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Effects of Decreased Renal Cortical Expression of G Protein–Coupled Receptor Kinase 4 and Angiotensin Type 1 Receptors in Rats

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Abstract

Abnormalities in renal angiotensin type 1 receptor (AT₁R), D₁ dopamine receptor (D₁R) and G protein–coupled receptor kinase 4 (GRK4) are present in polygenic hypertension. Selective renal reduction of AT₁R expression by intrarenal cortical infusion of antisense oligodeoxynucleotides (As-Odns) in conscious, uni-nephrectomized, sodium-loaded rats decreases proteinuria, normalizes the glomerular sclerosis index (GSI), increases the sodium excretion (UNaV), and modestly increases blood pressure (BP) in spontaneously hypertensive rats (SHR) but not in normotensive Wistar-Kyoto rats (WKY). In contrast, selective renal reduction of GRK4 expression by infusion of GRK4 As-Odns increases UNaV, attenuates the increase in arterial BP with age, and modestly decreases protein excretion in SHR, but not in WKY. In this study, we report that intrarenal cortical infusion of both GRK4 and AT₁R As-Odns decreased BP and increased UNaV in SHR; these effects were also noted in WKY to a lesser extent. Infusion of SHR with this combination of As-Odns resulted in a decrease in proteinuria and improvement of GSI similar to those by AT₁R As-Odn only. In contrast to the increased circulating angiotensin II and aldosterone levels induced by AT₁R As-Odn alone, the combination of As-Odns decreased both, contributing to greater natriuresis and amelioration of hypertension than by GRK4 or AT₁R As-Odn only. Our results indicate an interaction between GRK4-regulated receptors and the renin-angiotensin system in the regulation of renal function and BP.

Keywords

hypertension; kidney; receptors; blood pressure; G protein–coupled receptor kinase 4

Introduction

The renal dopaminergic and renin-angiotensin systems (RAS) have counter regulatory effects on cellular signal transduction, production of reactive oxygen species, renal sodium excretion, and blood pressure (1). The renal dopaminergic system is important in promoting sodium excretion during conditions of sodium excess (2, 3) while the RAS is preminent in sodium conservation (4–6). Indeed, under basal conditions, angiotensin II is responsible for more than 50% of renal sodium reabsorption (5).

Disruption of genes regulating the RAS, including the angiotensin type 1 receptor (AT₁R), in mice decreases blood pressure (7). We have reported, however, that in salt-loaded, uninephrectomized spontaneously hypertensive rats (SHR) the renal cortical inhibition of AT₁R expression by intrarenal cortical infusion of AT₁R-specific antisense oligodeoxynucleotides (As-Odns) increases blood pressure because the treatment increases circulating angiotensin II (Ang II), renin and aldosterone levels by interruption of the renin and Ang II negative feedback loop (8). The treatment also enhances sodium excretion in spontaneously hypertensive rats (SHR), but not in normotensive Wistar-Kyoto (WKY) rats. The major consequence of decreasing renal AT₁R expression in SHR is a decrease in proteinuria, probably as a result of amelioration in glomerular histopathology that is independent of blood pressure and circulating renin, Ang II, and aldosterone levels (8).

The dopamine produced by the kidney is natriuretic and antihypertensive (1–3). The D₁- (D₁R and D₅R) and D₂-like dopamine receptors (D₂R, D₃R, and D₄R) are responsible for more than 50% of sodium excreted under conditions of moderate sodium excess (1–3). Blockade of D₁- and/or D₂-like receptors in rodents (1–3, 9, 10) and humans (11) and disruption of any of the dopamine receptor genes in mice increases blood pressure (1–3, 12).

In humans with essential hypertension and rodents with genetic hypertension (SHR and Dahl salt-sensitive rats), the diuretic and natriuretic responses mediated by D₁-like receptor agonists are impaired (1–3). The ability of the D₁-like receptors to inhibit renal proximal sodium reabsorption is also impaired in humans with essential hypertension (1, 2). Further studies have shown that the impaired inhibitory effect of D₁-like receptors on epithelial sodium transport in genetic hypertension is due to an uncoupling of the D₁-like receptor from its G-protein/effector complex (1–3, 13, 14). The uncoupling is receptor-specific, organ-selective and nephron-segment-specific, precedes the onset of hypertension and co-segregates with the hypertensive phenotype (1–3). The defective transduction of the renal dopaminergic signal is caused by increased activity of G-protein-coupled receptor kinase type 4 (GRK4), which constitutively desensitizes D₁Rs, in part by increasing receptor phosphorylation. In genetically hypertensive rats, selective renal inhibition of the increased GRK4 expression ameliorates the hypertension (13). In humans, the presence of activating variants of the GRK4 gene, by themselves or in conjunction with variants of genes of the RAS, is associated with an increase in blood pressure with age (15) or mental stress (16), and with hypertension in several ethnic groups (Caucasians, Chinese, Africans, and Japanese) (1, 17–20). In mice, overexpression of human GRK4 gene variants causes hypertension either with or without salt sensitivity, depending upon the variant. GRK4 gene variants, by preventing the natriuretic function of the dopaminergic system, may be a cause of salt sensitivity (1, 14, 16, 20).

We have reported that selective inhibition of renal cortical GRK4 expression by the intrarenal cortical infusion of GRK4-specific As-Odns increases sodium excretion, decreases arterial blood pressure, and minimally decreases protein excretion in SHR, and that these effects are not observed in WKY rats (13). The current studies were conducted to determine the renal functional and blood pressure consequences of selective renal cortical

inhibition of both GRK4 and AT₁R protein expression using specific As-Odns. We hypothesized that the combined selective renal cortical inhibition of GRK4 and AT₁R protein expression would normalize blood pressure by ameliorating the blood pressure increase associated with AT₁R As-Odn alone. We also predicted that the combined inhibition would decrease blood pressure and increase sodium excretion to a greater extent than by either GRK4 or AT₁R As-Odn alone.

Methods

Animals

Four-week-old male WKY and SHR (Japan SLC Inc., Sendai, Japan) were fed 0.26% NaCl chow (Japan CLEA, Tokyo, Japan) and tap water. At 5 weeks of age, under intraperitoneal pentobarbital anesthesia (50 mg/kg), the right kidney was removed, and a catheter (an 8-mm PE 10 tube connected to 30 gauge, BB311-30 medical Teflon microtubing; Scientific Commodities, Inc., Lake Havasu City, USA) was implanted with Bipax epoxy resin glue 3- to 4-mm deep into the lower pole of the remaining left kidney (8, 13). Uninephrectomy was performed to obviate the interstitial infusion of the vehicle and oligodeoxynucleotides (Odns) to both kidneys, which would necessitate extra surgery. An osmotic mini-pump (1 μ L/h; Alzet Corporation, Palo Alto, USA) was positioned in the space previously occupied by the right kidney for the continuous interstitial infusion of lactated Ringer's solution. After the surgery the diet was increased to 4% NaCl in order to suppress the RAS, maintain a high level of AT_{1A}R expression in the kidney (5), increase renal dopamine production, and enhance the natriuretic effect of the D₁R (1–3).

One week later the rats were re-anesthetized and the implanted osmotic mini-pump was replaced with another that infused GRK4 and AT₁R As-Odns and scrambled Odns (Scr-Odns) (50 nmol/d) or vehicle (lactated Ringer's solution) at 0.2 μ L/h. The infusion was continued for 4 weeks.

Unanesthetized systolic blood pressures were measured twice a week by the tail-cuff method (Blood Pressure Analyzer model BP-8A; Softron, Tokyo, Japan) (8, 13). Urine was collected for 24 h, twice a week. Sodium, potassium and urinary protein concentrations were determined as described previously (8, 13). At the end of 4 weeks the rats were anesthetized, blood was obtained and the heart and remaining kidney were removed. All experimental procedures were approved by the Fukushima Medical University School of Medicine Animal Committee.

Design and Synthesis of Odns

The nucleotide sequences of purified rhodamine-conjugated propyne/phosphorothioate-modified rat GRK4 Odns (Greiner, Tokyo, Japan) were as follows: GRK4 As-Odn, 209-5' CATGAAGTTCTCCAGTTCCAT-3'-89; GRK4 Scr-Odn, 5' -ATTTTCCATACGCCGCATTAG-3'. GRK4 As-Odns but not GRK4 Scr-Odns have been shown to cause a 50% decrease in GRK4 protein in the kidney, as measured by immunoblotting (13). The phosphorothioate-modified AT₁R Odns (Bowman Gray School of Medicine of Wake Forest University, Winston-Salem, USA) were as follows: AT₁R As-Odn, 286-5' -AGAGTTAAGGGCCAT-3' -272; AT₁R Scr-Odn, 5' -CCCTTTGAAGGTTCC-3'. AT₁R As-Odns but not AT₁R Scr-Odns cause a 50% decrease in AT₁R protein, as measured by immunoblotting (8).

Quantitative Real-Time RT-PCR

Total RNA was prepared from renal cortex tissue using TRIzol (Invitrogen, Carlsbad, USA) and column-purified after DNase treatment with an RNase-free DNase set (Qiagen,

Valencia, USA). Subsequently, one microgram of total RNA was reverse transcribed into cDNA using the oligo(dT) method (iScript cDNA synthesis kit; Bio-Rad, Hercules, USA) in a 20 μ L reaction volume. cDNA in 1 μ L of reaction mix was used for real-time quantitative PCR using an iQ5 Real-Time PCR Detection System and iQ SYBR Green Supermix (Bio-Rad). Quantification of mRNA was based on the C_t value, normalized to β -actin, and expressed as relative mRNA. Primers for rat AT₁R, GRK4 and β -actin were used to replicate cDNAs reverse-transcribed from the experimental, and positive and negative control RNA samples. The primers used were as follows: for AT₁R, TGCCTGAATCCTCTGTTTTATGG (forward) and AGCCTGCGTGTGACTTGG (reverse); for GRK4, ACTTCAGCAGACTGGAAGCA (forward) and GGTGTCCAGGTTGACTCCTT (reverse); and for β -actin, GAGGGAAATCGTGCGTGAC (forward) and CCAGGGAGGAAGAGGATGC (reverse).

Immunoprecipitation and Immunoblotting

Renal cortical proteins were immunoblotted or immunoprecipitated as previously reported (8, 12–14, 21–23). Polyclonal rabbit anti-human AT₁R (sc173; Santa Cruz Biotechnology Inc., Santa Cruz, USA), polyclonal anti-rat GRK4, anti-human GRK4 γ/δ (Santa Cruz), anti-rat D₁R, anti-phospho-serine (Zymed Laboratories Inc., San Francisco, USA), and monoclonal β -actin antibodies (Santa Cruz) were used. The specificities of the anti-human AT₁R (8, 21, 22), anti-rat D₁R (13, 14, 22, 23), polyclonal anti-rat GRK4 (13, 14), anti-human GRK4 γ/δ (13, 14) and anti-phosphoserine antibodies (14, 23) have been established. The bands were visualized by ECL reagents (Amersham Biosciences, Piscataway, USA) and quantified by densitometry (Quantiscan, Ferguson, USA).

Histochemistry

Three μ m kidney sections were stained with periodic-acid-Schiff reagent and coded to remove evaluator bias (8). The degree of glomerulosclerosis was semi-quantified using a glomerular sclerosis index (GSI) from 0 to 4+. Sclerosis was defined as collapse and/or obliteration of the glomerular capillary tuft in association with increased hyaline matrix. In each single section of the kidney, all glomeruli (*i.e.*, superficial and juxtaglomerular) were assessed for glomerulosclerosis. The severity of sclerosis for each glomerulus was graded from 0 to 4+. No lesion was graded as 0, lesions constituting 10% or less of the glomerulus were graded as 1, lesions of up to 25% of the glomerulus were graded as 2, lesions of up to 50% of the glomerulus were graded as 3, and lesions of up to 100% of the glomerulus were graded as 4.

Hormone Determinations

EDTA-treated blood was centrifuged at $3,000 \times g$ for 15 min at 4°C and the plasma was stored at – 80°C until analyzed. Samples for measurement of Ang II were flash frozen immediately after collection and stored at – 80°C until analyzed. Plasma renin activity (PRA), Ang II, and aldosterone concentrations were measured by radioimmunoassay.

Statistical Analysis

The data are expressed as the mean \pm SEM. Comparisons within and among groups were made by repeated measures or factorial ANOVA, respectively, followed by Duncan's test. Two-group comparisons were performed using the Student's *t*-test. A value $p < 0.05$ was considered statistically significant.

Results

Baseline Data

At the beginning of the study, body weight, water and food intake, urine output, and sodium, potassium, and protein excretions, as well as the weights of the removed kidneys expressed as a percentage of body weight, were similar among the groups, but systolic blood pressures were higher in SHR than in WKY (Table 1).

Effect of Treatment with GRK4 and AT₁R Odns

Infusion of vehicle, combined GRK4 and AT₁R Scr-Odns (GRK4-AT₁R Scr-Odns), or combined GRK4 and AT₁R As-Odns (GRK4-AT₁R As-Odns) had no effect on body or heart weight, weight of the remaining kidney, or food and water intake (Table 2). Body weight and heart weight (as a percentage of body weight) were lower in WKY than in SHR. Serum creatinine was normal in all groups, although the values were lower in SHR than in WKY (Table 2).

GRK4 Expression

GRK4 mRNA was similar in vehicle- and GRK4-AT₁R Scr-Odn-treated WKY and SHR but was significantly decreased by GRK4-AT₁R As-Odn treatment (Fig. 1). GRK4 protein was greater in SHR than in WKY. GRK4 mRNA was not increased in these rats, suggesting that the increase in GRK4 protein was a consequence of post-transcriptional modification. Vehicle treatment or GRK4-AT₁R Scr-Odns had no effect on GRK4 protein in either WKY or SHR (Fig. 2A). GRK4-AT₁R As-Odns decreased renal GRK4 protein levels in WKY and SHR, but the absolute values remained higher in SHR than in WKY.

AT₁R Expression

The expression of AT₁R mRNA was not affected by either vehicle or GRK4-AT₁R Scr-Odn treatments but decreased in both groups of rats after treatment with GRK4-AT₁R As-Odns (Fig. 1). The level of AT₁R protein was greater in SHR than in WKY (Fig. 2A). Vehicle or GRK4-AT₁R Scr-Odns had no effect on AT₁R protein in either WKY or SHR. GRK4-AT₁R As-Odns decreased AT₁R in both groups; AT₁R protein in SHR decreased to the levels seen in vehicle- and Scr-Odn-treated WKY and became approximately the same as that observed in As-Odn-treated WKY.

D₁R Expression and Serine-Phosphorylation

D₁R protein was lower in SHR than in WKY (Fig. 2B). In a previous study, we did not find decreased D₁R protein in renal cortical homogenates in SHR relative to WKY (13). Probably depending upon membrane preparation, renal cortical or proximal tubule D₁R expression may (Hussain and Lokhand-wala (3) and the current study) or may not be (13) decreased in SHR relative to WKY. GRK4-AT₁R Scr-Odns or GRK4-AT₁R As-Odns had no effect on renal D₁R protein in either group, although there was a slight but nonsignificant tendency for D₁R protein to increase in SHR treated with GRK4-AT₁R As-Odns (Fig. 2B).

Serine-phosphorylated D₁R was greater in vehicle- and GRK4-AT₁R Scr-Odn-treated SHR than in WKY, as in a previous report (23). GRK4-AT₁R Scr-Odns had no effect on serine-phosphorylated D₁R in WKY but modestly decreased it in SHR, although the levels remained higher than those observed in WKY. GRK4-AT₁R As-Odns also had no effect on the level of serine-phosphorylated D₁R in WKY but decreased it in SHR so that the levels were no longer different between the two rat strains (Fig. 2B).

PRA, Ang II, and Aldosterone Levels

PRA and plasma aldosterone were higher in vehicle-treated SHR than in vehicle-treated WKY and their levels were not modified by treatment with GRK4-AT₁R Scr-Odns. Although samples for plasma Ang II were obtained in the absence of specific protease inhibitors and results should be interpreted with caution, plasma Ang II levels were similar in both rat strains treated with vehicle or with GRK4-AT₁R Scr-Odns and were also decreased in both strains by GRK4-AT₁R As-Odns treatment. Plasma aldosterone concentrations were also decreased in SHR but not in WKY (Table 2).

Blood Pressure

Blood pressure increased with age to a greater extent in SHR than in WKY (Table 1 and Fig. 3). Infusion of GRK4-AT₁R Scr-Odns had no effect on blood pressure in either group. In SHR, GRK4-AT₁R As-Odns treatment decreased blood pressure from 1.5 weeks of treatment (data not shown) until the end of the study (Fig. 3). In WKY the blood pressure-lowering effect of GRK4-AT₁R As-Odns was noted at 2.5 weeks (data not shown) after starting the treatment and was evident until the end of the study (Fig. 3).

Urine Flow, Sodium and Potassium Excretion, and Sodium Balance

Urine output was lower in vehicle- or GRK4-AT₁R Scr-Odn-treated SHR than in WKY (Table 2). In SHR, GRK4-AT₁R As-Odns increased urine output relative to vehicle and GRK4-AT₁R Scr-Odn treatment and approached the values observed in SHR. Urine volume was not affected by the treatments in WKY (Table 2).

Sodium (Table 1 and Fig. 4A) and potassium excretions increased with age in all groups (Tables 1 and 2). Potassium excretion was similar in WKY and SHR and unaffected by Odn treatment (Table 2).

At the end of the study sodium excretion was lower in vehicle- and GRK4-AT₁R Scr-Odn-treated SHR than in WKY (Fig. 4A). Sodium excretion increased in GRK4-AT₁R As-Odn-treated SHR after 1.5 weeks of treatment (data not shown) and remained increased until the end of the study; sodium excretion was also increased by GRK4-AT₁R As-Odn treatment in WKY, but to a lesser degree (Fig. 4A).

Average daily sodium balance during the last 2 weeks of the study was greater in vehicle- and GRK4-AT₁R Scr-Odn-treated SHR than in vehicle or Odn-treated WKY. In contrast, GRK4-AT₁R As-Odns decreased sodium balance in SHR to values lower than those observed in WKY (Fig. 4B).

Urine Protein Excretion

Protein excretion became greater in SHR than in WKY at 5.5 weeks of age (data not shown). GRK4-AT₁R As-Odn treatment substantially decreased protein excretion in SHR. The treatment also modestly decreased urinary protein excretion in WKY to levels that were slightly but not significantly lower than those observed in SHR (Fig. 5).

Histochemistry

WKY glomeruli appeared normal and were not affected by Odns (Fig. 6A–C). In contrast, glomerulosclerosis was evident in vehicle (Fig. 6D)- and GRK4-AT₁R Scr-Odn-treated SHR (Fig. 6E). However, in GRK4-AT₁R As-Odn-treated SHR, glomeruli appeared almost normal (Fig. 6F) (8, 13) and the glomerular sclerosis index was similar to that in WKY, with or without Odn treatment (Fig. 7); As-Odns minimally increased the glomerular sclerosis index in WKY.

Discussion

This study evaluated the contributions of the renal dopaminergic and angiotensin systems in the regulation of blood pressure and sodium excretion by the selective intrarenal cortical infusion of combined GRK4 and AT₁R As-Odns (8, 13). The D₁R is important in the regulation of blood pressure, because disruption of the D₁R gene in mice increases blood pressure (1, 2). However, the intrarenal cortical infusion of D₁R As-Odn only transiently increases sodium excretion and does not affect blood pressure in WKY (24). GRK4 desensitizes a few G protein-coupled receptors, including D₁R (25–31). Heterologous expression of GRK4 gene variants in Chinese hamster ovary cells impairs D₁R function (14). Overexpression of the human GRK4 γ gene variant, GRK4 γ A142V, but not the human GRK4 γ wild-type gene in mice impairs the natriuretic effect of D₁R and produces hypertension in human GRK4 γ A142V transgenic mice fed a normal NaCl diet (14). Moreover, overexpression of human GRK4 γ A486V, but not the human GRK4 γ wild-type gene, in mice causes hypertension in human GRK4 γ A486V transgenic mice fed a high NaCl diet (32).

Although the infusion of GRK4 As-Odns increases sodium excretion, and decreases sodium balance and blood pressure in SHR (13), blood pressure is not normalized, indicating the importance of renal factors (*e.g.*, AT₁R) not alleviated by inhibiting renal GRK4 function or non-renal mechanisms that participate in hypertension (7, 33–35). The intrarenal cortical infusion of GRK4 As-Odns also does not alter sodium excretion or blood pressure in salt-loaded WKY (13). Renal GRK4 expression is not increased in WKY and therefore, inhibition of renal GRK4 expression would not be expected to increase sodium excretion in this rat strain.

In this study, when the expressions of both GRK4 and AT₁R were inhibited by combined GRK4-AT₁R As-Odn treatment in SHR, sodium excretion was increased and sodium balance was decreased to a greater extent in SHR than when rats were treated with either As-Odn alone (8, 13). Combined GRK4-AT₁R As-Odn treatment increased sodium excretion not only in SHR but also in WKY. GRK4-AT₁R As-Odn treatment also decreased blood pressure in WKY, an effect not observed with either GRK4 As-Odn or AT₁R As-Odn treatments (8, 13). At first glance, one may conclude that in SHR, blood pressure decreases to a similar degree with GRK4-AT₁R As-Odns and with GRK4 As-Odn treatment alone (13). However, because AT₁R As-Odn treatment, alone, increases blood pressure in SHR (8) one can conclude that combined GRK4-AT₁R As-Odn treatment does decrease blood pressure in SHR to a greater extent than GRK4 As-Odn treatment alone. Therefore, these studies suggest that the interaction between GRK4 and AT₁R may be important in the overall regulation of sodium balance and blood pressure.

Selective renal inhibition of AT₁R expression in SHR does not decrease blood pressure because the interruption of the renin-angiotensin negative feedback loop results in increased circulating renin and Ang II (8). We did not study the effect of selective renal inhibition of GRK4 alone on the RAS. However, the combined inhibition of GRK4 and AT₁R expression unexpectedly decreased circulating PRA and Ang II in both WKY and SHR (and plasma aldosterone concentration in SHR but not in WKY). Renin activity and Ang II levels should have increased in both strains with AT₁R As-Odn treatment only (8). The inhibition of GRK4 expression should have allowed D₁R activity to become manifest, resulting in an increase in renin secretion; D₁R stimulates renin secretion under certain circumstances (33, 36). A greater effect should have occurred in the SHR whose D₁R function was impaired, because of the constitutively increased GRK4 activity in these animals (13). One possible explanation for these results is that they occur through an effect of the D₃R that inhibits renin secretion (37). We have preliminary data indicating that GRK4 also regulates the D₃R

(unpublished results). D₁R and D₃R co-localize in mouse juxtaglomerular cells (33, 37). The dopaminergic stimulation of renin secretion (*via* the D₁R) is more apparent under sodium deficit and the stimulatory effect is decreased during sodium excess (38, 39). If the inhibitory effect of D₃R on renin secretion becomes manifest during sodium excess, then the lack of desensitization of D₁R and D₃R that results from the decrease in the expression of GRK4 should decrease renin secretion and circulating Ang II. The decrease in Ang II levels could explain the decrease in plasma aldosterone concentrations with GRK4-AT₁R As-Odn treatment. An alternative explanation for our findings may be related to tubuloglomerular feedback (38); the decrease in sodium transport caused by enhanced D₁R and D₃R functions and decreased AT₁R function increases the amount of sodium presented to the macula densa, resulting in the inhibition of renin release. This assumes, however, that the GRK4 and AT₁R As-Odn treatment does not alter distal nephron transport.

The intrarenal cortical administration of GRK4 As-Odn in SHR prevents by more than 50% the increase in blood pressure with age but only modestly reduces the age-related increase in urinary protein excretion (13). In contrast, the intrarenal cortical administration of AT₁R As-Odns that results in an increase rather than in a decrease in blood pressure in SHR causes a dramatic reduction in protein excretion (8). These data suggest that the antiproteinuric effect of AT₁R As-Odn treatment may be a result of a reduction of AT₁R action in the glomerulus, as AT₁R As-Odns do reach the glomeruli (data not shown), while the modest decrease in proteinuria afforded by GRK4 As-Odns may be the result of the reduction in blood pressure. In the current studies, GRK4-AT₁R As-Odns decreased proteinuria to an extent similar to that obtained by AT₁R As-Odn only. Angiotensin-converting enzyme inhibitors, alone or in combination with AT₁R blockers, decrease urine protein excretion, even in normotensive rats and human subjects, independent of blood pressure (40, 41). In diabetic subjects whose aldosterone levels are increased during angiotensin-converting enzyme inhibition, the addition of an aldosterone blocker decreases urinary protein even further, without changes in blood pressure (42). Our earlier studies also indicate that the antiproteinuric effect of AT₁R suppression is independent of aldosterone levels and predominates over any proteinuria-promoting effect of aldosterone (8). The absence of an added antiproteinuric effect of GRK4 As-Odns with concomitant AT₁R As-Odn treatment is not surprising, because GRK4 is not expressed in glomeruli (13). Similarly, in the current studies, GRK4-AT₁R As-Odns improved the GSI to the same extent as observed by AT₁R As-Odn-treatment alone (8). Because GRK4 As-Odns do not affect GSI in SHR (13), it is likely that the AT₁R As-Odns are mainly responsible for the decrease in the GSI resulting from combined GRK4-AT₁R As-Odn treatment.

In conclusion, combined treatment with both GRK4 and AT₁ As-Odns decreased blood pressure and increased sodium excretion in both normotensive and hypertensive rats, and the effects on hypertensive rats appeared to be greater than those observed with either of the As-Odns alone. These effects may have been at least partly mediated by a decrease in circulating renin, Ang II and aldosterone, which would indicate an interaction among GRK4-regulated receptors and the RAS. These results may have important therapeutic implications in salt sensitivity and/or hypertension.

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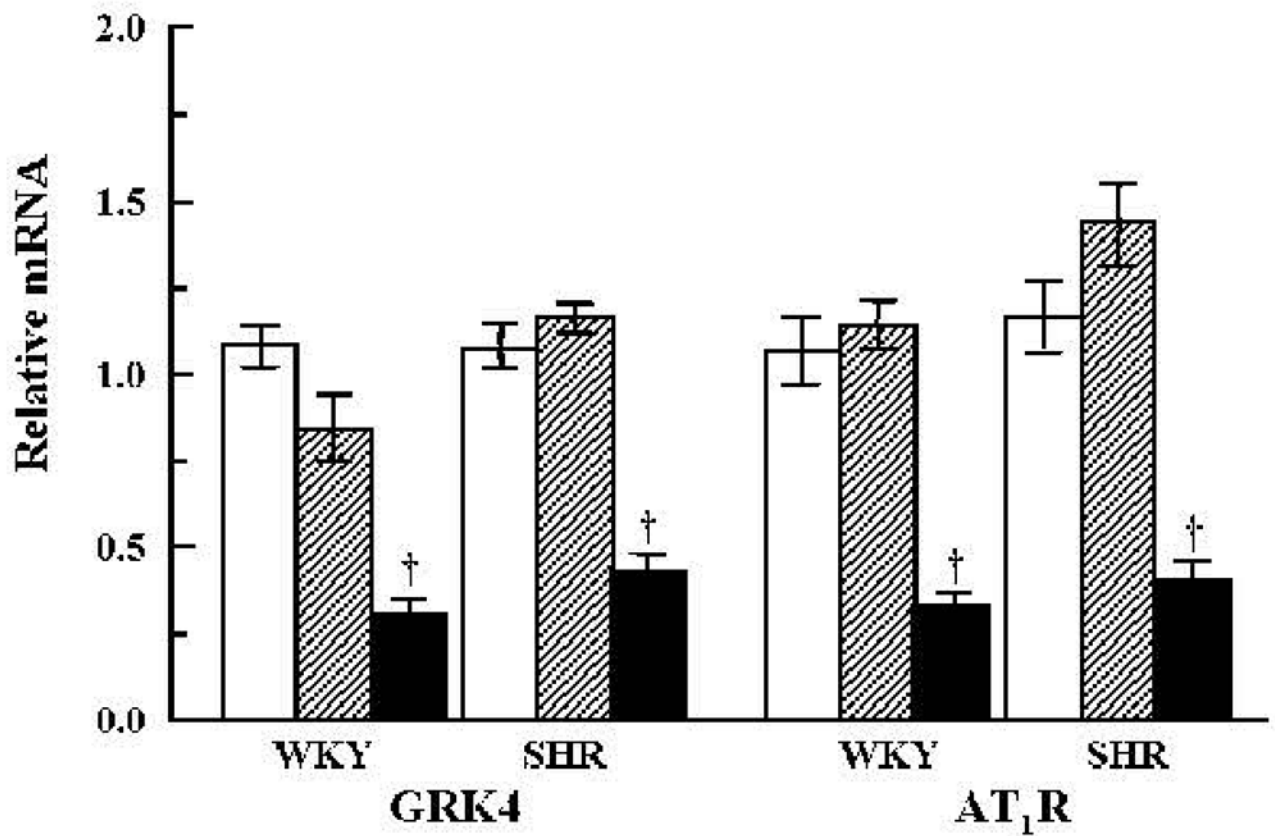


Fig. 1. Effect of vehicle or combined GRK4 and AT₁R oligodeoxynucleotides on the expression of GRK4 mRNA and AT₁R mRNA, as determined by quantitative RT-PCR in the renal cortex. Open bars: vehicle; hatched bars: Scr-Odns; closed bars: As-Odns. †p < 0.05 vs. all others in the same group by factorial ANOVA and Duncan's test; n = 5–6 per group.

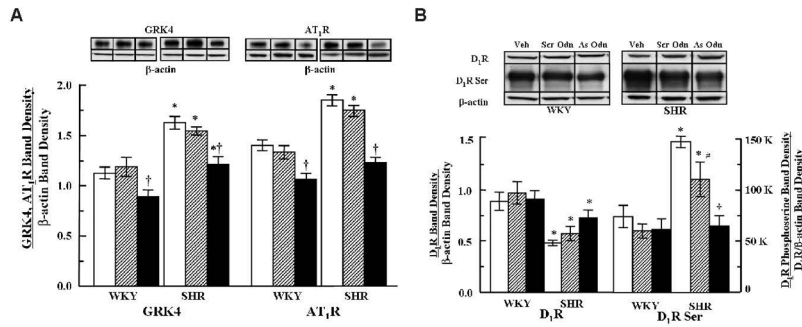


Fig. 2. Effect of vehicle or combined GRK4 and AT₁R oligodeoxynucleotides on the protein expression of GRK4, AT₁R, D₁R, and serine-phosphorylated D₁R expression in renal cortical membranes. A: Top: immunoblots of GRK4 (54 kDa), AT₁R, and β-actin are shown. Bottom: quantification of GRK4 and AT₁R protein expression in cortical membranes. GRK4 and AT₁R band densities were corrected by the respective band densities of β-actin. B: Top: immunoblots of D₁R protein and serine phosphorylated D₁R expression are shown. Bottom: quantification of D₁R protein and serine phosphorylated D₁R expression in cortical membranes. D₁R band densities were corrected by the respective band densities of β-actin. Serine-phosphorylated D₁R is expressed as a ratio of the respective D₁R band density corrected by actin. Open bars: vehicle; hatched bars: Scr-Odns; closed bars: As-Odns. *p < 0.05 vs. WKY, [†]p < 0.05 vs. all others in the same group, #p < 0.05 vs. SHR Veh (vehicle) by factorial ANOVA and Duncan's test; n = 5–6 per group.

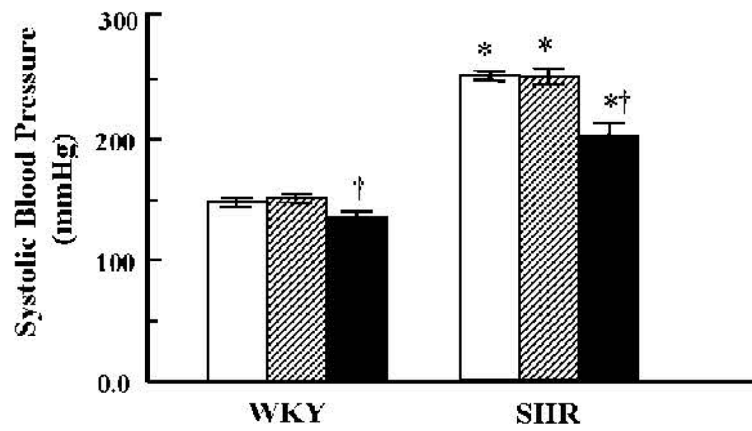


Fig. 3. Effect of vehicle or combined GRK4 and AT₁R oligodeoxynucleotides on systolic blood pressure. Blood pressures were measured by the tail-cuff method. * $p < 0.05$ vs. WKY, † $p < 0.05$ vs. all others in the same group by factorial ANOVA and Duncan's test; $n = 5-6$ per group. Open bars: vehicle; hatched bars: Scr-Odns; closed bars: As-Odns.

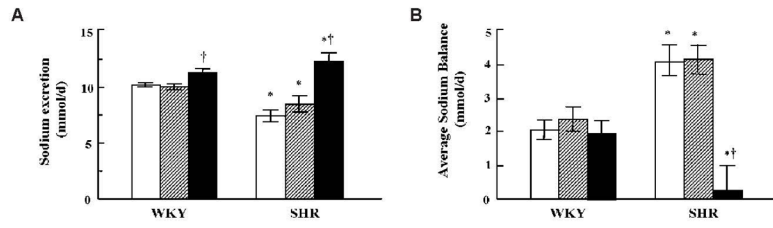


Fig. 4. Effect of vehicle or combined GRK4 and AT₁R oligodeoxynucleotides on urinary sodium excretion and sodium balance. A: Sodium concentrations were measured in urine collected over 24 h and expressed as mmol/d. B: Average daily sodium balance during the last 2 weeks of the study. Values are expressed in mmol/d. Open bars: vehicle; hatched bars: Scr-Odns; closed bars: As-Odns. * $p < 0.05$ vs. WKY, [†] $p < 0.05$ vs. all others in the same group by Factorial ANOVA and Duncan's test; $n = 5-6$ per group.

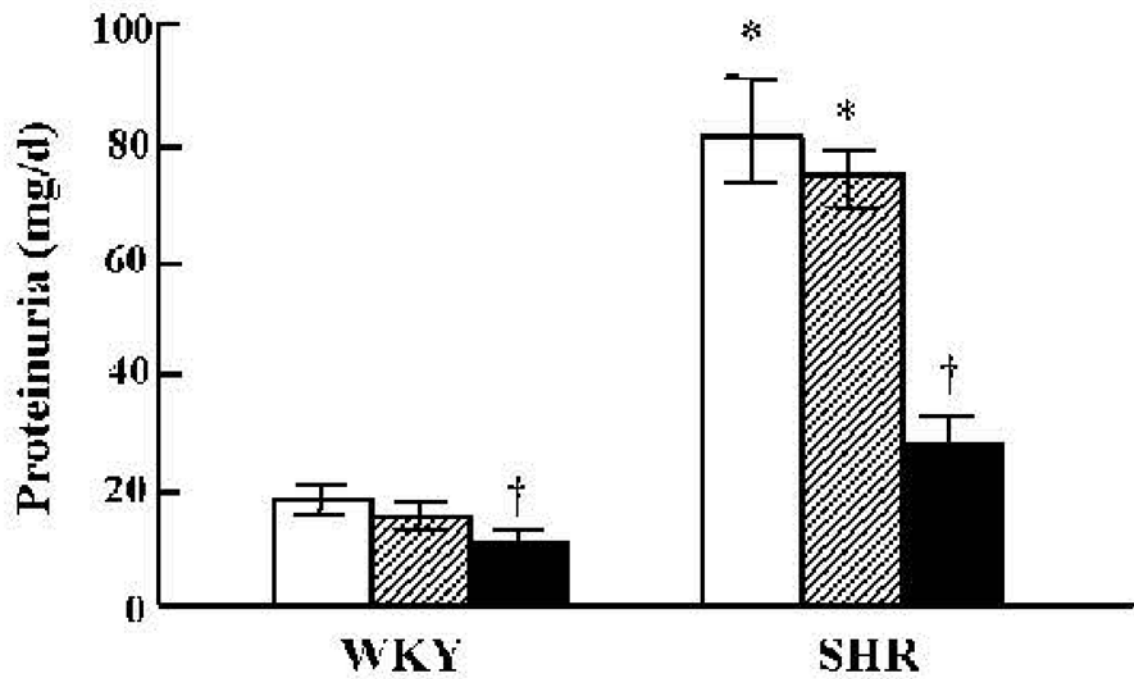


Fig. 5. Effect of vehicle or combined GRK4 and AT₁R oligodeoxynucleotides on urinary protein. Protein concentrations were measured in urine collected over 24 h and expressed as mg/d. Open bars: vehicle; hatched bars: Scr-Odns; closed bars: As-Odns. * $p < 0.05$ vs. WKY, † $p < 0.05$ vs. all others in the same group by factorial ANOVA and Duncan's test; $n = 5-6$ per group.

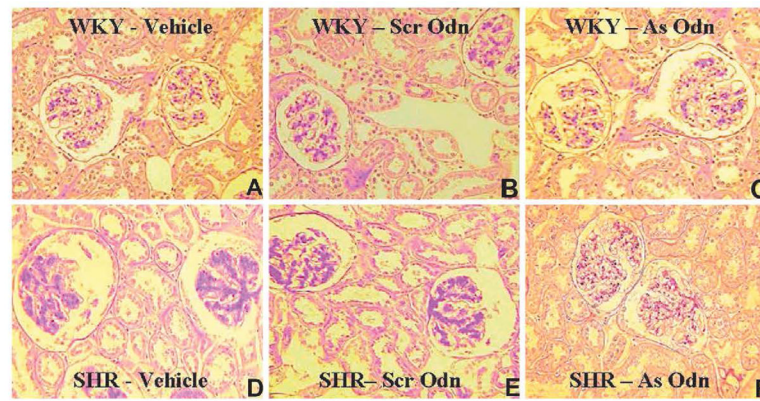


Fig. 6. Effect of vehicle or combined GRK4 and AT₁R oligonucleotides on renal glomerular morphology. Three μ m sections were stained with periodic-acid-Schiff (PAS) reagent. At 10 weeks of age, the glomeruli in WKY were normal in appearance and not affected by Odn treatment (A–C). In contrast, in SHR at 10 weeks of age, glomerulosclerosis was evident in vehicle (D)- and combined GRK4 and AT₁R Scr-Odn-treated SHR (E). However, GRK4 and AT₁R As-Odn-treated SHR had almost normal-appearing glomeruli (F).

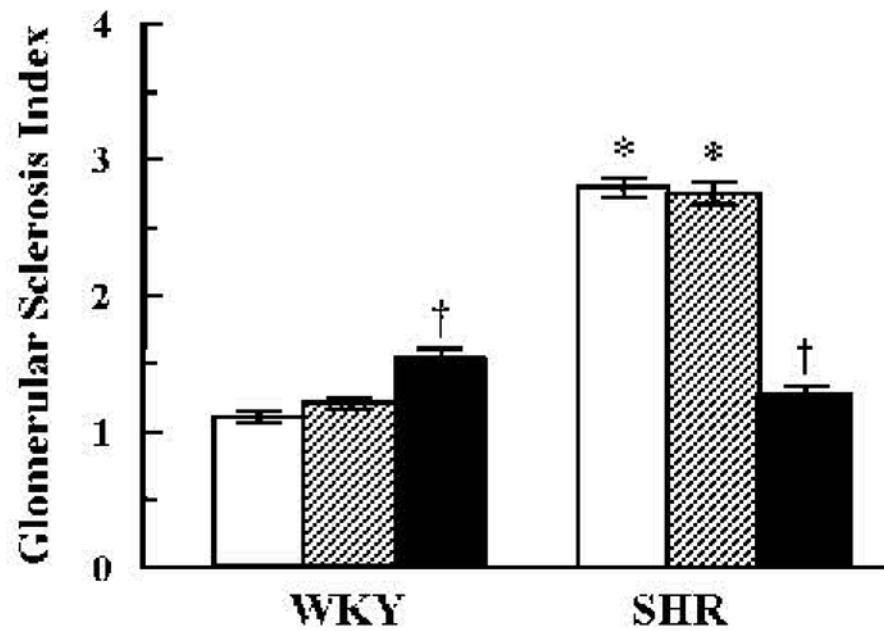


Fig. 7. Effect of vehicle or combined GRK4 and AT₁R oligodeoxynucleotides on the glomerular sclerosis index. The severity of sclerosis for each glomerulus was graded from 0 to 4+ as described in the Methods. Open bars: vehicle; hatched bars: Scr-Odns; closed bars: As-Odns. * $p < 0.05$ vs. WKY, † $p < 0.05$ vs. all others in the same group by factorial ANOVA and Duncan's test; $n = 5-6$ per group.

Table 1

Characteristics of 4.5 Week Old WKY and SHR rats Prior to Unilateral Nephrectomy and Insertion of an Intracortical Catheter into the Remaining Kidney

Variables	WKY rats			SHRs		
	Vehicle (n=6)	Scrambled-Odn (n=6)	Antisense-Odn (n=6)	Vehicle (n=5)	Scrambled-Odn (n=6)	Antisense-Odn (n=5)
BW(g)	75±3	78±4	73±1	77±3	77±4	75±5
Kidney weight (% BW)	0.83±0.06	0.83±0.08	0.88±0.05	0.72±0.04	0.70±0.04	0.72±0.06
Systolic BP	92±2	96±1	95±1	115±2*	111±2*	112±2*
Water intake (mL/d)	13.5±0.7	13.8±10.6	12.5±1.0	11.6±0.9	12.2±0.8	11.8±1.2
Food intake (g/d)	8.8±1.2	10.0±0.9	9.7±0.4	9.4±1.3	9.2±0.8	9.4±1.4
Urine output (mL/d)	2.36±0.43	1.77±0.43	1.63±0.46	3.05±0.76	2.94±0.51	2.44±0.38
Na ⁺ excretion (mmol/d)	0.35±0.05	0.37±0.08	0.35±0.04	0.48±0.04	0.47±0.06	0.45±0.04
K ⁺ excretion (mmol/d)	0.95±0.17	0.76±0.15	0.67±0.19	0.53±0.04	0.58±0.09	0.72±0.17
Proteinuria (mg/d)	0.52±0.10	0.46±0.06	0.41±0.12	0.46±0.10	0.40±0.08	0.34±0.04

* $p < 0.05$ vs. WKY.

BW, body weight; BP, blood pressure; WKY, Wistar Kyoto; SHR, spontaneously hypertensive rats; Odn, oligo-deoxynucleotide.

Table 2

Characteristics of WKY and SHRs at the End of the Study

Variables	WKY rats			SHRs		
	Vehicle (n=6)	Scrambled-Odn (n=6)	Antisense-Odn (n=6)	Vehicle (n=5)	Scrambled-Odn (n=6)	Antisense-Odn (n=5)
BW(g)	250±6	255±6	247±4	229±3*	225±3*	230±1*
Heart weight (%BW)	0.41±0.01	0.41±0.01	0.43±0.01	0.55±0.01*	0.55±0.02*	0.55±0.02*
Remaining kidney weight (%BW)	0.71±0.01	0.70±0.01	0.69±0.02	0.68±0.02	0.68±0.02	0.65±0.01
Water intake (mL/d)	48.8±2.5	52.2±4.1	49.5±4.4	54.8±0.9	50.5±1.2	51.4±2.6
Food intake (g/d)	17.8±0.9	18.7±1.4	19.2±0.6	18.2±1.0	17.2±0.6	17.7±1.0
Serum creatinine (mg/dL)	0.27±0.01	0.27±0.01	0.26±0.005	0.23±0.01*	0.23±0.01*	0.22±0.01*
Urine output (mL/d)	31.5±2.5	31.0±2.8	33.5±1.3	23.5±1.3*	24.2±1.9*	29.8±1.8 [†]
K ⁺ excretion (mmol/d)	3.38±0.31	3.45±0.30	3.86±0.55	2.77±0.25	2.92±0.15	3.08±0.14
PRA (ng/mL/h)	2.68 ±0.43	3.13±0.48	0.95±0.28 [†]	4.46±0.48*	5.02±0.68*	1.60±0.37 [†]
PAC (mg/dL)	50.7±2.8	60.4±4.5	49.5±3.6	74.1±6.5*	74.9±5.7*	47.5±3.1 [†]
Ang II (pg/mL)	9.0±1.3	9.3±1.7	3.0±0.07 [†]	6.2±0.7	6.8±0.8	4.20±.6 [†]

* $p < 0.05$ vs. WKY.

[†] $p < 0.05$ vs. others within their respective groups, ANOVA, Duncan's test.

BW, body weight; PRA, plasma renin activity; PAC, plasma aldosterone concentration; Ang II, angiotensin II; WKY, Wistar Kyoto; SHRs, spontaneously hypertensive rats; Odn, oligodeoxynucleotide.