroxyl radical. [Reprinted from Saito K, Takeshita K, Ueda J, and Ozawa T (2003) Two reaction sites of a spin label, TEMPOL (4-hydroxy-2,2,6,6-tetramethylpiperidine-*N*-oxyl), with hydroxyl radical. *J Pharm Sci* 92: 275–280. Copyright © 2003 Wiley-Liss, Inc., a subsidiary of John Wiley & Sons, Inc. Used with permission.]

C. Interaction with Reactive Oxygen Species

Nitroxides metabolize O_2^- to H_2O_2 by a catalytic action and are thereby termed “SOD mimetics” (Chateaufneuf et al., 1988; Samuni et al., 1988, 1990a,b, 2002; Krishna et al., 1992, 1996a; Damiani et al., 1999a; Zhang et al., 1999; Samai et al., 2007; Van Dyke et al., 2007). The catalytic nature of this reaction was challenged by results of stop-flow kinetics (Weiss et al., 1993). In contrast, a detailed EPR study concluded that nitroxides exert apparent catalytic activity above stoichiometric scavenging of O_2^- (Krishna et al., 1996a). Tempol is effective in metabolizing O_2^- generated in solutions of xanthine plus xanthine oxidase (Patel et al., 2006) or in cells stimulated by angiotensin (Ang) II (Luo et al., 2007).

The conversion of nitroxides to the hydroxylamine occurs principally intracellularly and is reversible (Onishi and Morales, 1976; Nothiglaslo and Bobst, 1991; Bobko et al., 2007). This reaction is facilitated by ascorbate (Marx et al., 2000) in erythrocytes (Saphier et al., 2003) and the liver (Keana et al., 1987). Ascorbate is oxidized by tempol to dehydroascorbate at a rate that is diffusion limited (Champion et al., 2004; Vislisis et al., 2007). Ascorbate is the preferred reductant in erythrocytes because incubation of human erythrocytes with

tempol over 2.5 h depleted 80% of intracellular ascorbate, without measurable effects on glutathione or α -tocopherol (May et al., 1998). Bobko et al. (2007) reported that the bimolecular rate constants of ascorbate-induced reduction are higher for six-member nitroxides than for five-member ring nitroxides. Tetraethyl-substituted imidazoline nitroxides are the most resistant to reduction by ascorbate.

However, nitroxides also can be reduced by glutathione (Finkelstein et al., 1984; Khramtsov et al., 1989; Schafer and Buettner, 2001; Kuppasamy et al., 2002; Glebska et al., 2003; Bobko et al., 2007). In the presence of thiols, O_2^- reacted with nitroxides to yield a *N*-hydroxy-*N*-hydroperoxyl intermediate that decomposed rapidly to the hydroxylamine and a compound believed to be sulfenyl hydroperoxide (Finkelstein et al., 1984). The latter reduced two additional nitroxide molecules to account for the unusual 3:1 stoichiometry of this reaction (Finkelstein et al., 1984).

Ascorbate can convert the nitroxide oxammonium cation rapidly to the hydroxylamine, whereas the nitroxide radical facilitates the dismutation of the ascorbate free radical. These reactions underlie a synergistic antioxidant effect of nitroxides and ascorbate (Bobko et al., 2007), which is facilitated further by scavenging of the ascorbate radical by glutathione. Clearly, there are extensive interactions between nitroxides, ascorbate, and glutathione.

Nitroxides such as tempol also metabolize, detoxify, or prevent the formation or action of a wide range of other ROS. These include H_2O_2 by a catalase-like action (Krishna et al., 1996b, 1998; Wu et al., 1997; Samuni et al., 2001), which can involve the metabolism of H_2O_2 by the oxammonium cation (Krishna et al., 1996b) or the hydroxylamine (Dikalov et al., 1998) and interaction with heme proteins (Krishna et al., 1996b). Nitroxides were shown to possess both catalytic and stoichiometric effects in metabolizing H_2O_2 (Krishna et al., 1998). Nitroxides metabolized or prevented the generation of $\cdot\text{OH}$ (Anastassopoulou and Rakintzis, 1984; Charloux et al., 1995; Wu et al., 1997; Risso-de Faverney et al., 2000; Zeltcer et al., 2002), singlet oxygen (Yoshino et al., 2002), peroxy radicals (Offer and Samuni, 2002; Gadjeva et al., 2005), nitroxyl anion (Wink et al., 1998; Bai et al., 2001; Hewett et al., 2005), peroxyxynitrite (ONOO^-) (Carroll et al., 2000; Cuzzocrea et al., 2001; El-Remessy et al., 2003; Fernandes et al., 2005; Song et al., 2007; Van Dyke et al., 2007), nitrogen dioxide generated by myeloperoxidase radicals (Borisenko et al., 2004; Dabrowska et al., 2005), and peroxidation products of lipids (Nilsson et al., 1989; Schnackenberg and Wilcox, 1999; Gadjeva et al., 2005) or phospholipids (Manevich et al., 2002). They prevented tissue damage by oxidizing reduced transition metals, including ferrous (Samuni et al., 1991b; Charloux et al., 1995; Zeltcer et al., 1997,

2002; Udassin et al., 1998; Risso-de Faverney et al., 2000; Glebska et al., 2001; Mehta et al., 2004; Murakami et al., 2005, 2006a,b,c; Nouri et al., 2007) and cuprous ions (Damiani et al., 1994; Zeltcer et al., 1997; Burlando and Viarengo, 2005; Murakami et al., 2006b, 2007; Persichini et al., 2006) or cadmium or chromium (Lewinska et al., 2008), thereby decreasing the availability of the reduced species for Fenton reactions (Monti et al., 1996; Glebska et al., 2001).

Tempol has been shown to protect lipids (Samuni and Barenholz, 1997; Samuni et al., 1997, 2000), DNA (Samuni et al., 1991a; Damiani et al., 1999b, 2000b), or proteins (Damiani et al., 2000a) from oxidative damage. Tempol interacted with other antioxidants to promote their ability to reduce oxidized lipids (Champion et al., 2004). Nitroxides prevented oxidative damage in many cellular or organ systems, for example, in the skin after UV radiation (Damiani et al., 2006; Shen et al., 2006), in cells after x-irradiation (Hahn et al., 1992b, 2000; Sasaki et al., 1998), or in tissues after incubation in a high glucose-containing medium (Xia et al., 2006).

Tempol has complicated effects on ONOO^- . Tempol prevented ONOO^- from nitrating phenol or tyrosine residues (Carroll et al., 2000) but increased nitrosation of phenol (Fernandes et al., 2005). Tempol decreased protein-3-nitrotyrosine formation while increasing the yield of protein nitrocysteine (Fernandes et al., 2005). Studies in solutions and cells implicated the oxammonium form of tempol in the oxidation of ONOO^- to NO. Thus, hydroxyl or carbonate radicals, derived from ONOO^- , oxidized tempol to the oxammonium cation that itself was reduced back to tempol while oxidizing further ONOO^- to O_2 and NO (Bonini et al., 2002). Thereafter NO reacted with nitrogen dioxide derived from ONOO^- to produce the nitrosating species, dinitrogen trioxide (Bonini et al., 2002).

Li et al. (2006) recently compared the IC_{50} values (potency) of nitroxides in protecting lipids from peroxidation by $\cdot\text{OH}$ [assessed from malondialdehyde (MDA) formation in tissue extracts stimulated with Fe^{2+} and ascorbic acid], in protecting cells from damage by H_2O_2 (assessed from red blood cell hemolysis by H_2O_2), and in enhancing O_2^- metabolism (assessed from formazan generation by the addition of nitroblue tetrazolium to zymosan A-stimulated leukocytes). Among eight 2,2,6,6-tetramethylpiperidine-1-oxyl (TEMPO) derivatives, 4-bis-TEMPO was the most potent. Interestingly, tempol had a lower IC_{50} value in the MDA assay for $\cdot\text{OH}$ ($0.8 \pm 0.2 \mu\text{mol/l}$) than in the hemolysis assay for H_2O_2 ($10.7 \pm 0.2 \mu\text{mol/l}$) or in the SOD assay for O_2^- ($326 \pm 18 \mu\text{mol/l}$). The authors concluded that the rank order of nitroxide scavenging of ROS was $\cdot\text{OH} > \text{H}_2\text{O}_2 > \text{O}_2^-$. This finding supports the rather weak SOD-mimetic action of tempol reported previously by Weiss et al. (1993). However, this conclusion is not secure because nitroxides were not tested against equimolar concentrations of ROS. Nevertheless, kinetic EPR studies in vitro demonstrated that

the rate constant for the reaction of nitroxides with $\cdot\text{OH}$ ($10^9 \text{ M}^{-1}\text{s}^{-1}$) was much higher than that for the reaction of nitroxides with O_2^- in the presence of cysteine (10^3 – $10^4 \text{ M}^{-1}\text{s}^{-1}$) (Takeshita et al., 2002). The rate of reaction of nitroxides with $\cdot\text{OH}$ was almost diffusion-limited (Takeshita et al., 2002).

The multiple antioxidant actions of nitroxides have two consequences. First, a functional response to a nitroxide (e.g., a fall in BP) should not be assumed to relate to metabolism of a single ROS. Second, the ability of nitroxides to inhibit three or more sequential sites in an oxidative chain (for example O_2^- , H_2O_2 , and $\cdot\text{OH}$) may underlie their efficacy in diverse models of oxidative stress.

These biochemical reactions have been widely studied in animal models, tissues, or cells. For example, the addition of tempol to aortas harvested from mice with oxidative stress reduced the lucigenin-enhanced chemiluminescence signal for vascular O_2^- but enhanced transiently the luminol signal for vascular H_2O_2 (Chen et al., 2007b). More prolonged incubation of endothelial cells (ECs) with tempol reduced the dihydrorhodamine signal for H_2O_2 . This result indicated that an increase in vascular H_2O_2 after tempol was a transient effect of metabolism of O_2^- to H_2O_2 . The H_2O_2 was later metabolized to O_2 and H_2O as a consequence of the catalase-mimetic effects of tempol. The addition of tempol to hepatoma cells blocked $\cdot\text{OH}$ signaling (Burlando and Viarengo, 2005). Tempol protected bacteria (Skórko-Glonek et al., 1999) and the stomach (Samuni et al., 1999) from the damaging ability of iron to generate $\cdot\text{OH}$. The hydroxylamine was not effective.

D. Pro-Oxidant Actions

High concentrations of tempol (10^{-4} – 10^{-2} M) can have pro-oxidant effects in vascular smooth muscle and endothelial cells (VSMCs) (Alpert et al., 2004; May et al., 2005). The paradoxical pro-oxidant effects of high concentrations of manganese superoxide dismutase (Mn-SOD) (Omar and McCord, 1990), Cu/Zn-SOD (Omar et al., 1990), or tempol (Offer et al., 2000) have been ascribed to the dual ability of O_2^- to both terminate and initiate lipid peroxidation (Nelson et al., 1994; Paller and Eaton, 1995; McCord and Edeas, 2005). These findings may account for the ability of low concentrations of tempol to protect cells from oxidant damage by paraquat, whereas very high concentrations of tempol of 10 mmol/l enhanced toxicity (Samai et al., 2007). The pro-oxidant action of tempol in ECs has been inconsistent and can be prevented by coincubation with antioxidants, for example, ascorbate (May et al., 2005).

A special feature of nitroxides is their conversion to the highly oxidizing oxammonium species (Goldstein et al., 2003), whose reduction to the hydroxylamine contributes to their pro-oxidant actions (Israeli et al., 2005).

E. Structure-Activity Relationships

The nitroxide moiety has been found to be essential for full antioxidant activity, whereas substitution at the 4-position affects potency (Samuni et al., 1988, 1990a; Krishna et al., 1998; Samuni and Barenholz, 2003; Anzai et al., 2006; Li et al., 2006). Extensive studies by Mitchell, Krishna, and colleagues using physicochemical methods coupled with EPR established that the one-electron redox cycling of six-member ring nitroxides such as tempol was enhanced by their ability to undergo reversible "boat-and-chair" conformational change (Krishna et al., 1996b). This was not possible with five-member ring nitroxides, which may account for their lesser biological activity (Patel et al., 2006). Krishna et al. (1998) reported a detailed structure-activity analysis of 58 nitroxides for protection against H₂O₂-induced cytotoxicity or ionizing radiation. Protection against H₂O₂ depended on the ring size, oxidation state (nitroxides > hydroxylamines > amines), and redox midpoint potentials (lowest potentials were most effective). A basic side chain enhanced radiation protection by facilitating the accumulation of the drug at the site of damage.

F. Metabolism and Pharmacokinetics

Nitroxides are stable organic free radicals without significant plasma protein binding (Okajo et al., 2006). The presence of a single unpaired electron on the radical yields unique insights into their pharmacokinetics because this species is detected by magnetic resonance imaging (MRI) via shortening of the relaxation time (T1) or by a characteristic spectrum on EPR (Hyodo et al., 2006; Swartz et al., 2007). These signals are lost after bioreduction of the nitroxide to the diamagnetic hydroxylamine (Yamaguchi et al., 1984). Tempol is converted to the hydroxylamine in liver microsomes, principally by NADPH and cytochrome *c* (Iannone et al., 1989a,b) but also can be reduced by sulfhydryl groups on proteins (Couet et al., 1985) or by ascorbate in the cell cytosol (Eriksson et al., 1987). Isolated keratinocytes use thioredoxin reductase to reduce tempol (Kroll et al., 1999).

The half-time ($t_{1/2}$) for the loss of the nitroxide EPR signal in blood in vivo is dose-dependent and very variable. The $t_{1/2}$ for the loss of signal from a fixed dose has been used to provide a measure of the rate of reduction of the nitroxide and thereby the redox state of the system. The $t_{1/2}$ in conscious rats or mice after acute intravenous injection was short (Komarov et al., 1994) with a mean residence time for radical tempol in the inferior vena caval blood, liver, and kidneys of 0.25 to 15 min (Kamataria et al., 2002). Ueda et al. (2003) reported a $t_{1/2}$ for radical tempol in the kidneys and livers of rats of 15 and 31 s after systemic injection, whereas a more prolonged $t_{1/2}$ of 148 and 278 s, respectively, was seen ex vivo in organ homogenates. The rate of tempol reduction in an organ was related to ROS production (Turrens, 2003). The $t_{1/2}$ for tempol reduction in the kidneys of rats

was shorter than that in the liver both in vivo (Kamataria et al., 2002; Ueda et al., 2003) and in cell homogenates and correlated with the greater mitochondrial density in the kidneys (Ueda et al., 2003). Hepatic reduction depended on the metabolic rate of the liver. Thus, the $t_{1/2}$ for tempol of 39 s in the liver of rats in vivo was reduced by 20% after ingestion of glucose, which was related to the development of mitochondrial oxidative stress because it was prevented by inhibition of mitochondrial function with sodium azide (Tada et al., 2001). Likewise, the reduction of tempol in the kidney depends on renal function. The administration of doxorubicin (Adriamycin) (Oteki et al., 2005) or puromycin (Ueda et al., 2002) to rats caused renal damage and proteinuria and prolonged the EPR decay of the tempol signal over the kidneys, indicating a diminished renal reducing ability. Subcellular fractionation revealed that the reducing activity of the kidneys was located primarily in mitochondria (Kamataria et al., 2002), whereas the liver also contained significant reducing actions in microsomes and cytosol (Ueda et al., 2003).

The rate of reduction of tempol has been used to assess the oxygenation or redox state of tissues (Mikuni and Tatsuta, 1998). The decay of the MRI or EPR signal after loading with tempol was reduced by hypoxia in vitro (Chen et al., 1989; Iannone et al., 1989b; Miura et al., 1990; Nakajima et al., 2002) and in vivo (Miura et al., 1992) and in neoplastic tissues, which are significantly hypoxic (Hyodo et al., 2006). The rate of reduction of tempol was increased in the livers of rats given ascorbic acid or glutathione (Tada et al., 2004), in the brains of rats fed vitamin E, vitamin C, or the free radical scavenging compound idebenone (Zs-Nagy, 1990; Matsumoto et al., 1998), or in cells deficient in glucose-6-phosphate dehydrogenase (Branca et al., 1988; Samuni et al., 2004). The time constant for the decay of the relaxation signal in a tissue was related to the initial reduction of the tempol radical to the hydroxylamine. The decay constant for tempol after injection into the mouse was 0.32 min⁻¹ in the leg and 1.2 to 1.5 min⁻¹ in the kidney (Hyodo et al., 2006).

The rate of reduction of six-member ring nitroxides in the presence of a reducing agent such as ascorbate has been shown to be 100-fold faster than that of five-member ring nitroxides (Samuni et al., 1990a; Nothiglaslo and Bobst, 1991). After intraperitoneal injection into mice, the oxidized (radical nitroyl) forms of six-member ring nitroxides such as tempol or 4-amino-2,2,6,6-tetromethylpiperidine-*N*-oxyl (tempamine) were reduced to 10% of peak values within 5 to 10 min, whereas the five-member ring nitroxides such as 3-carbamoyl-PROXYL (3-CP) and 3-aminomethyl-PROXYL remained at 10% or more of peak values for 30 to 60 min (Hahn et al., 1998). Thus, tempamine was considered to be an excellent redox probe, whereas 3-CP was recommended for EPR imaging (Matsumoto et al., 2004).

Takechi et al. (1997) used a continuous blood sampling technique for an in vivo EPR study in the rat to

determine the composite pharmacokinetic parameters of a range of nitroxides after intravenous bolus injection (Takechi et al., 1997). A rapid initial distribution phase was followed by a plasma clearance phase whose $t_{1/2}$ depended on the physical chemistry characteristics of the probe. Lipid-soluble agents had lower plasma clearance values, perhaps because of a greater volume of distribution. The decay of intravenously injected nitroxide radicals in mice had a biphasic curve with an initial rapid decay that was attributed to reduction to the hydroxylamine followed by a slow decay attributed to excretion (Matsumoto et al., 2004). The cationic nitroxide 4-trimethylammonium-2,2,6,6-tetramethylpiperidine-1-oxyl iodide (CAT-1) had a triphasic decay (Matsumoto et al., 2004). Both free radical and hydroxylamine species were excreted into the urine. Reduction to the hydroxylamine and reoxidation to the nitroxide soon reached equilibrium (Matsumoto et al., 2004).

Tempol has been found to enter cells rapidly and to be widely distributed in the body. It reacted with cellular O_2^- in the cytoplasm and in the mitochondria (van der Poel et al., 2006). Tempol penetrated the blood-brain barrier (Behringer et al., 2002) and accumulated in the brain (Matsumoto et al., 1998). Tempol penetrated intact skin (Herrling et al., 2002) where it accumulated in the lipid compartment of the stratum corneum (Li et al., 2001). It was distributed rapidly into the aqueous humor (Zamir et al., 1999) and diffused through cartilage into the underlying bone (Fischer et al., 1995).

The reduction of nitroxides occurs principally within cells, accounting for the much slower rate of reduction of hydrophilic than lipophilic nitroxides by intact cells or bacteria (Jung et al., 1998). Using erythrocytes as a test system, Gwoździński and coworkers concluded that cell membrane passage of tempol was limited by diffusion (Gwoździński, 1985), which was affected by SH- groups (Gwoździński et al., 1983; Gwoździński, 1985), adenine nucleotides (Jozwiak et al., 1983), and ionizing radiation (Gwoździński, 1986). Negatively charged or amphiphilic nitroxides such as tempol were concentrated in hydrophobic microdomains of cell membranes (Timoshin and Ruuge, 1994). Positively charged nitroxides such as CAT-1 had very little penetration into cells (Samuni et al., 2001; Okajo et al., 2006) unless they were incorporated into liposomes (Matsumoto et al., 2005). Consequently, tempol, but not CAT-1, protected cells against H_2O_2 -induced DNA damage (Samuni et al., 2001). Negatively charged nitroxides or probes with carboxyl moieties, such as carboxy-TEMPO and carboxy-PROXYL did enter cells, albeit slowly, via an anion transporter that was inhibited by 4-acetamido-4'-isothiocyano-stilbene-2,2'-disulfonic acid (Ross and McConnell, 1975; Pikula et al., 1994; Okajo et al., 2006). After intravenous injection, tempol produced additional EPR signals in the bile that were attributed to hepatic uptake and biliary excretion, whereas the highly hydrophilic CAT-1 was not present in bile. One study demonstrated that the

blood levels of membrane-permeable nitroxides were replenished by an active enterohepatic recirculation (Hahn et al., 1998), but this finding was not confirmed in another study (Okajo et al., 2006). After oral administration to the rabbit, the great majority of tempol in the plasma or aqueous humor was in the reduced form (Sasaki et al., 1998).

The $t_{1/2}$ for decay of the tempol radical in the blood pool of mice after intraperitoneal injection was approximately 50 min (Hahn et al., 1992a), which was much longer than the $t_{1/2}$ of 1 min after intravenous injection or of 5 min after intramuscular injection (Kuppusamy et al., 1998). The $t_{1/2}$ after subcutaneous injection was prolonged by coinjection with polynitroxyl-albumin (Kuppusamy et al., 1998). Tempol-H given at a very high dose of 1.45 mmol/kg i.p. to mice provided an early whole-body EPR peak within 1 to 2 min, demonstrating some rapid oxidation to tempol, but this was <10% of the signal produced by tempol itself (Hahn et al., 2000). An equilibrium was reached after 10 min. Thereafter, the two signals decayed at similar rates, largely because of renal excretion.

The $t_{1/2}$ for reduction of tempol was greatly increased by NO (Nakajima et al., 2002). Studies in hepatic microsomes (Nakajima et al., 2002) and cell lines (Samuni et al., 2004) showed that NO donors reduced both the reduction of nitroxides and the reoxidation of hydroxylamines, thereby limiting redox recycling perhaps by inhibition of mitochondrial function by NO (Wolin et al., 1999).

In addition to rapid and reversible redox reduction of nitroxides to hydroxylamines, nitroxide probes also were reduced by enzymic one-electron reduction reactions (Okajo et al., 2006). Liver microsomes were shown to metabolize tempol from a six- to a five-member ring in the presence of Fe^+ (Yin et al., 2003, 2004) or to sterically hindered secondary amines (Kroll and Borchert, 1999).

A slow-release formulation of tempol has been provided by incorporation into fluoroalkyl double-ended polyethylene glycol (R_f -PEG) micelles (Prabhutendolkar et al., 2006).

G. Modified Nitroxides

Nitroxides have been joined covalently to other compounds via the 4'-site. Tempol has been linked to drugs such as chlorpromazine to study the pharmacokinetics of the drug (Feldman et al., 1975), to agents such as acyl-coenzyme A to incorporate tempol into the mitochondrial membrane wherein acyl-coenzyme A interacts with a specific ADP carrier protein (Devaux et al., 1975), to agents such as serum albumin to prolong the duration of tempol in the plasma (Li et al., 2002), and to therapeutic agents to reduce their oxidative actions (Alayash, 1999; Buehler et al., 2000, 2004).

II. Mechanistic Basis of the Blood Pressure-Lowering Effect of Tempol

A. Signaling Studies in Cells and Tissues

Cellular signaling pathways activated by ROS have been reviewed (Griendling and Ushio-Fukai, 2000; Finkel, 2003; Griendling and FitzGerald, 2003; Touyz et al., 2003; Touyz, 2004; Cash et al., 2007).

1. *Protein Kinase G and cGMP.* Incubation of VSMCs with 30 mM glucose down-regulated the mRNA, protein, and activity of cGMP-dependent protein kinase G-1 (Liu et al., 2007b). This down-regulation was prevented by incubation with tempol (Liu et al., 2007b). Wang et al. (2003a,b) showed that tempol reversed the defective acetylcholine (ACh)-induced endothelium-dependent relaxations of renal afferent arterioles dissected from rabbits with oxidative stress caused by prolonged infusion of Ang II. This effect of tempol depended on cGMP. The authors proposed that tempol improved NO signaling via cGMP in models of oxidative stress.

2. *Protein Kinase A and cAMP.* Tempol did not alter isoproterenol-stimulated generation of cAMP in preglomerular microvessels (Jackson et al., 2004). Indeed activation of β_1 -adrenergic receptors in renal afferent arterioles from a rabbit model moderated oxidative stress. Only after blockade of cAMP was the contraction to norepinephrine (NE) enhanced by oxidative stress and normalized by coinubation with tempol (Wang et al., 2006b).

3. *Mitogen-Activated Protein Kinases.* Ang II is a potent activator of the MAPK cascade in cardiovascular tissue where it acts via a redox-sensitive mechanism. Tempol markedly suppressed Ang II-induced activation of vascular extracellular signal-regulated kinase (ERK) 1 and 2 and p38 (Zhang et al., 2007). This suppression was ascribed in part to an increase in NO bioactivity because it was prevented by NOS blockade (Zhang et al., 2007). Tempol prevented the phosphorylation of MAPKs, ERK1 and 2, c-Jun N-terminal kinase (JNK), and p38 in the aorta and heart of rats during infusions of Ang II or phenylephrine (PE) (Zhang et al., 2004a; Kimura et al., 2005a) and inhibited the phosphorylation of p38, MAPK, JNK, and ERKs in vascular tissue stimulated by Ang II or endothelin-1 (ET-1) (Touyz et al., 2004). Cerebral ischemia increased O_2^- generation and phosphorylation of ERK1 and 2, which were prevented by tamoxifen or tempol (Wakade et al., 2008).

Prolonged administration of tempol has been found to be very effective in preventing MAPK activation in the tissues of several animal models of hypertension (Iglarz et al., 2004; Nishiyama and Abe, 2004). For example, tempol (3 mmol/l in drinking water for 6 weeks) prevented the increased activities of ERK1 and 2 and JNK in the renal cortex of rats with aldosterone- and salt-induced hypertension (Nishiyama et al., 2004a). Dahl salt-sensitive (DSS) rats fed salt had a major increase in the glomerular MAPK activity, including ERK1 and 2 and JNK which was prevented by oral tempol (3 mmol/l

in drinking water for 4 weeks) (Nishiyama et al., 2004b). This effect was independent of BP reduction. The stimulation by a low-potassium diet of renal c-Jun phosphorylation and c-Src expression was prevented by 1 week of tempol administration (Babilonia et al., 2005).

Thus, tempol is very effective in preventing MAPK activation during oxidative stress both in vivo and in vitro.

4. *Nuclear Factor κ B.* Tempol or pyrrolidine dithiocarbamate prevented activation of NF- κ B in the aorta and kidney of rats with deoxycorticosterone acetate (DOCA)-salt induced hypertension (Beswick et al., 2001). Tempol also prevented activation of NF- κ B and protein kinase C (PKC) in rats with oxidative stress caused by feeding buthionine sulfoximine (BSO) to deplete glutathione (Banday et al., 2007a).

5. *Rho and Rho Kinase.* ROS generated by xanthine plus xanthine oxidase in rat aortic rings led to incorporation of Rho into membranes (Jin et al., 2004). The associated phosphorylation of the myosin light chain phosphatase target subunit-1 and vascular contraction were blocked by the Rho kinase inhibitor Y-27632. Tempol blocked the ROS-induced Ca^{2+} sensitization of these rings by preventing activation of Rho and Rho kinase (Jin et al., 2004). This may be an important component of the effect of tempol to reduce contractility of VSMCs during oxidative stress.

6. *Protein Kinase C.* Pretreating blood vessels from diabetic rats with the PKC inhibitor bisindolylmaleimide I improved endothelium-dependent relaxant factor (EDRF)/NO responses without moderating vascular O_2^- (Coppey et al., 2003). The authors concluded that activation of PKC was downstream from oxidative stress. Indeed, tempol prevented PKC activation, O_2^- generation (Coppey et al., 2003), downstream phosphorylation of target proteins (Banday et al., 2007a), and c-jun oncogene expression (Kuo et al., 1995) in proximal tubules from rats with glutathione depletion (Banday et al., 2007a) and in lung cells stimulated with the redox-cycling quinolone, paraquat (Kuo et al., 1995). However, the finding that tempol blocked increases in intracellular $[Ca^{2+}]$ and constriction of vasa recta pericytes after stimulation by the PKC agonist phorbol 12,13-dibutyrate demonstrated that tempol also can interrupt signaling downstream from PKC (Zhang et al., 2004c).

B. Antihypertensive Action in Animal Models

1. *Overview of Antihypertensive Response to Tempol.* Both acute and prolonged administration of tempol have been shown to reduce the BP in hypertensive models. However, two differences are apparent between these responses.

First, the acute response to intravenous tempol in hypertensive rodent models was very rapid in onset (maximal within 2 min of the intravenous bolus) and reversed fully within 15 min (Patel et al., 2006), whereas the response to tempol added to the drinking water has

been a delayed reduction in BP over 24 h that took 2 or more weeks to develop fully (Welch et al., 2005b). Second, acute administration of tempol reduced the heart rate (HR) (Patel et al., 2006) and renal sympathetic nerve activity (RSNA) of rats (Xu et al., 2002, 2004). This contributed to the fall in BP in the SHR after intravenous tempol because blockade of ganglionic transmission reduced the antihypertensive response (Chen et al., 2007a). In contrast, Welch et al. (2005b) reported that prolonged subcutaneous infusion of tempol to SHR over 2 weeks did not alter the HR or plasma NE or renal catecholamine excretion. Thus, either the sympatholytic actions of intravenous tempol are a unique response to acute administration or compensatory mechanisms to override this effect develop during prolonged tempol administration.

Despite these differences, >85% of hypertensive models studied have shown a reduction in BP with tempol, whether given acutely or by prolonged administration. The hypertensive models to which tempol has been administered acutely and by prolonged administration are detailed in Tables 1 and 2, respectively.

It is hard to compare responses to tempol among models with widely varying basal levels of hypertension. Because the absolute reduction in BP with antihypertensive agents increases with the basal levels of BP, one solution has been to assess the fractional (percent) changes in BP with tempol. However, clinicians require insight into the degree to which a new agent corrects established hypertension. These goals are better served by quantitating the fractional (percent) normalization of BP. Therefore, we have reported the effectiveness of tempol in Tables 1 and 2 both as percent reductions and percent normalizations of BP. We have used as "normal BP" that of a control group, for example, Wistar-Kyoto rats (WKY) in a study of SHR, when it has been provided by the investigator. For studies that have not reported data on a control model, we have estimated the normal level of BP from animals prepared under comparable conditions in other studies.

Of 26 studies in which tempol was given by acute intravenous injection or acute infusion to hypertensive rat models, 22 (85%) have recorded a fall in BP (Table 1, *Studies in hypertensive rats with intravenous tempol*). Of the four studies in which acute intravenous tempol failed to reduce the BP in a hypertensive rat model, three were in rats infused for only a few minutes with pressor doses of PE (Zhang et al., 2004a) or Ang II (Kimura et al., 2004; Zhang et al., 2004a). The fourth discordant study was in DSS rats fed a high-salt diet (Zicha et al., 2001). Two of these four negative studies used a dose of tempol of 15 $\mu\text{mol/kg}$ (Table 1, *Studies in hypertensive rats with intravenous tempol*), which is below the effective dose for intravenous tempol in the anesthetized SHR, which is 72 to 90 $\mu\text{mol/kg}$ (Patel et al., 2006). Parameters of ROS were not recorded in these four studies with negative results.

Isolated vessels incubated with Ang II took 10 to 20 min to develop a significant increase in ROS and a relaxation response to tempol (Wang et al., 2003b, 2004; Chen et al., 2007b). The two models in which hypertension was induced by prolonged infusion of Ang II into rats for 1 h to 2 weeks showed a 92% (Kimura et al., 2004) or 100% (Kimura et al., 2005a) normalization of BP with intravenous tempol. Thus, the failure of tempol to reduce the BP in studies in which PE (Zhang et al., 2004a) or Ang II (Kimura et al., 2004; Zhang et al., 2004a) was infused for only a few minutes may be explained by a failure of this protocol to induce vascular oxidative stress, but this hypothesis was not established.

Of two studies in hypertensive mice, intravenous tempol reduced the BP in *D5R(-/-)* mice but not in *GRK4 γ A142V(-/-)* mice (Wang et al., 2007) (Table 1, *Studies in mice with intravenous tempol*). Both of these models had modest hypertension yet only the *D5R(-/-)* mouse had evidence of increased ROS, and only this model had an acute antihypertensive response to tempol.

BP was reduced by acute intravenous administration of tempol in 9 of 13 studies (69%) of normotensive rats (Table 1, *Studies in normotensive rats with intravenous tempol*). Two studies that reported no fall in BP in normotensive rats included one that used a low dose of 15 $\mu\text{g/kg}$ (Zhang et al., 2004a) that is below the effective threshold (Schnackenberg et al., 1998; Campese et al., 2004; Patel et al., 2006). Thus, when given in an effective dose, acute intravenous tempol reduced the BP in all hypertensive models with evidence of oxidative stress, but in only 8 of 12 studies in normotensive models.

Intravenous tempol reduced mean arterial pressure (MAP) by 28% in hypertensive SHR, which was significantly more than the 11% reduction in normotensive WKY. Likewise, tempol caused a significantly greater reduction in renal vascular resistance (RVR) in SHR (Schnackenberg et al., 1998). Clearly, the effects of intravenous tempol are greater in hypertensive than in normotensive models. No study has reported adverse effects from hypotension when tempol was given to hypertensive or normotensive rodents.

When recorded, the HR was reduced with intravenous tempol in six of seven hypertensive rat models (Table 1, *Studies in hypertensive rats with intravenous tempol*) including one study in which NOS was blocked (Thakali et al., 2006). Four studies in normotensive models reported a modest increase in HR with intravenous tempol (Table 1, *Studies in normotensive rats with intravenous tempol*).

Multiple studies have investigated the effect of prolonged tempol administration. Of 68 studies, 59 (87%) recorded a significant reduction in BP for at least one time point after administration (Table 2, *Studies in hypertensive rats with systemic tempol*). The majority (58 of 68) used oral tempol, three used subcutaneous infusions

TABLE 1
Blood pressure and heart rate in response to acute tempol administration

Mean values are shown for systolic blood pressure (SBP) or mean arterial pressure (MAP) and percent normalization of blood pressure and percent heart rate response to acute tempol administration. Rat models: Acute Ang II, short term infusion of angiotensin II; Chronic Ang II, prolonged (d) infusion of angiotensin II; Acute PE, short-term (minutes or hours) infusion of phenylephrine; Capsaicin-salt, rats given capsaicin to induce sensory denervation and fed a high-salt diet; DSS, Dahl salt-sensitive rats fed a high-salt diet; DOCA-salt, deoxycorticosterone acetate plus salt; HTG, rat transgenic for human renin gene; Inducible malignant HTN, rats with an inducible renin gene to cause malignant hypertension; Lead, rats fed lead; *D5R*(-/-), dopamine-5 receptor deficient; GRK4A142V, G-coupled receptor for valine polymorphism at nucleotide 142.

Model	n	Tempol Maximum Dose or Dose Used to Compare Groups	Tempol Route of Delivery	Normotensive Control Group	SBP or MAP		BP Response	HR Response	Reference
					Experimental Hypertensive Group without Tempol	Experimental Group with Tempol			
		$\mu\text{mol/kg}$			mm Hg	% change	% normalization	% changed	
Studies in hypertensive rats with intravenous tempol									
Acute Ang II	4	15	i.v. over 5 min	93 [†]	153 [†]	-1	3	N.D.	Zhang et al., 2004a
Acute Ang II [200 (ng · kg/min)]	5	173	i.v. bolus then 43 $\mu\text{mol/kg}$	110 [†]	152 [†] (N.S.)	0	0	N.D.	Kimura et al., 2004
Acute Ang II [200 (ng · g/min) from 1–24 h]	4–6	173	i.v. bolus then 43 $\mu\text{mol/kg}$	N.D.	148 [†]	-33	N.D.	N.D.	Kimura et al., 2004
Chronic Ang II [200 (ng · kg/min) for 2 wk]	5	173	i.v. bolus then 43 $\mu\text{mol/kg}$ over 15 min	110 [†]	175 [†]	-37	100	N.D.	Kimura et al., 2004
Chronic Ang II [200 (ng · kg/min) for 2 wk]	4	175	i.v. bolus then 0.5 (mg · kg)/min	110 [†]	165 [†]	-33	92	N.D.	Kimura et al., 2005a
Acute PE	4	15	i.v. over 5 min	95 [†]	148 [†]	+3	0	N.D.	Zhang et al., 2004a
Capsaicin-4% salt (WKY)	?	216	i.v. bolus	N.D.	149	-12	N.D.	N.D.	Song et al., 2004
DSS 8% salt	7	145	i.v. bolus	129 [†]	197 [†]	-7	19	N.D.	Zicha et al., 2001
DSS 8% salt, young	7	60	i.v. bolus	125	150	-9	48	N.D.	Zicha et al., 2001
DSS 8% salt, old	7	60	i.v. bolus	145	170	-5	23	N.D.	Zicha et al., 2001
DOCA-salt	5	300	i.v. bolus	74 [†]	140 [†]	-43	91	-10*	Xu et al., 2004
HTG rat	145	145	i.v. bolus	95 [†]	124 [†]	-9	31	N.D.	Kunes et al., 2002
HTG female	?	145	i.v. bolus	101 [†]	120 [†]	-8	53	N.D.	Kunes et al., 2002
Inducible malignant HTN rat	5	300–400	i.v. over 1 h	123 [†]	184 [†]	-18	54	N.D.	Patterson et al., 2005
2K,1C	13	200	i.v. over 1 h	107 [†]	155 [†]	-15	50	N.D.	Guron et al., 2006
Lead	6	90	i.v. over 30 min	138	168	-18	100	N.D.	Vaziri et al., 2003b
Lewis rats, Zn-deficient	8	20	i.v. bolus and infusion	110	110	-26	100	N.D.	Kurihara et al., 2002
SD + L-NNA for 2 wk	7	300	i.v.	118 [†]	194 [†]	-28	72	-16*	Thakali et al., 2006
SHR	6	270	i.v. bolus	N.D.	140 [†]	-50	N.D.	-20*	Patel et al., 2006
SHR	6	173	i.v. over 1 min	108 [†]	166 [†]	-26	74	-13*	Shokoji et al., 2003
SHR	6	72	i.v. bolus	96 [†]	145 [†]	-28	84	N.D.	Schnackenberg et al., 1998
SHR	6	900	i.v. over 30 min	72 [†]	167 [†]	-57	100	N.D.	Schnackenberg et al., 1998
SHR	10	174	i.v. bolus	N.D.	178 [†]	-33	100	-12*	Chen et al., 2007a
SHR Zn-deficient diet	7	100	i.v. bolus	148 [†]	162 [†]	-20	100	N.D.	Sato et al., 2002
Zinc-fed rats	8	100	i.v.	107 [†]	128 [†]	-27	100	N.D.	Yanagisawa et al., 2004
Studies in normotensive rats with intravenous tempol									
DOCA-sham	5	300	i.v. bolus	N.D.	98 [†]	-24	N.D.	+9	Xu et al., 2004
Lewis rat, male	?	145	i.v. bolus	N.D.	100 [†]	-4	N.D.	N.D.	Kunes et al., 2002
Lewis rat, female	?	145	i.v. bolus	N.D.	105 [†]	-4	N.D.	N.D.	Kunes et al., 2002
4% salt (WKY)	?	216	i.v. bolus	N.D.	102	-14	N.D.	N.D.	Song et al., 2004
SD	5	90	i.v. over 1 h	N.D.	104 [†]	-27	N.D.	+8	Campese et al., 2004
SD	4	15	i.v. over 5 min	N.D.	100 [†]	-2	N.D.	+1	Zhang et al., 2004a
SD	6	90	i.v. over 30 min	N.D.	133 (N.S.)	-4	N.D.	N.D.	Vaziri et al., 2003b
SD	8	216	i.v. bolus	N.D.	116 [†]	-3	N.D.	N.D.	Nishiyama et al., 2001
WKY	12	200	i.v. over 1 h	N.D.	116 [†]	-8	N.D.	N.S.	Guron et al., 2006
WKY	6	173	i.v. over 1 min	N.D.	108	-19	N.D.	+7	Shokoji et al., 2003
WKY	6	72	i.v. bolus	N.D.	118 [†]	-19	N.D.	N.D.	Schnackenberg et al., 1998
WKY	6	900	i.v. over 30 min	N.D.	122 [†]	-41	N.D.	N.D.	Schnackenberg et al., 1998
WKY	6	90	i.v. over 30 min	N.D.	122 [†]	-12	N.D.	N.D.	Schnackenberg et al., 1998

TABLE 1—Continued.

Model	n	Tempol Maximum Dose or Dose Used to Compare Groups	Tempol Route of Delivery	SBP or MAP		BP Response	HR Response	Reference		
				Normotensive Control Group	Experimental Hypertensive Group without Tempol					
				mm Hg	% change	% normalization	% changed			
Studies in normotensive or hypertensive rats with intracerebroventricular tempol										
DDS + 8% NaCl	8	40 μmol	i.c.v.	100	146	116*	-21	65	-11*	Fujita et al., 2007
DSR NS vs. DSS NS	7	40 μmol	i.c.v.	103	119	109*	-10	63	-6	Fujita et al., 2007
SD	6	50 or 100	i.c.v.	106†	N.A.	75*	-29	100	-15*	Lu et al., 2004
SHR	4	1.5	i.c.v. over 1 min	108†	162†	163† (N.S.)	+1	0	+1	Shokoji et al., 2003
SHR ^{sp}	5	3.8	i.c.v. bolus RVLm	105†	170†	133†*	-22	57	-11*	Kishi et al., 2004
WKY	5	3.8	i.c.v. bolus RVLm	N.D.	112†	105† (N.S.)*	-6	N.D.	-1	Kishi et al., 2004
WKY	4	1.5	i.c.v. over 1 min	N.D.	108†	108† (N.S.)*	0	N.D.	+1	Shokoji et al., 2003
Studies in mice with intravenous tempol										
D5R(-/-) mice (ROS)	7	58	i.v. bolus	86	110	97*	-12	54	N.D.	Wang et al., 2007
GRK4γA142V mice (NO ROS)	4	58	i.v. bolus	99	117	117 (N.S.)	0	0	N.D.	Wang et al., 2007

? unknown; N.D., not done; L-NNA, L-nitroarginine; N.A., not applicable. DSR, Dahl salt-resistant rat; i.c.v., intracerebroventricular; NS, normal salt; wk, week(s).
 * Significant change or difference from without tempol.
 † Value is for MAP.

(Welch et al., 2003, 2005a; Dikalova et al., 2005), five used intraperitoneal injections (Schnackenberg et al., 1998; Vaziri et al., 2001; Hasdan et al., 2002; Adeagbo et al., 2003; Awe et al., 2003), and two used intravenous infusions (Meng et al., 2003; Sedeek et al., 2003). The BP during prolonged tempol seems to be dependent on the level of BP before tempol.

The antihypertensive effects of tempol were apparent across a wide range of models. Although all routes of tempol administration were effective, in all except one study in which tempol failed to reduce BP (Elmarakby et al., 2005), it was given orally. Of four studies in which prolonged administration of tempol was given to hypertensive mice, all recorded a fall in BP (Table 2, *Studies in hypertensive mice with systemic tempol*).

Of 63 studies in hypertensive rats reporting a measure of systemic, vascular, or renal ROS, 55 (87%) reported that tempol had reduced ROS, at least in some parameter of measurement (Table 3, *Studies in hypertensive rat models*).

Of the 45 studies in which measurements were made of the BP and some parameter of ROS, 34 (76%) reported a reduction in both, 7 (16%) reported a reduction in BP but not in ROS (Hasdan et al., 2002; Fortepiani et al., 2003; Sedeek et al., 2003; Zhang et al., 2003b; Williams et al., 2004; Dikalova et al., 2005; Sullivan et al., 2006), 3 (7%) reported unchanged BP despite a reduction in ROS (Song et al., 2004; Elmarakby et al., 2005; Whaley-Connell et al., 2007), and 1 (2%) reported no change in BP or ROS (Song et al., 2004). Thus, BP and ROS were directionally concordant in 35 of 45 studies (78%).

Tempol has been an effective antihypertensive agent in Ang II-dependent models (e.g., Ang II-infused rats), renin-dependent models [e.g., two-kidney, one-clip (2K,1C) Goldblatt hypertensive rats] and salt- and volume-dependent, low-renin models (e.g., DOCA-salt rats). Clearly, there is no absolute requirement for an activated systemic renin-angiotensin-aldosterone system (RAAS) or volume expansion for a model to be responsive to tempol. The antihypertensive action of tempol in DSS rats was additive with the mineralocorticoid receptor antagonist eplerenone, which suggests that tempol and eplerenone reduce BP by largely independent means in this model (Bayorh et al., 2006).

Tempol has been as effective in prevention as in reversal of established hypertension. This fact was illustrated in two studies from Zheng et al. (2003b, 2004b), who reported that tempol was equally effective in preventing or normalizing the elevation in BP in rats whether given 4 days before or 8 days after prolonged infusions of adrenocorticotropin or dexamethasone (DEXA). However, tempol generally has been most effective when administered before the onset of hypertension.

Of the seven reports in which prolonged administration of tempol failed to reduce BP, two were in models that were barely hypertensive (Williams et al., 2004; Elmarakby et al., 2005) and one showed reductions in

TABLE 2
Blood pressure response to prolonged tempol administration

Mean values are shown for systolic blood pressure (SBP) or mean arterial pressure (MAP) and percent blood pressure normalization of blood pressure. Tempol dose p.o. is concentration of tempol in the drinking water unless otherwise noted.

Model	Control Model	Tempol Route of Delivery and Duration	n	Tempol Maximum Dose or Dose Used to Compare Groups	SBP or MAP		BP Response		Reference	
					Control Normotensive	Experimental Hypertensive Group without Tempol	Change	Normalization		
Studies in hypertensive rats with systemic tempol										
ACTH (0.2 mg · kg/d s.c.)	Vehicle	p.o. from 4 d before to 8 d after ACTH	10	1 mM	119	134	4 d, 118* 8 d, 123 (N.S.)	4 d, -12 8 d, -8	4 d, 106 8 d, 73	Zhang et al., 2003b
Aldosterone (0.75 µg/h s.c. + salt 1% for 6 wk)	Vehicle	p.o. for 6 wk	8	3 mM	118	165	125*	-24	85	Nishiyama et al., 2004a
Aldosterone (0.75 µg/h s.c. for 6 wk)	Vehicle	p.o. for 6 wk	7	1 mM	123	170	149*	-12	45	Iglarz et al., 2004
Ang II [5 (ng · kg/min) i.v. for 15 d]	Vehicle	p.o. for 15 d	6	1 mM	119	151	119*	-21	100	Ortiz et al., 2001a
Ang II [100 (ng · kg/min) s.c. ± 8% salt diet for 12 d]	Vehicle	p.o. for 12 d	5	1 mM	127	184	150*	-18	60	Ogihara et al., 2002
Ang II [200 (ng · kg/min) s.c. for 2 wk]	Vehicle	s.c. minipump for 2 wk	8-11	200 (nmol · kg)/min	104 [†]	146 [†]	116 [†] *	-21	71	Welch et al., 2005a
Ang II [300 (ng · kg/min) s.c. for 7 d]	Vehicle	p.o. for 7 d	8	2 mM	125	186	142*	-24	72	Hattori et al., 2005
BSO (30 mM for 2 wk)	SD	p.o. for 2 wk	8	1 mM	100 [†]	123 [†]	104 [†] *	-15	82	Banday et al., 2007a
BSO (30 mM) + HS for 2 wk	HS	p.o. for 12 d	8	1 mM	112 [†]	143 [†]	107 [†] *	-25	116	Banday et al., 2007c
Capsaicin-4% salt (WKY)	None	p.o. (gavage) for 3 wk	5-6	1 (nmol · kg)/d	113	150	150 (N.S.)	0	0	Song et al., 2004
Cyclosporine [30 (mg · kg/d) s.c.]	Vehicle	p.o. for 3 wk	7	3 mM	119	145	115*	-21	115	Nishiyama et al., 2003
Dexamethasone [10 (µg · kg/d) s.c.]	Vehicle-infused	p.o. from 4 d before to 8 d after Dex	10	1 mM	122	136	4 d, 128* 8 d, 122*	4 d, -6 8 d, -10	4 d, 57 8 d, 100	Zhang et al., 2004b
DOCA-salt	Sham	p.o. for 3 wk	6	1 mM	107 [†]	161 [†]	108 [†] *	-33	100	Ghosh et al., 2004
DOCA-salt	SD/sham-salt	i.p. for 3 wk	8	87 (µmol · kg)/d	119 [†]	164 [†]	123 [†] *	-25	91	Adeagbo et al., 2003
DOCA-salt	Sham	i.p. for 3 wk	8	87 (µmol · kg)/d	130	203	151*	-26	71	Awe et al., 2003
DOCA-salt	Sham	p.o. for 5 wk	13	1 mM	118	200	176*	-12	29	Nakano et al., 2003
DOCA-salt	Sham	p.o. for 4 wk	10	1 mM	113	199	142*	-29	66	Beswick et al., 2001
DSS/8% salt	DSS LS and DSR	p.o. for 10 wk	9	3 mM	120	220	191*	-13	29	Guo et al., 2006
DSS/8% salt	DSS LS	p.o. for 5 wk	5	10 mM	144	224	186*	-17	47	Hisaki et al., 2005
DSS/8% salt	DSS LS	p.o. for 4 wk	8	3 mM	107	184	128*	-30	73	Kobori and Nishiyama, 2004
DSS/8% salt	DSS/NS	p.o. for 3 wk	34	1 mM	148	233	199*	-15	40	Bayorh et al., 2006
DSS + salt	DSS LS	i.v. for 3 wk	7	3 (mmol · kg)/d	122 [†]	140 [†]	118 [†] *	-16	102	Meng et al., 2003
DSS + 8% NaCl	DSS LS	p.o. for 4 wk	8	3 mM	113	185	128*	-31	79	Nishiyama et al., 2004b
DSS + 8% NaCl	DSR	p.o. for 8-10 wk	20	1 mM	124	179	132*	-26	85	Ozawa et al., 2004
ET-1 [5 (pmol · kg/min)-8% salt	Vehicle-treated, normal salt	p.o. for 12 d	7-10	1 mM	114 [†]	132 [†]	127 [†] (N.S.)	-4	28	Elmarakby et al., 2005

TABLE 2—Continued.

Model	Control Model	Tempol Route of Delivery and Duration	n	Tempol Maximum Dose or Dose Used to Compare Groups	SBP or MAP		BP Response		Reference	
					Control Normotensive	Experimental Hypertensive Group without Tempol	Change	Normalization		
ET-1 [5 (pmol · kg/min)]-8% salt	Tempol untreated	s.c. for 12 d	7–10	170 (μmol · kg/d)	114 [†]	138 [†]	134 [†] (N.S.)	-3	16	Elmarakby et al., 2005
ET-1 [5 (pmol · kg/min)] i.v.	Vehicle-infused	i.v. for 9 d	6	170 (μmol · kg/d)	125 [†]	141 [†]	127 ^{†*}	-10	87	Sedeek et al., 2003
ET-B antagonist (A-192621) p.o.	Vehicle-treated	p.o. for 1 wk	6	1 mM	100 [†]	117 [†]	117 [†] (N.S.)	0	0	Williams et al., 2004
ET-B antagonist-10% salt p.o.	Vehicle-treated, 10% salt	p.o. for 1 wk	6	1 mM	Day 3, 110 [†] Day 7, 110 [†]	Day 3, 135 [†] Day 7, 138 [†]	Day 3, 120 ^{†*} Day 7, 138 [†] (N.S.)	Day 3, -11 Day 7, 0	Day 3, 60 Day 7, 0	Williams et al., 2004
Fructose-fed	Fructose untreated	p.o. for 4 wk	7	1 mM	101 [†]	128 [†]	103 ^{†*}	-19	93	Onuma and Nakamishi, 2004
Hyperthyroid (s.c. thyroxine for 6 wk)	Vehicle-infused	p.o. for 6 wk	8	1 mM	120 [†]	147 [†]	127 ^{†*}	-14	74	Moreno et al., 2005
High salt 10%	Normal salt	p.o. for 1 wk	6	1 mM	98 [†]	112 [†]	100 ^{†*}	-11	85	Williams et al., 2004
Inducible renin transgene-8% salt	Pre-salt loading	p.o. for 10 d	6	2 mM	137	171	148 [*]	-13	63	Howard et al., 2005
Intermittent hypoxia (sleep apnea)	SD	p.o. for 2 wk	?	1 mM	101	118	107 ^{**}	-9	65	Troncoso Brindeiro et al., 2007
1K,1C	Sham UNX	p.o. for 2 wk	6	2 mM	95 [†]	159 [†]	139 ^{†*}	-13	31	Dobrian et al., 2001
1K,1C (10 wk)	Sham	p.o. for 5 wk	5	1 mM	130	170	135 ^{**}	-21	90	Christensen et al., 2007b
2K,1C	Sham	s.c. minipump for 13 d	8	288 (μmol · kg/d)	105 [†]	148 [†]	118 ^{**}	-20	70	Weich et al., 2003
Lead (100 ppm for 12 wk)	SD without lead in diet	i.p. for 2 wk	6	15 (mmol · kg/d)	122	173	143 [*]	-17	59	Vaziri et al., 2001
Leptin	Leptin untreated	p.o. for 7 d	8	2 mM	126	152	128 ^{**}	-16	92	Belkowski et al., 2005
Offspring of malnourished mothers	Offspring of normal mothers	p.o. for 13 wk including 3 wk pre-HTN	14–19	2 mM	130	143	130 [*]	-9	100	Stewart et al., 2005
Obese Zucker	Lean	p.o. for 15 d	10	1 mM	89 [†]	110 [†]	100 ^{†*}	-9	48	Banday et al., 2005
Five-sixths nephrectomy (infarction)	Sham	IP for 10 d	10	1.5 (mmol · kg/d) for 10 d	118	145	122 ^{**}	-8	85	Hasdan et al., 2002
Five-sixths nephrectomy	Sham	p.o. for 1 wk	6	1 mM	120	180	150 [*]	-17	50	Vaziri et al., 2003a
Five-sixths nephrectomy	Sham	IP for 10 d	10	1.5 (mmol · kg/d)	118	145	122 ^{**}	-16	85	Hasdan et al., 2002
Ren-2 transgenic rat	SD	p.o. for 3 wk	6	1 mM	115 [†]	197 [†]	194 [†] (N.S.)	-2	3	Whaley-Connell et al., 2007
SD + HS + BSO (30 mM for 12 d)	HS	p.o. for 12 d	8	1 mM	112 [†]	142 [†]	114 ^{**}	-20	93	Banday et al., 2007d
SHR	WKY	p.o. or s.c. for 2 wk	6–8	2 mM 200 (nmol · kg/min) by minipump	104 [†] 104 [†]	p.o., 147 [†] s.c., 150 [†]	p.o., 128 ^{†*} s.c., 126 ^{†*}	p.o., 13 [*] s.c., -16 [*]	p.o., 45 s.c., 52	Weich et al., 2005b
SHR	WKY	p.o. from wk 6–11	6	1 mM	143	171	137 [*]	-20 [*]	105	Nabha et al., 2005
SHR	SD	p.o. for 5 - 15 d	10	1 mM	5 d, 118 [†] 15 d, 124 [†]	5 d, 149 [†] 15 d, 179 [†]	5 d, 143 [†] (N.S.) 15 d, 165 [†] (N.S.)	5 d, -4 15 d, -8	20 25	de Richeieu et al., 2005

TABLE 2—Continued.

Model	Control Model	Tempol Route of Delivery and Duration	n	Tempol Maximum Dose or Dose Used to Compare Groups	Control Normotensive	SBP or MAP		BP Response		Reference
						Experimental Hypertensive Group without Tempol	Experimental Group with Tempol	Change	Normalization	
SHR	WKY	p.o. from 0–15 wk	6–12	170 ($\mu\text{mol} \cdot \text{kg}/\text{d}$)	100 [†]	181 [†]	156 ^{†*}	-14	30	Fortepiani and Reckelhoff, 2005
SHR	WKY	p.o. from 9–15 wk	6–12	170 ($\mu\text{mol} \cdot \text{kg}/\text{d}$)	100 [†]	195 [†]	163 ^{†*}	-16	34	Fortepiani and Reckelhoff, 2005
SHR	Untreated SHR	p.o. for 4 d at 13–14 wk	10	1 mM	108 [†] (WKY)	199 [†]	177 ^{†*}	-11	24	Feng et al., 2001
SHR	Untreated SHR	p.o. for 7 wk from 5–12 wk	7	1 mM	108 [†] (WKY)	187 [†]	167 ^{†*}	-11	25	Feng et al., 2001
SHR	WKY	p.o. for 2 wk	8	1 mM	118 [†]	162 [†]	134 ^{†*}	-17	63	Schnackenberg and Wilcox, 1999
SHR	WKY	i.p. for 7 d	7	1.5 (mmol \cdot kg/d)	97 [†]	133 [†]	120 ^{†*}	-10	36	Schnackenberg et al., 1998
SHR aging (16 mo)	Untreated SHR	p.o. for 8 mo from 8–16 mo	6	6 mM	108 [†]	185 [†]	160 ^{†*}	-14	34	Fortepiani et al., 2003
SHR aging (16 mo)	Untreated SHR	p.o. for 8 mo	10	1 mM	108 [†]	188 [†]	161 ^{†*}	-14	33	Payne et al., 2003
SHR aging female (16 mo)	Untreated SHR female	p.o. for 8 mo from 8–16 mo	6	6 mM	108 [†]	195 [†]	195 [†] (N.S.)	0	0	Fortepiani et al., 2003
SHR female	WKY	p.o. from 0–15 wk	6–12	170 ($\mu\text{mol} \cdot \text{kg}/\text{d}$)	101 [†]	172 [†]	127 ^{†*}	-26	63	Fortepiani and Reckelhoff, 2005
SHR female	WKY	p.o. from 9–15 wk	6–12	170 ($\mu\text{mol} \cdot \text{kg}/\text{d}$)	101 [†]	160 [†]	159 [†] (N.S.)	-1	1	Fortepiani and Reckelhoff, 2005
SHR _{SP} , Mg ²⁺ -deficient	Untreated SHR _{SP}	p.o. for 7 wk	6	1 mM	108	240	195 [*]	-19	30	Touyz et al., 2002
SHR _{SP} , 4% salt	Untreated SHR _{SP}	p.o. for 6 wk	6	1 mM	108	260	220 [*]	-15	25	Park et al., 2002
UNX, aldosterone (0.75 $\mu\text{g}/\text{h}$), s.c. salt 8% for 6 wk	Vehicle	p.o. for 4 wk	27	6 mM	139	236	131 [*]	-45	100	Shibata et al., 2007
UNX, aldosterone (75 $\mu\text{g}/\text{h}$), 1% NaCl for 3 wk	Vehicle	p.o. for 3 wk	?	2 mM	118	186	158 [*]	-8	41	Hirono et al., 2007
Studies in hypertensive rats with intracerebroventricular tempol	Untreated SHR	i.c.v. for 2 wk	6	13.2 $\mu\text{mol}/\text{d}$	163	209	210 (N.S.)	0	0	Kagiyama et al., 2000
Studies in hypertensive mice with systemic tempol	Ang II (0.7 (mg \cdot kg/d), WT mice	s.c. for 2 wk	6	28 (mmol \cdot kg/d)	95	154	128 [*]	-17	44	Dikalova et al., 2005
Ang II (0.7 (mg \cdot kg/d), Nox-1-overexpressing mice	Vehicle	s.c. for 2 wk	6	28 (mmol \cdot kg/d)	95	175	138 [*]	-21	46	Dikalova et al., 2005
ET-B-deficient; 8% NaCl	WT; 8% NaCl	p.o. for 1 wk	20	1 mM	134	183	143 [*]	-22	82	Sullivan et al., 2006
ET-B-deficient; 8% NaCl	WT; 8% NaCl	p.o. for 2 wk	20	1 mM	134	174	158 [*]	-9	40	Sullivan et al., 2006

HS, high salt; LS, low salt; DSR, Dahl salt-resistant rat; UNX, uninephrectomized; Dex, dexamethasone; NS, normal salt; HTN, hypertension; ?, unknown; i.c.v., intracerebroventricular; WT, wild type; ACTH, adrenocorticotropin; d, day(s); wk, week(s); mo, month(s).

* Significant change with tempol.

† Value is for MAP.

MAP of 8% (de Richelieu et al., 2005), but one study in rats transgenic for the renin-2 (*ren-2*) gene (Whaley-Connell et al., 2007) and two in the SHR (Fortepiani et al., 2003; de Richelieu et al., 2005) showed no changes in BP despite considerable baseline hypertension. These reports do not indicate a specific lack of effect of tempol for reducing BP in the Ren-2 or SHR models. Thus, another study at a somewhat earlier stage of Ren-2 hypertension showed a significant fall in BP, and 13 other studies in SHR (Table 2, *Studies in hypertensive rats with systemic tempol*) reported significant falls in BP with prolonged tempol administration (Howard et al., 2005). The response of these models to tempol is described in greater detail in sections II.B.2.a and II.B.2.d.

2. Action in Animal Models of Hypertension.

a. Spontaneously Hypertensive Rat. The SHR has been particularly well studied. In five studies, tempol was administered acutely by intravenous injection to SHR in doses of 72 to 900 $\mu\text{mol}/\text{kg}$ and reduced BP in all five (Schnackenberg et al., 1998; Sato et al., 2002; Shokoji et al., 2003; Patel et al., 2006; Chen et al., 2007a) with a 26% (Shokoji et al., 2004) to 100% (Schnackenberg et al., 1998) normalization of hypertension. Tempol has been added to the drinking water of SHR in nine studies (Schnackenberg et al., 1998; Schnackenberg and Wilcox, 1999; Feng et al., 2001; Fortepiani et al., 2003; Payne et al., 2003; de Richelieu et al., 2005; Fortepiani and Reckelhoff, 2005; Nabha et al., 2005; Welch et al., 2005b) in doses from 1 (Schnackenberg and Wilcox, 1999; Feng et al., 2001; Payne et al., 2003; de Richelieu et al., 2005; Nabha et al., 2005) to 6 (Fortepiani et al., 2003) mmol/l over 5 days (de Richelieu et al., 2005) to 8 months (Fortepiani et al., 2003). It reduced the BP in at least one group of SHR in all except one study (de Richelieu et al., 2005) in which it produced a 20 to 25% normalization of MAP over a short period of 5 days that was not statistically significant. The mean normalization of BP in the studies in SHR was 43%. Oral tempol prevented the age-dependent rise in BP in the SHR (Nabha et al., 2005). Tempol was also effective when given intraperitoneally to SHR at 1.5 $\text{mmol} \cdot \text{kg}^{-1} \cdot \text{day}^{-1}$ (Schnackenberg and Wilcox, 1999) or by subcutaneous infusion via an osmotic minipump at 200 $\text{nmol} \cdot \text{kg}^{-1} \cdot \text{min}^{-1}$ (Welch et al., 2005b).

Although a fall in BP during prolonged administration of tempol to male SHR has been a remarkably consistent finding, an exception was the absence of a significant fall in BP of 10- to 12-week-old SHR after 5 to 15 days of oral tempol (1 mmol/l in drinking water) (de Richelieu et al., 2005). BP was directly measured only after surgery and during mechanical ventilation and muscle paralysis, which might have obscured an earlier antihypertensive effect of tempol in this study.

A surprising finding has been the variable BP response to tempol in female SHR (Sartori-Valinotti et al., 2007). Fortepiani and coworkers reported that whereas

male SHR had an antihypertensive response to tempol (6 mmol/l in drinking water) (Fortepiani et al., 2003) as did female SHR administered tempol for the first 15 weeks of their life (Fortepiani and Reckelhoff, 2005), no response was observed in postmenopausal female SHR (Fortepiani et al., 2003) or premenopausal female SHR when dosed from 9 to 15 weeks of age (Fortepiani and Reckelhoff, 2005). These data demonstrate a complex interaction between gender or sex hormones and age in the response to tempol. Remarkably, tempol was more effective in lowering the BP of young female than young male SHR and became less effective after menopause. The finding that aged, postmenopausal female SHR, which lack estrogen, would lack an antihypertensive response to tempol was unexpected because other observations by this group attested to increased ROS generation in postmenopausal rats (Fortepiani et al., 2003). Disparate responses between male and female SHR have also been noted by these authors with an antioxidant regimen of vitamins C and E. However, in the vitamin study, the postmenopausal females had an antihypertensive response to the antioxidants, whereas the males were resistant (Fortepiani and Reckelhoff, 2005). No clear explanation for the opposite effects in these studies of age and gender on the antihypertensive response to tempol or vitamins is apparent presently (Sartori-Valinotti et al., 2007). Sullivan et al. (2006) also reported sex differences in the response to prolonged oral tempol administration to salt-fed endothelin type B receptor-deficient rats (Sullivan et al., 2006). Whereas oral tempol caused almost complete reversal of hypertension initially in both males and females, these effects waned over 2 weeks at which time the BP was higher in females. This higher BP was accompanied by elevated plasma levels of ET-1. The authors concluded that ET-1 may have caused the elevated BP in females given tempol. These results suggest that mechanisms compensating for the effects of tempol that are mediated by ET-1 may be more important in females. However, this explanation is unsatisfactory because tempol reduced the BP of rats made hypertensive by infusion of ET-1 (Sedeek et al., 2003).

b. Renovascular Effects. There is evidence of oxidative stress in renovascular disease. Patients with renal artery stenosis and renovascular hypertension had increased plasma levels of lipid peroxidation products that were corrected by a successful intervention to correct the renal artery stenosis (Higashi et al., 2002).

Intravenous tempol (200 $\mu\text{mol} \cdot \text{kg}^{-1} \cdot \text{h}^{-1}$) produced a 50% normalization of the hypertension that developed 1 month after clipping of one renal artery (2K,1C model) in the rat (Guron et al., 2006). Tempol given subcutaneously by minipump over 13 days at 288 $\mu\text{mol} \cdot \text{kg}^{-1} \cdot \text{day}^{-1}$ produced a 70% normalization of MAP in this model (Welch et al., 2003). Oral tempol given to the less renin-dependent 1K,1C rat model at 1 (Christensen et al., 2007b) or 2 (Dobrian et al., 2001) mmol/l in the

TABLE 3
 Response of indices of oxidative stress to prolonged tempol administration

Model	Control Model	Tempol Route of Delivery and Duration	n	Tempol Maximum Dose or Dose Used to Compare Groups	ROS Marker and Value in Hypertensive Group without Tempol	Control ROS Value in Normotensive Group	ROS Value in Hypertensive Group with Tempol	Change in ROS	Normalization of ROS	Reference
								%		
Studies in hypertensive rat models										
ACTH [0.2 (mg · kg/d s.c.)]	Vehicle	p.o. (4 d before to 8 d after ACTH)	10	1 mM	Plasma 8-iso, 12.9 nM	8.4	4 d pre, 16.3 8 d post, 13.2	+26/+2 8-iso d 4/8 (NS/NS)	-26/-2	Zhang et al., 2003b
Aldosterone (0.75 μg/h s.c. + salt 1% for 6 wk)	Vehicle	p.o. for 6 wk	8	3 mM	Renal cortex TBARS, 0.23 nmol/mg protein Urine TBARS, 0.39 μmol/d	0.10	0.11	-52*	92	Nishiyama et al., 2004a
Aldosterone (0.75 μg/h s.c. for 6 wk)	Vehicle	p.o. for 6 wk	7	1 mM	Plasma 8-iso, 16.8 ng/ml NADPH-generated O ₂ in heart/aorta/kidney/mesenteric artery, 230/930/1500/670 cpm/ mg tissue	13.1	10.6	-37*	166	Iglarz et al., 2004
Ang II [5 (ng · kg/min i.v. for 15 d)]	Vehicle	p.o. for 15 d	6	1 mM	Plasma/renal vein 8-iso, 193/353 pg/ml Plasma/renal vein TBARS, 1.7/1.9 nmol/ml	122/202	122/242	-37*/-31*	100/74	Ortiz et al., 2001a
Ang II [100 (ng · kg/min s.c. ± 8% salt for 12 d)]	Vehicle	p.o. for 12 d	5	1 mM	Plasma cholesterol ester hydroperoxide levels, 0.27 μM Kidney cortex NADPH oxidase activity, 3.6 nmol of O ₂ ⁻ /min · mg protein)	0.8/0.7	1.2/1.1	-33*/-36*	56/67	Ogihara et al., 2002
Ang II [200 (ng · kg/min s.c. for 2 wk)]	Vehicle	s.c. for 2 wk	8-11	200 (nmol · kg)/min	Plasma cholesterol ester hydroperoxide levels, 0.27 μM Kidney cortex NADPH oxidase activity, 3.6 nmol of O ₂ ⁻ /min · mg protein)	2.3	2.7	-15*	69	Welch et al., 2005a
Ang II [300 (ng · kg/min s.c. for 7 d)]	Vehicle	p.o. for 7 d	8	2 mM	Aortic O ₂ , 4.3 RLU	0.7	1.3	-70*	83	Hattori et al., 2005
BSO (30 mM for 2 wk)	Vehicle	p.o. for 2 wk	8	1 mM	Urine 8-Iso/Cre, 1.2 pg/mg	0.80	0.82	-33*	98	Banday et al., 2007a
BSO (30 + HS)	HS	p.o. for 12 d	8	1 mM	Urine 8-Iso/Cre, 1.3 pg/mg	0.84	0.86	-34*	96	Banday et al., 2007c
Capsaicin-4% salt	4% salt	p.o. (gavage) for 3 wk	5-6	1 (mmol · kg)/d	Mesenteric artery O ₂ ⁻ , 1125 cpm/ mg tissue	730	950	-16*	44	Song et al., 2004
Cyclosporine [30 (mg · kg/d s.c.)]	Vehicle	p.o. for 3 wk	7	3 mM	Kidney TBARS, 37 nmol/g Aortic O ₂ , 26 cpm	24	19	-49*	138	Nishiyama et al., 2003
DETC [7.5 (mg · kg/d) into medullary interstitium; SD]	Tempol only, no DETC	Infused into medullary interstitium for 8 d before and during DETC	8	58 (μmol · kg)/d	Urine 8-iso, 8.8 ng/d	17 4.1 ng/d	16 4.2 ng/d	-38* -52*	111 98	Makino et al., 2003
Dexamethasone [10 μg · kg/d s.c.]	Vehicle	p.o. (4 d before to 8 d after Dex)	10	1 mM	Plasma 8-iso, 12 nM	8.8	4 d before, 10.4 8 d after, 10	-13/17, d 4/8 (NS/NS)	50/63	Zhang et al., 2004b
DOCA + salt 0.9%	Sham	p.o. for 3 wk	6	1 mM	Aortic O ₂ , 3166 (mU · mg)/min	875	824	-74*	102	Ghosh et al., 2004 2004

Mean values are shown.

TABLE 3—Continued.

Model	Control Model	Tempol Route of Delivery and Duration	n	Tempol Maximum Dose or Dose Used to Compare Groups	ROS Marker and Value in Hypertensive Group without Tempol	Control ROS Value in Normotensive Group	ROS Value in Hypertensive Group with Tempol	Change in ROS	Normalization of ROS	Reference
DOCA + salt	SD/sham high salt	i.p. for 3 wk	8	87 ($\mu\text{mol} \cdot \text{kg}/\text{d}$)	Plasma 8-iso, 0.77 ng/ml	0.20	0.36	-53*	72	Adeagbo et al., 2003
DOCA + salt 1%	Sham	p.o. for 4 wk	10	1 mM	O ₂ ⁻ aortic rings, 7153 cpm/mg tissue	3055	2939	-59*	103	Beswick et al., 2001
DOCA + salt 1%	Sham	p.o. for 5 wk	13	1 mM	Aortic O ₂ ⁻ 1250 RLU/(min · mg)	525	750	-40*	69	Nakano et al., 2003
DSS + 8% salt	DSR	p.o. for 10 wk	9	3 mM	Plasma TBARS, 14 nmol/ml	7	7	-50*	100	Guo et al., 2006
DSS + 8% salt	DSS LS	p.o. for 5 wk	5	10 mM	Cardiac NADPH oxidase, 361 cpm/mg protein	245	185	-49*	152	
DSS + 8% salt	DSS LS	p.o. for 5 wk	5	10 mM	8-OHdG-positive cells, 347 cells/area	159	259	-25*	47	Hisaki et al., 2005
DSS + 8% salt	DSS LS	p.o. for 4 wk	8	3 mM	Urine TBARS, 0.66 $\mu\text{mol}/\text{d}$	0.14	0.24	-64*	81	Kobori and Nishiyama, 2004
DSS + 8% salt	DSS LS	p.o. for 2 wk	9	1 mM	Urine 8-iso, 14 ng/d	7.4	8	-43*	91	Hoagland et al., 2003
DSS + 8% salt	DSS LS	p.o. for 4 wk	8	3 mM	Kidney TBARS, 86 nmol/g	41	48	-46*	88	Nishiyama et al., 2004b
DSS + 8% salt + L-NAME	DSS LS	p.o. for 2 wk	9	1 mM	Urine 8-iso, 15.2 ng/d	7.4	7.5	-51*	99	Hoagland et al., 2003
DSS + 8% salt + HET-0016 (20-HETE blocker)	DSS LS	p.o. for 2 wk	9	1 mM	Urine 8-iso, 14.6 ng/d	7.4	8.5	-42*	85	Hoagland et al., 2003
DSS + salt	DSS LS	i.v. for 3 wk	7	3 (mmol · kg)/d	Renal cortical/medullary O ₂ ⁻ 72/35 cpm/mg protein	47/26	22/12	-69*/-66*	200/256	Meng et al., 2003
ET-1 [5 (pmol · kg)/min i.v. + 8% salt]	Vehicle-infused, normal salt	p.o. for 12 d	7-10	1 mM	Plasma 8-iso, 128 pg/ml	52	72	-44*	74	Elmarakby et al., 2005
ET-1 [5 (pmol · kg)/min i.v. + 8% salt]	Untreated	s.c. for 12 d	7-10	170 ($\mu\text{mol} \cdot \text{kg}/\text{d}$)	Aortic O ₂ ⁻ 740 cpm/mg	90	210	-72*	82	
ET-1 [5 (pmol · kg)/min i.v.]	Vehicle-infused	i.v. for 9 d	6	170 ($\mu\text{mol} \cdot \text{kg}/\text{d}$)	Aortic O ₂ ⁻ 740 cpm/mg	90	240	-68*	77	Elmarakby et al., 2005
ET-B antagonist (A-192621) p.o.	Vehicle-treated	p.o. for 1 wk	6	1 mM	Kidney TBARS, 462 ng/ μg of protein	48	287	-88 (N.S.)	42 (N.S.)	Sedek et al., 2003
ET-B antagonist + 10% salt p.o.	Vehicle-treated	p.o. for 1 wk	6	1 mM	Urine 8-iso, 11 ng/d	7.5	8.9	-19*	60	Williams et al., 2004
ET-B antagonist + 8% salt	Wild type; 8% NaCl	p.o. for 15 d	20	1 mM	Plasma 8-iso, 25 pg/ml	18	23	-8 (N.S.)	29	Williams et al., 2004
High salt 10%	Normal salt	p.o. for 1 wk	6	1 mM	Urine H ₂ O ₂ , 4 nmol/d	<1	<1	-75*	100	
Hypert thyroid (s.c. T4 for 6 wk)	Vehicle-infused	p.o. for 6 wk	8	1 mM	Plasma 8-iso d 3/7, 75/66 pg/ml	18	57/66	-24/0 (NS/NS)	32/0	Williams et al., 2004
					Urine TBARS, 1164 nmol/24 h	1314	1169	-11*	11	Sullivan et al., 2006
					Plasma 8-iso d 3/7, -64/50 pg/ml	18	60/63	-6/+26 (NS/NS)	9/26	Williams et al., 2004
					Urine H ₂ O ₂ , 3 nmol/d	<1	2	-33*	50	
					Plasma MDA, 10.2 μM	6.8	7.5	-26*	79	Moreno et al., 2005
					Urine 8-iso, 12.5 $\mu\text{g}/\text{d}$	6.5	7.5	-40*	83	

TABLE 3—Continued.

Model	Control Model	Tempol Route of Delivery and Duration	n	Tempol Maximum Dose or Dose Used to Compare Groups	ROS Marker and Value in Hypertensive Group without Tempol	Control ROS Value in Normotensive Group	ROS Value in Hypertensive Group with Tempol	Change in ROS	Normalization of ROS	Reference
1K, 1C	Sham uninephrectomized	p.o. for 2 wk 2 d	6	2 mM	Aortic rings/O ₂ , 80 (RLU 15 min/ mg)	42	63	-21*	45	Dobrian et al., 2001
					Renal nitrotyrosine, 59 ng/mg of protein	14	32	-46*	60	
1K, 1C (10 wk)	Sham	p.o. for 5 wk	5	1 mM	Plasma 8-iso, 240 pg/ml	305	340	-42*	0	Christensen et al., 2007b
					DHE fluorescence in mesenteric arteries	16	25	-38*	68	
2K, 1C	Sham	s.c. minipump for 13 d	8	288 (μmol · kg/d)	Urine 8-iso, 12.5 ng/d	8	9	-28*	78	Welch et al., 2003
					Urine MDA, 610 μmol/d	400	330	-46*	133	
Leptin	Leptin untreated	p.o. for 7 d	8	2 mM	Urine 8-iso, 325 ng/d	190	225	-31*	43	Beltowski et al., 2005
					Plasma 8-iso, 218 pg/ml	130	138	-37*	91	
Obese Zucker	Lean	p.o. for 15 d	10	1 mM	Renal MDA, 0.91 nmol/mg	0.55	0.59	-35*	89	Banday et al., 2005
Offspring of protein malnourished mothers	Offspring of normal mothers	p.o. for 13 wk	14–19	2 mM	Kidney nitrotyrosine, 1.42 (relative abundance)	1.0	0.45	-70*	100	Stewart et al., 2005
Ren-2 transgenic rat	Control	p.o. for 3 wk	6	1 mM	Cardiac MDA, 0.60 μm/mg of protein	47	0.33	-45*	207	Whaley-Connell et al., 2007
Ren-2 transgenic rat	SD	p.o. for 3 wk	6	1 mM	NADPH oxidase of mesenteric arteries, 18	11	13	-28*	71	Whaley-Connell et al., 2007
SD + HS (1% NaCl) + BSO (30 mM for 12 d)	HS	p.o. for 12 d	8	1 mM	Urine 8-iso, 54 pg/mg Cr	41	42	-22*	92	Banday et al., 2007d
SHR	WKY	s.c. for 2 wk	6–8	200 (nmol · kg/min)	Urine 8-iso, 13.2 ng/d	N.A.	9.6	-27*	N.A.	Welch et al., 2005b
SHR	Untreated SHR	p.o. for 2 wk	6–8	1 mM	Kidney cortex O ₂ ⁻ , 11,889 RLU	N.A.	9315	-22*	N.A.	Yanes et al., 2005
					Medulla, 6413 RLU	N.A.	5944 (N.S.)	-7 (N.S.)		
SHR	WKY	p.o. for 15 wk	6–12	170 (μmol · kg/d)	Plasma total anti-oxidant, 1.2 mM	1.7	1.57	-31*	100	Fortepiani and Reckelhoff, 2005
					Urine 8-iso, 2.21 ng/mg of creatinine	1.7	1.53	-31*	14	Fortepiani and Reckelhoff, 2005
SHR	WKY	p.o. for 6 wk	6–12	170 (μmol · kg/d)	Kidney 8-iso, 5.2 ng/mg of tissue	1.7	4.8	-9*	127	Schnackenberg and Wilcox, 1999
					Urine 8-iso, 9.8 ng/d	6.8	6.0	-39*	NA	Fortepiani et al., 2003
SHR aging (16 mo)	Untreated SHR	p.o. for 8 mo	6	6 mM	Urine 8-iso, 20 ng/d	N.A.	12	-40*	N.A.	Fortepiani et al., 2003
SHR aging female (16 mo)	Untreated SHR female	p.o. for 8 mo	6	6 mM	Urine 8-iso, 45 ng/d	N.A.	37	-18*	N.A.	Fortepiani et al., 2003

TABLE 3—Continued.

Model	Control Model	Tempol Route of Delivery and Duration	n	Tempol Maximum Dose or Dose Used to Compare Groups	ROS Marker and Value in Hypertensive Group without Tempol	Control ROS Value in Normotensive Group	ROS Value in Hypertensive Group with Tempol	Change in ROS	Normalization of ROS	Reference
SHR aging (16 mo)	Untreated SHR	p.o. for 8 mo	10	1 mM	Urine 8-iso, 1.84 (ng · mg Cr)/d	N.A.	1.28	-30*	N.A.	Payne et al., 2003
SHR female	WKY	p.o. for 15 wk	6–12	170 (μ mol · kg)/d	Kidney 8-iso, 3.7 ng/mg of tissue	2.2	1.7	-54*	133	Fortepiani and Reckelhoff, 2005
SHR female	WKY	p.o. for 6 wk	6–12	170 (μ mol · kg)/d	Kidney 8-iso, 3.6 ng/mg of tissue	2.2	3.7	+3 (N.S.)	0	Fortepiani and Reckelhoff, 2005
SHR + L-NAME	Untreated SHR	p.o. for 2 wk	6–8	1 mM	Kidney cortex O ₂ ⁻ , 10.423 RLU Medulla, 7422 RLU	N.A.	9506	-9*	N.A.	Yanes et al., 2005
					Plasma total antioxidant, 1.25 nM	5248	1.46	-29*		
					Urine 8-iso, 1.89 ng/mg creatinine		1.32	-30*		
SHR _{sp} low Mg ²⁺ diet	Untreated SHR _{sp}	p.o. for 7 wk	6	1 mM	Plasma TBARS, 2.7 μ mol/ml	N.A.	1.5	-44*	N.A.	Touyz et al., 2002
					Vascular O ₂ ⁻ , 1.2 (nmol · min)/mg tissue		0.6	-50*		
SHR _{sp} 4% salt	Untreated SHR _{sp}	p.o. for 6 wk	6	1 mM	Vascular O ₂ ⁻ , 19 RLU	N.A.	4	-79*	N.A.	Park et al., 2002
					Plasma total antioxidants, 0.85 nM		1.1	+29*		
Streptozotocin (DM)/L-NAME	SD, tempol untreated	i.v. for 2 wk	5	18 (μ mol · kg)/h	Urine 8-iso, 118 ng/d	23	40	-66*	82	Brands et al.,
Studies in hypertensive mouse models	Vehicle	s.c. for 2 wk			Aortic O ₂ ⁻ , 125 pmol/mg of tissue	50	85	-32*	53	Dikalova et al., 2005
Ang II [0.7 (mg · kg/d), WT mice]	Vehicle	s.c. for 2 wk			Aortic O ₂ ⁻ , 250 pmol/mg of tissue	75	160	-36*	51	Dikalova et al., 2005

8-iso, 8-isoprostane PGF_{2 α} ; TBARS, thiobarbituric acid reactive agent; RLU, relative light unit; HS, high salt; Dex, dexamethasone; DSR, Dahl salt-resistant rat; LS, low salt; 8OHdG, 8-hydroxy-2'-deoxyguanosine; HET-0016, N-hydroxy-N'-(4-butyl-2-methylphenyl)-formamide; N.A., not applicable; adrenocorticotropin; d, day(s); wk, week(s); mo, month(s).

* Significant change with tempol.

drinking water for 2 (Dobrian et al., 2001) to 5 (Christensen et al., 2007b) weeks produced a 31% (Dobrian et al., 2001) and 90% (Christensen et al., 2007b) normalization of MAP.

Rats at the early (2–4 weeks) phase of 2K,1C hypertension, which is strongly Ang II-dependent, had increased excretion of 8-iso-PGF_{2 α} and MDA and reduced glomerular filtration rate (GFR) and kidney weight downstream from the renal artery clip (clipped kidney). These were accompanied by reduced outer cortical pO₂ and reduced renal tubular Na⁺ transport (T_{Na}) per oxygen used (Q_{O_2}) by the clipped kidney (Welch et al., 2003). All of these parameters were prevented by 2 weeks of tempol infusion (200 nmol · kg⁻¹ · min⁻¹ s.c.) but not by 2 weeks of administration of an angiotensin receptor blocker (ARB) despite a similar moderation of hypertension (Welch et al., 2003). This result was remarkable because the 2K,1C rat is the quintessential model of Ang II-induced hypertension. These findings point to potential advantages of tempol over an ARB or an ACEI in renovascular disease that merit further study.

c. Angiotensin II-Infused and Angiotensin II-Dependent Hypertension. Incubation of many vascular tissues with Ang II increased O₂⁻ generation (reviewed in Wilcox, 2005). The addition of tempol to blood vessels in which O₂⁻ had been stimulated by prolonged incubation with Ang II generally prevented the increase in O₂⁻ and reduced the contraction (Cai et al., 2003; Wilcox, 2005). However, one study of rat aortic rings and mesenteric resistance vessels incubated acutely with Ang II, ET-1, PE, and KCl demonstrated that coincubation with 10⁻⁴ M tempol reduced the sensitivity and responsiveness to Ang II selectively in an endothelium-dependent manner. This reduction was associated with a quenching of vascular O₂⁻ and an enhancement of NO signaling by Ang II (Shastri et al., 2002). A selective effect of tempol on Ang II responses was also seen in a mild model of oxidative stress. Thus, Wang et al. (2003b) studied the contractility of perfused renal afferent arterioles isolated from rabbits with oxidative stress caused by a 2-week infusion of Ang II at two different rates. There was a selective enhancement of contractions to Ang II in vessels from rabbits infused with Ang II at the lower, nonpressor rate that was prevented by tempol but a more general enhancement of contraction to Ang II, ET-1, and U-46,619 in those infused with Ang II at a higher pressor rate that were all prevented by tempol.

Ang II treatment of porcine isolated coronary arterioles elicited Ang type 1 receptor (AT₁-R)-dependent contractions at low concentrations and Ang type 2 receptor (AT₂-R)- and NOS-dependent dilations at higher concentrations that were apparent after AT₁-R blockade (Zhang et al., 2003a). Tempol moderated the AT₁-R-dependent contraction, consistent with AT₁-R mediating ROS production (Chabrashvili et al., 2003). Incubation of mesenteric or renal afferent arterioles from rabbits

with oxidative stress with Ang II further impaired their EDRF/NO responses, which were restored by tempol (Wang et al., 2004, 2006a). Moreover, the relaxation responses to Ang II of aortas from diabetic rats in the presence of AT₁-R blockade that were mediated by AT₂-Rs were enhanced by tempol (Arun et al., 2004). However, elderly rats were shown to have enhanced expression of AT₂-Rs, which mediated a paradoxical endothelium-dependent contractile response that was prevented by tempol (Pinaud et al., 2007). Thus, tempol normally resets the balance of vasoconstriction: vasodilation induced by Ang II toward a moderation of vasoconstriction.

Tempol (3 μ mol · kg⁻¹ · min⁻¹) infused into the renal arteries of dogs pretreated with L-nitroarginine to block NOS attenuated reductions in renal blood flow (RBF), GFR, and sodium and fluid excretion in response to intra-arterial infusion of Ang II (Majid et al., 2005). Thus, tempol has an NO-independent component of action to blunt renal responses to Ang II.

The infusion of Ang II into rats (Chabrashvili et al., 2003; Welch et al., 2005a) or mice (Kawada et al., 2002; Dikalova et al., 2005; Welch et al., 2006) at a slow pressor rate increased the expression of NADPH oxidase components, increased O₂⁻ generation in the blood vessels and kidneys, and increased the excretion of 8-iso-PGF_{2 α} and MDA. These effects and the rise in BP were prevented by coinfusion of tempol (Kawada et al., 2002; Kimura et al., 2005a; Welch et al., 2005a). In a discordant study, tempol failed to reduce the BP of rats during a 2-week infusion of Ang II despite a reduction in aortic O₂⁻ unless the Ang II was given with enalapril to block angiotensin-converting enzyme (Elmarakby et al., 2007). Mice with vascular oxidative stress due to overexpression of neutrophil oxidase-1 (Nox-1) in VSMCs had an exaggerated increase in BP, vascular hypertrophy, and ROS during infusion of Ang II that were moderated by coinfusion of tempol (Dikalova et al., 2005).

Tempol given by acute intravenous injection at 15 (Zhang et al., 2004a) or 173 μ mol/kg followed by an infusion at 43 μ mol/kg (Kimura et al., 2004) did not reduce the BP of rats during a 5-min intravenous infusion of Ang II but, when given at a later stage at 1 to 24 h of Ang II infusion, tempol reduced the BP by up to 33% (Kimura et al., 2004). Tempol given intravenously at 43 (Kimura et al., 2004) or 170 μ mol/kg or infused at 3 μ mol · kg⁻¹ · min⁻¹ (Kimura et al., 2005a) produced 100 and 92% normalization of BP of rats infused with Ang II for 2 weeks at a slow pressor rate of 200 ng · kg⁻¹ · min⁻¹. When tempol was given in the drinking water to Ang II-infused rats (Ortiz et al., 2001a; Ogihara et al., 2002; Hattori et al., 2005) at 1 to 2 mmol/l or by subcutaneous infusion at 200 nmol · kg⁻¹ · min⁻¹ (Welch et al., 2005a) or 28 μ mol · kg⁻¹ · day⁻¹ (Dikalova et al., 2005) over 7 (Hattori et al., 2005) to 15 (Ortiz et al., 2001a) days, it produced a 44 (Dikalova et al., 2005) to 100% (Ortiz et al., 2001a) normalization of the BP. These data

demonstrate that tempol does not act as a direct antagonist of circulating Ang II but is highly effective in moderating the sustained increase in BP during prolonged Ang II infusion in rats and mice.

Transgenic rats overexpressing the *ren-2* gene develop extreme hypertension that is lethal unless they receive an ARB or an ACEI. In one study, the hypertension was not significantly moderated by 3 weeks of oral tempol (1 mmol/l) administration, despite normalization of cardiac levels of NADPH oxidase and MDA and p22^{phox} expression (Whaley-Connell et al., 2007). Administration of an ARB or tempol to this model prevented the activation of insulin-stimulated protein kinase B, which is required for phosphorylation and activation of phosphoinositol 3-kinase, and prevented coronary artery adventitial fibrosis (Whaley-Connell et al., 2007). This outcome is remarkable because in a second study in Ren-2 rats at a somewhat earlier stage with less severe hypertension, tempol (2 mmol/l of water) produced a robust 63% normalization of BP over 10 days (Howard et al., 2005). Moreover, in prehypertensive Ren-2 transgenic rats, intrarenal arterial infusions of tempol increased the RBF, GFR, and sodium excretion more than in control rats (Kopkan et al., 2007). Presumably the very high levels of renin and Ang II in the later stages of this severely hypertensive model can sustain hypertension even after inhibition of excessive ROS.

These animal studies could have clinical relevance because human brachial (Hussain et al., 2006) or coronary arteries (Püntmann et al., 2005) from subjects with cardiovascular disease had increased O_2^- generation in response to Ang II. Coincubation with tempol prevented the increase in O_2^- and reduced the contractions to Ang II (Püntmann et al., 2005; Hussain et al., 2006).

d. Deoxycorticosterone Acetate- or Aldosterone-Salt Hypertension. The administration of a mineralocorticosteroid such as DOCA with a high salt intake to uninephrectomized rats produces severe hypertension with suppression of circulating renin. Therefore, it is considered a model of human low-renin hypertension.

A high salt intake in the rat increased lipid peroxidation and NADPH oxidase activity and reduced the expression of Cu/Zn- and Mn-SOD in the kidneys (Kitiyakara et al., 2003). Therefore, an increase in dietary salt itself can cause oxidative stress. Tempol has been very effective in preventing or moderating hypertension in uninephrectomized rats given a high-salt diet and DOCA (Beswick et al., 2001; Adeagbo et al., 2003; Awe et al., 2003; Nakano et al., 2003; Ghosh et al., 2004) or aldosterone (Nishiyama et al., 2004a; Hirono et al., 2007; Shibata et al., 2007). Tempol given by intravenous infusion (300 $\mu\text{mol/kg}$) to DOCA-salt hypertensive rats produced a 91% normalization of BP (Xu et al., 2004). Tempol given in the water at 1 (Beswick et al., 2001; Nakano et al., 2003; Ghosh et al., 2004; Iglarz et al., 2004) to 6 (Shibata et al., 2007) mmol/l or infused subcutaneously at 87 $\mu\text{mol} \cdot \text{kg}^{-1} \cdot \text{day}^{-1}$ (Adeagbo et al., 2003; Awe et al., 2003) produced a 29

(Nakano et al., 2003) to 106% (Shibata et al., 2007) normalization of BP, which averaged 73% among these studies. Oral tempol (3 mmol/l for 6 weeks) was as effective as the mineralocorticosteroid antagonist eplerenone in preventing hypertension and proteinuria in rats given 1% NaCl to drink and infused with aldosterone (Nishiyama et al., 2004a). Tempol also reduced the BP of rats infused with aldosterone for 6 weeks without salt loading but did not reverse the remodeling of the resistance vessels in this model although it did prevent cardiac, renal, and aortic fibrosis and the associated oxidative stress (Iglarz et al., 2004). Aldosterone infusion for 3 weeks into uninephrectomized rats given saline to drink reduced plasma Ang II concentrations predictably, yet aortic tissue Ang II concentrations were increased (Hirono et al., 2007). Tempol and an ARB were equally effective in this model in moderating the hypertension and reducing the vascular expression of inflammatory mediators (Hirono et al., 2007). Tempol (3 mmol/l in water) prevented MAPK activation and glomerular sclerosis in the rat DOCA-salt model (Nishiyama and Abe, 2004). These results indicate that mineralocorticosteroid-salt models of low-renin hypertension in the rat are associated with rather severe oxidative stress, perhaps related to activation of a local tissue RAAS. The increased ROS signaling via MAPK and the hypertension can be largely prevented by the administration of tempol.

e. Dahl Salt-Sensitive Rat. As recently reviewed (Manning et al., 2005), the DSS rat is considered a model of salt sensitivity and nephrosclerosis. After 3 to 5 weeks of salt feeding, these rats developed severe oxidative stress, hypertension, and renal damage associated with reduced renal SOD activity. These defects were ameliorated by administration of tempol or vitamins E plus C (Manning et al., 2003, 2005). An intravenous bolus of tempol given to DSS rats (Zicha et al., 2001; Dobesová et al., 2002) at 60 (Dobesová et al., 2002) or 145 (Zicha et al., 2001) $\mu\text{mol/kg}$ produced a 48 and 19% normalization of the BP. The fall in BP with intravenous tempol (142 $\mu\text{mol/kg}$) was greater in young than in elderly DSS rats (Dobesová et al., 2002). Prolonged tempol administration for 3 (Bayorh et al., 2006) to 10 weeks (Ozawa et al., 2004; Guo et al., 2006) in seven studies (Meng et al., 2003; Kobori and Nishiyama, 2004; Nishiyama et al., 2004b; Ozawa et al., 2004; Hisaki et al., 2005; Bayorh et al., 2006; Guo et al., 2006) produced a 29 (Guo et al., 2006) to 102% (Meng et al., 2003) normalization of BP, which averaged 65% among these studies. Thus, tempol is an effective antihypertensive agent in this highly salt-sensitive rat model of hypertension.

f. Endothelin Models. An infusion of ET-1 into rats increased their lipid peroxidation, RVR, and BP, all of which were reduced by coinfusion of tempol (110 $\mu\text{mol} \cdot \text{kg}^{-1} \cdot 24 \text{ h}^{-1}$) (Sedeek et al., 2003). However, in another study of ET-1-infused rats, there was no significant reduction in MAP with a similar rate of tempol infusion or

with the addition of tempol to the drinking water (1 mmol/l) for 12 days (Elmarakby et al., 2005), perhaps because ET-1 produced only a modest increase in MAP of 15% in this protocol.

The administration of an endothelin type B receptor (ET-B) antagonist (A-192621) to normal rats for 1 week raised their BP by 17 to 25%. The coadministration of tempol for 1 week produced an initial 60% normalization of the BP, but this effect was lost after 1 week (Williams et al., 2004). ET-B-deficient rats given salt developed a more robust increase in BP of 37%, which was 40% normalized initially by oral tempol (Sullivan et al., 2006). However, over 15 days of tempol administration, the antihypertensive response again waned, especially in female rats, which had an increase in plasma ET-1. These results suggest that the modest and inconsistent effects of tempol to reduce the BP of rats infused with ET-1 or in ET-B-deficient rats may relate to a combination of the modest levels of hypertension, because tempol is not effective in reducing the BP of normotensive models, and sex-dependent compensatory changes in ET-1 generation. This result is surprising because tempol was quite effective in preventing increases in ET-1 generation both *in vitro* (An et al., 2007) and *in vivo* (Ortiz et al., 2001a; Fujii et al., 2005; Bell et al., 2007; Troncoso Brindeiro et al., 2007) and in moderating vasoconstriction of isolated blood vessels to ET-1 in several models of hypertension and oxidative stress (Wang et al., 2004, 2006c).

g. Lead- and Zinc-Induced Hypertension. Prolonged exposure of rats to lead *in vivo* or of ECs to lead *in vitro* generated $\cdot\text{OH}$ and O_2^- . These increases in ROS were prevented by tempol (Vaziri and Ding, 2001; Vaziri et al., 2003b). Tempol given intravenously or by subcutaneous injection moderated the oxidative stress and the hypertension of rats given a diet with added lead (Vaziri et al., 2001). Rats fed a diet with added zinc for 5 weeks also developed hypertension that was reversed by acute intravenous tempol (Yanagisawa et al., 2004). SHR given a Zn-free diet also had an increased MAP perhaps because of defective function of Cu/Zn-SOD. The component of hypertension that was related to Zn deficiency was abolished by intravenous tempol (100 $\mu\text{mol/kg}$) (Sato et al., 2002).

h. Nitric-Oxide Synthase Inhibitor Hypertension. Oral administration of L-nitroarginine methyl ester (L-NAME) to block NOS increased the MAP of conscious rats substantially (Elmedal et al., 2004; Thakali et al., 2006). The hypertension was 72% normalized by acute intravenous tempol but was not modified by prolonged administration of tempol in the water (1.3 mmol \cdot kg $^{-1}$ \cdot day $^{-1}$) (Elmedal et al., 2004). The hypertension of rats given L-NAME for 2 months was not prevented by coadministration of tempol (142 $\mu\text{mol} \cdot$ kg $^{-1}$ \cdot day $^{-1}$) plus vitamin C despite correction of cardiac indices of ROS (Bell et al., 2007). Thus, the hypertension accompanying acute, but not prolonged, NOS inhibition is responsive to

tempol. This finding is consistent with the concept that a component of the acute antihypertensive response to tempol entails an improvement in NO bioactivity (Chen et al., 2007a).

i. Reduced Renal Mass Models. Removal of one kidney and two-thirds of the other produces a five-sixths nephrectomy rat model of progressive chronic kidney disease (CKD) related to a reduced renal mass (RRM). The BP and plasma renin activity (PRA) rose steeply in rats in which the tissue of the remaining kidney was infarcted by ligating the upper and lower pole renal arteries to produce a renal ischemia model (Hasdan et al., 2002; Vaziri et al., 2003a). However, there was little change in BP or PRA of rats in which the tissue of the remaining kidney was surgically resected (Griffin et al., 1994, 2004; Ibrahim and Hostetter, 1998; Griffin et al., 2004) unless these rats were fed a high-salt diet in which case they had a steady rise in BP (Ylitalo et al., 1976; Bidani et al., 1987; Li et al., 2007). Oral tempol (1 mmol/l to the drinking water) (Hasdan et al., 2002; Vaziri et al., 2003a; Li et al., 2007) or tempol given by intraperitoneal injection at 1.0 to 1.5 mmol \cdot kg $^{-1}$ \cdot day $^{-1}$ for 10 days (Hasdan et al., 2002), 5 weeks (Vaziri et al., 2003a), or 12 weeks (Li et al., 2007) produced a 50 (Vaziri et al., 2003a) to 85% (Hasdan et al., 2002; Li et al., 2007) normalization of hypertension in these two models of CKD. Thus, the hypertension in the RRM rat model is highly responsive to tempol whether or not it is accompanied by a stimulated circulating RAAS.

j. Catecholaminergic and Dopaminergic Hypertension. Tempol did not affect contractions to NE in human brachial artery segments (Püntmann et al., 2005) or renal afferent arterioles from rabbits with Ang II-induced oxidative stress (Wang et al., 2003b, 2006b) or to PE in the SHR mesenteric vascular bed (Girouard and de Champlain, 2004). The failure of NE to enhance vascular O_2^- in vessels from Ang II-infused rabbits was due to inhibition of O_2^- generation by β_1 -adrenoceptor signaling via protein kinase A (Wang et al., 2006b).

The role of abnormal dopamine receptor (DR) signaling in hypertension has been studied extensively by José and colleagues (Albrecht et al., 1996; O'Connell et al., 1997; Felder and Jose, 2006; Wang et al., 2007) and Lokhandwala and colleagues (Banday et al., 2005, 2005a,b,c; Fardoun et al., 2006; Marwaha and Lokhandwala, 2006). The effect of tempol on the renal tubular actions of dopamine are reviewed in section II.E.6, where it is shown that the ability of dopamine-1-like receptors to decrease tubular NaCl transport is impaired in several hypertensive models because of the uncoupling of the D1R from the GRK type 4 γ receptor (Wang et al., 2007), which participates in D1R desensitization (Felder and Jose, 2006). GRK4 γ A142V transgenic mice have hypertension but do not have oxidative stress. These mice had no fall in BP with acute intravenous tempol (Wang et al., 2007). *D1R*($-/-$) (Wang et al., 2007) and *D5R*($-/-$) mice (Wang et al., 2007) also had

hypertension, but this model had extensive evidence of oxidative stress and a significant reduction of hypertension with acute intravenous tempol.

Lokhandwada and colleagues reported that rats with oxidative stress from the administration of BSO for 2 weeks had hypertension (Banday et al., 2007a) that was exacerbated by a high-salt diet (Banday et al., 2007c). Administration of tempol for 2 weeks produced an 85% normalization of hypertension and restored normal signaling via the D1R to inhibit renal Na^+/K^+ -ATPase (Banday et al., 2007c), G-protein coupling, NF- κ B translocation, PKC activation, GRK-2 sequestration, and D1 receptor phosphorylation (Banday et al., 2007a). Other studies by this group demonstrated potent effects of tempol to restore dopamine D1R signaling in the kidneys of old Fisher 344 rats (Fardoun et al., 2006), in insulinopenic diabetic rats given streptozotocin (STZ) (Marwaha and Lokhandwala, 2006), and in obese Zucker rats (Banday et al., 2005, 2007b). Tempol caused a 50% normalization of the elevated BP in the Zucker model (Banday et al., 2005).

Collectively, these results point to an important role for impaired renal D1R signaling in the kidneys of animal models of oxidative stress, hypertension, or type I or II DM. These effects are responsive to tempol administration.

k. Hypoxia. Rats subjected to intermittent hypoxia for 14 days to mimic sleep apnea developed oxidative stress and hypertension, which were prevented by coadministration of tempol (1 mmol/l in water) (Troncoso Brindeiro et al., 2007). However, prolonged administration of tempol to neonatal rats chronically exposed to hypoxia led to stunted growth and impaired cellular proliferation in the airspaces although pulmonary vascular remodeling was prevented (Jankov et al., 2008). This result raises a note of caution for the use of tempol in chronic hypoxia.

l. Blood Pressure Programming. The perinatal milieu can program subsequent levels of BP in the adult (Racasan et al., 2005). Tempol (1 mmol/l in the drinking water) or a mixture of vitamins C and E or the NO donor compound molsidomine given to the dam for the last 2 weeks of gestation and to the offspring for the first 4 weeks after birth each prevented age-related increases in BP and proteinuria in SHR (Racasan et al., 2005). The authors attributed these effects of tempol to prevention of O_2^- -induced activation of inducible NOS, because the inducible nitric-oxide synthase (iNOS) inhibitor, $L\text{-N}^6$ -(1-iminoethyl) lysine reduced the BP of SHR offspring (Racasan et al., 2002, 2005).

Feeding rats a low-protein diet during pregnancy caused oxidative stress, nitrotyrosine deposition, immune cell infiltration of the kidneys, and subsequent hypertension in the offspring (Stewart et al., 2005). Administration of tempol (2 mmol/l of water) or the anti-inflammatory agent mycophenolate mofetil to the pups

for 3 weeks prevented these changes (Stewart et al., 2005).

m. Oxidant Protocols. The addition of BSO (30 mM) to the drinking water of rats for 2 weeks reduced their cellular levels of glutathione, induced lipid peroxidation, and raised the BP (Banday et al., 2007a). These changes were prevented by oral tempol (1 mmol/l) (Banday et al., 2007a). When combined with a high-salt diet, oral tempol was fully effective in preventing the rise in BP (Banday et al., 2007c,d).

n. Other Hypertensive Models. Rats given cyclosporin A developed vascular and renal oxidative stress and increased renal concentrations of Ang II and hypertension, which were reversed by oral tempol (3 mmol/l in the water) (Nishiyama et al., 2003). Tempol (1 mmol/l in the water for 6 weeks) was fully effective in preventing the rise in BP, oxidative stress, and vascular remodeling with dietary magnesium deficiency in the stroke-prone spontaneously hypertensive rat (SHR_{SP}) (Touyz et al., 2002).

3. Mechanism of Antihypertensive Response to Acute Administration of Tempol. The fall in BP accompanying an acute intravenous dose of tempol (216 $\mu\text{mol/kg}$ i.v.) in Ang II-infused rats (Nishiyama et al., 2001) or miniature swine (Hahn et al., 1999) has been ascribed to a fall in total peripheral resistance with a maintained or increased cardiac output.

The short-lived fall in BP after bolus intravenous dosing of tempol has been related to a rapid conversion of the plasma concentrations of the nitroxide radical to the hydroxylamine, as assessed by EPR (Hahn et al., 1999). Early studies in mice (Hahn et al., 1998, 2000) and miniature pigs (Hahn et al., 1999) demonstrated that acute administration of tempol reduced the BP and the HR, whereas acute intraperitoneal administration of the reduced form, tempol-H, had no immediate effect on BP. However, there was a modest reduction in BP after a delay of 5 to 10 min at which time whole-body EPR studies demonstrated that some tempol-H had been oxidized to the nitroxide form (Hahn et al., 2000). These findings relate the acute antihypertensive response to tempol to the nitroxide radical and probably to its facility for reducing tissue levels of O_2^- . Indeed, Hahn et al. (1999) demonstrated that the catalytic rate constants for superoxide dismutation by a series of nitroxides predicted their effects on systemic NO. Patel et al. (2006) demonstrated that the effectiveness of six-member ring nitroxides to reduce the BP of anesthetized SHR was predicted by their in vitro SOD-mimetic activity. In contrast, five-member ring nitroxides such as 3-CP did not lower BP in SHR (Patel et al., 2006), miniature swine (Hahn et al., 1999), or mice (Hahn et al., 1998) despite in vitro SOD-mimetic activity (Patel et al., 2006). This finding is consistent with the hypothesis that the acute antihypertensive response is due to rapid metabolism of O_2^- , which is facilitated by a boat-and-chair conformational change that occurs with six- but not five-member

ring nitroxides. This conformational change greatly accelerates the dismutation reaction that is apparently required for effective antihypertensive action in vivo. Thus, the rapid reversal of hypertension after intravenous injections of tempol may relate to the reduction of the nitroxide to the hydroxylamine, which does not lower BP itself. In addition, tempol is highly permeable and will leave the plasma compartment as it partitions into cells (Patel et al., 2006).

An acute infusion of Ang II over 30 min into conscious rats increased the MAP and the activation of MAPKs in the aorta and heart (Zhang et al., 2004a). Whereas tempol prevented MAPK activation and lipid peroxidation, it did not prevent the early rise in BP during the first 5 min of an Ang II infusion in this model (Zhang et al., 2004a). Likewise, tempol did not relax blood vessels that had been contracted with Ang II, U-46,619, or ET-1 for 5 min, yet relaxed them after they had been exposed to these agonists for 10 to 30 min or more (Wang et al., 2003b, 2004; Chen et al., 2007b). Three conclusions follow from these findings. First, the acute antihypertensive effect of tempol can be dissociated from its effects on MAPK signaling. Second, the vascular effects of tempol are not due to interruption of agonist-receptor interactions. Third, a period of agonist stimulation is required to generate vascular O_2^- and create the condition for a BP-lowering effect of tempol.

Xu, Fink, and colleagues reported that the acute reduction in BP with intravenous tempol in normotensive (Xu et al., 2001) and hypertensive rats (Xu et al., 2002, 2004) was accompanied by inhibition of the renal sympathetic nerves that was independent of NOS (Xu et al., 2002). These studies are described further in section II.D.2.

Chen et al. (2007a) further probed the mechanism of the acute hypotensive response to graded doses of intravenous tempol in the anesthetized SHR (Chen et al., 2007a). The response was unaffected by blockade of catalase with 3-aminotriazole, by infusion of pegylated catalase, by glutathione depletion with BSO, by blockade of BK channels with iberiotoxin, or by inhibition of hemoxygenase with tin mesoporphyrin. Thus, the acute hypotensive response to tempol does not depend on the generation of H_2O_2 or the activation of BK channels or the generation of carbon monoxide or biliverdin by hemoxygenase. However, the hypotensive response was blunted by activation of K_{ATP} channels with cromakalim during maintenance of BP with infused NE or by blockade of these channels by glibenclamide. This finding implicated K_{ATP} channels in the hypotensive response. Moreover, the hypotensive response was reduced by blockade of NOS with L-NAME or by blockade of ganglionic transmission with hexamethonium (Chen et al., 2007a). Because L-NAME and hexamethonium were additive but glibenclamide and hexamethonium were less than additive, the authors concluded that the acute antihypertensive response to tempol depended on the in-

dependent effect of potentiation of NO and inhibition of the peripheral SNS and that the latter involved the activation of K_{ATP} channels.

4. Mechanism of Antihypertensive Response to Prolonged Tempol.

a. Studies of Dose, Duration, and Route of Administration. Tempol infused subcutaneously over 2 weeks into conscious SHR in doses of 50, 100, and 200 $nmol \cdot kg^{-1} \cdot min^{-1}$ reduced the MAP and the excretion of 8-iso-PGF_{2 α} at the higher rates of infusion (Welch et al., 2005b). When tempol was given to rats in the drinking water within a range of 1 to 6 mmol/l, there was no clear indication that the fall in BP increased with the dose of tempol or with the duration of tempol administration (Table 2, *Studies in hypertensive rats with systemic tempol*). These oral doses are approximately equivalent to 100 to 600 $nmol \cdot kg^{-1} \cdot min^{-1}$, which is within the effective dose range for infused tempol. Indeed, intraperitoneal and oral dosing of tempol yielded apparently similar reductions in BP in a single study. A 1.5 $mmol \cdot kg^{-1} \cdot day^{-1}$ intraperitoneal dose of tempol given to SHR produced a 36% normalization of MAP, which was comparable with the response to an equivalent oral dose of tempol (Schnackenberg et al., 1998). This result suggests that rats respond similarly to infused and to oral tempol and that the bioactivity of tempol is probably quite high, but these theories remain to be tested formally. Feng et al. (2001) reported an equivalent reduction in BP of SHR given tempol for 4 days or 7 weeks. Welch et al. (2005b) reported that the MAP of conscious SHR infused subcutaneously with tempol (200 $nmol \cdot kg^{-1} \cdot min^{-1}$) was reduced within the first 12 h and fell further over the subsequent 12 days. In contrast, Sullivan et al. (2006) reported that oral tempol (1 mmol/l) given to salt-loaded ET-B-deficient rats entirely prevented the rise in BP during the first week of a high-salt diet but was no longer effective after 15 days at which time the ET-B-deficient rats had enhanced excretion of 8-iso-PGF_{2 α} . These authors identified a delayed increase in plasma ET-1 as a potential compensatory mechanism that may have overridden the antioxidant and antihypertensive actions of tempol. However, tachyphylaxis to tempol has not been apparent in other models.

Some of the reports in which oral tempol failed to reduce the BP in hypertensive models may relate to loss of pharmacological activity of tempol in the drinking water. We have noted discoloration of tempol solutions exposed to light after approximately 2 days. We recommend protecting tempol from light in foil-wrapped drinking bottles and providing fresh tempol solutions daily to prevent this apparent degradation.

b. Relationships to Antioxidant Action. Tempol reduced the levels of markers of oxidative stress, such as lipid peroxidation products, in the kidney cortex, kidney medulla, renal blood vessels, plasma, and urine of many hypertensive rat and mouse models (Oberley et al., 1993; Ortiz et al., 2001a; Nishiyama et al., 2003; Welch et al.,

2003, 2005b). Prolonged infusions of Ang II at a slow pressor rate enhanced parameters of oxidative stress in the kidney and enhanced the renal cortical NADPH oxidase activity (Chabrashvili et al., 2003; Wang et al., 2003b; Welch et al., 2005a), both of which were prevented by coinfusion of tempol (Welch et al., 2005a). Thus, prolonged administration of tempol can reduce oxidative stress and can reset the endogenous redox machinery toward an antioxidant profile in the kidneys, which could be important for its prolonged hypotensive action.

Mitchell and coworkers demonstrated that tempol added to hamster lung fibroblasts interacts with heme proteins to exert a catalase-like metabolism of H_2O_2 (Krishna et al., 1996b). In contrast, Chen et al. (2007a) reported that the addition of tempol to the aorta of rats with oxidative stress caused an abrupt increase in H_2O_2 and showed further that H_2O_2 was required for the early, transient vasodilator response. H_2O_2 can produce vasodilation (Chen et al., 2007b), vasoconstriction (Schnackenberg et al., 2000) via activation of thromboxane-prostanoid receptors (TP-Rs) (Gao and Lee, 2001), or a biphasic response (Gao et al., 2003), depending on the vascular bed, the concentration, and the experimental conditions.

Pollock, Makino, and coworkers have demonstrated an accumulation of H_2O_2 in the urine and kidneys of rats given tempol over a prolonged period (Makino et al., 2003; Elmarakby et al., 2005). However, pretreatment

with intravenous PEG-catalase did not blunt the acute hypotensive response to intravenous tempol in the SHR (Chen et al., 2007b). Moreover, PEG-catalase was actually required to permit a hypotensive response to infusion of tempol into the renal medulla of the rat, suggesting that tempol-induced generation of H_2O_2 at this site prevented a fall in BP. Therefore, the present evidence suggests that the accumulation of H_2O_2 in the blood vessels after acute tempol administration may contribute to a transient vasodilation but probably is not required for the sustained antihypertensive response to tempol. However, tempol-induced increases in H_2O_2 in the renal medulla may enhance NaCl reabsorption and maintain hypertension.

Many studies have shown rather directly that tempol decreased tissue levels of ROS using assays that include lipid peroxidation, protein or DNA oxidation, dihydroethidium (DHE) fluorescence, or lucigenin-enhanced chemiluminescence (Beswick et al., 2001; Dobrian et al., 2001; Park et al., 2002; Touyz et al., 2002; Meng et al., 2003; Nakano et al., 2003; Nishiyama et al., 2003; Ghosh et al., 2004; Iglarz et al., 2004; Elmarakby et al., 2005; Hattori et al., 2005; Yanes et al., 2005). Figure 3 depicts values from individual studies in which tempol has been given by prolonged administration to hypertensive rat models. Significant correlations are apparent between the changes in BP and plasma indices of ROS (Fig. 3A) and especially in changes in renal excretion of lipid peroxidation products (Fig. 3B). Figure 4 depicts values

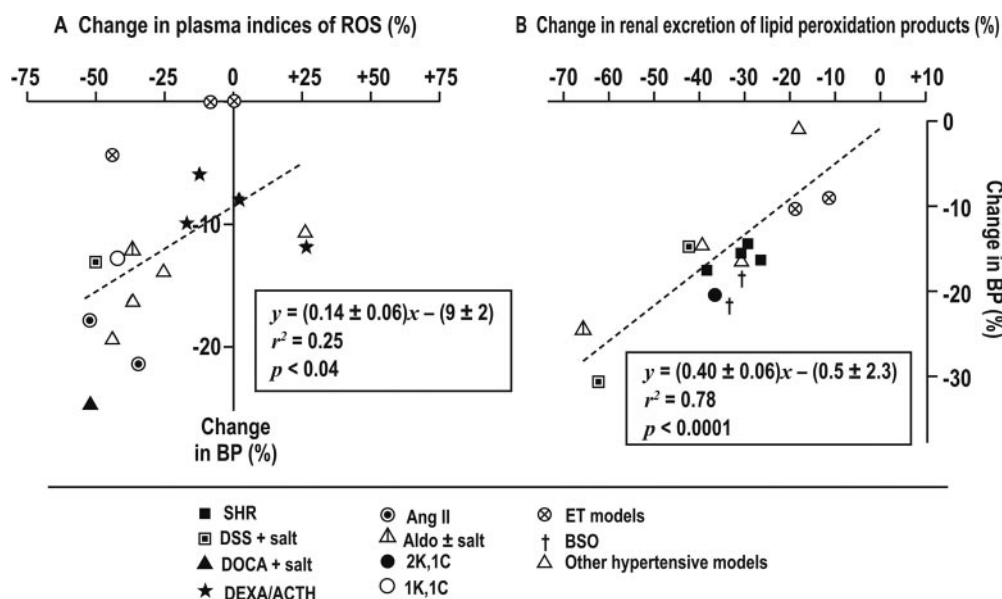


FIG. 3. Individual mean study values from hypertensive rat models showing the relationships between changes in plasma indices of ROS (A) or change in renal excretion of lipid peroxidation products (B) and changes in BP with prolonged tempol administration. The data were derived from the following animal models and studies: spontaneously hypertensive rats (SHR) (Fortepiani et al., 2003; Payne et al., 2003; Welch et al., 2005b; Yanes et al., 2005); Dahl salt-sensitive rats and fed salt (DSS + salt) (Hoagland et al., 2003; Kobori and Nishiyama, 2004; Guo et al., 2006); rats administered deoxycorticosterone acetate, uninephrectomized, and fed salt (DOCA + salt) (Adeagbo et al., 2003); rats infused with dexamethasone or adrenocorticotropic hormone (DEXA/adrenocorticotropic) (Zhang et al., 2003b, 2004b); rats infused with angiotensin II (Ang II) (Ortiz et al., 2001a; Ogihara et al., 2002); rats infused with aldosterone and fed a diet with or without extra salt (Aldo ± salt) (Iglarz et al., 2004; Nishiyama et al., 2004a); two kidney, one clip Goldblatt rat model of renovascular hypertension (2K,1C) (Welch et al., 2003); one kidney, one clip Goldblatt rat model of renovascular hypertension (1K,1C) (Dobrian et al., 2001); rats infused with endothelin-1 (ET models) (Sedeek et al., 2003; Elmarakby et al., 2005; Sullivan et al., 2006); rats administered buthionine sulfoximine (BSO) (Banday et al., 2007a,c); and other hypertensive models (Makino et al., 2003; Song et al., 2004; Beltowski et al., 2005; Moreno et al., 2005).

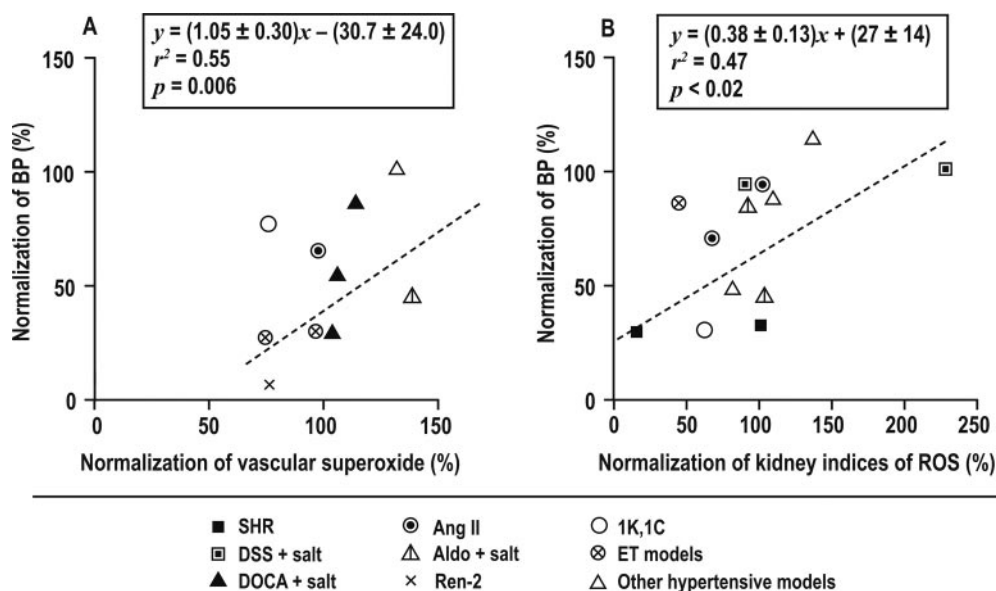


FIG. 4. Individual mean study values from hypertensive rat models showing the relationships between normalization of vascular superoxide (A) or normalization of kidney indices of ROS (B) and normalization of BP with prolonged tempol administration. The data were derived from the following animal models and studies: spontaneously hypertensive rats (SHR) (Fortepiani and Reckelhoff, 2005); Dahl salt-sensitive rats and fed salt rat (DSS + salt) (Meng et al., 2003; Nishiyama et al., 2004b); rats administered deoxycorticosterone acetate, uninephrectomized, and fed salt (DOCA + salt): (Beswick et al., 2001; Nakano et al., 2003; Ghosh et al., 2004); rats infused with angiotensin II (Ang II) (Ortiz et al., 2001a; Hattori et al., 2005; Welch et al., 2005a); rats infused with aldosterone and fed salt (Aldo + salt) (Iglarz et al., 2004; Nishiyama et al., 2004a); rats transgenic for the human renin-2 gene (Ren-2) (Whaley-Connell et al., 2007); one kidney, one clip Goldblatt rat model of renovascular hypertension (1K,1C) (Dobrian et al., 2001; Christensen et al., 2007b); rats infused with endothelin-1 (ET models): (Sedeek et al., 2003; Elmarakby et al., 2005); and other hypertensive models: (Nishiyama et al., 2003; Banday et al., 2005; Stewart et al., 2005).

from individual rat studies of the degree of normalization of BP during prolonged tempol administration and the normalization of vascular O_2^- (Fig. 4A) or of kidney tissue indices of ROS (Fig. 4B). The close correlations are remarkable, given the variability in the measurement of ROS in vivo. These relationships are quite compatible with the hypothesis that prolonged tempol administration reduced hypertensive levels of BP by reducing systemic, vascular, and/or renal oxidative stress. The observation that prolonged tempol administration did not lower BP in normotensive control animals with normal parameters of oxidative stress provides further support for this hypothesis.

On the other hand, some reports have dissociated the ability of tempol to reduce BP from its ability to reduce oxidative stress. Zhang et al. (2003a,b) reported that the administration of 1 mmol/l tempol in the drinking water of Sprague-Dawley (SD) rats made hypertensive by the administration of DEXA or adrenocorticotropin reduced their BP without an accompanying decrease in plasma levels of 8-iso-PGF_{2α}. It is notable that the systolic blood pressure was increased by only 15 mm Hg in these models. Hasdan et al. (2002) reported that 10 days of intraperitoneal administration of tempol (1.5 mmol · kg⁻¹ · day⁻¹) to rats subjected to five-sixths nephrectomy prevented the early increase in BP but did not reduce the plasma levels of advanced oxidation protein products. However, mesenteric arterioles dissected from these rats had an impaired relaxant response to ACh that was improved by tempol. This finding suggests that

tempol administration had corrected oxidative stress within the small blood vessels. Pollock and coworkers noted that oral tempol (1 mmol/l) decreased the hypertension of rats fed a high-salt diet despite no change in the excretion of 8-iso-PGF_{2α} (Williams et al., 2004; Elmarakby et al., 2005), whereas the same dose of tempol did not consistently decrease the hypertension of rats infused with ET-1 or with the ET-B receptor antagonist A-192621 despite decreasing plasma levels of 8-iso-PGF_{2α} and vascular O_2^- . These authors proposed that an increase in the renal production of H₂O₂ had compromised the reduction in BP with tempol. However, a prior study by Sedeek et al. (2003) reported that intravenous infusion of tempol (173 μmol · kg⁻¹ · day⁻¹) into rats infused with ET-1 caused a robust 87% normalization of BP. In this study, the fall in BP was coupled with significant reductions in the malonaldehyde content of the kidney and in the renal excretion of 8-iso-PGF_{2α}. These conflicting results of tempol administration in rat models of ET-1-induced hypertension may relate to the modest increase in BP with ET-1. Thus, Pollock and coworkers noted a 28% normalization of BP and 44% normalization of the excretion of 8-iso-PGF_{2α} with tempol in rats infused with ET-1, but the change in BP did not represent a significant change.

Recent studies in the Ren-2 rat model of malignant hypertension showed that 21 days of oral tempol (1 mmol/l) reduced parameters of ROS and the remodeling in the aorta (Wei et al., 2007) and heart (Whaley-Connell et al., 2007) without significantly reducing the BP. How-

ever, these studies used the tail-cuff method to assess BP, which provides a stressed measure that may not reflect the BP measured telemetrically in unrestrained rats (Sasser et al., 2002).

Tempol has been found to be ineffective as an antihypertensive agent in animal models that are not associated with heightened oxidative stress. The antihypertensive response to an intravenous infusion of tempol in SD rats infused with pressor doses of Ang II was negligible during the first 10 min but became increasingly effective as the duration of the Ang II infusion increased up to a maximum at 12 h at which time tempol prevented 96% of the increase in BP (Kimura et al., 2004). It has been reported that it takes 5 to 20 min of incubation with Ang II for isolated blood vessels to develop oxidative stress (Wang et al., 2003b). These studies show that tempol does not block the immediate pressor effects of Ang II, but diminishes, or even prevents, the effects that develop during a prolonged Ang II infusion that are accompanied by increased ROS.

Thus, the balance of evidence favors the hypothesis that prolonged administration of tempol reduces BP in hypertensive models as a consequence of its antioxidant actions, although some discordant results are apparent.

c. Interaction with Endogenous Oxidant/Antioxidant Pathways. Tempol can down-regulate the expression of the p22^{phox} subunit of NADPH oxidase and thereby reduce the activity of the enzyme in target tissues. Slow pressor infusions of Ang II increased p22^{phox} expression and NADPH oxidase activity in the kidney (Chabrashvili et al., 2003; Welch et al., 2005a; Modlinger et al., 2006) and the renal afferent arteriole (Wang et al., 2003b). Coinfusion of tempol with Ang II prevented the up-regulation of p22^{phox} in these models (Nishiyama et al., 2004a; Welch et al., 2005a). Hattori et al. (2005) reported that tempol prevented an increase in the expression in the aorta, cardiac, and adipose tissue of the NADPH oxidase components p47^{phox}, Nox-2, p22^{phox}, and Rac1 in rats infused with a slow pressor dose of Ang II for 7 days. Prolonged tempol administration to DSS rats normalized the NADPH oxidase activity and p22^{phox} and gp91^{phox} expression in the left ventricle (Guo et al., 2006).

Tempol also can promote endogenous antioxidant defense systems. For example, tempol administered to DOCA-salt rats challenged with the pro-oxidant *tert*-butyl hydroperoxide prevented the down-regulation of Cu/Zn-SOD in the kidneys and mesenteric vessels (Awe et al., 2003). A slow pressor infusion of Ang II reduced the expression of EC SOD in the kidneys, and reduced the SOD activity of the plasma, aorta, and kidneys (Welch et al., 2006), which was preserved by the administration of tempol (Welch et al., 2005a).

These effects of tempol administration to reduce endogenous O₂⁻ levels could be important in providing sustained reductions in oxidative stress in the tissues. They may thereby contribute to the reduction in oxidative

stress that is correlated with the reduction in BP (Figs. 3 and 4) and to the rather uniform reductions of BP throughout the day seen in SHR given tempol in the drinking water (Welch et al., 2005b), because these effects on endogenous O₂⁻ generation and metabolism may outlive the direct redox effects of circulating tempol. On the other hand, tempol prevented the vascular expression of the inducible (type 1) isoform of heme oxygenase, which is an important endogenous antioxidant and vasodilator pathway (Lee et al., 2005).

d. Role of Nitric-Oxide Synthase. Several studies have addressed the hypothesis that the antihypertensive effects of tempol entail restoration of an action of NO whose bioactivity in the blood vessels and kidneys is often curtailed in hypertension (Wilcox, 2005). Tempol could enhance the effects of NO by preventing its bioinactivation by O₂⁻ (Rubanyi and Vanhoutte, 1986; Zhang et al., 2005), by enhancing the stimulus to endothelial NO generation by stimulating blood flow and endothelial shear forces, by interrupting the incorporation of NO into glutathione to form S-nitrosoglutathione (Schrammel et al., 2003), by increasing the activity of the redox-sensitive dimethylarginine dimethylaminohydrolase, which metabolically inactivates the endogenous NOS inhibitor asymmetric dimethylarginine (Palm et al., 2007), or by recoupling NOS by improving the availability of the reduced form of tetrahydrobiopterin (BH₄) (Cai and Harrison, 2000). Indeed, vascular eNOS was uncoupled from NO formation by oxidation of BH₄ in the blood vessels from DOCA-salt rats (Zheng et al., 2003). Treatment of these blood vessels with apocynin or tempol reduced O₂⁻, increased BH₄, and restored NO activity and EDRF responses (Zheng et al., 2003).

Schnackenberg et al. (1998) first reported that acute intravenous infusions of tempol into anesthetized SHR caused substantial reductions in MAP that were blocked during inhibition of NOS by L-NAME. This effect was not due to the increase in BP with L-NAME because SHR infused with a pressor dose of NE retained a full hypotensive response to tempol. They concluded that the hypotensive response to short-term administration of tempol to the SHR depended on NOS. Nishiyama et al., (2001) showed further that L-NAME almost abolished the falls in BP and in systemic and renal vascular resistances produced by tempol infused intravenously into Ang II-infused hypertensive rats. Indeed, tempol increased NO activity measured electrochemically in vivo in rats infused with Ang II (López et al., 2003). Prolonged administration of tempol to aging SHR reduced their BP and the PE- induced "active stress" and increased the ACh-induced relaxations of aortic strips isolated from these rats (Payne et al., 2003). These vascular effects of tempol were prevented by inhibitors of NOS or cGMP, implying that tempol had restored vascular NO signaling. L-NAME given to aged SHR prevented the fall in BP produced by a prolonged 2-week administration of oral tempol (Yanes et al., 2005). Tempol (150 μmol/kg i.v.)

given acutely to hypertensive DSS rats with oxidative stress restored a pressor response to NOS inhibition with L-NAME, suggesting that tempol had restored vasoactive NO generation (Zicha et al., 2001). Thus, both the acute and the prolonged lowering of the BP by tempol has been related to enhancing the production or action of endogenous NO. Interestingly, the bradycardia that accompanies acute tempol administration has been little affected by NOS blockade (Table 1).

On the other hand, several studies have shown that tempol can reduce the BP of rats made hypertensive by prolonged blockade of NOS. Two weeks of NOS inhibition in normal rats by L-nitroarginine (0.5 g/l in the drinking water) increased their MAP by approximately 75 mm Hg (Thakali et al., 2006). The acute intravenous administration of tempol to rats in this study caused dose-dependent reductions in MAP of up to 54 mm Hg. Clearly, tempol given acutely can reduce the BP independent of NOS. However, the effect of prolonged NOS blockade to modify the hypotensive response to tempol has been inconsistent. The administration of L-NAME to rats over 7 days increased the MAP by approximately 70 mm Hg and increased the reactivity of the aorta to PE (Preti et al., 2005), neither of which was modified by oral administration of tempol ($1.2 \text{ mmol} \cdot \text{kg}^{-1} \cdot \text{day}^{-1}$) (Preti et al., 2005). NOS blockade did not affect the lowering of BP by acute intravenous tempol in one study (Xu et al., 2002) but blunted (Shokoji et al., 2003; Thakali et al., 2006) or blocked (Schnackenberg et al., 1998; Nishiyama et al., 2001) the response in other studies. Prolonged L-NAME administration to rats for 2 months caused hypertension and cardiac oxidative stress. Coadministration of tempol ($150 \text{ } \mu\text{mol} \cdot \text{kg}^{-1} \cdot \text{day}^{-1}$) and vitamin C prevented the oxidative stress but not the hypertension (Bell et al., 2007). NOS blockade blunted (Majid and Navar, 2001; Hoagland et al., 2003; Majid et al., 2005; Sainz et al., 2005) or blocked (Yanes et al., 2005) the hypotensive or renal responses to prolonged oral tempol in several studies. The reason for these discordant results requires further study. However, the reports that tempol retains efficacy in reducing the BP in some studies in which NOS has been blocked demonstrate the presence of NOS-independent pathways of BP reduction by tempol.

C. Vascular Actions of Tempol

1. Endothelium-Dependent Relaxant Factor/Nitric Oxide. The reaction of O_2^- with NO not only biodegrades NO (Rubanyi and Vanhoutte, 1986) but generates ONOO^- , which is a potent oxidant and nitrosating species that can modify protein structure and function. For example, ONOO^- inactivated vascular prostacyclin (PGI_2) synthase (Zou et al., 1997). Thus, a reduction of O_2^- in ECs by tempol should increase vascular NO and PGI_2 signaling, both of which should enhance EDRF responses. However, an increase in bioactive NO is not necessarily accompanied by an increase in the excretion

of NO metabolites [nitrite (NO_2) and nitrate (NO_3)] (Welch et al., 2005b). Moreover, tempol increased vascular NO bioactivity (Zhang et al., 2005) but reduced renal cortical nNOS protein expression in the 1K,1C rat model of Goldblatt hypertension (Dobrian et al., 2001; Dutta et al., 2006).

Several studies have shown that tempol added to vessels from hypertensive models can enhance NO generation (Park et al., 2002; Lu and Kassab, 2004; Arrick et al., 2007). NO activity measured with a catheter-type NO sensor placed in the aorta of rabbits was reduced during prolonged infusion of Ang II but was restored by tempol (Imanishi et al., 2006). Treatment of bovine aortic or atrial ECs with SOD, tempol, or two other nitroxides, 3-CP or 3-ethoxycarbonyl-peroxyl (Zöllner et al., 1997), or the addition of tempol to the bath of rat perfused vasa recta capillary blood vessels increased NO release or activity (Zhang et al., 2005).

The EDRF/NO response was diminished or absent in blood vessels from many hypertensive or diabetic models of oxidative stress (Didion et al., 2006; Viswanad et al., 2006; Blanco-Rivero et al., 2007) or from mice with deletion of the gene for EC SOD (Kitayama et al., 2006). The EDRF/NO responses in vessels from these models of oxidative stress were enhanced by tempol (Haj-Yehia et al., 1999). Tempol restored NO-dependent vasodilation of blood vessels perfused at high pressure, which enhanced vascular O_2^- generation (Christensen et al., 2007a). Tempol added to the bath of aortas from rats with enhanced ROS due to a high-salt diet restored NO bioactivity and relaxation responses to methacholine (cholinergic agonist) or histamine (Zhu et al., 2004) without moderating the increased EC $[\text{Ca}^{2+}]$ induced by these agonists (Zhu et al., 2006). This finding indicates that tempol preserved bioactive NO, whether generated in response to endothelial shear stress or agonist stimulation rather than raising EC calcium concentration to stimulate eNOS activity.

Multiple studies have demonstrated that tempol can increase the vascular relaxation response to ACh. This increase could contribute to the blunting by tempol of the constrictor responses to agonists in vessels from animals with hypertension or oxidative stress (Romanko and Stepp, 2005). Thus, incubation with tempol improved EDRF/NO responses and reduced contractile responses to agonists in mouse mesenteric arterioles (Wang et al., 2006a), in rabbit renal afferent arterioles from animals with oxidative stress due to Ang II infusion (Wang et al., 2003b, 2004), and in subcutaneous resistance arteries taken from patients with oxidative stress due to cardiovascular disease or hypertension (Hussain et al., 2006). Incubation with tempol of carotid arteries from DOCA-salt hypertensive rats increased the ratio of $\text{BH}_4/\text{dihydrobiopterin}$, thereby improving NO generation by recoupling NOS (Zheng et al., 2003). Tempol can do more than just prevent oxidation of BH_4 . Thus, the administration of tempol to rats with an un-

coupled eNOS from STZ-induced DM enhanced the expression of GTP cyclohydrolase 1, which is required for biopterin synthesis (Xu et al., 2007). Tempol protected blood vessels from impaired EDRF/NO responses produced by the oxidants homocysteine (Hucks et al., 2004) or C-reactive protein (Qamirani et al., 2005). Because the plasma levels of these compounds are increased in many patients with cardiovascular or inflammatory diseases (Qamirani et al., 2005), moderation of their vascular effects by tempol could be beneficial in these conditions.

Vaziri et al. (2001) reported that rats with lead-induced hypertension and oxidative stress had reduced excretion of nitrate and nitrite, despite an up-regulation of eNOS and iNOS in the kidneys. They attributed the up-regulation of NOS to reduced bioactive NO because it was restored by 2 weeks of tempol administration (Vaziri and Ding, 2001; Vaziri et al., 2001).

Thus, tempol may enhance EDRF responses in models of oxidative stress and inflammation by reducing metabolism of NO to ONOO⁻, preventing inactivation of PGI₂ synthase, by enhancing NOS expression, and by enhancing NOS activity by preventing the uncoupling of the enzyme during reduced availability of its cofactor, BH₄. Because blockade of NOS increases BP and RVR substantially (Gilani et al., 2007), an improvement in small vessel EDRF/NO by tempol could be an important component of its hypotensive action.

Studies have disclosed additional NO-independent mechanisms of endothelium-dependent relaxation with tempol. Thus, nitronyl nitroxides that trap NO blocked the coronary vasodilation response to NO donor compounds but not to tempol. This identified an NO-independent pathway (Konorev et al., 1995). Acute intravenous and 8- to 10-week prolonged oral administration of tempol improved both the defective NOS-dependent and NOS-independent ACh-induced vasodilation of renal afferent arterioles in hydronephrotic kidneys from DSS rats (Ozawa et al., 2004).

Rabbits developed nitrate tolerance, endothelial dysfunction, and a reduction in plasma NO activity after 7 days of treatment with nitroglycerin patches. Nitroglycerin responses were restored by tempol (10 mmol/l), whereas an ACEI or ARB was less effective (Imanishi et al., 2007). Nitrate tolerance developed after 90 min of incubation of aortic rings with nitroglycerin (Ghatta et al., 2007). This was prevented by coincubation with tempol or H₂O₂ but was exacerbated by catalase or ebselen. Because tempol released H₂O₂ from nitroglycerin-tolerant rings, the nitrate tolerance was ascribed to decreased endogenous formation of H₂O₂, which was restored by tempol (Ghatta et al., 2007).

2. Endothelium-Dependent Hyperpolarizing Factor/Hydrogen Peroxide. EDHF is released by Ca²⁺-mobilizing endothelial agonists or shear stress but is distinct from NO or PGI₂. It causes hyperpolarization and vasodilation of adjacent VSMCs (Miura et al., 2003). Studies

have shown that the EDHF response can depend on H₂O₂ (Matoba et al., 2000; Yada et al., 2008), epoxyeicosatrienoic acid (Wang et al., 2003a), endocannabinoids (Randall and Kendall, 1997), a local rise in extracellular [K⁺] (Edwards et al., 2001), or electromechanical coupling via gap junctions (Figuroa et al., 2006), depending on the species, conditions, and type of blood vessel.

Sainz et al. (2005) attributed the antihypertensive effects of tempol in rats with L-NAME-induced hypertension to increased EDHF activity. Likewise, the reduction in PE-induced contractions of mesenteric arteries from cholesterol-fed mice with oxidative stress by tempol also was attributed to the release of an EDHF (Kutala et al., 2006). Tempol restored the blunted EDHF-dependent vasodilation in mesenteric vessels from DOCA-salt hypertensive rats (Adeagbo et al., 2003).

Tempol may enhance EDHF by increasing the generation of H₂O₂ that Ghatta et al. (2007) and Chen et al. (2007a) demonstrated directly in rat aortic rings from Amplex red and luminol fluorometry, respectively. Tempol improved EDHF responses in blood vessels from several models of hypertension or oxidative stress including the coronary (Morikawa et al., 2003) and the mesenteric (Yada et al., 2008) arteries from Cu/Zn-SOD(-/-) mice in which endogenous H₂O₂ was severely compromised. Indeed, enhancement of EDHF responses by tempol was related to H₂O₂ formation because it was blocked by catalase (Yada et al., 2008).

However, it is unclear whether tempol generates functionally significant quantities of H₂O₂ in vivo (Kopkan et al., 2006). Moreover, some studies have dissociated H₂O₂-dependent relaxations to tempol from EDHF responses. Chen et al. (2007b) reported that the addition of tempol to the perfusate of rat isolated mesenteric resistance vessels precontracted with U-46,619 caused a transient dilation that was accompanied by increased H₂O₂. The relaxation was prevented by catalase but not by endothelium removal or by a high bath [K⁺]. This result related the vasodilator response to tempol to H₂O₂ but dissociated it from EDHF. The moderation by tempol of stretch-induced tone in aortic rings from DOCA-salt rats was prevented by catalase or SOD independent of the endothelium or of NOS. This result again identified an H₂O₂-dependent but endothelium-independent pathway for VSMC relaxation by tempol (Itoh et al., 2003; Ghosh et al., 2004). Presently, it is not clear how tempol generates H₂O₂ in VSMCs and how H₂O₂ elicits relaxation independent of K⁺ channels.

There are several pathways of interaction between NO and H₂O₂ that are potentiated by tempol. For example, H₂O₂ enhanced phosphoinositol-dependent phosphorylation of eNOS at Ser-1177 thereby increasing NOS activity (Douthwaite et al., 1999; Thomas et al., 2002) and up-regulated eNOS expression by transcriptional and post-transcriptional mechanisms (Drummond et al., 2000). Tempol prevented the reduction in calcium-

stimulated NO generation by H_2O_2 in ECs (Douthwaite et al., 1999). Both NO and H_2O_2 generated in mitochondria can mediate flow-dependent dilation in blood vessels (Liu et al., 2003; Gutterman, 2005). Small mesenteric arteries from Ang II-infused rats retained a vasodilator response to ACh that was mediated both via NO generated by a coupled eNOS and via H_2O_2 generated by an uncoupled eNOS, because relaxation responses were blunted by NOS blockade and by catalase (Kang et al., 2007). The vasodilation of rabbit mesenteric arterioles to an NO donor was enhanced by tempol and was prevented by catalase, indicating a role for H_2O_2 to increase NO signaling. The effect of tempol to enhance the response to the NO donor was attributed to a reduction in the generation of $\cdot OH$ from H_2O_2 by tempol (Douthwaite et al., 1999).

These vasodilator actions of tempol that are mediated by H_2O_2 in vitro must be contrasted with the prohypertensive effects of H_2O_2 produced by infusion of tempol into the renal medulla of rats with oxidative stress (Makino et al., 2003) and with the absence of an effect of PEG-catalase on the antihypertensive response to acute administration of tempol to the SHR (Chen et al., 2007a). Presently, it is unclear whether the effects of tempol on vascular H_2O_2 detected ex vivo are relevant to in vivo responses.

3. Endothelium-Dependent Contracting Factor. Blood vessels from some models of hypertension, when studied under spontaneous tone, display a paradoxical constrictor response to ACh that is abolished by endothelium removal. This EDCF response occurs in human coronary arteries at sites of atherosclerosis (Lavi et al., 2008) and may contribute to coronary spasm and myocardial ischemia.

Aortic rings (Jerez et al., 2005) or renal afferent arterioles (Wang et al., 2004) from rabbits infused with Ang II have an enhanced contraction to Ang II that is mediated in part by the endothelium. These endothelium-dependent responses were diminished by incubation with indomethacin to block cyclooxygenase, by SQ-29,548 to block TP-Rs, or by tempol to reduce ROS. These findings suggest that tempol prevented the endothelial generation of vasoconstrictor prostanoids that activated TP-Rs on VSMCs of blood vessels from animals with oxidative stress.

There are several other examples of tempol moderating contractile responses that were mediated by an EDCF. ACh or a low ambient pO_2 contracted blood vessels from DSS rats even when the rats were maintained on a low-salt diet. These contractions were reversed by bath addition of tempol (10^{-4} M) (Drenjancevic-Peric and Lombard, 2005). AT_2 -Rs mediated a paradoxical endothelium-dependent contractile response in mesenteric resistance vessels from aged rats that was prevented by incubation with tempol (Tatchum-Talom and Martin, 2004). Stretching of the aorta isolated from DOCA-salt rats increased the O_2^- generation via an en-

dothelium-dependent mechanism. This generation was prevented by 3 weeks of oral tempol administration (Ghosh et al., 2004).

Thus, tempol not only promotes responses mediated by EDRF/NO and EDHF but also prevents responses mediated by EDCF. The outcome should be vasodilation and a fall in BP, but it is not possible to study directly the role of the endothelium in hypertension. Indeed, caution is warranted because there are examples in which pharmacological treatment of a hypertensive model has improved the endothelial function of isolated blood vessels without a corresponding fall in BP (Teschfariam and Ogletree, 1995).

4. Endothelin-1. Ang II (Moreau et al., 1997; An et al., 2006., 2007), 8-iso-PGF $_{2\alpha}$ (Yura et al., 1999), H_2O_2 (Ruef et al., 2001), and a high-salt diet (Pollock and Pollock, 2001; Sasser et al., 2002) all increased ET-1 generation or release from VSMCs. ET-1 synthesis in rat cardiac fibroblasts was stimulated by ROS via an ERK pathway (Cheng et al., 2003). ET-1 generation in the rat aorta stimulated the generation of O_2^- via cooperative effects of endothelin type A receptors (ET-A-Rs) and endothelin type B receptors that engaged NADPH oxidase and an uncoupled NOS (Loomis et al., 2005). Thus, ET-1 can stimulate O_2^- generation in VSMCs and O_2^- can itself stimulate ET-1 release. Other studies demonstrate that this feed-forward mechanism could sustain ROS production in vascular tissue and could be interrupted by tempol (Pollock, 2005).

There are several examples of tempol reducing ET-1 generation. Bath addition of tempol reduced ET-1-induced contractions of renal afferent arterioles from rabbits with oxidative stress (Wang et al., 2003b). Tempol (10^{-5} M), 4,5-dihydroxy-1,3-benzene disulfonic acid (tiron), diphenylethylidenehydrazine, apocynin, and SOD all prevented the effect of Ang II to increase preproendothelin-1 mRNA and ET-1 release from vascular adventitial fibroblasts (An et al., 2007). A 2-month administration of L-NAME to rats increased cardiac ROS and mRNA for preproendothelin-1 (Bell et al., 2007). The coadministration of tempol ($200 \mu\text{mol} \cdot \text{kg}^{-1} \cdot \text{day}^{-1}$) and vitamin C prevented the ET-1 response without modifying the hypertension (Bell et al., 2007). Intravenous tempol caused dose-dependent ($55\text{--}600 \mu\text{mol/kg}$) reductions in renal venous ET-1 release after ischemia-reperfusion injury in the rat (Fujii et al., 2005). A 2-week infusion of Ang II into SD rats increased renal venous concentrations of 8-iso-PGF $_{2\alpha}$, MDA, and ET-1. These were all prevented by oral tempol (1 mmol/l) (Ortiz et al., 2001a). Rats subjected to intermittent hypoxia for 2 weeks developed oxidative stress, hypertension, and increased plasma ET-1 that were normalized by oral tempol (1 mmol/l) (Troncoso Brindeiro et al., 2007).

Tempol also blunts responses to ET-1. Thus, ET-1 increased O_2^- and intracellular $[Ca^{2+}]$ in VSMCs of the shark. These effects were blocked by inhibition of ROS by tempol, by inhibition of NADPH oxidase by apocynin,

or by inhibition of cyclic adenine diphosphate ribose cyclase with nicotinamide and Zn (Fellner and Parker, 2005). These authors concluded that tempol blocked the adenine diphosphate ribose cyclase-induced Ca^{2+} response initiated by ET-1. Tempol moderated the enhanced ET-1 contractions in blood vessels from several animal models of hypertension (Li et al., 2003; Wang et al., 2004, 2006a).

The ET-A-Rs of mesenteric resistance arteries from the *EC-SOD(-/-)* mouse were up-regulated and mediated enhanced O_2^- generation and contraction to ET-1 that were normalized by PEG-SOD or tempol (Wang et al., 2006c).

5. Potassium Channels. As recently reviewed by Liu, Gutterman, Harder, and coworkers, O_2^- and H_2O_2 both enhance BK channel activity in rat and cat cerebral arterioles (Liu and Gutterman, 2002; Gebremedhin et al., 2008). Tempol activated K^+ currents in cells transfected with the gene for the BK channel (Xu et al., 2005) and activated BK channels directly in VSMCs from mesenteric arteries of rats (Xu et al., 2006). Superfusion with tempol (1–3 mmol/l for 10 min) during patch-clamp studies increased by approximately 4-fold the peak outward current (I_o) through BK channels in VSMCs from control and DOCA-salt hypertensive rats. The authors concluded that tempol activated BK channels directly on VSMCs. However, some inconsistencies were apparent. Thus, tempol did not change the mean open time or the single channel conductance. The I_o was not increased in VSMCs from DOCA-salt rats, which have increased BK channel expression. The expression of the $\beta 1$ subunit of the BK channel is down-regulated in VSMCs from SHR and Ang II-infused rats (Amberg and Santana, 2003; Amberg et al., 2003), which are models that exhibit a strong antihypertensive response to tempol (Thakali et al., 2006). Pretreatment of SHR with iberiotoxin in a dose 3-fold higher than that required to block BK channels did not affect basal BP or the antihypertensive response to an acute intravenous dose of tempol (Chen et al., 2007a). Indeed, ROS generation by rat hypoxic cerebral VSMCs activated BK channels and this effect was actually blocked by tempol (Gebremedhin et al., 2008). Thus, the role of BK channel activation in the BP-lowering action of tempol requires further study.

Other studies have implicated tempol in the regulation of the K_{ATP} channel (Kir6.1) (Hanna et al., 2005). Superoxide enhanced K_{ATP} channel activity in guinea pig cardiac myocytes yet decreased the K_{ATP} channel opening probability in cerebral vessels (Liu and Gutterman, 2002; Gebremedhin et al., 2008). Other studies have shown that tempol can enhance relaxation responses of VSMCs via K_{ATP} channel activation. Thus, Ang II added to the aorta of rats with STZ-induced DM during blockade of AT_1 -Rs caused relaxation via the AT_2 -Rs that were augmented by 100 μM tempol. This effect of tempol was due to activation of K_{ATP} channels because it was prevented by the K_{ATP} channel antago-

nist glipizide (Arun et al., 2004). The K_{ATP} channel activator, cromakalim, reduced the BP of the SHR and, when given with NE to maintain the BP, cromakalim prevented approximately 40% of the reduction in BP and HR with intravenous tempol. Likewise, blockade of the K_{ATP} channel with glipizide prevented approximately 40% of the hypotensive response to tempol (Chen et al., 2007a). These studies related the BP-lowering action of acute, intravenous doses of tempol in the SHR to activation of K_{ATP} channels. Tempol may have activated K_{ATP} channels on sympathetic neurons in this preparation because the blunting of the hypotensive action of intravenous tempol by glibenclamide was diminished in SHR pretreated with the ganglion blocking drug, hexamethonium (Chen et al., 2007a). Activation of K_{ATP} channels also can generate ROS that were blocked by tempol (Hanna et al., 2005).

Thus, although tempol can activate BK channels on VSMCs, these channels do not seem to mediate the acute hypotensive response to intravenous tempol in the SHR. In contrast, the activation of K_{ATP} channels by tempol is implicated in the acute hypotensive response in this preparation. This effect of tempol to activate K_{ATP} channels may be explained by a reduction of vascular O_2^- or perhaps an increase in vascular H_2O_2 by tempol that removes an inhibitory influence on K_{ATP} channel activity. However, this hypothesis requires further study because tempol both activates and inhibits K_{ATP} channels in different preparations.

6. Contractility. Tempol can moderate vascular contractions due to O_2^- . Thus, the addition of the oxidant drug tert-butyl hydroperoxide to the perfusate of a rat kidney or mesenteric artery increased ROS and led to vasoconstriction, which were moderated by tempol (Awe et al., 2003) or the redox-cycling spin trap, nitroblue tetrazolium but not by catalase (Ghosh et al., 2002). Because nitroblue tetrazolium reduced vascular O_2^- without the formation of H_2O_2 (Chen et al., 2007b), these observations imply that tempol can reduce vascular contractility by reducing O_2^- .

There are several examples in which the addition of tempol to the bath of blood vessels from hypertensive models reduced their sensitivity and responsiveness to agonists (Tatchum-Talom and Martin, 2004). These findings have been variously ascribed to an enhancement of the effect of NO (Shastri et al., 2002), to release of an EDHF (Kutala et al., 2006), or to prevention of the generation of an EDCF (Wang et al., 2004). The enhanced contractile responses of mesenteric vascular beds isolated from 40-week-old (aged) SD rats to NE and 5-hydroxytryptamine were normalized by 3 weeks of oral tempol (1 mmol/l) (Tatchum-Talom and Martin, 2004). Moreover, the addition of tempol to the bath of blood vessels from rats or mice with oxidative stress moderated the contractions to Ang II (Shastri et al., 2002; Wang et al., 2003b, 2004, 2006a; Hussain et al., 2006), ET-1 (Wang et al., 2003b, 2004, 2006a), U-46,619

(Schnackenberg et al., 2000; Wang et al., 2003b, 2004), arginine vasopressin (Faraci et al., 2006), serotonin (Tatchum-Talom and Martin, 2004), or PE (Wang et al., 2006b) but generally did not moderate the contractions to NE (Wang et al., 2003b, 2004, 2006a,b). The discordant effects of NE were ascribed to its activation of β_1 adrenoceptors on VSMCs that prevented vascular O_2^- generation and thereby prevented a response to tempol (Wang et al., 2006b).

7. *Cyclooxygenase, Vasoconstrictor Prostaglandins, and Thromboxanes.* The addition of nitroxides to cells cultured with pro-oxidants increased prostaglandin (PG) synthesis, perhaps by increasing production of peroxide that is required for cyclooxygenase activity (Taylor et al., 1983; Smith and Marnett, 1991; Smith et al., 1996). Superoxide activated PKC and increased the expression of cyclooxygenase-2 (COX-2) (Cosentino et al., 2003; Kiritoshi et al., 2003; Li et al., 2005a) via the formation of peroxynitrate (Eligini et al., 2001; Chen et al., 2006). Administration of tempol to rats with STZ-induced DM moderated the increased expression of COX-2 in their kidneys (Li et al., 2005a; Chen et al., 2006).

Cyclopentane isoprostanes are generated nonenzymically by interaction of O_2^- with arachidonate. Tempol reduced the excretion of 8-iso-PGF_{2 α} in the SHR (Schnackenberg and Wilcox, 1999) and many rat models of oxidative stress (Ortiz et al., 2001a; Welch et al., 2003, 2005a). Tempol inhibited the production of 8-iso-PGF_{2 α} in RAW264.7 macrophage cells, in which it prevented the activation of NF- κ B, iNOS, and the generation of NO from iNOS (Musiek et al., 2005). Isoprostanes are agonists at TP-Rs (Wang et al., 2004). Prolonged infusions of Ang II into rats or mice increased renal excretion of thromboxane B₂ (Luft et al., 1989) and 8-iso-PGF_{2 α} (Kawada et al., 2002). Similar infusions of Ang II into rabbits increased the mRNA for COX-2 but not COX-1 in renal afferent arterioles, which had enhanced contractions to the TP-R agonist U-46,619 that were prevented by bath addition of 10^{-4} M tempol (Wang et al., 2004). Thus, tempol can moderate vascular contractility in oxidative stress by reducing TP-R signaling.

Lipoxygenase-1 requires ROS for activity. It was inhibited by tempol (Jang et al., 2007).

Rat cerebral VSMCs exposed to hypoxia developed oxidative stress, a reduction in 20-hydroxyeicosatetraenoic acid (20-HETE) formation and increased BK channel currents. Both tempol and 20-HETE blocked the activation of BK channels (Gebremedhin et al., 2008). It is possible that tempol may reduce the formation of 20-HETE because blocking 20-HETE generation prevented the antihypertensive response to tempol in the DSS rat (Hoagland et al., 2003).

COX-2 is up-regulated in the blood vessels (Wang et al., 2004), glomeruli (Jaimes et al., 2005), and kidney cortex of several models of hypertension and vascular disease, including the RRM model of CKD (Wang et al., 1998), the 2K,1C model of renal artery stenosis (Mann et

al., 2001), models of DM (Komers et al., 2001; Chen et al., 2006), SHR_{SP} (Suganami et al., 2003), DSS rats (Jaimes et al., 2008), and rats with slow pressor infusions of Ang II (Wang et al., 2003b, 2004; Jaimes et al., 2005). COX-1 products maintain hypertension in early 2K,1C hypertensive rats, but this enzyme is expressed constitutively (Welch et al., 2007).

Thromboxane A₂, prostaglandin endoperoxide, and isoprostanes are vasoconstrictor PGs that activate TP-Rs. The TP-R has been implicated in the vasoconstriction and the hypertension of Ang II-infused and Goldblatt hypertensive models of hypertension and oxidative stress (Lin et al., 1991; Wilcox and Lin, 1993; Welch et al., 2007). COX requires peroxide for full activity. Thus, H₂O₂ may contribute to the increased generation of COX-1 and -2 products activating TP-Rs in models of oxidative stress. ROS also can increase the expression of COX-2 (Smith and Marnett, 1991).

Several studies have shown that up-regulation of COX-2 during high salt intake is dependent on ROS and can be prevented by tempol. DSS rats fed salt had oxidative stress, up-regulation of renal cortical COX-2, and increased PGE₂ excretion that were prevented by the administration of candesartan or tempol (Jaimes et al., 2008). The microsomal fraction of aortas from DOCA-salt hypertensive rats produced excessive ROS when stimulated by arachidonate (Adeagbo et al., 2003). This effect was mediated by COX-2 products and was prevented by 3 weeks of tempol administration (90 μ mol/kg i.p.) (Adeagbo et al., 2003). Exposure of mouse CD cells to a hypertonic NaCl solution increased the phosphorylation of ERK1/2 and p38 within 20 min and, after 16 h, increased COX-2 expression by 6-fold. These increases were accompanied by increased PGE₂ release (Yang et al., 2005). Coincubation with 2 mM TEMPO reduced the levels of COX-2 by 80%. This is an interesting model because these effects of TEMPO to reduce COX-2 were shown to depend on a reduction in ROS generated from mitochondria, rather than from NADPH oxidase.

Tempol not only reduces PG generation but also reduces the response to TP-R agonists. Thus, tempol moderated the contractions of rabbit renal afferent arterioles to prolonged stimulation with a stable TP-R agonist, U-46,619 (Schnackenberg et al., 2000), and moderated EDCF responses in renal afferent arterioles from rabbits with oxidative stress. These EDCF responses were mediated by COX-2 products activating TP-Rs on VSMCs (Wang et al., 2004). Recent studies in mesenteric resistance vessels from *EC-SOD*(-/-) mice showed enhanced contractions to ET-1 mediated by COX-1-derived vasoconstrictor PGs activating TP-Rs that were normalized by bath addition of tempol (Wang et al., 2006c).

COX-derived PGs mediate constrictor responses in blood vessels and the kidneys of many models of hypertension and oxidative stress in contrast with the vasodilator responses that are characteristic of normal animals. Prostacyclin synthase can be nitrosated and

inactivated by low concentrations of peroxynitrite (Zou et al., 1997). The recycling of the TP-R from the membrane can be interrupted by H_2O_2 (Valentin et al., 2004). These are mechanisms that could contribute to a resetting of PG action by ROS. Moreover, studies in the TP-R knockout mouse by Kawada et al. (2004) have shown that this receptor is required for the generation of oxidative stress and thereby a response to tempol in Ang II-infused mice. Thus, ROS generated in hypertensive models can enhance activation of TP-Rs and thereby enhance vasoconstriction and further ROS generation. This process can be interrupted by tempol, which may contribute to its moderation of vasoconstriction and ROS production in blood vessels from hypertensive animal models.

8. Comparison with Other Antioxidants. It is beyond the scope of this review to detail the activity of other antioxidants. However, a brief description of studies that have compared antioxidants with tempol is included below.

Nitroxides inhibited lipid peroxidation and protein carbamylation better than the commercial antioxidant chemicals butylated hydroxytoluene and butylated hydroxyanisole or the natural phenolic antioxidants α -hydroxytyrosol, tyrosol, caffeic acid, and α -tocopherol (Damiani et al., 2003).

Cu/Zn-SOD and catalase were not taken up into alveolar cells in culture, even over 24 h of incubation, unless they were covalently linked to PEG, which provided cellular entry and defense against oxidant damage (Walther et al., 1991). The half-time for this uptake was approximately 4 h. This relatively slow time course of cellular uptake may explain that whereas intravenous tempol reduced MAP maximally in the SHR within 3 min, PEG-SOD had no immediate effect on BP but reduced MAP over 110 min (Patel et al., 2006) and reduced oxidative stress over 1 week (Mügge et al., 1991). SOD that was encapsulated in liposomes (Laursen et al., 1997) or bonded to heparin (Nakazono et al., 1991) was also effective in lowering BP or parameters of oxidative stress when given over several days but was no more effective than native SOD in reducing BP when given acutely by intravenous injection (Patel et al., 2006).

Mn(III)tetrakis[1-methyl-4-pyridyl] porphyrin (MnTMPyP) was the most effective agent studied for restoring nitregic neurotransmission in the bovine retractor penis muscle during oxidative stress (Mok et al., 1998). After incubation of aortic rings with diethyldithiocarbamate (DETC) to block SOD and xanthine plus xanthine oxidase to generate O_2^- , the most effective agents in restoring EDRF/NO responses were Cu(II)-[diisopropylsalicylate]₂, MnTMPyP, tempol, and 4,5-dihydroxy-1,3-benzene-disulfonic acid (MacKenzie and Martin, 1998). MacKenzie and Martin (1998) concluded that metal-based antioxidants were more effective than spin traps. This conclusion may reflect the specific experimental conditions in which ROS were generated by chelation of

metals with DETC (MacKenzie and Martin, 1998) and should not be generalized. Indeed, in a study of rat aorta, MnTMPyP caused graded enhancement of PE-induced contractions by destruction of NO via a paradoxical increase in O_2^- generation. This effect was blocked by SOD (MacKenzie et al., 1999).

Tiron has been used widely to scavenge O_2^- . However, studies in blood vessels and in solutions demonstrated that it chelated Ca^{2+} at concentrations well below those at which it scavenged O_2^- and that this effect on Ca^{2+} was responsible for its vasorelaxant properties (Ghosh et al., 2002). Moreover, its vasodilator action in the rat superior mesenteric vascular bed was not perturbed by coadministration of tempol (100 μ mol/l), which led to the conclusion that its biological effects may not be due to scavenging of O_2^- (Ghosh et al., 2002).

MnTMPyP (Day and Crapo, 1996; Mollace et al., 2003) and EUK-134 (Baudry et al., 1993; Sharpe et al., 2002) are SOD mimetics with catalase-like activity. They had efficacy similar to that of tempol in protection against oxidative stress induced by the redox-cycling quinolone paraquat (Samai et al., 2007).

Kruglov et al. (2008) compared the efficacy of antioxidants in preventing the generation of O_2^- in permeabilized mitochondrial membranes. Tempo was almost as effective as SOD and 8-fold more effective than a triphenylphosphonium-linked TEMPO compound termed mitoTEMPO that was designed to partition into mitochondria. 2,2,5,7,8-Pentamethyl-6-chromanol and 2,6-di-*tert*-butyl-4-methylphenol, two phenolic antioxidants, and α -tocopherol (vitamin E) were almost ineffective. Luo et al. (2007) reported preliminary results from a comparative study of the effectiveness and sensitivity of 11 drugs in extinguishing O_2^- (detected by lucigenin-enhanced chemiluminescence) generated by Ang II stimulation of SHR preglomerular VSMCs (Luo et al., 2007). The catalytic antioxidants, SOD, PEG-SOD, and tempol, were the most effective followed by *N*-acetylcysteine (NAC), Mn(III)tetrakis(4-benzoic acid)porphyrin, epicachin, nitroblue tetrazolium, and ebselen. Vitamins C or E or trilox (soluble form of vitamin E) were almost ineffective. Nitroblue tetrazolium and *N*-acetylcysteine elicited a paradoxical increase in O_2^- at low concentrations. The authors concluded that cell-permeable catalytic antioxidants such as PEG-SOD or tempol are the ideal agents for cellular dismutation of O_2^- .

D. Sympatholytic Actions

Tempol can interrupt the actions of the SNS at several sites. These effects are more prominent in response to acute than prolonged administration of tempol.

1. Afferent Actions. Intraperitoneal administration of tempol (300–1200 μ mol/kg) to mice reduced nociceptive responses to intraplantar injections of phenol (Hacımuftuoğlu et al., 2006). This result probably involved a spinal action because intrathecal injections of tempol were also highly effective. Studies in the rat by Campese

and Krol (2002) disclosed an important role for renal nociceptive responses in causing hypertension. Stimulation of rat renal afferent nerves by an intrarenal injection of phenol increased NADPH oxidase activity in the hypothalamus and brainstem and increased the RSNA and the BP (Ye et al., 2006). All of these effects were abolished in rats given intracerebroventricular injections of tempol or PEG-SOD.

2. Peripheral Sympathetic Nervous System. ROS can stimulate the peripheral SNS and the release of NE (Yoshino et al., 2002). Renal ischemia for 45 min in the rat, followed by reperfusion, increased renal venous NE spillover, which was reduced by pretreatment with tempol (55–550 $\mu\text{mol/kg}$ i.v.) (Fujii et al., 2005). Xu and coworkers first demonstrated that the acute fall in BP with intravenous tempol was accompanied by a sympatholytic action in normotensive (Xu et al., 2001) and DOCA-salt hypertensive rats (Xu et al., 2002; , 2004). They noted a robust, dose-dependent, and immediate reduction in the BP after tempol in DOCA-salt rats despite the absence of any change in DHE-induced fluorescence in the aorta or vena cava dissected from these rats, indicating a maintained level of vascular O_2^- (Xu et al., 2004). Because the administration of apocynin to inhibit the p47^{phox} component of NADPH oxidase or SOD or PEG-SOD all failed to reduce the BP acutely in this model, they concluded that the BP-lowering effect of acute intravenous tempol was independent of vascular SOD-mimetic effects. The fall in BP with tempol was blocked by inhibition of the SNS but not by inhibition of NOS; thus, they concluded that the response to tempol was mediated by direct inhibition of sympathetic nerve discharge independent of NOS. However, the failure of SOD or PEG-SOD to exert an abrupt hypotensive action in this study (Xu et al., 2004) may relate to the initial retention of these large molecular weight substances within the vascular system, thus limiting their diffusion to sites around the sympathetic nerves. Consistent with this concept, Patel et al. (2006) showed that SOD and liposome-encapsulated SOD do indeed reduce the BP of anesthetized SHR to a level comparable to that produced by intravenous tempol, but whereas the effects of tempol were maximal within 1 to 3 min, the hypotensive effects of SOD and even liposomal-encapsulated SOD were delayed more than 90 min, perhaps reflecting the time for these agents to escape from the bloodstream. The failure to detect a reduction in O_2^- in the blood vessels of rats shortly after tempol administration is surprising because tempol has an almost instantaneous effect to reduce O_2^- in isolated blood vessels and cultured VSMCs (Schnackenberg et al., 2000; Chen et al., 2007b). Finally, recent studies have concluded that apocynin inhibits phagocytic (Nox-2-dependent) but not vascular (Nox-1-dependent) NADPH oxidases (Stolk et al., 1994; Vejraska et al., 2005; Ximenes et al., 2007; Heumuller et al., 2008; Touyz, 2008) and so would not be anticipated to mimic the vascular effects of tempol.

Subsequent studies have confirmed that tempol given acutely inhibits the SNS. Direct application of tempol to renal sympathetic nerves reduced their activity (Shokoji et al., 2003). This was a manifestation of SOD-mimetic activity because inhibition of SOD activity by local application of DETC to renal nerves increased their spontaneous traffic, which was reversed by local application of tempol (Shokoji et al., 2004). Because blockade of voltage-gated potassium channels by local application of 4-aminopyridine prevented the increase in renal nerve activity induced by DETC, the authors suggested that tempol activated these channels, but this effect was not studied directly (Shokoji et al., 2004).

3. Baroreflex Inhibition. Inhibition of sympathetic nerves probably underlies the paradoxical slowing of the HR with acute intravenous administration of tempol despite a sharp fall in the BP that should engage a baroreflex activation of the SNS. The observation by Shokoji et al. (2003) that the acute reduction in HR with tempol was less prominent in normotensive WKY than in hypertensive SHR suggests further that this sympatholytic action of tempol is enhanced under conditions of hypertension and that the degree of oxidative stress may set the level of SNS activity. A deficiency of NO within the rostroventrolateral medulla (RVLM) has been implicated in baroreceptor dysfunction (Mayorov, 2005). However, although blockade of nNOS in the RVLM of conscious rabbits reduced sympathetic baroreflex transmission, this was unaffected by local microinjection of tempol (Mayorov, 2005). Thus, the baroreflex inhibition that accompanies intravenous tempol probably relates to its established actions to reduce the peripheral SNS discharge or the central sympathetic drive rather than to resetting of the baroreflex itself.

4. Central Actions. Although some studies have established that tempol can reduce the activity of the SNS by direct effects on postganglionic sympathetic nerves, others have documented additional central actions to reduce the SNS activity (Tables 1 and 2, *Studies in normotensive or hypertensive rats with intracerebroventricular tempol*). Thus, central infusions of Ang II increased the MAP only in male mice that had evidence of greater ROS in the brain (Xue et al., 2007). This effect of Ang II was prevented by the central administration of tempol (Xue et al., 2007). Infusions of tempol (20 or 40 μmol) into the lateral ventricle of DSS or Dahl salt-resistant rats reduced the BP, SNS activity, and HR (Fujita et al., 2007). Salt-sensitive rats fed salt had enhanced hypothalamic NADPH oxidase activity, enhanced hypothalamic mRNA expression of p22^{phox}, p47^{phox}, and Nox-2, and an enhanced response to central tempol (Fujita et al., 2007). The central administration of tempol at doses greater than 5 $\mu\text{mol/kg}$ caused dose-dependent reductions in BP, HR, and SNS discharge in baroreceptor-denervated, anesthetized rats (Lu et al., 2004). Campese et al. (2004) infused approximately 4 μmol of tempol over 1 h into the lateral cerebral ventri-

cle of SD rats. This infusion reduced MAP, HR, RSNA, and hypothalamic NE secretion, thereby demonstrating a central action of tempol to inhibit the SNS in normotensive rats. The authors contrasted this finding with the effects of intravenous infusions of a high dose of 20 μmol of tempol over 1 h, which also decreased MAP but increased HR, hypothalamic NE secretion, and RSNA. Although sinoaortic denervation and cervical vagotomy blunted the effects of intravenous tempol to reduce hypothalamic NE secretion, the reduction in BP after intracerebroventricular administration of tempol remained largely intact and was accompanied by an increase in HR. The authors concluded that tempol had contrasting actions: central effects to reduce SNS discharge and BP and peripheral effects to reduce the BP but reflexly activate the SNS. It is unclear why acute intravenous administration of tempol in this study increased SNS discharge and HR in contrast to the previously discussed examples in which the acute intravenous administration of tempol elicited the opposite effects.

The RVLM and the paraventricular nucleus (PVN) are important brainstem sites for regulation of the SNS. Both are responsive to local tempol microinjection. Microinjection of tempol into the RVLM attenuated pressor responses to local Ang II and attenuated the accompanying phosphorylation of ERK1 and 2 but not the phosphorylation of stress-activated protein kinase/Jun N-terminal kinase (Chan et al., 2005). Infusions of tempol (10–100 pmol) over 1 min into the RVLM of SHR_{SP} caused graded decreases in MAP and HR (Kishi et al., 2004). Consistent with this result was the observation by Kimura et al. (2005b) that overexpression of Mn-SOD in the RVLM of SHR_{SP} decreased MAP and SNS activity and that central infusions of tempol at 0.5 $\mu\text{mol}/\text{h}$ for 1 week attenuated the hypertension in rats with cerebral oxidative stress induced by overexpression of iNOS in the RVLM. Microinjection of tempol (200 nmol) or tiron (10 nmol) into the PVN of anesthetized rats blocked the reflex increase in RSNA after epicardial bradykinin injection and blocked the increase in RSNA and MAP accompanying injection of Ang II into the PVN (Han et al., 2005).

Ang II acting on AT₁ receptors has important effects within the brain to activate the SNS and raise BP, which are targets for tempol. Zimmerman et al. (2004) reported that injection of an adenovirus expressing Cu/Zn-SOD into the subfornical organ of the hindbrain of rats blunted the rise in MAP produced by a 2-week infusion of Ang II at a slow pressor rate. Ang II increased the rate of firing of neuronal cells cultured from the hypothalamus and brainstem by inhibiting the delayed rectifier potassium current (I_{KV}) (Sun et al., 2005). There were accompanying increases in neuronal cell ROS and NADPH oxidase activity that were blocked by gp91ds-tat, which inhibits NADPH oxidase/Nox-2, or by tempol (Sun et al., 2005).

Because Ang II activates NADPH oxidase (Chabrashvili et al., 2003; Wang et al., 2004), it is likely that increased O₂⁻ formation by Ang II caused the increase in SNS activity. Indeed, an intracerebroventricular injection of a relatively large dose of tempol (75 $\mu\text{mol}/\text{kg}$) prevented the increase in SNS discharge and BP after intracerebroventricular Ang II (Lu et al., 2004). These central effects of tempol reduced brain levels of markers of ROS and were specific for Ang II because intracerebroventricular tempol did not prevent the increased SNS discharge after acute heat stress (Lu et al., 2004). Moreover, the microinjection of tempol (20 nmol) into the rostroventral medulla of the rabbit reduced the hypertensive response to microinjection of Ang II but not glutamate (Mayorov et al., 2004) and reduced the hypertension and tachycardia with air-jet stress (De Matteo et al., 2006), whereas 3-CP, a nitroxide with little SOD-mimetic activity in vivo (Adler et al., 2003; Patel et al., 2006), was not effective.

Adrenomedullin is an endogenous antioxidant peptide. Fujita et al. (2005) reported that adrenomedullin knockout mice develop an exaggerated increase in BP and RSNA when fed a high-salt diet and infused intracerebroventricularly with hypertonic saline. These effects were prevented by intracerebroventricular tempol, which also prevented the NaCl-induced increase in brain O₂⁻. This result suggests that the effect of tempol to correct salt sensitivity could entail a central action (Meng et al., 2003; Kopkan and Majid, 2005; Welch et al., 2005b; Banday et al., 2007d).

In contrast to these studies that have documented the BP-lowering effects of centrally administered tempol in the rat, Patel et al. (2006) reported that whereas acute intravenous injection of tempol into anesthetized SHR elicited graded reduction in BP and HR, intracerebroventricular injections of 0.85 to 13.5 $\mu\text{mol}/\text{kg}$ (up to 5% of the effective intravenous dose) had no antihypertensive effect. As a positive control, these authors showed that intracerebroventricular injections of Ang II raised the BP in this model. Likewise, Shokoji et al. (2003) reported that intracerebroventricular doses of tempol up to approximately 1.7 μmol over 1 min did not alter MAP or RSNA of anesthetized SHR or WKY. Kagiya et al. (2000) infused tempol intracerebroventricularly at 0.55 $\mu\text{mol}/\text{h}$ into 12-week-old SHR and reported no reduction in MAP over 2 weeks.

Thus, although SOD and tempol reduced the BP by central actions in many studies, these effects have been inconsistent. This inconsistency may relate to insufficient passage of tempol from the lateral ventricle to brain sites that activate the SNS in the rostroventral medulla and RVLM wherein local application of tempol has been more effective. However, it remains unclear to what extent central effects of tempol contribute to its antihypertensive action. Because prolonged infusion of tempol lowers the BP in conscious SHR without changes in catecholamines or HR (Welch et al., 2005b), it is likely that central effects on the SNS are not a prominent part

of the antihypertensive response to prolonged tempol, at least in the SHR model.

E. Renal Actions

Prolonged administration of tempol has multiple effects on the kidney, which could contribute to its antihypertensive action.

1. *Renal Hemodynamics and Autoregulation.* Tempol can increase the RBF in models of oxidative stress by enhancing the renal actions of NO (Majid and Kopkan, 2007). Tempol increased tissue levels of NO in the renal medulla of rats infused with Ang II, as detected with an NO-sensitive electrode (Badzyńska et al., 2004) or by 3-amino-4-aminomethyl-2',7'-difluorescein fluorescence studies of isolated, perfused vasa recta capillaries (Zhang et al., 2005). Zinc deficiency increased RVR in rats, perhaps because it limited the activity of Cu/Zn-SOD. Tempol led to a steep reduction in RVR in this model (Kurihara et al., 2002). However, the effect of tempol to improve renal EDRF/NO responses has been dissociated from a reduction in RVR in a model of experimental atherosclerotic renovascular disease in swine (Chade et al., 2004).

Tempol (216 $\mu\text{mol/kg}$ i.v.) infused into rats during an Ang II infusion reduced the MAP, but did not change the RBF, implying that it had reduced the RVR (Nishiyama et al., 2001). This result may be more than a manifestation of an autoregulatory response to a fall in BP because, in other studies, a direct intrarenal arterial infusion of tempol into Ang II-infused rats increased the cortical, medullary, and total RBF and increased the GFR, urine flow, and Na^+ excretion without changing the BP (Kopkan et al., 2006).

Tempol also can increase RBF by NO-independent means. Oral tempol normalized the increased RVR and the exaggerated increase in RVR produced by an infusion of Ang II into SHR kidneys despite the administration of L-NAME (de Richelieu et al., 2005). Indeed, the acute effect of tempol to moderate renal vasoconstriction with Ang II was enhanced after NOS inhibition perhaps because of NOS-inhibition enhanced ROS generation (Just et al., 2007). The GFR and the RBF in models of DM were increased by tempol despite blockade of NOS (Brands et al., 2004).

Tempol has produced prominent renal vasodilation in Ang II-dependent models of hypertension. Guron et al. (2006) reported a sharp reduction in RVR by intravenous tempol (200 $\mu\text{mol} \cdot \text{kg}^{-1} \cdot \text{h}^{-1}$) in the clipped and contralateral kidneys of rats with early (3-week) 2K,1C Goldblatt hypertension. Tempol increased the GFR and the RBF of the clipped kidney, despite a fall in MAP (Guron et al., 2006). A 2-week administration of tempol (200 $\text{nmol} \cdot \text{kg}^{-1} \cdot \text{min}^{-1}$ s.c.) or an ARB to early 2K,1C rats moderated hypertension. However, only tempol increased the GFR and reduced the RVR of the clipped kidney (Welch et al., 2003).

Tempol has increased blood flow to the renal medulla more than to the renal cortex in several models. Interstitial infusion of tempol (30 $\mu\text{mol} \cdot \text{kg}^{-1} \cdot \text{min}^{-1}$) into the renal medulla increased medullary blood flow and sodium excretion by NOS-independent means (Zou et al., 2001; Chen et al., 2003). Oral tempol (1 mmol/l in the water for 4 weeks) reduced the BP and increased the medullary but not the cortical or total RBF of fructose-fed rats (Onuma and Nakanishi, 2004). Oral tempol (1 mmol/l over 4 days or 7 weeks) reduced the MAP of SHR by 20 mm Hg yet increased the medullary blood flow by 35 to 50% without changing cortical blood flow or total RBF (Feng et al., 2001). Thus, tempol produces pronounced vasodilation of medullary blood vessels in the rat (Feng et al., 2001; Onuma and Nakanishi, 2004). An increase in medullary blood flow in hypertensive models can contribute to natriuresis and a reduction in BP (Cowley et al., 2003).

An intrarenal infusion of tempol increased RBF in denervated kidneys from salt-depleted dogs (Dutta et al., 2006). This result indicates that although tempol can reduce RSNA (Xu et al., 2002, 2004), a reduction in RSNA is not required for tempol to reduce RVR.

Tempol given over 2 weeks to SHR, DSS, or Ang II-infused rats increased the GFR (Schnackenberg and Wilcox, 1999; Hoagland et al., 2003; Just et al., 2007). Presently, there are no studies of the effects of tempol on glomerular capillary pressure to assess the hemodynamic mechanism of this effect.

Impaired renal autoregulation and glomerular hypertension in CKD predisposes to progressive kidney damage (Kotchen et al., 2000; Bidani and Griffin, 2004). Renal damage is accompanied by an increase in the circulating levels and glomerular expression of transforming growth factor β (Sharma et al., 2005). Infusion of transforming growth factor β into rats increased ROS generation in renal blood vessels and prevented afferent arteriolar constrictor responses to increased renal perfusion pressure (autoregulation). Tempol or apocynin enhanced autoregulation in this model (Sharma et al., 2005). This finding is interesting because tempol impaired renal afferent arteriolar contractions to agonist drugs in several models of oxidative stress (Wang et al., 2003b, 2004) and impaired vasoconstriction during activation of the tubuloglomerular feedback (TGF) response (Welch and Wilcox, 2001) yet enhanced myogenic contractions to increased stretch in this model. On the other hand, oral tempol (1 mmol/l) given to SHR over 4 days to 7 weeks did not alter autoregulation (Feng et al., 2001) but blocked the enhanced autoregulation of RBF produced by Ang II in isolated perfused kidneys (Guan et al., 2003). Reports from studies in which tempol preserved or improved RBF, despite a fall in BP, are consistent with the conclusion that tempol preserved or enhanced renal autoregulation (Kawada et al., 2002; Welch et al., 2005b).

2. Afferent Arteriole and Tubuloglomerular Feedback Response. Schnackenberg et al. (2000) reported that isolated, perfused renal afferent arterioles dissected from normal rabbits developed strong contractions when incubated for 20 to 30 min with the TP-R-mimetic U-46,619. These contractions were moderated by the addition of 10^{-3} M tempol to the bath. The addition of tempol to the bath had no effect on the immediate contractions to Ang II or U-46,619 but moderated the contractions of arterioles incubated with these agonists for 20 to 30 min (Wang et al., 2003b). Chen et al. (2007b) demonstrated that it takes some minutes for Ang II or U-46,619 to increase O_2^- in rat mesenteric resistance vessels *ex vivo* or mouse cremasteric vessels *in vivo*, which may explain why normal vessels have to be incubated with Ang II or U-46,619 for some time before tempol becomes effective in moderating contractile responses. In contrast to these effects in vessels from normal animals, Wang et al. (2003b, 2004) have shown that tempol moderated the immediate contractions produced by Ang II, U-46,619, and ET-1 in afferent arterioles isolated from rabbits infused with slow pressor doses of Ang II for 2 weeks. The Ang II infusion had up-regulated the expression of p22^{phox} in the afferent arterioles (Wang et al., 2004) and the kidneys (Chabrashvili et al., 2003) and increased the renal cortical NADPH oxidase activity (Wang et al., 2003b). Apparently, the slow pressor infusion of Ang II had induced the machinery for O_2^- generation in the renal afferent arteriole, thereby creating the conditions for an abrupt increase in ROS when agonists were added to these arterioles, which now became responsive to the moderating effects of tempol.

The replacement of an EDRF/NO response in normal mesenteric and renal afferent arterioles (Wang et al., 2003a, 2006a) by an EDCF response in vessels from Ang II-infused rodents (Wang et al., 2003b, 2006a) enhanced their contractility to Ang II, ET-1, and TP-R activation. These effects were moderated by bath addition of tempol. Likewise, Ozawa et al. (2004) reported impaired EDRF/NO and EDHF responses of renal afferent arterioles from DSS rats fed a high-salt diet that were restored by oral tempol over 10 weeks or after acute bath addition of tempol to the vessel.

Guyton's model of body fluid and BP homeostasis predicts that the level of BP is sensed in the kidneys wherein appropriate changes in salt and fluid excretion stabilize the pressure despite perturbations caused by vasoconstriction or salt intake (Guyton et al., 1995). The pressure sensed within the kidney must represent the integrated effects of the perfusion pressure and the preglomerular tone that regulates the transmission of this pressure into the kidney. Thus, a reduction in renal afferent arteriolar vasoconstriction by tempol in hypertensive models should permit better transmission of pressure into the kidneys and thereby restore a normotensive set point for the regulation of BP, which should lead to a lowering of BP. However, set against this

result, is the finding that tempol can restore renal autoregulation in some models of hypertension (Sharma et al., 2005). This ability to enhance afferent arteriolar vasoconstriction during increased perfusion pressure should limit the transmission of the arterial pressure into the kidneys. A study of the effects of tempol on glomerular capillary pressure at different levels of perfusion pressure would be helpful in resolving these apparent contradictions.

NaCl delivery and reabsorption at the macula densa segment elicits an increase in renal afferent arteriolar tone mediated by the TGF response. The same signal also inhibits renin secretion. nNOS is heavily expressed in the macula densa cells. Generation of NO by nNOS in macula densa cells blunted TGF responses during NaCl reabsorption (Wilcox et al., 1992). Tempol (10^{-4} M) dampened TGF responses when perfused through the loop of Henle of normotensive Sprague-Dawley rats (Wilcox and Welch, 2000) and especially SHR (Welch and Wilcox, 2001) in which NADPH oxidase components were overexpressed in macula densa cells (Chabrashvili et al., 2002). Welch and Wilcox (2001) demonstrated further that the local microperfusion of tempol into the interstitium of the juxtaglomerular apparatus of the SHR blunted the TGF responses in adjacent nephrons. This effect was attributed to a restoration by tempol of NO signaling in the juxtaglomerular apparatus because a local interstitial infusion of tempol to this region restored the enhanced TGF response to microperfusion of the neuronal NOS inhibitor, 7-nitroindazole, into the macula densa segment, implying that tempol had restored the blunting of the TGF response by NO derived from nNOS. Further experiments were conducted in SHR given the ARB, candesartan, or equally antihypertensive therapy with hydralazine, hydrochlorothiazide, and reserpine for 2 weeks. Only candesartan prevented a TGF response to tempol microperfused into the interstitium and restored a TGF response to 7-nitroindazole microperfused into the macula densa segment (Welch and Wilcox, 2001). The authors concluded that tempol had reversed oxidative stress and restored local NO signaling in the juxtaglomerular apparatus of the SHR and that the oxidative stress was caused by prolonged AT_1 -receptor activation. This ability of tempol to blunt TGF responses was confirmed by Ichihara et al. (2001) in the juxtamedullary nephron preparation.

Microperfusion of tempol (10^{-4} M) via the tubular lumen of the macula densa segment or the addition of tempol to the bath of a perfused juxtaglomerular apparatus dissected from rabbit kidneys blunted the TGF responses (Ren et al., 2002). These effects of tempol were ascribed to actions within the macula densa cells rather than the afferent arterioles because perfusion of tempol via the lumen of the afferent arteriole was not effective. Furthermore, they were ascribed to an action of tempol to reduce O_2^- within the macula densa cells because the addition of the impermeable SOD to the bath was not

effective. Blockade of neuronal NOS in macula densa cells prevented the blunting of TGF by bath addition of tempol, thereby relating the effect of tempol to nNOS in the macula densa. The authors concluded that tempol preserved the effect of NO derived from nNOS to inhibit the luminal solute entry into macula densa cells via the $\text{Na}^+/\text{K}^+/\text{2Cl}^-$ transporter. NO generated within the macula densa inhibited solute transport by inactivating the $\text{Na}^+/\text{K}^+/\text{2Cl}^-$ transporter. Solute reabsorption by this pathway is the signal for activation of the TGF response (Ren et al., 2002). These conclusions were supported by the direct observation that tempol (10^{-4} M) blocked the increase in O_2^- , as detected by DHE fluorescence in the macula densa segment during luminal perfusion of NaCl (Liu et al., 2007a). This finding led to an intriguing hypothesis that NaCl reabsorption by macula densa cells enhanced O_2^- generation, which impaired NO bioactivity, thereby facilitating further NaCl reabsorption via the $\text{Na}^+/\text{K}^+/\text{2Cl}^-$ luminal transporter. Such a response would generate a strong signal to activate the TGF response that could be interrupted by tempol acting within macula densa cells. Later studies showed that tempol also can blunt TGF by moderating afferent arteriolar contractions in response to macula densa activation (Liu et al., 2004). These dual effects of tempol to moderate the TGF response via reducing the signal in the macula densa and reducing the response in the afferent arteriole could contribute to renal vasodilation and to a fall in BP.

3. Glomerulus and Podocyte. Glomerular podocytes are prominent sites for the expression of NADPH oxidase components whose expression was enhanced in the SHR (Chabrashvili et al., 2002) and a model of type I DM (Asaba et al., 2005, 2007; Tojo et al., 2007). Blockade of mineralocorticosteroid receptors with eplerenone or the administration of tempol to uninephrectomized rats infused with aldosterone and fed an 8% NaCl diet blocked the development of hypertension, proteinuria, oxidative stress, podocyte damage, and up-regulation of the aldosterone effector kinase-1 in glomerular podocytes (Shibata et al., 2007). Tempol was as effective as a mineralocorticosteroid antagonist in interrupting aldosterone signaling in podocytes. The effects of tempol on the glomerular podocytes and mesangial cells (Kwan et al., 2005) in models of oxidative stress might contribute to a reduction in proteinuria or glomerular damage.

4. Salt and Fluid Reabsorption and Excretion and Salt Sensitivity. Tempol increased Na^+ excretion in rats with oxidative stress due to infusion of Ang II (López et al., 2003). Some studies have related changes in Na^+ reabsorption with tempol to facilitation of NO-dependent actions. Thus, tempol blocked the effect of an intra-arterial infusion of xanthine plus xanthine oxidase to increase Na^+/K^+ -ATPase activity in the renal medulla. This effect was dependent on NOS and cGMP and was specific because tempol did not affect cortical Na^+/K^+ -ATPase or H^+/K^+ -ATPase (Bełtowski et al., 2004).

Studies by Majid and Navar (2001) have demonstrated the NOS-independent effects of tempol. Thus, blockade of NOS actually enhanced the effect of tempol to reduce the Na^+ reabsorption in the kidneys of anesthetized dogs. Tempol ($3 \mu\text{mol} \cdot \text{kg}^{-1} \cdot \text{min}^{-1}$) infused intrarenally into anesthetized dogs did not change renal hemodynamics or fluid excretion, but after blockade of NOS tempol increased urine flow and Na^+ excretion (Majid et al., 2004) and moderated the fall in Na^+ excretion during Ang II infusion (Majid et al., 2005). The authors proposed that NOS blockade enhanced renal ROS generation and thereby enhanced the renal response to tempol.

On the other hand, Sainz et al. (2005) failed to detect an effect of tempol on diuresis, natriuresis, kaliuresis, proteinuria, or creatinine clearance in rats made hypertensive with L-NAME. However, a preserved rate of Na^+ excretion, despite lower BP in the rats given tempol, led these authors to conclude that tempol had reset the pressure natriuresis, which contributed to the fall in BP.

Garvin, Ortiz, Stoos, and coworkers found that NO blocked luminal Na^+ uptake into isolated, perfused thick ascending limb (TAL) segments of the loop of Henle (García et al., 1999; Garvin and Hong, 1999; Ortiz and Garvin, 2001; Ortiz et al., 2001b; Herrera et al., 2006) and the CD (Stoos et al., 1992, 1994, 1995). In contrast, NO can enhance Na^+ and fluid reabsorption in the proximal tubule of the rat and mouse (Wang, 1997, 2000, 2002; Wang et al., 2000; Wu and Johns, 2004). However, the effects of NO on proximal reabsorption are controversial (Wilcox, 2000).

The effects of tempol on tubular reabsorption have been studied at several nephron sites. Although the effects of tempol on the proximal tubule have not been studied directly, Wu and Johns (2004) reported that luminal perfusion of SOD into the proximal tubule of the SHR increased fluid reabsorption. In contrast, Banday, Lokhandwala, Josè, and coworkers have shown that tempol restored proximal tubule dopamine D1 receptor signaling in hypertensive models. This effect was predicted to reduce proximal reabsorption via a cAMP-dependent mechanism (see section II.E.6) (Bek et al., 2001; Asghar and Lokhandwala, 2004; Banday et al., 2005; Fardoun et al., 2006; Felder and Jose, 2006; Marwaha and Lokhandwala, 2006; Yang et al., 2006; Banday et al., 2007a,b). Direct studies of proximal tubular fluid reabsorption are required to settle this controversy.

The addition of tempol ($50 \mu\text{mol/l}$) to isolated, perfused TAL segments increased the release of NO in response to L-arginine and inhibited Cl^- reabsorption (Ortiz and Garvin, 2002a). This result was ascribed to a reduction in O_2^- rather than to an increase in H_2O_2 , because H_2O_2 did not affect Cl^- reabsorption from this segment (Ortiz and Garvin, 2002b). Ortiz and Garvin (2002a) demonstrated a negative interaction between NO and O_2^- on tubular reabsorption from isolated TAL segments. An increase in tubular fluid reabsorption via

the $\text{Na}^+/\text{K}^+/\text{2Cl}^-$ luminal transporter in the perfused TAL segments increased tubular O_2^- , as assessed by DHE fluorescence (Hong and Garvin, 2007). The increase in O_2^- was prevented by luminal perfusion of tempol. Tempol inhibited Cl^- reabsorption in the isolated perfused TAL segments of SD rats by promoting the inhibitory action of NO on luminal Na^+ entry (Ortiz and Garvin, 2002a). This effect of tempol was mediated by the combined effects of blocking the luminal $\text{Na}^+/\text{K}^+/\text{2Cl}^-$ cotransporter (Ortiz and Garvin, 2002b; Juncos and Garvin, 2005), blocking the Na^+/H^+ exchange (Juncos et al., 2006), and blocking Na^+/K^+ -ATPase (Varela et al., 2004).

Juncos et al. (2006) studied the effects of ROS on Na^+/H^+ exchange in isolated perfused TAL segments dissected from rat kidneys (Juncos et al., 2006). Generation of O_2^- by xanthine plus xanthine oxidase doubled luminal Na^+/H^+ exchange but reduced basolateral Na^+/H^+ exchange. Tempol prevented both effects. The authors proposed that a primary effect of O_2^- to reduce basolateral Na^+/H^+ exchange increased intracellular $[\text{H}^+]$ and thereby stimulated luminal Na^+/H^+ exchange and NaHCO_3 absorption. Tempol inhibited this process and thereby inhibited Na^+ and HCO_3^- reabsorption.

An increase in luminal flow or an increase in tubular Na^+ absorption increased O_2^- in perfused TAL segments (Garvin and Hong, 2008). These effects were prevented by luminal tempol (Garvin and Hong, 2008). These authors demonstrated an important regulatory role for ROS in the TAL. An increase in tubular fluid flow enhanced tubular reabsorption, which increased O_2^- and facilitated luminal NaCl uptake and Na^+/K^+ ATPase activity. This process was interrupted by tempol, which therefore should prevent Na^+ reabsorption during high flow states. Recently, Garvin and Hong (2008) have controlled for the effects of Na^+ reabsorption to increase ROS by using a Na^+ -free perfusate and controlled for the effects of flow by obstructing the tubule. They demonstrated that stretch per se enhanced O_2^- production in TAL segments. This was prevented by luminal tempol. The authors proposed that an increase in tubular stretch accompanying an increase in tubular fluid filtration and nephron flow that can occur with hypertension, salt loading, or DM may contribute to O_2^- generation in the TAL. This generation would be anticipated to enhance tubular NaCl reabsorption and could thereby contribute to salt sensitivity and an increase in BP. Therefore, tempol can interrupt an increase in O_2^- generation in the nephron whether caused by increased tubular transport, luminal flow, or stretch. This effect could be an important component of the action of tempol to prevent salt sensitivity or hypertension.

Tempol has been found to reduce the open probability of the epithelial sodium channel (ENaC) in aldosterone-stimulated distal nephron cells (Yu et al., 2007). Reduced activity of ENaC is anticipated to reduce the luminal membrane potential and to reduce tubular K^+

secretion. However, this hypothesis is not consistent with the finding that 7 days of tempol administration to rats fed a low- K^+ diet prevented renal O_2^- generation and increased renal K^+ excretion (Babilonia et al., 2005). This kaliuretic response to tempol may be related to the observation that tempol prevented the phosphorylation and inactivation of the ROMK (Kir 1.1) channel in the CDs of rats with oxidative stress (Babilonia et al., 2006). Activation of luminal ROMK channels in the CDs by tempol would be anticipated to enhance tubular K^+ secretion. Further work is required to establish more clearly the effects of tempol on renal potassium excretion and K^+ transport in the collecting ducts.

Thus, a prominent effect of tempol is to reduce luminal Na^+ entry and thereby reduce Na^+ reabsorption in the TAL and CDs. However, the anticipated increases in net Na^+ and fluid excretion are not prominent with tempol. This might relate to opposite effects of tempol on reabsorption from the proximal tubule but has yet to be studied directly.

Guyton's theory predicts that the BP will rise with salt intake ("salt-sensitivity") if tubular NaCl reabsorption is not reduced appropriately by the high salt intake at a nephron segment at or beyond the macula densa (Guyton et al., 1995; Guyton and Coleman, 1999). Ongoing NaCl reabsorption in the distal nephron would impair the efficient elimination of the salt load and lead to increased blood volume, venous return, and cardiac output. After time, whole-body autoregulation would dictate a rise in peripheral resistance that would sustain the rise in BP during the high salt intake (Hinojosa-Laborde et al., 1992). Salt-sensitive hypertension develops if there is a failure to adjust nephron reabsorption or peripheral resistance appropriately to changes in dietary salt. A high-salt diet increased oxidative stress in the kidneys of the rat (Kitiyakara et al., 2003). This increase was accompanied by up-regulation of p47^{phox} and Nox-1, down-regulation of Cu/Zn-SOD, and increased activity of NADPH oxidase in the kidney cortex (Kitiyakara et al., 2003). ROS have been implicated in causing salt-sensitive hypertension (Manning et al., 2003; Welch et al., 2005b). The ability of tempol to reduce luminal entry of Na^+ into the TAL and to reduce ENaC activity in the CDs should combat salt sensitivity of BP. These effects have been studied in several models.

The administration of BSO to rats to deplete glutathione caused marked oxidative stress and salt-sensitive hypertension. Oral administration of tempol (1 mmol/l for 12 days) prevented the hypertension, oxidative stress, and endothelial dysfunction in this model (Banday et al., 2007d).

Tempol (50 μM) or apocynin restored both defective endothelial signaling and NO activity in mesenteric resistance vessels from rats fed a high-salt diet for 3 days. The high salt intake had provoked an increase in vascular O_2^- , as assessed from DHE fluorescence (Zhu et al., 2007).

Welch et al. (2005b) reported that a high salt intake led to a greater fall in MAP during administration of tempol to SHR (Welch et al., 2005b). This was not due to a natriuretic action of tempol because the cumulative balance for Na^+ and the body weight of the SHR were not perturbed. These observations imply that tempol had corrected the salt sensitivity in this model. Meng et al. (2003) reported that tempol prevented the salt-induced increase in BP in DSS rats, whereas Kopkan and Majid (2005) reported that tempol prevented the salt-induced increase in BP of rats given L-NAME. Thus, tempol corrects salt sensitivity independent of NOS, consistent with its natriuretic actions in the dog that also are independent of NOS (Majid et al., 2004, 2005; Majid and Kopkan, 2007). The observation in these studies that a high salt intake potentiated the reduction in BP with tempol contrasts with the response to all other antihypertensive agents whose effects are reduced during increases in dietary salt (Cappuccio, 2008).

5. Renin-Angiotensin-Aldosterone System. Navar, Nishiyama, and coworkers reported that 4 weeks of oral administration of tempol to DSS rats fed a high-salt diet prevented an increase in intrarenal angiotensinogen, whereas an equally antihypertensive dose of hydralazine was not effective (Kobori et al., 2003). Because intrarenal angiotensinogen correlated with the levels of Ang II in the renal tissues (Kobori et al., 2006), they concluded that tempol prevented Ang II generation in the kidneys in this salt-sensitive model. Indeed, direct measurement by Bayorh et al. (2006) have confirmed that 3 weeks of oral tempol administration to hypertensive DSS rats reduced the tissue levels of Ang II in the kidneys, but not in the heart. An effect of tempol to reduce renal tissue levels of Ang II could contribute to an NO-independent reduction in Na^+ reabsorption (Majid and Nishiyama, 2002). However, Welch et al. (2005b) reported that prolonged oral administration of tempol to SHR increased the PRA. The functional significance of an increase in circulating renin-angiotensin-aldosterone components is not clear because tempol prevented many of the effects of an activated renin-angiotensin system, including the ability of Ang II to raise BP (Ortiz et al., 2001a; Kawada et al., 2002; Dikalova et al., 2005; Hattori et al., 2005; Welch et al., 2005a) and RVR (Nishiyama et al., 2001; Kawada et al., 2002; Welch et al., 2005a) and to constrict renal afferent arterioles (Wang et al., 2003b, 2004).

6. Dopamine Receptor Signaling. D1-like receptors include the dopamine type 1 and 5 receptors whose activation moderated ROS and reduced the NaCl and fluid reabsorption in the proximal tubule, reduced the RSNA, and reduced the renal expression of AT_1 -Rs. These effects could have contributed to a fall in BP with dopamine infusion (Hollon et al., 2002; Zeng et al., 2005; Felder and Jose, 2006; Yang et al., 2006).

Tempol prevented the down-regulation and hyperphosphorylation of dopamine D1 receptors in the proxi-

mal tubules of rats with oxidative stress (Fardoun et al., 2006). Banday, Lokhandwala, and coworkers evaluated the effects of oral tempol (1 mmol/l for 2 weeks) in obese Zucker rats that had hypertension, hyperglycemia, and hyperinsulinemia, increased renal oxidative stress, and increased PKC activity in the proximal tubules (Banday et al., 2005), which inactivated the D1 receptor (Banday et al., 2007b). Tempol improved each of these defects, thereby restoring D1 receptor signaling and a natriuretic response to a D1 receptor agonist. These authors also reported that prolonged tempol administration to diabetic rats (Marwaha and Lokhandwala, 2006) or elderly Fischer 344 rats (Asghar and Lokhandwala, 2004, 2006) corrected renal lipid peroxidation and moderated hyperglycemia (Banday et al., 2007b). They proposed that tempol both normalized MAPK in renal proximal tubules and prevented D1-R inactivation, thereby restoring D1-R G-protein coupling and signaling via adenylate cyclase. These restorative effects of tempol on D1-R signaling in the proximal tubule are predicted to reduce proximal reabsorption of NaCl and fluid and moderate hypertension (Bek et al., 2001).

DR signaling has also been implicated in moderating oxidative stress. Activation of vascular D1-like receptors inhibited oxidative stress in VSMCs provoked by platelet-derived growth factor (Yasunari et al., 2000). The D5R knockout mouse had enhanced NADPH oxidase activity in proximal tubules and hypertension, both of which were reversed by tempol (Yang et al., 2006).

Thus, signaling via the D1- and D5-R in renal proximal tubules is reduced by oxidative stress and can itself reduce the generation of O_2^- . Correction of D1R and D5-R signaling in the proximal tubule by tempol may contribute to an antioxidant and natriuretic action.

7. Adenosine. Adenosine generated within the kidneys during Ang II infusion enhanced renal vasoconstriction and tubular NaCl reabsorption via activation of adenosine type 1 receptors (A_1 -Rs) (Welch, 2002). Activation of A_1 -Rs constricted the renal afferent arteriole, enhanced proximal tubule Na^+ and fluid reabsorption, activated the TGF response, and inhibited renin secretion (Welch, 2002). Adenosine released within the kidneys may contribute to the renal effects of ROS. Thus, O_2^- generation in renal tissue homogenates increased the maximal velocity of the adenosine-generating enzyme, 5'-nucleotidase, and doubled the release of adenosine (Chen et al., 2001). Moreover, blockade of SOD with DETC caused oxidative stress and renal vasoconstriction. These effects were mediated by adenosine and prevented by tempol (Chen et al., 2001). Interestingly, the increase in tissue concentrations of adenosine in kidneys of rats given DETC or subjected to ischemia and reperfusion were blocked by infusion of tempol ($30 \mu\text{mol} \cdot \text{kg}^{-1} \cdot \text{min}^{-1}$) (Chen et al., 2001). Long et al. (2007) reported that rats infused with Ang II for 2 weeks had an increased level of renal cortical interstitial adenosine that was corrected by coinfusion of tempol. Thus, tempol

blocks renal adenosine generation during oxidative stress. Because A_1 -Rs enhance proximal tubular Na^+ and fluid reabsorption and enhance renal vasoconstriction, a reduction in renal adenosine by tempol could contribute to natriuresis, vasodilation, and a fall in BP.

8. Renal and Systemic Oxygenation and Hypoxia-Inducible Factor. Welch and coworkers reported that a 2-week administration of tempol to rats corrected the reduced renal cortical pO_2 and the reduced tubular Na^+ transport per O_2 used (T_{Na^+}/Q_{O_2}) in the kidneys of rats with oxidative stress caused by a slow pressor infusion of Ang II (Welch et al., 2005a) or in the clipped kidneys of the early 2K,1C rat model of Goldblatt hypertension (Welch et al., 2003). The administration of tempol also increased the pO_2 of the renal cortex and the renal medulla of the SHR but not the WKY kidney when studied noninvasively by blood oxygen level-dependent MRI (Li et al., 2005b). The administration of bradykinin to the rat kidney increased NO generation and reduced O_2 usage. This effect of bradykinin was blunted in the SHR model of oxidative stress but was restored to levels of WKY kidneys by tempol (Adler and Huang, 2002).

The mechanisms whereby tempol improves renal oxygenation have not yet been established. They entail a direct renal action because tempol suppressed agonist-stimulated O_2 consumption in rat renal cortical homogenates and prevented increased O_2 consumption in the renal tissues from elderly Fisher 344 rats (Adler et al., 2004). Because the proximal tubule is the major site for Na^+ reabsorption and O_2 usage and the changes in T_{Na^+}/Q_{O_2} in the kidneys with tempol are profound, it is likely that tempol affects the energetics of proximal transport. The improvement in renal oxygenation with tempol could represent an increase in NO bioavailability because NOS blockade reduced renal cortical pO_2 and the T_{Na^+}/Q_{O_2} (Adler et al., 2001, 2004; Adler and Huang, 2002). Because NO competes with O_2 in the mitochondrial respiratory chain, an increase in bioactive NO with tempol should reduce mitochondrial O_2 usage (Wolin et al., 1999; Nisoli et al., 2007). Adler and Huang (2002) reported that bradykinin or enalaprilat administered to anesthetized rats stimulated endogenous NO and decreased renal O_2 consumption. The responses to these agents were impaired in the kidneys of SHR but were restored by inhibition of NADPH oxidase with apocynin or AT_1 -Rs with an ARB (Adler and Huang, 2004). They suggested that activation of AT_1 -R in the kidneys enhanced renal cortical ROS production via NADPH oxidase, limited bioactive NO, and thereby impaired renal O_2 usage. These results are consistent with the finding that the oral administration of tempol prevented the fall in renal cortical pO_2 and in T_{Na^+}/Q_{O_2} in the kidneys of rats infused with Ang II (Welch et al., 2005a).

The protein expression for HIF-1 α in renal medullary interstitial cells was reduced by O_2^- but not by $\cdot OH$ and was increased by tempol, PEG-SOD, or blockade of NADPH oxidase (Yang et al., 2003). Tempol increased

the expression of HIF-1 α , HIF-2 α , and hemoxygenase-1 in the outer medulla of diabetic kidneys (Rosenberger et al., 2008).

Thus, prominent effects of tempol in the kidney are to improve the efficiency of O_2 usage and increase tissue pO_2 . Because prolonged hypoxia in rats increases their BP (Mazzali et al., 2003), an improvement in kidney pO_2 with tempol may contribute to combating hypertension or progressive kidney disease, but this action is speculative. Despite an improvement in renal oxygenation, tempol increased HIF expression, presumably because of its ability to reduce renal O_2^- generation.

III. Toxicity of Tempol

The intraperitoneal doses of tempol or tempol-H causing death of 50 to 70% of mice (LD_{50-70}) were found to be 1.6 (Hahn et al., 1992a) and 2.0 mmol/kg, respectively (Hahn et al., 2000). A dose of 1.85 mmol/kg tempol-H given intraperitoneally was well tolerated (Hahn et al., 2000). The maximum tolerated doses of nitroxides after acute intravenous injection into the tail vein of mice were 0.25 to 1.5 mmol/kg (Matsumoto et al., 2004), which were similar to the maximum tolerated doses after intraperitoneal administration (Hahn et al., 1998). Of five nitroxides tested, the least toxic was tempol (Matsumoto et al., 2004). The toxicity of tempol-H was similar to that of tempol. These toxic or lethal doses of tempol are approximately 30-fold higher than the effective doses for reduction in BP.

The toxic manifestations of high doses of tempol entailed restlessness and seizures 10 to 20 min after intraperitoneal injections (Gallez et al., 1992; Hahn et al., 1992a). Nitroxides added to slices of guinea pig hippocampus in the concentration range of 1 to 5 mM increased the neural excitability, but tempol was less toxic than tempo or tempamine (Hahn et al., 1995).

Tempol has been reported either not to change (An and Hsie, 1993) or to decrease the frequency of gene mutations in Chinese hamster ovary cells stimulated by bleomycin (An and Hsie, 1992). Tempol and other nitroxides in concentrations up to 1 mM had no adverse effects on the growth and viability of these cells (Ankel et al., 1987). Even at concentrations of 50 mM, tempol did not induce chromosomal aberrations in cultured human peripheral blood lymphocytes (Johnstone et al., 1995). In another study, concentrations of tempol up to 100 $\mu mol/l$ protected lymphocytes from metal-induced toxicity but, in the absence of metals, tempol was toxic at more than 100 to 1000 $\mu mol/l$ (Lewinska et al., 2008). The nitroxides 2,2,5,5-tetramethyl-1-pyrrolidinyloxy-3-carboxylic acid and 2,2,6,6-tetramethyl-1-oxido-4-piperidinyloxy-1-succinic acid and their hydroxylamine and amine derivatives did not induce sister chromatid exchanges or mutations in Chinese hamster ovary cells (Afzal et al., 1984). The LD_{50} doses of these

nitroxides in rats exceeded 15 mmol/kg, suggesting that they had a very low toxicity (Afzal et al., 1984).

High concentrations of nitroxides decreased the osmotic fragility of erythrocytes and caused dysmorphic changes that might predispose to hemolysis (Bieri et al., 1974). A study of 58 nitroxides in V79 cells detected no cytotoxicity at doses of 100 $\mu\text{mol/l}$ (Krishna et al., 1998). The acute toxicity of nitroxides after intravenous injection in mice followed the order amino-TEMPO > tempone > tempol > carboxy-TEMPO = carbamoyl-PROXYL > carboxy-TEMPO. When given in vivo to mice, tempone is metabolized rapidly to the less toxic tempol (Kroll et al., 1999). These studies suggest that nitroxides are generally free from toxic effects except at exceptionally high concentrations.

IV. Conclusions Concerning Blood Pressure-Lowering Actions of Tempol

The available data are compatible with the hypothesis that the immediate reduction in BP with intravenous tempol is due predominantly to vasodilation that can be ascribed in part to potentiation of vascular NO by a reduction of its interaction with O_2^- . However, a component of the early fall in BP is independent of NOS and is accompanied by a fall in HR. This response represents an inhibition of the afferent, peripheral, and central activation of the SNS. The reduction in peripheral SNS activity may represent local action of tempol on the neurons to activate BK or K_{ATP} channel conductances, thereby leading to hyperpolarization that decreases neural discharge, but this hypothesis requires further study. The reduction in central sympathetic drive with tempol probably entails a reduction in O_2^- in the RVLM and PVN, which are brainstem nuclei that are very responsive to microinjection of tempol and that coordinate the central sympathetic drive.

The reason for the transient ability of an acute intravenous bolus of tempol to reduce BP, sympathetic tone, and HR may relate to the rapid reduction of tempol to the hydroxylamine that does not directly reduce BP. Tempol can cause a transient increase in vascular H_2O_2 and can activate BK and K_{ATP} channels on blood vessels and neurons that lead to hyperpolarization and thereby to vasorelaxation or reduced neural discharge.

Besides actions that promote vasodilation in models of oxidative stress, tempol also diminishes vasoconstriction by several mechanisms. Tempol diminishes the activation of AT_1 -Rs by Ang II, diminishes COX activity, diminishes vasoconstriction by PGs acting on TP-Rs, and diminishes the effects of ET-1 acting on ET-A-Rs and of catecholamines acting on α -adrenoceptors. Tempol prevents ET-1 release. Tempol corrects endothelial function by restoring EDRF/NO and EDHF responses and preventing EDCF responses in blood vessels from hypertensive models during agonist stimulation of the endothelium.

The constellation of acute vascular actions of tempol in hypertensive models demonstrates that it can function to potentiate NO and EDRF, as an EDHF mimetic, as an EDCF antagonist, as a potassium channel opener, and as a sympatholytic agent. This is a unique profile.

Although a bolus intravenous dose of tempol produces an abrupt fall in BP with a recovery over 10 to 15 min, an oral dose produces a gradual decline in BP that is maximal at 18 to 24 h. This probably represents the restoration of bioactive tempol nitroxide from the reduced hydroxylamine that occurs gradually in the circulation.

The effects of tempol that develop over days or weeks of administration to hypertensive models seem to be largely independent of the SNS, as indicated by the absence of any change in HR, plasma NE, or renal catecholamine excretion. The close correlation that is apparent in data from multiple studies of hypertensive rats between the reductions in BP and the reductions in systemic, renal, or vascular ROS with tempol supports the proposal that the antihypertensive response to prolonged tempol administration depends on a reduction in tissue O_2^- and oxidative stress.

Proposed mechanisms for the sustained fall in BP during prolonged tempol administration include resetting of the renal pressure natriuresis mechanism, correction of salt sensitivity, an increase in the rates of Na^+ and fluid excretion by NOS-dependent and -independent means, a reduction in renal adenosine release and intrarenal angiotensin II, prevention of phosphorylation and inactivation of DRs in renal proximal tubules, a reduction in NaCl reabsorption from the TAL of the loop of Henle and CDs, a reduction in the reactivity of the renal afferent arteriole to constrictor agonists and blunting of TGF responses leading to reduced RVR and better transmission of pressure into the kidneys, and improved renal usage of O_2 and increased renal oxygen tension yet increased levels of HIF-1 α . Presently, it is not clear which of these mechanisms is of predominant importance, but this probably varies among models.

Animal studies show that tempol is free of serious toxic effects at doses that reduce the BP. Despite these apparently beneficial effects in a wide range of animal models, tempol has yet to be developed as a drug for human hypertension.

Acknowledgments. C.W. holds patents for the use of tempol to treat hypertension, SOD deficiency, iron toxicity, and skin ulceration and is a member of the scientific advisory board of MitoS Inc., which is developing topical tempol to prevent radiation-induced alopecia.

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