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Role of nNOS in Regulation of Renal Function in Hypertensive Ren-2 Transgenic Rats

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Summary

The present study was performed to evaluate the role of neuronal nitric oxide synthase (nNOS)-derived nitric oxide (NO) during the developmental phase of hypertension in transgenic rats harboring the mouse *Ren-2* renin gene (TGR). The first aim of the present study was to examine nNOS mRNA expression in the renal cortex and to assess the renal functional responses to intrarenal nNOS inhibition by S-methyl-L-thiocitrulline (L-SMTC) in heterozygous TGR and in age-matched transgene-negative Hannover Sprague-Dawley rats (HanSD). The second aim was to evaluate the role of the renal sympathetic nerves in mediating the renal functional responses to intrarenal nNOS inhibition. Thus, we also evaluated the effects of intrarenal L-SMTC administration in acutely denervated TGR and HanSD. Expression of nNOS mRNA in the renal cortex was significantly increased in TGR compared with HanSD. Intrarenal administration of L-SMTC decreased the glomerular filtration rate (GFR), renal plasma flow (RPF) and sodium excretion and increased renal vascular resistance (RVR) in HanSD. In contrast, intrarenal inhibition of nNOS by L-SMTC did not alter GFR, RPF or RVR and elicited a marked increase in sodium excretion in TGR. This effect of intrarenal L-SMTC was not observed in acutely denervated TGR. These results suggest that during the developmental phase of hypertension TGR exhibit an impaired renal vascular responsiveness to nNOS derived NO or an impaired ability to release NO by nNOS despite enhanced expression of nNOS mRNA in the renal cortex. In addition, the data indicate that nNOS-derived NO increases tubular sodium reabsorption in TGR and that the renal nerves play an important modulatory role in this process.

Key words
Hypertension • Transgenic rat • Neuronal nitric oxide synthase • Renal nerves • Renal hemodynamics

Introduction

Although the hypertension that occurs in rats transgenic for the mouse *Ren-2* renin gene (TGR) is clearly due to the expression of the *Ren-2* renin gene, the exact pathophysiological mechanisms responsible for the development of hypertension in this model remain unclear (Mullins *et al.* 1990). Previous studies have

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shown that the hypertension in TGR is angiotensin II (ANG II)-dependent and that activation of ANG II receptor subtype 1 (AT_1) is largely responsible for the development of hypertension in this model (Hirth-Dietrich *et al.* 1994, Böhm *et al.* 1995, Gross *et al.* 1995, Mitchell and Mullins1995). However, it has been demonstrated that plasma and kidney ANG II levels are not elevated during prehypertensive and developmental phases of hypertension in TGR (Mitchell *et al.* 1997). Therefore, the development of hypertension in this model cannot be explained purely on the basis of increased production of ANG II. However, it has been reported that TGR exhibit exaggerated peripheral and renal vascular responsiveness to ANG II (Jacinto *et al.* 1999). The enhanced renal vascular responsiveness to ANG II could contribute to the inability of the kidney to maintain normal rates of salt and water excretion at normotensive pressures and, thereby, to the hypertension in this model. Nevertheless, the mechanisms responsible for the augmented vascular responsiveness to ANG II in this model remain uncertain.

It is well known that tonically produced nitric oxide (NO) plays an important role in the maintenance of systemic and vascular tone and that acute NO synthase (NOS) inhibition causes dose-dependent increases in blood pressure and renal vasoconstriction (for review see Wilcox 2001). In addition, it has been shown that enhanced NO formation counteracts the vasoconstrictor influences of ANG II in ANG II-dependent forms of hypertension (for review see Navar *et al.* 2000). It is therefore possible that NO deficiency could contribute to the development of hypertension. However, it has been reported that overall intrarenal NOS expression and NO production are increased in various models of hypertension (Dubey *et al.* 1998, Hayakawa and Raij 1998, Vaziri *et al.* 1998). Nevertheless, it has been reported that despite an increased overall intrarenal NO production, ANG II-infused hypertensive rats exhibit an impaired ability to produce NO by neuronal NOS (nNOS) (Ichihara *et al.* 1999, Červenka *et al.* 2001). Thus, it is possible that a selective deficit in nNOS function accounts for the diminished nNOS-derived NO renoprotective effects on renal hemodynamic function in TGR. This notion is supported by previous *in vitro* and *in vivo* studies that demonstrated a diminished role for nNOS-derived NO in counteracting ANG II-mediated vasoconstriction in ANG II-infused hypertensive and spontaneously hypertensive rats (SHR) (Ichihara *et al.* 1999, Welch *et al.* 1999, Červenka *et al.* 2001). The importance of NO derived from nNOS in the regulation of blood pressure is also supported by the findings that nNOS inhibition in Dahl salt-resistant rats caused the development of salt-sensitive hypertension (Tan *et al.* 1999). Taken together, these data are consistent with the possibility that decreased renal vascular effects of nNOS-derived NO contribute to the enhanced renal vascular responsiveness to ANG II in TGR. Such effects might contribute to a compromised ability of the kidney to respond to ANG II-mediated increase in arterial blood pressure with appropriate increase in sodium excretion and thereby contribute to the development of hypertension in TGR. However, the specific contribution of nNOS-derived NO to the impairment of renal function in TGR remains uncertain.

The present study was performed to evaluate the renal cortical expression of nNOS and to determine the renal hemodynamic and sodium excretory responses to selective intrarenal nNOS inhibition in hypertensive TGR. Since the renal sympathetic nerves are important in modulating renal hemodynamic and excretory function in both normotensive and hypertensive states (Lundin *et al.* 1984, Khraibi 1995), and given that nNOS is expressed in renal sympathetic nerves fibers (Liu and Barajas 1998), additional experiments were performed to determine whether renal denervation influences the renal functional responses to intrarenal nNOS inhibition.

Methods

Our studies were performed in accordance with guidelines and practice established by the Institute for Clinical and Experimental Medicine Animal Care and Use Committee and are in accordance with laws in the Czech Republic and Federal Republic of Germany. The experiments were performed in male heterozygous TGR rats of the hypertensive line TGR(mRen2)27 (37 to 38-day-old) and age-matched transgene-negative Hannover Sprague-Dawley rats (HanSD). All animals used in the present study were bred at the Center for Experimental Cardiovascular Research of Institute for Clinical and Experimental Medicine from stock animals supplied from the Max Delbrück Center for Molecular Medicine of Berlin, Germany. Animals were fed standard rat chow (SEMED, Prague, Czech Republic) with NaCl content 0.4% and tap water *ad libitum* and were kept on a 12-hour/12-hour light/dark cycle.

Experiment 1: Studies on nNOS mRNA expression in the renal cortex

Experiments were performed to compare the nNOS mRNA expression in TGR and HanSD. In previous studies, a close correlation between nNOS mRNA transcript abundance in the renal cortex and nNOS transcript abundance in macula densa (MD) has been demonstrated (Schricker *et al.* 1996, Welch *et al.* 1999). Thus, this study was performed in renal cortical tissue with the assumption that possible differences reflect changes in the MD region. TGR (n=5) and HanSD (n=5) were anesthetized with thiopental sodium (60 mg/kg i.p.), the abdomen was opened and a segment of renal cortex was removed and immediately frozen in liquid nitrogen. The samples were stored at –80 ^{o}C until isolation of total RNA, which was extracted from the frozen renal cortex using a commercially available kit (RNeasy; Qiagen, Germany). The semiquantitative RT-PCR was performed as described in detail previously (Schricker *et al.* 1996). The sense primer for nNOS was 5´-GAATACCAGCCTGATCCA.-3´, and the antisense primer was 5´-TCCAGGAGGGTGTCCACCGCA-3´. Glyceraldehyde-3-phosphate dehydrogenase (GAPDH) was used as an internal standard. The sequences of the GAPDH primers were as follows: sense, 5´-GATGCAGGGATGATGTTC-3´ and the antisense, 5´-CGCTAACATCAAATGGGGTG-3´. Because GAPDH mRNA levels did not differ between these groups, nNOS mRNA expression was evaluated as the nNOS mRNA/GAPDH mRNA ratio.

Experiment 2: Renal functional studies – effects of acute intrarenal nNOS inhibition

For studies designed to evaluate the effects of intrarenal blockade of nNOS on renal function, rats were fasted overnight. On the day of the experiment they were anesthetized with thiopental sodium (50 mg/kg i.p) and were placed on a thermoregulated table to maintain body temperature at 37-37.5 ^{o}C. A tracheostomy was performed to maintain a patent airway, and the exterior end of the tracheal cannula was placed inside a small plastic chamber into which humidified 95 % O_2/5 % CO_2 mixture was continuously passed. This procedure improves the stability of arterial pressure in anesthetized rats (Mitchell and Mullins 1995). The right jugular vein was catheterized with PE-50 tubing for infusion of solutions and additional anesthetic as required. The right femoral artery was cannulated to allow continuous monitoring of arterial blood pressure and blood sampling. Mean arterial pressure (MAP) was monitored with a pressure transducer (model MLT 1050) and recorded on a computer using a computerized data-acquisition system (PowerLab/4SP, ADInstruments, UK).

The left kidney was exposed *via* a flank incision, isolated from the surrounding tissue, and placed in a Lucite cup. A tapered PE-10 catheter was inserted into the left renal artery *via* the left femoral artery for selective intrarenal administration. This catheter was kept patent by a continuous infusion of heparinized isotonic saline at a rate of 4 μl/min throughout the experiment. In a previous study, it was demonstrated that this procedure allows selective administration of drugs without spillover into the systemic circulation (Červenka *et al.* 2001). During surgery, an isotonic saline solution containing bovine serum albumin (6 %) (Sigma Chemical Co., Prague, Czech Republic) was infused at a rate of 20 μl/min. After surgery, isotonic saline solution containing p-aminohippurate sodium (PAH; Merck, Sharp & Dohme West Point, PA) (1.5 %), and polyfructosan (Inutest, Laevosan, Linz/Donau, Austria) (7.5 %) was infused at the same infusion rate.

After completion of the surgical procedures, an equilibrium period of 45 min was allowed for the animals to establish steady state before initiating two 30-min control urine collections. Then, continuous intrarenal infusion of the nNOS inhibitor (S-methyl-L-thiocitrulline, L-SMTC; Sigma) at a rate of 0.3 mg/h (infusion rate 4 μl/min) was started. After a 10-min delay, two 30-min experimental urine collections were obtained. Simultaneously with the urine collections, arterial blood samples were collected to allow the determination of whole kidney hemodynamic function. The dose of L-SMTC used in the present study was chosen because we had previously demonstrated that it elicited substantial selective blockade of nNOS activity when infused directly into the renal artery of ANG II-infused hypertensive rats (Červenka *et al.* 2001). The effects of intrarenal L-SMTC administration on renal hemodynamics and excretory function were evaluated in both TGR and HanSD (n=10 in both cases). For control purposes, the effects of intrarenal infusion of isotonic saline were assessed in separate groups of TGR (n=7) and HanSD (n=8).

Experiment 3: Renal functional studies – effects of acute intrarenal nNOS inhibition after acute renal denervation

In order to evaluate the role of the renal sympathetic nerves in mediating the renal functional responses to intrarenal nNOS inhibition, additional experiments in which the effects of intrarenal L-SMTC

administration in TGR (n=7) and HanSD (n=8) subjected to acute renal denervation were examined. The rats were surgically prepared for renal clearance studies as described above. Renal denervation was performed as described and validated in previous studies (Bello-Reus *et al.* 1975, Quan *et al.* 2001). Briefly, all visible nerves were sectioned, the adventitia of the left renal artery was stripped by coating it with a solution of 10 % phenol in absolute alcohol. During the application of phenol, the left kidney and adjacent tissues were carefully protected from exposure to the chemical and damage of the major lymphatic vessels in the area was avoided. Rats that exhibited a spasm of the renal artery during or following phenol application were not included in the study. The experimental protocol was identical to that described in Experiment 2. For control purposes, the effects of intrarenal infusion of isotonic saline were assessed in acutely denervated TGR (n=8) and HanSD (n=7).

Urine volume was measured gravimetrically, inulin and PAH concentrations in urine and plasma were determined colorimetrically. Glomerular filtration rate (GFR) and renal plasma flow (RPF) were calculated from the clearances of inulin and PAH, respectively. Sodium and potassium concentrations in plasma and urine were determined by flame photometry. Renal vascular resistance (RVR) and fractional sodium excretion rate were calculated using standard formulae.

Statistical comparisons within groups were conducted by the ANOVA test for repeated measurements, followed by Newman-Keuls test. One-way ANOVA test was used for comparisons between groups. Values exceeding the 95 % probability limits ($P<0.05$) were considered statistically significant. All data are expressed as mean ± S.E.M.

Results

Experiment 1: studies on nNOS mRNA expression in renal cortex

As shown in Figure 1, semiquantitative analysis of RT-PCR products of nNOS mRNA demonstrated by expression of nNOS mRNA in the renal cortex was significantly increased in TGR compared with HanSD (0.35±0.04 vs. 0.22±0.02 densitometric units, $P<0.05$).

Experiment 2: renal functional studies – effects of acute intrarenal nNOS inhibition

Basal values for blood pressure, renal hemodynamics, and sodium excretion rates from Experiment 2 are summarized in Table 1. There were no significant differences in hemodynamic function and sodium excretion between TGR and HanSD.

Because experimental manipulations did not cause significantly different responses in experimental periods 1 and 2 in all groups, we decided, in order to highlight our results, to show only data from the experimental period 2.

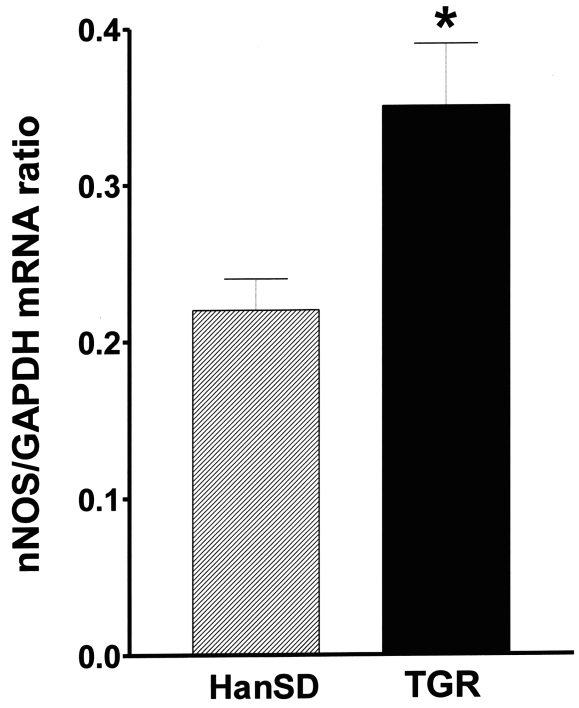

Fig. 1. *Expression of nNOS mRNA corrected for GAPDH expression (nNOS mRNA/GAPDH mRNA ratio) in renal cortical tissue of hypertensive TGR and normotensive HanSD. * P<0.05 compared with HanSD.*

Intrarenal infusion of L-SMTC did not alter MAP in either TGR or HanSD (131±5 vs.133±4 mm Hg and 119±3 vs.121±4 mm Hg, respectively). As shown in Figure 2, intrarenal administration of L-SMTC did not alter either GFR or RPF in TGR (–2±2 and –8±4 %, respectively). In contrast, L-SMTC administration elicited significant decreases in GFR and RPF in HanSD (–20±3 and –23±3 %, $P<0.05$ in both cases). Thus, L-SMTC infusion did not influence RVR in TGR (from 27±3 to 30±4 mm $Hg.ml^{-1}.g^{-1}$) but significantly increased RVR in HanSD (from 29±2 to 38±3 mm $Hg.ml^{-1}.g^{-1}$, $P<0.05$). Intrarenal infusion of L-SMTC caused marked increases in absolute and fractional sodium excretion in TGR (+565±88 and +573±64 %, $P<0.05$ in both cases). In HanSD, the L-SMTC-mediated reductions in GFR were associated with decreases in absolute and fractional sodium excretion (–23±7 and –24±5 %, $P<0.05$ in both

Table 1. Basal values for mean arterial blood pressure, renal hemodynamics and excretory function in TGR and HanSD with intact renal nerves (not subjected to acute renal denervation).

Group	n	MAP (mm Hg)	GFR (ml/min.g)	RPF (ml/min.g)	UV_{Na} (μEq/min.g)	FE_{Na} (%)	UF (μl/min.g)
HanSD + SAL	8	117±2	0.55±0.03	2.36±0.13	0.37±0.06	0.38±0.06	6.31±0.48
HanSD + L-SMTC	10	119±3	0.54±0.02	2.21±0.14	0.38±0.09	0.57±0.09	7.98±0.88
TGR + SAL	7	135±3*	0.65±0.05	2.87±0.33	0.58±0.14	0.56±0.13	7.61±0.39
TGR + L-SMTC	10	131±5*	0.57±0.03	2.60±0.18	0.48±0.25	0.55±0.25	6.75±0.44

*Values are mean ± SEM. SAL indicates intrarenal saline infusion; L-SMTC indicates intrarenal S-methyl-L-thiocitrulline. GFR, glomerular filtration rate; RPF, renal plasma flow; UV_{Na}, absolute sodium excretion; FE_{Na}, fractional sodium xcretion; UF, urine flow. *P<0.05 compared with other groups.*

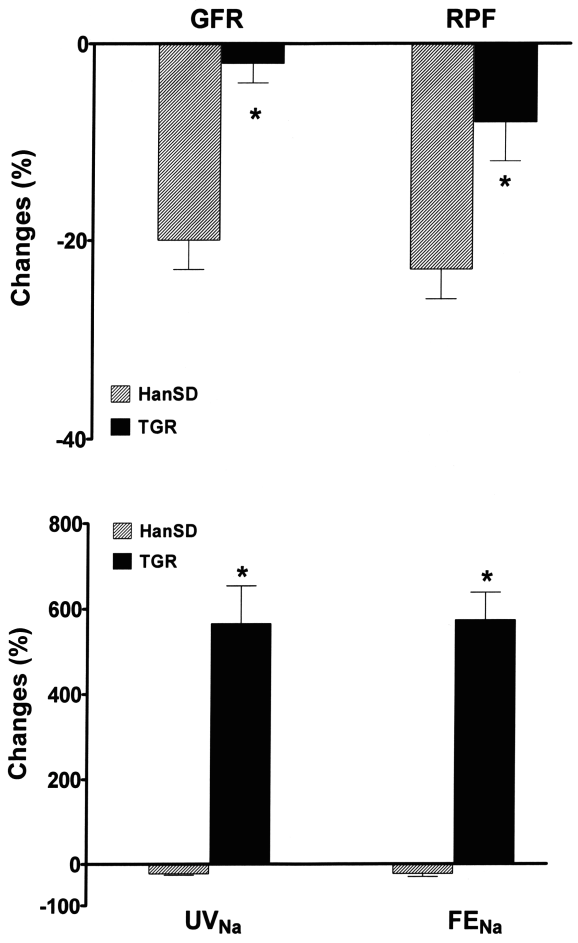

Fig. 2. *Renal hemodynamics (top) and sodium excretory responses (bottom) to intrarenal infusion of L-SMTC in hypertensive TGR and normotensive HanSD. Results are taken from the second experimental period of Experiment 2. GFR, glomerular filtration rate; RPF, renal plasma flow; UV_{Na}, absolute sodium excretion; FE_{Na}, fractional sodium excretion. * P<0.05 compared with HanSD.*

cases) (Fig. 2). Intrarenal infusion of the vehicle (isotonic saline) did not significantly influence MAP in either TGR (135±3 vs. 133±4 mm Hg) or in HanSD (117±2 vs. 115±2 mm Hg). In addition, intrarenal infusion of saline did not alter GFR or RPF in either TGR (+5±3 and +2±2 %, respectively) or in HanSD (–5±3 and –1±1 %, respectively). Thus, RVR did not exhibit any significant changes in response to intrarenal saline infusion in TGR or HanSD (25±3 vs. 24±3 mm Hg. $ml^{-1}.g^{-1}$ and 27±4 vs. 26±3 mm Hg. $ml^{-1}.g^{-1}$, respectively).

Experiment 3: Renal functional studies – effects of acute intrarenal nNOS inhibition after acute renal denervation

As summarized in Table 2, acute renal denervation resulted in about a two- to three-fold increase in absolute and fractional sodium excretion in both TGR and HanSD compared with denervated animals from Experiment 2 (Table 1). Intrarenal saline infusion did not significantly influence MAP in either denervated TGR (131±3 vs. 130±5 mm Hg) or in denervated HanSD (115±3 vs. 117±2 mm Hg). Similarly, intrarenal saline infusion did not alter GFR and RPF in either denervated TGR (+9±3 and +8±4 %, respectively) or in denervated HanSD (–6±2 and –4±2 %, respectively). However, intrarenal infusion of saline was associated with slight reductions in both absolute and fractional sodium excretion in denervated TGR (–27±11 % and –37±12 %, respectively, P<0.05 in both cases) and in denervated HanSD (–26±9 % and –38±14 %, respectively, P<0.05 in both cases). Intrarenal administration of L-SMTC did not significantly change GFR (+8±4 %) or RPF (–2±2 %) in acutely denervated TGR. However, L-SMTC administration elicited decreases in both GFR and RPF (–19±5 % and –26±7 %, respectively, P<0.05 in both cases) in denervated HanSD. Intrarenal infusion of

Table 2. Basal values for mean arterial blood pressure, renal hemodynamics and excretory function in TGR and HanSD subjected to acute renal denervation.

Group	n	MAP (mm Hg)	GFR (ml/min.g)	RPF (ml/min.g)	UV_{Na} (μEq/min.g)	FE_{Na} (%)	UF (μl/min.g)
HanSD + SAL	7	115±3	0.59± 0.05	2.66±0.22	1.27±0.25	1.69±0.41	12.65±1.11
HanSD +L-SMTC	8	112±3	0.61±0.04	2.83±0.28	1.65±0.16	1.24±0.14	15.55±1.24
TGR +SAL	8	131±3*	0.60±0.08	2.84±0.19	1.64±0.27	1.53±0.20	14.25±0.91
TGR + L-SMTC	7	133±4*	0.61±0.04	3.04±0.21	1.34±0.11	1.36±0.21	13.3±0.82

*Values are mean ± S.E.M. SAL indicates intrarenal saline infusion; L-SMTC indicates intrarenal S-methyl-L-thiocitrulline. GFR, glomerular filtration rate; RPF, renal plasma flow; UV_{Na}, absolute sodium excretion; FE_{Na}, fractional sodium excretion; UF, urine flow. *P<0.05 compared with other groups.*

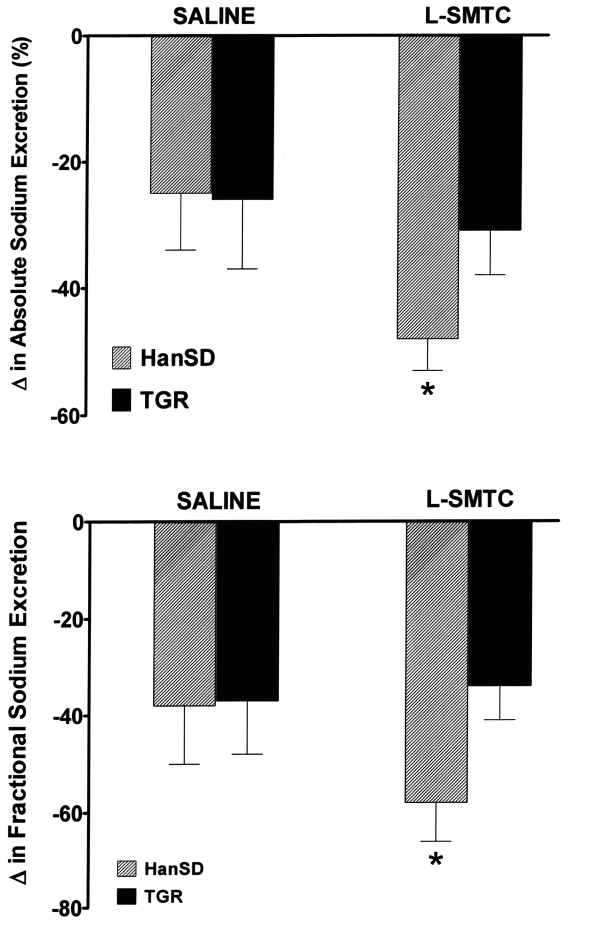

Fig. 3. *Absolute (top) and fractional (bottom) sodium excretion rate responses to intrarenal saline and L-SMTC infusion in acutely denervated TGR and HanSD. Results are taken from the second experimental period of Experiment 3. SALINE indicates intrarenal saline infusion; L-SMTC indicates intrarenal S-methyl-L-thiocitrulline. * P<0.05 compared with TGR.*

L-SMTC did not increase either absolute or fractional sodium excretion rates in denervated TGR or in denervated HanSD. Rather, intrarenal L-SMTC infusion elicited decreases in both absolute and fractional sodium excretion rates in denervated TGR (–32±7 % and –34±8 %, respectively, P<0.05 in both cases) as well as in denervated HanSD (–47±5 % and –58±8 %, respectively, P<0.05 in both cases) (Fig. 3).

Discussion

The present study was performed in order to evaluate the role of nNOS-derived NO in the regulation of renal function in TGR rats and its potential contribution to the development of hypertension in this model. We found that the expression of nNOS is increased in the kidney cortex of the TGR. Despite this enhanced nNOS expression in TGR the present data demonstrate that selective blockade of intrarenal nNOS with L-SMTC does not elicit marked alterations of renal hemodynamic function in hypertensive TGR. The reason for this apparent unresponsiveness to nNOS inhibition could be related to reduced enzyme activity of nNOS in the macula densa cells of TGR. Alternatively, it is possible that intrarenal production of superoxide anion (O_2^-) in TGR is increased. It is well known that O_2^- interacts with NO to yield peroxynitrate so that the biological half-life of NO could therefore be substantially reduced. This second explanation is supported by a recent study which demonstrated that enhanced production of O_2^- in the renal cortex is responsible for exaggerated tubuloglomerular feedback (TGF) responsiveness in SHR (Welch *et al.* 2000, for review see Schnackenberg 2002). In addition, it has been shown that the removal of O_2^- by superoxide dismutase mimetic tempol restored the

impaired afferent arteriole responses to nNOS inhibition in SHR (Ichihara *et al.* 2001). Moreover, it has been demonstrated that ANG II stimulates O_2^- production *via* an AT1 receptor-dependent mechanism (for review see Berry *et al.* 2001) and that AT_1 receptor blockade specifically attenuates oxidative stress and restores renal vascular responsiveness to NO in SHR (Welch and Wilcox 2001). In view of the findings that the hypertension in TGR is ANG II-dependent and activation of AT_1 receptors is largely responsible for the hypertension in this model (Hirth-Dietrich *et al.* 1994, Böhm *et al.* 1995, Gross *et al.* 1995, Mitchell and Mullins1995), it seems reasonable to assume that enhanced degradation of NO *via* interaction with O_2^- contributes to the diminished renal vascular responses to nNOS inhibition in TGR. However, additional studies are required to address this issue.

In the present study, selective intrarenal blockade of nNOS by L-SMTC markedly increased in absolute and fractional sodium excretion without influencing renal hemodynamics in TGR. These results imply that nNOS-derived NO increases tubular sodium reabsorption in TGR. This finding is apparently controversial to the results of previous studies showing that NO exerts a direct inhibitory action on sodium channels in the distal tubule and collecting duct epithelium (Stoos *et al.* 1994, 1995) and that NOS inhibition decreases sodium excretion when associated increases in renal perfusion pressure are prevented (Takenaka *et al.* 1993). However, it has been shown that luminal application of L-NAME decreased whereas NO donors increased fluid and HCO_3^- reabsorption in the rat proximal tubule (Wang 1997).

In addition, it has been reported that mice with targeted disruption of the nNOS gene exhibit lower tubular fluid and HCO_3^- reabsorption by the proximal tubule accompanied by lower blood pressure and metabolic acidosis compared with corresponding wild-type control mice (Wang *et al.* 2000). These findings imply that endogenously produced NO enhance tubular fluid and HCO_3^- reabsorption by the proximal tubule. Altogether, these data suggest that NO elicits disparate effects on the proximal and distal nephron reabsorptive function with NO stimulating proximal tubular reabsorption rate while inhibiting reabsorption by the distal tubule and collecting duct segments. The mechanism responsible for the inhibitory action of NO in distal and collecting duct nephron segments is well established and involves an inhibitory action of NO on the amiloride-sensitive apical membrane sodium channel as well as inhibition of the apical membrane sodium chloride cotransporter in the early distal tubule (Majid and Navar 1994, Stoos *et al.* 1994, 1995). In contrast, the mechanism underlying the stimulation of proximal tubular reabsorption by NO remains unclear. Whatever the mechanism, it is possible that nNOS-derived NO may act to maintain an inappropriately high proximal reabsorptive rate in TGR and, thereby, may contribute to the hypertension in this model.

It is generally recognized that the renal sympathetic nerves play an important role in the regulation of tubular sodium reabsorption (Quan and Baum 2001) and that increased renal sympathetic nerve activity contributes markedly to the renal functional derangements that appear to be necessary for the development of various forms of hypertension (for review see DiBona and Kopp 2001). In view of this and given that nNOS in the kidney is expressed in nerve bundles, especially in the region of the proximal tubules (Liu and Barajas 1998), we hypothesized that the effect of nNOS-derived NO to increase tubular reabsorption rate is mediated, at least in part, *via* increased activity of the renal sympathetic nerves. In order to address this hypothesis, we evaluated whether acute renal denervation alters the renal functional responses to intrarenal blockade of nNOS in both TGR and HanSD. Intrarenal administration of L-SMTC in acutely denervated HanSD decreased GFR and RPF to a similar extent as was observed in intact HanSD. These decreases of renal hemodynamic function in HanSD were accompanied by decreases in both absolute and fractional sodium excretion rates. In contrast, inhibition of nNOS by L-SMTC in acutely denervated TGR did not significantly alter renal hemodynamics and did not significantly increase sodium excretion. Indeed, the renal hemodynamic and excretory responses to nNOS inhibition in denervated TGR were similar to those elicited by intrarenal administration of isotonic saline. Thus, the present findings indicate that the increase in sodium excretion following nNOS inhibition is dependent on intact renal sympathetic nerves. This suggests that nNOS-derived NO acts to stimulate tubular reabsorption *via* an interaction with the renal sympathetic nerves. Whether this involves an increase in the release of norepinephrine from sympathetic nerve terminals or an increased tubular responsiveness to norepinephrine remains to be determined. Regardless of this , the effects of nNOS-derived NO to augment tubular reabsorption rate would likely contribute to an inappropriately high

reabsorptive status, to the elevation of arterial pressure and, thus to the hypertension in TGR.

On the basis of these results we conclude that during the developmental phase of hypertension, TGR exhibit an impaired renal vascular responsiveness to nNOS derived NO or an impaired ability to release NO by nNOS despite enhanced expression of nNOS mRNA in the renal cortex. In addition, our data indicate that nNOS-derived NO increases tubular sodium reabsorption in TGR. An attenuated vasodilator influence of nNOS-derived NO on renal hemodynamic function combined with nNOS-derived stimulation of sodium reabsorption may contribute to the impaired renal excretory function and, thus to the development of hypertension in this model.

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Selected Abbreviations and Acronyms

ANG II – angiotensin II
AT_1 – angiotensin II receptor subtype 1
GFR – glomerular filtration rate
GAPDH – glyceraldehyde-3-phosphate dehydrogenase
HanSD – transgene-negative Hannover Sprague-Dawley rats
L-SMTC – S-methyl-L-thiocitrulline, neuronal nitric oxide synthase inhibitor
MAP – mean arterial pressure
MD – macula densa
nNOS – neuronal nitric oxide synthase
NOS – nitric oxide synthase
O_2^- – superoxide anion
PAH – p-aminohippurate sodium
RPF – renal plasma flow
RVR – renal vascular resistance
SHR – spontaneously hypertensive rats
TGF – tubuloglomerular feedback
TGR – transgenic rats for the mouse Ren-2 renin gene

References

BELLO-REUS E, COLINDRES RE, PASTORIZA-MUŇOZ E, MUELLER R, GOTTSCHALK CW: Effects of acute unilateral renal denervation in the rat. *J Clin Invest* **56**: 208-217, 1975.

BERRY C, BROSNAN MJ, FENNELL J, HAMILTON CA, DOMINICZAK AF: Oxidative stress and vascular damage in hypertension. *Curr Opin Nephrol Hypertens* **10**: 247-255, 2001.

BÖHM M, LEE MA, KREUTZ MA, KIM S, SCHNINKE M, DJAVIDANI B, WAGNER J, KALING M, WIENEN W, BADER M, GANTEN D: Angiotensin II receptor blockade in TGR(mRen2)27: effects on renin-angiotensin-system gene expression and cardiovascular functions. *J Hypertens* **13**: 891-899, 1995.

ČERVENKA L, KRAMER HJ, MALY J, HELLER J**:** The role of nNOS in regulation of renal function in angiotensin II-induced hypertension. *Hypertension* **38**: 280-285, 2001.

DIBONA GF, KOPP UC: Neural control of renal function. In: *The Kidney, Physiology and Pathophysiology.* DW SELDIN, G. GIEBISCH (eds), Philadelphia, Lippincott Williams and Wilkins, 2001, pp 981-1005.

DUBEY RK, BOEGEHOLD MA, GILLESPIE DG, ROSSELLI M: Increased nitric oxide activity in early renovascular hypertension. *Am J Physiol* **270**: R118-R124, 1998.

GROSS V, LIPPOLDT A, SCHNEIDER W, LUFT FC: Effect of captopril and angiotensin II receptor blockade on pressure natriuresis in transgenic TGR(mRen2)27 rats. *Hypertension* **26**: 471-479, 1995.

HAYAKAWA H. RAIJ L: Nitric oxide synthase activity and renal injury in genetic hypertension. *Hypertension* **31**: 266-270, 1998.

HIRTH-DIETRICH C, STASCH JP, GANTEN D, LUFT FC: Renal effects of captopril and nitrendipine in transgenic rats with an extra renin gene. *Hypertension* **23**: 626-631, 1994.

ICHIHARA A, IMIG JD, NAVAR LG: Neuronal nitric oxide synthase-dependent afferent arteriolar function in angiotensin II-induced hypertension. *Hypertension* **33**: 462-466, 1999.

ICHIHARA A, HAYASHI M, HIROTA N, SARUTA T: Superoxide inhibits neuronal nitric oxide synthase influences on afferent arterioles in spontaneously hypertensive rats. *Hypertension* **37**: 630-634, 2001.

JACINTO SM, MULLINS JJ, MITCHELL KD: Enhanced renal vascular responsiveness to angiotensin II in hypertensive ren-2 transgenic rats. *Am J Physiol* **276**: F315-F322, 1999.

KHRAIBI AA: Role of renal nerves in natriuresis of L-NNMA infusion in SHR and WKY rats. *Am J Physiol* **269**: F17-F21, 1995.

LIU L, BARAJAS L: Evidence for NOS-containing renal neuronal somata transiently expressing a catecholaminergic phenotype during development in the rat. *Neurosci Lett* **251**: 161-164, 1998.

LUNDIN S, RICHSTEN SE, THOREN P: Renal sympathetic activity in spontaneously hypertensive rats and normotensive controls, as studied by three different methods. *Acta Physiol Scand* **120**: 265-272, 1984.

MAJID DSA, NAVAR LG: Blockade of distal nephron sodium transport attenuates pressure natriuresis in dogs. *Hypertension* **23**: 1040-1045, 1994.

MITCHELL KD, JACINTO SM, MULLINS JJ: Proximal tubular fluid, kidney, and plasma levels of angiotensin II in hypertensive ren-2 transgenic rats. *Am J Physiol* **273**: F246-F253, 1997.

MITCHELL KD, MULLINS JJ: ANG II dependence of tubuloglomerular feedback responsiveness in hypertensive ren-2 transgenic rats. *Am J Physiol* **268**: F821-F828, 1995.

MULLINS JJ, PETERS J, GANTEN D: Fulminant hypertension in transgenic rats harbouring the mouse Ren-2 gene. *Nature* **344**: 541-544, 1990.

NAVAR LG, ICHIHARA A, CHIN SY, IMIG JD: Nitric oxide-angiotensin II interactions in angiotensin II-dependent hypertension. *Acta Physiol Scand* **168**: 139-147, 2000.

QUAN A, BAUM M: The renal nerve is required for regulation of proximal tubule transport by intraluminally produced ANG II. *Am J Physiol* **280**: F524-F529, 2001.

SCHNACKENBERG CG: Physiological and pathophysiological roles of oxygen radicals in the renal microvasculature. *Am J Physiol* **282:** R335-R342, 2002.

SCHRICKER K, POTZL B, HAMANN M, KURTZ A: Coordinate changes of renin, and brain-type nitric oxide-synthase (b-NOS) mRNA levels in rat kidneys. *Pflügers Arch* **432**: 394-400, 1996.

STOOS BA, CARRETERO OA, GARVIN JL: Endothelial-derived nitric oxide inhibits sodium transport by affecting apical membrane channels in cultured collecting duct cells. *J Am Soc Nephrol* **4**: 1855-1860, 1994.

STOOS BA, GARCIA NA, GARVIN JL: Nitric oxide inhibits sodium reabsorption in the isolated perfused cortical collecting duct. *J Am Soc Nephrol* **6**: 89-94, 1995.

TAKENAKA T, MITCHELL KD, NAVAR LG: Contribution of angiotensin II to renal hemodynamic and excretory responses to nitric oxide synthesis inhibition in the rat. *J Am Soc Nephrol* **4**: 1046-1053, 1993.

TAN DY, MENG S, MANNING JR. RD: Role of neuronal nitric oxide synthase in Dahl salt-sensitive hypertension. *Hypertension* **33**: 456-461, 1999.

VAZIRI ND, NI Z, OVEISI F: Upregulation of renal and vascular nitric oxide synthase in young spontaneously hypertensive rats. *Hypertension* **31**: 1248-1254, 1998.

WANG T: Nitric oxide regulates HCO_3^- and Na^+ transport by a cGMP-mediated mechanism in the kidney proximal tubule. *Am J Physiol* **272**: F242-F248, 1997.

WANG T, INGLIS FM, KALB RG: Defective fluid and HCO_3^- absorption in proximal tubule of neuronal nitric oxide synthase-knockout mice. *Am J Physiol* **279**: F518-F524, 2000.

WELCH WJ, WILCOX CS: AT_1 receptor antagonist combats oxidative stress and restores nitric oxide signaling in the SHR. *Kidney Int* **59**: 1257-1263, 2001.

WELCH WJ, TOJO A, LEE JU, KANG DG, SCHNACKENBERG CG, WILCOX CS: Nitric oxide synthase in the JGA of the SHR: expression and role in tubuloglomerular feedback. *Am J Physiol* **277**: F130-F138, 1999.

WELCH WJ, TOJO A, WILCOX CS: Roles of NO and oxygen radicals in tubuloglomerular feedback in SHR. *Am J Physiol* **278**: F769-F776, 2000.

WILCOX CS: L-arginine-nitric oxide pathway. In: *The Kidney, Physiology and Pathophysiology* DW SELDIN, G GIEBISCH (eds), Philadelphia, Lippincott Williams and Wilkins, 2001, pp 849-872.

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