

Insulin's impact on renal sodium transport and blood pressure in health, obesity, and diabetes

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Tiwari S, Riaz S, Ecelbarger CA. Insulin's impact on renal sodium transport and blood pressure in health, obesity, and diabetes. *Am J Physiol Renal Physiol* 293: F974–F984, 2007. First published August 8, 2007; doi:10.1152/ajprenal.00149.2007.—Insulin has been shown to have antinatriuretic actions in humans and animal models. Moreover, endogenous hyperinsulinemia and insulin infusion have been correlated to increased blood pressure in some models. In this review, we present the current state of understanding with regard to the regulation of the major renal sodium transporters by insulin in the kidney. Several groups, using primarily cell culture, have demonstrated that insulin can directly increase activity of the epithelial sodium channel, the sodium-phosphate cotransporter, the sodium-hydrogen exchanger type III, and Na-K-ATPase. We and others have demonstrated alterations in the expression at the protein level of many of these same proteins with insulin infusion or in hyperinsulinemic models. We also discuss how this regulation is perturbed in type I and type II diabetes mellitus. Finally, we discuss a potential role for regulation of insulin receptor signaling in the kidney in contributing to sodium balance and blood pressure.

insulin resistance; ENaC; Zucker rats; streptozotocin

Overview

INSULIN HAS BEEN DEMONSTRATED to increase sodium reabsorption in the proximal tubule (9), the thick ascending limb (78, 102), and the distal tubule including the collecting duct (54, 144), using micropuncture and perfused tubule approaches. Over 10 years ago, Sechi (127), Butlen (30), and colleagues using I¹²⁵-labeled insulin demonstrated binding sites in multiple cell types along the renal tubular epithelium. However, because the kidney expresses other receptors similar to the classic insulin receptor (IR), including the insulin growth factor receptor (IGF) and the IR-related receptor (IRR), the precise nature of the “receptor” that labeled insulin was binding to was not absolutely certain. Nonetheless, more recent studies showed mRNA expression of the classic IR in whole homogenates of the kidney (31, 32, 130, 132), and our group (150) has recently found IR expression at the protein level using immunoblotting and immunohistochemistry in the proximal tubule, thick ascending limb, and collecting duct. In this review, we discuss studies that have investigated the regulation of the major renal sodium transporters in the kidney by insulin. In addition, we examine how this regulation is altered in insulin resistance, which is a hyperinsulinemic state, as well as in type I and type II diabetes. We also discuss what is known about the regulation of the IR in the kidney, how it is affected by insulin resistance and/or diabetes, and how this might ultimately affect sodium balance and blood pressure.

Localization of the IR in the Kidney

The IR in metabolic tissues such as liver, adipose, and muscle has been extensively studied; however, its role in the kidney is less well defined. Human IR cDNA was first cloned and reported in 1985 (43, 154). The characterization of the cDNA clones encoding IR indicated that the receptor is composed of two subunits, i.e., α and β , which are derived by the proteolytic processing of a 1,382-amino acid-long prepro-receptor. Subsequently, the exon-intron organization of the human IR gene and characterization of the 5'-flanking promoter sequence, which regulate its expression, was determined by Seino et al. (133). They have shown that the IR gene spans >120 kb and is encoded by 22 exons and 21 introns. Exon 11 is subjected to alternative splicing, leading to two isoforms, A and B, which differ in their affinity for insulin, with B being higher than the A isoform. Furthermore, they have also determined that there are three transcriptional initiation sites and an active promoter region that lacks the TATA-like sequence. IR is a member of the receptor tyrosine kinase superfamily. The α -subunit is primarily extracellular and contains the ligand-binding domain, whereas the β -subunit is intrinsic to the lipid bilayer and contains the tyrosine kinase signaling domain, which catalyzes the transfer of the γ -phosphate of ATP to tyrosine residues on protein substrates (37). In 2002, Hubbard et al. (72) reported the crystal structure of the tyrosine kinase domain of IR. The two subunits, α and β , are encoded by 11 exons each and are held together by disulfide bonds (72).

Several groups have characterized IR expression in the kidney (2, 17, 30, 127). Sechi and associates (127) utilized an autoradiographic technique with membrane preparations obtained from both glomeruli and tubules of the rat kidney. In the

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cortex, binding density was comparable in glomeruli and tubules. In the medulla, bound radioligand was found primarily in longitudinal structures traversing the outer portion, presumably corresponding to vascular bundles, and in the inner portion. Butlen et al. (30) examined ^{125}I -labeled insulin binding in microdissected rat renal tubules and found the greatest binding per length renal tubule in the proximal convoluted tubule and distal convoluted tubule (DCT), followed by the cortical and outer medullary collecting duct (CD) and cortical thick ascending limb (TAL) (Table 1). The thin limbs and outer medullary TAL were lower. Using autoradiography, Bisbis et al. (17) characterized the IR in chicken kidney. They demonstrated that renal IRs in the chicken are upregulated by fasting and downregulated by refeeding. We recently localized the IR in rat kidney using immunofluorescence and commercially available polyclonal antibodies against the α - and β -subunits (150). These antibodies were designed not to overlap with receptors of similar structure also expressed in the kidney including the insulin-like growth factor receptor (IGF1) and the IRR found in renal intercalated cells (8, 115). We found that classic IR was expressed in the proximal tubule, TAL, distal convoluted tubule, and CD.

Regulation of Sodium Transport Proteins Along the Renal Tubule by Insulin

Based on the expression pattern of IR, it is not surprising to find that insulin increases the sodium reabsorptive activity of major renal sodium transporter proteins in nearly every cell type that reabsorbs NaCl, from the proximal tubule throughout the collecting duct (19, 49–51, 54, 60, 96, 100, 117).

Proximal tubule. Two decades ago, Baum (9) demonstrated that insulin added to the bath increased volume flux in isolated perfused proximal tubules from rabbit kidney. This suggested that insulin directly stimulated sodium reabsorption in this segment. The regulation of a variety of major sodium transporters and exchangers in the proximal tubule by insulin has been examined. First of all, the sodium hydrogen exchanger type III (NHE3) is responsible for ~60–65% of the apical sodium reabsorption in the proximal tubule, which as a segment is responsible for reabsorbing ~65% of the total filtered sodium load (157). Thus regulation of NHE3 activity, if not compensated for downstream, has the potential to greatly affect overall sodium balance. Insulin has been shown to increase NHE3 activity both acutely and chronically in a cell line derived from opossum kidney with proximal tubule-like characteristics (OKP cells) (96). Recently, this same group reported

that the mechanism for chronic induction involved activation of phosphatidylinositol 3-kinase (PI-3K) and serum- and glucocorticoid-regulated kinase 1 (SGK1), as transport was inhibitable by wortmannin, a PI-3K antagonist, or coexpression of dominant-negative SGK1 (60).

Insulin has also been shown to be antiphosphaturic (35). The major tubule segment responsible for the regulation of phosphorus reabsorption in the kidney is the proximal tubule. The sodium-phosphate cotransporter type II (NaPi-2) is responsible for apical phosphorus reabsorption coupled to sodium in the proximal tubule (14). In 1984, Hammerman et al. (69) showed insulin-mediated increased phosphate uptake by isolated renal proximal tubule brush border from dog kidney. In later studies performed in OKP cells, the increased reabsorptive activity due to insulin was demonstrated to be influenced by calcium, as active transport was facilitated by the calcium channel blockers verapamil and nifedipine (1). Increased phosphate reabsorption by NaPi-2 would simultaneously increase sodium reabsorption in the proximal tubule given no changes in other apical transporter activity.

Feraile and associates (50–54) have performed many elegant studies demonstrating and characterizing the ability of insulin to activate the basolateral Na-K-ATPase (50–54). This pump is essential in establishing the electrochemical gradient for sodium reabsorption in all cells of the renal tubule that reabsorb NaCl. In proximal tubule suspensions from rats, as well as in microdissected tubules, activation appears to involve phosphorylation of a tyrosine residue on the α -subunit of Na-K-ATPase (52).

TAL. TAL is critical in determining water concentrating and diluting capacity of the kidney but may also affect sodium balance (157). Several groups (53, 78–80, 95, 102, 144, 148) have demonstrated increased sodium or chloride reabsorption in response to insulin in this segment. This transport was blockable by furosemide or ouabain, thus implying increased activity of the bumetanide-sensitive Na-K-2Cl cotransporter (NKCC2) and/or Na-K-ATPase (144). Mandon et al. (102) showed that insulin stimulated not only NaCl reabsorption in mouse cortical and medullary TAL, but calcium and magnesium, as well, in the cortical TAL. Insulin increased cAMP production at this site (102), but other studies indicate the sodium reabsorptive activity of insulin may be independent of cAMP (79). Studies by Ito and colleagues (78) suggest that insulin increases sodium reabsorption in the TAL by activation of tyrosine kinase, PI-3K, and protein kinase C.

DCT. The actions of insulin in the DCT are less clear as this segment is short and thus not readily perfusable in most species. Nonetheless, the DCT has been demonstrated to have a fairly high abundance of insulin binding sites (30, 108), suggesting that there is the clear potential for regulation of electrolyte transport. Dai et al. (34) used an immortalized mouse distal convoluted cell line to demonstrate that insulin can increase both magnesium reabsorption and cAMP in this cell type. Direct evidence that insulin increases sodium reabsorption in the DCT or activity of the major apical sodium transporter, i.e., the thiazide-sensitive Na-Cl cotransporter (NCC), is sparse. We have shown that chronic infusion of insulin to Sprague-Dawley rats increased the natriuretic response to an acute dose of hydrochlorothiazide, an NCC antagonist, suggesting that chronic hyperinsulinemia may up-regulate the activity of this protein (140). However, we saw no

Table 1. ^{125}I -labeled insulin binding in microdissected rat renal tubule segments and glomeruli

Glomeruli	2.5 ± 0.3
Proximal convoluted tubule	12.6 ± 0.6
Pars recta	4.0 ± 2.6
Thin descending limb	0.6 ± 0.2
Thin ascending limb	0.6 ± 0.2
Medullary thick ascending limb	0.8 ± 0.1
Cortical ascending limb	2.1 ± 0.1
Distal convoluted tubule	5.6 ± 1.1
Cortical collecting tubule	3.2 ± 0.3
Outer medullary collecting tubule	2.3 ± 0.1

Values are means ± SE expressed as 10^{-18} mol [^{125}I]-insulin bound per glomerulus or per mm tubule length.

change in protein expression for NCC. We (140) did find reduced protein levels of WNK4 [for “with no lysine” (K) kinase], a kinase that has been shown to decrease NCC activity, purportedly by reducing its levels in the plasma membrane (165, 167).

CD. Studies by DeFronzo et al. in humans (35) and dogs (36) suggested that the major site for sodium reabsorption by insulin was the distal tubule. Moreover, insulin has been shown to increase the activity of Na-K-ATPase (53, 54) and the epithelial sodium channel (ENaC) (19, 20, 49, 85, 117, 135, 136, 161, 163) in CD or distal tubule cell systems. Nonetheless, at least one study showed decreased sodium reabsorption and potassium secretion in perfused rabbit cortical CD in response to insulin (59). The mechanisms by which insulin increases activity of Na-K-ATPase in the CD vs. the proximal tubule are slightly different. In the cortical CD, insulin increases $V_{\max}$ (or maximal turnover activity) of Na-K-ATPase, whereas in the proximal tubule, insulin appears to affect the affinity of the pump for sodium without affecting $V_{\max}$ (54).

ENaC activation by insulin in cell culture has been fairly extensively characterized. The A6 cell line derived from toad kidney cells has been widely exploited to elucidate cellular mechanisms involved (48, 104, 118). Insulin’s ability to increase short-circuit current, a measure of NaCl flux across a membrane, in A6 cells is additive to that of aldosterone (119). However, most studies suggest a convergence between signaling cascades at the level of SGK1. Insulin increases phosphorylation of existing SGK1, and aldosterone increases SGK expression (49, 161); thus the actions of the hormones together, at least acutely, would be expected to be additive. Insulin treatment has been shown to increase the sodium channel density on the apical surface of these cells (48), which implies regulated trafficking. Blazer-Yost and associates (19, 118) were able to demonstrate that both increased sodium transport and trafficking of ENaC subunits in response to insulin in the A6 cells required PI-3K. We have recently shown, by immunobased approaches, increased apical localization of all three subunits of ENaC in native kidney tissue from rats treated for 28 days with insulin by an osmotic minipump (Fig. 1) (140), as well as in kidneys harvested from mice given a single intraperitoneal injection of insulin (149). Furthermore, insulin may acutely alter ENaC activity by mechanisms in addition to trafficking, perhaps by changing open probability or activity of existing channels. For example, Canessa and associates (136, 169) have demonstrated that insulin phosphorylates ENaC subunits in both transfected Madin-Darby canine kidney cells and endogenously ENaC-expressing A6 cells. In A6 cells, this phosphorylation occurred on all three subunits and was associated with increased activity (169). Figure 2 illustrates how insulin might increase sodium reabsorption in the CD principal cell by activating both ENaC and Na-K-ATPase.

Altered Expression of Sodium Transporter and Channels in Diabetes

An absence of circulating insulin or a substantial decrease in the activity of insulin at the cell level leads to impaired glucose uptake from plasma, hyperglycemia, and glucosuria, i.e., diabetes mellitus. Type I diabetes mellitus is due to the destruction of insulin-producing β cells in the pancreatic islets. It is primarily a T cell-mediated autoimmune disease directed

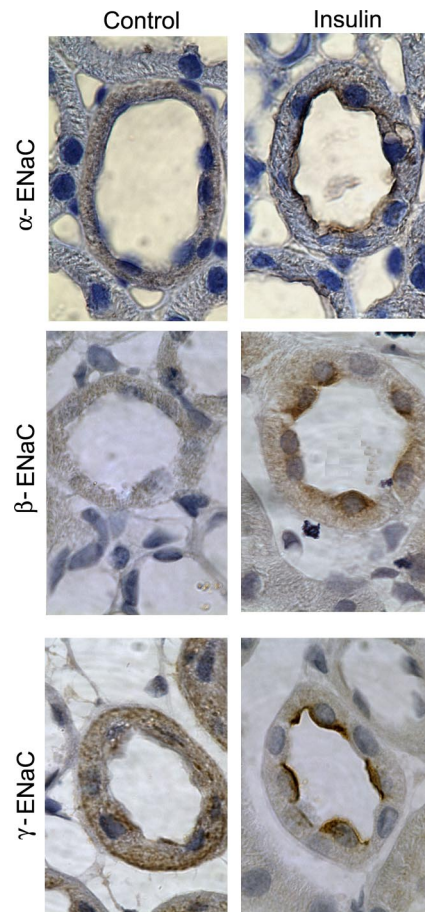


Fig. 1. Epithelial sodium channel (ENaC) subunit subcellular distribution in rat cortical collecting duct in response to chronic insulin infusion. Male Sprague-Dawley rats were infused with insulin by osmotic minipump ($20 \text{ U} \cdot \text{kg body wt}^{-1} \cdot \text{day}^{-1}$) or maintained as control rats for 28 days. Kidneys were perfusion fixed and prepared for immunoperoxidase-based staining as described (140). Sections were probed with our own polyclonal antibodies against α (top)-, β (middle)-, and γ -ENaC (bottom). Staining was localized more apically in the insulin-infused rats relative to control rats for all 3 subunits. Shown are cortical collecting ducts at $\times 1,000$ magnification. (Figure is a modification of staining published in Ref. 140.)

against a β cell autoantigen (101). In humans, this is treated with various cocktails of insulin that are designed to release slowly or quickly to try to mimic endogenous patterns. However, this regulation is clearly not perfect, and periods of hyper- and hypoglycemia develop. A major portion of the morbidity and mortality arising from diabetes mellitus is due to the high incidence of progressive kidney dysfunction and diabetic nephropathy.

Type I diabetes has been associated with natriuresis and diuresis, as well as activation of vasopressin and the renin-angiotensin-aldosterone system (7, 84). Several groups have examined the regulation of sodium transporters in type I diabetic rodents (47, 57, 91, 92, 97, 109, 124). Animal models which have been employed to study different aspects of diabetes and its complications, such as diabetic nephropathy and altered sodium and water balance, include treatment with alloxan (138) or streptozotocin (STZ), which directly destroy pancreatic β cells (44, 91–94, 103, 109, 142, 162). Siddiqui et al. (138) showed a significant increase in renal Na-K-ATPase activity in alloxan-induced diabetic rats compared

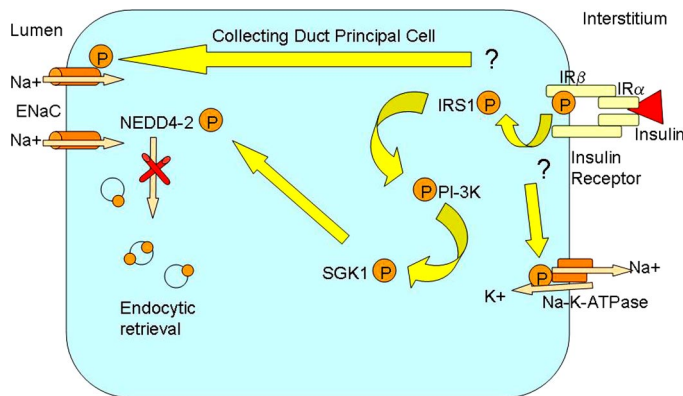


Fig. 2. Insulin receptor (IR) signaling in the renal collecting duct principal cell. Insulin binds to the basolateral IR, resulting in autophosphorylation of the β -subunit tyrosine residues. In most epithelial cells that reabsorb NaCl, autophosphorylation of the IR initiates a phosphorylation cascade including phosphorylation of insulin receptor substrate (IRS) and phosphatidylinositol 3-kinase (PI-3K). In collecting duct principal cells and possibly proximal tubule cells, PI-3K phosphorylates serum- and glucocorticoid-regulated kinase (SGK1). SGK1 phosphorylates NEDD4-2 in principal cells, which has been shown to reduce endocytic retrieval of ENaC from the apical membrane, increasing its activity. Furthermore, ENaC subunits and Na-K-ATPase may also be directly phosphorylated, although intermediary steps are not well understood, which has been postulated to increase the activity of these proteins while they are in the plasma membrane.

with control rats. In STZ-treated Sprague-Dawley rats, we found increased protein expression of the majority of primary apical sodium transport proteins of the TAL through the CD, including NKCC2, NCC, and the α -, β -, and γ -subunits of ENaC, relative to control rats (142). Kim and associates (91–93) similarly found a 244% increase in NKCC2 in the outer medulla of STZ-induced rats (Fig. 3). This compensatory upregulation might have a prominent role in reducing volume depletion and sodium losses.

Sex steroids may also influence the course of diabetes in type I models. For example, 17- β estradiol (E_2) and its analogs have been demonstrated to be protective against the progression of kidney disease and diabetic nephropathy (4, 65, 103). We (124, 164) conducted a study to examine the chronic (12-wk) effects of estrogen (E_2) replacement to ovariectomized female rats in the setting of STZ-induced diabetes on the expression of sodium transport proteins. E_2 replacement in STZ-diabetic rats altered the ratio of estrogen receptor subtypes in the kidney (164) and also attenuated the severity of the diabetes, as measured by a reduction in the hyperglycemia in these rats (124). With regard to changes in transport proteins, NKCC2 abundance was increased by diabetes in the whole kidney and outer medulla, as in our earlier short-term (4-day) study previously described (142). E_2 replacement normalized NKCC2 expression. Similarly, E_2 replacement normalized the expression of α - and β -ENaC, which were reduced in diabetic female rats. Decreased α - and β -ENaC in the chronically diabetic female rats contrasted with our earlier finding in male rats, which were diabetic for only 4 days (142). The reasons underlying these discrepancies are not clear. It is possible that the sex of the rats modulates these responses, or that the differing lengths of the two studies played a role. In other words, the ability to compensate with upregulation of all distal proteins may be compromised long term. Finally, in this chronic study in female rats, we also saw increased NHE3 and

decreased NaPi-2 protein levels in the STZ-treated rats (124). E_2 replacement reduced the abundance of NHE3 but did not restore the abundance of NaPi-2. Thus estrogen status is clearly modulatory with regard to the regulation of major sodium transport proteins in the STZ-induced diabetic rat. The cellular mechanisms underlying the up- or downregulation of specific transporters, and whether insulin or insulin signaling plays a role, are in need of further study.

In contrast to type I, in type II diabetes (non-insulin-dependent diabetes mellitus), circulating plasma insulin levels may be high, normal, or low depending on how advanced pancreatic β cell exhaustion has progressed. Type II diabetes is an expanding problem worldwide and is the manifestation of extreme “insulin resistance.” Insulin resistance refers to blunted IR signaling in the major metabolic tissues including liver, adipose, and skeletal muscle to a given level of circulating insulin, and is commonly associated with obesity. Approximately 40% of the patients with type II diabetes will develop diabetic kidney disease (99). Many animal models of type II diabetes have been used to examine metabolic abnormalities and diabetic complications including, the *ob/ob* mouse (leptin deficient), the *db/db* mouse (leptin receptor deficient) (6), the nonobese Goto-Kakizaki rat (153, 168), the obesity-prone Sprague-Dawley rat (38–40), the diabetic Zucker rat (70), and the obese Zucker rat (77). The diabetic Zucker rat is a substrain of the obese Zucker rat (leptin receptor mutation) and develops diabetes at a fairly young age (156). The obese Zucker rat is highly insulin resistant, hyperinsulinemic, and obese but does not develop type II diabetes until 5–6 mo of age (86).

The obese Zucker rat has been used extensively to examine the regulation of sodium and water balance in insulin resistance and/or type II diabetes (15, 16, 58, 67, 74, 75, 89, 116, 120–123, 134, 152). In young (2- to 4-mo-old), insulin-resistant, nondiabetic male rats, we (16) found increased whole kidney abundances of the α_1 -subunit of Na-K-ATPase, NCC, and β -ENaC. When we examined slightly older (6-mo-old), overtly diabetic obese Zucker rats, the renal sodium transporter profile was remarkably altered (15). In these rats, we found a marked decrease in several proximal tubule, TAL, and CD apical sodium transporters or channel subunits including NKCC2, α -ENaC, NHE3, and NaPi-2 relative to lean age mates. Thus these proteins may be sensitive to factors relating to diabetes and/or nephropathy but not strictly to insulin resistance.

In agreement with our findings in young Zucker rats, Lokhandwala et al. (74) demonstrated increased activity of Na-K-ATPase and also NHE3 in proximal tubules of obese Zucker rats. They attributed this relatively increased activity to impaired dopamine D1 receptor function. Dopamine activity via the D1 receptor normally should inhibit these proteins. Defective natriuresis due to impaired dopamine function and elevated proximal tubule Na-K-ATPase activity has also been



Fig. 3. Bumetanide-sensitive Na-K-2Cl cotransporter (NKCC2/BSC1) protein in outer medulla of control (CTR) vs. 20-day streptozotocin (STZ)-induced diabetic rats. Samples from 6 different control rats and 6 different diabetic rats probed with an antibody to NKCC2/BSC1 are shown. There was a 244% increase in NKCC2/BSC1 abundance in rats with diabetes mellitus (DM) (91).

demonstrated in another model of salt-sensitive hypertension associated with obesity, i.e., the Wistar fatty rat (152).

Several investigators (15–20), have demonstrated the remarkable effectiveness of “insulin-sensitizing” drugs, i.e., peroxisome proliferator-activated receptor subtype- γ (PPAR- γ) agonists such as rosiglitazone (RGZ) in the treatment and attenuation of insulin resistance and type II diabetes in the obese Zucker rat. We (89, 122) found that chronic (12-wk) treatment of the obese Zucker rat with RGZ not only reduced the diabetes and proteinuria but normalized the expression of several renal sodium transport proteins. For example, RGZ abrogated the downregulation of NKCC2 and α -ENaC in the aging Zucker rats. Similarly, Umrani and associates (155) showed that RGZ treatment of obese Zucker rats restored dopamine signaling in the proximal tubule and attenuated the increased Na-K-ATPase activity. Thus treatment of insulin resistance clearly protects renal sodium reabsorptive and urine concentrating capacity in these rats.

High-fructose (5, 31, 46, 76, 137, 145, 160) or -fat (81, 90) diets fed to rats have been used as additional models of insulin resistance. We (141) have shown that 28-day dietary fructose (65%) resulted in an upregulation of NaPi-2 abundance but decreased NKCC2, and no change in ENaC subunits, NCC, NHE3, or α_1 -Na-K-ATPase in Sprague-Dawley rats.

The Dahl salt-sensitive rat is also insulin resistant (151), as are ANG II-infused rats (111), and high-NaCl-fed rats (112). The cellular mechanisms underlying insulin sensitivity in these models are not well understood but have been proposed to involve later components of IR signaling, i.e., post-PI-3K activation, perhaps involving increased oxidative stress (113). No studies, to our knowledge, have examined IR signaling in the kidney in these models.

Insulin and Blood Pressure

Hyperinsulinemia, as a result of insulin resistance, is clearly associated with hypertension in humans and animal models (23, 42, 55, 83). However, whether increased insulin signaling in any tissue is the cause of increased blood pressure is uncertain. Moreover, the effects of insulin infusion on blood pressure and hemodynamics in general, in mammals, are variable. Brands et al. (26) demonstrated over 15 years ago that insulin infusion into rats increases blood pressure. The rise in blood pressure was not associated with any sustained increase in sodium balance but did correspond to a transient fall in glomerular filtration rate (GFR). They went on to pursue the mechanism for these effects and subsequently reported that hyperinsulinemia, in the rat, was not salt sensitive but led to a rightward shift in the pressure-natriuretic curve as sodium balance was maintained at a higher blood pressure (25). They (87) found no evidence for increased adrenergic activity as blockade of either α - or β -adrenergic receptors with selective antagonists did not abolish the blood pressure rise. On the contrary, thromboxane and an intact renin-angiotensin system (RAS) were found to be important in mediating the rise in blood pressure in the rat. A thromboxane synthesis inhibitor, U63557A (87), or coinfusion with an angiotensin-converting enzyme inhibitor (24) significantly attenuated the rise in blood pressure and fall in GFR.

Other investigators (45, 106) including ourselves (140) have confirmed the rise in blood pressure in rats with insulin

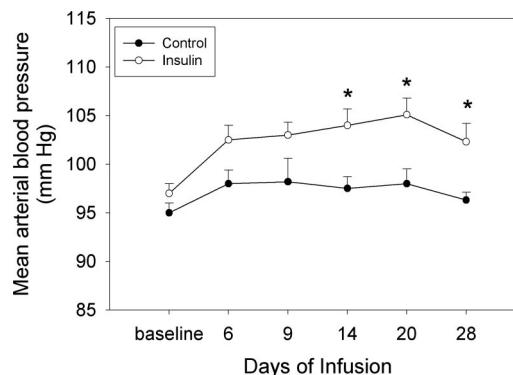


Fig. 4. Mean arterial blood pressure (MAP) measured by radiotelemetry in male Sprague-Dawley rats infused with insulin by osmotic minipump ($20 \text{ U} \cdot \text{kg body wt}^{-1} \cdot \text{day}^{-1}$) for 28 days vs. control (untreated same age, rats). *Insulin infusion resulted in significantly ($P < 0.05$, by unpaired t -test) higher MAP after 14 days of infusion. (Figure is a modification of a figure published in Ref. 140.)

infusion (Fig. 4). We (140) found the rise in blood pressure using radiotelemetry was associated with increased natriuretic response to benzamil and hydrochlorothiazide and increased apical localization of ENaC subunits in rat kidney, as described above. Edwards et al. (45) found increased blood pressure in rats infused with insulin was blockable by chemical denervation. Meehan et al. (106) demonstrated that the rise in blood pressure and heart rate in rats infused with insulin was preventable by coinfusion with the sympatholytic agent clonidine.

On the contrary, insulin infusion into mixed-breed dogs using a similar approach does not appear to increase blood pressure, even when renal mass is reduced (27, 68). However, in the dogs insulin did result in peripheral vasodilatation, leading to reduced peripheral vascular resistance as well as sodium retention (27). Few studies have reported the effects of insulin infusion into mice. Huang et al. (71) reported that acute insulin infusion into mice on a mixed Sv129/C57BL background did not raise blood pressure or alter GFR, but it did produce antinatriuresis. Insulin infusion into human subjects, normal or with borderline hypertension, has been reported to raise (88, 125), decrease (3), or not change mean arterial blood pressure (11, 63, 110). It is likely that species/strain or underlying conditional differences in the blood pressure response to insulin infusion reflect the fact that insulin signaling is associated with both factors that may reduce blood pressure, e.g., nitric oxide generation, and factors that may increase blood pressure, e.g., increased sodium reabsorption, activation of the sympathetic nervous system, augmentation of the RAS, and/or thromboxane synthesis. Ultimately, insulin may be viewed as more of a facilitator rather than a cause of increased blood pressure and clearly dependent on other factors. This facilitation, however, may be particularly important in disease states such as insulin resistance, type II diabetes, and obesity.

Insulin Resistance and Blood Pressure

It is fairly clear that insulin resistance in humans and animals models is associated with hypertension. Insulin resistance, however, is clearly a complex disorder, and the role of hyperinsulinemia per se on blood pressure control in the context of other metabolic disturbances is uncertain. One problem is that it is difficult to alleviate hyperinsulinemia without affecting

other metabolic parameters. We (89) showed that treatment of young obese Zucker rats with the PPAR- γ agonist RGZ normalized blood pressure for nearly the entire 12-wk study. Treatment with RGZ also markedly reduced circulating insulin levels. However, RGZ also reduced plasma triglycerides and urine albumin excretion. Thus we clearly cannot ascribe the normalization in blood pressure solely to the fall in circulating insulin levels in this complex disease model. However, it did seem to relate overall to the amelioration of the insulin-resistant state in these rats. These findings were in agreement with those of Umrani et al. (155), who found RGZ treatment of the obese Zucker rat resulted in increased sodium excretion and reduced blood pressure.

Furthermore, in the obese Zucker rat, Hussain and colleagues (10, 134) found upregulation of both major classes of ANG II receptors, i.e., AT₁ and AT₂, in the proximal tubule. ANG II is not only a potent vasoconstrictor, but it also increases sodium reabsorption in the renal tubule primarily via increased activity at the AT₁ receptor (126). AT₂, on the other hand, is coupled to natriuresis and vasodilation. Increased expression levels of both receptors may increase the sensitivity of obese rats to ANG II, but the opposing downstream actions of these receptors may be the reason for only modestly elevated blood pressure. Hussain (73) in his review suggests that increased circulating insulin levels may be responsible for upregulation of both AT₁ and AT₂ receptors.

The fructose-fed rat has also been demonstrated to develop hypertension (13, 61, 62, 139, 160). McNeil and colleagues (13, 62, 139, 158, 160) have used this model extensively to examine mechanisms underlying hypertension associated with insulin resistance. Vanadyl sulfate supplementation of the diet was found to reduce insulin resistance and increased blood pressure in these rats (13). Furthermore, a role for increased activity of the sympathetic nervous system (160) and thromboxane (61) in the blood pressure response was demonstrated. Finally, sex hormones appear to influence the blood pressure response to fructose, as female rats were not found to have an increase in blood pressure with these diets (62, 139, 158).

Nonetheless, not all researchers have found that a diet high in fructose raises blood pressure in normal rats (12, 22, 33, 141). Brands et al. (22) saw no effect of fructose on blood pressure in Sprague-Dawley rats after 11 days. We also saw little effect of fructose to raise blood pressure in Sprague-Dawley rats even when coupled with high NaCl or high fat, which did tend to marginally raise blood pressure (141). In our study, a high-fructose diet did increase kidney size and raise plasma triglycerides (141). One difference between the latter studies and many of the McNeill studies is that tail-cuff measurements were made in the former and direct arteriography or radiotelemetry was used in the latter cases. The act of performing tail-cuff plethysmography even in trained rats is likely stimulating and may raise blood pressure in susceptible animals. We might speculate that fructose feeding makes these animals more sensitive with regard to the blood pressure response to small stressors such as routine tail cuff measurements, which might increase sympathetic activity.

Therefore, in the case of insulin resistance with high circulating plasma insulin levels, often 10 times higher than normal levels (16), it is not clear whether it is increased IR signaling, decreased IR signaling, or neither one which increases blood pressure. Furthermore, while insulin resistance is clearly asso-

ciated with impaired IR signaling in major metabolic tissues, signaling in the kidney, the organ perhaps most critical in blood pressure regulation, has not been well defined.

Renal IR: Altered Regulation in Diabetes, Role in Blood Pressure Control

The etiology of insulin resistance has not been definitively elucidated, but most studies agree that reduced IR signaling relative to circulating insulin levels is decidedly a key determinant (56). Downregulation of the level of IR protein itself, with insulin resistance, has been demonstrated in various insulin-target tissues central to energy metabolism, e.g., liver, skeletal muscle, and adipose tissue (21, 64, 159), but not specifically in the kidney. We have recently found reduced IR protein in the kidney of obese Zucker rats and rats fed a high-fat diet for several weeks, relative to respective controls (147, 150). The reduction occurred in both subunits, and the intensity tended to be correlated with the severity of the insulin resistance. The inner medulla appeared to be the most sensitive to insulin resistance-associated downregulation. Immunoperoxidase labeling of IR- β revealed that the downregulation in the inner medulla was associated with CD cells. We suggest that this downregulation could impact the regulation of CD proteins such as ENaC, Na-K-ATPase, and possibly even aquaporin-2. Downregulation of CD IR might also affect nitric oxide (NO) generation in this cell type; insulin has been shown to increase NO in many tissues (143). CD nitric oxide synthase

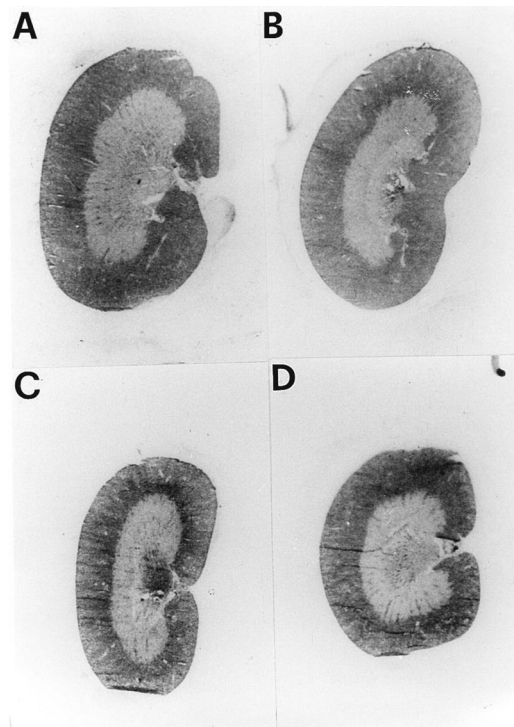


Fig. 5. Distribution of ¹²⁵I-insulin binding sites in longitudinal sections of kidney obtained from Wistar-Kyoto rats fed a low-salt (A) or high-salt (B) diet and spontaneously hypertensive rats fed low-salt (C) or high-salt (D) diets. In all rats, radioligand binding was more abundant in renal cortex than medulla. In the cortex, binding intensity was comparable in glomeruli and tubules. In the medulla, bound radioligand was found primarily in longitudinal structures traversing the outer medulla, presumably corresponding to medullary vascular bundles (128). (Used by permission of Lippincott Williams & Wilkins.)

(NOS) activity has been shown to be among the highest relative to other renal segments (166). Furthermore, all three isoforms, i.e., neuronal, endothelial, and inducible NOS, have been localized to the CD, at least at the mRNA level (166). However, decreased expression of the IR does not necessarily equate to reduced IR binding and signal transmission.

In contrast, Sechi et al. (127, 130) and Catena and colleagues (31, 32) determined that while IR binding and mRNA in the liver and skeletal muscle was decreased in fructose-fed, insulin-resistant rats, there was no difference in the kidney, compared with control rats. Similarly, corticosterone-induced insulin resistance in the chicken did not change renal IR number, affinity, or tyrosine kinase activity (18). Therefore, they suggested that the relative absence of renal insulin resistance would allow for the greater circulating levels of insulin to bind to renal IR and increase sodium reabsorption, for instance, and thus blood pressure. Furthermore, based on mRNA expression and insulin binding studies, Sechi's group (129, 132) also reported increased renal IR in STZ-induced diabetic rats, and, in this context, IR binding remained unaffected by ANG II.

Moreover, Sechi and colleagues (31, 128, 130, 131) demonstrated that both the density and level of mRNA encoding IRs in the kidney are inversely related to the dietary sodium content by using in situ autoradiography and mRNA analysis (Fig. 5). Based on these findings, they suggest that this reduced IR is a feedback mechanism that limits the insulin-induced sodium retention when extracellular fluid volume is expanded. Furthermore they have also demonstrated that this feedback mechanism is lost in fructose-fed Sprague-Dawley rats (32) and also in spontaneously hypertensive rats (SHR) (128). This abnormality might be implicated in the pathophysiology of insulin resistance and hypertension in these models. However, this inverse relationship between salt intake and renal IR could not be found in the mildly insulin-resistant Dahl salt-sensitive rat (131).

Nonetheless, renal IR signaling per se has not been examined in the kidney to determine whether IR binding and expression relate to signal intensity. In insulin resistance, reduced IR signal transmission has been postulated to involved post-receptor-related events, such as increased serine phosphorylation of the IR substrate (41). Additional studies are needed to determine whether renal cellular signaling is similarly impaired.

Ultimately, the study of the role of insulin in the kidney has been hampered by the fact that insulin, as a hormone, is primarily a regulator of energy homeostasis in liver, adipose tissue, and muscle. Its putative roles in the kidney to affect reabsorption of electrolytes and minerals, as well as in NO generation, and blood pressure control are considered secondary actions. IR antagonists cannot be used in whole-animal experiments because of their obvious major impact on glucose metabolism. Furthermore, whole-body knockout of the IR results in early postnatal death due to diabetic ketoacidosis (82, 114). Kahn and colleagues (98, 105) have used the Cre-loxP-mediated recombination strategy to selectively knock out the IR from a variety of tissues including the liver (107), muscle (29), brown adipose tissue (66), and brain (28). Currently, we are developing several renal cell-selective knockouts of the IR, and phenotyping is ongoing (146, 148). The generation of these renal epithelial cell-specific knockouts of IR will allow us to

further elucidate the role of the IR in the kidney and more broadly to derive insight into the mechanism(s) underlying insulin resistance-associated hypertension.

Conclusions

Overall, insulin actions on renal epithelial cells are ubiquitous and likely play a more important role in the physiological control of nutrient reabsorption and blood pressure than currently appreciated. Insulin levels vary throughout the day with meals and may facilitate efficient reabsorption of filtered nutrients at the renal level. Pathologically high plasma insulin levels are a fact of life for millions of insulin-resistant individuals worldwide. Other persons, with type I or type II diabetes, have fluctuating insulin levels due to less than ideal insulin replacement or other treatment strategies. Normal regulation of renal salt, water, and other nutrient reabsorption might be expected to be altered in these individuals. However, the way in which the IR signals in this context and the resulting impact on physiology such as blood pressure control have not been sufficiently elucidated. Further studies are warranted to evaluate the regulation of insulin signaling in the kidney in health, obesity, and diabetes.

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DISCLOSURES

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REFERENCES

1. Abraham MI, McAteer JA, Kempson SA. Insulin stimulates phosphate transport in opossum kidney epithelial cells. *Am J Physiol Renal Physiol* 258: F1592–F1598, 1990.
2. Ammar A, Schmidt A, Semmekrot B, Roseau S, Butlen D. Receptors for neurohypophyseal hormones along the rat nephron: ¹²⁵I-labelled d(CH₂)₅[Tyr(Me)₂, Thr₄, Orn₈, Tyr-NH(2)₉] vasotocin binding in microdissected tubules. *Pflügers Arch* 418: 220–227, 1991.
3. Anderson EA, Balon TW, Hoffman RP, Sinkey CA, Mark AL. Insulin increases sympathetic activity but not blood pressure in borderline hypertensive humans. *Hypertension* 19: 621–627, 1992.
4. Antus B, Liu S, Yao Y, Zou H, Song E, Lutz J, Heemann U. Effects of progesterone and selective oestrogen receptor modulators on chronic allograft nephropathy in rats. *Nephrol Dial Transplant* 20: 329–335, 2005.
5. Anuradha CV, Balakrishnan SD. Taurine attenuates hypertension and improves insulin sensitivity in the fructose-fed rat, an animal model of insulin resistance. *Can J Physiol Pharmacol* 77: 749–754, 1999.
6. Arakawa K, Ishihara T, Oku A, Nawano M, Ueta K, Kitamura K, Matsumoto M, Saito A. Improved diabetic syndrome in C57BL/KsJ-db/db mice by oral administration of the Na⁺-glucose cotransporter inhibitor T-1095. *Br J Pharmacol* 132: 578–586, 2001.
7. Bankir L, Bardoux P, Ahloulay M. Vasopressin and diabetes mellitus. *Nephron* 87: 8–18, 2001.
8. Bates CM, Merenmies JM, Kelly-Spratt KS, Parada LF. Insulin receptor-related receptor expression in non-A intercalated cells in the kidney. *Kidney Int* 52: 674–681, 1997.
9. Baum M. Insulin stimulates volume absorption in the rabbit proximal convoluted tubule. *J Clin Invest* 79: 1104–1109, 1987.
10. Becker M, Umrani D, Lokhandwala MF, Hussain T. Increased renal angiotensin II AT₁ receptor function in obese Zucker rat. *Clin Exp Hypertens* 25: 35–47, 2003.

11. Berne C, Fagius J, Pollare T, Hjemdahl P. The sympathetic response to euglycaemic hyperinsulinaemia. Evidence from microelectrode nerve recordings in healthy subjects. *Diabetologia* 35: 873–879, 1992.
12. Bezerra RM, Ueno M, Silva MS, Tavares DQ, Carvalho CR, Saad MJ, Gontijo JA. A high-fructose diet induces insulin resistance but not blood pressure changes in normotensive rats. *Braz J Med Biol Res* 34: 1155–1160, 2001.
13. Bhanot S, McNeill JH, Bryer-Ash M. Vanadyl sulfate prevents fructose-induced hyperinsulinemia and hypertension in rats. *Hypertension* 23: 308–312, 1994.
14. Biber J, Murer H, Forster I. The renal type II Na⁺/phosphate cotransporter. *J Bioenerg Biomembr* 30: 187–194, 1998.
15. Bickel CA, Knepper MA, Verbalis JG, Ecelbarger CA. Dysregulation of renal salt and water transport proteins in diabetic Zucker rats. *Kidney Int* 61: 2099–2110, 2002.
16. Bickel CA, Verbalis JG, Knepper MA, Ecelbarger CA. Increased renal Na-K-ATPase, NCC, and β -ENaC abundance in obese Zucker rats. *Am J Physiol Renal Physiol* 281: F639–F648, 2001.
17. Bisbis S, Derouet M, Simon J. Characterization of insulin receptors in chicken kidneys: effect of nutritional status. *Gen Comp Endocrinol* 96: 37–49, 1994.
18. Bisbis S, Taouis M, Derouet M, Chevalier B, Simon J. Corticosterone-induced insulin resistance is not associated with alterations of insulin receptor number and kinase activity in chicken kidney. *Gen Comp Endocrinol* 96: 370–377, 1994.
19. Blazer-Yost BL, Esterman MA, Vlahos CJ. Insulin-stimulated trafficking of ENaC in renal cells requires PI 3-kinase activity. *Am J Physiol Cell Physiol* 284: C1645–C1653, 2003.
20. Blazer-Yost BL, Liu X, Helman SI. Hormonal regulation of ENaCs: insulin and aldosterone. *Am J Physiol Cell Physiol* 274: C1373–C1379, 1998.
21. Boden G, Chen X, Ruiz J, Heifets M, Morris M, Badosa F. Insulin receptor down-regulation and impaired antilipolytic action of insulin in diabetic patients after pancreas/kidney transplantation. *J Clin Endocrinol Metab* 78: 657–663, 1994.
22. Brands MW, Garrity CA, Holman MG, Keen HL, Alonso-Galicia M, Hall JE. High-fructose diet does not raise 24-hour mean arterial pressure in rats. *Am J Hypertens* 7: 104–109, 1994.
23. Brands MW, Hall JE, Van Vliet BN, Alonso-Galicia M, Herrera GA, Zappe D. Obesity and hypertension: roles of hyperinsulinemia, sympathetic nervous system and intrarenal mechanisms. *J Nutr* 125: 1725S–1731S, 1995.
24. Brands MW, Harrison DL, Keen HL, Gardner A, Shek EW, Hall JE. Insulin-induced hypertension in rats depends on an intact renin-angiotensin system. *Hypertension* 29: 1014–1019, 1997.
25. Brands MW, Hildebrandt DA, Mizelle HL, Hall JE. Hypertension during chronic hyperinsulinemia in rats is not salt-sensitive. *Hypertension* 19: 183–89, 1992.
26. Brands MW, Hildebrandt DA, Mizelle HL, Hall JE. Sustained hyperinsulinemia increases arterial pressure in conscious rats. *Am J Physiol Regul Integr Comp Physiol* 260: R764–R768, 1991.
27. Brands MW, Mizelle HL, Gaillard CA, Hildebrandt DA, Hall JE. The hemodynamic response to chronic hyperinsulinemia in conscious dogs. *Am J Hypertens* 4: 164–168, 1991.
28. Bruning JC, Gautam D, Burks DJ, Gillette J, Schubert M, Orban PC, Klein R, Krone W, Muller-Wieland D, Kahn CR. Role of brain insulin receptor in control of body weight and reproduction. *Science* 289: 2122–2125, 2000.
29. Bruning JC, Michael MD, Winnay JN, Hayashi T, Horsch D, Accili D, Goodyear LJ, Kahn CR. A muscle-specific insulin receptor knockout exhibits features of the metabolic syndrome of NIDDM without altering glucose tolerance. *Mol Cell* 2: 559–569, 1998.
30. Butlen D, Vadrot S, Roseau S, Morel F. Insulin receptors along the rat nephron: [¹²⁵I] insulin binding in microdissected glomeruli and tubules. *Pflügers Arch* 412: 604–612, 1988.
31. Catena C, Cavarape A, Novello M, Giacchetti G, Sechi LA. Insulin receptors and renal sodium handling in hypertensive fructose-fed rats. *Kidney Int* 64: 2163–2171, 2003.
32. Catena C, Giacchetti G, Novello M, Colussi G, Cavarape A, Sechi LA. Cellular mechanisms of insulin resistance in rats with fructose-induced hypertension. *Am J Hypertens* 16: 973–978, 2003.
33. D'Angelo G, Elmarakby AA, Pollock DM, Stepp DW. Fructose feeding increases insulin resistance but not blood pressure in Sprague-Dawley rats. *Hypertension* 46: 806–811, 2005.
34. Dai LJ, Ritchie G, Bapty BW, Kerstan D, Quamme GA. Insulin stimulates Mg²⁺ uptake in mouse distal convoluted tubule cells. *Am J Physiol Renal Physiol* 277: F907–F913, 1999.
35. DeFronzo RA, Cooke CR, Andres R, Faloona GR, Davis PJ. The effect of insulin on renal handling of sodium, potassium, calcium, and phosphate in man. *J Clin Invest* 55: 845–855, 1975.
36. DeFronzo RA, Goldberg M, Agus ZS. The effects of glucose and insulin on renal electrolyte transport. *J Clin Invest* 58: 83–90, 1976.
37. De Meyts P. Insulin and its receptor: structure, function and evolution. *Bioessays* 26: 1351–1362, 2004.
38. Dobrian AD, Davies MJ, Schriver SD, Lauterio TJ, Prewitt RL. Oxidative stress in a rat model of obesity-induced hypertension. *Hypertension* 37: 554–560, 2001.
39. Dobrian AD, Schriver SD, Khraibi AA, Prewitt RL. Pioglitazone prevents hypertension and reduces oxidative stress in diet-induced obesity. *Hypertension* 43: 48–56, 2004.
40. Dobrian AD, Schriver SD, Lynch T, Prewitt RL. Effect of salt on hypertension and oxidative stress in a rat model of diet-induced obesity. *Am J Physiol Renal Physiol* 285: F619–F628, 2003.
41. Draznin B. Molecular mechanisms of insulin resistance: serine phosphorylation of insulin receptor substrate-1 and increased expression of p85 α : the two sides of a coin. *Diabetes* 55: 2392–2397, 2006.
42. Dustan HP. Mechanisms of hypertension associated with obesity. *Ann Intern Med* 98: 860–864, 1983.
43. Ebina Y, Ellis L, Jarnagin K, Edery M, Graf L, Clauser E, Ou JH, Masiar F, Kan YW, Goldfine ID. The human insulin receptor cDNA: the structural basis for hormone-activated transmembrane signalling. *Cell* 40: 747–758, 1985.
44. Ecelbarger CA, Kim GH, Wade JB, Knepper MA. Regulation of the abundance of renal sodium transporters and channels by vasopressin. *Exp Neurol* 171: 227–234, 2001.
45. Edwards JG, Tipton CM. Influences of exogenous insulin on arterial blood pressure measurements of the rat. *J Appl Physiol* 67: 2335–2342, 1989.
46. Elkayam A, Mirelman D, Peleg E, Wilchek M, Miron T, Rabinkov A, Oron-Herman M, Rosenthal T. The effects of allicin on weight in fructose-induced hyperinsulinemic, hyperlipidemic, hypertensive rats. *Am J Hypertens* 16: 1053–1056, 2003.
47. el-Seifi S, Freiberg JM, Kinsella J, Cheng L, Sacktor B. Na⁺-H⁺ exchange and Na⁺-dependent transport systems in streptozotocin diabetic rat kidneys. *Am J Physiol Regul Integr Comp Physiol* 252: R40–R47, 1987.
48. Erlij D, De Smet P, Van Driessche W. Effect of insulin on area and Na⁺ channel density of apical membrane of cultured toad kidney cells. *J Physiol* 481: 533–542, 1994.
49. Faletti CJ, Perrotti N, Taylor SI, Blazer-Yost BL. sgk: an essential convergence point for peptide and steroid hormone regulation of ENaC-mediated Na⁺ transport. *Am J Physiol Cell Physiol* 282: C494–C500, 2002.
50. Feraille E, Carranza ML, Gonin S, Beguin P, Pedemonte C, Roussetot M, Caverzasio J, Geering K, Martin PY, Favre H. Insulin-induced stimulation of Na⁺-K⁺-ATPase activity in kidney proximal tubule cells depends on phosphorylation of the α -subunit at Tyr-10. *Mol Biol Cell* 10: 2847–2859, 1999.
51. Feraille E, Carranza ML, Roussetot M, Favre H. Insulin enhances sodium sensitivity of Na-K-ATPase in isolated rat proximal convoluted tubule. *Am J Physiol Renal Fluid Electrolyte Physiol* 267: F55–F62, 1994.
52. Feraille E, Carranza ML, Roussetot M, Favre H. Modulation of Na⁺-K⁺-ATPase activity by a tyrosine phosphorylation process in rat proximal convoluted tubule. *J Physiol* 498: 99–108, 1997.
53. Feraille E, Marsy S, Cheval L, Barlet-Bas C, Khadouri C, Favre H, Doucet A. Sites of antinatriuretic action of insulin along rat nephron. *Am J Physiol Renal Fluid Electrolyte Physiol* 263: F175–F179, 1992.
54. Feraille E, Roussetot M, Rajerison R, Favre H. Effect of insulin on Na⁺-K⁺-ATPase in rat collecting duct. *J Physiol* 488: 171–180, 1995.
55. Ferrannini E. Insulin and blood pressure: possible role of hemodynamics. *Clin Exp Hypertens* 14: 271–284, 1992.
56. Flier JS. Insulin receptors and insulin resistance. *Annu Rev Med* 34: 145–160, 1983.
57. Fujisawa G, Okada K, Muto S, Fujita N, Itabashi N, Kusano E, Ishibashi S. Spironolactone prevents early renal injury in streptozotocin-induced diabetic rats. *Kidney Int* 66: 1493–1502, 2004.

58. Fujiwara K, Hayashi K, Matsuda H, Kubota E, Honda M, Ozawa Y, Saruta T. Altered pressure-natriuresis in obese Zucker rats. *Hypertension* 33: 1470–1475, 1999.
59. Furuya H, Tabei K, Muto S, Asano Y. Effect of insulin on potassium secretion in rabbit cortical collecting ducts. *Am J Physiol Renal Fluid Electrolyte Physiol* 262: F30–F35, 1992.
60. Fuster DG, Bobulescu IA, Zhang J, Wade J, Moe OW. Characterization of the regulation of renal Na^+/H^+ exchanger NHE3 by insulin. *Am J Physiol Renal Physiol* 292: F577–F585, 2007.
61. Galipeau D, Arikawa E, Sekirov I, McNeill JH. Chronic thromboxane synthase inhibition prevents fructose-induced hypertension. *Hypertension* 38: 872–876, 2001.
62. Galipeau D, Verma S, McNeill JH. Female rats are protected against fructose-induced changes in metabolism and blood pressure. *Am J Physiol Heart Circ Physiol* 283: H2478–H2484, 2002.
63. Gans RO, vd Toorn L, Bilo HJ, Nauta JJ, Heine RJ, Donker AJ. Renal and cardiovascular effects of exogenous insulin in healthy volunteers. *Clin Sci (Lond)* 80: 219–225, 1991.
64. Garvey WT, Olefsky JM, Marshall S. Insulin induces progressive insulin resistance in cultured rat adipocytes. Sequential effects at receptor and multiple postreceptor sites. *Diabetes* 35: 258–267, 1986.
65. Gross ML, Adamczak M, Rabe T, Harbi NA, Krtil J, Koch A, Hamar P, Amann K, Ritz E. Beneficial effects of estrogens on indices of renal damage in uninephrectomized SHRsp rats. *J Am Soc Nephrol* 15: 348–358, 2004.
66. Guerra C, Navarro P, Valverde AM, Arribas M, Bruning J, Kozak LP, Kahn CR, Benito M. Brown adipose tissue-specific insulin receptor knockout shows diabetic phenotype without insulin resistance. *J Clin Invest* 108: 1205–1213, 2001.
67. Hakam AC, Hussain T. Renal angiotensin II type-2 receptors are upregulated and mediate the candesartan-induced natriuresis/diuresis in obese Zucker rats. *Hypertension* 45: 270–275, 2005.
68. Hall JE, Coleman TG, Mizelle HL. Does chronic hyperinsulinemia cause hypertension? *Am J Hypertens* 2: 171–173, 1989.
69. Hammerman MR, Rogers S, Hansen VA, Gavin JR, 3rd. Insulin stimulates P_i transport in brush border vesicles from proximal tubular segments. *Am J Physiol Endocrinol Metab* 247: E616–E624, 1984.
70. Harker CT, O'Donnell MP, Kasiske BL, Keane WF, Katz SA. The renin-angiotensin system in the type II diabetic obese Zucker rat. *J Am Soc Nephrol* 4: 1354–1361, 1993.
71. Huang DY, Boini KM, Osswald H, Friedrich B, Artunc F, Ullrich S, Rajamanickam J, Palmada M, Wulff P, Kuhl D, Vallon V, Lang F. Resistance of mice lacking the serum- and glucocorticoid-inducible kinase SGK1 against salt-sensitive hypertension induced by a high-fat diet. *Am J Physiol Renal Physiol* 291: F1264–F1273, 2006.
72. Hubbard SR, Wei L, Ellis L, Hendrickson WA. Crystal structure of the tyrosine kinase domain of the human insulin receptor. *Nature* 372: 746–754, 1994.
73. Hussain T. Renal angiotensin II receptors, hyperinsulinemia, and obesity. *Clin Exp Hypertens* 25: 395–403, 2003.
74. Hussain T, Becker M, Beheray S, Lokhandwala MF. Dopamine fails to inhibit Na^+/H^+ -exchanger in proximal tubules of obese Zucker rats. *Clin Exp Hypertens* 23: 591–601, 2001.
75. Hussain T, Beheray SA, Lokhandwala MF. Defective dopamine receptor function in proximal tubules of obese Zucker rats. *Hypertension* 34: 1091–1096, 1999.
76. Hwang IS, Ho H, Hoffman BB, Reaven GM. Fructose-induced insulin resistance and hypertension in rats. *Hypertension* 10: 512–516, 1987.
77. Iida M, Murakami T, Ishida K, Mizuno A, Kuwajima M, Shima K. Phenotype-linked amino acid alteration in leptin receptor cDNA from Zucker fatty (fa/fa) rat. *Biochem Biophys Res Commun* 222: 19–26, 1996.
78. Ito O, Kondo Y, Oba M, Takahashi N, Omata K, Abe K. Tyrosine kinase, phosphatidylinositol 3-kinase, and protein kinase C regulate insulin-stimulated NaCl absorption in the thick ascending limb. *Kidney Int* 51: 1037–1041, 1997.
79. Ito O, Kondo Y, Takahashi N, Kudo K, Igarashi Y, Omata K, Imai Y, Abe K. Insulin stimulates NaCl transport in isolated perfused MTAL of Henle's loop of rabbit kidney. *Am J Physiol Renal Fluid Electrolyte Physiol* 267: F265–F270, 1994.
80. Ito O, Kondo Y, Takahashi N, Omata K, Abe K. Role of calcium in insulin-stimulated NaCl transport in medullary thick ascending limb. *Am J Physiol Renal Fluid Electrolyte Physiol* 269: F236–F241, 1995.
81. Iwashita S, Tanida M, Terui N, Ootsuka Y, Shu M, Kang D, Suzuki M. Direct measurement of renal sympathetic nervous activity in high-fat diet-related hypertensive rats. *Life Sci* 71: 537–546, 2002.
82. Joshi RL, Lamothe B, Cordonnier N, Mesbah K, Monthieux E, Jami J, Bucchini D. Targeted disruption of the insulin receptor gene in the mouse results in neonatal lethality. *EMBO J* 15: 1542–1547, 1996.
83. Juan CC, Fang VS, Kwok CF, Perng JC, Chou YC, Ho LT. Exogenous hyperinsulinemia causes insulin resistance, hyperendothelinemia, and subsequent hypertension in rats. *Metabolism* 48: 465–471, 1999.
84. Kalantarina K, Okusa MD. The renin-angiotensin system and its blockade in diabetic renal and cardiovascular disease. *Curr Diab Rep* 6: 8–16, 2006.
85. Kamynina E, Staub O. Concerted action of ENaC, Nedd4-2, and Sgk1 in transepithelial Na^+ transport. *Am J Physiol Renal Physiol* 283: F377–F387, 2002.
86. Kasiske BL, O'Donnell MP, Keane WF. The Zucker rat model of obesity, insulin resistance, hyperlipidemia, and renal injury. *Hypertension* 19: 110–115, 1992.
87. Keen HL, Brands MW, Alonso-Galicia M, Hall JE. Chronic adrenergic receptor blockade does not prevent hyperinsulinemia-induced hypertension in rats. *Am J Hypertens* 9: 1192–1199, 1996.
88. Kern W, Peters A, Born J, Fehm HL, Schultes B. Changes in blood pressure and plasma catecholamine levels during prolonged hyperinsulinemia. *Metabolism* 54: 391–396, 2005.
89. Khan O, Riaz S, Hu X, Song J, Wade JB, Ecelbarger CA. Regulation of the renal thiazide-sensitive Na-Cl cotransporter, blood pressure, and natriuresis in obese Zucker rats treated with rosiglitazone. *Am J Physiol Renal Physiol* 289: F442–F450, 2005.
90. Khoursheed M, Miles PD, Gao KM, Lee MK, Moossa AR, Olefsky JM. Metabolic effects of troglitazone on fat-induced insulin resistance in the rat. *Metabolism* 44: 1489–1494, 1995.
91. Kim D, Sands JM, Klein JD. Changes in renal medullary transport proteins during uncontrolled diabetes mellitus in rats. *Am J Physiol Renal Physiol* 285: F303–F309, 2003.
92. Kim D, Sands JM, Klein JD. Role of vasopressin in diabetes mellitus-induced changes in medullary transport proteins involved in urine concentration in Brattleboro rats. *Am J Physiol Renal Physiol* 286: F760–F766, 2004.
93. Kim DU, Klein JD, Meireles CL, Sands JM. Regulation of renal transporter abundances in diabetic rats (Abstract). *J Am Soc Nephrol* 13: 67A, 2002.
94. Kim GH, Ecelbarger C, Knepper MA, Packer RK. Regulation of thick ascending limb ion transporter abundance in response to altered acid/base intake. *J Am Soc Nephrol* 10: 935–942, 1999.
95. Kirchner KA. Insulin increases loop segment chloride reabsorption in the euglycemic rat. *Am J Physiol Renal Fluid Electrolyte Physiol* 255: F1206–F1213, 1988.
96. Klisic J, Hu MC, Nief V, Reyes L, Fuster D, Moe OW, Ambuhl PM. Insulin activates Na^+/H^+ exchanger 3: biphasic response and glucocorticoid dependence. *Am J Physiol Renal Physiol* 283: F532–F539, 2002.
97. Ku DD, Sellers BM, Meezan E. Development of renal hypertrophy and increased renal Na,K-ATPase in streptozotocin-diabetic rats. *Endocrinology* 119: 672–679, 1986.
98. Kulkarni RN, Bruning JC, Winnay JN, Postic C, Magnuson MA, Kahn CR. Tissue-specific knockout of the insulin receptor in pancreatic beta cells creates an insulin secretory defect similar to that in type 2 diabetes. *Cell* 96: 329–339, 1999.
99. Lewis EJ, Lewis JB. Treatment of diabetic nephropathy with angiotensin II receptor antagonist. *Clin Exp Nephrol* 7: 1–8, 2003.
100. Li H, Ren P, Onwochei M, Ruch RJ, Xie Z. Regulation of rat Na^+/P_i cotransporter-1 gene expression: the roles of glucose and insulin. *Am J Physiol Endocrinol Metab* 271: E1021–E1028, 1996.
101. Li RJ, Qiu SD, Chen HX, Tian H, Wang HX. The immunotherapeutic effects of *Astragalus* polysaccharide in type 1 diabetic mice. *Biol Pharm Bull* 30: 470–476, 2007.
102. Mandon B, Siga E, Chabardes D, Firsov D, Roinel N, De Rouffignac C. Insulin stimulates Na^+ , Cl^- , Ca^{2+} , and Mg^{2+} transports in TAL of mouse nephron: cross-potentialiation with AVP. *Am J Physiol Renal Fluid Electrolyte Physiol* 265: F361–F369, 1993.
103. Mankhey RW, Bhatti F, Maric C. 17β -Estradiol replacement improves renal function and pathology associated with diabetic nephropathy. *Am J Physiol Renal Physiol* 288: F399–F405, 2005.

104. Marunaka Y, Hagiwara N, Tohda H. Insulin activates single amiloride-blockable Na channels in a distal nephron cell line (A6). *Am J Physiol Renal Fluid Electrolyte Physiol* 263: F392–F400, 1992.
105. Mauvais-Jarvis F, Virkamaki A, Michael MD, Winnay JN, Zisman A, Kulkarni RN, Kahn CR. A model to explore the interaction between muscle insulin resistance and beta-cell dysfunction in the development of type 2 diabetes. *Diabetes* 49: 2126–2134, 2000.
106. Meehan WP, Buchanan TA, Hsueh W. Chronic insulin administration elevates blood pressure in rats. *Hypertension* 23: 1012–1017, 1994.
107. Michael MD, Kulkarni RN, Postic C, Previs SF, Shulman GI, Magnuson MA, Kahn CR. Loss of insulin signaling in hepatocytes leads to severe insulin resistance and progressive hepatic dysfunction. *Mol Cell* 6: 87–97, 2000.
108. Nakamura R, Emmanouel DS, Katz AI. Insulin binding sites in various segments of the rabbit nephron. *J Clin Invest* 72: 388–392, 1983.
109. Nejsum LN, Kwon TH, Marples D, Flyvbjerg A, Knepper MA, Frokiaer J, Nielsen S. Compensatory increase in AQP2, p-AQP2, and AQP3 expression in rats with diabetes mellitus. *Am J Physiol Renal Physiol* 280: F715–F726, 2001.
110. O'Callaghan CJ, Komersova K, Krum H, Louis WJ. 'Physiological' hyperinsulinaemia increases distal artery systolic blood pressure without changing proximal blood pressure. *Clin Sci (Lond)* 93: 535–540, 1997.
111. Ogihara T, Asano T, Ando K, Chiba Y, Sakoda H, Anai M, Shojima N, Ono H, Onishi Y, Fujishiro M, Katagiri H, Fukushima Y, Kikuchi M, Noguchi N, Aburatani H, Komuro I, Fujita T. Angiotensin II-induced insulin resistance is associated with enhanced insulin signaling. *Hypertension* 40: 872–879, 2002.
112. Ogihara T, Asano T, Ando K, Chiba Y, Sekine N, Sakoda H, Anai M, Onishi Y, Fujishiro M, Ono H, Shojima N, Inukai K, Fukushima Y, Kikuchi M, Fujita T. Insulin resistance with enhanced insulin signaling in high-salt diet-fed rats. *Diabetes* 50: 573–583, 2001.
113. Ogihara T, Asano T, Fujita T. Contribution of salt intake to insulin resistance associated with hypertension. *Life Sci* 73: 509–523, 2003.
114. Okamoto H, Nakae J, Kitamura T, Park BC, Dragatsis I, Accili D. Transgenic rescue of insulin receptor-deficient mice. *J Clin Invest* 114: 214–223, 2004.
115. Ozaki K, Takada N, Tsujimoto K, Tsuji N, Kawamura T, Muso E, Ohta M, Itoh N. Localization of insulin receptor-related receptor in the rat kidney. *Kidney Int* 52: 694–698, 1997.
116. Pamidimukkala J, Jandhyala BS. Effects of salt rich diet in the obese Zucker rats: studies on renal function during isotonic volume expansion. *Clin Exp Hypertens* 26: 55–67, 2004.
117. Pearce D. The role of SGK1 in hormone-regulated sodium transport. *Trends Endocrinol Metab* 12: 341–347, 2001.
118. Record RD, Froelich LL, Vlahos CJ, Blazer-Yost BL. Phosphatidylinositol 3-kinase activation is required for insulin-stimulated sodium transport in A6 cells. *Am J Physiol Endocrinol Metab* 274: E611–E617, 1998.
119. Record RD, Johnson M, Lee S, Blazer-Yost BL. Aldosterone and insulin stimulate amiloride-sensitive sodium transport in A6 cells by additive mechanisms. *Am J Physiol Cell Physiol* 271: C1079–C1084, 1996.
120. Reddy SR, Kotchen TA. Dietary sodium chloride increases blood pressure in obese Zucker rats. *Hypertension* 20: 389–393, 1992.
121. Riazi S, Khan O, Hu X, Ecelbarger C. Aldosterone infusion with high-NaCl diet increases blood pressure in obese but not lean Zucker rats. *Am J Physiol Renal Physiol* 291: F597–F605, 2006.
122. Riazi S, Khan O, Tiwari S, Hu X, Ecelbarger CA. Rosiglitazone regulates ENaC and Na-K-2Cl cotransporter [NKCC(2)] abundance in the obese Zucker rat. *Am J Nephrol* 26: 245–257, 2006.
123. Riazi S, Madala Halagappa VK, Hu X, Ecelbarger CA. Sex and body-type interactions in the regulation of renal sodium transporter abundance, urinary excretion, and activity in lean and obese Zucker rats. *Genet Med* 3: 309–327, 2006.
124. Riazi S, Maric C, Ecelbarger CA. 17-beta Estradiol attenuates streptozotocin-induced diabetes and regulates the expression of renal sodium transporters. *Kidney Int* 69: 471–480, 2006.
125. Rowe JW, Young JB, Minaker KL, Stevens AL, Pallotta J, Landsberg L. Effect of insulin and glucose infusions on sympathetic nervous system activity in normal man. *Diabetes* 30: 219–225, 1981.
126. Sandberg K, Ji H. Kidney angiotensin receptors and their role in renal pathophysiology. *Semin Nephrol* 20: 402–416, 2000.
127. Sechi LA, De Carli S, Bartoli E. In situ characterization of renal insulin receptors in the rat. *J Recept Res* 14: 347–356, 1994.
128. Sechi LA, Griffin CA, Giacchetti G, Zingaro L, Catena C, Bartoli E, Schambelan M. Abnormalities of insulin receptors in spontaneously hypertensive rats. *Hypertension* 27: 955–961, 1996.
129. Sechi LA, Griffin CA, Grady EF, Grunfeld C, Kalinyak JE, Schambelan M. Tissue-specific regulation of insulin receptor mRNA levels in rats with STZ-induced diabetes mellitus. *Diabetes* 41: 1113–1118, 1992.
130. Sechi LA, Griffin CA, Schambelan M. Effect of dietary sodium chloride on insulin receptor number and mRNA levels in rat kidney. *Am J Physiol Renal Fluid Electrolyte Physiol* 266: F31–F38, 1994.
131. Sechi LA, Griffin CA, Zingaro L, Catena C, De Carli S, Schambelan M, Bartoli E. Glucose metabolism and insulin receptor binding and mRNA levels in tissues of Dahl hypertensive rats. *Am J Hypertens* 10: 1223–1230, 1997.
132. Sechi LA, Griffin CA, Zingaro L, Valentin JP, Bartoli E, Schambelan M. Effects of angiotensin II on insulin receptor binding and mRNA levels in normal and diabetic rats. *Diabetologia* 40: 770–777, 1997.
133. Seino S, Seino M, Nishi S, Bell GI. Structure of the human insulin receptor gene and characterization of its promoter. *Proc Natl Acad Sci USA* 86: 114–118, 1989.
134. Shah S, Hussain T. Enhanced angiotensin II-induced activation of Na⁺, K⁺-ATPase in the proximal tubules of obese Zucker rats. *Clin Exp Hypertens* 28: 29–40, 2006.
135. Shane MA, Nofziger C, Blazer-Yost BL. Hormonal regulation of the epithelial Na⁺ channel: from amphibians to mammals. *Gen Comp Endocrinol*, 2006.
136. Shimkets RA, Lifton R, Canessa CM. In vivo phosphorylation of the epithelial sodium channel. *Proc Natl Acad Sci USA* 95: 3301–3305, 1998.
137. Shinozaki K, Ayajiki K, Nishio Y, Sugaya T, Kashiwagi A, Okamura T. Evidence for a causal role of the renin-angiotensin system in vascular dysfunction associated with insulin resistance. *Hypertension* 43: 255–262, 2004.
138. Siddiqui MR, Moorthy K, Taha A, Hussain ME, Baquer NZ. Low doses of vanadate and *Trigonella* synergistically regulate Na⁺/K⁺-ATPase activity and GLUT4 translocation in alloxan-diabetic rats. *Mol Cell Biochem* 285: 17–27, 2006.
139. Song D, Arikawa E, Galipeau D, Battell M, McNeill JH. Androgens are necessary for the development of fructose-induced hypertension. *Hypertension* 43: 667–672, 2004.
140. Song J, Hu X, Riazi S, Tiwari S, Wade JB, Ecelbarger CA. Regulation of blood pressure, the epithelial sodium channel (ENaC), and other key renal sodium transporters by chronic insulin infusion in rats. *Am J Physiol Renal Physiol* 290: F1055–F1064, 2006.
141. Song J, Hu X, Shi M, Knepper MA, Ecelbarger CA. Effects of dietary fat, NaCl, and fructose on renal sodium and water transporter abundances and systemic blood pressure. *Am J Physiol Renal Physiol* 287: F1204–F1212, 2004.
142. Song J, Knepper MA, Verbalis JG, Ecelbarger CA. Increased renal ENaC subunit and sodium transporter abundances in streptozotocin-induced type 1 diabetes. *Am J Physiol Renal Physiol* 285: F1125–F1137, 2003.
143. Steinberg HO, Brechtel G, Johnson A, Fineberg N, Baron AD. Insulin-mediated skeletal muscle vasodilation is nitric oxide dependent. A novel action of insulin to increase nitric oxide release. *J Clin Invest* 94: 1172–1179, 1994.
144. Takahashi N, Ito O, Abe K. Tubular effects of insulin. *Hypertens Res* 19, Suppl 1: S41–S45, 1996.
145. Tay A, Ozelikay AT, Altan VM. Effects of L-arginine on blood pressure and metabolic changes in fructose-hypertensive rats. *Am J Hypertens* 15: 72–77, 2002.
146. Tiwari S, Madala-Halagappa VK, Ecelbarger CA. Increased systolic blood pressure and defective pressure-natriuresis in mice lacking the insulin receptor in the thick ascending limb through collecting duct (Abstract). *FASEB J* 21: 892.7, 2007.
147. Tiwari S, Madala-Halagappa VK, Riazi S, Hu X, Ecelbarger CA. Insulin receptor protein abundance in the kidney is reduced in insulin-resistant rats and upregulated by candesartan. *J Am Soc Nephrol*. In press.
148. Tiwari S, Madala Halagappa VK, Igarashi P, Kahn CR, Kohan DE, Ecelbarger CA. Sodium handling in response to insulin in renal thick ascending limb through collecting duct insulin receptor knockout mice (Abstract). *J Am Soc Nephrol* 17: 298A, 2006.

149. Tiwari S, Nordquist L, Halagappa VK, Ecelbarger CA. Trafficking of ENaC subunits in response to acute insulin in mouse kidney. *Am J Physiol Renal Physiol* 293: F178–F185, 2007.
150. Tiwari S, Wade JB, Ecelbarger CA. Insulin receptor localization and regulation in rat kidney (Abstract). *FASEB J* 20: A1169, 2006.
151. Tomiyama H, Kushiro T, Abeta H, Kurumatani H, Taguchi H, Kuga N, Saito F, Kobayashi F, Otsuka Y, Kanmatsuse K. Blood pressure response to hyperinsulinemia in salt-sensitive and salt-resistant rats. *Hypertension* 20: 596–600, 1992.
152. Tsuchida H, Imai G, Shima Y, Satoh T, Owada S. Mechanism of sodium load-induced hypertension in non-insulin dependent diabetes mellitus model rats: defective dopaminergic system to inhibit Na-K-ATPase activity in renal epithelial cells. *Hypertens Res* 24: 127–135, 2001.
153. Ueta K, Ishihara T, Matsumoto Y, Oku A, Nawano M, Fujita T, Saito A, Arakawa K. Long-term treatment with the Na⁺-glucose co-transporter inhibitor T-1095 causes sustained improvement in hyperglycemia and prevents diabetic neuropathy in Goto-Kakizaki Rats. *Life Sci* 76: 2655–2668, 2005.
154. Ullrich A, Bell JR, Chen EY, Herrera R, Petruzzelli LM, Dull TJ, Gray A, Coussens L, Liao YC, Tsubokawa M. Human insulin receptor and its relationship to the tyrosine kinase family of oncogenes. *Nature* 313: 756–761, 1985.
155. Umrani DN, Banday AA, Hussain T, Lokhandwala MF. Rosiglitazone treatment restores renal dopamine receptor function in obese Zucker rats. *Hypertension* 40: 880–885, 2002.
156. Van Zwieten PA, Kam KL, Pijl AJ, Hendriks MG, Beenen OH, Pfaffendorf M. Hypertensive diabetic rats in pharmacological studies. *Pharmacol Res* 33: 95–105, 1996.
157. Vander AJ. Basic renal processes for sodium, chloride, and water. In: *Renal Physiology* (5 ed.). New York: McGraw-Hill, 1995, p. 89–115.
158. Vasudevan H, Nagareddy PR, McNeill JH. Gonadectomy prevents endothelial dysfunction in fructose-fed male rats, a factor contributing to the development of hypertension. *Am J Physiol Heart Circ Physiol* 291: H3058–H3064, 2006.
159. Venkatesan N, Davidson MB. Insulin resistance in rats harboring growth hormone-secreting tumors: decreased receptor number but increased kinase activity in liver. *Metabolism* 44: 75–84, 1995.
160. Verma S, Bhanot S, McNeill JH. Sympathectomy prevents fructose-induced hyperinsulinemia and hypertension. *Eur J Pharmacol* 373: R1–R4, 1999.
161. Wang J, Barbry P, Maiyar AC, Rozansky DJ, Bhargava A, Leong M, Firestone GL, Pearce D. SGK integrates insulin and mineralocorticoid regulation of epithelial sodium transport. *Am J Physiol Renal Physiol* 280: F303–F313, 2001.
162. Ward DT, Yau SK, Mee AP, Mawer EB, Miller CA, Garland HO, Riccardi D. Functional, molecular, and biochemical characterization of streptozotocin-induced diabetes. *J Am Soc Nephrol* 12: 779–790, 2001.
163. Weisz OA, Wang JM, Edinger RS, Johnson JP. Non-coordinate regulation of endogenous epithelial sodium channel (ENaC) subunit expression at the apical membrane of A6 cells in response to various transporting conditions. *J Biol Chem* 275: 39886–39893, 2000.
164. Wells CC, Riaz S, Mankhey RW, Bhatti F, Ecelbarger C, Maric C. Diabetic nephropathy is associated with decreased circulating estradiol levels and imbalance in the expression of renal estrogen receptors. *Gend Med* 2: 227–237, 2005.
165. Wilson FH, Disse-Nicodeme S, Choate KA, Ishikawa K, Nelson-Williams C, Desitter I, Gunel M, Milford DV, Lipkin GW, Achard JM, Feely MP, Dussol B, Berland Y, Unwin RJ, Mayan H, Simon DB, Farfel Z, Jeunemaitre X, Lifton RP. Human hypertension caused by mutations in WNK kinases. *Science* 293: 1107–1112, 2001.
166. Wu F, Park F, Cowley AW Jr, Mattson DL. Quantification of nitric oxide synthase activity in microdissected segments of the rat kidney. *Am J Physiol Renal Physiol* 276: F874–F881, 1999.
167. Yang CL, Angell J, Mitchell R, Ellison DH. WNK kinases regulate thiazide-sensitive Na-Cl cotransport. *J Clin Invest* 111: 1039–1045, 2003.
168. Yasuda K, Okamoto Y, Nunoi K, Adachi T, Shihara N, Tamon A, Suzuki N, Mukai E, Fujimoto S, Oku A, Tsuda K, Seino Y. Normalization of cytoplasmic calcium response in pancreatic beta-cells of spontaneously diabetic GK rat by the treatment with T-1095, a specific inhibitor of renal Na⁺-glucose co-transporters. *Horm Metab Res* 34: 217–221, 2002.
169. Zhang YH, Alvarez de la Rosa D, Canessa CM, Hayslett JP. Insulin-induced phosphorylation of ENaC correlates with increased sodium channel function in A6 cells. *Am J Physiol Cell Physiol* 288: C141–C147, 2005.