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ACUTE ANGIOTENSIN II INFUSION PROMOTES LOCAL PRODUCTION OF PROSTAGLANDIN E2 AND REACTIVE OXYGEN SPECIES IN THE RENAL CORTEX

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Abstract

Angiotensin II (ANGII) is a systemic vasoconstrictor and body fluid regulator. Virtually all released ANGII binds to AT1 receptors, which stimulates cell signaling pathways in various cell types. In kidneys, ANGII enhances arachidonic acid release by increasing the activity of phospholipase enzymesand cyclooxygenase-2 (COX-2), leading to an elevation in prostaglandinlevels where prostaglandin E2 (PGE2) is predominately generated. Male Sprague Dawley rats (200 – 300g) were acutely injected for four hours by vehicle or ANGII (7ng/kg/min, IV). Blood pressure was elevated significantly during ANGII infusion from (109.82 ± 0.085mmHg) to (129.36 ± 0.1mmHg). Urine flow(8.98 ± 0.23µl/min), glomerular filtration rate (1.93 ± 0.18ml/min/gm), and renal blood flow(1.75 ± 0.064ml/min) were decreased in comparison with control(14.03 ± 0.26µl/min), (2.71 ± 0.075ml/min) and (3.8 ± 0.14ml/min/gm) respectively. Through renal microdialysis, we found that PGE2 levels were significantly induced by ANGII treatment (358.27 ± 35.3pg/ml)when compared to control(234.39 ± 11.72pg/ml).Also, acute ANGII infusion augmented the present oxidative stress in renal tissues. Superoxide and peroxynitrite levels were increased by two fold during ANGII treatment when evaluated by EPR using CMH and CPH as spin traps.In the current study, we found that local effects of acute ANGII infusion stimulate PGE2 and free reactive species, which produce a disruption in normal renal hemodynamic function. In summary, the present study demonstrates a potential mechanism by which acute elevations in ANGII promotes hypertension induced end organ kidney damage.

INTRODUCTION

Angiotensin II (ANGII) is the dominant effector peptide of the renin angiotensin system (RAS). It regulates blood pressure and renal hemodynamics through the control of vascular function and electrolyte balance[1], [2], [3], [4].Systemically, ANGII has a well-recognized role in many cardiovascular diseases such as vascular endothelial dysfunction and hypertension[2], [3], [4]. In recent investigations, the localfunction of ANGII has been identified in a variety of body tissues including theheart, kidney, brain and the adrenal glands[2], [3], [4], [5], [6]. In the kidneys, long-termactivation of AT1 receptorsdisturbs renal hemodynamic function by inducing various mechanisms that enhance the development of glomerular sclerosis leading to renal dysfunction[7].

In response to ANGII and other stimuli, prostanoidsincluding prostaglandins (PGs) and thromboxanes are released to induce various physiological and pathophysiological functions in the reno-cardiovascular system [12]. PGs are a group of lipid compounds that have been identified in various tissues, where some of them have the same or opposite function.PGs are involved in several biological and pathological functionssuch as platelet aggregation, and regulation of immune responses and hemodynamic functions in nearly all body systems[8], [9].Prostanoid formation is turned on by the interaction between phospholipid cell membranes and phospholipase A2 which causes arachidonic acid release. Arachidonic acid is then converted by COX1 and COX2 througha cyclooxygenase site to unstable PGG2, which isreduced by a peroxidase site and hemeto stable PGH2, the precursor of all prostanoids[8], [9], [10].PGE2 is the predominant prostanoidin body tissuesand is produced by three types of PGE2 synthases (mPGES-1, cPGES, and mPGES-2)[11].PGE2 has been reported as a vasoconstrictor and vasodilatory agent in the renal microcirculation based on the activationof different functional glomerular arteriolereceptors (EP1, 2, 3, and 4) [16], [17]. In renal vascular smooth muscle cells, EP2 andEP4 receptors are activatedto cause smooth muscle relaxation, and lead to vasodilation in the renal arterioles, whereas EP1 andEP3 receptors are activatedto induce renal vasoconstriction [16], [17], [18], [19].

In addition to vasoconstrictor and antidiuretic effectsof ANGII, it also has a cardinal role inPG formation. ANGII

enhancesthe activity of PLA2 enzyme and induces the expression of the inducible cyclooxygenase enzyme (COX2)[12], [20], [21].In renal tissues, PGE2 has a pivotal role in the filtration system by controlling water/ions reabsorption and counteracting the vasoconstrictor effect of ANGII through EP4receptor activation [13], [14], [15].Therefore, PGE2 mediates an antihypertensive effectinthe face of ANGII-induced high blood pressure.

Moreover, ANGII inducesoxidative stress by enhancingreactive oxygen species (ROS) generationthrough coupling tovarious signaling moleculesmainly NADPH oxidase [22], [23].NADPH oxidase has been labeled as a major source of superoxide (O2-) inliving cells. NADPH oxidase subunits are widely distributed inphagocytes, endothelial cells, smooth muscle cells, and mesangial cells in the renal tissues [22], [23],[24]. Previous reports have demonstrated the potent role of ANGII in NADPH oxidase activation in various organs including the heart, kidney, and brain [23]. In the presence of nitric oxide (NO), O2- produces peroxynitrite (ONOO-) which is known as the most potent free radical in the cells. It avidly oxidizes a broad range of biological molecules whichcausessevere cellular damage[25].

The current study was performed to evaluate the effect of ANGII on the renal filtration system, free radicalsrelease, and renal PGE2 production by using a microdialysis technique. Taken together,acute infusion of low-dose ANGIIinduced renal damage which was evaluated by fractional excretion of sodium (FENa) as a pre-acute kidney injury (pre-AKI)biomarker[26], [34].

MATERIALS AND METHODS

Animals:

Male Sprague-Dawley rats (200-300g, n=12, Harlan, Indianapolis, IN) were housed in a controlled room temperature and light facility. Rats had free access to standard rat chow and water during a week of acclimatization. All animal experiments were approved by the University of Louisiana at Monroe Institutional Animal Care and Use Committee (IUCAC).

Materials:

CMA 30 linear microdialysis probes were obtained from CMA/Microdialysis (Harvard Apparatus, Holliston, MA). ANGII, para-aminohippuric acid (PAH), Inactin (thiobutabarbital sodium), and 2-Methylbutane (isopentane) were purchased from Sigma-Aldrich (St. Louis, MO). Albumin and inulin were obtained from EMD Biosciences Inc. (San Diego, CA) and Fresenius Kabi UK Ltd. (Runcorn, Cheshire) respectively. PGE2 ELISA kits, CPH, and CMH were purchased from Enzo Life Sciences (Farmingdale, NY). All other chemicals were purchased from Fisher Scientific (Houston, TX).

Experimental procedure:

Male rats (200-300g) were anesthetized throughout the experiments by using Inactin (120mg/kg IP). The trachea was cannulated by polyethylene tube (PE 240) to maintain an open airway. PE-50 tube was filled with heparinized saline solution and inserted into the carotid artery to monitor the hemodynamic function during the experiment. Then, the jugular vein was cannulated to infusestock solution with or without ANGII (7ng/kg/min, IV). PE240 tube was implanted into the bladder forurine sampling to evaluate renal filtration system and electrolytelevels. During the experiment, blood pressure was measured by connecting the arterial catheter to a pressure transducer (model TSD104A, Biopac Systems, Santa Barbara, CA). The stock solution was infused at a rate of 0.02 ml/min throughthe venous catheter that was attached to a microinfusion pump (kd Scientific). Formicrodialysis probe implantation, the rat was placed on its right side to make a small midsagittal incision to expose the kidney. As previously described, CMA 30 linear microdialysis probes were inserted into the renal cortex and connected to a microinfusion pump (HARVARD apparatus, PHD 2000,) for saline infusion at a rate of 3µl/min[35]. However, the corticalmicrodialysis samples were collected to investigate alterations in PGE2 during control, vehicle, and ANGII treatments. At the end of the experiment, kidneys and blood were harvested for oxidative stress measurement and renal function evaluation respectively.

ANGII treatment and sample collection:

After a stabilization period (45min), the control period was started and performed for four hours. Then, a stock solution with or without ANGII(7ng/kg/min, IV) was acutely infused for 4 hours. During the entire 8 hour experiment, urine samples were collected to assess renal function, and renal microdialysis samples were harvested to evaluate the local release of PGE2 in the renal cortex.The changes in mean arterial pressures (MAP), systolic blood pressures (SBP), diastolic blood pressures (DBP), and heart rates (HR) were monitored during the experiment. At the end of the experiment, blood was collected in EDTA tubes, centrifuged and plasma was extracted. Plasma samples were aliquoted and stored at -80°C until analyzed. Kidneys were harvested and stored at -80°C until analyzed.

SAMPLES ANALYSIS

Glomerular Filtration Rate (GFR) and Renal Blood Flow (RBF) measurement:

During the experiment, para-aminohippuric acid (PAH) and inulin were infused with stock solution through the jugular vein in the control period (4hrs), and vehicle or ANGII treatmentperiods (4hrs). Urine and plasma samples were analyzed for PAH and inulin concentrations through use of colorimetricmethods to calculate renal blood flow (RBF) and glomerular filtration rate (GFR) respectively.In addition, Na^+ and K^+ were analyzed in urine and plasma by using Flame photometer.Based on the urinary and plasma concentrations of Na^+, fractional sodium excretion (FENa) was calculated to evaluate renal function in all groups as a Pre-AKI biomarker.

PGE2 measurement by ELISA:

PGE2 levels in the previously frozen renal interstitial samples were measured by using commercial colorimetric competitiveenzyme immunoassay kits during control and treatment periods. The unknown PGE2 concentrations were calculated from the standard curves.

Superoxide and peroxynitrite measurement by Electron Paramagnetic Resonance (EPR) spectrometer:

In the current study, EPR spectrometer was used to evaluate O2- and ONOO- levels in the renal cortex. Spin trapping reagents CMH (1-hydroxy-3-methoxycarbonyl-2,2,5,5-tetramethylpyrrolidine.HCl) and CPH (1-hydroxy-3-carboxy-2,2,5,5-tetramethylpyrrolidine.HCl) were used to trap the short-lived free radicals (O2- and ONOO-). At the end of the experiment, kidneys were collected and then flash frozen in isopentane and stored at -80˚C until analysis. At the time of EPR experiments, kidneys were sliced equally into 2 mm and immersed in 0.5 ml DF/DETC/HEPES buffer containing 5mM of CMH or CPH and incubated for 1 hour at 37˚C. After incubation, a glass micropipette was filled with the sample solution to be

placed in the resonator cavity of the EPR spectrometer as previously described [36], [37].

Statistics:

Data were expressed as mean ± SE and analyzed by one-way or two-way analysis of variance (ANOVA) followed by Tukey-Kramer multiple comparison test when appropriate (INSTAT 3). ($P < 0.05$) was accepted as statistically significant.

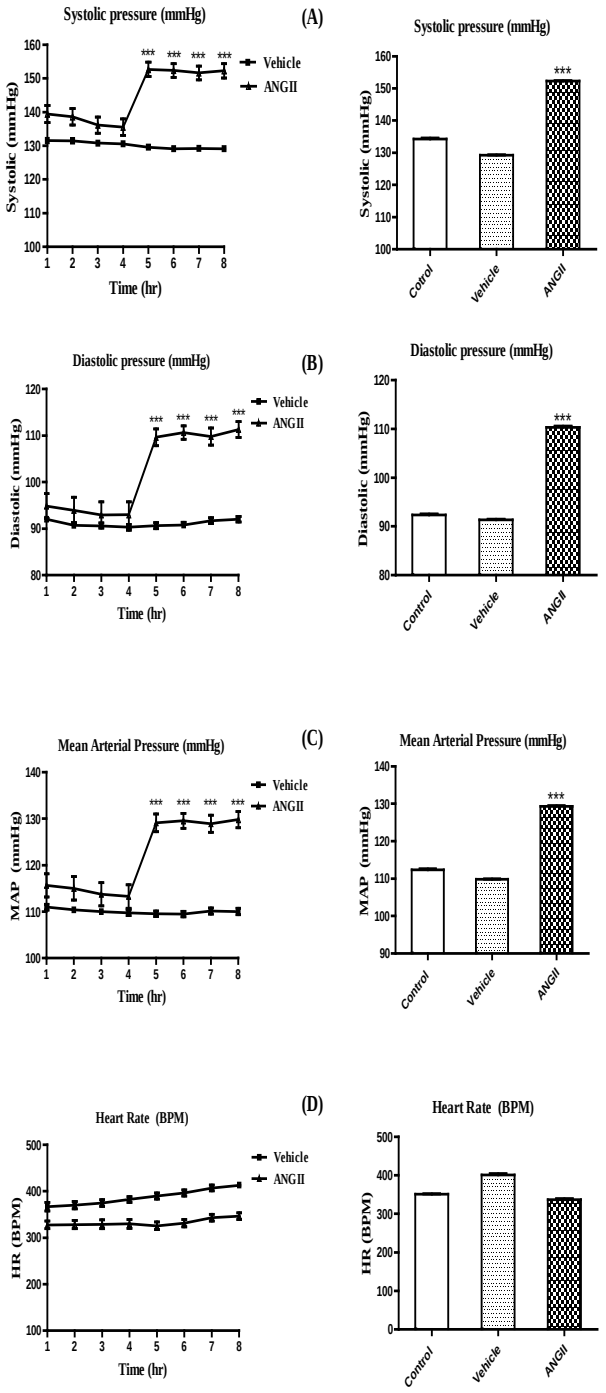

Figure 1: Acute ANGII infusion effects on systemic hemodynamic function. Fig.1 (A), SBP was significantly elevated (152.25 ± 0.11mmHg) by ANGII when compared with control (134.28 ± 0.30mmHg) and vehicle (129.26 ± 0.057mmHg) (***P<0.001). Fig.1 (B), DBP also was increased during ANGII infusion for 4 hrs (110.35 ± 0.19mmHg) in comparison with control (92.30 ± 0.20mmHg) and vehicle (91.30 ± 0.17mmHg) (***P<0.001). Fig.1 (C), MAP was elevated continuously for 4hrs of ANGII infusion (129.36 ± 0.1mmHg) as compared to control (112.36 ± 0.20mmHg) and vehicle (109.82 ± 0.085mmHg) (***P<0.001). Fig.1 (D), HR during ANGII infusion wasn't changed significantly (401.64 ± 2.60 BPM) as compared to control (351.25 ± 0.96 BPM) and vehicle (336.76 ± 2.44 BPM).

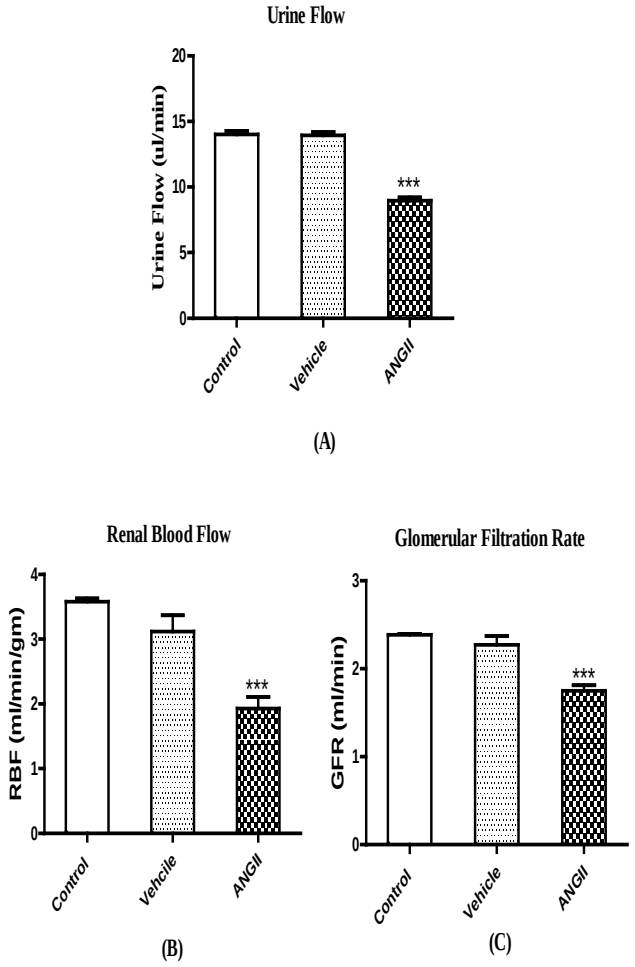

Figure 2: Urine flow, renal blood flow, and glomerular filtration rate calculations. During intravenous ANGII infusion, urinary flow rate (UFR) was reduced significantly (8.98 ± 0.23µl/min) in comparison with vehicle (13.94 ± 0.25µl/min) (figure.2A) (***P<0.001). RBF and GFR was evaluated during ANGII treatment and it showed significant reduction in RBF (1.93 ± 0.18ml/min/gm) and GFR (1.75 ± 0.064ml/min) when compared to control (3.8 ± 0.14ml/min/gm) and (2.71 ± 0.075ml/min) respectively (figure.2B and 2C) (***P<0.001).

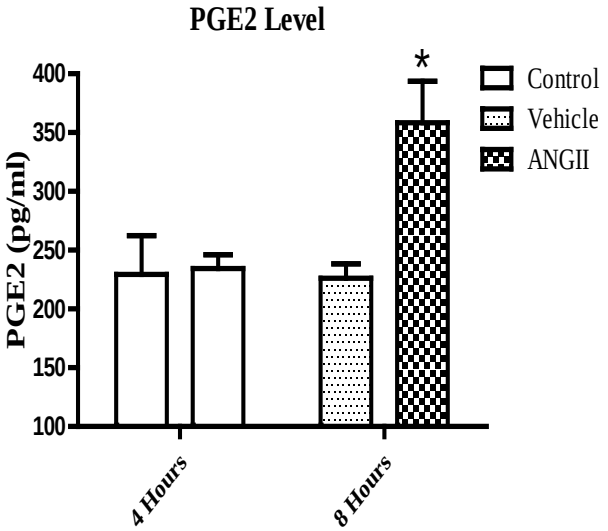

Figure 3: Interstitial PGE2 measurement in the renal cortex.Renal PGE2 level was induced significantly (358.27 ± 35.30pg/ml) by ANGII as compared to vehicle (226.18 ± 12.30pg/ml), and control (229 ± 33.03and234.39 ± 11.72pg/ml) (*P<0.05).

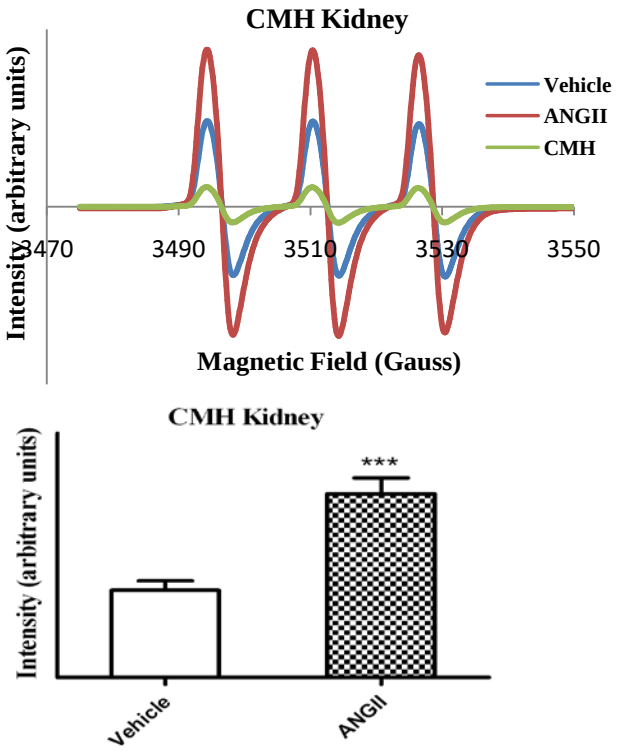

Figure 4: Superoxide measurement in renal tissues. EPR spectrum was obtained from EPR spectroscopy after renal slice incubation for one hour with CMH. Superoxide was significantly induced by ANGII as compared to vehicle. Bar graph was derived from the EPR spectra peaks to compare superoxide production in vehicle and ANGII treatment (***P<0.001).

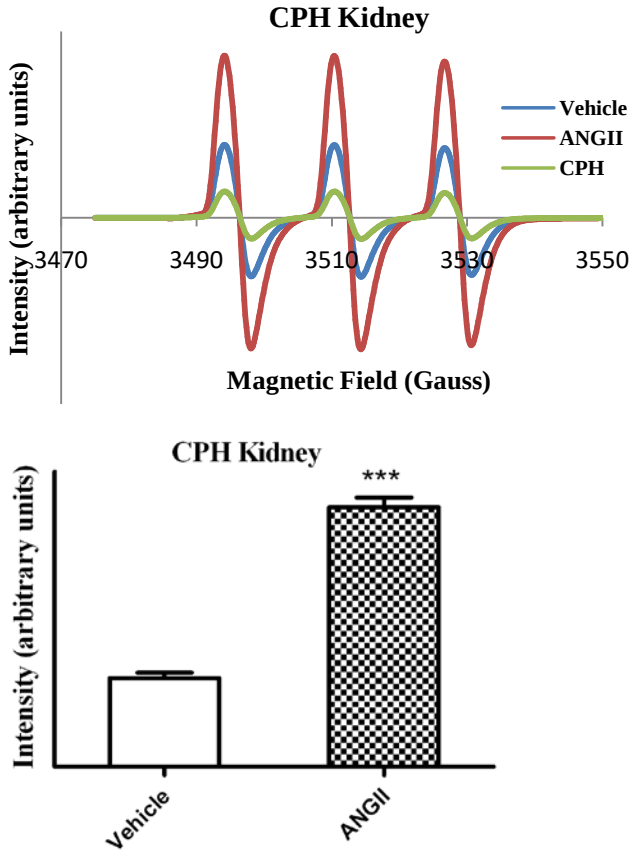

Figure 5: Peroxynitrite measurement in renal tissues. EPR spectrum was obtained from EPR spectroscopy after renal slice incubation for one hour with CPH. Peroxynitrite was significantly induced by ANGII as compared to vehicle. Bar graph was derived from the EPR spectra peaks to compare peroxynitrite production in vehicle and ANGII treatment (***P<0.001).

Table I: Effect of ANGII on urinary sodium (UNaV) and potassium (UKV) excretion, fractional sodium excretion (FENa)

Treatment	*UNaV (umol/min)*	*UKV (umol/min)*	*FENa (%)*
Control	1.04 ± 0.076	2.2 ± 0.05	0.013 ± 0.001
Vehicle	0.97 ± 0.04	2.0 ± 0.18	0.014 ± 0.0007
ANGII	0.17 ± 0.009***	0.37 ±0.017***	0.007 ± 0.0008**

Table I:ANGII inhibited Na+ and K+ excretion in renal tubules. Urinary Na+ and K+ excretion were decreased significantly in ANGII treated animals (0.17 ± 0.009µmol/min and 0.37 ± 0.017µmol/min respectively) when compared to control (1.04 ± 0.076µmol/min and 2.2 ± 0.05µmol/min) and vehicle (0.97 ± 0.04µmol/min and 2.00 ± 0.18µmol/min) (***P<0.001). FENa was calculated as a pre AKI biomarker, FENawas reduced in ANGII treated animals (0.007 ± 0.0008%) when compared to control (0.013 ± 0.001%) and vehicle (0.014 ± 0.0007%) (**P<0.01). Values are expressed as mean ± SE.

RESULTS

Hemodynamic effects of ANGII:

Acute ANGII infusion at 7ng/kg/min led to a significant increase in MAP (129.36 ± 0.1mmHg) with no significant differencesduring vehicle infusion(109.82 ± 0.085mmHg) as compared to control (112.35 ± 0.20mmHg). SBP and DBP were also dramatically increased by ANGII infusion with no changes during vehicle infusion in comparison with control.However, there was no significant change in HR during vehicle (401.64 ± 2.6 BPM) and ANGII infusion (336.76 ± 2.44 BPM) in comparison with control (351.25 ± 0.96 BPM).The heart ratewas slightly declined within the first hours of ANGII infusion due to the baroreceptor reflex, and then returned to the normal level until the end of the experiments (Figure 1).

Renal response:

Urinary flow rate (UFR) was reduced significantly during ANGII infusion (8.98 ± 0.23µl/min) with no significant change during vehicle infusion (13.94 ± 0.25µl/min) when compared to control (14.03 ± 0.26µl/min) (Figure 2A). ANGII reduced GFR and RBF with no significant differences during vehicle infusionas compared to control (Figure2B and 2C). Moreover, ANGII decreased urinary sodium (UNaV) and potassium (UKV) excretion, with no changes in their levels duringvehicle treatment when compared to control(Table I). Furthermore, FENa was measured as a pre-AKI biomarker, and it was reduced significantly during ANGII infusionwith no significant change duringvehicle infusionwhen compared to control (Table I).

PGE2 measurement in the interstitial fluid of the renal cortex:

Microdialysis samples were analyzed by using enzyme-linked immunosorbent assay (ELISA) to determine cortical PGE2 release during ANGII treatment. 4 hours of acute ANGII infusion increased PGE2 levels significantly in the renal cortex (358.27 ± 35.3pg/ml) with no significant changes during vehicle infusion (226.18 ± 12.3pg/ml) when compared to control (229 ± 33.03pg/ml)(Figure 3).

Oxidative stress measurement by EPR spectroscopy:

Acute intravenous ANGII infusion augmented free radical formation in renal tissues. O2- and ONOO- levels were measured by electron paramagnetic resonance (EPR) spectroscopy after 60 minute incubation of similar-sized renal slices in spin traps. O2- and ONOO- were elevated significantly by ANGII as compared tovehicle as shown in Figures4 and 5, respectively.

DISCUSSION

In the present study, the microdialysis technique was adapted as a sampling method for accurateevaluation of interstitial PGE2 levelsin the renal cortex. For several decades, microdialysishas been employed to providean

adequate prediction of extracellular peptides and drug concentrations. This technique has several advantages over other sampling methods, whereas it provides continuous monitoring of analytes with minimal invasiveness in the target tissue. Moreover, it overcomes undesirable changes in the hemodynamic function due to frequent blood sampling as well as it evaluates local changes within the target organ while avoiding any systemic factors that could alterthe final results (35). The obtained samples are directly analyzed and an additional cleanup process and/or enzyme inhibitors is unnecessary.

Current literature has identified various potential pathways which lead to ANGII systemically promoting hypertension. Recently, our lab has been found that ANGII elevated blood pressure by increasing carbonmonoxide (CO)through heme oxygenase-1 (OH-1) induction, whereasit was returned to the normal level by ZnDPBG(HO-1 inhibitor) [27].In the current study, ANGII functions were evaluated in the renal cortex since previous reports have identifiedthat renalANGII concentrations were 1000 fold higher than its plasma concentrations[28]. Our lab has found that acute infusion of low-dose ANGII can increase the blood pressure and promote hypertension[27].The current study succeeded in getting sustained hypertension over four hours of continuous infusion oflow-dose ANGII. However, enough time was not available to collect renal interstitial samples and see the role of ANGII in PGE2 production in the renal cortex.A previous report has demonstrated that continuous intravenous infusion of high dose ANGII(30ng/kg/min) augmented PGE2 production in the renal interstitial fluid with no data evaluating ANGII effects on hemodynamic and excretory functions [31].The renal induction of PGE2 during ANGII infusion is a counteracting mechanism which tries toattenuate the vasoconstrictor actions of ANGIIby activating EP4 receptorsto promote vasodilation of the renal afferent arteriole[30].On the other hand, activation of Gi coupled EP3 receptors by PGE2 antagonizes the vasodilatory effects of cAMP coupled EP4 receptors to promote vasoconstriction of the renal afferent arteriole [16], [38]. The vasoconstrictor and vasodilator actions of PGE2 in pre-glomerular and glomerular microcirculation have been reported in previous studies [39], [40], [41]. However, the role of PGE2 within the renal microvasculature depends on the expression and distribution of EP receptor subtypes.In the renal tissues, EP4 receptor mRNA expression was observed mainly in the glomerulus and afferent arterioles, whereas EP4 agonistsinduced vasodilation as shown previously with no evidence for the expression of EP2 receptorsin the afferent arterioles[16], [42].In the current study, the endogenous cortical PGE2 induction during ANGII infusion was not enough tooffset the renal vasoconstrictor action of ANGII and maintain normal excretory function. In addition to EP4 activation, the endogenous PGE2 binds to EP3 receptorsin the renal circulation which antagonizes the EP4-induced vasodilation.

The disturbancebetween the desirable and undesirable functions of ROSdue toover activation of ROS sourcescausessevere vascular damage that lead to various cardiovascular diseases namely, hypertension, atherosclerosis, and congestive heart failure [31], [32], [33]. The current study observed the role of acute infusion of low-dose ANGII in inducing O2- and ONOO- production in the renal cortex. As previously demonstrated, ANGII is a potent mediator of ROS mainly by enhancing NADPH oxidase activityand uncoupled nitric oxide synthase production, where both mechanisms augment O2- release [22], [23], [43].Recently, the role of PGE2 as a promoter of NADPH oxidase during enhanced ANGII productionhas been detected in thesubfornical organ, whereas ANGII-induced PGE2 production mediates superoxide release by NADPH oxidase through EP1 receptor activation [44]. However, EP receptors (EP1, EP2, EP3, and EP4) are interesting targets to modulate the role of ANGII in inducing renal vasoconstriction and oxidative stress that lead to end-stage renal damage in diabetes mellitus and cardiovascular diseases.

CONCLUSION

In summary, the present study examined the role of acute ANGII infusion in cortical PGE2 production by using the microdialysis technique. Four hours of ANGII infusion caused sustained hypertension, a massive increase in the cortical PGE2 release, oxidative stress, and renal dysfunction.Acute ANGII infusion induced renal damage was evaluated by the pre-AKI biomarker (FENa). In addition, PGE2 could have a potential therapeutic role in renal microcirculation through targeting EP3 and EP4 receptors to prevent ANGII mediated end-organ damage.

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