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NO and cGMP mediate angiotensin AT2 receptor-induced renal renin inhibition in young rats

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Siragy HM, Inagami T, Carey RM. NO and cGMP mediate angiotensin AT2 receptor-induced renal renin inhibition in young rats. Am J Physiol Regul Integr Comp Physiol 293: R1461–R1467, 2007. First published August 1, 2007; doi:10.1152/ajpregu.00014.2007.—We hypothesized that angiotensin subtype-2 receptor (AT2R) inhibits renal renin biosynthesis in young rats via nitric oxide (NO). We monitored changes in renal NO, cGMP, renal renin content (RRC), and ANG II in 4-wk-old rats in response to low sodium (LNa-) intake alone and combined with 8-h direct renal cortical administration of AT1 receptor blocker valsartan (VAL), AT2R blocker PD123319 (PD), NO synthase inhibitor Nω-nitro-l-arginine methyl ester (l-NAME), NO donor S-nitroso-N-acetyl penicillamine (SNAP), or guanlyl cyclase inhibitor 1H-[1,2,4] oxadiazolo[4,2-α] quinoxaline-1-one (ODQ). In addition, we monitored renal endothelial nitric oxide synthase (eNOS) and neuronal nitric oxide synthase (nNOS) in response to VAL or PD. LNa-, VAL, PD, l-NAME, and ODQ increased RRC, ANG II, and renin mRNA. PD and l-NAME decreased NO and cGMP, while SNAP reduced RRC, ANG II, renin mRNA, and reversed the effects of PD. PD also reduced eNOS and nNOS protein and mRNA. Combined treatment with PD, l-NAME, or ODQ and VAL reversed the effects of VAL and caused further increase in RRC. ANG II, renin mRNA, and protein. ODQ reversed the effects of SNAP. These data demonstrate that the renal AT2 receptor decreases renal renin biosynthesis and ANG II production in young rats. Reversal of the PD effects by SNAP and SNAP effects by ODQ confirms that NO and cGMP mediate the AT2 receptor inhibition of renal renin production.

ANGII; AT1 receptor; nitric oxide; valsartan

NO and cGMP mediate angiotensin AT2 receptor-induced renal renin inhibition in young rats. All components of the renin-angiotensin system (RAS) are present within the kidney and local renal production of ANG II is well documented (26). ANG II is the major effector hormone of the RAS. Most of the known physiological functions and pathological effects associated with ANG II are mediated by the angiotensin subtype-1 receptor (AT1R), including direct renin inhibition at the juxtaglomerular (JG) cells. In contrast, the functions of the angiotensin subtype-2 receptor (AT2R) are still being elucidated. Previously, we demonstrated that angiotensin subtype-2 receptor (AT2R) is expressed in young rats. We hypothesized that angiotensin subtype-2 receptor (AT2R) inhibits renal renin biosynthesis in young rats via nitric oxide (NO). We monitored changes in renal NO, cGMP, renal renin content (RRC), and ANG II in 4-wk-old rats in response to low sodium (LNa-) intake alone and combined with 8-h direct renal cortical administration of AT1 receptor blocker valsartan (VAL), AT2R blocker PD123319 (PD), NO synthase inhibitor Nω-nitro-l-arginine methyl ester (l-NAME), NO donor S-nitroso-N-acetyl penicillamine (SNAP), or guanlyl cyclase inhibitor 1H-[1,2,4] oxadiazolo[4,2-α] quinoxaline-1-one (ODQ). In addition, we monitored renal endothelial nitric oxide synthase (eNOS) and neuronal nitric oxide synthase (nNOS) in response to VAL or PD. LNa-, VAL, PD, l-NAME, and ODQ increased RRC, ANG II, and renin mRNA. PD and l-NAME decreased NO and cGMP, while SNAP reduced RRC, ANG II, renin mRNA, and reversed the effects of PD. PD also reduced eNOS and nNOS protein and mRNA. Combined treatment with PD, l-NAME, or ODQ and VAL reversed the effects of VAL and caused further increase in RRC. ANG II, renin mRNA, and protein. ODQ reversed the effects of SNAP. These data demonstrate that the renal AT2 receptor decreases renal renin biosynthesis and ANG II production in young rats. Reversal of the PD effects by SNAP and SNAP effects by ODQ confirms that NO and cGMP mediate the AT2 receptor inhibition of renal renin production.

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min by a rat tail cuff (Rat Tail Manometer-Tachometer System, Natsume model KN-210; Peninsula Laboratories, Belmont, CA), and the recorded values were averaged for each study.

Renal microdialysis technique. For the determination of renal interstitial fluid (RIF), NO, and cGMP, we used a microdialysis technique as described previously (2–4).

Renal total, cortical, and medullary blood flow determination. For determination of renal total (RBF), cortical (RCBF), and medullary (RMBF) blood flow changes (9) in response to local cortical interstitial administration of Na+ depletion, AT1R, or AT2R blockade, individually and combined with manipulations of NO-cGMP, as per treatment protocols. A midline celiotomy incision was made. The treated kidney renal artery was exposed by careful dissection. Monitoring of RBF was accomplished by inserting a renal artery flow probe on the renal artery by a dual-channel flowmeter (Transonic Systems, Ithaca, NY). RCBF and RMBF were monitored by using a laser flowmeter (Advance Laser Flowmeter ALF 21D). After infusion of pharmacological agents, the renal artery was exposed once again, and RBF was measured.

Protocols

Effects of Na+ depletion, AT1R, or AT2R blockade individually and combined with manipulations of NO-cGMP on RIF, NO, cGMP, ANG II, RRC, and renal expression of renin. One week before starting the studies, all animals were instrumented with renal cortical interstitial microdialysis catheters, as previously described (23, 24, 27). While animals were consuming normal Na+ diet (0.28% NaCl; BioServe Biotechnologies, Frenchtown, NJ), rats (n = 8 each group) were placed in metabolic cages for baseline 24-h urine collections and measurement of 24-h urinary Na+ excretion (UNaV). In one group of animals (n = 8), while consuming a normal Na+ diet, RIF was collected over 8 consecutive hour periods for measurements of NO during renal cortical administration of 5% dextrose in water (vehicle). Animals were then euthanized, and the infused kidneys were removed, weighed, placed on ice, and divided into sections for RNA extraction and measurements of RRC and ANG II contents. Previously, we demonstrated that animals that were fed normal Na+ diet did not respond to pharmacological manipulations of the AT1R or AT2R (23, 24). To enhance the renal RAS activity, the remaining rats were placed on a low-Na+ diet (0.05% NaCl) for 8 days. At day 7, we monitored 24-h UNaV. While rats (n = 8 each treatment group) continued to consume the low-Na+ diet (day 8), we sampled RIF NO during intrarenal cortical interstitial administration (3 μl/min for 8 h) in random order of vehicle; AT1R blocker valsartan (VAL) at 10 μg/kg·h−1 (Novartis, East Hanover, NJ); AT2R blocker PD-123319 (PD) at 10 μg·kg−1·min−1 (Parke-Davis-Warner Lambert, Auburndale, FL); combined VAL and PD; NOS inhibitor Nω-nitro-L-arginine methyl ester (l-NNAME) at 100 ng·kg−1·min−1; NO donor S-nitroso-N-acetyl-penicillamine (SNAP) at 1.2 μM·kg−1·min−1, guanylate cyclase inhibitor 1H-[1,2,4]oxadiazolo[4,2-α] quinoxaline-1-one (ODQ) at 0.12 mg·kg−1·min−1 (9) and combined VAL or PD with l-NNAME, SNAP, or ODQ. At the end of each study, infused kidneys were removed and weighed for measurements of RRC, ANG II, and renin mRNA and protein expression. These studies were repeated with the exception that the utilized pharmacological agents were infused systemically instead of intrarenally.

Renal eNOS and nNOS mRNA and protein responses to low-Na+ intake and AT1R or AT2R blockade. Five groups of rats (n = 8 each group) were studied. Measurements of renal eNOS and nNOS mRNA, protein expression, and immunostaining in response to normal or low-Na+ intake alone and combined with VAL or PD individually and combined were performed.

Molecular Methods

Quantitative real-time RT-PCR and Western blot analysis. Kidneys were weighed promptly and homogenized on ice, and the total renal RNA was extracted using Trizol reagent (Invitrogen, Carlsbad, CA). RNA was quantified by ethidium bromide staining in 2% agarose gel. Single-stranded cDNA was synthesized using iScript cDNA Synthesis Kit (Bio-Rad, Hercules, CA). Gene-specific primers were designed using the GenBank. The exon-intron boundaries were determined using the University of California Santa Cruz Genome Bioinformatics site (http://genome.ucsc.edu). The corresponding cDNA primer for renin was selected as described previously (27). Other corresponding cDNA primers were selected from AF085195, AF037071, and BC063166, the gene codes for rat eNOS, nNOS, and β-actin sequences. The specificity of the primers was verified by melting curves (iCyler, Bio-Rad, Hercules, CA) and amplified product sizes using agarose gel electrophoresis. Quantitative real-time PCR was performed using an iCyler (Bio-Rad), and the threshold cycle number was determined using iCyler software version 3.1 (Bio-Rad). Reactions were performed in triplicate, and threshold cycle numbers were averaged. A nontemplate control was used as a negative control. Samples were calculated with normalization to β-actin. Fold down-expression or up-expression was calculated according to the formula 2(Rt−En)/2(Rn−Et), where Rt is the threshold cycle number for the reference gene observed in the test sample, Et is the threshold cycle number for the experimental gene observed in the test sample, Rn is the threshold cycle number for the reference gene observed in the control sample, and En is the threshold cycle number for the experimental gene observed in the test sample. Western blot analysis (9) was used for measurements of renal eNOS and nNOS. Monoclonal antibodies for eNOS and nNOS were purchased from Santa Cruz Biotechnology (Santa Cruz, CA).

eNOS and nNOS immunohistochemistry

Kidneys were fixed in 4% paraformaldehyde in PBS and embedded in paraffin. Four-micrometer sections were stained for eNOS and nNOS using monoclonal antibodies and methods described previously (9). Negative controls included mouse IgG for primary antibodies. Kidney sections were immunostained in the same run.

Analytical Methods

Urinary Na+ levels were measured by a NOVA analyzer (NOVA Biochemical, Waltham, MA). RRC, ANG II, and RIF NO, and cGMP levels were measured according to previously published methods (23, 24, 27, 31).

Statistical Analysis

Comparisons among different treatment groups were examined by ANOVA, including a repeated-measures term, using the general linear models procedure of the Statistical Analysis System (SAS Software, SAS Institute, Cary, NC). Data are expressed as means ± SE. P < 0.05 was considered statistically significant.

RESULTS

BP, RBF, RCBF, or RMBF Responses to Sodium Depletion, AT1R, or AT2R Blockade Individually or Combined With Manipulation of NO-cGMP

On the study day, mean body and kidney weights were 122 ± 2 g and 0.68 ± 0.04 g, respectively. During normal Na+ intake, UNaV was 583 ± 42 μmol/day and decreased to 33 ± 9 μmol/day (P < 0.001) after 7 days of low-Na+ intake. BP in rats on normal Na+ intake was 110 ± 2 mmHg and did not change significantly during low-Na+ diet alone or combined with systemic or intrarenal cortical administration of VAL or PD (alone or combined). Systemic or intrarenal administration of l-NNAME, SNAP, or ODQ individually or combined with VAL or PD did not cause any significant changes in BP.
There were no significant changes in RBF, RCBF, and RMBF in response to local cortical interstitial administration of AT₁R or AT₂R blockade individually and combined with manipulations of NO-cGMP in the treated or contralateral kidneys (data not shown).

**Renal NO, cGMP, Renin, and ANG II Contents in Response to Low-Na⁺ Intake, AT₁R, or AT₂R Blockade Individually or Combined With Manipulation of Renal NO-cGMP**

During normal Na⁺ intake, RIF NO and cGMP were 0.09 ± 0.005 μmol/μl and 1.1 ± 0.1 pmol/μl, respectively. RRC was 28 ± 4 μg/g kidney weight, and ANG II contents were 138 ± 12 fmol/g kidney weight (Fig. 1). Low-Na⁺ intake increased RIF NO, cGMP, RRC, and ANG II levels (P < 0.01). Systemic administration of VAL, PD, L-NAME, SNAP, or ODQ did not influence renal NO, cGMP, renin, or ANG II (data not shown). These results suggest that the employed doses of these pharmacological agents were too low to cause any significant changes in the kidney. During low-Na⁺ intake, intrarenal cortical administration of VAL further increased RIF NO, cGMP, RRC, and ANG II content (P < 0.001 vs. low Na⁺ alone). Intrarenal administration of PD or L-NAME reduced RIF, NO, and cGMP (P < 0.001) and increased RRC and ANG II (P < 0.001). Combined intrarenal administration of PD or L-NAME with VAL reduced RIF, NO, and cGMP (P < 0.001), while causing further increase in RRC and ANG II (P < 0.001). Intrarenal infusion of ODQ alone and combined with VAL, PD, or SNAP reduced RIF cGMP and increased RRC and ANG II (P < 0.001). In contrast, intrarenal SNAP alone and combined with VAL increased RIF, NO, and cGMP (P < 0.001) and decreased RRC and ANG II (P < 0.001). SNAP but not ODQ reversed PD effects on renal RRC and ANG II (P < 0.001). There were no differences in RRC and ANG II responses to PD, L-NAME, or ODQ individually and combined (Fig. 1). There were no significant changes in the contralateral kidney levels of NO, cGMP, RRC, or ANG II with any of the administered treatments (data not shown).

**Renal Renin mRNA and Protein Expression in Response to Low-Na⁺ Intake, Intrarenal AT₁R, or AT₂R Blockade Individually or Combined With Manipulation of Renal NO-cGMP Production**

Systemic administration of the doses of the pharmacological agents used did not influence renal renin mRNA and protein (data not shown). Similarly, intrarenal cortical administration of different treatments did not cause any significant changes in renin mRNA levels of the contralateral kidney. In the treated kidney, renin mRNA and protein expression increased (P < 0.01) in response to low-Na⁺ intake (Fig. 2). Combined low-Na⁺ intake and intrarenal administration of VAL or PD individually and combined caused further increase in renal renin mRNA and protein expression (P < 0.001). Similarly, L-NAME or ODQ alone and combined with VAL or PD increased expression of renin mRNA and protein (P < 0.01). Intrarenal SNAP administration decreased reninal renin mRNA and protein (P < 0.01) and reversed the VAL or PD effects (P < 0.01). Intrarenal ODQ treatment reversed SNAP effects on renin mRNA and protein expression. The responses to combined PD and L-NAME or ODQ were not different from responses to PD treatment alone (Fig. 2).

**Renal eNOS and nNOS mRNA and Protein Expression in Response to Low-Na⁺ Intake, Intrarenal AT₁R, or AT₂R Blockade Individually and Combined**

Systemic administration of VAL or PD did not influence renal eNOS or nNOS. Renal eNOS (Fig. 3, A and B) and nNOS (Fig. 3, C and D) mRNA and protein increased significantly during low-Na⁺ intake (P < 0.01), respectively. AT₁R blockade with intrarenal administration of VAL caused further increase in eNOS and nNOS mRNA and protein by 50% and 16% (P < 0.05), respectively. In contrast, AT₂R blockade with intrarenal administration of PD caused significant reduction in eNOS and nNOS mRNA and protein by 52% and 68% (P < 0.01), respectively. Combined intrarenal administration of VAL and PD reversed the increase in eNOS (P < 0.01) and nNOS associated with VAL alone (P < 0.01). There were no significant changes in the contralateral kidney levels of eNOS.
or nNOS mRNA with any of the administered treatments (data not shown).

Renal eNOS and nNOS Immunostaining in Response to Low-Na\(^+\) Intake, Intrarenal AT\(_2\)R, or AT\(_2\)R Blockade Individually or Combined

Immunostaining for eNOS or nNOS (Fig. 4) was absent in kidney sections when IgG preimmune serum was used as a control (Fig. 4, A and G). During normal Na\(^+\) intake, the immunostaining of eNOS (Fig. 4B) and nNOS (Fig. 4H) was minimum and was localized mainly in glomeruli and tubules, respectively. This immunostaining did not change in response to VAL or PD treatment during normal Na\(^+\) intake or systemic administration of VAL or PD (data not shown). Low-Na\(^+\) intake caused significant increase in eNOS (Fig. 4C) in glomeruli, JGA apparatus and vasculature, and nNOS (Fig. 4J) in macula densa and tubules. Compared with normal or low-Na\(^+\) intake, combined low-Na\(^+\) intake with intrarenal administration of VAL or PD were associated with decreased eNOS (Fig. 4F) and nNOS (Fig. 4L) staining compared with combined low-Na\(^+\) and VAL alone.

DISCUSSION

This study demonstrates the regulation of the renal renin production by the AT\(_2\)R. We hypothesized that in young rats, AT\(_2\)R inhibits renal renin production through its mediators NO and cGMP. In this study, we present data to support this hypothesis. First, we confirmed the inhibitory action of AT\(_2\)R of VAL caused further increase in eNOS (Fig. 4D) in glomeruli, JGA, some tubes, and blood vessels, and it increased nNOS (Fig. 4J) immunostaining in macula densa and tubules. In contrast, eNOS (Fig. 4E) and nNOS (Fig. 4K) staining was decreased in response to combined low Na\(^+\) and intrarenal infusion of PD. Similarly combined low-Na\(^+\) intake and intrarenal administration of VAL and PD were associated with decreased eNOS (Fig. 4F) and nNOS (Fig. 4L) staining compared with combined low-Na\(^+\) and VAL alone.

Fig. 2. Renal renin mRNA (A and C) and protein response to (B and D) low-Na\(^+\) intake alone and combined with intrarenal cortical administration of vehicle (5% dextrose in water), VAL, PD123319 (PD), nitric oxide synthase inhibitor (L-NAME), and NO donor SNAP (B), guanylate cyclase inhibitor ODQ, individually or combined. *P < 0.01 vs. normal Na\(^+\), **P < 0.001 vs. low Na\(^+\), †P < 0.001 vs. VAL, ***P < 0.001 vs. PD, and ††P < 0.001 vs. SNAP.

Fig. 3. Renal eNOS mRNA (A) and protein (B) and nNOS mRNA (C) and protein (D) response to low Na\(^+\) intake alone and combined with intrarenal cortical administration of VAL or PD 123319 (PD), individually and combined. n = 8 each group. †P < 0.01 vs. normal Na\(^+\); *P < 0.05; **P < 0.01 vs. low Na\(^+\) vs. VAL.
activation on renin biosynthesis and ANG II production. Second, we demonstrated the role of NO and cGMP in AT2R-induced renin inhibition based on the observed concurrent increase in renal renin mRNA and protein expression and ANG II levels, as well as a decrease in constitutive NOS isof orm expression in response to AT2R blockade. Third, our data demonstrated the inhibitory role of NO and cGMP on renin biosynthesis and ANG II formation within the kidney.

Recently, we showed that the AT2R inhibits renin biosynthesis (27). However, this study (27) did not offer an explanation to the mechanism through which the AT2R influences renin biosynthesis. Our current study differs from our previous report (27) in that it explains the modulation of renin production by the AT2R second messengers NO and cGMP. Both the AT1R and AT2R inhibit renin, and the effects are additive, suggesting that these receptors manipulate renal renin production through multiple mechanisms (14), including NO. These effects were observed during low-Na\(^+\) intake, suggesting that activation of the RAS may be required to induce the AT2R effects. Reversing the effects of VAL on eNOS and nNOS by PD provides evidence for AT2R involvement in mediating an increase in NO production during AT1R blockade. AT2R inhibition, while stimulating renin, decreased RIF, NO, cGMP, and renal NOS expression. NO donor reversal of the increase in renal renin induced by the AT2R blockade confirms the involvement of NO in mediating this receptor effect on renin inhibition. These results are consistent with our previous studies of AT2R stimulation of NO release (23) and are in agreement with the reported vasodilator and cardiovascular protective effects of the AT2R (31, 25). Since there were no changes in BP and the infused dose of PD was small—one-fifth of previously reported doses (9, 23, 24, 25)—the observed results are mediated via local renal, and not by systemic, mechanisms. This is further supported by the absence of changes in the contralateral kidney levels of NO, cGMP, RRC, ANG II, mRNA for renin, eNOS, or nNOS, with any of the administered treatments. These results suggest that the administered drugs were confined to the site of administration and did not enter the systemic circulation to influence the contralateral kidneys. In addition, the PD infusion rate employed in this study was effective in blocking the AT2R, as it decreased NO and cGMP production (23) and does not interact with the AT1R (3).

Our studies demonstrate complex interactions between AT1R and AT2R on renin gene and protein expression. Previous studies have suggested generally opposite functions for the AT1R and AT2R (28). Because AT1R blockade enhances renin expression, our studies uncover one of the few reported instances in which both AT1R and AT2R perform a similar function, namely, regulation of renin biosynthesis. Combined AT1R and AT2R blockade doubled the increase in renin expression that was observed with either blocker alone, suggesting that these receptors may have different mechanisms of regulation of renal renin production. AT1R receptors are believed to influence renin secretion directly via calcium-dependent mechanisms (14). These mechanisms may include the activation of PKC and chloride channels (14).

In the present study, we provide evidence for AT2R inhibition of renin production through its mediators NO and cGMP. First, AT2R inhibition increased renin mRNA and protein, while decreasing RIF, NO, and cGMP, as well as renal eNOS and nNOS mRNA and protein expression. Second, NO donor SNAP decreased while NOS inhibitor L-NAME and guanylate cyclase inhibitor ODQ increased renal renin and ANG II production and renin mRNA and protein expression. Third, combined inhibition of AT2R and NOS or guanylate cyclase did not produce any further increase in renal renin, ANG II, or renin mRNA and protein, suggesting that both of these manipulations are mediated by the same mechanism contributing to renin production. Fourth, NO donor SNAP reversed the effects of AT2R blockade on renin, ANG II production, and renin mRNA and protein, confirming that the regulation of renin production by the AT2R involves its mediator NO. Fifth, guanylate cyclase inhibitor ODQ reversed the effects of NO donor SNAP, suggesting that NO inhibits renin production via cGMP. Finally, there were no changes in RBF, RCBF, or RMBF in response to any of the administered treatments. These results are consistent with our previous studies showing no changes in RCBF or RMBF during renal cortical administration of L-Arginine or cGMP (9) and suggest that the changes in renin biosynthesis with different treatments are not due to local changes in renal hemodynamics.

**Fig. 4.** Representative immunohistochemistry staining for eNOS and nNOS in the kidney. A: no staining is seen in IgG negative control for eNOS. B: eNOS staining was minimum during normal Na\(^+\) intake, localized mainly in glomeruli and vasculature and increased during low Na\(^+\) intake (C). D: eNOS staining increased further during combined low Na\(^+\) intake and Valsartan (VAL). E: eNOS expression was reduced during low Na\(^+\) and PD or combined low Na\(^+\), VAL and PD (F). G: no staining is seen in IgG negative control for nNOS. H: nNOS staining increased further during combined low Na\(^+\) intake and Valsartan (VAL). I: nNOS expression was reduced during low Na\(^+\) and PD123319 (PD) or combined low Na\(^+\), VAL and PD L: arrowheads point to macula densa. Magnification at \(\times 63\) oil.
During the last decade, a number of studies were performed to characterize the physiological role of NO and its second messenger cGMP, in the control of renin synthesis and secretion. These studies, conducted under a variety of different experimental conditions and using different animal models, have led to controversy concerning the role of NO and cGMP in control of renin secretion (7, 18, 19). Acute inhibition of NO production decreased (15), while its chronic inhibition increased renin secretion (4). Other studies reported that inhibition of NO production did not change plasma renin activity (5, 11). Intrarenal infusion of the neuronal NOS inhibitor, 7-nitroindazole (7-NI), did not alter basal renin secretion during normal Na\(^+\) intake, but its chronic administration reversed the stimulation of renin induced by dietary Na\(^+\) restriction (1). In contrast to these studies, in anesthetized dogs and pigs, intrarenal administration of NOS inhibitor increased renin secretion (20, 29). Furthermore, plasma renin concentration in the eNOS\(^{-/-}\) mice is nearly twice that of eNOS\(^{+/+}\) mice (22).

These divergent data suggest that there may be inhibitory and stimulatory pathways for NO on renin secretion. Similarly, the contribution of the NO second messenger cGMP to the regulation of renal renin production is poorly understood. Reported studies on the effect of cGMP on renin secretion are as conflicting as those obtained for the effect of NO itself. Both the stimulatory effects of NO in isolated kidneys (13), isolated JG cells (21), and freshly dispersed renal cortical cells (16), as well as the acute inhibitory effect of NO in isolated JG cells (6, 12, 30) and isolated perfused kidney (8), were found to be abrogated by guanylyl cyclase inhibitors, suggesting involvement of cGMP in both stimulation and inhibition of renin secretion by NO. At lower concentrations, cGMP analogs had either no effect on renin secretion from kidneys slices or slightly increased renin secretion from dispersed renal cortical cells, whereas at higher concentrations, it inhibited renin secretion (16). Similarly, activation of cGMP-dependent protein kinase II inhibited renin secretion from renal JG cells (8). Overall, the effect of cGMP on renin secretion in JG cells is variable, ranging from inhibition to stimulation of renin secretion, similar to the effects of NO donors. Maximal activation of cGMP formation is associated with inhibition of renin secretion, whereas renin secretion appears to increase when cGMP formation is reduced (16). Therefore, previous studies offer neither a clear explanation to the physiological impact of NO and its mediator cGMP in the regulation of renin biosynthesis and secretion nor its mode of action. The controversy surrounding the influence of NO and cGMP on renin may be related to the fact that those studies were performed in vitro in isolated cells or in kidney slices in which many physiological mechanisms involved in the regulation of renin synthesis and secretion were eliminated. The present study differs from previous studies in that it was conducted in conscious young rats with intrarenal manipulation of the AT\(_1\)R, AT\(_2\)R, NO, and cGMP activities. Because age and salt intake contribute greatly to AT\(_2\) receptor expression, these factors might have influenced previous studies’ outcomes, namely affecting renin biosynthesis. AT\(_2\) receptor expression is higher at a young age and declines with advancing age (17). This is consistent with the reports of decreased NO production with aging (2). Similarly, low-sodium increases, while high-sodium diet decreases AT\(_2\) receptor expression (17). In the present study, we report that AT\(_1\)R blockade increased renal eNOS and nNOS mRNA and protein and NO-cGMP production. The reversal of these effects by AT\(_2\)R blockade confirms our previous reports that during AT\(_1\)R blockade there is an increase in AT\(_2\)R activity to produce NO-cGMP (23, 24, 27). In addition, the present study demonstrates that the AT\(_2\)R stimulates eNOS and nNOS mRNA and protein expression leading to renin inhibition.

In conclusion, we provide evidence for AT\(_2\)R regulation of renal renin and ANG II production. Because our studies were conducted in 4-wk-old rats, the interpretation of our findings should be limited to the conclusion that AT\(_2\)R inhibits renal renin production via its mediators NO and cGMP in young rats.

GRANTS

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