Effects of chronic treatment with 7-nitroindazole in hyperthyroid rats

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Wangensteen, Rosemary, Isabel Rodríguez-Gómez, Juan Manuel Moreno, Miriam Álvarez-Guerra, Antonio Osuna, and Félix Vargas. Effects of chronic treatment with 7-nitroindazole in hyperthyroid rats. Am J Physiol Regul Integr Comp Physiol 291: R1376–R1382, 2006.—This study analyzed the contribution of neuronal nitric oxide synthase (nNOS) to the hemodynamic manifestations of hyperthyroidism. The effects on hyperthyroid rats of the chronic administration of 7-nitroindazole (7-NI), an inhibitor of nNOS, were studied. Six groups of male Wistar rats were used: control, T4-NI (30 mg·kg⁻¹·day⁻¹ by gavage), T₄, T₅₀, T₇₅ (50 or 75 μg thyroxine·rat⁻¹·day⁻¹, respectively), T₅₀+7-NI, and T₇₅+7-NI. All treatments were maintained for 4 wk. Body weight, tail systolic blood pressure (SBP), and heart rate (HR) were recorded weekly. Finally, SBP, pulse pressure (PP), and HR were measured in conscious rats, and morphological, metabolic, plasma, and renal variables were determined. Expression of nNOS in the hypothalamus of T₇₅ and control rats was analyzed by Western blot analysis. The response of mean arterial pressure (MAP) to pentolinium (10 mg/kg iv) was used to evaluate the sympathetic activity in the T₄75 and T₄75+7-NI rats. T₄ produced an increased hypothalamic nNOS expression and dose-related increases in blood pressure (BP), HR, and PP vs. control rats. 7-NI did not modify BP or any other hemodynamic variable in normal rats. However, 7-NI produced a marked reduction in BP, HR, PP, and food and water intake in both hyperthyroid groups and improved creatinine clearance in the T₇₅ group. Pentolinium produced a greater MAP decrease in the T₇₅+7-NI than in the T₅₀ group. In conclusion, administration of 7-NI attenuates the hemodynamic and metabolic manifestations of hyperthyroidism, suggesting that nNOS contributes to the hyperdynamic circulation of this endocrine disease by modulating sympathetic activity.

blood pressure; heart rate; pulse pressure; neuronal nitric oxide synthase inhibition

HEMODYNAMIC, CARDIAC, AND RENAL alterations are prominent manifestations of hyperthyroidism (9, 25). The hyperthyroid state courses with hyperdynamic circulation characterized by increased cardiac output, heart rate (HR), pulse pressure (PP), and decreased peripheral resistance (9, 25). The administration of thyroxine to rats produces dose-related increases in blood pressure (BP) (16, 25), cardiac and renal hypertrophy, proteinuria, and a decreased renal ability to excrete sodium after several stresses (16, 20, 25).

NO can be produced by the enzymatic activity of a family of three nitric oxide synthase (NOS) isoforms: neuronal (nNOS), inducible (iNOS), and endothelial (eNOS). These NOS isoforms are present in tissues primarily related to cardiovascular regulation and in the kidney (23). Our group recently observed that administration of the nonspecific NO inhibitor Nω-nitro-L-arginine methyl ester (L-NAME), at a dose without pressor activity in normal rats, or administration of the iNOS inhibitor aminoguanidine increased BP in thyroxine-treated rats (20, 21). However, the effects of nNOS blockade in the cardiovascular manifestations of hyperthyroidism have not been investigated.

Nitrogen heterocyclic compounds represent an important group of NOS inhibitors that bind at the sixth coordination position of the heme iron atom (31). Thus it has been demonstrated that indazole derivatives and especially 7-nitroindazole (7-NI) are potent NOS inhibitors (14, 28, 31, 32). 7-NI is more selective for nNOS than methyl- or nitro-aromatic-based inhibitors, and the administration of this indazole agent to experimental animals inhibits nNOS in the brain without changes in BP or endothelium-dependent relaxation (2, 11, 32). Moreover, 7-NI has been used as an effective nNOS blocker in many different experimental settings, including studies of renal function (1, 3, 30), learning (13), or penile erection (22).

Several studies have demonstrated an important role for nNOS in central and peripheral modulation of sympathetic activity and in the regulation of drinking behavior (18). Thus 7-NI inhibited the sympathoexcitatory cardiovascular response produced in several circumstances (5, 6, 24), and it also inhibited the increase in plasma vasopressin (AVP) produced by salt loading in rats (26).

Because the cardiovascular manifestations of hyperthyroidism are suggestive of an increased sympathetic activity (10) and are associated with polydipsia and polyuria (8), we hypothesized that nNOS may participate in these alterations. With this background, we analyzed the effects of the chronic nNOS blockade with 7-NI on the long-term control of BP and other variables in hyperthyroid rats.

METHODS

Animals

Forty-eight male Wistar rats born and raised in the experimental animal service of the University of Granada were used. The experiment was performed according to European Union guidelines for the ethical care of animals and was approved by the Ethical Committee for Animal Experimentsation of the University of Granada. Rats initially weighing 280 ± 4 g were randomly assigned to the different experimental groups. Each experimental group comprised eight animals, except where stated. All rats had free access to food and tap water. 7-NI (30 mg·kg⁻¹·day⁻¹) was given by gavage because of the low solubility of this compound. Thyroxine (T₄; Merck) was dissolved in isotonic saline plus 0.5 N NaOH (1:100 vol/vol), buffered to pH 7, and subcutaneously injected. The doses of T₄ and 7-NI are in accordance with previous protocols used in our laboratory (16, 29).

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Experimental Protocols

Experiment I. Effects of chronic administration of 7-NI to hyperthyroid rats. The groups were as follows: control, treatment with 7-NI, treatment with thyroxine at 50 (T450) or 75 (T475) µg·rat⁻¹·day⁻¹, and treatment with thyroxine at 50 and 75 µg·rat⁻¹·day⁻¹ plus 7-NI (T450+7-NI and T475+7-NI). The treatments were administered for 4 wk. Body weight, tail systolic BP (SBP), and heart rate (HR) were measured once a week. Tail SBP and HR were measured with the use of tail-cuff plethysmography in unanesthetized rats (LE 5001 pressure meter; Letica, Barcelona, Spain). When the experimental period was completed, all rats were housed in metabolic cages (Panlab, Barcelona, Spain) with free access to food and water and to their respective treatments for 4 days (2 days for adaptation + 2 experimental days), to measure the food and water intake and collect urine samples. Twenty-four-hour urine volume, proteinuria, creatinine, and total excretion of sodium and potassium were measured. The mean values of all intake and urinary variables obtained during the 2 experimental days were used for statistical analyses among the groups.

After completion of the metabolic study, the rats were anesthetized with ethyl ether. A polyethylene catheter (PE-50) containing 100 units of heparin in isotonic sterile NaCl solution was inserted into the femoral artery for intra-arterial BP and HR measurement in conscious rats and for extraction of blood samples. The catheter was tunneled subcutaneously and brought out through the skin at the dorsal side of the neck. Intra-arterial BP was measured at 24 h after implantation of the femoral catheter. Direct BP and HR were recorded continuously for 60 min with a sampling frequency of 400/s (MacLab; AD Instruments, Hastings, UK). The values obtained during the last 30 min were averaged to obtain the BP and HR values used for intergroup comparisons. Subsequently, blood samples taken with the femoral catheter were used to determine total protein, electrolytes, and creatinine concentration. Finally, the rats were killed by exsanguination. The kidneys and ventricles were then removed and weighed. The heart was divided into right ventricle and left ventricle plus septum.

Experiment II. Expression of nNOS was determined by Western blot analysis in the hypothalamus of hyperthyroid (T475) rats treated for 4 wk and in control rats (n=4, each). Hypothalami were homogenized and centrifuged at 12,000 g for 5 min at 4°C. Proteins (30 µg) from the supernatant were subjected to SDS-PAGE using 4–7.5% gels. After electrophoresis, proteins were transferred to a nitrocellulose membrane. The membrane was stained with Ponceau stain, which verified the uniformity of protein load and transfer efficiency across the test samples. After blocking, the membrane was probed with rabbit anti-nNOS polyclonal antibody (BD Biosciences, Fig. 1. Time course of systolic blood pressure (SBP) measured using tail-cuff method (top) and final SBP measured by direct recording (femoral artery) in conscious rats (bottom). Groups are as follows: control, treatment with 7-nitroindazole (7-NI), treatment with thyroxine at 50 (T450) or 75 (T475) µg·rat⁻¹·day⁻¹, and treatment with thyroxine at 50 and 75 µg·rat⁻¹·day⁻¹ plus 7-NI (T450+7-NI and T475+7-NI). Data are means ± SE. *P<0.05; **P<0.01 compared with controls. ++P<0.01 compared with respective T4 group.

Fig. 2. Time course of heart rate (HR) measured using tail-cuff method (top) and final HR measured by direct recording (femoral artery) in conscious rats (bottom). Data are means ± SE. *P<0.05; **P<0.01 compared with controls. ++P<0.01 compared with respective T4 group.
Erembodegen, Belgium). Bound antibodies were detected with a secondary horseradish peroxidase-conjugated goat anti-rabbit antibody (BD Biosciences). The bands were visualized using the enhanced chemiluminescence system ECL Plus (Amersham, Amsersh, UK), and chemiluminescence intensity was quantified with a Kodak Image Station (New Haven, CT).

**Experiment III.** This experiment was performed to evaluate the contribution of sympathetic activity to arterial BP and HR in hyperthyroid and hyperthyroid 7-NI-treated rats. The acute BP and HR responses to an intravenous injection of the sympathic blocker pentolinium (10 mg/kg; Sigma) were analyzed in conscious control, T4, thyroxine; FBW, final body weight; KW/BW, kidney weight-to-body weight ratio; LVW/BW, left ventricular weight-to-body weight ratio; LVW/HVW, left ventricular weight-to-heart ventricular weight ratio. *P < 0.05; †P < 0.01 compared with control group.

**Analytical Procedures**

Proteinuria was measured using the Bradford method (4). Plasma and urinary electrolytes, plasma protein, and creatinine were measured with an autoanalyzer (Beckman CX4; Breca, CA).

**Statistical Analysis**

The evolution of SBP and HR with time was compared with the use of a nested design, with groups and days as fixed factors and rat as a random factor. When the overall difference was significant, Bonferroni’s method with an appropriate error was used. Comparisons of each variable at the end of the experiments were done by performing a one-way ANOVA. When the overall ANOVA was significant, we performed pairwise comparisons using Bonferroni’s methods. The differences were considered significant when P < 0.05. The Western blot analysis data and the acute responses to pentolinium were determined by 10.220.33.6 on April 19, 2017 http://ajpregu.physiology.org/ Downloaded from

**RESULTS**

**Blood Pressure and Heart Rate**

Figures 1 and 2, top, show the time course of the tail SBP and HR, respectively, and Figs. 1 and 2, bottom, show the final SBP and HR measured by direct recording in the experimental groups, respectively. T4 administration produced a dose-related increase in SBP and HR and in final SBP, final HR, and PP compared with control rats. 7-NI administration to normal rats at the dose used in this experiment did not modify the time course evolution of BP, HR, final SBP, final HR, or PP. However, 7-NI administration to hyperthyroid rats reduced BP, HR, and PP in both T4-treated groups. Thus the T50+7-NI group showed SBP and HR evolution and final SBP, final HR, and PP similar to those of control rats, and these variables were significantly attenuated in the T75+7-NI group. PP values in the groups were as follows: control, 36.8 ± 1.6; 7-NI, 34.9 ± 1.6; T450, 48.2 ± 1.0 (P < 0.01 compared with control); T475, 52.1 ± 2.1 (P < 0.01 compared with control); T450+7-NI, 34.1 ± 2.6 (P < 0.01 compared with respective T4-treated group), and T75+7-NI, 39.1 ± 2.6 (P < 0.01 compared with respective T4-treated group).

**Morphological Variables**

All T4-treated groups showed a significant reduction in body weight compared with the control groups. Kidney-weight-to-body weight ratios and left ventricular-weight-to-body weight ratios were significantly increased in T450 and T475 groups compared with controls. The left ventricular-weight-to-heart weight ratio was not significantly modified in T4-treated groups compared with controls. None of these morphological variables were affected by 7-NI treatment in control or T4-treated rats (Table 1).

**Plasma, Metabolic, and Urinary Variables**

There were no significant differences in plasma sodium and potassium among the groups. Total plasma protein concentration was decreased in both T4 groups, and 7-NI administration reversed these decreases and did not significantly modify this variable in control rats. Plasma urea and creatinine were increased in the T75 group and similar to control values in the T75+7-NI group (Table 2).

**Table 1. Morphological variables in experimental groups**

<table>
<thead>
<tr>
<th>Groups</th>
<th>FBW, g</th>
<th>KW/BW, mg/g</th>
<th>LVW/BW, mg/g</th>
<th>LVW/HVW</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>394.3±7.8</td>
<td>2.37±0.05</td>
<td>1.60±0.04</td>
<td>0.80±0.01</td>
</tr>
<tr>
<td>7-NI</td>
<td>370.7±4.6</td>
<td>2.61±0.11</td>
<td>1.70±0.05</td>
<td>0.79±0.01</td>
</tr>
<tr>
<td>T50</td>
<td>338.6±6.8*</td>
<td>3.08±0.07*</td>
<td>2.29±0.06*</td>
<td>0.80±0.01</td>
</tr>
<tr>
<td>T50+7-NI</td>
<td>340.3±5.7*</td>
<td>3.13±0.13</td>
<td>2.06±0.09*</td>
<td>0.78±0.02</td>
</tr>
<tr>
<td>T75</td>
<td>296.3±5.3*</td>
<td>3.21±0.04*</td>
<td>2.36±0.05*</td>
<td>0.79±0.01</td>
</tr>
<tr>
<td>T75+7-NI</td>
<td>305.3±6.5†</td>
<td>3.01±0.05*</td>
<td>2.25±0.05*</td>
<td>0.81±0.01</td>
</tr>
</tbody>
</table>

Data are expressed as means ± SE. 7-NI, 7-nitroindazole; T4, thyroxine; FBW, final body weight; KW/BW, kidney weight-to-body weight ratio; LVW/BW, left ventricular weight-to-body weight ratio; LVW/HVW, left ventricular weight-to-heart ventricular weight ratio. *P < 0.05; †P < 0.01 compared with control group.

**Table 2. Plasma variables in experimental groups**

<table>
<thead>
<tr>
<th>Groups</th>
<th>Na*, meq/l</th>
<th>K*, meq/l</th>
<th>Creatinine, mg/dl</th>
<th>Total Protein, g/dl</th>
<th>BUN, mg/dl</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>140.8±0.30</td>
<td>4.63±0.10</td>
<td>0.58±0.03</td>
<td>5.97±0.08</td>
<td>37.8±1.76</td>
</tr>
<tr>
<td>7-NI</td>
<td>140.5±0.37</td>
<td>4.61±0.14</td>
<td>0.52±0.01</td>
<td>6.12±0.07</td>
<td>33.3±2.00</td>
</tr>
<tr>
<td>T50</td>
<td>139.5±0.52</td>
<td>4.70±0.17</td>
<td>0.60±0.02</td>
<td>5.03±0.11*</td>
<td>34.2±2.91</td>
</tr>
<tr>
<td>T50+7-NI</td>
<td>142.1±0.10</td>
<td>4.80±0.15</td>
<td>0.58±0.01</td>
<td>5.79±0.07†</td>
<td>43.6±4.78</td>
</tr>
<tr>
<td>T75</td>
<td>140.8±0.17</td>
<td>4.62±0.09</td>
<td>0.96±0.06+</td>
<td>4.55±0.18</td>
<td>68.6±5.88*</td>
</tr>
<tr>
<td>T75+7-NI</td>
<td>141.2±0.29</td>
<td>4.64±0.17</td>
<td>0.54±0.02†</td>
<td>5.46±0.11†</td>
<td>41.1±2.10‡</td>
</tr>
</tbody>
</table>

Data are expressed as means ± SE. *P < 0.05; †P < 0.01 compared with control group. ‡P < 0.05 compared with respective T4 group.
Metabolic studies at the end of treatment showed increased food and fluid intake (g/100 g body wt) in all T4-treated groups compared with controls. Food intake was significantly reduced in the T475 group compared with the T475 group. The 7-NI group showed increased fluid intake compared with the T475 group. Thus 7-NI administration produced a reduction in water intake in both T4-treated groups. However, 7-NI administration produced a reduction in water intake in both T4-treated groups. These data are consistent with the greater food intake of T4-treated rats. 7-NI did not significantly affect natriuresis or kaliuresis in control or T4-treated groups (data not shown).

Water and sodium balances, proteinuria, and creatinine clearance (CrC) values are summarized in Table 4. Water and sodium balances significantly increased in both T4-treated groups, whereas significance was not reached in the T4-7-NI-treated groups compared with controls. 7-NI administration reduced diuresis in both T4-treated groups. Total sodium and potassium excretion was higher in the T4-treated animals. These data are consistent with the greater food intake of T4-treated rats. 7-NI did not significantly affect natriuresis or kaliuresis in control or T4-treated groups (data not shown).

Proteinuria was significantly increased in all T4-treated groups and was unaffected by 7-NI treatment. However, 7-NI produced a mild increase in proteinuria in control rats. Creatinine clearance normalized per gram of kidney weight was similar in all experimental groups, with the exception of a lower value in the T475 group.

Hypothalamic nNOS Expression

Hypothalamic nNOS expression was higher in hyperthyroid rats than in control rats (Fig. 3).

Blood Pressure Response to Ganglionic Blockade

Figure 4 summarizes the data concerning the sympathetic component of BP in the experimental groups. The values are expressed as the percentage of maximal inhibition on the variables induced by pentolinium injection. Ganglionic blockade produced a similar decrease in mean arterial pressure (MAP) in control and hyperthyroid rats, whereas the T475+7-NI group showed a greater decrease in MAP. Pentolinium administration did not significantly modify HR in control rats but produced a significant increase of HR in hyperthyroid rats, which was similar between T475 and T475+7-NI rats.

DISCUSSION

This study shows that hyperthyroid rats had an increased hypothalamic nNOS expression and that the administration of the nNOS inhibitor 7-NI prevented or attenuated the BP, HR, and PP increases produced by increasing doses of thyroxine. Moreover, 7-NI administration attenuated the increased food intake and polydipsia of hyperthyroid rats in this study. Therefore, our results suggest that nNOS may participate in developing the characteristic manifestations of hyperdynamic circulation and in the food and fluid intake behavior of hyperthyroid rats. The hemodynamic data clearly contrast with previous studies from our laboratory showing that the nonspecific blockade of NOS activity with L-NAME (20) or the specific blockade of iNOS with aminoguanidine (21) aggravates the time course of hypertension in hyperthyroid rats. Together, these findings suggest that prohypertensive effects predominate after nonspecific NOS blockade, overriding the antihypertensive effects of nNOS blockade.

Table 3. Food and fluid intake in experimental groups

<table>
<thead>
<tr>
<th>Groups</th>
<th>Food Intake, g/100 g body wt·24 h⁻¹</th>
<th>Water Intake, ml/100 g body wt·24 h⁻¹</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>5.01±0.16</td>
<td>5.35±0.38</td>
</tr>
<tr>
<td>7-NI</td>
<td>5.21±0.24</td>
<td>7.26±0.39*</td>
</tr>
<tr>
<td>T450</td>
<td>7.58±0.35*</td>
<td>8.57±0.28*</td>
</tr>
<tr>
<td>T450+7-NI</td>
<td>7.58±0.43*</td>
<td>6.59±0.45†</td>
</tr>
<tr>
<td>T475</td>
<td>8.16±0.31†</td>
<td>10.9±1.17†</td>
</tr>
<tr>
<td>T475+7-NI</td>
<td>7.22±0.26‡</td>
<td>6.32±1.02‡</td>
</tr>
</tbody>
</table>

Data are expressed as means ± SE. *P < 0.05 compared with control group. †P < 0.01 compared with T475 group.

Table 4. Water and sodium balances, proteinuria, and creatinine clearance in experimental groups

<table>
<thead>
<tr>
<th>Groups</th>
<th>Water Balance, ml/100 g body wt·24 h⁻¹</th>
<th>Sodium Balance, mmol/100 g body wt·24 h⁻¹</th>
<th>Protein Excretion Rate, mg/mg creatinine</th>
<th>Creatinine Clearance, ml/min · g kidney⁻¹</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>3.49±0.33</td>
<td>0.18±0.04</td>
<td>5.41±0.17</td>
<td>0.49±0.06</td>
</tr>
<tr>
<td>7-NI</td>
<td>3.28±0.14</td>
<td>0.14±0.02</td>
<td>7.92±0.97*</td>
<td>0.47±0.02</td>
</tr>
<tr>
<td>T450</td>
<td>5.58±0.53*</td>
<td>0.29±0.05*</td>
<td>9.54±0.41*</td>
<td>0.42±0.03</td>
</tr>
<tr>
<td>T450+7-NI</td>
<td>4.41±0.73</td>
<td>0.24±0.04*</td>
<td>9.57±0.52*</td>
<td>0.48±0.021</td>
</tr>
<tr>
<td>T475</td>
<td>5.85±0.45*</td>
<td>0.26±0.03*</td>
<td>11.7±0.71*</td>
<td>0.30±0.02*</td>
</tr>
<tr>
<td>T475+7-NI</td>
<td>4.75±0.43</td>
<td>0.18±0.02†</td>
<td>11.0±0.44*</td>
<td>0.52±0.04†</td>
</tr>
</tbody>
</table>

Data are expressed as means ± SE. *P < 0.05 compared with control group. †P < 0.05 compared with T475 group.
Furthermore, 7-NI inhibited the reflex sympathetic pressor response to bradykinin (24). In addition, indazole derivatives and the α2-adrenergic agonist clonidine are structurally similar. Based on this observation, Venturini et al. (27) analyzed clonidine as a nNOS inhibitor and reported that this drug competitively inactivated nNOS without affecting iNOS or eNOS activities in vitro. Clonidine inhibits central sympathetic activity, reducing BP and HR, effects that resemble those produced by 7-NI in hyperthyroid rats.

Another possible explanation for the antihypertensive effects of 7-NI in hyperthyroid rats is its action on water and sodium handling, probably mediated by an increased renal sympathetic tone. Thus water and sodium balances were significantly increased in both T4-treated groups, whereas the comparison between T4-7-NI-treated groups and controls did not reach significance. These results suggest that 7-NI treatment attenuates these positive balances, which might contribute to its antihypertensive effect in hyperthyroid rats.

The absence of changes in BP or HR after chronic administration of 7-NI to control rats is consistent with previous observations in rats and mice. Chronic administration of 7-NI did not modify BP or HR in normal, sensitized saline-drinking, or deoxycorticosterone acetate (DOCA)-treated rats (29) or in male or female rats studied in different experimental settings (1, 3). Moreover, absence of the gene for the neural isoform of NOS had no effects on the BP of knockout mice (17). Together, all these data indicate that the hemodynamic effects of 7-NI administration (nNOS blockade) in hyperthyroid rats are not due to nonspecific antithyroid effects of this drug.

Results show that ganglionic blockade produces a similar decrease in MAP in hyperthyroid and control rats and an increase in HR in hyperthyroid rats, whereas it did not change HR in control rats. These data are similar to those reported by Foley et al. (7) in hyperthyroid rats after ganglionic blockade with trimethaphan. These authors suggest that the increase in HR induced by the autonomic blockade indicates an increased parasympathetic influence on this variable in hyperthyroid rats, which may be a compensatory response against the direct stimulating effects of thyroid hormones on HR. In any case, our results in the T4-7-NI group clearly indicate a reduced sympathetic contribution to resting MAP, as reported above, despite the increase in HR induced by the ganglionic blockade.

One critical issue in this study concerns the specificity of 7-NI as an inhibitor of nNOS. Several studies have indicated that 7-NI selectively inhibits nNOS without affecting eNOS or iNOS (2, 11, 32). In fact, 7-NI was able to block NOS activity in the cerebellum, a region with the highest levels of NOS in the central nervous system, without altering BP (2, 14, 15). Moreover, many laboratories have reported that acute and chronic 7-NI administration to normal rats does not affect BP or endothelium-dependent vasodilatation (1, 3, 11, 14, 29), although Zagvazdin et al. (33) observed a pressor response after 7-NI administration (50 mg/kg ip) to conscious rats. Other studies indicate that 7-NI has little effect on iNOS (32). Thus 7-NI produced greater inhibition of nNOS than of iNOS in lung cells (15) and renal medulla (12) of rats.

The 7-NI group showed a mild polyuria-polydipsia syndrome, in agreement with previous observations by our group (29). This phenomenon has been attributed to an inhibitory effect of 7-NI on the release of AVP, because nNOS and AVP are colocalized in the supraoptic and paraventricular nuclei.
(18) and other forebrain structures that participate in the regulation of drinking behavior. In fact, the systemic administration of 7-NI was recently shown to inhibit the increase in plasma AVP produced by salt loading in rats (26). However, 7-NI administration to hyperthyroid rats produced a decreased fluid intake. We lack a reasonable explanation for this last result, but it is consistent with the inability of 7-NI to increase fluid intake in saline-drinking or DOCA-treated rats (29).

Hyperthyroidism produces cardiac hypertrophy (9, 20, 25). However, the left ventricular-weight-to-heart weight ratio, an index of absolute left ventricular hypertrophy, was not affected by T₄ treatment, indicating that cardiac hypertrophy in hyperthyroidism involves both ventricles as previously reported (20). 7-NI treatment did not modify either ratio in the hyperthyroid groups, despite its antihypertensive effect. These data confirm previous findings (16, 20) indicating that ventricular hypertrophy in hyperthyroidism is unrelated to the BP level.

Total plasma protein concentration, an index widely used, is decreased in hyperthyroid rats, indicating intravascular volume expansion. This alteration is in agreement with previous findings by our group (20) and may be related to the positive water and sodium balances of these animals. Plasma protein concentration was significantly increased by 7-NI treatment in hyperthyroid rats. The basis for this effect is not clear, although the ability of 7-NI to reduce plasma urea and creatinine levels and CrCl and to attenuate the positive water and sodium balances in hyperthyroid rats indicates that this drug may improve renal function, and therefore blood volume, suggesting the possible participation of nNOS in the renal dysfunction of these animals. The proteinuria observed in the present T₄-treated rats was not affected by 7-NI treatment, despite its antihypertensive effect. However, the administration of 7-NI to normal rats unexpectedly increased proteinuria. These observations agree with previous reports that the proteinuria of hyperthyroid rats is not related to BP levels (16, 20, 21).

In conclusion, the present study has shown that chronic 7-NI treatment suppresses or attenuates the characteristic hemodynamic manifestations of hyperthyroidism, suggesting that nNOS contributes to developing the hyperdynamic circulation of hyperthyroidism. This study also has demonstrated that 7-NI administration attenuates the polyphagia and polydipsia of hyperthyroid rats and improves their glomerular filtration rate.

Perspectives

This study has shown that 7-NI administration prevents hemodynamic and other characteristic manifestations of hyperthyroidism by a mechanism that is unrelated to a nonspecific antithyroid effect. The data reported indicate that nNOS participates in the pathophysiology of hyperthyroidism. To our knowledge, this is the first report that assesses the effects of the blockade of nNOS isoform on hemodynamic and renal abnormalities in hyperthyroidism, opening up a new line for the study of cardiovascular abnormalities in thyroid disorders. This study also suggests that 7-NI may be of therapeutic value in the hyperthyroid state.

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