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Effects of Angiotensin II on Renal Dopamine Metabolism: Synthesis, Release, Catabolism and Turnover

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Key Words
Angiotensin II · Dopamine · Catechol-*o*-methyl transferase · Monoamine oxidase · L-Dopa decarboxylase · Aromatic acid decarboxylate

Abstract
Background/Aims: Dopamine (DA) uptake inhibition in the renal cortex, elicited by angiotensin II (ANG II), is mediated by AT_1 receptors and signals through the phospholipase C pathway and activation of protein kinase C and CaM-kinase II. By this indirect way, ANG II stimulates renal Na^+,K^+-ATPase activity through DA intracellular reduction. In the present work, we continued to study different aspects of renal DA metabolism in DA-ANG II interaction, such as DA synthesis, release, catabolism and turnover. ***Methods:*** ANG II effects on DA synthesis, release, catabolism and turnover were measured in samples from the outer renal cortex of Sprague-Dawley rats. ***Results:*** ANG II reduced renal aromatic acid decarboxylate activity without affecting basal secretion of DA or its KCl-induced release. Moreover, ANG II enhanced monoamine oxidase activity without altering catechol-*o*-methyl transferase activity and increased DA turnover. ***Conclusion:*** Current results as well as previous findings show that ANG II modifies DA metabolism in rat renal cortex by reducing DA uptake, decreasing DA synthesis enzyme activity and increasing monoamine oxidase activity, and DA turnover. Together, all these effects may reduce DA accumulation into renal cells and decrease its endogenous content and availability. This would prevent D1 receptor recruitment and stimulation, while diminishing DA inhibition of Na^+,K^+-ATPase activity and stimulating sodium reabsorption.

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Introduction

Renal functions are modulated by several endocrine, autocrine and neuronal factors. It has been recognized for many years how critical a role the circulating renin-angiotensin system (RAS) plays in the regulation of arterial pressure and sodium homeostasis. Biologically, angiotensin II (ANG II) is the most powerful active member of the RAS. It regulates sodium transport by epithelial cells in the kidney, acting as an endocrine, paracrine or autocrine factor [1–3]. ANG II contributes to the increase of proximal tubular sodium reabsorption through diverse mechanisms. Most of them are due to direct effects on the

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regulation of sodium transporters, increasing the activity of the Na^+/H^+ antiporter in the luminal membrane and the activities of the Na^+/HCO_3^- co-transporter and Na^+,K^+-ATPase enzyme in the basolateral membrane, along with stimulation of Na^+-ATPase activity on the proximal tubules [4, 5]. Moreover, ANG II could enhance sodium reabsorption through indirect mechanisms mediated by renal dopamine (DA) [6, 7].

The role of DA as an important autocrine and paracrine regulator of renal function was first recognized in 1964, when it was found that DA increases the glomerular filtration rate and sodium excretion [8]. The kidney has all the enzymatic machinery to synthesize and catabolize DA [9]. Renal DA is synthesized from neuronal and extraneuronal sources. The neuronal sources are noradrenergic and dopaminergic [10]. Extraneuronal sources involve L-Dopa decarboxylation, after amino acid has been taken up from the tubular fluid, and DA uptake by tubular cells from the blood [11]. The main source of renal DA is the proximal tubular cell, which has a high concentration of aromatic acid decarboxylate (AADC), also named L-Dopa decarboxylase [12]. DA is catabolized in the kidney mainly by monoamine oxidase (MAO) and catechol-*o*-methyl transferase (COMT) [13, 14]. The activities of both enzymes are high in renal tissue compared with the activity in other peripheral tissues. COMT is abundantly expressed in proximal tubular cells of the kidney and appears to play an important role in the physiological regulation of the renal DA tonus [13]. Deamination by MAO was found to be the major metabolic pathway for renal DA [15].

Intrarenal DA acts together with other natriuretic hormones and antagonizes the effects of antinatriuretic agents [16]. We have previously reported that ANG II modifies DA uptake in the outer renal cortex by binding to AT_1 receptors, which are coupled to phospholipase C (PLC). This leads to the generation of second messengers, 1,4,5-inositol triphosphate (IP_3) and diacylglycerol (DAG), the release of intracellular calcium, and the activation of protein kinase C (PKC) and CaM-kinase II [6, 7]. By this way, the interaction between circulating as well as locally synthesized ANG II and locally produced DA in the kidney could contribute to the regulation of sodium reabsorption and excretion.

Based on previous findings and considering that ANG II regulates DA uptake in renal tissues, the aim of the present work was to further explore the ANG II-DA relationship and to study ANG II effects on DA synthesis, release, catabolism and turnover in samples of the renal outer cortex.

Methods

Male Sprague-Dawley rats weighing between 250 and 300 g (from the animal care room of the Faculty of Pharmacy and Biochemistry of Buenos Aires) were used. The animals were housed in cages, with a 12-hour light/dark cycle, where temperature and humidity were controlled. All animals were given free access to water and food (Rodents Purina Chow, Cooperación SRL, Argentina). Experiments were conducted in accordance with institutional guidelines for the care and use of research animals.

The following drugs were used in the experiments: ^3H-DA (28.0 Ci/mmol specific activity) and ^3H-S-adenosine methionine (SAM) (74.7 Ci/mmol specific activity) (New England Nuclear, Boston, Mass., USA); ANG II (American Peptide Company, Calif., USA); nomifensine, 3,4-dihydroxybenzoic acid, SAM, bovine seroalbumin fraction V of Cohn, 4-aminoantipyrine, vanillic acid, peroxidase type II (4 U/ml), tyramine, clorgyline, pargyline, tropolone, picrylsulfonic acid solution 5% w/v (TNB), pyridoxal 5′-phosphate hydrate (PLP), L-Dopa, DA (all from Sigma-Aldrich Inc., St. Louis, Mo., USA), benzene, Folin-Ciocalteus phenol reagent (Merck Co., USA), potassium cyanide (Fluka BioChemika, Germany), carbidopa (kindly supplied by Dr. Victor Nahmod, Buenos Aires, Argentina), and EcoLite for liquid scintillation (ICN Pharmaceutical Inc., Calif., USA).

The standard Krebs bicarbonate (SKB) solution composition (mM) was: 118 NaCl, 4.7 KCl, 1.2 $MgSO_4 \cdot 7H_2O$, 1.0 NaH_2PO_4, 2.4 $CaCl_2$, 0.004 EDTA, 11.1 glucose, 0.11 ascorbic acid, 26.0 $NaHCO_3$.

The depolarizing 25 mM KCl solution had the same composition as the SKB solution, except that the NaCl concentration was decreased to keep the osmolality constant.

Experimental Protocols

Rats were anesthetized with 10% ethyl urethane (1.3 mg/kg i.p.). Both kidneys were removed and slices of the outer cortex were cut, minced and weighed.

ANG II Effects on AADC Activity

Experiments were carried out according to the techniques previously outlined by Fernández et al. [17]. Minced samples of approximately 50 mg of the outer cortex were placed in a 2.0-ml SKB medium in a Dubnoff incubator and pre-incubated at 37°C, pH 7.40, bubbled with a gaseous mixture of 95% O_2 and 5% CO_2 for 15 min. Afterwards, the samples were transferred to a fresh SKB medium and incubated for 30 min in the absence (control group) or presence of 200 μM carbidopa or 100 nM ANG II (experimental groups). After the incubation period, the tissues were washed with cold SKB for 5 min, blotted, weighed, and homogenized in a cool 0.1 M phosphate buffer (1:10 w/v), pH 7.10, and then centrifuged at 21,420 *g* at 4°C for 15 min. The supernatants were centrifuged one more time at 24,480 *g* at 4°C for 30 min. AADC activity was determined in the supernatant by the modified spectrophotometric UV method (UV-VIS Beckman DB-GT) of Sherald et al. [18]. Briefly, the reaction was based on the colorimetric complex generated between TNB and DA, employing L-Dopa (5 μM/ml) as a substrate and PLP (1 μM/ml) as a cofactor. Reagent concentrations were chosen from preliminary experiments to obtain optimal assay conditions. In order to avoid DA degradation by MAO A, MAO B, and COMT, the assays were performed in the presence of clorgyline (500 μM), pargyline (500 μM), and tropolone (100

μM), respectively. Tissue protein concentration was measured according to Lowry et al. [19]. Results of AADC activity are expressed as nmol/mg protein/min.

ANG II Effects on ^{3}H-DA Release

Tissue samples of approximately 40 mg of the outer cortex were minced and preincubated for 15 min as previously described, and then incubated for 30 min with 0.625 μCi/ml ^{3}H-DA. After labeling, tissue samples were transferred to a fresh medium for 120 min, where the tested drugs were added. The medium was changed every 10 min.

In order to examine ANG II effects on ^{3}H-DA-induced release, the following groups were studied: (a) control (basal spontaneous ^{3}H-DA release), only incubated with SKB; (b, c) experimental groups, incubated with 100 nM ANG II, and 100 nM ANG II plus 25 mM KCl, respectively.

Medium samples corresponding to the first 3 min were collected at 0 (basal), 30, 60, 90 and 120 min and saved for measurement of tritium activity by the usual scintillation counting method. Nomifensine (50 μM during pre-incubation and then 17 μM during incubation) was added to avoid neuronal DA uptake.

Renal ^{3}H-DA release was calculated as log dpm/g fresh tissue ± SEM and compared by the slope of the regression line between the different groups.

Effects of ANG II on MAO and COMT Activities

Samples of approximately 40 mg of the outer cortex were minced and preincubated during 15 min as described previously, and then incubated for 30 min in the absence (control group) or presence of 100 nM ANG II (experimental group). After the incubation period, the tissues were washed with cold SKB for 5 min, dried, weighed, and homogenized in a cool 0.2 M phosphate buffer (1:10 w/v), pH 7.60, and then centrifuged at 1,000 *g* at 4°C for 30 min.

MAO activity was determined in the supernatant by the modified spectrophotometric UV method (UV-VIS Beckman DB-GT) of Holt et al. [20]. Briefly, 100 μl of tissue supernatant was incubated for 45 min with 50 μl of chromogenic solution, 200 μl of tyramine (500 μM) and 650 μl of 0.2 M potassium phosphate buffer (pH 7.60). The chromogenic solution contained 1 mM vanillic acid, 500 μM 4-aminoantipyrine and 4 U/ml peroxidase in potassium phosphate buffer. All reagent concentrations were chosen after a preliminary experiment to obtain optimal assay conditions.

COMT activity was determined in the supernatants by the modified radioenzymatic method of Jarrot [21]. ^{3}H-SAM was used as a methyl donor and tritium activity was measured by the liquid scintillation method. Tissue protein concentration was measured according to Lowry et al. [19]. Results of COMT and MAO activities are expressed as pmol/mg protein/min and nmol/mg protein/h, respectively.

Effects of ANG II on ^{3}H-DA Turnover

The animals were treated with carbidopa, a DA synthesis inhibitor (200 μg/kg i.p.), 24 and 2 h before being sacrificed. Tissues from the renal outer cortex were obtained, preincubated, and incubated as described above, but in the presence of 100 μM carbidopa. Labeling was followed by incubation with either SKB (control group) or 100 nM ANG II (experimental group) for 0 (basal, without ANG II), 30, 60, 90, 120 and 150 min. Nomifensine was used under the same conditions as described in the experiments of DA release. At the end of the mentioned periods, tissue samples were blotted, weighed, and then homogenized with 10% trichloroacetic acid and centrifuged at 1,700 *g* for 30 min at 4°C. Tritium activity was determined in the supernatants by the usual scintillation counting methods. Results are expressed as dpm/mg fresh tissue and compared by the linear regression analysis and the slope (k) of control and experimental groups.

Statistical Analysis

All values are expressed as mean ± SEM. Data were processed using Graph Pad InStat Software (San Diego, Calif., USA). Statistical analysis was performed by the Student's t test and one-way ANOVA. p values of 0.05 or less were considered statistically significant.

Results

In a previous study, we showed that ANG II was able to regulate renal DA uptake [6]. In the current study, we examined other aspects of DA metabolism that could also be regulated by ANG II, such as synthesis, release, catabolism, and turnover.

Initially, we investigated if ANG II could regulate DA renal synthesis through the modulation of AADC activity, which represents the enzyme responsible for DA production in the renal outer cortex. Therefore, we measured AADC-specific activity in the absence and presence of 100 nM ANG II. The enzyme activity decreased from 5.01 ± 0.06 (control) to 2.62 ± 0.14 (nmol/mg/min) in the presence of ANG II (fig. 1). To be certain that the measured activity actually belongs to AADC, and not to other decarboxylating enzymes, we employed 200 μM of carbidopa, which is a specific inhibitor of AADC. The DA synthesis inhibitor suppressed approximately 96% of the basal enzymatic activity in the outer renal cortex samples: 5.01 ± 0.06 (control) versus 0.21 ± 0.05. As shown in figure 1, this result suggests that ANG II decreases renal DA synthesis through the inhibition of AADC-specific activity.

As previously demonstrated, ANG II inhibited DA renal uptake [6]. In the next group of experiments, we investigated if ANG II could also regulate the DA release process. We proceeded by examining the effects of 100 nM ANG II on ^{3}H-DA basal and KCl-induced release in the outer renal cortex from 0 to 150 min of incubation. The peptide altered neither spontaneous nor KCl-induced ^{3}H-DA release, which is represented by the slope of the lineal regression curve: R^2 = control 0.91; ANG II 0.92; ANG II plus KCl 0.94; k (log dpm/g) = control –0.022 ± 0.004; ANG II –0.021 ± 0.003; ANG II plus KCl –0.021 ± 0.002 (fig. 2). These results suggest that under this experimental condition ANG II did not affect renal DA basal or KCl-induced release.

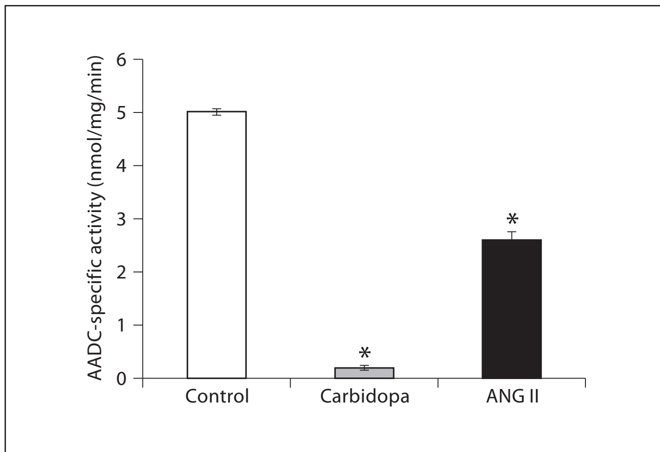

Fig. 1. Effects of 100 nM ANG II on AADC-specific activity (nmol/mg of protein/min ± SEM) in outer renal cortex. * $p < 0.001$ compared with control. Number of cases = 6–8.

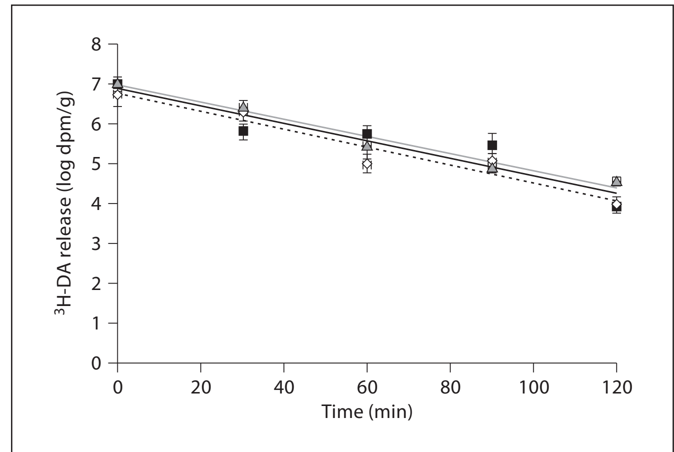

Fig. 2. Effects of 100 nM ANG II on spontaneous and induced (25 mM KCl) release of ^{3}H-DA (log dpm/g ± SEM) in outer renal cortex. Dotted line = control; black line = 100 nM ANG II; gray line = 100 nM ANG II plus 25 mM KCl. The slope of the linear regression curve represents DA release. Number of cases = 9–11.

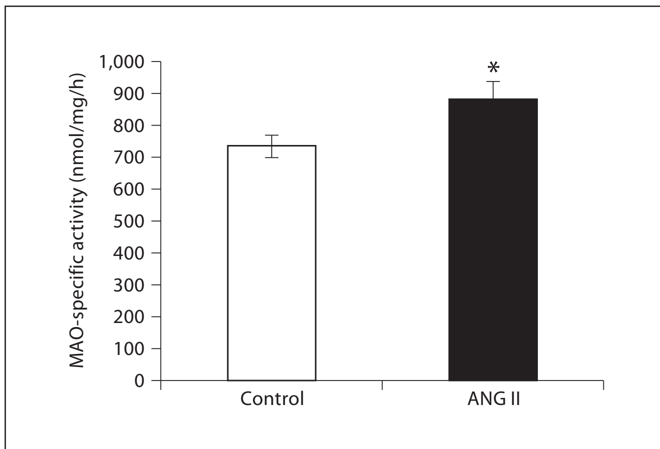

Fig. 3. Effects of 100 nM ANG II on MAO-specific activity (nmol/mg of protein/h ± SEM) in the outer renal cortex. * $p < 0.05$ compared with control. Number of cases = 12–14.

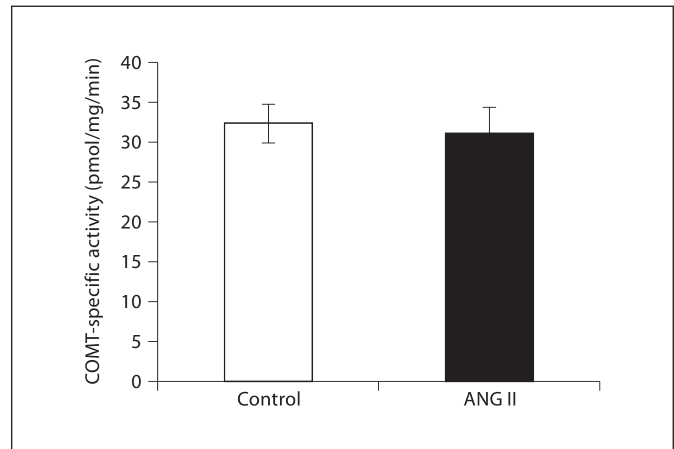

Fig. 4. Effects of 100 nM ANG II on COMT-specific activity (pmol/mg of protein/min ± SEM) in outer renal cortex. Number of cases = 7–8.

To test if ANG II could affect the catabolism of renal DA, we measured the effects of 100 nM ANG II on the specific activity of MAO and COMT in the renal outer cortex. In the presence of ANG II, MAO-specific activity increased from 737 ± 35 (control) to 884 ± 57 (nmol/mg/h) (fig. 3), while COMT-specific activity remained unchanged: 32.4 ± 2.4 (control) versus 31.2 ± 3.2 pmol/mg/min (fig. 4). These results indicate that ANG II altered the specific activity of only one DA-catabolic enzyme, enhancing MAO activity by 20%.

Finally, 100 nM ANG II increased ^{3}H-DA turnover within 0–150 min of incubation (fig. 5). DA turnover is represented by the slope of the lineal regression curve. R^2 = control 0.99; ANG II 1.00; k (dpm/mg $\cdot 10^{-2}$) = control $(-0.44 \pm 0.01) \cdot 10^{-2}$; ANG II $(-1.16 \pm 0.01) \cdot 10^{-2}$. A time-dependent reduction of ^{3}H-DA content elicited by ANG II can be observed from 0 to 150 min.

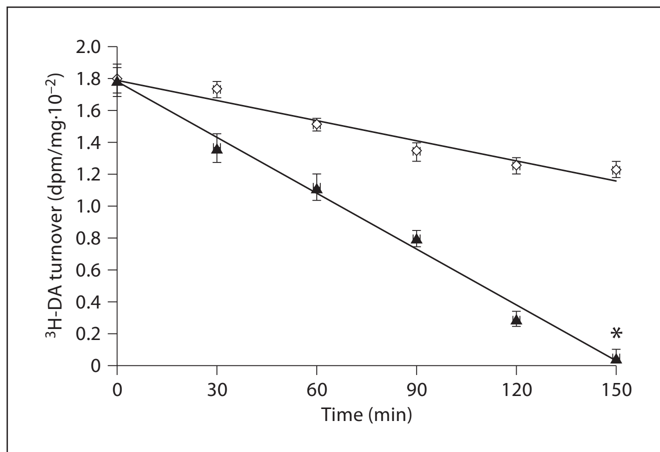

Fig. 5. Effects of 100 nM ANG II on ^{3}H-DA turnover in outer renal cortex (dpm/mg ± SEM). ◇ = Control; ▲ = ANG II. The slope of linear regression curve represents DA turnover. * $p < 0.001$ compared with control. Number of cases = 8–9.

Discussion

Little information is available about the cellular machinery that lies beneath ANG II actions on the different steps of DA metabolism in renal tubular cells. Our previous results demonstrated that ANG II inhibits renal DA uptake and stimulates renal Na^+,K^+-ATPase activity, while DA inhibits this enzyme. DA uptake inhibition in renal tubular cells elicited by ANG II is mediated by AT_1 receptors and signals through PLC pathway generating second messengers (IP_3 and DAG), releasing intracellular calcium, and activating PKC and CaM-kinase II [6, 7]. This way, ANG II diminishes intracellular accumulation of renal DA, which in turn might favor sodium reabsorption and reduce natriuresis linked to Na^+,K^+-ATPase activity in renal tubules [7].

We continued exploring the possibility that ANG II, besides its effect on DA uptake, could also affect different aspects of renal DA metabolism, such as DA synthesis, release, catabolism and turnover.

DA synthesis has been demonstrated in the isolated perfused kidneys, renal cortical slices, and isolated tubules [22–24]. There are no previous reports regarding the effects of ANG II on DA production by tubular cells. In our experiments, we found that ANG II inhibited the DA-forming enzyme, which suppressed AADC activity and resulted in DA synthesis inhibition. It must be pointed out that 200 μM of carbidopa suppressed the basal AADC-specific activity. This suggests that the decarboxylation activity belonged to AADC, and not to other decarboxylating enzymes. While keeping in mind that ANG II inhibited renal DA uptake and DA synthesis, we can conclude that both effects contribute to decrease renal DA content.

ANG II neither altered the basal secretion of DA nor its KCl-induced release in the outer renal cortex, suggesting that DA release is not controlled by ANG II. We have previously reported that atrial natriuretic peptide inhibits both spontaneous and induced norepinephrine release in the central nervous system [25, 26], but does not affect basal secretion or KCl-induced DA release in the kidney [27]. DA release seems to be a metabolic process that is not regulated by the vasoactive peptides. Considering that DA is not stored in granular compartments and lacks an exocytotic release mechanism as it occurs in the sympathetic nervous system, our results coincide with the fact that membrane-depolarizing agents such as KCl or neurotransmitter-releasing factors such as ANG II did not affect DA secretion in the kidney. It remains unknown which carriers could mediate ANG II effects on DA transport in renal tubular cells. Three subtypes of polyspecific cation transporters, OCT1, OCT2 and OCT3, are present in renal tubular epithelial cells and mediate the membrane potential entry of organic cations. This includes monoamine neurotransmitters like DA from the blood into the cells [28]. Moreover, OCTN1 and OCTN2 transporters have been detected at the apical membrane of proximal tubular epithelial cells and could mediate the release of endogenous organic cations, such as DA [29, 30]. In addition, Gründemann et al. [31] reported that OCT2 induces specific transport of tritiated DA. Further experiments have to be carried out to test the possibility that OCTs could be implied in DA uptake and release in renal tubular cells.

The MAO and COMT enzymes are the main regulators of DA catabolism and are widely distributed in the renal cortex and the medulla [9]. The activity of both catabolic enzymes is high in renal tissue compared to that of peripheral organs, the mitochondrial enzyme MAO being the major metabolic pathway for biogenic amine degradation [9, 32, 33]. COMT is abundantly expressed within the proximal tubular cells of the kidney and its inhibition significantly increased the DA content in the renal interstitial fluid as well as in urine [13, 32, 33]. We examined the effects of ANG II on MAO- and COMT-specific activities, showing that ANG II stimulates MAO activity in the outer renal cortex without altering COMT activity. This way, ANG II stimulates DA degradation and contributes to diminish the availability of renal DA.

Finally, DA turnover is the result of diverse combined metabolic processes such as uptake, release, synthesis and degradation. The current results demonstrate that ANG II increases DA turnover. This can be the consequence of its own actions on DA uptake (inhibited), synthesis (diminished), and catabolism (stimulated). If we consider that ANG II inhibits renal DA uptake [6], inhibits DA synthesis, and stimulates DA catabolism, then all these effects contribute to decrease renal DA content, as can be seen in figure 5.

Renal DA located at the luminal side of the proximal tubules, increases sodium and water excretion through the stimulation of D1-like receptors (D2-like receptors acting synergistically) that are localized in the plasma membrane [34]. To exert these effects, renal DA must reach the luminal compartment first, a process that depends on intratubular DA concentration. DA tissue concentration depends on the presence and activity of the amine-synthesizing or amine-degrading enzymes, as well as on its uptake.

Three different mechanisms are involved in the stimulation of D1 receptors. In the first process, D_1 receptors are mainly located intracellularly under basal conditions [35] and these receptors can be recruited to the luminal side of the proximal tubules either by D1 agonists or by the increase in intracellular DA availability [15, 36, 37]. This process is known as homologous regulation. Secondly, in a process known as heterologous regulation, peptide hormones like ANP or ANG II, can regulate sodium homeostasis indirectly via sensitization or desensitization of the D1 receptors [38, 39]. Aperia et al. [15] have described that both types of regulation increased renal silent D1 receptor recruitment to the cellular membrane. Homologous regulation is a consequence of the augmented DA availability, as observed when incubating with DA, L-DOPA or inhibiting the COMT. A third mechanism involves the functionality of D1 receptors in caveolar plasma membranes [40] and potentiates a DA-induced inhibition of Na^+,K^+-ATPase in the proximal tubules [41].

Current findings, as well as previous reports from our laboratory [6, 7], show that ANG II decreased endogenous ^{3}H-DA content in the renal outer cortex as a result of effects evoked by ANG II, including the reduction of ADCC activity, inhibition of ^{3}H-DA uptake, stimulation of MAO activity and unaltered DA secretion. Those effects may prevent DA accumulation into tubular renal cells, resulting in a decrease of DA availability inside the cell and at the luminal side of the nephron. These mechanisms would prevent homologous D1 receptor recruitment and stimulation, which in turn would result in Na^+,K^+-ATPase activity disinhibition and, consequently, increased sodium reabsorption and decreased natriuresis.

In conclusion, ANG II modifies DA metabolism in the outer renal cortex of the rat by affecting the amine storage, uptake and catabolism pathways without altering its release.

Acknowledgments

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