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"VIRGEN DE LAS NIEVES"

INTERACCIÓN ÓXIDO NÍTRICO-HORMONAS TIROIDEAS. PAPEL HOMEOSTÁTICO DE LAS ISOFORMAS DE LA ÓXIDO NÍTRICO SINTASA (NOS)

TESIS DOCTORAL

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ABREVIATURAS

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- PDN — Presión-diuresis-natriuresis
- T_4 — Tiroxina
- RAS — Sistema renina-angiotensina
- NO — Óxido nítrico
- L-NAME — N^{ω}-nitro-L-arginina metil éster
- NOS — Óxido nítrico sintasa
- nNOS — Óxido nítrico sintasa neuronal
- iNOS — Óxido nítrico sintasa inducible
- eNOS — Óxido nítrico sintasa endotelial
- AG — Aminoguanidina
- 7NI — 7-nitroindazol
- AVP — Vasopresina

ÍNDICE

INTRODUCCIÓN

1.-INTRODUCCIÓN

1.1.- Manifestaciones cardiovasculares y renales en la disfunción tiroidea.

El estado hipertiroideo es un desorden endocrino asociado con importantes cambios en la función hemodinámica, renal y cardiaca (Larsen PR et. al1998; Klein I et. al, 1990). El hipertiroidismo cursa con circulación hiperdinámica caracterizada por un incrementado gasto cardiaco, frecuencia cardiaca, presión de pulso y reducida resistencia periférica (Klein et. al, 1990; Vargas et. al, 2006), mientras que el estado hipotiroideo está asociado con un bajo gasto cardiaco, bradicardia y elevada resistencia periférica (Bradley et. al, 1974; Klein et. al, 1995). El hipertiroidismo incrementa la respuesta vascular a vasodilatadores endotelio-dependientes como la acetilcolina, mientras que el hipotiroidismo inducido por metimazol atenúa la respuesta endotelio-dependiente (Vargas et. al, 1995). El hipertiroidismo cursa con reducción en la capacidad de excreción de sodio. Así, se ha podido observar que las ratas hipertiroideas presentan un descenso en la excreción de sodio tras una sobrecarga salina así como una atenuada respuesta de presión-diuresis-natriuresis (PDN) (Vargas et. al, 1994; Vargas et. al, 1991) mientras, en el hipotiroidismo, se ha observado una excreción normal de sodio (Vargas et. al, 1991) ó una tendencia a excretar menos sodio (Bradley et. al, 1974). La administración de tiroxina a ratas produce un incremento en la presión sanguínea relacionado con la dosis, hipertrofia cardiaca, renal y proteinuria (Moreno et. al, 2005; Rodríguez-Gómez et. al, 2003; Vargas et. al, 2006).

Las anomalías de la función tiroidea se conocen desde hace más de un siglo (Parry, 1825). Los desórdenes del tiroides son alteraciones endocrinas comunes en humanos y animales (Feldman et. al, 1995). Cambios del estado del eutiroides afectan a todos los sistemas fisiológicos pero los efectos en el sistema cardiovascular son más notables. El hipertiroidismo acelera, mientras que el hipotiroidismo previene y revierte, algunos modelos de hipertensión experimental (Vargas et. al, 1988; Andrade et. al, 1992). El hipertiroidismo también está asociado con un incremento en el volumen sanguíneo, y lo contrario ocurre con el hipotiroidismo (Graettinger et. al, 1957; Graettinger et. al, 1959). La eritropoyesis y los niveles de eritropoyetina en suero cambian directamente con los niveles de tiroxina (T_4) en suero.

1.2.- Papel del sistema renina-angiotensina (RAS) en el hipertiroidismo

El hipertiroidismo se acompaña por hiperactividad del sistema renina-angiotensina (RAS) (Ganong et. al, 1982; García del Río et. al, 1997). De este modo, la actividad de renina plasmática y los niveles de aldosterona están directamente relacionados con los niveles plasmáticos de hormonas tiroideas (Ganong et. al, 1982; Jiménez et. al, 1982). Además, estudios previos de nuestro laboratorio han demostrado que el bloqueo a corto plazo del RAS desciende la presión arterial y mejora la hemodinámica renal y excreción de sodio en ratas hipertensas hipertiroideas (García-Estañ et. al, 1995); y que a largo plazo la administración de captopril previene la hipertensión inducida por tiroxina (T_4) (García del Río et. al, 1997). Conjuntamente, estos resultados indican que el RAS juega un papel importante en el incremento de la presión sanguínea y en las alteraciones renales del hipertiroidismo.

La actividad plasmática de renina muestra un patrón heterogéneo en la hipertensión por inhibición de la síntesis de NO (Zatz et. al, 1998). Sin embargo, hay evidencias considerables de que el RAS puede jugar un papel importante en este tipo de hipertensión. La participación del RAS está apoyada por datos que demuestran que el bloqueo del sistema previene o atenúa el desarrollo de la hipertensión L-NAME (Ribeiro et. al, 1992; De Gracia et. al, 2000). Además se sabe que, existe un balance funcional entre la angiotensina II y el NO en condiciones normales (Raij et. al, 2001).

1.3.- Óxido nítrico sintasa (NOS): acciones e inhibidores.

El oxido nítrico (NO) es producido a través de la transformación de L-arginina a L-citrulina por una familia de enzimas conocidas como óxido nítrico sintasa (NOS): neuronal (nNOS), inducible (iNOS), y endotelial (eNOS). Estas isoformas de las NOS están presentes en tejidos importantes relacionados con la regulación cardiovascular y en el riñón (Thorup et. al, 1998). Es bien sabido que el NO es un factor importante regulador del tono vascular (Gardier et. al, 1990), la excreción renal de sodio y la respuesta de PDN (Romero et. al, 1992; Cowley et. al, 1995) y por lo tanto de la presión arterial (Moncada et. al, 1991). Ambas administraciones, aguda y crónica, de inhibidores de NOS atenúan severamente la respuesta PDN, incrementan la presión arterial (Baylis et. al, 1996; Fernández-Rivas et. al, 1995), y ratones transgénicos que carecen del gen de la NOS se hacen hipertensos (Huang et. al, 1995).

La administración oral crónica de un inhibidor activo de la síntesis de NO, el N^{ω}-nitro-L-arginina metil éster (L-NAME), promueve una hipertensión permanente y daño renal, que son parcialmente revertidos por administración intravenosa de L-arginina (Ikeda et al., 1992) o por interrupción en la administración del inhibidor

(Gardier et. al., 1992). Baylis et al. (1992) demostraron que la administración de L-NAME durante 8 semanas provoca una hipertensión estable y glomeruloesclerosis mientras que Ribeiro et al. (1992), debido al uso de dosis más elevadas de L-NAME, obtuvieron una forma de hipertensión severa y progresiva asociada a isquemia glomerular, glomeruloesclerosis y expansión intersticial renal. Otros grupos de investigación demostraron simultáneamente, que el tratamiento crónico con L-NAME (Vargas et. al, 1996) provoca un predominio de los agentes vasoconstrictores con reducción de la respuesta a vasodilatadores endotelio-dependientes (Arnal et. al, 1992; Lahera et. al, 1992). Además, varios trabajos indican que la angiotensina II endógena es un importante factor en la génesis de este tipo de hipertensión (Jover et al., 1993; Pollock et al., 1993; De Gracia et. al, 2000).

La aminoguanidina (AG) es un inhibidor selectivo de la iNOS "in vitro" e "in vivo" (Griffiths et. al, 1993; Mismo et. al, 1993). Varios autores sostienen que la iNOS puede jugar un papel en el control de la presión sanguínea (Mattson et. al, 1998; Ortiz et. al, 1996; Tan et. al, 2000). Así, recientes estudios han demostrado que el NO producido por iNOS podría jugar un papel significativo en la prevención de la hipertensión en ratas sensibles a la sal (Mattson et. al, 1998; Tan et. al, 2000) y que la inhibición aguda de iNOS incrementa de forma marcada la presión sanguínea en ratas cirróticas hipotensas (Ortiz et. al, 1996).

Los compuestos heterocíclicos de nitrógeno representan un importante grupo de inhibidores de la nNOS que se unen a la sexta posición de coordinación del átomo de hierro del grupo hemo (Wolf et. al, 1993). Se ha demostrado que derivados del indazol y especialmente el 7-nitroindazol (7NI) son potentes inhibidores de la isoenzima neuronal (Moore et. al, 1993; Wang et. al, 1995; Wolf et. al, 1993; Wolf et. al, 1994). El

7NI es más selectivo para la nNOS que inhibidores basados en metil o nitro arginina, y la administración de este agente indazólico a animales de experimentación inhibe la nNOS en el cerebro sin cambios en la presión sanguínea o en la relajación dependiente del endotelio (Babbedge et. al, 1993; Mackenzie et. al, 1994; Wolf et. al, 1994). Por otro lado, el 7NI ha sido utilizado como un bloqueante eficaz de la nNOS en diferentes condiciones experimentales; incluyendo estudios de función renal (Abram et. al, 2001; Beierwaltes et. al, 1997; Welch et. al, 2000), aprendizaje (Mejer et. al, 1998) o erección del pene (Spiess et. al, 1996).

Varios estudios han demostrado un papel importante de la nNOS en la modulación central y periférica de la actividad simpática; y en la regulación de la conducta ante la ingesta de bebida (Nylen et. al, 2001). Así, el 7NI inhibió la respuesta excitatoria simpática cardiovascular producida por la administración de mevinfos, el cual activó los receptores muscarínicos M2 en la médula ventrolateral rostral de las ratas (Chan et. al, 2004), y por la administración de bradiquinina que provocaba elevaciones transitorias de la presión arterial en gatos (Tjen-A-Looi et. al, 2001) y también inhibió el incremento de vasopresina (AVP) en plasma producido por sobrecarga salina en ratas (Ventura et. al, 2005). Además, las manifestaciones cardiovasculares del hipertiroidismo sugieren la presencia de una incrementada actividad simpática (Levey et. al, 1971) y están asociadas con un síndrome de polidipsia y poliuria (García del Río et. al, 1997).

OBJETIVOS

2.- OBJETIVOS

1º-Dada la importancia de la actividad de la óxido nítrico sintasa (NOS) en la homeostasis cardiovascular y renal, el objetivo del primer trabajo fue analizar si las alteraciones de la función tiroidea cursan con cambios en la actividad de la NOS como posible factor implicado en las manifestaciones cardiovasculares y renales del hiper- e hipotiroidismo.

2º-El segundo estudio fue diseñado para evaluar si el óxido nítrico (NO) tiene efectos protectores homeostáticos en la presión sanguínea y otras variables en el estado hipertiroideo y además, analizar la importancia del sistema renina-angiotensina (RAS) en la hipertensión inducida por la administración simultanea y crónica de dosis supresoras de T_4 y L-NAME.

3º-Un aumento de producción de óxido nítrico (NO) mediado por la enzima iNOS, isoforma que juega un papel importante en la función renal y regulación de la presión sanguínea en varias situaciones fisiopatológicas, sugiriendo que el NO producido por esta isoenzima, podría ejercer un efecto homeostático en el hipertiroidismo del NO en esta enfermedad. En consonancia con esta hipótesis, el tercer estudio se diseñó para valorar el papel de la óxido nítrico sintasa inducible (iNOS) en el control a largo plazo de la presión sanguínea y otras variables en el estado hipertiroideo, para lo cual se analizó el efecto del bloqueo crónico de iNOS con AG en ratas hipertiroideas.

4º-Las manifestaciones cardiovasculares del hipertiroidismo sugieren que esta enfermedad se acompaña de una elevada actividad simpática y también está asociada a un síndrome de polidipsia y poliuria. Por ello, el objetivo del cuarto estudio fue analizar la contribución de la óxido nítrico sintasa neuronal (nNOS) a las manifestaciones hemodinámicas y metabólicas del hipertiroidismo mediante el bloqueo crónico de la nNOS con 7NI.

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EXPERIMENTAL STUDY

Nitric oxide synthase activity in hyperthyroid and hypothyroid rats

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Abstract

Objective: Thyroid disorders are accompanied by important changes in haemodynamic and cardiac functions and renal sodium handling. Since nitric oxide (NO) plays a crucial role in regulating vascular tone and renal sodium excretion, the present paper was designed to determine whether changes in the activity of NO synthase (NOS) participate in the cardiovascular and renal manifestations of thyroid disorders.

Methods: We measured NOS activity in the heart (left and right ventricles), vessels (aorta and cava) and kidney (cortex and medulla) of euthyroid, hyperthyroid and hypothyroid rats after 6 weeks of treatment. NOS activity was determined by measuring the conversion of L-[^{3}H]-arginine to L-[^{3}H]-citruline.

Results: NOS activity was higher in all tissues from hyperthyroid rats when compared with controls, except in the right ventricle. In the hypothyroid group, NOS activity showed a more heterogeneous pattern, with significant increases in both ventricles but significant reduction in the aorta, while in the vena cava, renal cortex and medulla the enzyme activity also tended to be higher, but significance was not reached.

Conclusions: These data indicated that NOS activity was upregulated in tissues primarily related to blood pressure control in hyperthyroid rats, suggesting that an increased NO production may contribute to the hyperdynamic circulation in hyperthyroidism and may have a protective homeostatic effect in the target organs of the hypertension that accompanies this endocrine disease. The aortic and renal findings in hypothyroid rats suggested a possible role for NOS in the increased peripheral resistance and the normal pressure–diuresis–natriuresis response of these hypotensive animals, although hypothyroidism produced a heterogeneous tissue response in NOS activity.

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Introduction

Thyroid disorders are common endocrine disorders in humans and animals (1) and are accompanied by important changes in haemodynamic, cardiac and renal function (2–4). Disturbances in the regulation of systemic arterial blood pressure are seen in both hypo- and hyperthyroid states in man and other animals (3–5). Hyperthyroidism manifests a hyperdynamic circulation with increased cardiac output, increased heart rate and decreased peripheral resistance, whereas the hypothyroid state is associated with low cardiac output, bradycardia and raised peripheral resistance (2–4). Hyperthyroidism increases the responsiveness of resistance vessels to the endothelium-dependent vasodilator, acetylcholine, whereas methimazole-induced hypothyroidism attenuates the endothelium-dependent response (5). Thyroid dysfunctions also affect cardiac and renal weight as well as renal sodium handling (2, 6, 7). Thus, hyperthyroidism occurs with a reduced sodium excretion after a saline load (7) and a blunted pressure–diuresis–natriuresis (PDN) response (6, 7) whereas, in hypothyroidism, a normal sodium excretion (7) or a tendency to sodium loss (2) have been reported.

Nitric oxide (NO) is produced through the transformation of L-arginine to L-citruline by a family of enzymes known as NO synthases (NOS). It is well known that NO is an important factor regulating vascular tone (8), renal sodium excretion and the PDN response (9, 10) and therefore arterial blood pressure (11). Both acute and chronic administration of NOS inhibitors severely attenuate the PDN response and increase systemic arterial blood pressure (12, 13), and transgenic mice that lack the NOS gene are hypertensive when compared with wild-type controls (14).

Given the importance of NOS activity in cardiovascular and renal homeostasis, the aim of the present paper

was to analyze whether changes in NOS activity participate in the cardiovascular and renal manifestations of thyroid disorders. To this end, we determined NOS activity by measuring the rate of formation of radiolabelled L-[^{3}H]-citruline from L-[^{3}H]-arginine in tissues primarily involved in blood pressure control from hyper- and hypothyroid rats. Moreover, the tissues selected for study are also the target organs in hypertensive disease.

Materials and methods

Animals

Male Wistar rats born and raised in the experimental animal service of the University of Granada were used. The experiments were performed according to European Union guidelines for the ethical care of animals. Rats initially weighing 180–200 g were maintained on standard chow and tap water *ad libitum* except where stated. The animals were divided into three groups: control, hyperthyroid and hypothyroid rats ($n = 16$ in each group). Hyperthyroidism was induced by injecting s.c. thyroxine (T_4; 300 μg/kg per day dissolved (1 μg/μl) in isotonic saline (100 ml) plus 1 ml 0.5 M NaOH). Hypothyroidism was induced by the continuous administration of 0.03% methimazole via the drinking water. Control rats were injected with the same solution as the hyperthyroid rats but without T_4. These treatments were administered for 6 weeks.

The effectiveness of these treatments was assessed by comparing serum T_4, serum tri-iodothyronine (T_3), mean arterial pressure, heart rate, pulse pressure and final thyroid, renal, ventricular and body weights of control and treated rats ($n = 8$ in each group). Blood pressure and heart rate were recorded using a TRA-121 transducer connected to a two-channel Letigraph 2000 recorder (Letica SA, Barcelona, Spain) in conscious rats through a polyethylene catheter inserted into the femoral artery and exteriorized at the dorsum of the neck. Blood pressure was measured 24 h after catheter implantation and blood samples were then taken via the arterial catheter to determine serum T_3 and T_4 levels, which were measured by ELISA (Immunoassay System; Baxter, Miami, FL, USA).

Measurement of NOS activity

The tissues chosen to measure NOS activity were: the heart (left and right ventricles), the vessels (aorta and cava) and the kidney (cortex and medulla). The heart was studied because of the important cardiac manifestations of both thyroid disorders (1–4), and left and right ventricles were analyzed to determine if the possible changes in NOS activity affect high and low pressure circulations. The vessels were studied because of the important abnormalities in vascular function in both thyroid diseases (5) and the analysis of arteries and veins was for the reason given above with respect to heart ventricles. NOS activity was measured in the renal cortex and medulla of hyper- and hypothyroid animals to assess the possible role of NO in the abnormalities in renal sodium handling of these animals (6, 7), since NO is essential in renal haemodynamics and renal sodium excretion (9), especially in the renal medulla, a structure that plays an important role in volume and blood pressure control (10).

The tissues were removed and homogenized with the aid of a tissue grinder (Omni International, Warrenton, VA, USA) at 3000 r.p.m. in ice-cold homogenization buffer (25 mM Tris, 0.5 mM D,L-dithiothreitol (DTT), 10 μg/ml leupeptin, 10 μg/ml pepstatin, 10 μg/ml aprotinin, 1 mM phenylmethylsulphonyl fluoride (PMSF), pH 7.6). The homogenized tissues were placed in 1 ml of the same buffer and sonicated (six times for 10 s). The crude homogenate was centrifuged 5 min at 3000 ***g*** and aliquots of the supernatant were either stored at −20 °C for total protein determination (15) or used immediately for NOS activity measurement. NOS activity was determined following the method of Bredt & Snyder (16), monitoring the conversion of L-[^{3}H]-arginine to L-[^{3}H]-citruline. The final incubation volume was 100 μl and consisted of 10 μl crude homogenate added to buffer to give a final concentration of 25 mM Tris, 1 mM DTT, 30 μM 5,6,7,8-tetrahydro-L-biopterin dihydrochloride (H_4-biopterin), 10 μM flavin adenine dinucleotide (FAD), 0.5 mM inosine, 0.5 mg/ml BSA, 0.1 mM $CaCl_2$, 10μM L-arginine and 50 nM L-[^{3}H]-arginine, at pH 7.6. The reaction was started by the addition of 10 μl NADPH (0.75 mM final) and maintained for 30 min at 37 °C. Control incubations were performed by omitting NADPH. The reaction was stopped by the addition of 400 μl cold 0.1 mM Hepes, 10 mM EGTA and 0.175 mg/ml L-citruline, pH 5.5. The reaction mixture was decanted into a 2 ml column packed with Dowex-50 W ($50 \times 8 - 200$) ion exchange resin (Na^+ form) eluted with 1.2 ml water. L-[^{3}H]-citruline was quantified by liquid scintillation spectroscopy. The retention of L-[^{3}H]-arginine in this process was greater than 98%. Specific enzymatic activity was determined by subtracting the control value, which was usually less than 1% of the radioactivity added. NOS activity is expressed as pM L-[^{3}H]-citruline produced/mg protein per min.

Drugs

The following drugs were used: heparin from Leo, Madrid, Spain, pentobarbital sodium (Nembutal) from Serva, Heidelberg, Germany, L-thyroxine from Merck, Darmstadt, Germany and methimazole, L-arginine, L-citruline, HEPES, DTT, leupeptin, aprotinin, pepstatin, PMSF, BSA, Dowex-50 W, FAD, NADPH, H_4-biopterin from Sigma Química, Madrid, Spain. L-[^{3}H]-arginine

(58 Ci/ mmol) was obtained from Amersham International plc, Amersham, Bucks, UK. Tris–HCl and calcium chloride were obtained from Merck, Darmstatd, Germany.

Statistical analysis

The results for each variable were compared with one-way ANOVA. When the overall ANOVA was significant, pairwise comparisons were performed with Bonferroni's method.

Results

Biological variables

The effects of T_4 or methimazole administration on biological variables are shown in Table 1. Animals given T_4 or methimazole for 6 weeks gained significantly less weight than their age-matched controls during this period. Mean arterial pressure, pulse pressure, heart rate, renal weight, ventricular weight, and serum T_3 and T_4 levels were increased and decreased in hyper- and hypothyroid rats respectively. Thyroid weight was decreased in hyperthyroid and increased in hypothyroid rats. Therefore, rats given T_4 for six weeks developed characteristic manifestations of hyperthyroidism, whereas those given methimazole for a similar period developed hypothyroidism.

NOS activity

Apart from the right ventricle, increased NOS activity was found in those tissues analysed from hyperthyroid rats (Figs 1–3). In the hypothyroid group, NOS activity showed a more heterogeneous pattern. It was significantly increased in both ventricles (Fig. 1) but significantly reduced in the aorta, while in the vena cava (Fig. 2), renal cortex and medulla (Fig. 3) the enzyme activity also tended to be higher, but significance was not reached. NOS activity was greater in the left than in the right ventricle in hyperthyroid rats ($P < 0.01$) and no difference between the left and right ventricle was observed in control and hypothyroid groups (Fig. 1). The comparison of NOS activity between arteries and veins showed a higher activity in the aorta ($P < 0.05$) in the control group, similar levels in the aorta and cava in hyperthyroid rats and a higher activity ($P < 0.05$) in the veins of hypothyroid rats (Fig. 2). NOS activity was greater in the medulla than in the cortex in the control ($P < 0.01$) and hypothyroid rats ($P < 0.05$) and similar in both renal tissues from hyperthyroid rats (Fig. 3).

Discussion

The present study provides new evidence that NOS activity is higher in most tissues primarily related to blood pressure control in hyperthyroid rats. The mechanism responsible for the enhancement of NOS activity in hyperthyroid rats is not known and various factors may participate alone or in combination. This could be due to the following: a compensatory response to the high arterial pressure of these animals (17); an increased release of vasoactive substances such as angiotensin II (18) or endothelin (19), which increase NO production and are increased in hyperthyroid rats (20, 21); or to the shear stress mechanism induced by the hyperdynamic circulation of these animals. Shear stress regulates the expression of NOS (22) and a putative shear stress response element has been described in the promoter sequence of the NOS gene (23). An upregulation of constitutive NOS has been reported in the aorta of other diseases that occur with hyperdynamic circulation, such as liver cirrhosis (24) and iron-deficiency anaemia (25). Alternatively, the hyperdynamic circulation of hyperthyroidism may be secondary to a direct effect of thyroid hormones on NOS activity. Stimulation of NOS activity via a non-genomic signal generation (10–30 s) has been observed in synaptosomes prepared from adult cerebral cortex after the addition of T_3 (26). However, T_3 administration was unable to stimulate inducible NOS activity in mesangial cells and tubular epithelial cells (27). These results seem to indicate that the direct effects of thyroid hormones vary depending on the NOS isoform and the tissue studied.

Table 1 Biological variables. Body weight (BW), ventricular weight (VW), renal weight (RW), thyroid weight (TW), mean arterial pressure (MAP), heart rate (HR), pulse pressure (PP), T_4 and T_3 plasma levels in control, hyperthyroid (treated with 300 μg T_4/kg per day, s.c.) and hypothyroid (treated with 0.03% methimazole in the drinking water) rats. Values are means±S.E.M. ($n = 8$ in all groups).

Groups	BW (g)	VW (mg)	RW (mg)	TW (mg)	MAP (mmHg)	HR (beats/min)	PP (mm/Hg)	T_4 (μg/dl)	T_3 (ng/dl)
Hyperthyroid	332**	1145*	1620*	22.3*	147**	407*	72*	40.1**	320**
	±13	±30	±38	±1.0	±3	±10	±4	±1.5	±7.1
Control	405	960	1200	34.5	118	370	43	4.2	50
	±2	±32	±36	±2.5	±2	±5	±4	±0.3	±7.2
Hypothyroid	251**	565**	860*	136**	103**	320**	28**	0.4**	4.3**
	±3	±22	±37	±8.5	±2	±10	±1	±0.1	±2.1

*$P < 0.05$, **$P < 0.01$ compared with control group.

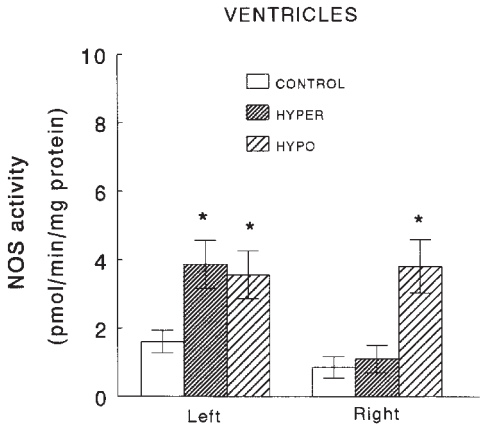

Figure 1 NOS activity in left and right ventricles from control, T_4-treated (hyperthyroid, HYPER) and methimazole-treated (hypothyroid, HYPO) rats ($n = 8$ in each group). *$P < 0.05$ compared with the control group. Data are means±S.E.M.

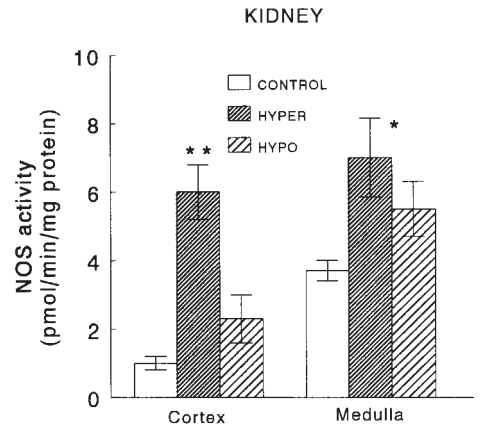

Figure 3 NOS activity in renal cortex and medulla from control, T_4-treated (hyperthyroid, HYPER) and methimazole-treated (hypothyroid, HYPO) rats ($n = 8$ in each group). *$P < 0.05$, **$P < 0.01$ compared with the control group. Data are means± S.E.M.

The heterogeneous pattern of NOS activity in the tissues from the hypothyroid group is difficult to reconcile with a common explanation or hypothesis, but it may be the result of changes in the expression of the different isoforms of NOS or even related to changes in the NOS activity of subcellular fractions. In fact, it has recently been reported (28) that liver and skeletal muscle mitochondrial NOS is increased in hypothyroidism and inversely correlated with serum T_3, whereas in neural tissues hypothyroidism is associated with a reduced NOS activity (29).

Considering the importance of NO in the control of vascular tone (10), the increased and decreased NOS activities in the aorta (in hyper- and hypothyroid rats respectively) suggests that this alteration may play a role in the changes in total peripheral vascular resistance previously reported in these animals (2–4). These data are consistent with the increased and decreased responsiveness to acetylcholine, the endothelium-dependent vasodilator, in perfused kidneys and isolated aortas from hyperthyroid and hypothyroid rats respectively (5). Our data in the hypothyroid group contrast with the increased staining of endothelial NOS using immunohistochemical techniques in the aortic endothelium of hypothyroid rats (30). However, it is well known that immunohistochemistry is a suitable technique to detect the presence of proteins in a given tissue but it is not a very accurate method to detect differences between groups. In fact, these observations were not accompanied by a quantitative analysis (30).

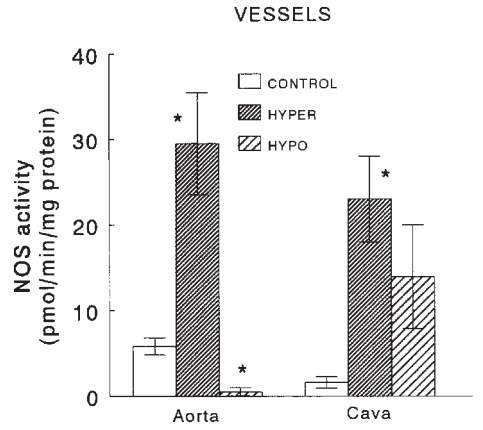

Figure 2 NOS activity in aorta and vena cava from control, T_4-treated (hyperthyroid, HYPER) and methimazole-treated (hypothyroid, HYPO) rats ($n = 8$ in each group). *$P < 0.05$ compared with the control group. Data are means±S.E.M.

The upregulation of constitutive NOS activity in hypertension may contribute to forestalling left ventricular and aortic hypertrophy (31) and to maintaining an adequate renal function (32). The increased NOS activity in these same organs of our hypertensive hyperthyroid group supports these roles and is consistent with the hypothesis that increased constitutive NOS activity may have protective homeostatic effect in all target organs of hypertensive disease (32).

Renal NOS activity is usually lower in the cortex than in medulla, as observed in the control group. Medullary NO plays a major role in the renal regulation of sodium and water excretion and therefore in the control of arterial blood pressure (8, 9). The normal values in medullary NOS activity in our hypothyroid rats indicate that the medulla has an adequate capacity to synthesize NO, which is consistent with the normal sodium handling of hypothyroid rats under normal conditions and after several challenges (6, 7). The high NOS activity in the renal cortex of hyperthyroid rats may be secondary to the hyperdynamic circulation of these animals, because cortical NOS activity is mainly produced by constitutive endothelial NOS (33). The similar levels of NOS activity in the renal cortex and medulla of our hyperthyroid rats might produce a defective NO

generation in renal medulla, which could participate in the blunted PDN response of these animals (6).

NOS activity in the left ventricle of hyperthyroid rats was twofold that in controls. This alteration would be secondary to the high blood pressure of these rats rather than caused by the hyperdynamic circulation or increased heart rate, because NOS activity in the right ventricle was similar to controls. These results agree with previous observations of higher NOS activity in the left heart of adult SHR rats and no differences between the left and right side of hearts from WKY rats (34). The increased NOS activity in both ventricles of the hypothyroid group is in consonance with the increased mitochondrial NOS reported in the skeletal muscle of ^{131}I-thyroidectomized rats (28), which has been implicated in thyroid-dependent regulation of O_2 uptake.

The increased NOS activity in the selected tissues of hyperthyroid rats agrees with published findings in other tissues. A study using quantitative *in situ* hybridization histochemistry with a specific oligodeoxynucleotide probe showed that T_3-induced hyperthyroidism more than doubled the prevalence of NOS gene transcript in the paraventricular (PVN) and supraoptic (SON) nuclei, whereas hypothyroidism produced a highly significant reduction in NOS gene transcripts in the PVN and SON (29). Fernandez *et al.* (35) demonstrated that hyperthyroidism leads to a significant and reversible enhancement in rat liver NOS activity.

In summary, our data have demonstrated that NOS activity is upregulated in tissues primarily related to blood pressure control in hyperthyroid rats. Thus, an increased NO production may participate in cardiovascular manifestations of this disease. NOS activity in the aorta and renal medulla of hypothyroid rats might participate in the increased peripheral resistance and the normal sodium handling of these hypotensive animals. However, the tissue response to NOS activity in hypothyroidism was heterogeneous.

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Increased Pressor Sensitivity to Chronic Nitric Oxide Deficiency in Hyperthyroid Rats

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Abstract—We studied the effects of a possible interaction between partial nitric oxide deficiency and thyroid hormone excess on the long-term control of blood pressure (BP) and morphological and renal variables and examined the role of the renin-angiotensin system in the increased BP of this interaction. Eight groups (n=8 each) of male Wistar rats were used: a control group; 3 groups that were treated with thyroxine (50 μg/d), N^{ω}-nitro-L-arginine methyl ester (L-NAME; subpressor dose, 1.5 mg · kg^{-1} · d^{-1}), or thyroxine plus L-NAME; and another 4 similarly treated groups that received losartan (20 mg · kg^{-1} · d^{-1}) in their drinking fluid. All treatments were maintained for 3 weeks. The time course of tail systolic BP was recorded once a week. At the end of the experimental period, we measured mean arterial pressure in conscious rats and assessed the morphological, metabolic, plasma, and renal variables. Thyroxine produced a mild BP increase from the second week of treatment and an increase in plasma angiotensin II and plasma nitrates/nitrites by the end of the study. Simultaneous administration of thyroxine and a subpressor dose of L-NAME produced a marked BP increase that reached significance from the first week of treatment. Losartan produced normotension in thyroxine-treated rats and attenuated the BP elevation in thyroxine+L-NAME–treated rats. Hyperthyroid rats showed relative renal and ventricular hypertrophy, absence of absolute left ventricular hypertrophy, and proteinuria. These alterations were not changed by losartan. We conclude that an impaired nitric oxide system might have a counterregulatory homeostatic role against the prohypertensive effects of thyroid hormone and that the renin-angiotensin system plays an important role in thyroxine+L-NAME hypertension. **(*Hypertension*. 2003;42:220-225.)**

Key Words: blood pressure ■ hypertrophy, cardiac ■ losartan ■ nitric oxide ■ hyperthyroidism

The hyperthyroid state is an endocrine disorder associated with important changes in hemodynamic, renal, and cardiac function.[1–3] Hyperthyroidism manifests a hyperdynamic circulation, with increased cardiac output, increased heart rate, and decreased peripheral resistance.[2–4] These characteristic cardiovascular manifestations of hyperthyroidism have been reproduced in rats by thyroid hormone treatment.[1–3] Animal studies have reported a dose- and time-related increase in arterial pressure[5,6] and have shown that the hyperthyroid state affects cardiac and renal weight and reduces renal sodium excretion.[2,5,6] It is well known that nitric oxide (NO) plays a major role in the regulation of vascular tone,[7] renal sodium excretion,[8,9] and therefore, of arterial blood pressure (BP).[10] Both acute and chronic administration of NO synthase (NOS) inhibitors increase systemic arterial BP.[11,12]

Hyperthyroidism in rats increases the responsiveness of resistance vessels to the endothelium-dependent vasodilator acetylcholine.[13] Fernández et al[14] demonstrated that hyperthyroidism leads to a significant and reversible enhancement in rat liver NOS activity. More recently, our group reported[15] that NOS activity is upregulated in tissues primarily related to BP control in hyperthyroid rats. Our finding suggested that increased NO production might contribute to the hyperdynamic circulation in hyperthyroidism and might have a protective homeostatic effect on the increased BP that accompanies this endocrine disease.

Hyperthyroidism is accompanied by hyperactivity of the renin-angiotensin system (RAS).[16–18] Thus, plasma renin activity and aldosterone are directly related to plasma levels of thyroid hormones.[16,17] Moreover, previous studies from our laboratory have demonstrated that short-term RAS blockade markedly decreases arterial pressure and improves renal hemodynamics and excretion in hypertensive hyperthyroid rats[19] and that long-term administration of captopril prevents thyroxine (T_4)-induced hypertension.[18] These results indicate that the RAS plays an important role in the increased BP and renal alterations of hyperthyroidism.

Although plasma renin activity shows a heterogeneous pattern in NO inhibition hypertension,[11] there is considerable evidence of the important role of the RAS in this type of hypertension. The participation of the RAS is supported by data showing that RAS blockade prevents or attenuates the development of N^{ω}-nitro-L-arginine methyl ester (L-NAME)

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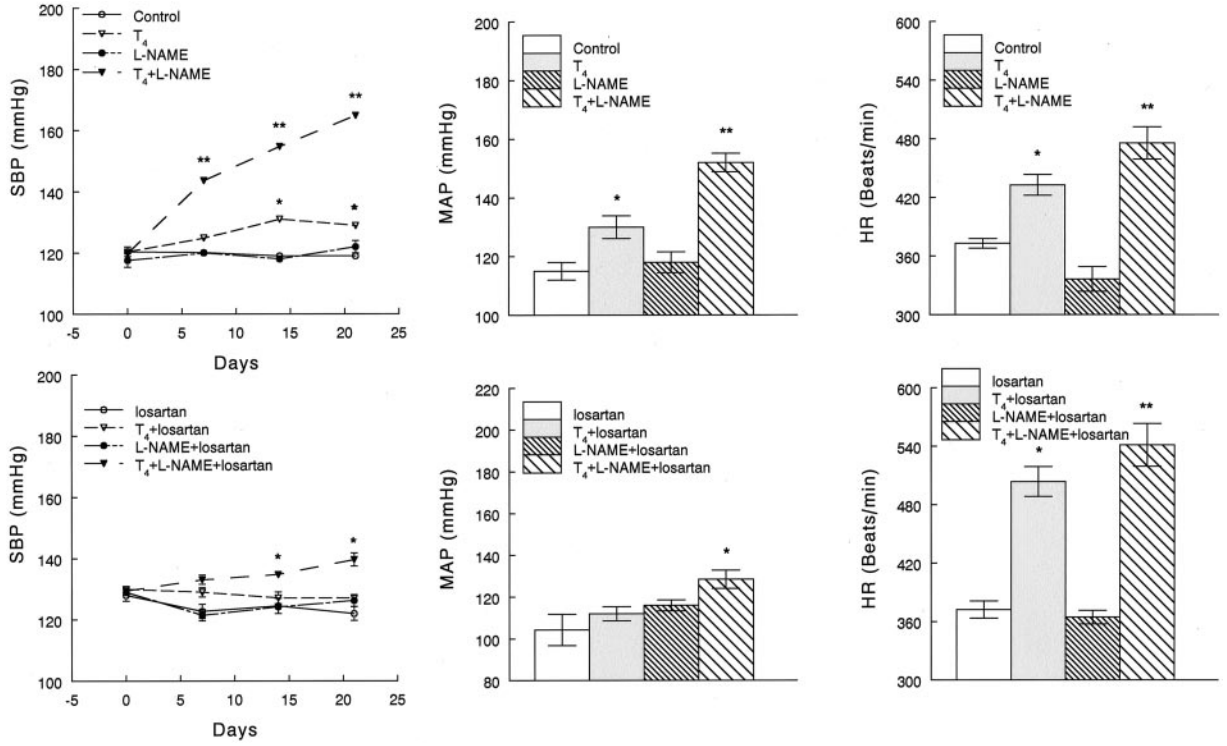

Time course of SBP measured by the tail-cuff method and final mean arterial pressure (MAP) and heart rate (HR), as measured by direct recording (femoral artery) in conscious rats. *$P<0.05$, **$P<0.01$ compared with control or losartan group.

hypertension.[20,21] In fact, a functional balance exists between angiotensin II (Ang II) and NO in normal conditions.[22]

With this background, the present study was designed to evaluate whether NO has homeostatic protective effects on BP and other variables in the hyperthyroid state. Moreover, given the importance of the RAS in the hypertension induced by both long-term administration of T_4 and long-term blockade of NO, we also determined the effects of long-term RAS blockade on the hypertension induced by the simultaneous administration of subpressor doses of T_4 and L-NAME.

Methods

Animals

Sixty-four 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. Rats initially weighing 150 to 175 g were randomly assigned to 1 of 2 experiments and were further assigned to 4 groups. In the first experiment, the groups were as follows: control, L-NAME, T_4, and T_4+L-NAME. In the second experiment, groups similar to those in experiment 1 were treated with losartan: control+losartan, L-NAME+losartan, T_4+losartan, and L-NAME+T_4+losartan. Each experimental group comprised 8 animals. All rats had free access to food and tap water, except where stated. Losartan (200 mg/L; $\approx$20 mg $\cdot$ kg^{-1} $\cdot$ d^{-1}) was given in the drinking water and L-NAME (1.5 mg $\cdot$ kg^{-1} $\cdot$ d^{-1}) by gavage. The concentration of losartan in the drinking fluid was adjusted every 2 days according to the fluid intake of the animals to ensure that a similar dose was administered to both T_4-treated and control groups. Hyperthyroidism was induced by injecting T_4 (Merck), 50 μg/d SC, dissolved in 0.5N NaOH isotonic saline. This dose was previously used and does not produce marked arterial hypertension.[5]

Experimental Protocol

The treatments were administered for 3 weeks. Body weight and tail systolic blood pressure (SBP) were measured once a week. Tail SBP was measured by tail-cuff plethysmography in unanesthetized rats. When the experimental period was completed, all rats were housed in metabolic cages with free access to food and their respective drinking fluids, and treatments were continued for 4 days (2 days for adaptation and 2 experimental days) to measure food and fluid intake and to collect urine samples. Twenty-four-hour urine volume, proteinuria, creatinine, and total excretion of sodium and potassium were measured. The means of the values obtained for each intake or urinary variable during the 2 experimental days were used for statistical analyses between groups.

After completion of the metabolic study, the rats were anesthetized with ethyl ether. A polyethylene catheter (PE-50) containing 100 U heparin in isotonic, sterile, NaCl solution was inserted into the femoral artery for intra-arterial BP and heart rate measurement in conscious rats and for extraction of blood samples. The catheter was tunneled subcutaneously, brought out through the skin at the dorsal side of the neck, and protected with a silver spring. Twenty-four hours after implantation of the femoral catheter, intra-arterial BP was measured with a TRA-021 transducer connected to a 2-channel recorder (Letigraph 2000, Letica SA). After 30 minutes of stabilization, values from the last 5 minutes recorded were averaged and used for comparisons between groups. Subsequently, blood samples taken with the femoral catheter were used to determine total protein, electrolytes, creatinine, Ang II, and nitrate and nitrite (NO_x) concentrations. Blood samples were taken as follows: the first 1.5 mL drawn was used for Ang II determination, the next 3 mL was used for measurement of the remaining biochemical variables, and finally the rats were exsanguinated. Subsequently, the kidneys and ventricles were removed and weighed. The heart was divided into right ventricle and left ventricle plus septum.

Analytical Procedures

Proteinuria was measured by the method of Bradford.[23] Plasma and urinary electrolytes and creatinine were measured in an autoanalyzer

TABLE 1. Morphological Variables

Groups	FBW, g	KW/BW, mg/g	VW/BW, mg/g	LVW/RVW
Control	352±14	2.38±0.02	2.11±0.09	3.71±0.18
T_4	316±9	3.13±0.11*	2.89±0.09*	3.26±0.16
L-NAME	355±13	2.31±0.05	2.01±0.05	4.24±0.27
T_4+L-NAME	330±7	3.13±0.09*	2.88±0.12*	3.77±0.12
Losartan	347±7	2.48±0.07	2.03±0.15	3.55±0.20
T_4+losartan	313±7	3.05±0.06*	2.78±0.06*	3.73±0.15
L-NAME+losartan	338±19	2.37±0.11	2.00±0.05	3.88±0.18
T_4+L-NAME+losartan	314±13	3.25±0.07*	2.73±0.11*	3.52±0.18

Data are expressed as mean±SEM. FBW indicates final body weight; KW/BW, kidney weight/body weight ratio; VW/BW, ventricular weight/body weight ratio; and LVW/RVW, left ventricular weight/right ventricular weight ratio.

*$P<0.05$ vs controls.

(Beckman CX4, USA). Plasma Ang II levels were measured in methanol-extracted samples with a radioimmunoassay kit (Eurodiagnostica) purchased from Izasa SA. Plasma NO_x concentration was measured by using nitrate reductase and the Griess reaction.[24]

Statistical Analysis

The evolution of SBP 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, the Bonferroni method with an appropriate error was used. Comparisons of each variable at the end of the experiments were done by performing a 1-way ANOVA. When the overall ANOVA was significant, we then performed pairwise comparisons with the Bonferroni and Newmann-Keuls methods.

Results

BP and Heart Rate

The Figure shows tail SBP, final mean arterial pressure, and heart rate in the groups from both experiments. Experiment 1 showed that T_4 administration produces a mild increase in BP by the second week of treatment when compared with control rats. L-NAME administration to normal rats at the dose used in this experiment did not modify the time-course evolution of BP, as expected. However, simultaneous administration of T_4 and a subpressor dose of L-NAME produced a marked increase in BP, which reached significance by the first week of treatment. Heart rate was significantly increased in both T_4-treated groups. In experiment 2, T_4+losartan–treated rats showed an SBP evolution similar to that of control rats; the T_4+L-NAME+losartan group showed a mild elevation in BP, which was significant from the second week of treatment when compared controls but highly attenuated in comparison with the T_4+L-NAME group. Both T_4-treated groups in experiment 2 also showed an increased heart rate. Tail SBP values were confirmed by mean arterial pressure measurements at the end of the experimental period, which were directly recorded in conscious animals in both experiments.

Morphological Variables

Both T_4-treated groups in experiment 1 showed a tendency to reduced body weight compared with the control groups, although these differences did not reach significance. Relative ventricular and renal weights were significantly increased in both T_4-treated groups in comparison with their control counterparts. The ratio of left ventricular to right ventricular weights, considered as an index for absolute left ventricular hypertrophy, was similar in all 4 groups of experiment 1 and 2. Losartan treatment had no significant effect on any morphological variable in either control or hyperthyroid rats (Table 1).

Plasma, Metabolic, and Urinary Variables

Plasma Ang II and plasma NO_x levels were significantly increased in the T_4 group. The L-NAME group showed

TABLE 2. Urine Variables and Creatinine Clearance Measured at the End of the Experimental Period

Groups	Urinary Flow Rate, $mL \cdot 100\ g^{-1} \cdot 24\ h^{-1}$	Sodium Excretion Rate, $\mu mol \cdot 100\ g^{-1} \cdot 24\ h^{-1}$	Potassium Excretion Rate, $\mu mol \cdot 100\ g^{-1} \cdot 24\ h^{-1}$	Protein Excretion Rate, $mg \cdot 100\ g^{-1} \cdot 24\ h^{-1}$	Creatinine Clearance, $mL \cdot min^{-1} \cdot g^{-1}$ kidney
Control	2.46±0.33	285±26	452 ±41	3.01±0.33	1.182±0.10
T_4	4.34±1.12*	352±45	572 ±68	8.65±0.93*	0.971±0.04
L-NAME	5.11±1.42*	243 ±9	582 ±42	4.40±0.61	1.204±0.10
T_4+L-NAME	6.37±1.12*	300±47	633 ±82	8.82±1.56*	0.900±0.15
Losartan	4.60±0.48	404±43	666 ±23	3.35±0.29	0.959±0.35
T_4+losartan	6.92±1.13	565±60	846 ±93	8.19±0.97*	1.046±0.03
L-NAME+losartan	3.54±0.85	385±67	565±106	3.67±0.95	0.903±0.16
T_4+L-NAME+losartan	7.47±1.27	387±17	769 ±58	8.54±1.09*	0.926±0.33

Data are expressed as mean±SEM. Each value is the mean of the variable on 2 consecutive days of urine collection.

*$P<0.05$ vs controls.

TABLE 3. Plasma Variables

Groups	Na, mEq/L	K, mEq/L	Creatinine, mg/dL	Total Protein, g/dL	Angiotensin II, pmol/L	NO_x, μmol/L
Control	144.9±0.3	4.14±0.15	0.65±0.02	6.01±0.07	24.6±3.5	6.30±0.20
T_4	145.1±0.8	4.47±0.07	0.62±0.02	5.56±0.09*	40.4±4.6*	9.82±0.30*
L-NAME	144.0±0.2	4.42±0.12	0.63±0.01	5.94±0.08	17.3±3.0	4.50±0.22*
T_4+L-NAME	143.6±0.5	4.84±0.17	0.65±0.02	5.50±0.09*	30.3±3.9*	5.19±0.28*
Losartan	143.5±0.8	4.35±0.19	0.53±0.06	5.46±0.08	258±10	6.29±0.25
T_4+losartan	144.8±0.7	4.48±0.15	0.56±0.02	5.44±0.09	290±13	8.74±0.32*
L-NAME+losartan	144.2±1.0	4.90±0.41	0.50±0.01	5.72±0.06	266±17	3.79±0.30*
T_4+L-NAME+losartan	144.9±0.7	5.23±0.21	0.73±0.09	5.26±0.08†	329±13	6.08±0.30†

Data are expressed as mean±SEM.
*$P<0.05$ vs control; †$P<0.05$ vs L-NAME or L-NAME-losartan.

unchanged Ang II levels and reduced NO_x concentrations, and the T_4+L-NAME group showed a reduction in both of these variables when compared with the T_4 group. All losartan-treated groups showed a marked increase in plasma Ang II levels, with no significant differences among then. Plasma NO_x concentration was unaffected by losartan treatment in all groups. There were no significant differences in plasma sodium, potassium, or creatinine levels between the groups in either experiment. Total plasma proteins were significantly decreased ($P<0.05$) in the T_4 and T_4+L-NAME groups compared with controls and in the T_4+L-NAME+losartan group when compared with the L-NAME–losartan group (Table 2). Metabolic studies at the end of treatment showed increased food intake (g/100 g body weight) in both T_4-treated groups (T_4=7.3±0.6, T_4+L-NAME=7.39±0.5; $P<0.01$) in comparison with their corresponding controls (control=5.2±0.2, L-NAME=4.8±0.2). T_4-treated groups drank more fluid than did the control groups ($P<0.05$). Fluid intake values were as follows: control, 7.7±0.5; L-NAME, 9.8±1.1; T_4, 11.7±1.0; and T_4+L-NAME, 12.8±1.8 (all mL/100 g body weight). Long-term treatment with losartan did not affect food or fluid intake in either control or hyperthyroid rats (data not shown).

Urine variables and creatinine clearance are summarized in Table 3. Urine volume was significantly higher in the T_4-treated groups versus controls in both experiments. Total sodium and potassium excretion showed a tendency to be higher in the T_4-treated animals of both experiments, although significance was not reached because of the variability of results. These data are consistent with the greater food intake of T_4-treated rats. Proteinuria was significantly increased in the T_4-treated groups in both experiments but was unaffected by L-NAME or losartan treatment in control and T_4-treated rats. Creatinine clearance, normalized per gram of kidney weight, was not significantly affected by the T_4 or L-NAME treatment. Losartan treatment did not significantly change creatinine clearance in the experimental groups.

Discussion

The main finding of this study is that T_4-treated rats became hypertensive after partial NOS inhibition with a dose of L-NAME that did not modify BP in control rats. Various mechanisms or sets of mechanisms might participate in the increased sensitivity to partial NOS blockade in hyperthyroid rats. This study and several reports[13–15] provide evidence that the hyperdynamic circulation of hyperthyroidism is accompanied by increased NO production. Anemia[25] and cirrhosis of the liver[26] are also associated with hyperdynamic circulation and increased NO production, and cirrhotic rats also show an increased pressor responsiveness to NO blockade.[27] These observations indicate that the increased pressor responsiveness to L-NAME in hyperthyroid rats might be secondary to an augmented production of NO, which might have an important homeostatic role in these animals.

A functional feedback balance exists between both Ang II and NO under normal conditions.[22,28] However, the present data show that the administration of losartan did not alter NO_x levels in T_4-treated rats, which had increased plasma levels of NO_x and Ang II. These data indicate that T_4 simultaneously stimulates both antagonist factors, Ang II and NO, and might interfere with the normal balance between then.

The increased plasma levels of Ang II in T_4-treated rats are consistent with the widely reported hyperactivity of the RAS[16–18] in hyperthyroidism, which plays an essential role in T_4-induced hypertension.[18,19] Therefore, because NO might interfere with the prohypertensive effects of Ang II,[22,28] partial NO blockade might result in predominance of the pressor effects of Ang II. The marked reduction in BP that losartan produced in T_4+L-NAME–treated rats indicates that Ang II plays an important role in this type of hypertension. However, the fact that losartan did not normalize BP in T_4+L-NAME–treated rats indicates that other factors besides Ang II also contribute to the increased BP in these animals. Thus, it is well known that NO blockade increases the responsiveness to vasoconstrictors, especially in resistance vessels,[29] and that this effect is greater in hyperthyroid rats.[30] This mechanism might increase peripheral resistance and therefore increase BP. Moreover, hyperthyroidism affects renal sodium handling in rats,[2,5,6] reduces sodium excretion after a saline load,[5] and blunts the pressure-diuresis-natriuresis response.[6] These antinatriuretic effects might be aggravated by NO deficiency[9,10] and contribute to producing a displacement to the right in the set point of the pressure-diuresis-natriuresis relation, as indicated by the normal sodium excretion with increased BP in the T_4+L-NAME–treated group.

Interestingly, losartan treatment suppressed the mild pressor effect of T_4 at the dose administered in our study. This observation is in line with previous findings that short- or long-term blockade of the RAS reduces BP to normal values in hypertensive hyperthyroid rats.[18,19] Therefore, the present report adds new evidence of the importance of the RAS in the elevation of BP in the hyperthyroid state.

It is well known that the hyperthyroid state is associated with cardiac hypertrophy.[3,4,18] The present results show that hyperthyroidism leads to an increased heart-to-body weight ratio, a measure of relative ventricular hypertrophy. In addition, treatment with T_4 had no influence on the left-to-right ventricular-weight ratio, a measure of absolute left ventricular hypertrophy. Therefore, cardiac hypertrophy in hyperthyroidism affects both ventricles to a similar extent. These results agree with previous observations in T_4-hypertensive rats.[18]

Kobori et al[31] suggested that the activated RAS, especially the cardiac RAS, of hyperthyroidism plays a role in the development of the cardiac hypertrophy of this disease. This same group reported that administration of RAS inhibitors suppressed the cardiac RAS and contributed to the regression of cardiac hypertrophy in the hyperthyroid state.[32] In the present study, however, chronic Ang II type 1 receptor blockade with losartan did not significantly alter the relative ventricular hypertrophy in hyperthyroid rats, indicating that the RAS plays no role in this type of cardiac hypertrophy. These findings agree with previous observations in normotensive[33] and hypertensive[19] hyperthyroid rats. Unfortunately, we can find no convincing explanation that accounts for the discrepancies between our data and the observations of Kobori et al.[32]

Although the present study was not designed to address the mechanisms by which hyperthyroidism produces cardiac hypertrophy, the data we report above indicate that cardiac hypertrophy in hyperthyroidism is unrelated to the BP or RAS. Bedotto et al[33] reported that cardiac hypertrophy produced by thyroid hormone is independent of loading conditions and β-adrenoceptors. Taking these observations together, it could be proposed that a direct trophic effect of thyroid hormones on the heart might be responsible for cardiac hypertrophy in hyperthyroidism. In support of this proposal, studies of cultured cardiomyocytes have demonstrated that thyroid hormone directly controls gene expression and cell growth.[34]

The present study shows that hyperthyroid rats have increased proteinuria, consistent with the presence of proteinuria in patients with Graves' disease.[35] This alteration might be secondary to the increased production in hyperthyroid rats of NO, a vasodilator that impairs the glomerular permeability barrier,[36] although the fact that the proteinuria of T_4-treated rats was unaffected by partial NO blockade argues against this possibility. Moreover, because proteinuria was also unrelated to BP or losartan administration, we suggest that proteinuria in the hyperthyroid state might be produced by a direct action of thyroid hormones, increasing the permeability of the glomerular barrier. In this context, Tanwani et al[37] reported a possible association between thyrotoxic patients and a nephrotic syndrome attributable to minimal change nephropathy, a clinical entity defined by selective proteinuria that occurs in the absence of lesions in the glomerular capillary wall. The only detectable abnormalities involve the epithelial visceral cells with effacement of foot processes.

Creatinine clearance, as normalized per gram kidney weight, was similar in all experimental groups of experiment 1 and was unaffected by losartan. The normal creatinine clearance of the T_4-treated rats contrasts with the reduced glomerular filtration rate previously reported by our group.[6,18] These discrepancies might be a consequence of the larger dose of T_4 (75 μg/d) and the longer period of treatment (6 weeks) used in the earlier studies, which produced full hypertension.[6,18]

In conclusion, the present study shows that impaired NO synthesis results in increased sensitivity to the chronic pressor effect of T_4, which is severely attenuated by losartan administration. These observations indicate that (1) NO contributes to the adaptive hemodynamic response to hyperthyroidism and (2) the RAS plays an important role in the hypertension induced by long-term, simultaneous administration of subpressor doses of T_4 and L-NAME. In addition, our data demonstrate the presence of relative renal and ventricular hypertrophy and the absence of absolute left ventricular hypertrophy and proteinuria in hyperthyroid rats, which was unchanged by losartan.

Perspectives

The present study, considered alongside our recent studies on NOS activity of tissues that are primarily related to BP control in hyperthyroid rats, strongly suggests that increased NOS activity might play a protective homeostatic role in hyperthyroidism against the prohypertensive effects of thyroid hormone. An important factor in the putative antihypertensive mechanisms of NO is its antagonistic effect on the pressor actions of Ang II, because administration of losartan severely attenuated the BP increase in rats treated with T_4+L-NAME. However, losartan did not normalize BP in these rats, indicating that partial NOS blockade also potentiates unknown factors that contribute to the development of this type of hypertension. Precise knowledge of the participation of different NOS isoenzymes in this adaptive hemodynamic response could allow a common pathophysiologic mechanism to be established for the hyperdynamic circulation that appears, regardless of BP level, in different cardiovascular diseases.

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Effects of chronic inhibition of inducible nitric oxide synthase in hyperthyroid rats

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Rodríguez-Gómez, Isabel, Rosemary Wangensteen, Juan Manuel Moreno, Virginia Chamorro, Antonio Osuna, and Félix Vargas. Effects of chronic inhibition of inducible nitric oxide synthase in hyperthyroid rats. *Am J Physiol Endocrinol Metab* 288: E1252–E1257, 2005. First published January 11, 2005; doi:10.1152/ajpendo.00279.2004.—We hypothesized that nitric oxide generated by inducible nitric oxide synthase (iNOS) may contribute to the homeostatic role of this agent in hyperthyroidism and may, therefore, participate in long-term control of blood pressure (BP). The effects of chronic iNOS inhibition by oral aminoguanidine (AG) administration on BP and morphological and renal variables in hyperthyroid rats were analyzed. The following four groups ($n = 8$ each) of male Wistar rats were used: control group and groups treated with AG (50 $mg \cdot kg^{-1} \cdot day^{-1}$, via drinking water), thyroxine (T_4, 50 $\mu g \cdot rat^{-1} \cdot day^{-1}$), or AG + T_4. All treatments were maintained for 3 wk. Tail systolic BP and heart rate (HR) were recorded weekly. Finally, we measured BP (mmHg) and HR in conscious rats and morphological, plasma, and renal variables. T_4 administration produced a small BP (125 ± 2, $P < 0.05$) increase vs. control (115 ± 2) rats. AG administration to normal rats did not modify BP (109 ± 3) or any other hemodynamic variable. However, coadministration of T_4 and AG produced a marked increase in BP (140 ± 3, $P < 0.01$ vs. T_4). Pulse pressure and HR were increased in both T_4- and T_4 + AG -treated groups without differences between them. Plasma NOx ($\mu mol/l$) were increased in the T_4 group (10.02 ± 0.15, $P < 0.05$ vs. controls 6.1 ± 0.10), and AG reduced this variable in T_4-treated rats (6.81 ± 0.14, $P < 0.05$ vs. T_4) but not in normal rats (5.78 ± 0.20). Renal and ventricular hypertrophy and proteinuria of hyperthyroid rats were unaffected by AG treatment. In conclusion, the results of the present paper indicate that iNOS activity may counterbalance the prohypertensive effects of T_4.

blood pressure; aminoguanidine; thyroxine; rat

HYPERTHYROIDISM MANIFESTS IMPORTANT CHANGES in hemodynamic, renal, and cardiac function (9, 18, 19). It is hemodynamically characterized by a hyperdynamic circulation with increased cardiac output, increased heart rate, and decreased peripheral resistance (4, 17, 18). In animal studies, thyroxine (T_4) produces a dose- and time-related rise in arterial blood pressure (BP; see Refs. 36 and 37), increases cardiac and renal weight, and reduces renal sodium excretion (4, 36, 37).

NO is known to play a major role in the regulation of vascular tone (12) and renal sodium excretion (6, 28) and, consequently, of BP (23). NO can be generated by the activity of neuronal (nNOS), inducible (iNOS), and endothelial (eNOS) nitric oxide synthase isoforms, which are all widely distributed in organs related to BP control and present in normal rat kidney (34).

Aminoguanidine (AG) is a selective iNOS inhibitor in vitro (14, 22) and in vivo (21, 25, 32). Several authors have supported a role for iNOS in BP control (21, 25, 32). Thus recent studies showed that NO produced by iNOS may play a significant role in preventing salt-sensitive hypertension in rats with normal salt sensitivity (21, 32) and that acute iNOS inhibition markedly increases BP in cirrhotic hypotensive rats (25).

Fernández et al. (9) demonstrated that hyperthyroidism leads to a significant and reversible enhancement in rat liver NOS activity, and our group reported (26) that NOS activity is upregulated in tissues primarily related to BP control in hyperthyroid rats. More recently, we observed that the simultaneous administration of T_4 and a suppressor dose of L-NAME produced a marked BP increase, indicating that NO may have a counterregulatory homeostatic role against the prohypertensive effects of thyroid hormone (27).

At the present time, the role of iNOS in hyperthyroidism is not clear, and the mechanisms responsible for the elevated NO activity of hyperthyroidism are not completely established. Moreover, no data have been reported on the contribution of iNOS to the antihypertensive effect of NO in this endocrine disease. With this background, we hypothesized that increased activity of iNOS, an isoform that plays an important role in renal function and BP regulation in various pathophysiological situations, might increase NO production and contribute to the homeostatic role of this factor in the hyperthyroid state. Therefore, the present study was designed to assess the role of iNOS to the long-term control of BP and other variables in the hyperthyroid state. To this end, we studied the effects of the chronic blockade of iNOS with AG in hyperthyroid rats.

METHODS

Animals. Thirty-two 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. Rats initially weighing 200–250 g were randomly assigned to one of four groups. The groups were as follows: control, AG, T_4, and T_4 plus AG (T_4 + AG) rats. Each experimental group comprised eight animals. All rats had free access to food and tap water except where stated. AG (50 mg/dl; ~50 $mg \cdot kg^{-1} \cdot day^{-1}$) was given in the drinking water. The AG concentration in the drinking fluid was adjusted every 2 days according to the fluid intake of the animals to ensure that a similar dose of iNOS inhibitor was administered to the T_4-treated and control groups.

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Hyperthyroidism was induced by injecting T_4 subcutaneously (Merck) at 50 $\mu g \cdot rat^{-1} \cdot day^{-1}$ dissolved in 0.5 N NaOH isotonic saline. This dose was chosen because it produces a slight BP increase of <15 mmHg (27, 37).

Experimental protocol. The treatments were administered for 3 wk. Body weight and tail systolic BP (SBP) were measured once a week. Tail SBP was measured with the use of tail-cuff plethysmography in unanesthetized rats. When the experimental period was completed, all rats were housed in metabolic cages with free access to food and their respective drinking fluids and treatments for 4 days (2 days for adaptation + 2 experimental days) to measure food and fluid 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 two 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 in the femoral artery for intra-arterial BP, heart rate, and pulse pressure (pulse pressure = SBP − diastolic BP) measurements in conscious rats and for extraction of blood samples. The catheter was tunneled subcutaneously, brought out through the skin at the dorsal side of the neck, and protected with a silver spring. After implantation of the femoral catheter (24 h), intra-arterial BP was measured by a TRA-021 transducer connected to a two-channel Letigraph 2000 recorder (Letica, Barcelona, Spain). After 30 min of stabilization, values from the last 5 min recorded were averaged and used for comparisons among groups. Subsequently, blood samples taken with the femoral catheter were used to determine total protein, electrolytes, and creatinine concentration. The kidneys and ventricles were then removed and weighed. The heart was divided into right ventricle and left ventricle plus septum.

Analytic procedures. Proteinuria was measured by the method of Bradford (3). Plasma and urinary electrolytes and creatinine were measured in an autoanalyzer (Beckman CX4). Plasma NOx (nitrites + nitrates) concentration was measured using nitrate reductase and the Griess reaction (13).

Statistical analysis. The evolution of SBP with time was compared by use of a nested design, with groups and days as fixed factors and rat as 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 with Bonferroni's and Newmann-Keuls methods.

RESULTS

BP and heart rate. Figure 1 shows the time course of tail SBP. T_4 administration produced a mild increase in SBP at *week 2* of treatment vs. control rats. AG administration to normal rats at the dose used in this experiment did not modify the time course evolution of SBP or any other hemodynamic variable. However, the simultaneous administration of T_4 and AG produced a marked increase in SBP, which reached significance at *week 1* of treatment. These data were confirmed by the final mean arterial pressure measurement in the conscious animals at the end of the experimental period (Fig. 1). Final heart rate and pulse pressure were significantly and similarly increased in both T_4- and T_4 +AG-treated groups (Fig. 2).

Morphological variables. T_4-treated groups showed a significant reduction in body weight gain during the course of the experiment compared with the control groups. AG treatment

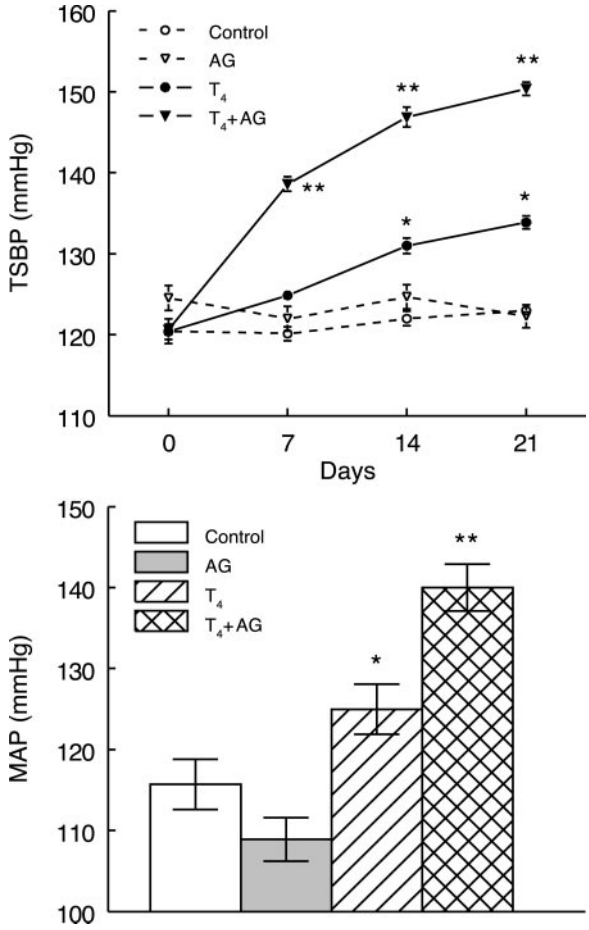

Fig. 1. Time course of systolic blood pressure (SBP) measured by tail-cuff method and final mean arterial pressure (MAP) measured by direct recording (femoral artery) in conscious rat. *$P < 0.05$ compared with controls.**$P < 0.05$ compared with T_4. AG, aminoguanidine; T_4, thyroxine; TSBP, tail SBP.

did not significantly affect the body weight increase in control or T_4-treated rats (Table 1). Absolute kidney weight and absolute left ventricular weight were similar in all groups. However, absolute right ventricular weight was increased in both T_4-treated groups (Table 1). The kidney-to-body weight ratio and left ventricular-to-body weight ratio were significantly increased in both T_4-treated groups compared with their respective controls and were unaffected by AG treatment (Table 1). The left ventricular-to-right ventricular weight ratio was reduced in all T_4-treated groups with respect to their controls (Table 1), indicating that the cardiac hypertrophy of the hyperthyroid state affects the right ventricle more than the left one.

Plasma, metabolic, and urinary variables. Plasma NOx concentration was unaffected by AG treatment in control rats. Plasma NOx was significantly increased in the T_4 group. AG treatment reduced plasma NOx in T_4-treated rats, suggesting that iNOS is partly responsible for the increased NO production induced by thyroid hormones. There were no significant differences in plasma sodium, potassium, or creatinine levels among the groups. Total plasma protein values were significantly decreased in the T_4 and T_4 + AG groups vs. controls (Table 2).

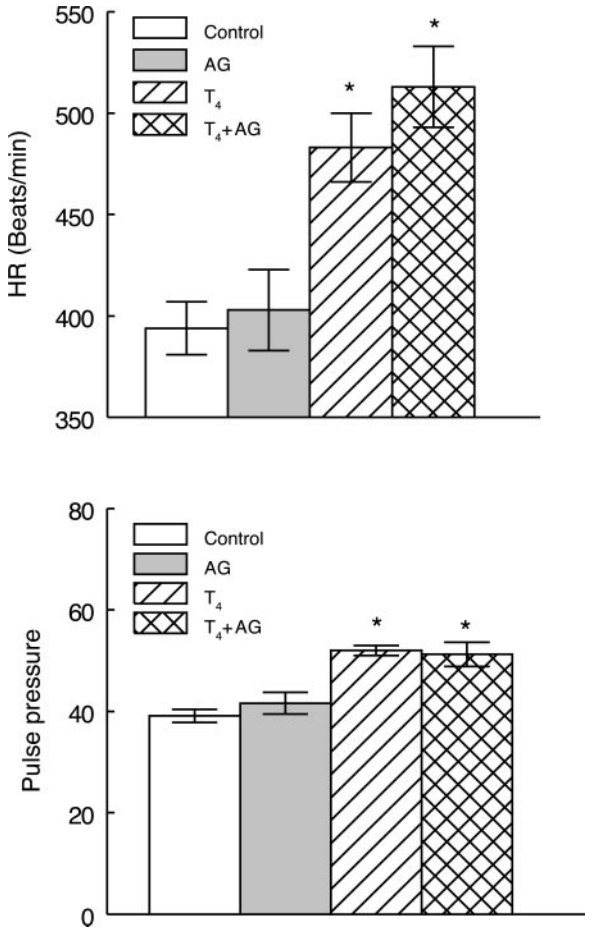

Fig. 2. Final heart rate (HR) and pulse pressure measured by direct recording (femoral artery) in conscious rats. *$P < 0.05$ compared with controls.

Metabolic studies at the end of treatment showed increased food and fluid intake (g/100 g body wt) in all T_4-treated groups compared with their corresponding controls (data not shown).

Urine variables and creatinine clearance values are summarized in Table 3. Urine volume was significantly higher in the T_4-treated groups. Total sodium and potassium excretion was not significantly modified by T_4 or AG treatments. Proteinuria was significantly increased in all T_4-treated groups and was unaffected by AG treatment in control and T_4-treated rats. Creatinine clearance, normalized per gram kidney weight, was similar in all experimental groups.

DISCUSSION

One of the main findings of this study was that AG increased BP in the hyperthyroid rats at doses that had no pressor effect in the control animals. This suggests that these hyperthyroid animals have an elevated sensitivity to AG. At the same time, the elevated plasma levels of nitrates plus nitrites (an index of NO production) in the hyperthyroid animals decreased with the administration of the drug, indicating that this important pressor effect is related to inhibition of NO production. Because AG has been reported to inhibit iNOS (14, 21, 22, 25, 32), these results indicate that the inducible isoform is activated in hyperthyroid animals. Interestingly, the BP increase induced by AG administration in hyperthyroid rats is approximately one-half that produced by L-NAME (27), the nonspecific NOS inhibitor. This suggests that NO derived from eNOS also contributes to counterbalancing the pressor response to T_4 administration, since nNOS blockade with 7-nitroindazole did not modify BP in hyperthyroid rats (unpublished observations).

At the concentration used in this study, AG did not significantly affect BP in normal rats, suggesting that only the upregulated NO production was blocked by AG. This proposition is supported by findings that hyperthyroidism causes an upregulation of NOS activity (26) and NO production (27 and Table 3) and that AG decreases plasma NOx only in hyperthyroid rats. The absence of effect of AG on BP in normal rats is in agreement with previous observations (7, 31).

This study and several reports (9, 26, 27) provide evidence that the hyperdynamic circulation of hyperthyroidism is accompanied by increased NO production. Cirrhosis of the liver (25) is also associated with hyperdynamic circulation and increased NO production, and cirrhotic rats show an increased pressor responsiveness to iNOS blockade (25). Taken together, these observations indicate that iNOS is activated in rats with hyperdynamic circulation, which may have an important homeostatic role in these animals.

Various mechanisms may participate in the increased BP sensitivity to iNOS blockade in hyperthyroid rats. First, the reduction in plasma NOx induced by AG in hyperthyroid rats may be an index of a reduced NO availability that might produce an imbalance in the NO-ANG II interaction, facilitating prohypertensive vascular and renal actions of ANG II. This phenomenon may be active in hyperthyroidism that produces stimulation of the renin-angiotensin system (11). This possibility is supported by our group's previous finding in hyperthyroid rats that ANG II type 1 receptor blockade suppressed the BP increase produced by partial NO inhibition with the nonspecific NOS inhibitor L-NAME (27). Second, renal iNOS activity may decrease because of AG administration, potentially contributing to enhanced sodium reabsorption in hyperthyroid rats. Finally, changes in renal iNOS activity can produce important effects on renal sodium excretion (21, 32). iNOS mRNA has been identified in tubular and vascular sections of the kidney. The greatest amount of iNOS mRNA

Table 1. *Morphological variables in the experimental groups*

Groups	ΔBW, g	FBW, g	KW, mg	KW/BW	LVW, mg	LVW/BW	RVW, mg	LVW/RVW
Control	110±15	379.4±9.99	985±28	2.651±0.070	680±24	1.789±0.070	140±15	4.928±0.423
AG	90±10	340.0±20.6	842±30	2.331±0.070	590±21	1.720±0.037	139±20	4.289±0.235
T_4	60±9*	316.0±9.50	995±19	3.131±0.110*	700±20	2.210±0.073*	218±22*	3.261±0.163*
T_4 + AG	55±7*	303.0±9.10	985±45	3.253±0.106*	674±27	2.062±0.099*	180±19*	3.709±0.096*

Data are expressed as means ± SE. AG, aminoguanidine; T_4, thyroxine; ΔBW, absolute increase in body weight; FBW, final body weight; KW, kidney weight; KW/BW, ratio of kidney weight to body weight; LVW, left ventricular weight; LVW/BW, ratio of left ventricular weight to body weight; LVW/RVW, ratio of left ventricular weight to right ventricular weight. *$P < 0.05$ vs. control group.

Table 2. *Plasma variables in the experimental groups*

Groups	Na, meq/l	K, meq/l	Creatinine, mg/dl	Total Protein, g/dl	NOx, μmol/l
Control	142.2±0.55	4.34±0.13	0.60±0.02	6.06±0.06	6.10±0.10
AG	144.1±0.35	4.03±0.16	0.65±0.01	6.00±0.11	5.78±0.20
T_4	145.1±0.85	4.47±0.07	0.62±0.02	5.56±0.09*	10.02±0.15*
T_4 + AG	144.9±0.70	4.51±0.09	0.64±0.02	5.53±0.08*	6.81±0.14

Data expressed as means ± SE. *$P < 0.05$ vs. control group.

was observed in the medullary thick ascending limb and the inner medullary collecting duct (1, 30), the major sites of sodium reabsorption. Mattson et al. (21) reported that chronic intravenous infusion of AG to uninephrectomized Sprague-Dawley rats maintained on a high-salt diet produced a decrease in renal medullary iNOS activity and in urinary sodium excretion and caused hypertension. Moreover, studies in hypertensive rats provide evidence that iNOS is connected with salt sensitivity and BP regulation (8, 32). Therefore, it is reasonable to assume that, in the present study, the AG-induced inhibition of medullary iNOS in the hyperthyroid rat may have increased sodium retention in the distal part of the nephron and aggravated the antinatriuretic effects of thyroid hormones (4, 36, 37), thereby increasing the blood volume and BP, which in turn elevated sodium excretion but at the expense of an increased BP. Indeed, our hyperthyroid rats required an increased BP to achieve a normal sodium excretion during AG administration.

Previous studies have shown that AG is a selective iNOS inhibitor (14, 21, 22, 25, 32). In vitro, the inhibition constant value of AG is 32- to 52-fold lower for iNOS than for eNOS (22). In vivo, AG is 40-fold less potent than L-NMMA to acutely increase BP in rats (5). AG had no effect on ACh-induced relaxation in intact vessels of sham-treated rats but competitively inhibited relaxation by L-arginine of artery rings from endotoxin-treated rats (14). A 6-day intravenous infusion of AG at 10 $mg \cdot kg^{-1} \cdot h^{-1}$ to normal rats on high sodium intake decreased renal medullary Ca-independent NOS activity without effect on Ca-dependent activity (21). Prevention of the long-term effects of AG on BP in sodium-loaded rats by administration of excess NOS substrate (2% L-arginine in drinking water) argues against nonspecific effects of this drug (21). All of these studies indicate that AG can be used as a selective inhibitor of iNOS in vivo. In fact, AG has been used as a reference compound to analyze the activity of new iNOS inhibitors in vivo and in vitro (16).

Moreover, several groups have shown that oral administration of AG at similar doses to those in the present study can selectively inhibit iNOS activity in different experimental settings. Chronic AG treatment (15 $mg \cdot kg^{-1} \cdot day^{-1}$ po) significantly suppressed the development of hypertension in spontaneously hypertensive rats and inhibited the increase in aortic iNOS expression, NO production, and superoxide anion formation of these rats (15). Sarthy and Kern (29) observed that retinal homogenates from diabetic rats produced greater amounts of NO and iNOS that were inhibited by oral AG administration.

Cardiac hypertrophy (17, 18, 27) is associated with hyperthyroidism. The ventricular-to-body weight ratio, a measure of relative ventricular hypertrophy, was increased in T_4-treated rats. However, the left-to-right ventricular weight ratio was reduced by T_4 treatment, indicating that the trophic effect of thyroid hormones affects both ventricles and predominates in the right ventricle. These results agree in part with previous observations (27), although the left ventricular-to-right ventricular weight ratio did not reach a statistically significant difference with that of controls in that study. Both ratios were unaffected by AG treatment in the present study. These data suggest that ventricular hypertrophy in hyperthyroidism is unrelated to the BP and agree with previous observations by our group (27) that increases or reductions in BP, induced by L-NAME or losartan, respectively, did not modify ventricular hypertrophy in hyperthyroid rats. Therefore, the present data add further support to our previous suggestion that a direct trophic effect of thyroid hormones on the heart may be responsible for cardiac hypertrophy in hyperthyroidism (27). This proposal is in agreement with the observations of Bedotto et al. (2), who reported that cardiac hypertrophy in hyperthyroid rats is independent of loading conditions, and with studies of cultured cardiomyocytes in which the thyroid hormone promoted cell growth (24).

All T_4-treated groups showed increased proteinuria, as previously reported in hyperthyroid rats by our group (27) and as observed in patients with Graves' disease (38). The proteinuria is not related to the BP, because it was similar in all T_4-treated rats, as found in the previous study (27), providing further evidence that proteinuria in the hyperthyroid state may be produced by a direct action of thyroid hormones, increasing the permeability of the glomerular barrier. These observations agree with clinical reports (33) of a nephrotic syndrome in thyrotoxic patients.

Several authors have reported that long-term oral administration of AG attenuates renal injury and reduces proteinuria in

Table 3. *Urine variables in the experimental groups*

Groups	Urinary Flow Rate, $ml \cdot 100\ g^{-1} \cdot 24\ h^{-1}$	Sodium Excretion Rate, $\mu mol \cdot 100\ g^{-1} \cdot 24\ h^{-1}$	Potassium Excretion Rate, $\mu mol \cdot 100\ g^{-1} \cdot 24\ h^{-1}$	Protein Excretion Rate, $\mu mol \cdot 100\ g^{-1} \cdot 24\ h^{-1}$	Creatinine Cl (100 g)
Control	3.70±0.43	310.5±27.1	417.9±51.1	3.65±0.43	0.332±0.020
AG	3.82±0.19	290.1±12.8	377.1±23.0	4.68±0.37	0.311±0.013
T_4	4.34±1.12*	352.0±45.0	572.0±68.0	8.65±0.93*	0.305±0.021
T_4 + AG	4.65±0.39*	381.0±31.6	540.1±54.8	7.91±0.71*	0.323±0.017

Data expressed as means ± SE. *$P < 0.05$ vs. control group.

several experimental chronic renal diseases, including lupus (39), diabetes (31), 5/6 nephrectomy (10), and aging-related nephropathies (20). The mechanism underlying these protective effects is unclear, although some authors reported that the beneficial effects of AG are associated with an inhibition of iNOS (10, 31, 39). However, our results show that AG was unable to reduce proteinuria in hyperthyroid rats. The inability of AG to reduce the proteinuria of hyperthyroid rats may be due to the short duration of our experiment, since the protective effects of AG on proteinuria have been observed after months of treatment (20, 31, 39). Therefore, our data do not rule out that a longer period of AG treatment can reduce proteinuria in hyperthyroid rats.

Creatinine clearance normalized per gram kidney weight was similar in all experimental groups, as observed in a previous study using the same dose and duration of T_4 treatment (27). However, this finding contrasts with the reduction in glomerular filtration rate in T_4-treated rats previously reported by our group (11, 36), when a larger T_4 dose (75 $\mu g \cdot rat^{-1} \cdot day^{-1}$) and a longer treatment period (6 wk) were used. In conclusion, the present study shows that iNOS plays a role in long-term control of BP of hyperthyroid rats, indicating that NO generated by iNOS contributes to the homeostatic role of this factor in hyperthyroidism. Moreover, AG treatment did not modify the cardiac hypertrophy or proteinuria of hyperthyroid rats.

iNOS has been implicated in the control of sodium excretion and consequently in BP regulation. The present study analyzed the role of iNOS in the long-term BP control of hyperthyroid rats. The data reported herein provide evidence that iNOS may counterbalance the prohypertensive effects of T_4. The present study is, to our knowledge, the first to assess the effects of the blockade of iNOS on hemodynamic and renal abnormalities in the hyperthyroid state and, therefore, opens up new perspectives for the assessment of cardiovascular abnormalities in thyroid disorders.

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Effects of chronic treatment with 7-nitroindazole in hyperthyroid rats

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[1]*Departamento de Ciencias de la Salud, Universidad de Jaén, Jaén;* [2]*Departamento de Fisiología, Facultad de Medicina, Granada; and* [3]*Servicio de Nefrología, Unidad Experimental, Hospital Virgen de las Nieves, Granada, Spain*

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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. First published June 15, 2006; doi:10.1152/ajpregu.00722.2005.—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, 7-NI (30 mg·kg^{-1}·day^{-1} by gavage), $T_4$50, $T_4$75 (50 or 75 μg thyroxine·rat^{-1}·day^{-1}, respectively), $T_4$50+7-NI, and $T_4$75+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 $T_4$75 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 contribution to BP in $T_4$75 and $T_4$75+7-NI rats. T_4 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 $T_4$75 group. Pentolinium produced a greater MAP decrease in the $T_4$75+7-NI than in the $T_4$75 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 N^{ω}-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 nNOS inhibitors (14, 28, 31, 32). 7-NI is more selective for nNOS than methyl- or nitro-arginine-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 Experimentation 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 (T_4; 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 T_4 and 7-NI are in accordance with previous protocols used in our laboratory (16, 29).

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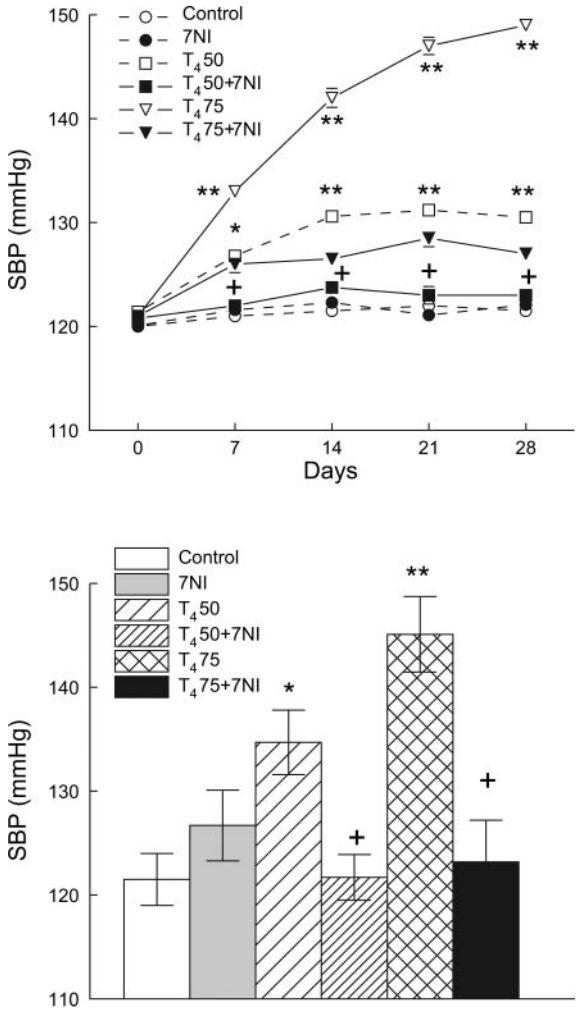

Fig. 1. Time course of systolic blood pressure (SBP) measured using tail-cuff method (*top*) and final SBP measured by direct recording (femoral artery) in conscious rats (*bottom*). Groups are as follows: control, treatment with 7-nitroindazole (7-NI), treatment with thyroxine at 50 ($T_4$50) or 75 ($T_4$75) $\mu g \cdot rat^{-1} \cdot day^{-1}$, and treatment with thyroxine at 50 and 75 $\mu g \cdot rat^{-1} \cdot day^{-1}$ plus 7-NI ($T_4$50+7-NI and $T_4$75+7-NI). Data are means ± SE. $*P < 0.05$; $**P < 0.01$ compared with controls. $+P < 0.05$; $++P < 0.01$ compared with respective T_4 group.

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 ($T_4$50) or 75 ($T_4$75) $\mu g \cdot rat^{-1} \cdot day^{-1}$, and treatment with thyroxine at 50 and 75 $\mu g \cdot rat^{-1} \cdot day^{-1}$ plus 7-NI ($T_4$50+7-NI and $T_4$75+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 ($T_4$75) 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,

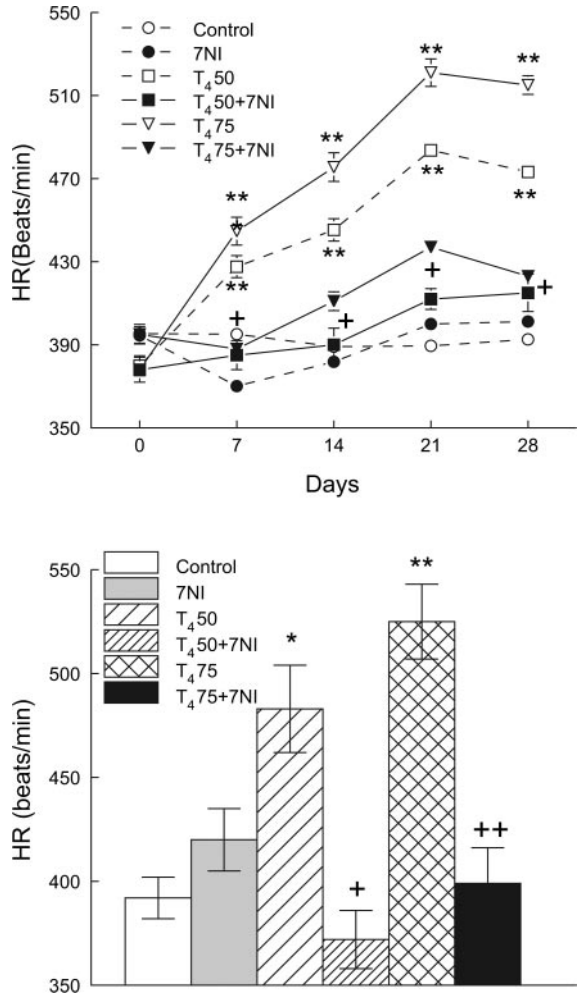

Fig. 2. Time course of heart rate (HR) measured using tail-cuff method (*top*) and final HR measured by direct recording (femoral artery) in conscious rats (*bottom*). Data are means ± SE. $*P < 0.05$; $**P < 0.01$ compared with controls. $+P < 0.05$; $++P < 0.01$ compared with respective T_4 group.

Table 1. *Morphological variables in experimental groups*

Groups	FBW, g	KW/BW, mg/g	LVW/BW, mg/g	LVW/HVW
Control	394.3±7.8	2.37±0.05	1.60±0.04	0.80±0.01
7-NI	370.7±4.6	2.61±0.11	1.70±0.05	0.79±0.01
$T_4$50	338.6±6.8*	3.08±0.07*	2.29±0.06*	0.80±0.01
$T_4$50+7-NI	340.3±5.7*	3.13±0.13*	2.06±0.09*	0.78±0.02
$T_4$75	296.3±5.3†	3.21±0.04*	2.36±0.05*	0.79±0.01
$T_4$75+7-NI	305.3±6.5†	3.01±0.050*	2.25±0.05*	0.81±0.01

Data are expressed as means ± SE. 7-NI, 7-nitroindazole; T_4, 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.

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, Amersham, 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 sympathetic blocker pentolinium (10 mg/kg; Sigma) were analyzed in conscious control, $T_4$75, and $T_4$75+7-NI rats. After 4 wk of treatment, the femoral artery and vein were catheterized, allowing a 24-h recovery period to perform the experiment. The dose of pentolinium selected was previously reported (19) to produce maximal sympathetic inhibition.

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 analyzed using unpaired Student's *t*-test.

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. T_4 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 T_4-treated groups. Thus the $T_4$50+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 $T_4$75+7-NI group. PP values in the groups were as follows: control, 36.8 ± 1.6; 7-NI, 34.9 ± 1.6; $T_4$50, 48.2 ± 1.0 ($P < 0.01$ compared with control); $T_4$75, 52.1 ± 2.1 ($P < 0.01$ compared with control); $T_4$50+7-NI, 34.1 ± 2.6 ($P < 0.01$ compared with respective T_4-treated group), and $T_4$75+7-NI, 39.1 ± 2.6 ($P < 0.01$ compared with respective T_4-treated group).

Morphological Variables

All T_4-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 $T_4$50 and $T_4$75 groups compared with controls. The left ventricular-weight-to-heart weight ratio was not significantly modified in T_4-treated groups compared with controls. None of these morphological variables were affected by 7-NI treatment in control or T_4-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 T_4 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 $T_4$75 group and similar to control values in the $T_4$75+7-NI group (Table 2).

Table 2. *Plasma variables in experimental groups*

Groups	Na^+, meq/l	K^+, meq/l	Creatinine, mg/dl	Total Protein, g/dl	BUN, mg/dl
Control	140.8±0.30	4.63±0.10	0.58±0.03	5.97±0.08	37.8±1.76
7-NI	140.5±0.37	4.61±0.14	0.52±0.01	6.12±0.07	33.3±2.00
$T_4$50	139.5±0.52	4.70±0.17	0.60±0.02	5.03±0.11*	34.2±2.91
$T_4$50+7-NI	142.1±0.10	4.80±0.15	0.58±0.01	5.79±0.07‡	43.6±4.78
$T_4$75	140.8±0.17	4.62±0.09	0.96±0.06*	4.55±0.18†	68.6±5.88*
$T_4$75+7-NI	141.2±0.29	4.64±0.17	0.54±0.02‡	5.46±0.11‡	41.1±2.10‡

Data are expressed as means ± SE. *$P < 0.05$; †$P < 0.01$ compared with control group. ‡$P < 0.05$ compared with respective T_4 group.

Table 3. *Food and fluid intake in experimental groups*

Groups	Food Intake, g·100 g^{-1}·24 h^{-1}	Water Intake, ml·100 g^{-1}·24 h
Control	5.01±0.16	5.35±0.38
7-NI	5.21±0.24	7.26±0.39*
$T_4$50	7.58±0.35*	8.57±0.28*
$T_4$50+7-NI	7.58±0.43*	6.59±0.45*‡
$T_4$75	8.16±0.31†	10.9±1.17†
$T_4$75+7-NI	7.22±0.26*‡	6.32±1.02‡

Data are expressed as means ± SE. *$P < 0.05$; †$P < 0.01$ compared with control group. ‡$P < 0.05$ compared with $T_4$75 group.

Metabolic studies at the end of treatment showed increased food and fluid intake (g/100 g body wt) in all T_4-treated groups compared with controls. Food intake was significantly reduced in the $T_4$75+7-NI group compared with the $T_4$75 group. The 7-NI group showed increased fluid intake compared with controls. However, 7-NI administration produced a reduction in water intake in both T_4-treated groups. Thus $T_4$50+7-NI and $T_4$75+7-NI groups showed a significantly reduced water intake compared with their respective T_4-treated groups (Table 3).

Water and sodium balances, proteinuria, and creatinine clearance (CrC) values are summarized in Table 4. Water and sodium balances significantly increased in both T_4-treated groups, whereas significance was not reached in the T_4-7-NI-treated groups compared with controls. 7-NI administration reduced diuresis in both T_4-treated groups. Total sodium and potassium excretion was higher in the T_4-treated animals. These data are consistent with the greater food intake of T_4-treated rats. 7-NI did not significantly affect natriuresis or kaliuresis in control or T_4-treated groups (data not shown). Proteinuria was significantly increased in all T_4-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 $T_4$75 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 $T_4$75+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 $T_4$75 and $T_4$75+7-NI rats.

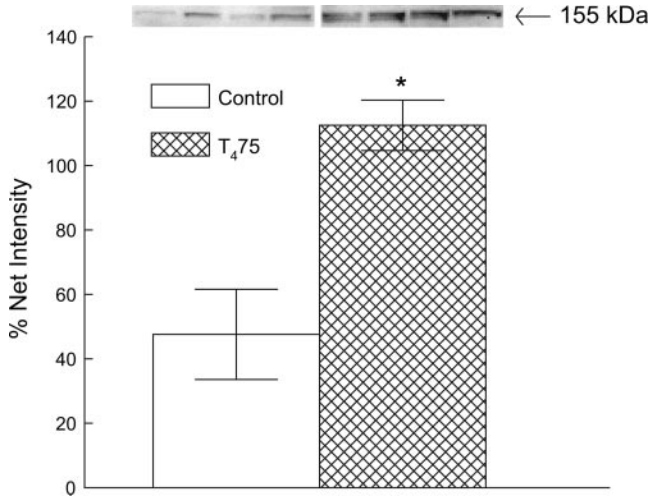

Fig. 3. Expression of neuronal nitric oxide synthase (nNOS) determined by Western blot analysis in the hypothalamus of hyperthyroid and control rats. Data are expressed as percentages of net intensity vs. commercial positive control band. Data are means ± SE. *$P < 0.01$ compared with controls.

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

Groups	Water Balance, ml·100 g^{-1}·24 h^{-1}	Sodium Balance, mmol·100 g^{-1}·24 h^{-1}	Protein Excretion Rate, mg/mg creatinine	Creatinine Clearance, ml·min^{-1}·g kidney^{-1}
Control	3.49±0.33	0.18±0.04	5.41±0.17	0.49±0.06
7-NI	3.28±0.14	0.14±0.02	7.92±0.97*	0.47±0.02
$T_4$50	5.58±0.53*	0.29±0.05*	9.54±0.41*	0.42±0.03
$T_4$50+7-NI	4.41±0.73	0.24±0.04*	9.57±0.52*	0.48±0.021
$T_4$75	5.85±0.45*	0.26±0.03*	11.7±0.71*	0.30±0.02*
$T_4$75+7-NI	4.75±0.43	0.18±0.02†	11.0±0.44*	0.52±0.04†

Data are expressed as means ± SE. *$P < 0.05$ compared with control group. †$P < 0.05$ compared with $T_4$75 group.

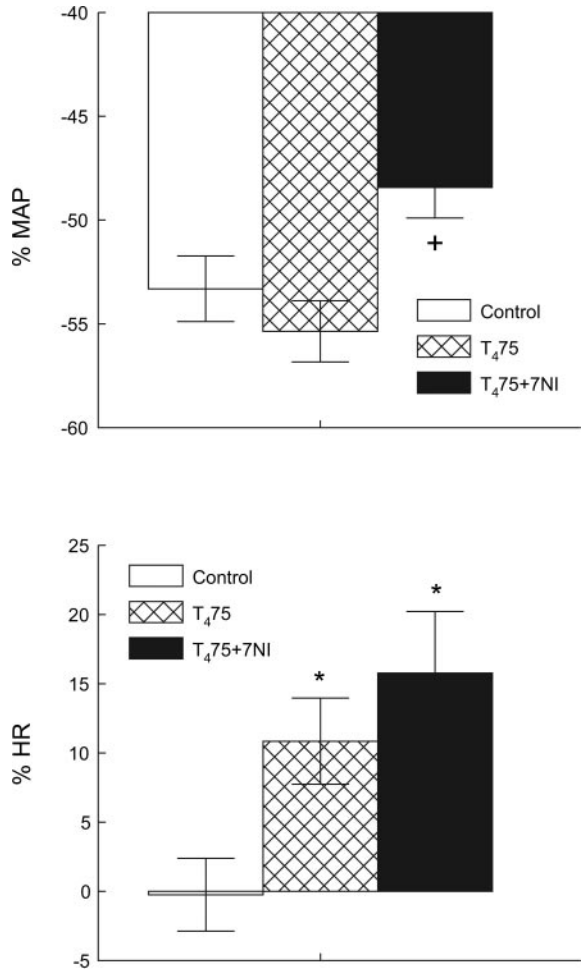

Fig. 4. Percentage of changes induced in mean arterial pressure (MAP) and HR by administration of acute pentolinium (10 mg/kg iv) in conscious rats in experimental groups. Data are means ± SE. *$P < 0.05$ compared with controls. +$P < 0.01$ compared with T_4 group.

The mechanism by which chronic 7-NI administration to T_4-treated rats suppresses the cardiovascular manifestations of hyperthyroidism remains to be defined, but we propose the possibility that 7-NI may produce a central or peripheral blockade of sympathetic discharge in these animals. In this regard, the data showed that the sympathetic blockade with pentolinium produced a greater BP decrease in hyperthyroid than in 7-NI-treated or control rats, suggesting a reduced contribution of the sympathetic tone in the resting blood pressure of hyperthyroid rats treated with 7-NI. Moreover, a number of studies have demonstrated an important role for NO in central and peripheral modulation of sympathetic activity. Thus microinjection of 7-NI bilaterally into the rostral ventrolateral medulla of rat, where sympathetic vasomotor tone originates, produced a reduction in systemic BP and HR and in the power density of the vasomotor components in the spectrum of arterial BP signals, which is the experimental index of sympathetic neurogenic vasomotor tone (6). 7-NI administration also inhibited the sympathoexcitatory cardiovascular response produced during intoxication by the cholinesterase inhibitor mevinphos, which was accompanied by increased nNOS mRNA levels in the rostral ventrolateral medulla (5). Furthermore, 7-NI inhibited the reflex sympathetic pressor response to bradykinin (24). In addition, indazole derivatives and the α_2-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 T_4-treated groups, whereas the comparison between T_4-7-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 $T_4$75+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 >80% of 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 T_4 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 CrC 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 T_4-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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GRANTS

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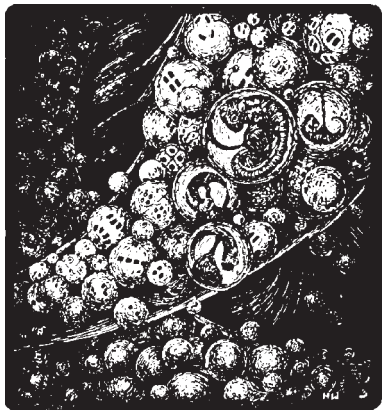

CONCLUSIONES

4.- CONCLUSIONES

1ª- La actividad NOS está aumentada en el corazón y riñón de las ratas hipertiroideas, tejidos relacionados de forma primaria con el control de la presión sanguínea. De este modo, un incremento en la producción de NO podría participar en manifestaciones cardiovasculares de esta enfermedad.

2ª- La actividad NOS mostró un patrón heterogéneo en los diversos tejidos estudiados en las ratas hipotiroideas. La baja actividad NOS en aorta y normal actividad en médula renal de estos animales, sugiere la participación de este factor en el aumento de la resistencia periférica y el manejo normal de sodio de las ratas hipotiroideas.

3ª- Un déficit de la síntesis de NO, produce en un incremento en la sensibilidad al efecto presor de la T_4, el cual se atenúa mediante la administración de losartan. Estas observaciones indican:

- Que el NO contribuye a la respuesta homeostática de adaptación hemodinámica al hipertiroidismo.
- El SRA juega un importante papel en la hipertensión inducida, por administración simultánea y crónica de una dosis supresora de T_4 y L-NAME.

4ª- Los datos aportados en esta tesis indican que la iNOS juega un papel en el control a largo plazo de la presión sanguínea en ratas hipertiroideas, indicando que el NO generado por iNOS contribuye a la acción homeostática de este factor en el hipertiroidismo, interfiriendo en la acción prohipertensiva de la tiroxina.

5ª- El tratamiento crónico de 7NI suprime o atenúa las manifestaciones hemodinámicas características del hipertiroidismo, sugiriendo que la nNOS contribuye al desarrollo de la circulación hiperdinámica del hipertiroidismo.

6ª- La administración de 7NI atenúa la polifagia y polidipsia de las ratas hipertiroideas y mejora su tasa de filtración glomerular. Indicando de nuevo la participación de la nNOS en estas alteraciones.

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