Salt sensitivity of nitric oxide generation and blood pressure in mice with targeted knockout of the insulin receptor from the renal tubule

Lijun Li,1 R. Mayuri Garikepati,1 Susanna Tsukerman,1 S. Tiwari,1,2 and Carolyn M. Ecelbarger1

1Department of Medicine, Georgetown University, Washington, District of Columbia; and 2Department of Molecular Medicine and Biotechnology, Sanjay Gandhi Postgraduate Institute of Medical Sciences, Lucknow, India

Submitted 23 January 2012; accepted in final form 13 July 2012

Li L, Garikepati RM, Tsukerman S, Tiwari S, Ecelbarger CM. Salt sensitivity of nitric oxide generation and blood pressure in mice with targeted knockout of the insulin receptor from the renal tubule. Am J Physiol Regul Integr Comp Physiol 303: R505–R512, 2012. First published July 18, 2012; doi:10.1152/ajpregu.00033.2012.—To elucidate the role of the insulin receptor (IR) on kidney nitric oxide generation and blood pressure (BP) control, we generated mice with targeted deletion of renal tubule IR usingloxP recombination driven by a Ksp-cadherin promoter. Male knockout (KO) and wild-type (WT) littermates (~4 mo old) were transitioned through three 1-wk treatments: 1) low-NaCl diet (0.085%); 2) high-NaCl diet (HS; 5%); and 3) HS diet plus 3 mM tempol, a superoxide dismutase mimetic, in the drinking water. Mice were then switched to medium-NaCl (0.5%) diet for 5 days and kidneys harvested under pentobarbital anesthesia. Twenty-four-hour urinary nitrates plus nitrites were significantly higher in the WT mice under HS (2,067 ± 280 vs. 1,550 ± 230 nmol/day in WT and KO, respectively, P < 0.05). Tempol attenuated genotype differences in urinary nitrates plus nitrites. A rise in BP with HS was observed only in KO mice and not affected by tempol (mean arterial pressure, dark period, HS, 106 ± 5 vs. 119 ± 4 mmHg, for WT and KO, respectively, P < 0.05). Renal outer medullary protein levels of nitric oxide synthase (NOS) isoforms by Western blot (NOS1–3 and phosphorylated-S1177-NOS3) revealed significantly lower band density for NOS1 (130-kDa isoform) in the KO mice. A second study, when mice were euthanized under HS conditions, confirmed significantly lower NOS1 (130 kDa) in the KO, with an even more substantial (>50%) reduction of the 160-kDa NOS1 isoform. These studies suggest that the loss of renal IR signaling impairs renal nitric oxide production. This may be important in BP control, especially in insulin-resistant states, such as the metabolic syndrome.

Address for reprint requests and other correspondence: C. M. Ecelbarger, Associate Professor, Dept. of Medicine, Georgetown Univ., 4000 Reservoir Rd., NW, Washington, DC 20007 (e-mail: ecelbarc@georgetown.edu).

Metabolic syndrome is a common and escalating constellation of clinically associated disorders, including dyslipidemia, insulin resistance, visceral adiposity, and hypertension (24). Recent estimates are that it may affect as many as 32–35% of the United States population (35). The relationships between the clinical factors that make up metabolic syndrome are unclear. However, impaired or blunted insulin receptor (IR) signaling in target tissues, including liver, muscle, and adipose, is associated with the metabolic syndrome (26). Moreover, altered IR signaling at the level of the kidney has been implicated in increased blood pressure (BP) and sodium retention (2, 6, 13, 22).

While IR are expressed along renal tubules (10, 11, 33), their physiological role in the maintenance of cellular function in the kidney, and whether they undergo “resistance”, is still not clear. Insulin has been associated with anti-natriuresis at the whole-organ level (7), sodium retention in isolated, perfused tubule studies (4, 25, 45), and sodium uptake in cell culture (5, 38). In addition, specific sodium transporters, exchangers, and channels have been demonstrated to be activated by insulin, including the epithelial sodium channel, among others (5, 19, 43, 52). Thus high circulating insulin levels, such as in the metabolic syndrome, might be expected to result in sodium retention and hypertension. However, IR signaling cascades are also linked to the production of nitric oxide (NO), which has been shown to oppose sodium retention at the renal level (36) and hypertension. Thus the integrative effects of high circulating insulin levels on sodium retention and BP are not crystal clear and may relate to fine tuning of IR signal transmission.

Recently, our laboratory showed targeted knockout (KO) of IR from the renal tubule of mice using Ksp-cadherin-targeted Cre-recombinase led to a phenotype of delayed clearance of orally administered saline, slightly elevated BP, and 20–50% reduced urine nitrates plus nitrites (UNOx) excretion (48). In these mice, the KO of IR was found to be most efficient in the thick ascending limb (TAL) through collecting duct (CD) and less efficient in the proximal tubule, although a significant reduction in IR protein on Western blots was found in both medulla and cortex (48). KO mice were produced via crossing Ksp-cadherin-driven Cre-recombinase carrier mice (23), with mice homozygous forloxP sites flanking the IR (9). Mice with brain, adipose tissue, liver, heart, or muscle KO of IR all have been determined to have phenotypes associated in some way with the metabolic syndrome (1, 8, 9, 21, 30). For example, while KO of IR from the muscle results in dyslipidemia, but normal glucose and insulin levels, mice with IR knockout of the liver were severely insulin resistant (9, 30). Our mice had normal glucose tolerance and circulating insulin levels (48).

The combination of reduced UNOx and elevated BP in our IR KO mice led to our hypothesis that the two might be causally linked. Other studies support signaling through IR may have a role in BP control via its effects on NO generation in the kidney (58). For example, fructose-fed hypertensive mice provided with nitric oxide synthase (NOS) 3 [endothelial NOS (eNOS)] gene therapy had reduced BP and attenuated insulin resistance, compared with untreated controls (58). IR signaling is linked to activation of the serine/threonine kinase Akt (protein kinase B) via serial phosphorylation of IR, IR substrate, and phosphoinositide 3-kinase (57). Akt has been demonstrated to phosphorylate and activate NOS in a variety of tissues, including kidney (58). NOS3 molecular sites, thought to be important in activation by the IR signaling cascade, are
serines 1, 177, and 617 (40). We have shown that acute administration of insulin to mice results in a decrease in sodium excretion, but an increase in UNOx, a surrogate marker for renal activity of the enzyme (47).

The renal medulla appears to be a primary site for NO production (28, 56). NO in the kidney can be produced by one or more of three isoforms (NOS1, NOS2, and NOS3). These isoforms have been demonstrated to be expressed at various sites along the renal tubule. NOS1 (neuronal NOS or brain NOS) exists as a splice variant (NOS1-α, NOS1-β) (34) and is expressed in macula densa cells, TAL, and in cortical and medullary CD (29). NOS2 (inducible NOS) is found in TAL (20, 32), and NOS3 (eNOS) is expressed in TAL and CD, in addition to renal endothelial cells (3, 51).

Therefore, the main aims of this study were to 1) determine whether the elevation in BP and 2) the reduction in UNOx that we observed previously in our KO mice under basal dietary NaCl conditions were salt sensitive; 3) to determine whether the three major NOS isoforms were differentially expressed in renal outer medulla; and 4) to determine whether we could attenuate differences in UNOx or BP by treatment with the superoxide dismutase mimetic tempol. Our overall hypothesis is that reduced renal expression of the IR in TAL and distal tubule will increase salt sensitivity of BP via a mechanism involving blunted production of NO. If tempol, an antioxidant, is able to abolish or attenuate genotype differences, it will then be explored whether the elevation in BP and UNOx can be amplified by HS.

METHODS

KO mice. The animal procedures described herein were fully approved by the Georgetown University Animal Care and Use Committee and were carried out in the Georgetown University Department of Comparative Medicine Animal Facility, a USDA and Association for Assessment and Accreditation of Laboratory Animal Care International approved facility. Mice with renal epithelial cell-selective KO of the IR were generated at Georgetown University by crossing mice that were homozygous for floxed IR gene, in which loxP sites flanked exon 4 of the IR gene (floxed IR mice) (9), with mice carrying Cre-recombinase driven by the kidney-specific, ksp-cadherin promoter (ksp-Cre mice) (53), as previously described (48). In subsequent generations, KO females were bred back to floxed IR male mice to produce progeny, of which about 50% were heterozygous for ksp-Cre (KO) and 50% were homozygous “wild-type” for this allele (WT). All offspring were homozygous for floxed IR. KO mice were determined by standard polymerase chain reaction genotyping of tail DNA using previously described Cre-recombinase primers (48). Our laboratory previously demonstrated reduced expression of IR in the medullary TALs and CD from these mice (48). To better define the cellular localization of active Cre-recombinase, KO females were also crossed with male mice homozygous for a transgene of the LacZ gene, with a DNA stop sequence flanked by loxP sites (JAX Laboratories, catalog no. 002073 B6, 129-Gtros26tm1Sor, Soriano Line). Removal of the stop sequence by Cre-recombinase allowed for expression of β-galactosidase reporter assay. To determine regions of the kidney with active Cre-recombinase, LacZ/ksp-Cre doubly heterozygous mice were deeply anesthetized with ketamine and xylazine, a laparotomy was performed, then they were perfused transcardially with 1× phosphate-buffered saline until clear of blood, followed by 20 ml of 2% gluteraldehyde. The kidneys were then removed and bisected and further exposed, exposing the medulla and cortex. The bisected sections were immediately stained for the product of β-galactosidase at 37°C for 1 h using a Beta-Gal Staining Kit (MIR 2600, Mirus Bio, Madison, WI). The sections were then photographed using a Zeiss dissecting scope and a Canon A590 camera with a fitted objective at ×1.2 and ×5 magnification.

Urinary sodium, potassium, 8-isoprostane, and nitrates plus nitrates. Twenty-four-hour urine collections were obtained with the addition of 10 μl of an antibiotic cocktail. Volumes were recorded, and then urine was centrifuged at a slow speed to remove food particles and other small debris. Urinary nitrates plus nitrates (NOx) concentration was measured using an ELISA assay (Cayman Chemical, Ann Arbor, MI). Urinary sodium and potassium concentrations were determined by use of a Medica EasyLyte analyzer (Bedford, MA). Urine 8-isoprostane was measured using an ELISA assay from Oxford Biomedical Research (Oxford, MI).

Western blotting. ISOM were sonicated in ice cold isolation solution containing protease inhibitors in a buffered 250 mM sucrose solution, as previously described (16). Western blotting of the ISOM was performed by the methods established in our laboratory (44). After determining the protein concentrations of the homogenates, the protein was solubilized in Laemmli sample buffer. Quality of tissue sample preparation was assessed by staining loading gels with Coomassie blue (Gelcode Blue, Pierce Endogen, Rockford, IL), and then examining the sharpness and intensity of the bands. To assess the alterations in the protein abundances of NOS1, NOS2, and NOS3, and p-S1177-NOS3, semiquantitative immunoblotting was performed. For immunoblotting, 15 μg of protein from each sample were loaded onto individual lanes of minigels of 7.5, 10, or 12% polyacrylamide (precast; BioRad, Hercules, CA). Blots were probed with commercial antibodies, including a rabbit polyclonal against NOS2 (sc-651, Santa Cruz Biotechnology, Santa Cruz, CA) made against an NH2-terminal portion of the molecule, a mouse monoclonal against NOS3 (BD Transduction Laboratories, San Jose, CA), made against amino acids 1025–1203 of human eNOS, a mouse monoclonal anti-Ser 1177 p-NOS3 (Cell Signaling, Beverly MA), and rabbit polyclonal NOS1 (SA-227 against amino acids 1414–1434, BIOMOL Research Laboratories, Plymouth Meeting, PA). Loading accuracy was evaluated by probing the lower portion of nitrocellulose membranes with GAPDH mouse monoclonal antibody (Abcam). Band densities for the NOS
isoforms and GAPDH were determined by IMAGE J software (National Institutes of Health, Bethesda, MD), analyzing an equivalently sized area of band for each lane. NOS band densities were then divided by GAPDH densities in each lane. Blocking peptides were used for NOS1 (BML SP227–0100) and NOS2 (sc-651 peptide, Santa Cruz) to determine whether NOS bands were ablated with the peptide sequence used to immunize the rabbits for these polyclonal antibodies. To do this, we loaded, in the last three lanes of the NOS1 and NOS2 gels in study 1, duplicate ISOM (15 µg) from three randomly chosen WT mice. After blotting, these three lanes of membrane were cut from the main piece and probed overnight with a solution containing the same concentration of primary antibody, with the addition of 5 µg/ml of the respective control peptide. At the end, the pieces were developed to the same piece of film as the rest of the blot using the same exposure time.

**Statistical analysis.** Quantitative data are expressed as means ± SE. Differences between WT and KO were determined by unpaired t-test, if there were no differences in variability or distribution of data. When data were not normally distributed or differences in variability existed, data were compared by the Mann-Whitney rank sum test. In study 2, data were compared by one- and two-way (genotype × treatment) ANOVA. A significant one-way ANOVA was followed by a multiple-comparisons test. P values < 0.05 were considered significant for all tests.

**RESULTS**

**Localization of Cre-recombinase activity.** To better define the renal tubular location of Cre-recombinase activity using Ksp-cadherin as a targeting promoter, we crossed KO female mice with male loxP-flanked LacZ reporter mice. Mice were genotyped for Ksp-cadherin, and carriers and “WT” littermates were evaluated for β-galactosidase activity in kidney (Fig. 1). Mice positive for Ksp-cadherin expressed blue staining concentrated in the ISOM, the inner medulla, extending up into the cortex of the kidney, consistent with the strongest activity of Cre-recombinase in TAL and distal tubule, including CDs (Fig. 1B). Staining was absent in the WT littermates (Fig. 1A).

**Urine volume and NOx.** There were no differences in body weights or kidney weights between KO and WT mice in studies 1 or 2. In study 1 (n = 20 WT, 18 KO), the final mean body weight for WT mice was (mean ± SE): 31.6 ± 0.6 g, compared with 31.8 ± 0.7 g for KO (P = 0.81). Kidney weights were 0.21 ± 0.01 (WT) and 0.22 ± 0.01 g (KO; P = 0.40). Twenty-four-hour urine volumes were slightly, but significantly, lower in the KO mice under LS conditions (P = 0.032), but not different under HS or HS + T treatments (Fig. 2A). No differences were seen in UNOx excretion in the LS period; however, under HS diet, WT mice showed a sharper increase in UNOx, leading to 69% more UNOx excretion, compared with the KO (P = 0.043, Fig. 2B). Tempol did not reduce UNOx in either genotype. In fact, UNOx continued to rise in the KO with the addition of tempol, so that it was no longer significantly different than the WT (P = 0.36). The WT mice were basically unresponsive to tempol with regard to UNOx.

**Urine sodium and potassium.** Urine sodium and potassium excretion and the ratio of the two are shown under each treatment in Fig. 3. As we predicted, there were no significant differences between genotypes for these parameters, likely due to the fact that mice were in sodium balance toward the end of each dietary period. Nonetheless, we were surprised to see a strong trend (P = 0.053) for WT mice to excrete more sodium than the KO mice when tempol was added to the HS diet (Fig. 3A). However, both KO and WT showed an increase in both urinary sodium and potassium in this week (Fig. 3, A and B), perhaps suggesting greater overall consumption as they adjusted to the HS diet in the second week. The sodium-to-potassium ratio, which would normalize for food intake, was slightly higher in the WT with tempol, but not significantly so (P = 0.46, Fig. 3C).

**Urine 8-isoprostane.** Urine levels of 8-isoprostane (8-isopGF2α), an end-product of free-radical peroxidation, were measured as an index of overall “oxidative stress” (Fig. 4). Overall, HS diet elevated 8-isoprostane excretion, but, surprisingly this effect was delayed in the KO mice. Tempol did not reduce 8-isoprostane levels in either group of mice when offered in conjunction with the HS diet. Excretion was significantly higher in the WT vs. the KO mice in the HS period and significantly elevated in both groups of mice in the HS + T vs. the LS periods.

**Salt sensitivity of BP and HR.** In Fig. 5A, 12-h (day and night) mean arterial pressure (MAP) averages on the final 2 days of each dietary period are shown. There were no differ-
ences in MAP between KO and WT mice in the LS dietary period; however, switching to the HS diet led to significantly higher MAP in the KO mice, which was not attenuated by the addition of tempol to the drinking water in the subsequent week. Similar findings were found when diastolic and systolic BPs were evaluated separately (not shown). As expected, MAP was higher in the night (dark period) for both genotypes, with no discernible differences in diurnal rhythm of MAP, either between genotypes or as a result of the diets or tempol. Mean change (delta) in MAP between the LS and HS period and the HS and HS conditions are plotted in Fig. 5B. KO mice had a significant rise in MAP with the HS diet; WT mice did not. Tempol did not affect MAP in either genotype in the following week. Mean HRs in 12-h periods on the final 2 days of each dietary period are shown in Fig. 5C. No significant differences in HR due to genotype in the night or day were found. HR fell in both genotypes with the addition of HS to the diet (Fig. 5D), with no effect of tempol.

Western blots of NOS isoforms. We next tested whether there were any differences in NOS isoforms protein expression levels between KO and WT mice in study 1 in which the mice had been euthanized under the MS diet. For NOS1, the 130-kDa band was significantly denser (25%) in the WT vs. the KO mice. There were no other significant differences between genotypes for NOS isoforms; however, there was a trend for NOS2 to be elevated in the KO (P = 0.27). There was also no difference in the absolute band density of p-S1177-NOS3 (the “activated” phosphorylated form of NOS3) or ratio of p-NOS3 to NOS3 in the same samples (Fig. 6).

In study 2, we tested whether isoform expression would vary when kidneys from mice were harvested under the conditions in which we saw differences in UNOx and BP, i.e., under HS or HS + T conditions (Fig. 7). HS led to larger variability in NOS expression than under the MS conditions of study 1 (especially for NOS2). Nonetheless, WT mice had significantly greater NOS1, both the 160- and 130-kDa bands [two-way ANOVA, P = 0.007 (160-kDa band) and P = 0.014 (130-kDa band) for genotype]. The 160-kDa band for NOS1 [possibly the NOS1-α isoform (20)] appeared to be virtually absent in the KO mice, even with a longer exposure (Fig. 7A). However, there was a significant interactive term for NOS1 in that tempol caused a reduction in NOS1 expression in the WT, but not in the KO mice (P = 0.029 for interaction). NOS3 abundance was
also marginally, but not significantly, lower in the KO vs. the WT \((P = 0.148 \text{ for genotype})\). There were no genotype differences in the band density for p-S1177-NOS3 or the ratio of p-NOS3 to total NOS3.

**DISCUSSION**

In this study, we utilize an animal model in which the IR has been knocked out of major portions of the renal tubule to evaluate the role of insulin signaling in the kidney on BP control, salt sensitivity, and renal excretion of metabolites of NO (nitrate and nitrite). Our major findings included increased salt sensitivity of BP, as well as a blunted rise in UNOx excretion with HS diet in KO vs. WT mice. Tempol did not affect BP differences, but it had some attenuating effects of the difference in UNOx. Furthermore, KO mice had reduced levels of outer medullary NOS1 protein levels.

In this set of studies, we chose to focus mainly on the ISOM as the source of deficit NO production, as the TAL is a renal segment of high-energy demands and subject to oxidative stress (18, 31). Moreover, previously, we found that the greatest relative decrease in IR mRNA and protein in the KO vs. WT was in this region, which is enriched in TAL, but also contains vascular elements, CD, and small portions of S3 segment of the proximal tubule. In those studies, IR mRNA in ISOM was \(\sim 25\%\) of the WT (48). To better visually characterize where the activity of Ksp-cadherin was greatest, in the present report, we crossed KO females with lacZ reporter mice (Fig. 1). In doubly heterozygous mice, when \(\beta\)-galactosidase was expressed upon removal of the stop sequence, we found the greatest production of blue stain in the inner and outer medulla, indicative of the greatest Cre-recombinase activity at these sites, with lower activity in the proximal tubule. This agrees with previous reports regarding renal relative expression of Ksp cadherin (23), and with our previous characterization of the reduction of mRNA and protein for IR in our KO mice (48).

KO mice had a significantly blunted rise in UNOx with a switch from LS to HS diet. These studies are in agreement with others (27) that demonstrate HS diet increase NO excretion. This increase in NOS activity may be due to a rise in the activity of NOS1, NOS2, or NOS3 isoforms. Mattson and Higgins (28) demonstrated increased protein expression of all three major isoforms of NOS with HS diet in rat medulla. The attenuated rise in NOx excretion in the KO mice could reflect reduced production or activity of NOS or increased loss of existing NO by superoxide radicals. In support of reduced NO production rather than increased loss, we showed significantly lower protein levels for NOS1, but minimal effect of tempol.

NOS isoforms have a variety of roles in diabetes and diabetic nephropathy (12, 14, 37) conditions in which insulin levels and signaling are altered. NOS2 KO mice have been shown to have increased severity of symptoms associated with
diabetic nephropathy compared with C57Bl6 controls (50), suggesting a protective role of NO production in this model. Insulin, per se, has been shown to increase the phosphorylation and activation of NOS3 (42). In our studies, whole cell ISOM levels of NOS3 were not significantly lower in the KO (although it trended in this direction). We also found no difference in the band densities for the phosphorylated Ser-1177 form of NOS3, which has been suggested as the key posttranslational modification in insulin-stimulated NOS3 activity (40), or the ratio of this form to total NOS3 on immunoblots. Thus we do not have evidence for a role of this pathway in explaining differences in UNOx. Nonetheless, Ritchie et al. (40) have also suggested Ser-615 may be another key site for insulin modulation of NOS3 (eNOS) activity, which we did not evaluate in this set of studies. We also attempted to measure NOS activity in aliquots of the ISOM homogenates from study 2; however, unfortunately, overall activity was too low to determine definitively whether genotypic differences existed.

In our studies, we showed no differences in BP between KO and WT under LS conditions; however, on switching to the HS diet, KO mice responded with an ~10-mmHg rise, while BP did not change in the WT mice. This was actually very similar to the difference we found in BP between KO and WT mice in our first study using medium-NaCl rat chow (~1% NaCl). Our mice are on a mixed background strain that contains mainly 129/Sv (main lineage of the IRloxP mice) and C57Bl6/J (main lineage of the Ksp-cadherin mice). However, as a result of successive breeding of KO females back to floxed males, the genetic background more strongly resembles the paternal lin-
eage. Salt sensitivity of BP is highly dependent on background strain of mice. Escano et al. (17) recently reported a 30% rise in BP using telemetry in C57Bl6/6 mice on 6% NaCl diet for 1 wk, but no rise in mice of the SJL/J strain. Therefore, further studies may be warranted to evaluate the effect of the loss of renal tubular IR in an alternative background strain.

Taken together, since urinary NO metabolite levels are suggested to reflect the activity of the endogenous NO system (55), our results support the view that the salt sensitivity of BP that we found may, in part, be determined by their inability to increase or to sustain NO production at a threshold level in response to the salt challenge. They are also supported by a study carried out by Tolins and Shultz (49), in which the authors have demonstrated that rats became sensitive to the pressor effect of salt when the expected increase in NO production was blocked by administration of an exogenous NOS inhibitor. In agreement, studies by Kopkan and Majid (27) in NOS3 KO mice demonstrated a role for increased superoxide activity as a determinant of salt-sensitive hypertension.

Tempol, an antioxidant and superoxide dismutase mimetic (41), was added to the drinking water to assess whether first it would attenuate differences in UNOx between the genotypes, and, if so, whether it could ameliorate BP differences. Tempol is a redox cycling nitrooxide that increases bioavailability of NO (54). In the Kopkan and Majid (27) studies, tempol with HS diet led to an increase in UNOx above what was observed with HS alone in WT, but not NOS3 KO mice. It also attenuated the rise in BP in the NOS3 KO mice. In our study, tempol led to a modest rise in UNOx, with the rise being somewhat larger in the KO, but it did not attenuate BP differences, nor reduce HS-induced rises in 8-isoprostane excretion in either genotype of mice. In fact, we found, surprisingly significantly reduced 8-isoprostane excretion in the HS period in KO mice.

It is not clear why tempol did not reduce 8-isoprostane excretion, nor cause a greater rise in UNOx in, at least, the WT mice on HS. Our concentration of tempol in the drinking water (3 mM) was slightly higher than what Kopkan and Majid (27) had offered to their mice (400 mg/l or 2.3 mM), showing an ~33% increase in UNOx over a 1-wk period. In our study, UNOx excretion under either LS or HS diet without tempol was, in general, about two times higher than in their study, which may have had some impact on the ability of the mice to elicit a further increase with tempol. We speculate (based on our urine sodium and potassium data) that both WT and KO mice in the second week of HS feeding were simply eating more diet, which may have increased oxidative stress, such that the tempol dose was not sufficient to reduce 8-isoprostane. Nonetheless, 8-isoprostane levels overall were lower in the KO mice, suggesting that oxidative stress did not account for genotype differences. In fact, it might suggest a protective effect of the reduced NO with regard to oxidative stress.

Overall, these data suggest that the loss of renal tubular IR signaling impairs renal NO production, leading to BP changes. These effects of IR were also sensitive to the level of dietary NaCl, suggesting that proper and effective signaling through the IR may assist in the renal processes needed to rapidly and efficiently excrete NaCl, under a HS diet. Our laboratory previously showed reduced renal levels of IR in obese Zucker rats and F344 × Brown Norway (F1) hybrid rats fed a high-fat diet, both models of the metabolic syndrome (46). Our labora-

ratory has also shown reduced NOS activity in the obese Zucker rat kidney (39). Thus impaired or reduced IR signaling in the kidney may contribute to hypertension in the metabolic syndrome.

ACKNOWLEDGMENTS

The authors thank Karishma Sitapara, Daniel Ecelbarger, and Tiana Woods, students working in the laboratory, for technical assistance.

Portions of this work were presented at the Experimental Biology Meeting in 2011 and the Annual Meeting of the American Society of Nephrology in 2011 and were published in abstract form for those meetings.

GRANTS

This work was primarily supported by National Institute of Diabetes and Digestive and Kidney Diseases Grant DK082507 (to C. M. Ecelbarger), as well as the American Heart Association Established Investigator Award (to C. M. Ecelbarger). S. Tiwari is partially supported by a DBT-Ramalingaswami grant.

DISCLOSURES

No conflicts of interest, financial or otherwise, are declared by the author(s).

AUTHOR CONTRIBUTIONS

Author contributions: L.L., S. Tiwari, and C.M.E. conception and design of research; L.L., R.M.G., and C. Tsukerman performed experiments; L.L., S. Tsukerman, and C.M.E. prepared figures; L.L., C.M.E. edited and revised manuscript; L.L., S. Tiwari, and C.M.E. approved final version of manuscript.

REFERENCES


Downloaded from http://ajpregu.physiology.org/ by 10.220.32.247 on October 15, 2017


SALT SENSITIVITY IN RENAL IR KO MICE