Insulin Regulates Nitric Oxide Production in the Kidney Collecting Duct Cells*

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Background: Renal nitric oxide (NO) production is important for long-term blood pressure regulation. Insulin stimulates NO production via the insulin receptor (IR)/PI3K/endothelial NO synthase-dependent pathway in inner medullary collecting duct (IMCD) cells, the largest source of NO in the kidney.

Conclusion: IR is crucial for insulin-induced NO generation in IMCD.

Significance: This study clarifies the implication of reduced IR in insulin-resistance associated hypertension.

The kidney is an important organ for arterial blood pressure (BP) maintenance. Reduced NO generation in the kidney is associated with hypertension in insulin resistance. NO is a critical regulator of vascular tone; however, whether insulin regulates NO production in the renal inner medullary collecting duct (IMCD), the segment with the greatest enzymatic activity for NO production in kidney, is not clear. Using an NO-sensitive fluorescent dye, we found that insulin increased NO production in mouse IMCD cells (mIMCD) in a time- and dose-dependent manner. A concomitant dose-dependent increase in the NO metabolite (NOx) was also observed in the medium from insulin-stimulated cells. NO production peaked in mIMCD cells at a dose of 100 nM insulin with simultaneously increased NOx levels in the medium. At this dose, insulin significantly increased p-eNOSSer1177 levels in mIMCD cells. Pretreatment of cells with a PI 3-kinase inhibitor or insulin receptor silencing with RNA interference abolished these effects of insulin, whereas insulin-like growth factor-1 receptor (IGF-1R) silencing had no effect. We also showed that chronic insulin infusion to normal C57BL/6J mice resulted in increased endothelial NOS (eNOS) protein levels and NO production in the inner medulla. However, insulin-infused IRKO mice, with targeted deletion of insulin receptor from tubule epithelial cells of the kidney, had ~50% reduced eNOS protein levels in their inner medulla along with a significant rise in BP relative to WT littermates. We have previously reported increased baseline BP and reduced urine NOx in IRKO mice. Thus, reduced insulin receptor signaling in IMCD could contribute to hypertension in the insulin-resistant state.

In insulin resistance is defined as inefficient sensitivity of major metabolic tissues, including liver, muscle, and adipose tissue, toward insulin (1–5). Insulin receptor modulation could be an important mechanism that may directly affect target cell sensitivity to insulin (2, 6, 7).

In kidney, the insulin receptor (IR) is expressed in nearly all absorptive epithelial cells along the renal tubule. Using mice with targeted deletion of IR gene from different segments of the kidney tubule, we have demonstrated a physiologic role for renal IR in proximal and distal tubules (8, 9). In addition, we have demonstrated reduced IR protein levels in renal epithelial cells in rat models of insulin resistance (10). Moreover, the observations that IRS-1 knock-out mice are hypertensive suggest that insulin signaling contributes importantly to regulation of cardiovascular physiology (31). Our equivalent observation of hypertension along with reduced urinary NOx levels, a measure of renal NO production, in mice with targeted deletion of IR from distal tubule cells of the kidney (IRKO) not only supports the existing idea of IR signaling as a regulator of cardiovascular physiology, but also adds a novel function for the IR in kidney in blood pressure (BP) and NO regulation (8, 11).

NO has been suggested to play an important role in long-term BP regulation by its autocrine and paracrine actions (12). Moreover, a role of local NO production in the kidney has been implicated in impaired renal blood flow found in patients with chronic congestive heart failure (13). Such a paracrine NO system has been postulated to reside within the renal medulla (14). In addition, the inner medullary collecting duct (IMCD) has been shown to exhibit the greatest enzymatic activity for NO production, about 3–6-fold higher than other segments (15). NO has also been suggested...

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The abbreviations used are: IR, insulin receptor; IMCD, inner medullary collecting duct; mIMCD, mouse IMCD; BP, blood pressure; NOx, NO metabolite; eNOS, endothelial NOS; nNOS, neuronal NOS; IGF-1R, insulin-like growth factor-1 receptor; PI, phosphatidylinositol; DAF-FM, 4-amino-5-methylamino-2',7'-difluorofluorescein; MAP, mean arterial blood pressure; p, phosphorylated; DAF-2DA, 4,5-diaminofluorescein diacetate; DAF-2T, fluorescent triazole derivative of DAF-2.
to modulate sodium transport (12, 16) and attenuate superoxide-stimulated urea permeability in this segment of the kidney (17).

Insulin is a known regulator of NO production in vasculature (18). In kidney, hyperinsulinemia has been shown to induce NO-dependent vasodilation and affect renal blood flow (19). This effect was found to be impaired in the insulin-resistant state (20–22). Thus, hemodynamic effects of insulin in the kidney are at least partially dependent on local NO synthesis (19). Moreover, reduced renal NO production has also been reported in rat models of diabetes (23–25) and in patients with chronic kidney disease and end-stage renal disease (26, 27). It is important to note that insulin resistance is a common finding in the above models/populations.

Overall, it appears that reduced insulin signaling impairs NO generation in kidney. Furthermore, as the IMCD is the largest source of NO generation in the kidney, it is of vital importance to determine whether insulin induces NO generation in these cells, and if so, then how reduced insulin action impairs NO production. This may improve our understanding of the pathophysiological association between insulin resistance and hypertension by clarifying the implication of reduced IR expression in renal epithelial cells reported in the insulin-resistant state (10).

**EXPERIMENTAL PROCEDURES**

*Cells and Culture Conditions—*The mouse inner medullary collecting duct cell line (mIMCD-3 cells) was received as a gift from Dr. Peter Igarashi (UT Southwestern Medical Center). Cells were maintained in DMEM/F12 supplemented with 10% FBS (Invitrogen) as described (28, 29). Cells were grown at 37 °C in 5% CO2. For experiments, cell cultures were switched from FBS (Invitrogen) as described (28, 29). Cells were grown at 37 °C in 5% CO2. For experiments, cell cultures were switched to serum-free medium for 6 h and then stimulated with insulin or vehicle for 15–20 min. In some cases, cells were pretreated with 500 nM wortmannin (Tocris Biosciences) before stimulation. Unless otherwise mentioned, all the chemicals were from Sigma-Aldrich.

*siRNA Transfection—*Cells were transfected in 6-well plates at 50% confluence with 100 nM siRNA (Ambion) using Lipofectamine RNAiMAX (Invitrogen), according to the manufacturer's instructions with the following modification; full growth medium was removed and the transfection complexes were added directly onto the cells in serum-free medium for 4–6 h before the addition of complete growth medium for 48 h. Cells were harvested for further analysis. Dual knockdown of insulin-like growth factor-1 receptor (IGF-1R) and IR was achieved by sequential transfection of 100 nM IR siRNA followed by 100 nM IGF-1R siRNA (30) from Dharmacon. The following primers were used: IR siRNA, sense, 5'-CGGUAAGAGGUGUAAUUUGAT-3', and antisense, 5'-UCAGUUGACAGUCUUAACCGca-3'; IGF-1R siRNA, sense, 5'-UACACGCGUGUAGUCUC-AATT-3', and antisense, 5'-UUUGAAGAUCCGGCGUGU-CAT-3'; and non-targeted siRNA, sense, 5'-UGGGUUUCAUG-UCGACUAA-3', and antisense, 5'-UGGGUUUCAUG-UGUGUGA-3'.

*Measurement of NO Production in Cultured Cells with DAF-FM Diacete—*Insulin-stimulated intracellular NO production was measured using NO-sensitive 4-amino-5-methylamino-2',7'-difluorofluorescein (DAF-FM) fluorescent dye (31). Briefly, serum-starved cells were first loaded with 10 μM DAF-FM diacetate dye (Molecular probe) in 96-well black plates for 60 min at 37 °C. After 1 h, cells were rinsed and supplemented with 100 μM L-arginine. Baseline fluorescence (excitation 492 nm, emission 515 nm) was measured by a fluorimeter (Biotek Synergy) for 2 min followed by fluorescence recording for 20 min in response to insulin or vehicle stimulation.

*Measurement of NO Metabolites—*At the end of incubations, the culture supernatants were collected for nitrate/nitrite estimation by a modification of the Griess reaction (32) using nitrate/nitrite colorimetric assay kit (Cayman).

*Nitric Oxide Production in Mouse Kidney—*To determine the effect of insulin on NO production in inner medulla, direct NO production was measured in inner medullary cell suspension by the method established by Kojima et al. (33). Briefly, the inner medullary collecting duct suspension obtained from wild type mouse kidney was incubated with DAF-2DA (10 μmol/liter; Calbiochem-Novabiochem Co) for 1 h at room temperature, washed, and incubated for 30 min in the experimental buffer with either 1) insulin (10 nM) or 2) vehicle. The changes in DAF-2T fluorescence were recorded.

*Immunocytochemistry—*mIMCD-3 cells, grown in slide chambers, were treated with vehicle or 100 nM insulin for 15 min. Immunostaining was performed by a standard method using anti-p-eNOSSer1177 antibody raised in rabbit (Abcam) followed by anti-rabbit Alexa Fluor 488-conjugated secondary antibody (Invitrogen). Cells were viewed and photographed in six random fields using a UV fluorescence microscope (Nikon Eclipse 80i).

*Immunohistochemical Examination of Kidney Tissues from Mice with Targeted Deletion of IR from Distal Renal Tubule (IRKO Mice)—*Paraffin blocks of kidney tissues from untreated IRKO mice and their WT littermates (n = 3/genotype) were obtained. Male mice were given an intraperitoneal injection of insulin (0.5 units/kg of body weight) in 300 μl of saline plus 300 μl of 25% dextrose in saline after 4 h of fasting to amplify the difference between KO and WT mice. After 20 min, mice were anesthetized, and perfusion-fixed kidneys were removed for paraffin block preparation. IRKO mice were generated as described previously (8). Three-μm thin sections of the blocks were used for immunohistochemical analysis for p-eNOSSer1177 using a standard method as described previously (10).

*Chronic Insulin Infusion in Mice—*C57BL/6J male mice at ∼5 months of age were anesthetized with isoflurane and subcutaneously implanted with osmotic minipumps (ALZET model 1007D; DURECT Corp.) preloaded with insulin (Humulin-R, Eli Lilly). Insulin was delivered at 50 units/kg of body weight per day for 28 days. A similar dose of insulin was used previously by us (34) Moreover, chronic doses of insulin between 1 and 50 units/kg of body weight per day have been reported by us and others (10, 35–37). Insulin-infused mice had ad libitum access to 20% glucose drinking water and 0.5% NaCl pelleted chow during the infusion. Control mice had free access to 0.5% NaCl chow and plain drinking water, but received no infusion. In a separate study, male IRKO mice and their wild type littermates (n = 7/genotype) were both chronically infused with insulin for 2 weeks as described above. At the end of 2 weeks, mice were placed in metabolic cages for a 24-h urine collection. All mice
were maintained under protocols approved by the Institutional Animal Care and Use Committee (IACUC).

Electrolyte and Metabolite Measurements—Urinary sodium and potassium were determined by ion-selective electrodes (ELISE electrolyte system; Beckman Instruments).

Blood Pressure Measurement—Blood pressure measurement was done using radiotelemetry. Transmitters were implanted in mice and blood pressure measurement was done at George-town University using the protocol approved by the George-town University Animal Care and Use Committee (GUACUC). Mice were anesthetized with ketamine plus xylazine and equipped with radiotelemetry transmitters (Data Sciences Inc., St. Paul, MN) as described previously (8, 34, 38). After a 1-week recovery, BP was recorded first at the baseline and then during the course of insulin infusion.

Western Blot Analysis—Protein from cell lysate and mouse inner medulla homogenates was measured, and immunoblotting was performed as described previously (8–10, 39). Before immunoblotting, Coomassie Blue-stained loading gels were prepared for all sample sets, and densitometry was scanned to assess the quality of the proteins by the sharpness of the bands. The gel was also used to confirm precision of protein concentration measurements, as described previously (9, 40–42). The following antibodies raised in rabbit were used: anti-phospho-Akt (Thr308), anti-AKT, anti-phospho-eNOS (Ser1177), and anti-eNOS (Abcam); anti-IR-β and anti-IGF-1R (Santa Cruz Biotechnology); and anti-β-actin (Cell Signaling). Signals were detected using a chemiluminescence-based detection system (Amersham Biosciences).

Statistics—Results are expressed as mean ± S.E. for the number of experiments indicated in the figure legends. Compari-
sions within groups were made using paired Student’s t tests, and comparisons between groups were made using unpaired Student’s t tests as appropriate. p < 0.05 was considered statistically significant. The n values represent the number of independent experiments.

RESULTS

Infusion of Insulin Increased eNOS Protein Levels and NO Production in Inner Medulla—To determine the effect of insulin on renal NO metabolism and BP, WT mice were chronically infused with insulin for a period of 28 days. We found a substantial increase in eNOS protein levels in inner medullary homogenate, as compared with control mice (Fig. 1, A and B). BP was not significantly different between treatments (Fig. 1C). We also measured direct NO production in IMCD-enriched suspensions from mouse kidney in response to insulin (10 nm) or vehicle. The changes in DAF-2T fluorescence were significantly increased by insulin in the inner medulla collecting duct suspension (Fig. 1D). To determine the mechanism and how reduced insulin action impairs NO production, the next set of experiments was carried out in IMCD cells.
We examined the time course of NO production in IMCD cells stimulated with increasing doses of insulin. With 10–200 nM concentration of insulin, we observed an increase in DAF-2 fluorescence by 2 min, with a peak at ~10 min followed by a rapid return to basal levels by 18 min (Fig. 2A). The fold change was greater with 100 nM. The area under the curve of increase in fluorescence over the period of 18 min was significant in insulin-treated cells at doses 10, 100, and 200 nM relative to vehicle. There was no detectable production of NO in response to vehicle or 1 nM insulin.

We next examined the accumulation of NO metabolites (nitrates plus nitrites; NOx) in the medium from these cells after 15 min of vehicle or insulin stimulation and compared these results with the medium from the same cells before stimulation. Insulin stimulation increased NOx levels in the medium in a dose-dependent manner as compared with vehicle (Fig. 2B). The rise in NOx levels by 1 nM insulin was not significantly different from vehicle. The rise in NOx was similar for 100 nM and 200 nM over the period of 15 min.

**PI 3-Kinase Is Required for Insulin-stimulated eNOS Activation and NO Production in IMCD Cells**—To determine the role of PI 3-kinase in insulin-stimulated eNOS regulation, IMCD cells were pretreated with wortmannin (500 nM). Immunoblotting revealed that insulin-stimulated eNOS phosphorylation at Ser-1177 was attenuated in wortmannin-pretreated cells (Fig. 4A). In addition, the rise in NOx levels in response to 15 min of insulin stimulation was also blocked by wortmannin pretreatment (Fig. 4B).

**IR Is Required for Insulin-induced NO Production**—To further confirm the role of insulin receptor in NO production, we silenced IR in IMCD cells. We found that insulin-induced eNOS phosphorylation at Ser-1177 was blocked in IR-silenced cells. In addition, intracellular nitric oxide production in response to insulin was also significantly attenuated in IR-silenced IMCD cells relative to control cells (Fig. 5B). The rise in NOx levels in response to insulin in the medium from these cells was also absent in IR-silenced cells (Fig. 5C).

**IGF-1R Is Not Crucial in Insulin-induced eNOS Activation**—Insulin and IGF-1 are capable of binding to each other’s receptors. Moreover, insulin, at a dose of 100 nM, may cross-activate with IGF-1R and insulin/IGF-1R hybrid receptors (43). To address this, we compared the relative transcript levels of IGF-1R and IR receptors in IMCD cells. Using real time PCR, we found 4.3-fold higher IGF-1R transcript levels as compared with IR in IMCD cells (p < 0.0001). We next silenced IGF-1R alone and also IGF-1R in combination with IR. The insulin-induced increase in p-eNOS to eNOS ratio was not significantly different between IGF-1R-silenced and control IMCD cells (Fig. 6A and B). Moreover, the rise in NOx levels in response to insulin was also similar in the media from IGF-1R-silenced and control IMCD cells (Fig. 6C). Silencing both receptors, however, significantly attenuated insulin-stimulated eNOS phosphorylation (Fig. 7), supporting a role of IR...
Impaired eNOS Regulation in Inner Medulla from IRKO Mice—An indication that the renal IR may have a role in insulin-induced NO production in IMCD cells originated from our earlier study done in IRKO mice (8). These IRKO mice had significantly (2-fold) lower urinary NOx levels as compared with WT littermates, both in response to acute insulin and in the basal state (24-h collection) (8). Based on data in IMCD cells in the present study, impaired eNOS regulation was a strong candidate to explain the reduced urinary NOx production in the IRKO mice (8). To address this, we performed immunohistochemistry for eNOS and p-eNOS<sup>Ser1177</sup> on kidney sections from untreated IRKO mice (<i>n</i> = 3/genotype). We found substantially lower immunoperoxidase-based staining for p-eNOS<sup>Ser1177</sup> in IMCD of IRKO mice as compared with WT littermates (Fig. 8A). In addition, when these mice were infused with insulin chronically (2 weeks), we found a significant rise in mean arterial blood pressure in IRKO mice (<i>p</i> = 0.03) along with lower overall protein levels of eNOS in the IRKO, <i>p</i> = 0.04; Fig. 8, B–D). Both groups had similar 24-h sodium and potassium excretion at the end of 2 weeks of insulin infusion (Table 1).

**DISCUSSION**

We have demonstrated for the first time that insulin induces eNOS activation and NO generation in IMCD cells. We have also shown that insulin stimulation of IMCD cells resulted in a dose-dependent rise in nitrate-nitrite levels in the medium, which furthermore illustrates the paracrine potential of NO produced in IMCD cells. Wortmannin and IR silencing abolished these effects of insulin, suggesting a role of the IR/PI3K pathway in insulin-stimulated NO generation in IMCD cells. Previously, we have reported increased BP and reduced urine NOx in IRKO mice, including collecting duct cells (8). We also

![FIGURE 3. Insulin stimulates eNOS phosphorylation in IMCD cells. IMCD cells were treated with vehicle or insulin (100 nm) for 15 min. A, representative lanes are shown from immunoblots of cell lysate probed with the antibody against p-Akt<sup>Thr308</sup>, Akt, p-eNOS<sup>Ser1177</sup>, eNOS, and IR-β. β-actin was used as a loading control. B, summary of band densities, normalized to β-actin (<i>n</i> = 5/treatment). *, <i>p</i> < 0.05 versus vehicle for same time of incubation. Error bars indicate mean ± S.E. C, representative microscopic images evaluating insulin-stimulated phosphorylation of eNOS at Ser-1177 by immunofluorescence using antibody against p-eNOS<sup>Ser1177</sup> (red color). Hoechst stain (blue color) was used for nuclear staining (100× and 600×).](image)
showed that this attenuation in urine NOx and BP was salt-sensitive (11). In this study, we demonstrated reduced p-eNOS<sup>Ser1177</sup> in the inner medulla of these mice as compared with WT. In addition, chronic insulin infusion resulted in significant rise in BP and significantly lower eNOS abundance in inner medullary homogenates from IRKO mice as compared with WT littermates. Taken together these findings suggest that insulin stimulates inner medullary eNOS expression (chronically), as well as activity (acutely and chronically), and that a reduction in the number of active receptors affects this feed-forward response on expression, and therefore, affects activity via altered NO production.

Our time course- and dose-dependent experiments for insulin-induced NO production in IMCD cells suggest that insulin at a dose of 100 nM could maximally generate NO in IMCD cells within 15 min of stimulation. This is significantly above the physiological range for insulin in mice or humans (44); however, an increase was also observed at lower doses. Our studies do indicate that IMCD cells have the capacity to respond in some manner to greater agonist levels of insulin, if available. Insulin at 100 nM concentration has repeatedly been shown to strongly stimulate phosphorylation of Akt, eNOS, and other insulin-activated pathways (45, 46).

In IMCD cells, constitutively phosphorylated eNOS<sup>Ser1177</sup> was observed in vehicle-treated cells as indicated by immunoblotting as well as immunofluorescence. Insulin significantly increased phosphorylation by ~2.5-fold in 15 min. Generation of significantly higher levels of NO within the first 2 min suggests a rapid phosphorylation of eNOS at Ser-1177 (Fig. 2). This insulin-induced eNOS activation and NO production appeared to be dependent on PI 3-kinase in IMCD cells (Fig. 4A). In addition, to PI 3-kinases, calcium/calmodulin-dependent protein kinase II (CaMKII) (47) and PKA (47) have also been shown to phosphorylate Ser-1177 and increase eNOS activity and NO production, at least in endothelial cells. We have not directly assessed the activation of these kinases; however, studies done in other cell systems have not shown insulin stimulation of eNOS via calcium/calmodulin-dependent protein kinase II or PKA (47, 48).
In endothelial cells, IGF-1R has been suggested to reduce the sensitivity of cells toward insulin and has also been indicated as a negative regulator of insulin-induced NO production (49, 50). In other cell systems, IGF-1R may activate the same pathways, i.e. PI3K, and may potentially amplify the signal (51, 52). To address the potential role of IGF-1R in mediating insulin-induced NO production in IMCD, we compared the transcript levels of IGF-1R in IMCD cells using RT-PCR. We found ~4-fold higher IGF-1R in IMCD cells relative to IR levels. This compares reasonably with what has been reported in an IMCD Transcriptome Database (53). The higher levels of IGF-1R did not appear to affect sensitivity of IMCD toward insulin, at least with regard to eNOS activation. That is, IGF-1R silencing did not significantly affect insulin-stimulated eNOS phosphorylation. This is in contrast to the microvascular endothelium, where silencing of IGF-1R resulted in further increase in insulin-induced eNOS activation (49). This could be attributed to a greater -fold difference in IGF-1R versus IR (20 times more than IR) in endothelium as compared with IMCD (54) because a greater predominance of IGFR-1R may result in the formation of more hybrid receptors along with the IR, which responds more to IGF-1 than to insulin (50). However, this may not be the case in IMCD, at least for NO production, because IGF-1 stimulation in IMCD cells (0.1–200 nM) was not found to increase or decrease nitrate + nitrite levels in the medium (data not shown). Thus, IGF-1R or IGF-1 ligand acting through hybrid or IGF-1R receptors may not be crucial for NO production in IMCD cells.

IMCD cells express all three known isoforms of NOS (15, 55); however, in this study, we have focused on the eNOS (NOS3) isoform. eNOS regulation has been suggested as a candidate link in the pathogenesis of hypertension in the insulin-resistant state (Shankar et al. (56)). Moreover, eNOS null mice were shown to have impaired insulin stimulation of blood flow and substrate delivery to skeletal muscle tissue, both NO-dependent processes. On the other hand, nNOS null mice had well preserved vascular function including arterial BP (57). Furthermore, even in the absence of nNOS, the insulin-stimulated blood flow was found to be preserved, suggesting that insulin-induced vascular tone is mediated by eNOS and not nNOS (57). However, an important role of inducible NOS as a BP modulator in a case of salt-sensitive hypertension has been suggested (58). Finally, most studies report that IR signaling is linked to the activation of NOS, especially the eNOS isoform (30). Here we report a potential role for Akt in collecting duct cells in mediating eNOS regulation by IR.

One of the reasons we set out to study the effect of insulin on IMCD-generated NO was the fact that we observed reduced urine NOx and elevated BP in renal epithelial cell-targeted (Ksp-cadherin promoter) IR KO mice (8). In the current study, we found significantly lower p-eNOSSer1177 in IMCD in kidney sections from IRKO mice as compared with WT littermates in the basal state (Fig. 8A). Moreover, chronic insulin infusion led to rise in blood pressure and an ~55% lower band density for eNOS protein in IRKO mice as compared with WT littermates (Fig. 8, B–D). In fact, WT mice infused with insulin, as compared with those maintained in the basal state, showed a marked increase in eNOS protein in inner medulla (Fig. 1, A

**FIGURE 6.** siRNA-mediated knockdown of IGF-1R in IMCD did not affect insulin-induced eNOS activation in IMCD cells. Cells were transfected with scrambled control siRNA or IGF-1R siRNA for 48 h followed by vehicle or insulin (100 nM) stimulation for 15 min. A, representative lanes are shown from immunoblots of cell lysate probed with the antibody against IGF-1R, p-eNOSSer1177, eNOS, and β-actin. B, summary of band densities for p-eNOSSer1177 normalized to eNOS. (n = 3/treatment). C, after 15 min of vehicle or insulin stimulation, medium from these cells were analyzed for increase in NO metabolite (nitrate + nitrite) levels over the medium from the same cells before stimulation. Treatment was plotted as the mean ± S.E. of three independent experiments; *, p < 0.05 as compared with vehicle. ns, not significant.

**FIGURE 7.** siRNA-mediated dual knockdown of IR and IGF-1R in IMCD reduced insulin-induced eNOS activation in IMCD cells. Cells were transfected with scrambled control siRNA or IR plus IGF-1R siRNA for 48 h followed by vehicle or insulin (100 nM) stimulation for 15 min. Representative lanes are shown from immunoblots of cell lysate probed with the antibody against IR-β, p-eNOSSer1177, followed by re-probing with IGF-1R, eNOS, and β-actin.
and B). Thus, the reduced capacity to respond to insulin at the level of the IMCD in the IRKO might very well have contributed to reduced urinary NOx and high BP in these mice. Moreover, normalized BP in insulin-infused WT mice, along with increased insulin-induced NO production in renal medulla (Fig. 1, C and D), further suggests that NO metabolism in renal inner medulla may have a role in normalizing BP in the insulin-sensitive state, despite putative sodium retention.

In summary, we provide direct evidence that in mouse IMCD cells, insulin augments NO production through increased eNOS expression and activity via PI3K-dependent pathway. This impairment in NO production in the setting of insulin resistance may further contribute to hypertension.

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