CALL FOR PAPERS | Integrative and Translational Physiology: Inflammation and Immunity in Organ System Physiology

Blood pressure and renal hemodynamic responses to acute angiotensin II infusion are enhanced in a female mouse model of systemic lupus erythematosus

Marcia Venegas-Pont,1 Keisa W. Mathis,1 Radu Iliescu,1,2 William H. Ray,1 Porter H. Glover,1 and Michael J. Ryan1

1Department of Physiology and Biophysics and the Center for Excellence in Cardiovascular Renal Research, University of Mississippi Medical Center, Jackson, Mississippi; and 2Department of Physiology, “Gr. T. Popa” Biomedical Research Center, “Gr. T. Popa” University of Medicine and Pharmacy, Iasi, Romania

Submitted 14 February 2011; accepted in final form 3 September 2011

Venegas-Pont M, Mathis KW, Iliescu R, Ray WH, PH, Ryan MJ. Blood pressure and renal hemodynamic responses to acute angiotensin II infusion are enhanced in a female mouse model of systemic lupus erythematosus. Am J Physiol Regul Integr Comp Physiol 301: R1286–R1292, 2011. First published September 7, 2011; doi:10.1152/ajpregu.00079.2011.—Inflammation and immune system dysregulation are recognized as prominent contributors to the development and progression of cardiovascular and renal disease. Systemic lupus erythematosus (SLE) is a chronic autoimmune inflammatory disorder associated with a high risk for the development of renal and cardiovascular disease, which are major causes of mortality in these patients (43). Almost all patients with SLE have at least some evidence of renal damage on biopsy (4, 11), and measures of declining renal function, including elevated serum creatinine and blood urea nitrogen or reduced filtration fraction, are commonly reported (11, 20, 28).

The renin-angiotensin system (RAS) is well known for its critical role in renal hemodynamic control and blood pressure regulation and is a common therapeutic target in patients with SLE. Although renal dysfunction and blockade of the RAS system are common to patients with SLE, renal hemodynamic responses to ANG II have not been previously investigated. In the present study, we tested the hypothesis that during SLE, there is an enhanced renal hemodynamic response to acute infusion of ANG II. This hypothesis was tested using an experimental mouse model of SLE.

The New Zealand Black/White F1 hybrid (NZBWF1) is an established and widely used model of lupus nephritis (8). Like humans with SLE, female NZBWF1 mice exhibit declining renal function with age (5, 23, 29, 42), and we previously reported that these mice develop hypertension with impaired endothelial dependent relaxation and enhanced smooth muscle contraction (40). In addition, chronic treatment of these animals with angiotensin-converting enzyme inhibitors delays the onset of renal injury (7, 12), making NZBWF1 mice ideal for examining the renal hemodynamic responses to ANG II.

METHODS

Animals. Thirty to forty-week-old female NZBWF1 (SLE) with albuminuria ≥300 mg/dl (measured by albustix) and NZW/LacJ (control) obtained from Jackson Laboratories (Bar Harbor, ME) were included in the study. All of the studies were performed with the approval of the University of Mississippi Medical Center Institutional Animal Care and Use Committee and in accordance with National Institutes of Health guidelines.

In vivo experimental set up. Mice were maintained under gas anesthesia (isoflurane) on a heating pad servo-controlled to the animal’s rectal temperature at 37°C. A catheter was placed in the left common carotid artery to monitor mean arterial pressure as previously described. The catheter used for infusions was made by pulling Renapulse 040 catheter tubing (Braintree Scientific, Braintree, MA) over heat tapered to an approximate 010 size. Up to 3.5 cm of tapered catheter was advanced via the left femoral artery into the abdominal...
aorta in control and SLE mice. The placement of the catheter did not cause any obvious signs of necrosis or cyanosis in the hind limbs of the animals. At the conclusion of the experiment, the location of the catheter in the abdominal aorta was determined relative to the right renal artery. The mean location of the catheter tip was 0.9 ± 0.6 mm below the right renal artery. With the animal on its ventral side, the right kidney was approached retroperitoneally and a perivascular flow probe (0.5 PSB; Transonic Systems, Ithaca, NY) was positioned around the right kidney and held in place using a micromanipulator, as previously described (39, 46).

Experimental protocol. Baseline mean arterial pressure (MAP) and renal blood flow (RBF) were collected at a sampling rate of 100 Hz for at least 30 min of stable recording. Following this period, the acute responses to bolus (10 μl via a Hamilton syringe) intra-arterial infusions of ANG II (1 and 10 ng) or the angiotensin receptor blocker, losartan (1 μg), were recorded in the same animal using a Powerlab (ADInstruments, Boulder, CO). The total volume of 60 μl (the dead space volume of the catheter was ~50 μl) was infused in less than 10 s. Hemodynamic responses to losartan and different doses of ANG II were separated by a 10-min recovery period. RBF is reported as flow per gram of kidney weight. Renal vascular resistance is calculated as the renal perfusion pressure (in mmHg) divided by the renal blood flow (ml·min⁻¹·g kidney wt⁻¹). The peak change in response to ANG II and losartan are presented as a percent change from baseline. Volume replacement was not used in this study, as blood pressure was constant throughout the protocol.

Urinary albumin. Mice were placed in metabolic cages overnight for collection of urine. Albuminuria was assessed using commercially available sticks, Albustix (Bayer), as previously described (40). SLE mice are typically considered to have positive albuminuria at a threshold of ≥120 mg/dl, and no control mouse had detectable urinary albumin.

Real-time PCR. Renal expression of angiotensin type 1 receptors (AT1R) was measured using real-time RT-PCR. RNA was isolated from renal cortex homogenates using the RNeasy Protect Minikit (Qiagen) per the manufacturer’s instructions. The RT reaction was carried out using the iScript cDNA synthesis kit (Bio-Rad, Hercules, CA), and real-time PCR for AT1R was performed using SYBR Green Supermix (Bio-Rad) with the iCycler (Bio-Rad). The primer sequences for mouse AT1R were as follows: sense 5'-ATCGTAC-TCTGGCATTGTGC-3' and antisense 5'-GTGACATTGCCCCACCCAGCAT-3'. The primers for the AT1R (or the antibody listed below) do not discern between the AT1a and AT1b subtype; however, it is known that AT1a is the major mediator of cardiovascular and renal actions of ANG II in mice (15). Primers for 18S ribosomal RNA were used to normalize the data (sense 5'-TAAGTCCCTGCTTTGCTACA-3' and antisense 5'-GATCCCGGCGCTACTAAAC-3').

A product-melt curve was generated at the end of the experiment to confirm the presence of a single PCR product. Data are presented as the degree of change relative to control expression levels, calculated as previously described (40).

Western blot analysis. Renal cortical protein expression of AT1R, ANG II receptor type-2 (AT2R) and renin were determined using standard Western blot analysis methods, as previously described (53, 54). Rabbit polyclonal anti-AT1R antibody (1:500; Alomone Laboratories, Jerusalem, Israel), rabbit polyclonal anti-AT2R antibody (1:500; Alomone Laboratories), goat polyclonal anti-renin (1:250; Santa Cruz Biotechnology, Santa Cruz, CA) and mouse anti-β-actin (1:5,000; Abcam, Cambridge, MA) were used. The secondary antibody was IR700-conjugated donkey anti-rabbit IgG (1:5,000; Rockland Immunologicals, Gilbertsville, PA), IR700-conjugated donkey anti-rabbit IgG (1:2,000; Rockland Immunologicals) and IR800-conjugated donkey anti-mouse IgG (1:2,000; Rockland Immunologicals) for AT1R/AT2R, renin, and β-actin, respectively. Antibody labeling was visualized using the Odyssey Infrared Scanner (LI-COR). Data are presented in arbitrary units of protein optical density band normalized to β-actin.

Data and statistical analysis. To determine maximal changes in flow and pressure, a moving average feature of the ADInstruments Chart Software was used to smooth the data. The maximal response was determined as the lowest or highest point in pressure or flow relative to the flow at the time of the bolus infusion. Statistical differences between SLE mice and control animals were determined using a Student’s t-test. Data are presented as means ± SE and were considered different at P < 0.05.

RESULTS

Baseline arterial pressure was not different between anesthetized SLE and control mice (91 ± 6 mmHg vs. 88 ± 2 mmHg, SLE vs. control, respectively; P = 0.46). Renal blood flow was significantly lower in SLE mice (3.4 ± 0.4 ml·min⁻¹·g kidney wt⁻¹) compared with control mice (4.5 ± 0.3 ml·min⁻¹·g kidney wt⁻¹; P = 0.03) and calculated renal vascular resistance (RVR) was significantly greater in SLE mice compared with control animals (SLE = 37 ± 6, control = 22 ± 6 mmHg·ml⁻¹·min⁻¹·g; P = 0.03) (Fig. 1).

Acute blood pressure and renal hemodynamic responses to an intra-arterial infusion of 1 ng of ANG II were tested in control and SLE mice. As expected, MAP pressure was increased in both groups; however, the change in pressure caused by ANG II was significantly greater in SLE mice (increase of 33 ± 4%) compared with controls (increase of 16 ± 2%, P = 0.0001). The change in RBF caused by ANG II infusion was also significantly greater in SLE mice (−72 ± 4%) compared with controls (−57 ± 4%, P < 0.04). As a result of the enhanced blood pressure and RBF response in SLE mice, calculated RVR was significantly greater in SLE mice (RVR: SLE = 410 ± 76, control= 208 ± 38 P < 0.02) (Fig. 2).

Experiments repeated using a 10-ng bolus dose of ANG II demonstrated a dose-dependent response; however, at this higher dose of ANG II, there was no difference in the blood pressure or blood flow response to ANG II between SLE and control mice (data not shown).

We subsequently tested whether the acute hemodynamic response to an angiotensin receptor blocker was altered in mice with SLE (Fig. 3). The decrease in blood pressure resulting from a bolus intra-arterial infusion of losartan (1 μg) was not different between SLE and control mice (SLE −12 ± 8% vs. control −12 ± 2%, P = 0.97). The predicted increase in RBF caused by losartan was markedly blunted in mice with SLE (SLE: 0 ± 3%) compared with control animals (31 ± 5%, P < 0.01). As a result, RVR decreased to a lesser degree in mice with SLE (−12 ± 5%) compared with controls (31 ± 2%, P < 0.01).

On the basis of the hemodynamic data, we measured the expression of AT1R in control and SLE mice. Renal cortical AT1R mRNA expression was significantly lower in SLE mice (0.56 ± 0.08, n = 11, P = 0.01) compared with control animals (1.19 ± 0.21, n = 10) (Fig. 4A). Consistent with the mRNA expression, renal cortex AT1R protein expression, presented as the ratio of AT1R to β actin, was significantly lower in SLE mice (0.20 ± 0.01, n = 7, P = 0.01) compared with controls (0.30 ± 0.03, n = 7) (Fig. 4B). In addition to AT1R protein expression, renal cortical expression of AT2R and renin was assessed. Fig. 5 shows an increase in renal cortical AT2R expression in SLE mice. Renal cortical expres-
Renin expression was lower in the SLE mice compared with control animals (Fig. 6).

**DISCUSSION**

In the present study, we tested whether renal hemodynamic responses to ANG II were enhanced in a mouse model of chronic inflammation, SLE. The major findings of the study are 1) direct measurements of renal hemodynamics indicate that renal blood flow is lower in mice with active SLE; 2) the renal blood flow and blood pressure response to an acute infusion of ANG II is exaggerated in mice with SLE; 3) the renal blood flow response to acute infusion of the AT1R blocker losartan, is blunted in mice with SLE; 4) renal cortical mRNA and protein expression of the AT1R are lower in mice with SLE; 5) renal cortical renin protein expression is lower in mice with SLE; and 6) AT2R protein expression is increased in the renal cortex of SLE mice.

**Renal hemodynamics and SLE.** The progression of renal disease during SLE, not surprisingly, correlates with decrements in renal function. For example, indicators of renal hemodynamic function, such as effective renal plasma flow, glomerular filtration, filtration fraction, and serum creatinine levels have all been reported to be abnormal in patients with SLE (11, 20, 28). Similar to data in humans, estimates of renal function have been made in the NZBWF1 model of SLE. Numerous reports show that as lupus nephritis progresses, serum creatinine and blood urea nitrogen levels are increased in NZBWF1 mice and that estimates of renal plasma flow are decreased in these mice (5, 18, 24, 42). The present study provides direct evidence that renal blood flow is reduced in SLE mice with renal disease and is consistent with early work using indirect measures of renal hemodynamics.

Impaired renal blood flow has important implications, not only as an indicator of advancing renal disease, but also as a potentially important factor in impaired blood pressure regulation during SLE. The pivotal role for the kidney in the...
long-term control of blood pressure is well known and a decreased renal blood flow can mechanistically contribute to the hypertensive shift in the pressure natriuresis relationship. Importantly, prevalent hypertension contributes to the greater cardiovascular risk in patients with SLE (25, 33, 35, 51), and we previously demonstrated that, similar to humans with SLE, NZBWF1 mice develop hypertension (40, 41, 53, 54). Under the current experimental conditions, the hypertension that is observed in conscious animals is largely muted as a result of the anesthesia. Nevertheless, these data show that even when blood pressure is equivalent, mice with SLE have increased renal vascular resistance (less renal blood flow for given level of renal perfusion pressure) and supports the concept that impaired renal hemodynamics could contribute to the development of hypertension during SLE.

**RAS in SLE.** Although it is a common therapeutic target, surprisingly little is understood about the RAS during SLE, and there is limited data related to circulating components of RAS. In small cohorts of patients with glomerulonephritis or nephrotic syndromes, including SLE, there is evidence for both elevated and low plasma renin activity. Whether these changes contribute to the progression of renal disease in SLE or occur...
secondarily to changes in renal function is not well defined (10, 14, 19, 49). Renin substrate is reportedly increased in both the plasma and urine in some patients with SLE and can be reduced with RAS blockade (26, 52). While circulating components of RAS during SLE have not been widely studied, the potential for genetic variation in components of the RAS and their association with SLE has been examined extensively. The common angiotensin-converting enzyme insertion/deletion polymorphism has been the most frequently studied. While some reports suggest a genetic association between SLE and with the deletion polymorphism or frequency of the deletion polymorphism, there are just as many studies that do not (1, 21, 27, 31, 34, 50). Fewer studies have examined the genetic link between SLE and common genetic variants of angiotensinogen or angiotensin receptors, and these results have also been equivocal (30, 44, 45).

Similar to data from humans, there is limited information related to RAS components in mouse models of SLE. Early studies suggest that plasma renin activity is low in both the NZBWF1 model used in this study, as well as the MRL/lpr model of SLE (another common murine model of SLE) (37, 38). Adding another layer of complexity, a recent study by Crowley et al. (6) showed that angiotensin receptor knockout in the MRL/lpr mouse model exacerbates renal injury (6), a response that may be mediated by the remaining AT1bR subtype in the glomerulus. To date, the most consistent evidence for the involvement of the RAS in the disease progression of murine models with SLE comes from studies in which animals are treated with RAS antagonists. For example, treatment of either the NZBWF1 mice or MRL/lpr mice with captopril or enalapril delays the onset of albuminuria and reduces chemokines in the kidney (7, 12, 13, 32). This is consistent with human SLE, where ACE inhibitors and ARBs are effectively used to reduce albuminuria and control blood pressure (9, 17). Therefore, the use of RAS blockade in disease management for both humans and animal models appears to be the one common piece of evidence supporting a possible contribution of RAS to SLE. Renal hemodynamic changes in response to ANG II have not been previously tested during SLE.

Renal hemodynamic responses to ANG II. Hemodynamic changes are not uncommon to SLE, with reports showing that brachial artery blood flow is impaired in patients with SLE (16, 22, 36, 47). Impaired hemodynamic responses have also been recently extended to the coronary circulation in patients with SLE (2). Consistent with data from human studies, we previously demonstrated peripheral vascular changes in NZBWF1 mice, including impaired endothelial dependent relaxation and enhanced smooth muscle cell contraction (40). The impaired endothelial dysfunction was recently confirmed by Kaplan’s group (48). In the present study, the data show that the renal blood flow and pressor response to acute ANG II infusion is greater in mice with SLE compared with controls. While renal hemodynamic responses to ANG II have not been reported in human or murine SLE, a relatively recent study using skeletal muscle arterioles from MRL/lpr mice demonstrated an exaggerated vasconstriction response to ANG II (3). Therefore, in mouse models of SLE, there may be a generalized increase in vascular sensitivity to ANG II among different vascular beds.

The enhanced blood flow and blood pressure response to ANG II in the NZBWF1 model is consistent with a reduced RAS in this model of SLE. For example, if RAS is lower in SLE mice, the infusion of ANG II will have greater potential to increase blood pressure and reduce renal blood flow. The fact that the response to the higher dose of ANG II (10 ng, not shown) was not different between SLE and control mice is also consistent with this concept and suggests that the upper end of the renal vascular and hemodynamic responses to ANG II is similar. Our data showing that renal cortical expression of renin is lower in mice with SLE further supports the concept that RAS is reduced in this model and is consistent with early measurements showing that NZBWF1 mice have low plasma renin activity (38).

On the basis of the enhanced ANG II responsiveness, we initially hypothesized that the RBF response to an acute bolus infusion of the AT1R blocker losartan would be enhanced in mice with SLE. However, the blood pressure response to losartan was not different between SLE and control mice, and the renal blood flow response to losartan was markedly blunted during SLE. One interpretation of these data is that the attenuated response to acute bolus losartan infusion under these baseline conditions is consistent with a reduced renal AT1R expression and that when ANG II is increased (as occurs with the infusion), the enhanced sensitivity of the existing receptors is unmasked. The fact that the blood pressure response to losartan is not different between NZBWF1 and NZW mice may reflect the possibility that extrarenal organs and the vasculature have similar AT1R expression, although the enhanced blood pressure response in mice SLE suggests that extrarenal AT1Rs are also sensitized.

On the basis of mRNA and protein analysis, we show that renal cortical AT1R mRNA and protein expression are reduced in mice with SLE. These data further support not only the idea that SLE mice have fewer AT1R, but also that the sensitivity of the existing receptors is enhanced. Interestingly, renal cortical expression of the AT2R was significantly increased in SLE mice, suggesting a possible compensatory change in response to the enhanced renal vascular sensitivity to ANG II. To fully appreciate the mechanistic role that altered angiotensin receptor expression has in the renal hemodynamic changes during
SLE, future experiments will be required to test the effect of long-term receptor blockade on renal hemodynamic function and receptor signaling.

**Perspectives and Significance**

On the basis of the high prevalence of cardiovascular and renal disease and the success of RAS blockade in both humans and murine models of SLE for blood pressure control and renal protection, we speculated that renal hemodynamic responses to ANG II may be altered in the kidneys. The data show that renal hemodynamic and blood pressure responses to ANG II are exaggerated in an established mouse model of SLE and that renal expression of ANG II receptors and renin is reduced. This supports the concept that ANG II receptor sensitivity is enhanced and could be a possible contributing mechanism to the progression of renal disease and hypertension in patients with SLE. These data may also provide insights as to the mechanisms by which RAS blockade can be an effective therapy in many patients with SLE.

**GRANTS**

M. Venegas-Point and K. W. Mathis are recipients of American Heart Association Greater Southeast Affiliate Postdoctoral Fellowship (2260874 and 4350019, respectively). This work was supported by the National Heart Lung and Blood Institute Grants HL085907, HL085907S, and HL092284 to M. J. Ryan, as well as P01HL5197.

**DISCLOSURES**

No conflicts of interest, financial or otherwise, are declared by the authors.

**REFERENCES**


14. Palsara A, Peden E, Lum RF, Seligman VA, Olson JL, Li H, Seldin MF, Criswell LA. Association of angiotensin-converting enzyme polymor-


