Superoxide Formation and Interaction with Nitric Oxide Modulate Systemic Arterial Pressure and Renal Function in Salt-Depleted Dogs

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To determine the role of superoxide (O_2^-) formation in the kidney during alterations in the renin-angiotensin system, we evaluated responses to the intra-arterial infusion of an O_2^--scavenging agent, tempol, in the denervated kidney of anesthetized salt-depleted (SD, $n=6$) dogs and salt-replete (SR, $n=6$) dogs. As expected, basal plasma renin activity was higher in SD than in SR dogs (8.4 ± 1.0 vs. 2.3 ± 0.6 ng angiotensin 1/ml/hr). Interestingly, the basal level of urinary F_2-isoprostanes excretion (marker for endogenous O_2^- activity) relative to creatinine (Cr) excretion was also significantly higher in SD compared to SR dogs (9.1 ± 2.8 vs. 1.6 ± 0.4 ng F_2-isoprostanes/mg of Cr). There was a significant increase in renal blood flow (4.3 ± 0.5 to 4.9 ± 0.6 ml/min/g) and decreases in renal vascular resistance (38.2 ± 5.8 to 33.2 ± 4.7 mm Hg/ml/min/g) and mean systemic arterial pressure (148 ± 6 to 112 ± 10 mm Hg) in SD dogs but not in SR dogs during infusion of tempol at 1 mg/kg/min for 30 mins. Glomerular filtration rate and urinary sodium excretion ($U_{Na}V$) did not change significantly during tempol infusion in both groups of dogs. Administration of the nitric oxide synthase inhibitor nitro-L-arginine (50 μg/kg/min) during tempol infusion caused a reduction in $U_{Na}V$ in SR dogs (47% ± 12%) but did not cause a decrease in SD dogs. These data show that low salt intake enhances O_2^- activity that influences renal and systemic hemodynamics and thus may contribute to the regulation of arterial pressure in the salt-restricted state. Exp Biol Med 231:269–276, 2006

Key words: oxidative stress; sodium restriction; renal blood flow; tempol

Introduction

It is generally recognized that the relationship between salt intake, extracellular fluid volume, and the renin-angiotensin system (RAS) plays an important role in the regulation of blood pressure (1, 2). Oxidative stress has been critically linked to the RAS and to variations in salt intake (3, 4) and is thus considered to play an important role in the regulation of blood pressure, possibly through the regulation of kidney function (5). Angiotensin II is a known stimulus for the formation of superoxide (O_2^-) via activation of NAD(P)H oxidase (6, 7). Salt restriction leads to activation of the RAS (1, 2) and thus may alter the basal levels of O_2^- and other reactive oxygen species in the body. However, the relation between low salt intake and the development of oxidative stress is not yet clearly defined.

Both O_2^- and nitric oxide (NO) have opposite actions in the kidney; NO is a known potent vasodilator and natriuretic factor, while O_2^- has been shown to exert vasoconstrictor and antinatriuretic actions (8–11). It is increasingly evident that O_2^- activity and interaction with NO contribute to the physiologic regulation of renal hemodynamics and excretory function (10–13). Recently we have demonstrated that endogenous NO has a renoprotective effect against O_2^- by acting as an antioxidant (10, 11). Chronic inhibition of NO synthesis increases angiotensin II–induced O_2^- production in the rat aorta (14). It was reported that the NO activity in the kidney was lower in rats fed a low-salt diet compared to rats fed a high-salt diet (13). This may indicate that the NO bioactivity is diminished, likely as a result of enhancement of the O_2^- formation during low salt intake. It is known that changes in the RAS and salt intake alter basal O_2^- formation (6, 13, 15, 16) and NO activity (13, 17). Thus, it is important to examine the exact role of changes in O_2^- level and interaction with NO in regulating renal hemodynamics and renal function in order to understand how such changes could influence blood pressure.

We hypothesize that enhanced production of O_2^- as a

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result of augmented RAS activity plays a role in the kidney and the maintenance of systemic blood pressure during salt restriction. To examine this hypothesis, the present study was carried out to determine the role of O_2^- and its interaction with NO with regard to renal hemodynamics and excretory function during alterations in salt intake. One group of dogs was maintained on a low-salt diet (containing 0.01% NaCl) for 5 days to induce a salt-depleted (SD) state (18). The other group of dogs was fed a normal diet with added salt (1.5 mg/kg/day) for 3 days to cause a salt-replete (SR) state (11, 19). Responses to intra-arterial administration of the O_2^- scavenger tempol (4-hydroxy-2,2,6,6-tetramethyl-piperidine1-oxyl) (11, 19) were evaluated before and during NO synthase inhibition induced by nitro-L-arginine (NLA) (10–12, 19) in anesthetized SD dogs compared to responses observed in SR dogs.

Materials and Methods

These experiments were performed in accordance with the guidelines and practices established by the Tulane University Animal Care and Use Committee. Mongrel dogs of either gender (12–23 kg body wt) were used for these experiments in which chronic manipulations were imposed to alter the RAS status by changing dietary salt intake. To stimulate the RAS, sodium depletion was produced in one group of dogs (SD group) with a very low-salt diet (0.01% NaCl) for 5 days, as described previously (18). Experiments were also conducted in a group of SR dogs (SR group) that were given a normal laboratory diet (canine diet 5LI8; Lab Diet, St. Louis, MO) with the addition of extra salt (1.5 g/kg body wt/day for 3–5 days) to increase sodium balance, as described previously (10, 11, 19). On the day of an experiment, the dogs were anesthetized with pentobarbital sodium (30 mg/kg, iv). Additional anesthetic was given as needed to maintain an appropriate level of anesthesia.

The surgical preparation and basic experimental techniques used were similar to those previously described (10–12, 18, 19). These procedures include insertion of a cuffed endotracheal tube that is connected to a respirator for assisted ventilation during anesthesia. Catheters were inserted into both femoral arteries for the collection of blood samples and the measurement of arterial pressure. The right femoral and left jugular veins were cannulated for infusion of a saline or 5% dextrose and inulin solutions. To avoid salt loading, a 5% dextrose solution instead of saline was infused in SD dogs throughout the experimental period, as described previously (18). Systemic arterial pressure (SAP) was measured with a femoral arterial catheter advanced into the abdominal aorta. The left kidney was exposed retroperitoneally by a flank incision and was denervated by cutting all visible nerves to the kidney from the adjacent nerve plexus. Renal blood flow (RBF) was measured with an electromagnetic flow probe (Carolina Medical Electronics) placed around the renal artery. A 23-gauge curved needle was inserted into the renal artery distal to the electromagnetic flow probe and used to measure renal arterial pressure. Two other perfusion catheters were connected to this needle to allow intrarenal infusion of drugs and heparinized saline solution. Laser-Doppler needle flow probes (LDF; Perimed, Inc., Jarfalla, Sweden) were introduced in the midcortex and midmedullary regions to measure relative blood flow in different regions (cortex and medulla) of the kidney, as described previously (10, 18). For technical reasons, LDF recording was not possible in two dogs in each group. Thus, results from four dogs in each group were included in this study. The ureter was catheterized for the collection of urine.

After completion of the surgical procedures, a bolus dose (1.6 ml/kg) of 2.5% solution of inulin in normal saline (SR dogs) or in 5% dextrose (SD dogs) was administered into the jugular vein and was followed by a continuous infusion of inulin (0.03 ml/kg/min) for the entire protocol period to determine glomerular filtration rate (GFR). Responses to increasing doses of tempol were assessed in both groups of dogs (SD and SR) using the same protocol. Following completion of surgery and an hour-long stabilization period, the experimental protocol started with two consecutive 10-min control collections of urine and blood samples. Two milliliters of arterial blood were collected at the midpoint of each urine collection period. Then a continuous infusion of O_2^- scavenger tempol (20, 21) at three doses (0.5, 0.75, and 1.0 mg/kg/min; Sigma Chemical Co., St. Louis, MO) was made intraarterially (11, 19). During each infusion period, 10 mins were allowed for stabilization before collection of two 10-min urine samples. At the end of the collection period with the highest dose (1.0 mg/kg/min) of tempol, the NO synthase inhibitor NLA (50 μg/kg/min) (10–12, 19) was infused in addition to tempol. After a 30-min stabilization period, another two 10-min urine samples were collected during the combined infusion of NLA and tempol.

To examine the specificity of the O_2^--scavenging effects of tempol in these experiments, a similar protocol was also carried out in three other dogs with infusion of a control nitroxide compound, 3-carbamyol proxyl (3-CP; 0.5. 0.75, and 1.0 mg/kg/min; Sigma Chemical) instead of tempol. 3-CP is structurally similar to tempol but has minimal O_2^--scavenging activity (11, 21).

To examine the time-dependent effect of tempol, we conducted another series of experiments in SD dogs with a long-term infusion of tempol. In these experiments ($n = 6$), we infused tempol at a dose of 0.5 mg/kg/min continuously for an hour after two consecutive control 10-min urine collections. Ten minutes after the initiation of the tempol infusion, urine samples were collected for five consecutive 10-min periods. After the fifth collection during the tempol infusion, NLA (50 μg/kg/min) was administered into the infusion line. Following a 30-min stabilization period during NLA infusion, two more urine collections were made during the combined infusion of NLA and tempol.

Sodium, potassium, and inulin concentrations in plasma

and urine were determined by flame photometry and spectrophotometry, respectively, as previously described (10–12, 18, 19). Baseline levels of plasma renin activity (PRA) in the control arterial samples in both groups of dogs (SD and SR) were determined by radioimmunoassay (Gamma coat kit; DiaSorin Co., Stillwater, MN) as described previously (12, 22). Baseline levels of F_2-isoprostane and creatinine (Cr) were measured in urine samples from these dogs using mass spectrometry (23). As the comparison of the baseline values of F_2-isoprostane excretion was made between two groups (SD and SR dogs) that have differences in water intake and urine output due to variation in dietary salt, it is essential to normalize F_2-isoprostane to Cr concentration, which is usually filtered at a constant rate into urine (24). F_2-isoprostane excretion was determined in the urine samples collected at the control period, during infusion of the highest dose of tempol (1 mg/kg/min), and during combined infusion of tempol and NLA to examine the effect of O_2^- scavenging before and during NO inhibition. Urine samples collected at lower doses of tempol (0.5 and 0.75 mg/kg/min for Iso.

Values are reported as mean ± SE. Statistical comparisons of differences in the values were conducted using one-way repeated measure analysis of variance (ANOVA) followed by a post-hoc Newman-Keul test (in case of absolute changes). Student's paired *t* test was used to determine the statistical significance of the percent change in response. Differences in the mean values were considered significant at $P \leq 0.05$.

Results

Basal Level of PRA and Urinary F_2-Isoprostane Excretion. Figure 1 illustrates these values obtained in both SD and SR dogs ($n = 5$ in both groups). As expected, the PRA was higher in SD dogs compared to the values obtained in SR dogs (8.4 ± 1.0 ng vs. 2.3 ± 0.6 ng angiotensin 1/ml/hr; $P < 0.001$). The basal level of urinary F_2-isoprostane excretion relative to Cr excretion was also higher in SD dogs than in SR dogs (9.1 ± 2.8 vs. 1.6 ± 0.4 ng Iso/mg of Cr; $P < 0.05$).

Effects of Intraarterial Infusion of Tempol and NLA on SAP and Renal Hemodynamics. Figures 2 through 4 illustrate the results obtained in these SD ($n = 6$) and SR ($n = 6$) groups of dogs. During the control period, there was no significant difference in mean systemic arterial pressure (MAP) between the groups (SD; 148 ± 6 mm Hg; SR; 143 ± 4 mm Hg), although SD dogs showed slightly higher MAP compared to SR dogs. However, similar baseline values of MAP were usually observed in dogs under anesthetic conditions, as reported previously (18, 25). Although tempol was administered intraarterially into the kidney, its effect on SAP was observed as a result of spillover from the renal circulation. During infusions of tempol, there were marked decreases in MAP in SD dogs. At infusion rate of 1 mg/kg/min, tempol decreased MAP to 112 ± 10 mm Hg ($P < 0.05$) in SD dogs. However, in SR dogs, this high infusion rate of tempol caused a smaller change in MAP (143 ± 4 to 125 ± 4; $P = 0.063$). The co-infusion of NLA and tempol prevented any further decrease in MAP in both groups of dogs (Fig. 2A).

The baseline value of RBF was slightly lower (Fig. 3A) in SD dogs than in SR dogs (4.3 ± 0.5 vs. 5.1 ± 0.5 ml/min/g), although this difference was not statistically significant. RBF did not change significantly during tempol infusion in SR dogs, but there was an increase in RBF (4.3 ± 0.5 to 4.9 ± 0.6 ml/min/g; $P < 0.05$) in SD dogs at the high infusion rate. Similar changes were also observed in renal cortical (19% ± 13% $n = 4$) and medullary blood flow (21% ± 12%, $n = 4$) in SD dogs but not in SR dogs. During infusion of NLA and tempol, cortical and medullary blood flow as well as total RBF decreased to the same levels in both SD and SR dogs (Fig. 3A). The baseline value for renal vascular resistance (RVR) was also higher in SD than in SR dogs (Fig. 2B). Tempol infusion caused dose-dependent decreases in RVR (38.2 ± 5.8 to 33.2 ± 4.7 mm Hg/ml/min/g) in SD dogs but not in SR dogs. During infusion of tempol and NLA, RVR increased in both groups of dogs. The basal level of GFR in SD dogs was also significantly lower ($P < 0.05$) compared to the values in SR dogs (Fig. 2B). However, there were no significant changes in GFR during infusion of tempol or during infusion of NLA and tempol.

Effects of Intraarterial Infusion of Tempol and NLA on Renal Function. Figure 4 illustrates the changes in urine flow (V; Fig. 4A) and urinary sodium excretion ($U_{Na}V$; Fig. 4B) in response to tempol and NLA infusions in SR and SD dogs. As expected, basal levels of V and $U_{Na}V$ were lower in SD dogs than in SR dogs. Infusion of tempol did not cause significant changes in V or $U_{Na}V$ in either group of dogs. As RBF was increased in response to tempol in SD dogs, the absence of significant increases in V or $U_{Na}V$ could be due to marked decreases in arterial pressure in this group of dogs.

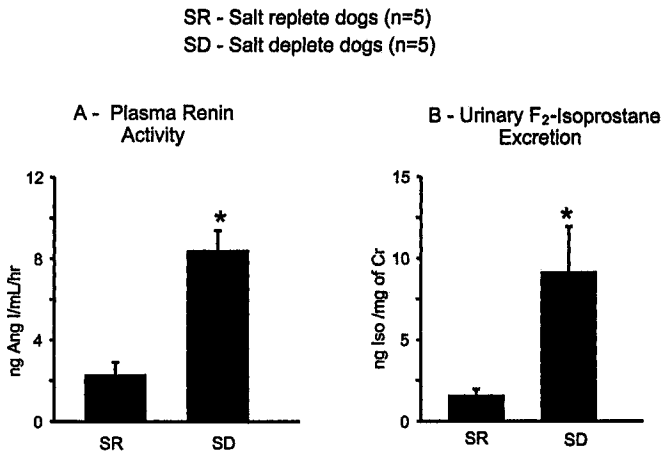

Figure 1. Basal plasma renin activity (A) and urinary F_2-isoprostane excretion (B) relative to creatinine (Cr) excretion in anesthetized dogs. *, $P < 0.05$ versus values in SR dogs.

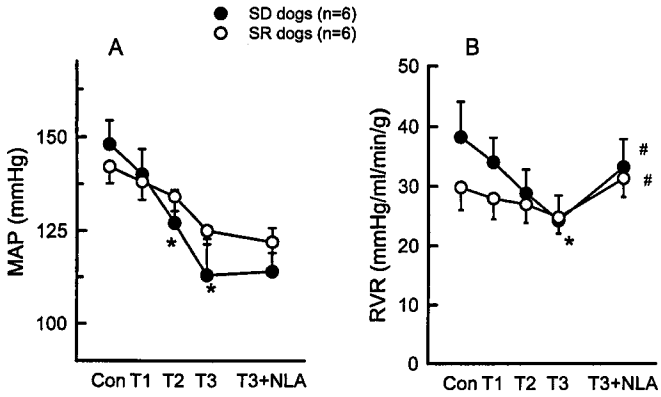

Figure 2. Mean arterial pressure (MAP; A) and renal vascular resistance (RVR; B) responses to tempol and nitro-L-arginine (NLA) in dogs. *, $P < 0.05$ versus control values; #, $P < 0.05$ versus values obtained during tempol dose infusion before NLA administration.

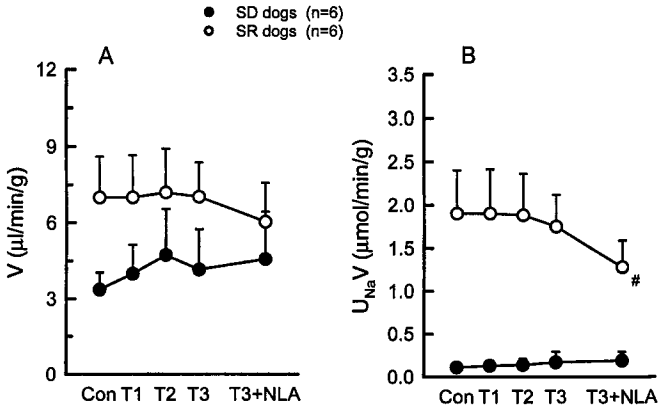

Figure 4. Urine flow (V; A) and sodium excretion ($U_{Na}V$; B) responses to tempol and nitro-L-arginine (NLA) in dogs. #, $P < 0.05$ versus values obtained during tempol dose infusion before NLA administration.

Inhibition of NO synthase in tempol-treated SD dogs did not cause a decrease in V or $U_{Na}V$. Responses to NLA were different when compared to responses observed in animals not treated with tempol, as reported earlier (9, 11). Although there were reductions in V (23% ± 8%; $P < 0.05$) and $U_{Na}V$ (47% ± 12%; $P < 0.05$) during NLA infusion in tempol-treated SR dogs, these reductions were comparatively less than those that were observed in animals not pretreated with tempol (10–12, 19).

In SD dogs, urinary Iso excretion was decreased during the high-dose (1.0 mg/kg/min) infusion of tempol (from 9.1 ± 2.8 to 5.5 ± 1.9 ng Iso/mg of Cr; $n = 5$) and then remained unchanged during co-administration of NLA (4.9 ± 3.2 ng Iso/mg of Cr; $n = 5$). On the other hand, in SR dogs, Iso excretion did not change significantly from the control value during infusion of tempol (1.6 ± 0.4 to 2.4 ± 1.2 ng Iso/mg of Cr, respectively). However, during NLA infusion in SR dogs, Iso excretion was variable, and values (7.4 ± 3.6 ng Iso/mg of Cr) were not statistically different from values determined before NLA administration.

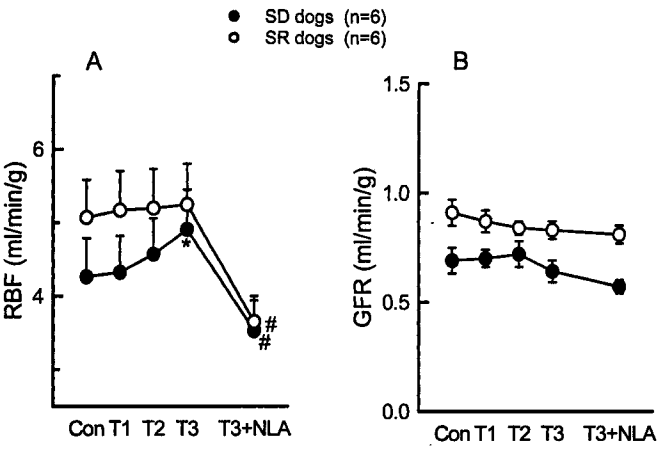

Figure 3. Renal blood flow (RBF; A) and glomerular filtration rate (GFR; B) responses to tempol and nitro-L-arginine (NLA) in dogs. *, $P < 0.05$ versus control values; #, $P < 0.05$ versus values obtained during tempol dose infusion before NLA administration.

Responses to 3-CP Infusion. In three SD dogs, the effects of infusion of increasing doses of 3-CP (similar to the doses of tempol used) were examined. Although tempol infusion produced an increase in RBF and decreases in RVR and MAP in SD dogs (Figs. 2–4), no significant changes were observed with 3-CP infusion. There were no appreciable changes in MAP (143 ± 5 to 146 ± 7, 146 ± 7 and 147 ± 6 mm Hg), RBF (4.8 ± 0.8 to 4.7 ± 1.1, 4.6 ± 1.1, and 4.6 ± 1.1 ml/min/g), GFR (0.79 ± 0.15 to 0.76 ± 0.13, 0.72 ± 0.13, and 0.77 ± 0.13 ml/min/g), V (3.2 ± 0.6 to 2.7 ± 0.5, 2.9 ± 0.5, and 3.1 ± 0.7 µl/min/g), and $U_{Na}V$ (0.44 ± 0.12 to 0.35 ± 0.04, 0.35 ± 0.4, and 0.37 ± 0.06 µmol/min/g) in response to infusions of 3-CP (0.5, 0.75, and 1.0 ml/min/g, respectively). However, infusion of NLA increased MAP (147 ± 6 to 157 ± 7 mm Hg) and decreased RBF (4.6 ± 1.0 to 2.9 ± 0.5 ml/min/g), V (3.1 ± 0.7 to 2.6 ± 0.6 µl/min/g), and $U_{Na}V$ (0.37 ± 0.06 to 0.23 ± 0.5 µmol/min/g) in 3-CP–treated dogs. Thus, unlike tempol, 3-CP did not prevent or attenuate the usual antidiuretic and antinatriuretic responses to NLA. Similar findings with 3-CP were also reported in previous studies (11, 19).

Effects of Tempol Infusion for Longer Periods in SD Dogs. Table 1 summarizes the responses to infusion of tempol for longer periods on MAP, renal hemodynamics, and excretory function in SD dogs. Tempol infusion at a rate of 0.5 mg/kg/min caused no significant change in RBF, GFR, V, and $U_{Na}V$, even after 60 mins of infusion. There was no indication of a delayed effect of tempol on these renal parameters. The change in MAP was significant only during the final collection period. These data indicate that responses observed in SD dogs with increasing doses of tempol were due to the dose-dependent O_2^--scavenging effects of tempol. Infusion of NLA with tempol in SD dogs also showed similar responses that were observed in earlier groups (Table 1 and Figs. 2–4).

Table 1. *Responses to Long-Term Infusion of Tempol (0.05 mg/kg/min), $n = 6$*[a]

	Control period (20-min collection)	Tempol infusion period (every collection made for 10 mins; collection starts after 10-min stabilization period)					Tempol + NLA period (20-min collection after 30-min stabilization period)
		Period 1	Period 2	Period 3	Period 4	Period 5	
Systemic arterial pressure (mm Hg)	141 ± 6	137 ± 7	135 ± 6	134 ± 6	127 ± 5	126 ± 8*	129 ± 7
Renal blood flow (ml/min/g)	4.6 ± 0.6	4.7 ± 0.6	4.8 ± 0.6	4.8 ± 0.6	4.9 ± 0.7	5 ± 0.9	3.7 ± 0.6**
Renal vascular resistance (mm Hg/ml/min/g)	31.4 ± 3.0	30.3 ± 2.9	29.3 ± 2.6	28.3 ± 2.4	27.1 ± 2.4	26.4 ± 3.0***	37.4 ± 3.7**
Glomerular filtration rate (ml/min/g)	0.76 ± 0.06	0.72 ± 0.07	0.76 ± 0.08	0.80 ± 0.04	0.88 ± 0.09	0.71 ± 0.08	0.72 ± 0.03
Urine flow (μl/min/g)	6.1 ± 2.6	6.6 ± 2.2	5.7 ± 1.4	5.7 ± 1.2	6.1 ± 1.3	6.3 ± 1.6	6.6 ± 1.4
Sodium excretion (μmol/min/g)	0.14 ± 0.03	0.18 ± 0.05	0.18 ± 0.04	0.19 ± 0.04	0.20 ± 0.05	0.19 ± 0.06	0.24 ± 0.07
Potassium excretion (μmol/min/g)	0.61 ± 0.10	0.62 ± 0.12	0.63 ± 0.11	0.68 ± 0.11	0.75 ± 0.13	0.68 ± 0.18	0.60 ± 0.12
Fractional excretion of sodium (%)	0.15 ± 0.05	0.18 ± 0.05	0.19 ± 0.04	0.17 ± 0.04	0.16 ± 0.04	0.18 ± 0.05	0.24 ± 0.08

[a] Values are given as mean ± SE. NLA, nitro-L-arginine.
*$P < 0.05$ versus control period; **$P < 0.05$ versus tempol period 5; ***$P = 0.06$ versus control period.

Discussion

The results of the present investigation demonstrate that urinary excretion of F_2-isoprostane was 3- to 4-fold higher in SD dogs compared to comparable values measured in SR dogs. PRA was also higher in SD dogs (Fig. 1). An increase in F_2-isoprostane excretion is considered to be an indicator of oxidative stress, as reported in many previous studies (4, 11, 19, 23, 24). Low salt intake activates the RAS, and it is reasonable to speculate that endogenous O_2^- production would increase as a result of increased angiotensin II formation (6, 16). This study further demonstrates that intraarterial administration of the O_2^--scavenging agent tempol increased RBF and decreased RVR and arterial pressure in SD but not in SR dogs. These data provide support for the notion that O_2^- generation is enhanced in SD dogs. Tempol, as an effective O_2^- scavenger, has been used in recent studies by different investigators (8, 19–21, 26) to examine the effects of reduced endogenous O_2^- activity. The specificity of tempol as an O_2^- scavenger was tested in *in vitro* (21) as well as *in vivo* experiments (27), which showed that enhanced O_2^- activity by superoxide dismutase (SOD) inhibition was attenuated by tempol administration. This action of tempol was also examined in previous studies (11, 19) that compared responses with those elicited by 3-CP, a compound structurally similar to tempol with minimal O_2^--scavenging activity (20, 21). In the present study, it was also observed that 3-CP had no effect in SD dogs. Thus, it is reasonable to hypothesize that the renal and systemic vasodepressor actions of tempol observed in SD dogs were related to O_2^--scavenging effects (11, 19–21).

Tempol administration caused marked reductions in RVR and arterial pressure in SD but not in SR dogs (Fig. 2). These findings indicate that enhanced O_2^- generation is involved in increasing vascular tone and in maintaining arterial pressure at a normal level in SD dogs that have low circulatory volume due to sodium depletion (1, 2, 4). It is known that angiotensin II (AngII) is an essential component in the regulation of blood pressure in salt-depleted subjects (1, 2, 4, 18). However, we observed previously (25) that intraarterial administration of candesartan (AT1 receptor antagonist) increased renal blood flow by 21% but caused minimal change in arterial pressure. In an earlier study (19), we demonstrated in dogs pretreated with tempol that there was marked attenuation of the effects of acute AngII administration on renal blood flow and sodium excretion, indicating that AngII-induced O_2^- generation is a significant component in the mechanism of the vasoconstrictor and antinatriuretic effects of AngII. AngII exerts its vascular effect directly and indirectly via alterations in vasoactive factors including O_2^- (4–7, 19). It is possible that an increase in O_2^- activity due to RAS enhancement plays an important role in modulating arterial pressure and vascular resistance during salt depletion. It may be argued that a lower baseline value of RVR in SR dogs poses a limiting factor that could be related to the minimal RVR response

observed in SR dogs. However, previous studies in SR dogs (9, 28) demonstrated that RVR with a similar basal value was decreased in response to reductions in renal arterial pressure. Thus, these findings would argue against the existence of such a limiting factor that may be involved in causing minimal responses to tempol in SR dogs. O_2^- was shown to cause vasoconstrictor effects either directly and/or by reducing NO bioactivity in the vessels (10, 29). The prevention of a further fall in arterial pressure in tempol-treated animals with co-administration of NLA indicates an involvement of NO in tempol-induced changes in vascular resistance. However, such changes in vascular tone could also be due in part to a decrease in sympathetic activity (27, 30), as the increase in O_2^- generation has been suggested to enhance sympathetic activity (27). It is also reported that salt depletion enhances sympathetic activity (1, 2). Thus, these results indicate that enhanced O_2^- formation may be a factor in the adjustment of sympathetic tone involved in the maintenance of normal arterial pressure with expected lower circulatory volume due to sodium depletion.

It was observed that administration of the NO synthase inhibitor NLA in tempol-treated SD dogs did not cause the usual decrease in urinary excretion of salt and water. NLA also caused a similar decrease in RBF in tempol-treated SD and SR dogs, although the baseline RBF values were different in both groups of dogs, indicating a lesser effect of NO blockade in SD dogs. This indicates that the bioactivity of NO could be reduced in SD dogs, as endogenous O_2^- production was enhanced in the condition of low salt intake. Although O_2^- is a constant product of cellular metabolism under normal conditions, the basal tissue concentration is maintained at a minimal level as a result of the efficient activity of various endogenous antioxidant systems. In addition to SOD and other antioxidative enzymes, endogenous NO is also known to inactivate O_2^-. It is hypothesized that an appropriate physiologic balance in oxidative status of the kidney during normal conditions is critically dependent on endogenous NO generation. In an earlier study (11), we demonstrated that tempol did not cause a significant change in renal function in intact dogs but did cause diuretic and natriuretic responses after NO blockade. Renal responses to inhibition of SOD in dogs were also greatly enhanced by NO synthase inhibition (10). These findings indicate that endogenous NO provides an important renoprotective effect by interacting with O_2^- at the cellular level and reduces NO bioactivity in SD dogs. This hypothesis is further supported by the findings in the present study that the O_2^- scavenger tempol did not cause significant effects in the SR state, during which the bioactivity of NO remains intact. However, under conditions that lead to an increase in O_2^- production or a decrease in NO production or both, this balance is shifted to a pathologic state of oxidative stress. The renal excretory effect of NLA in tempol-treated SR dogs was smaller compared to the effect of NO synthase inhibition in dogs in other studies from our laboratory (10–12, 19). Pretreatment with 3-CP did not prevent NLA-induced decreases in urine flow and sodium excretion in the present study, confirming the hypothesis that O_2^--scavenging activity was associated with reduced effects of NO blockade during tempol infusion (11). These findings indicate that an interaction between NO and O_2^- may serve a regulatory role in maintaining sodium balance and arterial pressure when salt intake is reduced.

Although the results of the present and previous studies (10, 11) indicate a renoprotective role for the NO-O_2^- interaction, the exact mechanism of this protective effect is not yet understood. The reaction between NO and O_2^- results in the formation of peroxynitrite ($ONOO^-$) which is a powerful cytotoxic agent (31–33). As an oxidant radical, $ONOO^-$ can induce a cytotoxic effect by sulfhydryl oxidation, protein tyrosine nitration, and membrane lipid peroxidation, as well as DNA damage leading to cellular injury and death (31–37). Thus, the possible formation of $ONOO^-$ would argue against a renoprotective role of the NO-O_2^- interaction. However, it is also reported that $ONOO^-$, when injected into the blood, caused vascular relaxation (38, 39) and provided protection against ischemia-reperfusion injury *in vivo* (40). The exact mechanism involved in the vascular actions of $ONOO^-$ is not yet clear and needs further investigation. It is to be considered that the cytotoxic effect of $ONOO^-$ usually occurs at high concentrations (micromole to millimole), as shown mainly in *in vitro* studies (34–37). On the other hand, $ONOO^-$-induced vascular relaxation has been demonstrated usually at low concentrations (nanomole to low micromole), which could be more physiologically relevant (38–40). Although no direct study has been conducted to examine the role of $ONOO^-$ in regulating kidney function, it is possible that the renoprotective effect of NO-O_2^- interaction can be due to the conversion of $ONOO^-$ to a NO donor compound (39). Further studies are required to examine the role of $ONOO^-$ in the regulation of kidney function in the intact animal to understand the exact nature of this NO-O_2^- interaction.

It is known that O_2^- can influence tubular reabsorptive function leading to sodium retention (5, 10, 11, 19, 26). However, in the present study, it was observed that tempol infusion in the SD dogs increased RBF but did not cause expected increases in UV and $U_{Na}V$. The reason for the lack of increases in excretory function could be due to counteracting antidiuretic and antinatriuretic effects of a decrease in SAP observed in response to tempol administration. The effects of changes in arterial pressure on tubular reabsorption have been demonstrated in many previous studies from our laboratory (9, 28) and from other laboratories (1, 2). The absence of significant changes in renal hemodynamics and excretory function in response to tempol in SR dogs indicates that O_2^- activity remains minimal in the SR condition (10, 19). An enhancement of O_2^- activity in the condition of salt deficiency decreases NO bioactivity, as indicated by the present results. It has to be emphasized that such enhancement of O_2^- activity can directly as well as indirectly (via reduction in NO bioactivity) play a role in

preventing further sodium loss by modulating renal hemodynamics and excretory function. In the same manner, sodium retention is increased by activation of the RAS during low salt intake (1, 2, 4). The results of the present investigation indicate that O_2^- is also involved in the mechanisms by which RAS-induced changes in kidney function occur. It should be emphasized that these results do not mean that enhanced O_2^- activity is good for SD dogs; rather, the present findings demonstrate that lower-than-normal salt intake can also induce a pathophysiologic condition of oxidative stress as a result of activation of the RAS and can thus aggravate the pathologic processes that depend on the disregulation of reactive oxygen radicals.

The basal level of RBF and GFR was lower in SD dogs than in SR dogs in this study. The reason for such reduced blood flow and glomerular filtration in SD dogs could be related to the involvement of many factors, including reduced circulatory volume, enhanced RAS, and increased sympathetic activity (1, 2). However, these experiments were conducted in denervated kidneys, indicating that changes in renal sympathetic activity would not have a major involvement in causing reduced blood flow in SD dogs. The results of the present investigation indicate that enhanced O_2^- activity could be listed as one of the contributory factors in decreasing renal perfusion during low salt intake. It has been reported recently that F_2-isoprostane can act as a potent vasoconstrictor in the brain and retina (41). Since salt depletion increases F_2-isoprostane level, it is likely that such increases in F_2-isoprostane could occur in the kidney and reduce blood flow in SD dogs. It was also seen in the present investigation that tempol-induced increases in RBF in SD dogs were associated with decreases in urinary Iso excretion.

Although low salt intake enhanced oxidative stress in dogs in the present study, it should be noted that a very high salt intake in rats was also shown to induce oxidative stress (42, 43). The mechanism of increasing oxidative stress may be different in each condition. High salt intake in rats was shown to enhance mRNA expression for NAD(P)H oxidase (42). The present study was not designed to examine the enzymatic mechanism involved in the increased oxidative stress condition in SD dogs. However, in a recent study in our laboratory (44), we observed that the enzymatic mechanisms involved in chronic AngII-induced oxidative stress were different depending on salt intake. In those experiments in rats (44), AngII-induced oxidative stress was associated with increases in the protein expression of NAD(P)H oxidase subunits in rats that were given a high-salt diet, whereas enhanced oxidative stress induced by AngII was associated with reductions in protein expression of catalase and glutathione peroxidase in rats that were given a normal salt diet. On the other hand, the changes in protein expression in these oxidative and antioxidative enzymes were heterogeneous in rats that were given a low-salt diet. Rugale *et al.* (45) recently showed that exogenous AngII-induced oxidative stress and its associated myocardial and renal injuries were less significant in rats given a low-salt diet than in rats fed a high-salt diet. However, the findings from the study of Rugale *et al.* (45) may not be comparable to those of the present study, as the species used and the experimental conditions as well as experimental protocols were not similar in the two studies. Thus, it seems reasonable that further studies are needed to explain the apparent contradictory results that could be related to differences in various enzymes activities in dogs and in rats.

In conclusion, the results of the present investigation indicate that low salt intake enhanced O_2^- generation and that elevated O_2^- interacts with NO to modulate renal hemodynamics and excretory function. These data indicate that enhanced production of O_2^- plays a physiologic role in maintaining sodium balance and regulates systemic blood pressure during reduction in extracellular fluid volume in the salt-restricted state.

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