Effects of methimazole in the early and established phases of N⁶-nitro-L-arginine methyl ester hypertension

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In the present study we evaluated the effects of methimazole, an antithyroid drug, on blood pressure and other variables in the early and established phases of hypertension induced by the inhibition of nitric oxide synthesis with the oral administration of N⁶-nitro-L-arginine methyl ester (l-NAME), 75 mg/100 ml in the drinking water. Moreover, we also evaluated the acute pressor effect of l-NAME on systemic blood pressure in control and rats treated chronically with methimazole, administered via drinking water (30 mg/100 ml). Oral administration of methimazole maintained the blood pressure of l-NAME-treated rats at normal levels 25 days after induction of hypertension. However, after 25 days of methimazole treatment in rats made hypertensive with l-NAME (for 25 days), high blood pressure was similar in methimazole-treated and non-treated l-NAME rats, despite the fact that a hypothyroid state had been achieved in the methimazole-treated rats. Acute intravenous injection of l-NAME caused a similar increase in mean arterial pressure in control and methimazole-treated rats at the lowest dose; however, smaller pressor responses were observed with increasing doses in hypothyroid rats. These results clearly demonstrate that hypothyroidism induced by methimazole prevents, but does not reverse, l-NAME hypertension and reduces the acute pressor responsiveness to l-NAME administration.

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Acute inhibition of NO synthesis with analogues, such as N⁶-nitro-L-arginine methyl ester (l-NAME), produces a dose-dependent increase in systemic blood pressure in anaesthetized and conscious rats (1, 2). Long-term oral administration of this inhibitor induces a dose- and time-dependent arterial hypertension (3, 4).

Thyroidectomy and antithyroid drugs reduce high blood pressure in several models of experimental hypertension (5–7). Recent studies have demonstrated that antithyroid treatments prevent spontaneous (8, 9), DOCA salt (10, 11), Goldblatt two-kidney–one-clip and low renal mass (12, 13) hypertension, whereas thyroxine accelerates its appearance in the DOCA salt model (11). To date, however, the effects of hypothyroidism in hypertension induced by the inhibition of nitric oxide synthesis with l-NAME have not been evaluated. In this study we determined the effects of methimazole, an antithyroid drug, on blood pressure and other variables in the early and established phases of l-NAME hypertension. We also evaluated the effect of methimazole on the systemic blood pressure increase induced by acute intravenous administration of l-NAME.

Methods

Animals

Male Wistar rats born and raised in the animal house of the University of Granada were used in the present studies. All experiments were performed according to the guidelines for the ethical care of the animals of the European Union. Rats weighing 100–125 g were assigned randomly to the different experimental protocols. All animals had free access to a standard rat diet (D04-UAR, Panlab S.L., Barcelona, Spain) and tapwater ad libitum, except where stated.

Experimental protocol

Experiment 1: Pressor responsiveness to acute l-NAME administration. The animals were divided into two groups: control and hypothyroid rats (n = 8 in each group). Hypothyroidism was induced by the continuous administration of (30 mg/100 ml) methimazole via drinking water for 28 days (Fig. 1). The estimated daily intake corresponded to a dose of approximately 17 mg·kg⁻¹·day⁻¹. The effectiveness of this treatment was assessed by comparing serum thyroxine (T₄), serum triiodothyronine (T₃), heart rate and the final heart and body weights of control and treated rats. After 28 days of treatment, the rats were anaesthetized 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 either direct blood pressure and heart rate measurement or extraction of blood samples. An additional catheter
was inserted into the femoral vein for the injection of L-NAME. Both catheters were tunneled subcutaneously and exteriorized at the dorsum of the neck.

After a 24-h recovery period, direct mean arterial pressure (MAP) was recorded through the femoral artery catheter in conscious rats (TRA-121 transducer connected to a two-channel Letigraph 2000 recorder, Letica SA, Barcelona, Spain). When the blood pressure had stabilized, the pressor responses to a series of intravenous bolus injections of L-NAME were measured. The doses of L-NAME were always given in ascending order (1, 10, and 30 mg/kg). The minimum time between successive doses was 5 min, and the MAP was not allowed to recover after each dose. The results obtained represent cumulative responses. N\textsuperscript{\textcircled{\textnormal{O}}}2-Nitro-L-arginine methyl ester was dissolved in isotonic saline and administered in 0.1-ml boluses. The venous catheter was flushed with 0.1 ml of saline after each injection. A control injection of saline (0.1 ml) was given to each rat to ensure that the responses to drug injections were not artifacts. Thirty minutes after the last dose, blood samples from the arterial catheter were taken to determine serum T\textsubscript{3} and T\textsubscript{4} levels.

Experiment 2: Effects of methimazole in the acute phase of L-NAME hypertension. The rats were divided randomly into four groups: control (N = 8), L-NAME (N = 8), methimazole-treated (MTZ, N = 8) and L-NAME plus methimazole-treated (L-NAME + MTZ, N = 8). N\textsuperscript{\textcircled{\textnormal{O}}}2-Nitro-L-arginine methyl ester was given to both L-NAME groups in the drinking water at a concentration of 75 mg/100 ml, resulting in a daily intake of approximately 65 mg kg\textsuperscript{-1} day\textsuperscript{-1}. Drinking water was replaced with 30 mg/100 ml (approximately 26 mg kg\textsuperscript{-1} day\textsuperscript{-1}) methimazole solution for MTZ rats, and with methimazole plus L-NAME solution for L-NAME + MTZ rats, while the control rats drank tapwater ad libitum. The concentration of L-NAME in the L-NAME + MTZ group was adjusted every 2 days according to the fluid intake (ml/100 g) of these animals, to ensure that a similar dose in both L-NAME and L-NAME + MTZ groups was administered. Methimazole treatment was started 14 days before the induction of L-NAME hypertension, and L-NAME treatment was maintained for 25 days (Fig. 1).

Body weight and tail systolic blood pressure (SBP) were determined twice a week during the course of the experiment. Systolic blood pressure was measured by tail-cuff plethysmography in unanaesthetized rats (LE 5001 Pressure Meter, Letica SA, Barcelona, Spain). At least seven determinations were made in every session and the mean of the lowest three values within 5 mm Hg was used to obtain the SBP level.

After the time course study, all animals were housed in metabolic cages with free access to food and their respective drinking fluids. After 2 days of adaptation, food and water intake and excretion of water, sodium and potassium were determined every 24 h during three consecutive days. The values obtained on each experimental day were averaged for statistical purposes.

After the metabolic study was completed, the femoral artery was cannulated. After a 24-h recovery period, direct SBP, pulse pressure (PP) and heart rate (HR) were recorded continuously for 60 min. The values obtained during each of the last 30 min were averaged to obtain the mean blood pressure value. Blood samples from the femoral catheter were taken to determine serum urea, creatinine, electrolytes and thyroid hormones. Plasma T\textsubscript{4} and T\textsubscript{3} levels were determined by ELISA (immunoassay system, Baxter, Miami, USA) with intra- and interassay coefficients of variation of 4.8 and 7.3%, respectively (13). Sodium, potassium, urea and creatinine were measured on the day by an autoanalyser (Beckman CX4, USA). Body weight (BW), heart weight (HW) and kidney weights (KW) were also measured at the end of the study.

Experiment 3: Effects of methimazole in the established phase of L-NAME hypertension. Twenty-five days after the induction of hypertension, 14 L-NAME hypertensive rats were divided into two groups L-NAME and L-NAME + MTZ rats (N = 7 in each group). Methimazole treatment was maintained for 25 days (Fig. 1) at the same concentration (30 mg/100 ml), and the concentration of L-NAME was adjusted according to the fluid intake related to body weight. The variables measured were the same as those in Experiment 2. Tail SBP and BW were measured during the course of the experiment. At the end of the time course study, metabolic and plasma variables were measured and SBP, HR and PP were determined by direct recording. Finally, the body, heart and kidney were weighed.

Statistical analysis

Analysis of the nested design was carried out with
Fig. 2. (Upper panel) Line graph showing the time course of changes in systolic blood pressure (SBP), measured by the tail-cuff method, in the early and established phases of N^G^-nitro-L-arginine methyl ester (l-NAME) hypertension. (Lower panel) Bar graph showing the final SBP measured by direct recording (femoral artery) at the end of the early and established phases of l-NAME hypertension in conscious control rats (C), rats treated with l-NAME (N), rats treated with methimazole (M) and rats treated with l-NAME and MTZ (NM). Values are means±SEM: *p < 0.01 and **p < 0.001 versus control: +++p < 0.001 versus l-NAME group.

groups and doses to compare the dose–response curve to l-NAME. The design had two fixed effect factors (group and dose) and one random effect factor (the rat), this factor being nested in the group. When the different tests for factors and the group–dose interaction were significant, groups at different doses were compared.

The evolution of tail SBP and BW over time in the early and established phases was compared using the nested design, with groups and days as fixed factors and the rat as the random factor. When the overall difference was significant, comparisons were made using Bonferroni’s method with an appropriate error.

The rest of the variables were compared at the end of the experiments with one-way ANOVA analysis and subsequent pairwise comparisons with Newmann–Keuls and t-Tukey schemes (15, 16). The statistical package used was BMDP with 2V and 8V programs.

Results

Animals given methimazole gained significantly less weight than their matched controls during the experimental period. Direct SBP (Fig. 2), HR and PP (Fig. 3), BW, HW and KW (Table 1) and serum T3 and T4 levels
Fig. 3. Final heart rate (HR, upper panel) and pulse pressure (PP, lower panel) measured by direct recording (femoral artery) at the end of the early and established phases of N°-nitro-L-arginine methyl ester (l-NAME) hypertension in conscious control rats (C), rats treated with l-NAME (N), rats treated with methimazole (M) and rats treated with l-NAME and MTZ (NM). Values are means ± SEM; *p < 0.01 versus control; **p < 0.01 and ***p < 0.001 versus l-NAME group.

Table 1. Morphological variables measured at the end of the experimental periods.*

<table>
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<th>Early phase</th>
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<th>Established phase</th>
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<tbody>
<tr>
<td></td>
<td>Control (N = 8)</td>
<td>MTZ (N = 8)</td>
<td>l-NAME (N = 8)</td>
</tr>
<tr>
<td>BW (G)</td>
<td>378 ± 9</td>
<td>274 ± 9*</td>
<td>381 ± 14</td>
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<tr>
<td>KW (mg)</td>
<td>1122 ± 35</td>
<td>781 ± 42*</td>
<td>1320 ± 29</td>
</tr>
<tr>
<td>HW (mg)</td>
<td>949 ± 12</td>
<td>682 ± 17*</td>
<td>1150 ± 29*</td>
</tr>
<tr>
<td>KW/BW (mg/g)</td>
<td>3.07 ± 0.07</td>
<td>2.84 ± 0.15</td>
<td>3.51 ± 0.19</td>
</tr>
<tr>
<td>HW/BW (mg/g)</td>
<td>2.49 ± 0.07</td>
<td>2.50 ± 0.10</td>
<td>3.04 ± 0.08*</td>
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* Values are expressed as means ± SEM; *p < 0.01 versus control and †p < 0.01 versus corresponding l-NAME group. Abbreviations: KW/BW = kidney weight/body weight ratio; HW/BW = heart weight/body weight ratio; control = control rats; MTZ = methimazole-treated rats; l-NAME = rats treated with N°-nitro-L-arginine methyl ester (l-NAME); l-NAME + MTZ = rats treated with l-NAME and methimazole.
were decreased in hypothyroid rats (Table 2). These results indicate that methimazole-treated rats developed the characteristic manifestations of hypothyroidism in the different experimental protocols.

![Graph](image-url)

Fig. 4. Effects of cumulative bolus doses of N^6^-nitro-l-arginine methyl ester (l-NAME) on mean arterial pressure (MAP) in conscious control (CONT) and methimazole-treated hypothyroid (MTZ) rats. Data are means ± SEM; *p < 0.05 and **p < 0.01 compared with the control group.

Table 2. Plasma variables measured in conscious rats at the end of the experimental periods.a

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<th>Early phase</th>
<th>Established phase</th>
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<tr>
<td></td>
<td>Control (N = 8)</td>
<td>MTZ (N = 8)</td>
</tr>
<tr>
<td>Sodium (mmol/l)</td>
<td>141.5 ± 2.3</td>
<td>141.2 ± 1.2</td>
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<tr>
<td>Potassium (mmol/l)</td>
<td>4.1 ± 0.05</td>
<td>3.7 ± 0.09</td>
</tr>
<tr>
<td>Urea (mg/dl)</td>
<td>27.3 ± 1.0</td>
<td>41.5 ± 1.5*</td>
</tr>
<tr>
<td>Creatinine (mg/dl)</td>
<td>0.28 ± 0.04</td>
<td>0.31 ± 0.02</td>
</tr>
<tr>
<td>T(_1) (µg/l)</td>
<td>550 ± 73</td>
<td>50 ± 35*</td>
</tr>
<tr>
<td>T(_2) (ng/l)</td>
<td>42.5 ± 1.8</td>
<td>2.5 ± 2.1*</td>
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</table>

\(^{a}\) Values are expressed as means ± sem; *p < 0.01 versus control and \(^{\dagger}\)p < 0.01 versus corresponding l-NAME group. Abbreviations: control = control rats; MTZ = methimazole-treated rats; l-NAME = rats treated with N^6^-nitro-l-arginine methyl ester (l-NAME); l-NAME + MTZ = rats treated with l-NAME and methimazole.

The pressor responses of control and hypothyroid rats to the intravenous bolus administration of l-NAME are shown in Fig. 4. In both groups, acute NO inhibition with l-NAME was associated with increases in arterial pressure: MAP rose in a dose-dependent fashion in both groups of animals. The lowest dose of l-NAME produced similar increases in MAP in treated and control rats. However, with higher doses of l-NAME the increase in arterial pressure was smaller in hypothyroid animals than in controls.

Figure 2 shows the evolution of systolic blood pressure recorded by the tail-cuff method. The results clearly demonstrate that treatment with methimazole before the administration of l-NAME prevented the development of hypertension. However, when methimazole was administered in the established phase, blood pressure remained at hypertensive values. These data were later confirmed by the direct recordings made at the end of the early (control = 124 ± 4.3, l-NAME = 199 ± 6.7, MTZ = 108 ± 4.3 and l-NAME + MTZ = 130 ± 4.2 mmHg) and established (l-NAME = 204 ± 8.2 and l-NAME + MTZ = 191 ± 2.7 mmHg) phases of hypertension.

Pulse pressure was significantly increased in l-NAME hypertensive rats in comparison with control rats 25 days after induction of hypertension (Fig. 3). Significant reduction in HR and PP were observed in both l-NAME + MTZ groups when compared with the corresponding l-NAME groups (Fig. 3).

Weekly increases in BW (Fig. 5) and final BW (Table 1) were similar in control and l-NAME groups (early phase). Absolute and relative HW were significantly elevated in l-NAME-treated rats compared with the control group (Table 1). The body weights both during (Fig. 5) and at the conclusion of the experiment (Table 1) were reduced significantly in both l-NAME + MTZ groups (i.e. during both early and established phases) relative to the rats receiving l-NAME alone. Absolute and relative HW and KW were also decreased significantly in both l-NAME + MTZ groups when compared with the l-NAME groups (Table 1).

No significant differences in plasma sodium and potassium were observed between the different experimental groups, but plasma urea and creatinine were increased significantly in both l-NAME hypertensive groups (Table 2), and plasma urea was higher in the MTZ group.

Metabolic studies at the end of the treatment showed similar food and fluid intakes in all groups, although a tendency to lower food and fluid intake was observed in all methimazole-treated groups (Table 3). Urinary volume and sodium and potassium excretion were not significantly modified in the different experimental groups in comparison with their respective control groups. However, a tendency to lower values for the urinary variables was observed in all the methimazole-treated groups (Table 4).
Fig. 5. Time course of body weight changes in the experimental groups. Values are means ± SEM: * p < 0.01 versus L-NAME group.

Discussion

Acute in vivo administration of L-arginine analogues markedly increases MAP, presumably as a consequence of an abrupt inhibition of NO biosynthesis (1, 17). In the present study we investigated the pressor responsiveness to increasing doses of the L-arginine analogue L-NAME to conscious control and hypothyroid rats. The findings of this study show that hypothyroid rats were less responsive than control animals to the pressor effect of NO inhibition. Because NO exerts a tonic vasorelaxing effect on the microcirculation, its inhibition allows vasoconstrictor agents to predominate, resulting in increased arterial pressure. These results might indicate that the reduced pressor responsiveness to L-NAME in thyroid disorders results from reduced basal NO activity, or from decreased responsiveness to endogenous vasoconstrictor agents. The latter situation may arise in hypothyroid rats, in which decreased sensitivity to vasoconstrictors was reported both in conscious animals (18) and in isolated vascular preparations (19).

Methimazole administration via the drinking water effectively prevented the development of L-NAME hypertension. Similar results have been observed in rats after interfering with the function of the thyroid gland by surgical or chemical means (propylthiouracil, radioactive iodine, methimazole) in other models of experimental hypertension (5–13). However, the results of the present work demonstrate that hypothyroidism induced by methimazole did not reduce significantly the blood pressure in established L-NAME hypertension. These results agree with those obtained by previous authors who failed to normalize BP with antithyroid treatments in the established phase of genetic (8), DOCA salt (6) and Goldblatt two-kidney–one-clip (20) hypertension. However, our findings contrast with previous observations in the low renal mass model, where the hypothyroidism induced by methimazole was shown to be equally effective in decreasing BP in both the early and established phases of hypertension (12). The reasons for the discrepant

<table>
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<th>Table 3. Food and fluid intake measured at the end of the experimental periods.</th>
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<tr>
<td><strong>Early phase</strong></td>
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<tr>
<td><strong>Control (N = 8)</strong></td>
</tr>
<tr>
<td>Food intake (g/100 g)</td>
</tr>
<tr>
<td>Fluid intake (ml/100 g)</td>
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</table>

*Values are expressed as means ± SEM. Abbreviations: control = control rats; MTZ = methimazole-treated rats; L-NAME = rats treated with N^o-,o-nitro-L-arginine methyl ester (L-NAME); L-NAME + MTZ = rats treated with L-NAME and methimazole.*
effects of hypothyroidism in the established phase of hypertension have not been investigated, although morphological changes as well as modifications in collagen synthesis or distribution in the vascular wall may be involved (21).

One important cardiovascular manifestation of hypothyroidism is reduced HR, which can contribute to the hypotensive effect of hypothyroidism and thus decrease cardiac output. However, the rats given methimazole in the established phase of L-NAME hypertension showed a marked reduction in HR, whereas their BP was not modified. These results indicate that the reduction in HR is not in itself responsible for the hypotensive effect of hypothyroidism in experimental hypertension. In this regard, Rioux and Berkowitz (8) also observed that HR may be severely reduced without restoring normotension in 10-week-old, thyroidectomized rats with spontaneous hypertension.

Arterial hypertension is commonly associated with relative cardiac hypertrophy (20, 22). Controversy exists, however, regarding the presence of cardiac hypertrophy in the L-NAME model (3, 4, 23). Our results indicate that both groups of L-NAME-treated animals (early and established phases) show an increased HW both absolute and relative to BW. These findings are compatible with previous data from our laboratory (4) and from Jover et al. (24). However, Arnal et al. did not find cardiac hypertrophy in several groups of rats treated with different doses of the NO inhibitor (25). These differences are not easy to explain, especially considering that the BP levels and duration of treatment were very similar in these studies. Marked reduction in HW as well as in the HW/BW ratio were noted in both groups of L-NAME + MTZ rats, giving rise to a dissociation between cardiac hypertrophy and hypertension in the established phase. This dissociation was also observed in Goldblatt two-kidney–one-clip hypertension (20) and in DOCA salt (6) hypertension, where hypothyroidism reversed cardiac hypertrophy regardless of the effect on BP.

It is known that gains in BW are reduced in rats in which a hypothyroid state has been induced (26). In fact, our L-NAME + MTZ rats in the early and established phases showed decreased BW when compared with the corresponding rats given L-NAME alone. Furthermore, it is also well known that reduction in BW is accompanied by a decrease in BP (27). Hence, the reduction in BW produced by methimazole in rats might play a role in its antihypertensive effect. However, the reduced BW with high levels of BP in the L-NAME + MTZ group of the established phase suggests that the reduction in BP observed after methimazole treatment is not due to a non-specific effect on BW.

Several studies seem to indicate that hypothyroidism in rats is accompanied by increased diuresis and natriuresis (26). This tendency toward sodium loss has been implicated as a mechanism by which hypothyroidism prevents experimental arterial hypertension (27). However, although we do not have information on the changes in sodium balance that may have occurred in the initial days of both time course studies, the data reported here (Table 4) and in previous reports indicate that treatment with methimazole of normal (14) or hypertensive rats (13) does not modify significantly the urinary sodium excretion under normal conditions or after different stresses (28). Thus, the hypotensive effect of methimazole is difficult to explain in terms of increased sodium loss.

In summary, the results of this study demonstrate that treatment with methimazole reduced the acute pressor response to L-NAME, prevented but did not reverse L-NAME hypertension in rats and reduced relative cardiac hypertrophy. However, the mechanism by which hypothyroidism produces these effects requires further investigation.

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**Table 4. Urinary variables measured at the end of the experimental periods.**

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<tr>
<th></th>
<th>Control (N = 8)</th>
<th>MTZ (N = 8)</th>
<th>L-NAME (N = 8)</th>
<th>L-NAME + MTZ (N = 8)</th>
<th>Established phase</th>
</tr>
</thead>
<tbody>
<tr>
<td>Urine volume (ml/100 g)</td>
<td>4.20 ± 0.50</td>
<td>3.26 ± 0.26</td>
<td>4.04 ± 0.64</td>
<td>2.96 ± 0.33</td>
<td>9.12 ± 2.5</td>
</tr>
<tr>
<td>Sodium excretion (µmol/100 g)</td>
<td>278 ± 27</td>
<td>220 ± 15</td>
<td>255 ± 18</td>
<td>201 ± 13</td>
<td>301 ± 24</td>
</tr>
<tr>
<td>Potassium excretion (µmol/100 g)</td>
<td>821 ± 73</td>
<td>660 ± 20</td>
<td>833 ± 49</td>
<td>720 ± 30</td>
<td>879 ± 59</td>
</tr>
</tbody>
</table>

a Values are expressed as means ± SEM. Abbreviations: control = control rats; MTZ = methimazole-treated rats; L-NAME = rats treated with Nω-nitro-L-arginine methyl ester (L-NAME); L-NAME + MTZ = rats treated with L-NAME and methimazole.

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