Tempol improves renal hemodynamics and pressure natriuresis in hyperthyroid rats

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HYPERTHYROIDISM is an endocrine disease associated with important hemodynamic, cardiac, and renal alterations (17, 36). The cardiovascular manifestations of hyperthyroidism are suggestive of an increased sympathetic activity (18). Thus, the hyperthyroid state courses with a hyperdynamic circulation characterized by increased cardiac output, heart rate (HR), pulse pressure, and decreased peripheral resistance (14, 36). Administration of thyroxine (T4) to rats accelerates the course of experimental hypertension (35) and produces dose-related increases in blood pressure (BP) (23, 36). T4-induced hypertension produces cardiac and renal hypertrophy and proteinuria (11, 23, 36) and is not associated with endothelial dysfunction (34).

There is considerable evidence that oxidative stress from superoxide and other reactive oxygen species (ROS) plays an important role in the development of cardiovascular diseases and hypertension and in the pathogenesis of renal diseases, producing vascular, glomerular, tubular, and interstitial injury (13, 37). It is known that ROS can be utilized in signal transduction events and play an important role in the regulation of cell biology and physiology. Thus, it has been demonstrated that ROS participate in BP control and kidney function (39, 40). In this regard, ROS can influence vascular reactivity either directly or via intermediate pathways. ROS also increase tubular reabsorption of sodium by inhibition of the natriuretic effects of NO and/or by affecting the activities of sodium transporters in the kidney (7, 12, 25).

Hyperthyroidism in rats is associated with reduced antioxidant enzyme activities in renal and cardiac tissues, and a dose-dependent increase in plasma malondialdehyde and urinary 8-isoprostane, markers of increased endogenous O2− activity (5, 8, 15, 16, 30), was observed in T4-treated rats (23) compared with euthyroid controls. Moreover, tempol, a superoxide dismutase mimetic (22), which reduces endogenous O2− levels in vitro and in vivo (19, 21, 29), attenuates the development of T4-induced hypertension and reduces plasma and urine levels of the oxidative stress markers (23). All of these observations indicate that hyperthyroidism produces an increased oxidative stress.

Hyperthyroid rats show increased blood volume (27), polydipsia, and polyuria (10), and a reduced ability to excrete sodium after isotonic or hypertonic saline loading (33), and they exhibit a blunted pressure-diuresis-natriuresis (PDN) relationship (32). The latter was markedly improved by angiotensin II (ANG II) inhibitors (11), recently shown to have antioxidant properties (2). Hence, renal sodium handling abnormalities in hyperthyroid rats may be related to increased ROS activity in this disease. The purpose of the present study was to examine the role of ROS in systemic and renal hemodynamics and in the blunted PDN curve of hypertensive hyperthyroid rats. To this end, we studied the effects of the acute administration of tempol, O2− scavenging agent, on these renal function abnormalities in T4-treated rats. To our knowledge, no previous study has specifically addressed the role of ROS in modulating renal function in T4-treated hypertensive rats.

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METHODS

Animals

Male Wistar rats born and raised in the Experimental Animal Service of the University of Granada were used. All experiments were performed according to European Union Guidelines for the Ethical Care of Animals and following the American Physiological Society’s “Guiding Principles in the Care and Use of Animals.” The experimental protocols were approved by the Institutional Review Board.

Rats initially weighing 200–225 g were assigned to the different experiments and were divided among the corresponding groups. Each experimental group comprised eight animals. All rats had free access to food and tap water. Hyperthyroidism was induced in by injecting T4 subcutaneously (Merck; 300 μg·kg⁻¹·day⁻¹ dissolved in 0.5 NaOH isotonic saline). The treatment was administered for 6 wk. We previously showed that this treatment increases serum T4 and triiodothyronine levels and BP (11, 23, 32). All rats were fasted for 16 h before the experiment. To assess the effectiveness of T4 treatment to induce hyperthyroidism and increase oxidative stress, the following biological variables were measured at the end of the experimental period: body weight, kidney weight, mean arterial pressure (MAP), HR, plasma thyroid hormone levels, plasma malondialdehyde, and total urinary excretion of 8-isoprostane, a marker of endogenous O₂ activity. Rats were housed for 24 h in metabolic cages for urine collection. These variables were measured in the animals of experiment 2 (controls and T4 rats, n = 8 each group).

Experimental Protocols

Experiment 1: effects of acute tempol administration on systemic and renal hemodynamics. Changes in MAP, HR, and renal hemodynamics induced by intravenous bolus injection of increasing doses of tempol were compared between anesthetized control and hyperthyroid rats. Control and T4-treated rats were anesthetized with thiobutabarbital (100 mg/kg ip; Inactin; Research Biochemicals International) and maintained at 37°C on a servocontrolled heated rodent operating table. A tracheostomy was performed, and polyethylene (PE)-240 tubing was inserted in the trachea. The left femoral vein and artery were cannulated with PE-50 tubing. The vein was used for infusions, and the artery was connected to a pressure transducer (MacLab; AD Instruments, Hastings, UK) for BP measurements. To maintain a euvoletic state, 1% albumin dissolved in isotonic NaCl solution was intravenously infused at 2 ml/h. A midline incision was made, and the left renal artery was isolated. The kidney was placed in a metal holder to eliminate respiratory movements. A perivascular blood flow probe (1RB) was placed around the renal artery for renal blood flow (RBF) measurement with a T106 flowmeter (Transonic Systems). Two separate laser-Doppler flowmeters were used to simultaneously measure relative changes in cortical and medullary blood flow (MBF). Cortical blood flow (CBF) was measured using a superficial 2.5-mm diameter probe (model PFd1; Perimed, Stockholm, Sweden). For MBF measurement, the laser-Doppler probe (0.45 mm diameter; model PF3; Perimed) was inserted into the kidney from the surface along the corticopapillary axis. The flow probes were calibrated by using a colloidal suspension of latex particles (Perimed motility standard), which gives a signal of 250 Units (2.5 V ± 5%) at room temperature. At the end of the experiment, the renal artery was completely occluded to obtain a zero-flow reading in the laser Doppler flowmeter, and these values were subtracted from signals recorded during the experiment. MAP, RBF, CBF, and MBF were continuously recorded. After 60 min of equilibration, basal values of hemodynamic variables were recorded during a 30-min period. Tempol was then injected at 25, 75, and 150 μmol·kg⁻¹·h⁻¹ during the stabilization period and maintained during the experimental procedure.

Analytical Techniques

Plasma levels of thyroid hormones (total circulating T₃ and T₄) were determined using rat radioimmunoassay kits according to the manufacturer’s instructions (Diagnostic Products, Los Angeles, CA). An enzyme immunoassay kit for 8-isoprostane EIA Kit, Cayman) was used to measure urinary 8-isoprostane levels, and samples were previously purified using the Affinity purification kit (Cayman). Plasma malondialdehyde levels were assessed using the method described by Esterbauer and Cheeseman (6).

[¹⁴C]Inulin in plasma and urine was measured by counting aliquots of the samples dissolved in scintillation fluid in a beta counter (Betamatic Basic; Kontron, Madrid, Spain). GFR was calculated as the clearance of radioactive inulin (urine-to-plasma concentration ratio × urine flow). Urine flow was determined gravimetrically.
Sodium concentration was measured by flame photometry (Corning 435; Izasa, Barcelona, Spain).

Statistical Methods

Data are presented as means ± SE. Biological variables were analyzed with one-way ANOVA. Dose-response curves were compared between the two groups using a nested design with three factors, with groups and doses as fixed factors and rat as random factor. The interaction between groups and doses was tested and, when significant, pairwise comparisons (between groups, dose by dose; among doses group by group) were performed using Tukey’s method. In the pressure natriuresis experiment, RPP and RBF values are the means of the values measured every minute during each experimental period. A repeated-measures ANOVA was used to obtain differences between groups and among different RPP levels within groups. When the global analysis was significant, pairwise comparisons were carried out using Tukey’s method. Slopes of the relationships between RPP and diuresis and natriuresis were computed for each rat, and the mean of slopes was obtained for each group. One-way ANOVA was performed to compare means of slopes followed by pairwise comparisons using Tukey’s method. Differences were considered statistically significant at P < 0.05.

RESULTS

Biological Variables

Except for final body weight, which was significantly reduced, the remaining biological variables measured and the markers of oxidative stress (plasma malondialdehyde and urinary 8-isoprostanate) were markedly increased in T4-treated rats (Table 1), indicating that a hyperthyroid state was achieved and was accompanied by an increased oxidative stress. Thyroid hormone levels resembled those observed in severe hyperthyroid patients without malignant thyrotoxicosis.

Hemodynamic Responses to Increasing Doses of Tempol Infusion

Figure 1 illustrates the dose-response relationship between increasing bolus doses of tempol (at 25, 75, and 150 μmol/kg) and MAP, HR, renal vascular resistance (RVR), total renal blood flow (RBF), CBF, and MBF in control and T4-treated rats. Baseline MAP was significantly (P < 0.01) higher in hyperthyroid rats (138 ± 4 mmHg) than in controls (110 ± 3.4 mmHg). Tempol dose-dependently decreased MAP in control and T4-treated rats, with the T4 group showing a greater responsiveness to tempol injection. The highest dose of tempol (150 μmol/kg) decreased MAP by 60 ± 5 and 35 ± 4 mmHg in T4 and control rats (P < 0.01), respectively. Baseline HR was significantly (P < 0.01) higher in hyperthyroid rats (495 ± 16 beats/min) than in controls (380 ± 10 beats/min). Tempol produced a slight dose-dependent increase in this variable in both groups, but statistical significance was not reached.

Baseline RVR was also significantly higher in T4-treated rats than in controls (25 ± 2 vs. 18.3 ± 1.5 mmHg·ml⁻¹·min⁻¹, respectively; P < 0.05). Tempol produced a dose-related decrease in this variable in both groups, which was greater in the T4 group (Fig. 1). The highest dose of tempol decreased the RVR by 13.5 ± 2.1 and 5.5 ± 1.2 mmHg·ml⁻¹·min⁻¹ in hyperthyroid (P < 0.01) and control rats, respectively. Basal RBF was 5.1 ± 0.3 in hyperthyroid rats and 6 ± 0.3 in controls (not significant). These values were not significantly modified by tempol injections in controls and were slightly increased in the T4 group, reaching significance at the highest dose (P < 0.05).

Tempol produced an increase in cortical RBF in control and T4-treated rats, and this effect was markedly higher in the hyperthyroid group (Fig. 1). The highest tempol dose increased CBF by 183 ± 15% in T4 and by 130 ± 12% in controls. MBF of hyperthyroid rats also showed increased responsiveness to tempol administration. The SOD mimetic, when injected at the highest dose, produced a small and nonsignificant rise in MBF in controls (122 ± 17%) and a marked increase in hyperthyroid rats (223 ± 14%).

Administration of bolus doses of 3-CP in hyperthyroid rats was not followed by appreciable changes in the systemic and renal hemodynamic variables studied (data not shown), indicating the specificity of tempol’s effects, as previously reported in dogs (5).

Effects of Continuous Infusion of Tempol on Pressure Natriuresis

Acute tempol administration significantly increased RBF and GFR in hyperthyroid rats but did not significantly affect these variables in control rats (Fig. 2). RPP-RBF and RPP-GFR relationships were significantly increased in the hyperthyroid group infused with the SOD mimetic compared with the nontreated hyperthyroid group. Overall, except for the nontreated hyperthyroid group, RBF and GFR were well autoregulated when RPP was varied in the experimental groups, and both variables were significantly increased by tempol in the hyperthyroid rats (Fig. 2). Tempol did not alter the absolute or fractional diuretic and natriuretic responses to RPP changes in the normal rats (Figs. 3 and 4). However, PDN responses, both absolute and fractional, were significantly improved in hyperthyroid rats, although without attaining normal values (Figs. 3 and 4). Thus, the slopes of the relationship between RPP and urine flow and between RPP and sodium excretion were significantly (P < 0.05) steeper in tempol-treated hyperthyroid rats (1.01 ± 0.03 μl·min⁻¹·g⁻¹·mmHg⁻¹ and 0.17 ± 0.05 μeq·min⁻¹·g⁻¹·mmHg⁻¹, respectively) than in the nontreated hyperthyroid group (0.58 ± 0.17 μeq·min⁻¹·g⁻¹·mmHg⁻¹ and 0.09 ± 0.03 μeq·min⁻¹·g⁻¹·mmHg⁻¹, respectively). In control animals, the slopes of these relationships were not significantly changed by tempol treatment (control: 2.17 ± 0.23; 0.35 ± 0.04; tempol: 2.05 ± 0.38 and 0.30 ± 0.04, respectively).

Total 8-isoprostanate excretion was determined in the urine samples collected in the period with highest BP level (last

Table 1. Biological variables in the experimental groups

<table>
<thead>
<tr>
<th>Variables</th>
<th>Control</th>
<th>T4</th>
</tr>
</thead>
<tbody>
<tr>
<td>Final body weight, g</td>
<td>395.5±6.5</td>
<td>315±10*</td>
</tr>
<tr>
<td>Left kidney weight, mg</td>
<td>930±21</td>
<td>1016±31</td>
</tr>
<tr>
<td>Kidney weight-to-body weight ratio, mg/g</td>
<td>2.35±0.4</td>
<td>3.06±0.10*</td>
</tr>
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<td>MAP, mmHg</td>
<td>108±2.0</td>
<td>140±5.5</td>
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<tr>
<td>Heart rate, beats/min</td>
<td>375±10</td>
<td>510±15.9</td>
</tr>
<tr>
<td>T4, μg/dl</td>
<td>4.7±0.4</td>
<td>50±5.5</td>
</tr>
<tr>
<td>T4, ng/dl</td>
<td>75±3.5</td>
<td>229±8.6</td>
</tr>
<tr>
<td>Plasma MDA, μM/l</td>
<td>5.7±1.5</td>
<td>11.2±1.1*</td>
</tr>
<tr>
<td>Urinary 8-isoprostanate, ng/24 h</td>
<td>5.8±0.4</td>
<td>9.3±0.5*</td>
</tr>
</tbody>
</table>

Data expressed as means ± SE. T4, thyroxine treated; MAP, mean arterial pressure; MDA, malondialdehyde. *P < 0.05, †P < 0.01 versus the control group.
period) in tempol-treated and untreated control and hyperthyroid rats. Urinary 8-isoprostane excretion rate was higher in hypertensive \((P < 0.01)\) than control rats \((4.2 \pm 0.4 \text{ vs. } 2.29 \pm 0.2 \text{ pg·min}^{-1} \cdot \text{g}^{-1}, \text{respectively})\), in consonance with the 24-h total urinary excretion of this marker in these animals (Table 1). Urinary 8-isoprostane excretion levels were lower in the T4+tempol group than in the T4 group \((2.73 \pm 0.3 \text{ pg·min}^{-1} \cdot \text{g}^{-1}) \quad (P < 0.01)\). However, in control rats, the tempol-induced reduction in this excretion rate did not reach statistical significance \((1.99 \pm 0.3 \text{ pg·min}^{-1} \cdot \text{g}^{-1})\).

**DISCUSSION**

This study shows that acute treatment with the antioxidant tempol markedly improves renal hemodynamics and resets the pressure natriuresis response to lower pressures in hyperthyroid rats in association with a reduction in urinary 8-isoprostane excretion. Our group previously demonstrated that chronic treatment with tempol attenuated the development of hypertension in rats administered with T4 for 6 wks (23). Taken together, these data indicate that enhanced O2\(^•\) activity modulates renal hemodynamics and excretory function during thyroid hormone excess, thereby contributing to the pathophysiology of the hypertension of hyperthyroidism.

Acute injection of tempol was followed by a greater decrease in MAP and RVR and a higher increase in cortical and MBF in hyperthyroid rats than in normal rats (Fig. 1). These data clearly demonstrate that the previously reported (23) hyperactivity of ROS in hyperthyroid animals may play an

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**Fig. 1.** Effects of the acute administration of increasing bolus doses of tempol (25, 75, and 150 \(\mu\text{mol/kg iv} \)) on mean arterial pressure (MAP), heart rate (HR), renal vascular resistance (RVR), total renal (RBF), cortical (CBF), and medullary (MBF) blood flow in control and hyperthyroid rats. CBF and MBF responses are expressed as a percentage of the control value (taken as 100%) because these are relative measures of local blood flow. T4, thyroxine treated. \(*P < 0.05, \,**P < 0.01 \text{ vs. baseline levels; } +P < 0.05 \text{ vs. control group.} \)
important role in the increased BP produced by T4 treatment. These results are in consonance with the antihypertensive effect of tempol given chronically in hyperthyroid rats (23), and similar results have been reported in other experimental hypertension models. Thus, Schnackenberg et al. (29) reported that acute tempol administration reduces MAP and RVR in spontaneously hypertensive rats (SHR) to a greater extent than observed in control Wistar Kyoto rats. More recently, Kopkan and colleagues reported that acute intrarenal administration of tempol decreases RVR and increases cortical and MBF in nitric oxide (NO)-deficient (16) and ANG II-infused (15) hypertensive rats.

In the present study, local RBF responses to tempol infusion in T4-treated rats showed higher increases (40% at maximum dose) in MBF than in CBF. This indicates that the generation of O$_2^\cdot$ during chronic T4 administration had a greater effect on the medullary circulation than on the cortical circulation. Previous studies to assess regional blood flow responses to tempol also suggested a greater involvement of O$_2^\cdot$ in the renal medulla (9, 40).

Oxidative stress has been implicated in the pathogenesis of arterial hypertension in genetic and secondary models in rats, and tempol decreases BP in SHR (29, 30), DOCA-salt (1), and NO inhibition-induced hypertension (29). ROS are also known to participate in renal hemodynamics and sodium excretion (7, 12, 25, 39, 40). Thus, Ortiz and Garvin (25) first showed that endogenous O$_2^\cdot$ might act as a physiological regulator of tubular NaCl transport. Other studies in rats demonstrated that the production of O$_2^\cdot$ in renal medulla had vasoconstrictor as well as antidiuretic and antinatriuretic effects (40). Lu and Wang (20) found that O$_2^\cdot$ modulates the effect of NO on basolateral K$^+$ channels in the collecting duct, and Ortiz and Garvin (26) observed that superoxide stimulates NaCl absorption in the thick ascending limb, which was reduced by tempol administration. It has also been reported (38) that O$_2^\cdot$ participates in the regulation of tubuloglomerular feedback. In addition, T3 administration increased intracellular superoxide con-
The shift to the left in the relationship between sodium excretion and RPP induced by tempol in hyperthyroid rats may result from a decrease in sodium reabsorption produced directly via tubular mechanisms and indirectly via dynamic effects. Although tempol administration did not change the autoregulation of RBF and GFR, it significantly increased both variables in hyperthyroid rats, which might contribute to the improved PDN curve in these animals. However, changes in sodium reabsorption appeared to predominate, since tempol produced a decrease in tubular reabsorption, as indicated by the increase in fractional excretion of water and sodium. This effect of tempol on tubular reabsorption might be secondary to the blockade of the multiple antinatriuretic tubular actions of O$_2^-$ (7, 12, 20, 25, 26, 38, 40).

The PDN curve of tempol-treated control rats was similar to that observed in untreated controls (Figs. 3 and 4). The lack of effects of tempol in normal rats may be secondary to the reduced renal hemodynamic response to tempol of these animals (Fig. 1). This is especially evident in the renal medulla, which plays a key role in the control of sodium excretion, as reported above (3). Moreover, because the PDN study was performed at servocontrolled BP levels, the systemic effects of tempol should not affect the renal response in this experiment.

Hyperthyroidism courses with increased sympathetic activity, activation of the renin-angiotensin system, augmented endothelin levels, and increased vasopressin production (36). Moreover, resistance vessels from hyperthyroid rats show increased responsiveness to vasoconstrictors, and, hemodynamically, this model is considered a prototype of cardiogenic hypertension (36). All of these alterations, alone or in combination, are recognized prohypertensive and prooxidant factors. In a previous report, we showed that T$_4$ administration produced a dose-dependent decrease in antioxidant enzymes and an increase in oxidative stress markers (23). The above-reported factors and thyroid hormones may be directly responsible for the increased oxidative stress in hyperthyroidism. Among these factors, the contribution of the renin-angiotensin system may be important, since ANG II increases O$_2^-$ production (13, 15, 19) and the renal results with tempol reported here resemble those previously reported with losartan (32). Unfortunately, however, it cannot be elucidated from the present data whether the protective effects of tempol in this model are secondary to blockade of the O$_2^-$ that can be generated by ANG II or other prooxidant factors present in this model.

The PDN response of hyperthyroid rats was significantly shifted to the left by the antioxidant treatment but did not completely revert to normal values. Therefore, additional factors must be responsible for the differences between hyperthyroid-tempol treated rats and controls. These may include intrinsic defects in renal microvasculature or tubular function, differences in the responsiveness of renal tubules of hyperthyroid rats to sodium- and water-retaining compounds of the hormone cocktail, or endogenous circulating and intrarenal factors known to modulate the PDN response but not analyzed in the present study (4). In conclusion, the present study shows that acute infusion of tempol induced a marked decrease in MAP, improved renal hemodynamics, and produced a leftward shift in the blunted PDN curve of hypertensive hyperthyroid rats. Therefore, these results indicate that ROS may play an important role in the increased BP and blunted PDN curve of hyperthyroid rats.
Perspectives and Significance

These findings and our previous observations indicate that the interaction between $O_2^-$ and $T_4$ plays an important role in the regulation of renal function and BP. To our knowledge, this is the first report to assess the effects of oxidative stress blockade on systemic and renal hemodynamics and sodium excretion in hyperthyroidism. This study opens up a new research approach to renal dysfunction in thyroid diseases and to the physiological and pathophysiological processes induced by $O_2^-$-$T_4$ interaction in other tissues. This study also suggests that antioxidants can be a potential therapeutic tool in hyperthyroidism.

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