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Thyroid hormone stimulates renin synthesis in rats without involving the sympathetic nervous system

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Abstract

The present study was performed to examine whether renal expression of the renin gene is regulated by thyroid hormone. Thirty male Sprague-Dawley rats were divided into hypothyroid, control, and hyperthyroid groups by use of daily intraperitoneal administration of methimazole, saline vehicle, or thyroxine, respectively. Each group was further subdivided into sympathetic innervated and sympathetic denervated subgroups by use of intraperitoneal administration of saline vehicle or 6-hydroxydopamine. Plasma renin activity and renal levels of renin were measured by radioimmunoassays after 8 wk. Renal expression of renin mRNA was evaluated by a semiquantitative reverse transcriptase-polymerase chain reaction. Compared with control animals, plasma renin activity, renal level of renin, and renal expression of renin mRNA were reduced (82, 94, and 71%, respectively) in hypothyroid animals and elevated (155, 1,182, and 152%, respectively) in hyperthyroid animals. Sympathetic denervation had no independent effect on these renin values. Our results indicate that thyroid hormone stimulates renin synthesis without involving the sympathetic nervous system.

Keywords

renin-angiotensin system; chemical sympathectomy; polymerase chain reaction

HYPERTHYROIDISM is associated with increased plasma renin activity (PRA) (10). The increased PRA activates the renin-angiotensin system and elevates the plasma level of angiotensin II. The elevated angiotensin II subsequently promotes cardiac hypertrophy, systemically through hemodynamic effects and regionally through proliferative effects. Cardiac hypertrophy is a late and often fatal symptom of hyperthyroidism (22). Therefore, increased PRA is considered to be an important clinical finding in hyperthyroidism. It is believed that augmented sympathetic nerve activity causes the increased PRA in hyperthyroidism (10). However, it was recently shown that hypothyroidism is associated with high sympathetic nerve activity and low PRA (19). In addition, in vitro studies have demonstrated that renin mRNA increases with treatment of thyroid hormone and decreases with treatment of propylthiouracil in the submandibular gland of mice (2,16). It was also shown that thyroid hormone suppresses forskolin-induced stimulation of promoter activity of the human renin gene by use of a pituitary cell line to which the 5' flanking regions of the human renin gene were transfected (7). These data suggest that thyroid hormone by itself may regulate expression of the renin gene. However, there is no evidence that expression of the renin reverse transcriptase-polymerase chain reaction (RT-PCR) to observe changes in renin mRNA expression.

METHODS

Animal preparation

Thirty male Sprague-Dawley rats (Charles River Japan, Kanagawa, Japan) weighing 150–200 g were used. They received standard laboratory chow containing 110 $\mu\text{mol/g}$ of sodium (Oriental Yeast, Tokyo, Japan), with tap water ad libitum. They were individually caged with 12:12-h light-dark cycles. They were divided into hypothyroid (Hypo), control (Cont), and hyperthyroid groups (Hyper) by daily intraperitoneal injections of methimazole (10 $\mu\text{g/g}$), saline vehicle, or thyroxine (T_4 , 1 $\mu\text{g/g}$), respectively, for 8 wk, as previously described (23). These groups were then subdivided into a sympathetic innervated group (IN) and a sympathetic denervated group (DX) by intraperitoneal injections of saline vehicle or 6-hydroxydopamine (100 $\mu\text{g/g}$), respectively, on *days 1, 3, 27, and 47*, as previously described (14). Systolic blood pressure and heart rate were measured weekly by tail-cuff plethysmography. Body weight was checked daily. All rats were decapitated at 8 wk. Blood was collected and centrifuged for plasma and serum that were then stored at -20°C . The kidneys were dissected free, decapsulated, washed in ribonuclease-free saline, weighed, snap-frozen in liquid nitrogen, and stored at -80°C until assayed.

Hormone measurement

Serum free 3,5,3'-triiodothyronine (T_3) and PRA were determined by radioimmunoassays with the Amarex-MAB free T_3 kit (Ortho-Clinical Diagnostics, Tokyo, Japan) and the Renin-Riabeat kit (Dainabot, Tokyo, Japan), respectively. Plasma angiotensin II was determined by a combined solid-phase extraction high-performance liquid chromatography-radioimmunoassay (25,27) with slight modifications. Briefly, after a mixed inhibitor solution (5 mmol/l EDTA, 10 $\mu\text{mol/l}$ pepstain, 20 $\mu\text{mol/l}$ enalapril, and 1.25 mmol/l 1,10-Phenanthroline, final concentration) was added to 1 ml of plasma, the sample was immediately applied to an octadecasilyl-silica solid-phase extraction column (Sep-Pak Plus C18 cartridge, Millipore, Bedford, MA) that had been moistened with 3 ml of methanol followed by 10 ml of 0.1 mol/l HCl. After washing with 10 ml of 0.1 mol/l HCl, the sample was eluted with a mixture of methanol-distilled water-trifluoroacetic acid (80: 19.1:0.1; vol/vol/vol). The eluate was evaporated to dryness in a vacuum centrifuge and resuspended in 1 ml of a buffer that contained 0.1 mol/l tris(hydroxymethyl)aminomethane (Tris)-acetate, 2.6 mmol/l EDTA, 1 mmol/l phenylmethylsulfonyl fluoride, and 0.1% bovine serum albumin (BSA). Angiotensin II was separated from other angiotensin peptides in the resuspended extract by reverse phase chromatography by use of the Nucleosil C₁₈ column (Alltech Associates, Deerfield, IL) and a mobile phase consisting of 0.085% orthophosphoric acid, 0.02% sodium azide, and methanol. The retention time of angiotensin II was 7.5 min. Fractions were collected and neutralized to pH 7.4. The sample was lyophilized, reconstituted in a radioimmunoassay buffer containing 50 mmol/l Tris-HCl (pH 7.4) and 0.3% BSA, and measured directly by the Angiotensin-II Radioimmunoassay Kit (Nichols Institute Diagnostics, San Juan Capistrano, CA).

Frozen kidneys were divided into three pieces; one was used for the measurement of renal level of renin as previously described (3). Briefly, kidney was homogenized with the polytron (Kinematica, Littau, Switzerland) in 10 ml of buffer containing 2.6 mmol/l EDTA, 1.6 mmol/l dimercaprol, 3.4 mmol/l 18-hydroxyquinoline sulfate, 0.2 mmol/l phenylmethylsulfonyl fluoride, and 5 mmol/l ammonium acetate. The homogenate was frozen and thawed four times and spun at 5,000 rpm for 30 min at 4°C , and the supernatant was removed. An aliquot of the supernatant was diluted 1:1,000. In addition, 500 μl of plasma obtained from nephrectomized male rats were added to an equal volume of the dilute supernatant as a substrate for the enzymatic reaction. Renin activity was determined as previously described (12) with a radioimmunoassay kit (Renin-Riabeat). Renal level of renin was calculated by the following

formula: renal level (μg of angiotensin $\text{I}\cdot\text{h}^{-1}\cdot\text{g}$ kidney $^{-1}$) = renin activity (μg of angiotensin $\text{I}\cdot\text{h}^{-1}$) $\times$ dilution rate ($1,000 \times 2 = 2,000$) $\times$ volume of buffer ($10 \times 10^{-3}\text{l}$)/weight of the aliquot of kidney assayed (g).

The second piece of kidney was used for determination of the renal level of norepinephrine, as previously described (15). Briefly, the kidney was homogenized in 10 ml of cooled phosphate buffer containing 5 mmol/l reduced glutathione. The homogenate was spun at 5,000 rpm for 30 min at 4°C and the supernatant was removed. The concentration of norepinephrine was determined using a kit for high-performance liquid chromatography according to the manufacturer's instructions (Cat-a-Kit assay system, Amersham, Buckinghamshire, UK). Renal level of norepinephrine was calculated from the following formula: renal level (ng/g of kidney) = norepinephrine concentration (ng/ml) $\times$ volume of buffer (10 ml)/weight of the aliquot of kidney assayed (g).

Total RNA isolation

Total RNA was extracted from the third piece of kidney by use of the Total RNA Separator Kit according to the manufacturer's instructions (Clontech, Palo Alto, CA). Extracted RNA was suspended in ribonuclease-free water and quantified by measuring the absorbance at 260 nm. In preliminary investigations, the integrity of the purified RNA was confirmed by visualization of the 28s and 18s ribosomal RNA bands after electrophoresis of the RNA sample on a 1% agarose-formaldehyde ethidium bromide gel.

RT of RNA

Total RNA from each kidney was reverse transcribed using the GeneAmp RNA PCR Core Kit (Perkin-Elmer Cetus, Norwalk, CT). Each sample contained 0.5 μg total RNA, 100 nmol MgCl₂, 1,000 nmol KCl, 200 nmol Tris-HCl (pH 8.3), 20 nmol of each dNTP (dATP, dTTP, dGTP, dCTP), 20 units of ribonuclease inhibitor, 50 pmol of random hexamers, and 50 units of murine leukemia virus RT in a final volume of 20 μl . After incubation at 42°C for 15 min, the samples were heated for 5 min at 99°C to finish the reaction and were stored at 5°C until assayed.

Primer preparation

Oligonucleotide primers were assembled from the published cDNA sequences of renin (24) and glyceraldehyde-3-phosphate dehydrogenase (GAPDH) (26). GAPDH was used as an internal standard. As previously reported (17), the sequences of the renin primers were 5' TGCCACCTTGTTGTGTGAGG 3' (sense), which corresponded to bases 851 to 870 (from exon 7) of the cloned full-length sequence, and 5' ACCCGATGCGATTGTTATGCCG 3' (anti-sense), which annealed to bases 1203 to 1224 (from exon 9). As previously reported (21), the sequences of the GAPDH primers were 5' TCCCTCAAGATTGTCAGCAA 3' (sense), which corresponded to bases 492 to 511 of the cloned full-length sequence and 5' AGATCCACAACGGATACATT 3' (antisense), which annealed to bases 780 to 799. The predicted sizes of the amplified renin and GAPDH cDNA products were 374 and 308 bp, respectively. The sense primers in each reaction were radiolabeled using gamma-[³²P]ATP (Amersham) and T₄ polynucleotide kinase, as described in the Kination kit (Toyobo, Osaka, Japan).

Semiquantitative PCR

Semiquantitative PCR was performed as previously described (21). Briefly, 5 μl of RT mixture were used for amplification; 25 nmol of MgCl₂, 1,000 nmol of KCl, 200 nmol of Tris-HCl (pH 8.3), 3.75 pmol and 10⁶ counts/min of each sense primer, 3.75 pmol of each antisense primer, and 0.625 units of AmpliTaq DNA polymerase were added to each sample, as described

in the GeneAmp RNA PCR Core Kit. To minimize nonspecific amplification, we used a “hot start” procedure in which PCR samples were placed in a thermocycler (PerkinElmer Cetus) that was prewarmed to 94°C. After 2 min, PCR was performed for 15–35 cycles with a 30-s denaturation step at 94°C, a 60-s annealing step at 62°C, and a 75-s extension step at 72°C. We added a 5-min extension step at 72°C. After completion of RT-PCR, DNA was electrophoresed on an 8% polyacrylamide gel. Gels were dried on filter paper, exposed to the BAS 2000 imaging plate (Fuji Film, Tokyo, Japan) for 1 min, and quantified with the BAS 2000 Laser Image Analyzer (Fuji Film) (1,13). The expression of renin mRNA was evaluated as the renin-to-GAPDH (renin./GAPDH) ratio, because the amount of GAPDH did not differ between groups.

PCR product conformation

The PCR products were sequenced to confirm that they were renin and GAPDH cDNA. Fragments of PCR products were gel-purified and inserted into pBS(+) (Stratagene, La Jolla, CA). Subclones were analyzed in an automated fluorescence-based GENESIS 2000 sequencer (Applied Biosystems, Foster City, CA) with the dideoxynucleotide-chain termination reaction, as previously described (20). The sequences obtained were identical to those previously reported (24,26).

Statistical analysis

Results are presented as means $\pm$ SE. We compared multiple groups by one-way factorial analysis of variance with post hoc Scheffé's *F* test to determine the significance of the differences between groups. A level of probability (*P*) < 0.05 was considered a statistically significant difference.

RESULTS

Effects of administration of methimazole, thyroxine, and 6-hydroxydopamine

Serum free T₃ decreased significantly with intraperitoneal administration of methimazole (Hypo-IN 1.2 $\pm$ 0.2 ng/l vs. Cont-IN 2.5 $\pm$ 0.1 ng/l, *P* < 0.05; Hypo-DX 1.2 $\pm$ 0.2 ng/l vs. Cont-DX 2.4 $\pm$ 0.2 ng/l, *P* < 0.05) and increased significantly with administration of thyroxine (Hyper-IN 6.6 $\pm$ 0.3 ng/l, *P* < 0.05; Hyper-DX 6.5 $\pm$ 1.5 ng/l, *P* < 0.05).

Renal levels of norepinephrine decreased significantly with administration of 6-hydroxydopamine (Hypo-DX 10 $\pm$ 2 ng/g vs. Hypo-IN 229 $\pm$ 26 ng/g, *P* < 0.05; Cont-DX 4 $\pm$ 1 ng/g vs. Cont-IN 107 $\pm$ 21 ng/g, *P* < 0.05; Hyper-DX 6 $\pm$ 1 ng/g vs. Hyper-IN 116 $\pm$ 9 ng/g, *P* < 0.05).

Effects of RT on amplification of renin and GAPDH mRNA

With RT, we found two clear bands that had predicted sizes of 374 bp for renin and 308 bp for GAPDH. When the PCR procedure was accomplished without RT, these bands were not observed, and no other bands were noted. This indicated that the 374 and 308 bp bands originated from mRNA, not from genomic DNA.

Relationship between PCR cycle number and quantity of amplified products for renin and GAPDH mRNA

We evaluated the cycle dependency of the radioactivity of RT-PCR product (Fig. 1). A linear relationship between the number of PCR cycles and the amount of PCR product (renin and GAPDH) was obtained between cycles 23 and 28. We chose 25 cycles of PCR for analysis.

Hemodynamic changes produced by methimazole and thyroxine treatment and by sympathetic denervation

Heart rate increased significantly in Hyper-IN vs. Cont-IN but was not elevated in Hyper-DX vs. Cont-DX. No difference in systolic blood pressure was observed among groups (Table 1).

Changes in the renin-angiotensin system caused by methimazole and thyroxine treatment and by sympathetic denervation

PRA was reduced by 82% (Hypo-IN $2 \pm 0 \mu\text{g}\cdot\text{h}^{-1}\cdot\text{l}^{-1}$) and 86% (Hypo-DX $1 \pm 0 \mu\text{g}\cdot\text{h}^{-1}\cdot\text{l}^{-1}$) in hypothyroid rats and elevated by 155% (Hyper-IN $28 \pm 4 \mu\text{g}\cdot\text{h}^{-1}\cdot\text{l}^{-1}$) and 157% (Hyper-DX $18 \pm 3 \mu\text{g}\cdot\text{h}^{-1}\cdot\text{l}^{-1}$) in hyperthyroid rats compared with control rats (Cont-IN $11 \pm 2 \mu\text{g}\cdot\text{h}^{-1}\cdot\text{l}^{-1}$ and Cont-DX $7 \pm 2 \mu\text{g}\cdot\text{h}^{-1}\cdot\text{l}^{-1}$; Fig. 2). PRA was reduced by 50% (Hypo), 36% (Cont), and 35% (Hyper) in DX vs. IN.

Renal levels of renin were reduced by 94% (Hypo-IN $2 \pm 0 \mu\text{g}\cdot\text{h}^{-1}\cdot\text{g}^{-1}$) and 97% (Hypo-DX $1 \pm 0 \mu\text{g}\cdot\text{h}^{-1}\cdot\text{g}^{-1}$) in hypothyroid animals and elevated by 1,182% (Hyper-IN $423 \pm 29 \mu\text{g}\cdot\text{h}^{-1}\cdot\text{g}^{-1}$) and 866% (Hyper-DX $280 \pm 15 \mu\text{g}\cdot\text{h}^{-1}\cdot\text{g}^{-1}$) in hyperthyroid animals compared with control animals (Cont-IN $33 \pm 2 \mu\text{g}\cdot\text{h}^{-1}\cdot\text{g}^{-1}$ and Cont-DX $29 \pm 1 \mu\text{g}\cdot\text{h}^{-1}\cdot\text{g}^{-1}$; Fig. 3). Renal levels of renin were reduced by 50% (Hypo), 12% (Cont), and 34% (Hyper) in DX vs. IN.

Figure 4 shows the changes in the renin/GAPDH ratio induced by methimazole, thyroxine, and sympathetic denervation. In sympathetic innervated rats, the renin/GAPDH ratio was reduced by 71% with methimazole (Hypo-IN 0.17 ± 0.02) and increased by 152% with thyroxine (Hyper-IN 1.46 ± 0.21) compared with saline vehicle (Cont-IN 0.58 ± 0.09). The magnitude of these changes was significantly inhibited by sympathetic denervation in Hypo (Hypo-DX 0.10 ± 0.02) and Hyper (Hyper-DX 0.95 ± 0.12) but not in Cont (Cont-DX 0.57 ± 0.07). However, the relationship between thyroid function and the renin/GAPDH ratio did not change in sympathetic denervated rats. Overall, in sympathetic denervated rats, the renin/GAPDH ratio was reduced by 82% with methimazole and increased by 67% with thyroxine compared with saline vehicle.

Plasma levels of angiotensin II were significantly reduced by 43% (Hypo-IN $25 \pm 2 \text{ ng/l}$) and 45% (Hypo-DX $21 \pm 1 \text{ ng/l}$) in Hypo and significantly elevated by 82% (Hyper-IN $80 \pm 8 \text{ ng/l}$) and 79% (Hyper-DX $68 \pm 7 \text{ ng/l}$) in Hyper compared with Cont (Cont-IN $44 \pm \text{ ng/l}$ and Cont-DX $38 \pm 5 \text{ ng/l}$). Plasma levels of angiotensin II were significantly reduced by 13% (Hypo), 14% (Cont), and 15% (Hyper) in DX vs. IN.

DISCUSSION

The principal finding in the present study is that PRA, plasma angiotensin II, renal renin levels, and renal expression of renin mRNA are all affected by thyroid function despite denervation, suggesting that thyroid hormone regulates renin synthesis without involving the sympathetic nervous system. Because most of the renin in blood is synthesized in and secreted from the kidney, it appears that thyroid hormone stimulates renin synthesis in the kidney through the enhancement of the expression of renin mRNA, subsequently increasing renal levels of renin, PRA, and plasma levels of angiotensin II. Thyroid function-dependent changes in plasma levels of angiotensin II parallel changes in PRA. Each multiple effect in plasma levels of angiotensin II was approximately one-half that of PRA. This result suggests that thyroid hormone primarily influences PRA in the circulating renin-angiotensin system and secondarily elevates plasma levels of angiotensin II through the increase in PRA. It seems that the effect of thyroid hormone on plasma levels of angiotensin II depends on PRA, even if thyroid hormone may influence synthesis of angiotensinogen.

Regulators other than the sympathetic nervous system, such as angiotensin II in a negative feedback loop (9), plasma levels of sodium (4), and renal perfusion pressure (9), can affect renin secretion. Therefore, it is difficult to determine from the present study whether thyroid hormone alone regulates renin secretion from the kidney; however, the present results suggest that possibility. Renal levels of renin are significantly more elevated in Hyper-IN and Hyper-DX relative to Cont-IN and Cont-DX, whereas the relatively small increase in PRA in Hyper vs. Cont indicates that thyroid hormone may influence renin secretion, processing of renin, or both, independently of its stimulation of renin synthesis. Because renal levels of renin measured by the present method indicate the summation of active and inactive renin, we cannot distinguish a thyroid effect on renin secretion from renin processing. In addition, the relatively small increase in PRA may be caused in part by regulation of the negative feedback loop in the circulating renin-angiotensin system, because plasma levels of angiotensin II were simultaneously increased in Hyper.

The present results also suggest additional interpretations. One possibility is that denervation affected PRA in all groups, although the expression of renin mRNA did not change with denervation in Cont. This may indicate a decrease in secretion due to the absence of sympathetic tone as well as decreased synthesis. Alternatively, the larger difference in Hyper-IN vs. Hyper-DX for renal levels of renin compared with Cont-IN vs. Cont-DX suggests a synergistic interaction of the sympathetic nervous system and thyroid hormone in upregulating renin synthesis. Namely, addition of thyroid hormone can enhance upregulation of renin synthesis induced by the sympathetic nervous system.

The sympathetic nervous system stimulates renin secretion and enhances renin synthesis in the kidney. Holmer et al. (11) and El-Dahr et al. (6) have shown that renal denervation by painting 10% phenol around the renal artery decreased renal expression of renin mRNA. The present study found that sympathetic denervation inhibited expression of renin mRNA only in Hypo and Hyper but not in Cont. This difference may be explained by differences in the method of sympathetic denervation. We used 6-hydroxydopamine for sympathetic denervation as previously described (14). This denervation does not injure the renal artery or induce vasoconstriction that may decrease renal blood flow. In contrast, in previous reports (6,11), sham denervation by painting the renal artery with vehicle may cause some renal vasoconstriction. Such vasoconstriction generally enhances sympathetic nerve activity and results in an increase in sympathetic-dependent renin synthesis (6,10,11,19). This explanation is supported by our finding that attenuation of renin synthesis by denervation occurred only in hypothyroid and hyperthyroid animals, which have augmented sympathetic nerve activity (19). Thus the sympathetic effect on renal renin synthesis is not dominant under normal conditions. This effect may be more significant under conditions causing an increase in sympathetic nerve activity.

Although painting phenol is specific for local denervation, intraperitoneal injections of 6-hydroxydopamine cause systemic denervation. Therefore, the present method of sympathetic denervation cannot specify which component of the sympathetic nervous system, the central nervous system, the peripheral (including renal) nerves, or circulating catecholamines is most important for stimulation of renin synthesis. It is known that systemic sympathetic denervation can decrease circulating catecholamines (18). If no compensatory mechanism is involved in maintaining systemic hemodynamics, a decrease in circulating catecholamines may reduce blood pressure, which would disturb peripheral blood flow, leading to inhibition of protein synthesis, including renin. However, such a scenario is unlikely in this study, since systolic blood pressure did not change with sympathetic denervation.

Although the multiple effects of sympathetic denervation on renal levels of norepinephrine were similar in Hypo, Cont, and Hyper, the reduction in the absolute value was most marked

in Hypo. In contrast, whereas the multiple effects of sympathetic denervation on PRA, renal renin, and renal expression of renin mRNA were similar among the three groups (except for the renin/GAPDH ratio in Cont), the reduction in their absolute values was most marked in Hyper. This suggests that renal renin synthesis does not depend on the renal level of norepinephrine, even though the sympathetic nervous system regulates renal renin synthesis. Renal levels of norepinephrine only reflect static sympathetic conditions. Therefore, in the present study, renal levels of norepinephrine indicate the efficacy of sympathetic denervation.

Although hypothyroidism is classically associated with high sympathetic nerve activity (19), heart rate and systolic blood pressure failed to increase in Hypo-IN vs. Cont-IN in this study. Hypothyroidism is also associated with hyporeactivity due to hypothyroidism-induced hypometabolism. Thus heart rate or systolic blood pressure may not always reflect sympathetic nerve activity. Renal levels of norepinephrine in this study were higher in Hypo-IN than in Cont-IN. In the present study, therefore, it remains undetermined whether sympathetic nerve activity in Hypo was as high as in the previous report (19). However, the principal finding, that thyroid hormone regulates renin synthesis in sympathetic denervated rats, does not depend on that determination.

Recent in vitro studies have demonstrated the effects of thyroid hormone on renin-related genes. Renin mRNA is elevated by thyroid hormone and is reduced by propylthiouracil in the submandibular gland of female mice (2,16). However, renin gene expression is not influenced by thyroid hormone in the submandibular gland of male mice, since testosterone regulates renin gene expression more strongly than thyroid hormone (2). In mice, renin production and secretion are more dependent on the submandibular gland than the kidney (2). Therefore, the present in vivo results are consistent with mouse in vitro data with respect to thyroid hormone stimulating expression of renin mRNA in the principal organ of synthesis and secretion. However, the relationship between thyroid hormone and testosterone on renin synthesis in the rat kidney is unknown, since all rats used in the present study were male. The 5' flanking regions of the human renin gene have some hormone-responsive elements, for example, glucocorticoids and estrogen (5,8), but a thyroid hormone-responsive element has not been found. Because a single injection of thyroid hormone significantly increases renin mRNA within 1 h in the submandibular gland of the female mouse (16), it has been suggested that thyroid hormone has a direct nuclear action on stimulating transcription of the renin gene and stabilization of nuclear precursor renin mRNA. Currently, only an indirect effect of thyroid hormone on the 5' flanking regions of the human renin gene is known. In an in vitro study using a pituitary cell line to which the 5' flanking regions of the human renin gene were transfected, thyroid hormone suppressed stimulation of human renin gene promoter activity induced by forskolin, but thyroid hormone had no direct effect on promoter activity (7). Our study implies that the 5' flanking regions of the rat renin gene have thyroid hormone-responsive elements. However, in vitro studies are needed for that determination.

In conclusion, thyroid hormone enhanced the renal expression of renin mRNA independently of the sympathetic nervous system. Renal levels of renin and PRA also increased. Accordingly, thyroid hormone is one factor regulating the renin-angiotensin system through the stimulation of renal expression of renin mRNA.

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Fig. 1. Cycle-dependent amplification of renin and glyceraldehyde-3-phosphate dehydrogenase (GAPDH) mRNA in a semiquantitative reverse transcriptase-polymerase chain reaction. A linear relationship between no. of cycles and amount of product (renin and GAPDH) was obtained between cycles 23 and 28.

Fig. 2. Plasma renin activity (means $\pm$ SE) by radioimmunoassay in each group; $n = 5$. Hypo, hypothyroid group; Cont, control group; Hyper, hyperthyroid group; IN, sympathetic innervated group; DX, sympathetic denervated group. * $P < 0.05$ vs. Cont; † $P < 0.05$ vs. IN.

Fig. 3. Renal levels of renin (means $\pm$ SE) by radioimmunoassay in each group; $n = 5$. Abbreviations are as in Fig. 2. * $P < 0.05$ vs. Cont; † $P < 0.05$ vs. IN.

Fig. 4. Ratio of renal expression of renin mRNA to GAPDH mRNA by a semiquantitative reverse transcriptase-polymerase chain reaction in each group; $n = 5$. Abbreviations are as in Fig. 2. * $P < 0.05$ vs. Cont; † $P < 0.05$ vs. IN.

Table 1
Hemodynamic changes produced by methimazole and thyroxine treatment and by sympathetic denervation

	Hypo		Cont		Hyper	
	IN	DX	IN	DX	IN	DX
Heart rate, beats/min	333 ± 21	363 ± 24	353 ± 24	383 ± 26	470 ± 28*	347 ± 23†
Systolic blood pressure, mmHg	125 ± 6	120 ± 5	132 ± 4	125 ± 2	140 ± 7	131 ± 8

Values are expressed as means ± SE; n = 5. Hypo, hypothyroid group; Cont, control group; Hyper, hyperthyroid group; IN, sympathetic innervated group; DX, sympathetic denervated group.

* $P < 0.05$ vs. Cont

† $P < 0.05$ vs. IN.