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Renal nerves and nNOS: roles in natriuresis of acute isovolumetric sodium loading in conscious rats

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Kompanowska-Jezierska E, Wolff H, Kuczeriszka M, Gramsbergen JB, Walkowska A, Johns EJ, Bie P. Renal nerves and nNOS: roles in natriuresis of acute isovolumetric sodium loading in conscious rats. *Am J Physiol Regul Integr Comp Physiol* 294: R1130–R1139, 2008. First published January 30, 2008; doi:10.1152/ajpregu.00908.2007.—It was hypothesized that renal sympathetic nerve activity (RSNA) and neuronal nitric oxide synthase (nNOS) are involved in the acute inhibition of renin secretion and the natriuresis following slow NaCl loading (NaLoad) and that RSNA participates in the regulation of arterial blood pressure (MABP). This was tested by NaLoad after chronic renal denervation with and without inhibition of nNOS by S-methyl-thiocitrulline (SMTC). In addition, the acute effects of renal denervation on MABP and sodium balance were assessed. Rats were investigated in the conscious, catheterized state, in metabolic cages, and acutely during anesthesia. NaLoad was performed over 2 h by intravenous infusion of hypertonic solution (50 μmol·min^{-1}·kg body mass^{-1}) at constant body volume conditions. SMTC was coinfused in amounts (20 μg·min^{-1}·kg^{-1}) reported to selectively inhibit nNOS. Directly measured MABPs of acutely and chronically denervated rats were less than control (15% and 9%, respectively, $P < 0.005$). Plasma renin concentration (PRC) was reduced by renal denervation (14.5 ± 0.2 vs. 19.3 ± 1.3 mIU/l, $P < 0.005$) and by nNOS inhibition (12.4 ± 2.3 vs. 19.6 ± 1.6 mlU/l, $P < 0.005$). NaLoad reduced PRC ($P < 0.05$) and elevated MABP modestly ($P < 0.05$) and increased sodium excretion six-fold, irrespective of renal denervation and SMTC. The metabolic data demonstrated that renal denervation lowered sodium balance during the first days after denervation ($P < 0.001$). These data show that renal denervation decreases MABP and renin secretion. However, neither renal denervation nor nNOS inhibition affects either the renin downregulation or the natriuretic response to acute sodium loading. Acute sodium-driven renin regulation seems independent of RSNA and nNOS under the present conditions.

plasma renin concentration; total body sodium; blood pressure; sodium excretion

THE INTERRELATIONSHIP BETWEEN blood pressure changes and acute variations in water and sodium excretion is not well defined. Arterial blood pressure is controlled by multiple systems, which differ widely in their time of activation. Neural systems react within seconds, while the hormonal and paracrine systems are more sluggish, requiring minutes to hours, or even days, to respond with full capacity. Neurogenic factors have long been hypothesized to be important in the initiation and maintenance of high blood pressure. There is considerable evidence suggesting that enhanced sympathetic drive to the heart and peripheral circulation is involved in the development of persistent blood pressure elevation or maintains the blood pressure elevation originally induced by nonneurogenic mechanisms (11). Moreover, it is known that electrical stimulation of the renal nerves in adult rats increases renin release and augments the expression of the renin gene within the kidney (22). Many neurophysiological and functional studies, reviewed by DiBona (7) and Malpas and Leonard (17), suggest that, the renal sympathetic nerves serve as the critical link between the nervous system and the kidney in control of fluid volume and composition.

It can be expected, therefore, that a decrease in renal sympathetic nerve activity (RSNA) and the associated decrease in plasma renin concentration participate in the excretion of a sodium load. A recent study showed that plasma volume expansion caused a modest reduction in RSNA (25%) and a significant increase in renal blood flow (RBF) in conscious rabbits, a response that was absent in renal-denervated animals (17). In dogs, a small sodium load may decrease renin in the absence of any increase in arterial blood pressure or glomerular filtration rate (28), apparently leaving RSNA as the sole controller of renin secretion. On the other hand, in studies by Morita and Vatner (19), also in conscious dogs, a reduction of RSNA of 18–87% observed during blood volume expansion did not change RBF, and it was concluded years ago that renin secretion induced by sodium deprivation is not primarily mediated by the sympathetic nerves (5). As yet, it is not clear whether the excretion of a physiological sodium load is related to a change in renal sympathetic nerve activity; likewise, the nature of the specific stimulus inducing the change remains

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unclear. We hypothesized that after renal denervation, the natriuretic response to slow sodium loading is blunted.

The natriuretic response to a sodium load could be mediated, at least in part, by increases in the generation of nitric oxide (NO) by the neuronal isoform of NO synthase (nNOS, NOS I). Solid evidence supports the view that NO limits the sensitivity of the tubuloglomerular feedback and, in general, inhibits and/or counterbalances the effects of RSNA on renal excretion, thereby promoting the disposal of excess sodium (33, 37, 38). Nonspecific inhibition of NOS enzymes blunts the natriuretic response to volume expansion (24), and NO may mediate this natriuresis (1). The relationship between nNOS in the macula densa and renin secretion has been investigated by several groups, but the results seem inconsistent. We hypothesized that nNOS-generated NO, via its inhibitory influence on sympathetic activity and possibly also renin secretion, contributes to the natriuresis generated by a moderate sodium load. It was anticipated that inhibition of nNOS in intact and denervated rats would help distinguish between the role of nNOS tightly connected with sympathetic nerves (prejunctional modulation of RSNA per se or postjunctional effects of nNOS-derived NO on the RSNA-mediated control of renal function) in intact rats and the role of nNOS located in macula densa, which affects renin release independent of RSNA (30) and, therefore, is operative in both intact and renally denervated rats.

The specific aims of the study were to determine *1)* whether absence of the renal nerves affects the natriuretic response to modest sodium load provided over hours without additional fluid (isovolumetric loading), *2)* whether the renal denervation procedure used for this purpose is associated with changes in blood pressure, plasma renin concentration, or fluid balance, and *3)* the role of nNOS-derived NO in the natriuretic response to isovolumetric sodium loading.

Any increase in total body sodium increases the extracellular volume. During isovolumetric sodium loading, this increase is caused only by a net flux of water from the intracellular to the extracellular compartment at constant total body water. Therefore, the procedure of isovolumetric loading minimizes the extracellular volume expansion inevitably associated with salt loading.

MATERIAL AND METHODS

Effect of Slow Sodium Loading on Blood Pressure and Renal Functions

Female Sprague-Dawley rats purchased from Harlan Scandinavia (Allerod, Denmark) and were fed a standard diet (Altromin 1324, Altromin, Lage, Germany) with free access to water. Prior to the experiments, the rats were trained to rest quietly in a plastic tube for more than 4 h. Each rat participated in two different experiments at intervals of 1 wk, in random order. The experiments were performed in conscious rats in accordance with the National Institutes of Health *Guide for the Care and Use of Laboratory Animals*, and the protocol was approved by the Danish Animal Experiments Inspectorate.

At least 1 wk before the first experiment, rats were surgically prepared as follows. Three groups of rats (DNX: control, $n = 4$; Hyp, $n = 12$; Hyp SMTC, $n = 6$) were renally denervated using standard techniques (34). Each kidney was exposed by a dorsal flank incision. Under a dissecting microscope, the kidneys were denervated by stripping all visible nerves, fat, and connecting tissue from the renal vessels and by coating the renal arterial wall with 10% phenol in ethanol to ensure the destruction of any remaining nerves. The efficacy of the denervation procedure was tested by measurement of the level of norepinephrine in the kidney tissue (see RESULTS). Other groups of rats (Sham: control, $n = 6$; Hyp, $n = 12$; Hyp SMTC, $n = 6$) were subject to the same dissection, except that renal denervation was not performed.

Chronic catheters were inserted during anesthesia with a mixture of Hypnorm (fentanyl, 0.315 mg/ml + fluanisone 10 mg/ml; VetaPharma, Leeds, UK; 0.75 ml/kg body wt) and midazolam (Hameln Pharmaceuticals, Hameln, Germany; 3.75 mg/kg body wt sc). Under antiseptic conditions Tygon arterial and venous catheters were inserted via the left femoral vessels. The arterial line was used for measurement of mean arterial pressure (MABP), heart rate (HR), and for blood sampling, while solutions were infused intravenously. The catheters were exteriorized at the nape of the neck. A sterile chronic suprapubic bladder catheter was introduced and exteriorized through an incision in the ventral abdominal wall. Between experiments, the bladder catheter was kept closed with a screw. Catheters were constructed and inserted as described by Petersen et al. (23). Temgesic (buprenorphine, 0.04 mg/kg body wt) was used for postoperative analgesia after all procedures.

Experimental Procedures

On the day of the experiment, the arterial line was connected to a pressure transducer (TBD-1222, Föhr Medical Instruments, Seeheim, Germany). An intra-arterial infusion of 5% glucose containing heparin (100 IU/ml) at a rate of 8 $\mu l \cdot kg^{-1} \cdot min^{-1}$ (1.5–2.0 μl/min) kept the arterial line patent. This infusion does not change the blood pressure signal measurably. MABP and HR were measured continuously and recorded using a personal computer running custom-designed software written in LabView (National Instruments, Austin, TX).

The venous line was connected to a multisyringe, programmable infusion pump. Para-aminohippurate (PAH; 20% solution, Merck, Whitehouse Station, NJ; 12 $\mu l \cdot kg^{-1} \cdot min^{-1}$) for renal plasma flow determination (RPF), inulin (Inutest, 25% solution, Fresenius Kabi Austria, Graz, Austria; 8 $\mu l \cdot kg^{-1} \cdot min^{-1}$) for measurement of glomerular filtration rate (GFR) and isotonic saline (0.9% NaCl; 24 $\mu l \cdot kg^{-1} \cdot min^{-1}$) were infused continuously during the experiment. In some experiments, hypertonic saline (2 M NaCl, 24 $\mu l \cdot kg^{-1} \cdot min^{-1}$) was infused instead of (but at the same rate as) the isotonic saline for 120 min, delivering 48 $\mu mol \cdot kg^{-1} \cdot min^{-1}$ of NaCl compared with 3.1 $\mu mol \cdot kg^{-1} \cdot min^{-1}$ during isotonic infusion. Therefore, the total rate of infusion of fluids was always constant, 52 $\mu l \cdot kg^{-1} \cdot min^{-1}$ (arterial, 8, PAH, 12, inulin, 8 and saline, 24 $\mu l \cdot kg^{-1} \cdot min^{-1}$), that is, around 12 μl/min; only the amount of dissolved NaCl varied between experiments.

During experiments, the chronic bladder catheter was connected to a drainage tube containing a thin flushing line, so that the tip of the flushing line protruded a few millimeters beyond the chronic catheter inside the bladder. At the end of each urine collection period, the bladder was flushed 3 times with 0.5 ml of sterile water.

Urine was collected in eight consecutive 30-min periods. Arterial blood samples (0.75 ml) were taken for measurement of plasma electrolytes, osmolality, PAH, inulin, and renin concentration. At the end of each experiment, the erythrocytes of the blood samples were suspended in saline and reinfused. After completing the experiments, each rat was euthanized with an overdose of pentobarbital, and both kidneys were removed for measurement of tissue norepinephrine content. The harvested kidneys were weighed, frozen rapidly in liquid nitrogen, and stored at −80°C until analyzed.

In sodium loading experiments, hypertonic saline replaced the isotonic saline for the four 30-min experimental periods. The sodium loading was performed in sham-denervated and denervated rats during blockade of nNOS with SMTC (S-methyl-L-thiocitrulline, 20 μg/kg/min; Sigma Chemicals, St. Louis, MO). The SMTC was dissolved in the saline, isotonic as well as hypertonic, and infused from the very beginning of the experiment (equilibration period), so that the administration of SMTC did not change the volume infused to the rats.

Analyses

The concentration of sodium and potassium ions in plasma and urine was measured by flame photometry (Model 943, Instrumentation Laboratory, Lexington, MA). Plasma and urine osmolalities were determined by freezing point depression (Osmomat 030-D, Gonotech, Berlin, Germany). The concentration of PAH in urine and plasma was measured by a colorimetric method using *N*-ethyldiamine-dihydrochloride. Inulin was measured by an enzymatic method (9). Briefly, inulin was hydrolyzed by inulinase (Inulinase I-2017, Sigma-Aldrich Denmark, Vallensbaek, Denmark) to fructose, which was converted to sorbitol by sorbitol dehydrogenase; the associated decrease in NADH concentration was measured at 340 nm. The analyses of PAH and inulin were adapted to microtiter plates, and the results were quantified by a microplate reader (VERSAmax, Molecular Devices, Sunnyvale, CA). PRC was determined by the antibody-trapping method of Poulsen and Jorgensen (26). The intra- and interassay coefficients of variation were 6% and 7%, respectively.

Norepinephrine determination in kidneys. Kidneys were stored at −80°C until HPLC analysis. Before analysis, whole kidneys (about 0.8 g) were homogenized (Polytron PT3100, Glen Mills, Clifton, NJ) in 5 ml 0.1 M perchloric acid (PCA, Merck, Darmstadt, Germany) with antioxidants (0.2 g/l $Na_2S_2O_5$, 0.05 g/l EDTA, Merck) at 0°C. After centrifugation, the norepinephrine content of the supernatant was determined by HPLC with electrochemical detection, essentially as described for dopamine analysis in brain tissue (10), but using a modified mobile phase [0.1 M sodium acetate (Sigma Chemicals), 2.0% methanol, (Merck, Darmstadt, Germany); 144 mg/l n-octyl sodium sulfate (Merck) and 13 mg/l disodium ethylenediaminetetraacetate (Na_2EDTA; Sigma) adjusted to pH 4.1 by glacial acetic acid (Merck)]. The kidney extracts were diluted 2–16-fold in mobile phase before injection (10 or 20 μl). Norepinephrine peaks were quantified by comparison with an external norepinephrine standard (Sigma Chemicals; 0.5 pmol) using chromatographic data software (model D-7000 HPLC system software manager ver. 2.0, Merck-Hitachi).

Early Effects of Chronic Renal Denervation on Water and Sodium Balance

Female Sprague-Dawley rats weighing 199 ± 3 g, were fed a standard diet, 0.25% Na (ssniff, Soest, Germany) and had free access to water. The experiments were performed in accordance with the National Institutes of Health's *Guide for the Care and Use of Laboratory Animals* and were approved by Ethical Committee of the Medical Research Centre, Polish Academy of Sciences, Warsaw.

One group of rats was denervated (DNX, $n = 6$) using the technique described in part I. Rats were anesthetized with intraperitoneal pentobarbital sodium (50 mg/kg body wt; Biowet, Pulawy, Poland). For postoperative analgesia, Metacam (0.2 mg/kg body wt; Boehringer Ingelheim, Ingelheim, Germany) was given subcutaneously. Sham-denervated rats ($n = 6$) had similar surgery but without mechanical or chemical kidney denervation.

Experimental Procedure

At the start of experiments, the rats were placed in metabolic cages (Tecniplast Gazzada, Buguggiote, Italy). The cages were cleaned with ethanol and rinsed with distilled water before the start of a control period and on the day of surgery. Body weight, water, and food intake, volume, and osmolality of collected urine, as well as its sodium concentration, were measured daily. Fecal sodium excretion was ignored, since rats kept on an ordinary diet excrete only 10 to 15% of the ingested sodium in the feces (20, 31). After three control days, the rats were renally denervated as described above. After return to the metabolic cages, the usual procedures were performed for three postoperative days. Subsequently, the animals were anesthetized with sodium thiopental (Sandoz, Kundl, Austria; 100 mg/kg ip), and both kidneys were removed for tissue norepinephrine determination (identical to *protocol I*).

Analyses

Urine osmolality was determined by freezing-point depression using a semi-microosmometer (Knauer ML, Bad Homburg, Germany) and sodium concentrations by flame photometry (Jenway PFP7; Essex, England).

Acute Effect of Renal Denervation on MABP

Female Sprague-Dawley rats ($n = 6$) weighing 270–280 g were fed a standard diet (ssniff, Soest, Germany) and had free access to tap water. The experiments were performed in accordance with the National Institutes of Health's *Guide for the Care and Use of Laboratory Animals* and were approved by Ethical Committee of the Medical Research Centre, Polish Academy of Sciences, Warsaw.

Animals were anesthetized with sodium thiopental (Sandoz, Kundl, Austria) (100 mg/kg ip) and placed on a heated device so that rectal temperature was maintained at about 37 C. After intubation and arterial and venous cannulation, 3% bovine serum albumin in saline was infused intravenously during surgery at 2.7 ml/h. After completion of surgery, 0.9% saline was infused at the same rate. Both kidneys were accessed by flank incisions. Loops of stainless steel wire (0.1 mm diameter) were placed bilaterally around the fat and connective tissue located cephalad to the junction of the renal artery and aorta. On each side, both ends of each loop were threaded through a segment of thick-walled polyethylene tubing, and left loose. The left kidney was immobilized in a plastic holder to allow measurement of local perfusion in the cortex, outer and inner medulla by the laser Doppler Periflux 4001 system (Perimed AB, Jarfalla, Sweden).

To denervate the kidney, the loop was tightened and fixed with a bulldog clamp. The tissue within the loop, including renal nerve fibers was electrocoagulated by neurosurgical cautery device (Famed, Warsaw, Poland) until the loop could be removed (14). For sham denervation ($n = 6$), fat and connective tissue in the area caudal to the kidneys were sequentially coagulated. After a 1-h equilibration period and control measurements, the right or left kidney (in random order) was denervated, and the effects on MABP and HR measured for 30 min. Subsequently, the remaining innervated kidney was denervated, and measurements were obtained for another 30-min period.

Statistics

Data are presented as means ± SE. The results were evaluated by one-way ANOVA for repeated measurements. If the results of ANOVA were significant ($P < 0.05$), differences between means were investigated with Newman-Keuls' test or by a modified Student's *t*-test for independent variables (35). Specific differences were tested by Student's *t*-test as indicated in the text.

RESULTS

Norepinephrine Content in Denervated Kidneys

Renal tissue norepinephrine was reduced to about 5% of control concentrations in denervated rats compared with sham-denervated rats both in *protocol I* (0.10 ± 0.10 vs. 2.1 ± 0.1 nmol/g kidney, $P < 0.001$) and in *protocol II* (0.06 ± 0.02 vs. 1.30 ± 0.07 nmol/g kidney, $P < 0.001$). Therefore, it is reasonable to assume that the denervation procedure was effective.

Effect of Salt Loading on MABP and Renal Functions

Effect of renal denervation per se on MABP, plasma renin concentration, and renal hemodynamics. The procedures for the time control and salt-loading experiments in innervated and

denervated rats were identical until the initiation of the sodium load. Therefore, the individual means of the MABP control period values for each rat in the isotonic (control) and the hypertonic (Hyp) series (Fig. 1) were pooled to give the best estimate of the basal MABP for denervated and for sham rats ($n = 16$ and 18, respectively). Basal MABP in DNX rats was significantly less than in the sham rats (103 ± 2 vs. 113 ± 2 mmHg, respectively, $P < 0.002$).

The basal plasma renin concentration [similarly pooled from control values for each rat in isotonic and hypertonic infusion group (Fig. 2)] was also significantly lower in DNX than in sham rats (15 ± 1 vs. 19 ± 1 mIU/l, respectively, $P < 0.005$).

In contrast to MABP and PRA, the basal values of GFR, RPF, and the urinary excretion rates of water and sodium were not significantly altered by renal denervation. Basal sodium excretion ranged from 1.1 ± 0.3 to 3.3 ± 0.7 μmol/min (Fig. 3). The basal GFR values were 2.3 ± 0.2 ml/min for DNX and 2.5 ± 0.1 ml/min for sham rats (Table 1), as well as basal RPF of 7.9 ± 0.5 ml/min and 8.1 ± 0.2 ml/min for DNX and SHAM, respectively (Fig. 4).

Effect of Slow Salt Loading

The slow salt loading increased MABP by 5–7 mmHg ($P < 0.05$), similarly in DNX and sham rats ($n = 12$ in each group, Fig. 1), surprisingly, without any significant changes in HR (Table 1). In association with the sodium loading, plasma renin concentration decreased significantly in both groups of rats (Fig. 2). The relative changes in plasma renin were very similar irrespective of the starting level; in sham-operated rats, the renin concentration decreased by 49%, and the decrease in DNX rats averaged 43%. In the time control group, PRC tended to decrease; however, this decrease (3.6 ± 1.4 mIU/l was not significant per se, but was significantly smaller than the decrease occurring during saline infusion 9.4 ± 1.3 mIU/l, $P = 0.014$, Student's t-test). The decrease in the control experiments in the renal denervated animals remains unexplained.

Salt loading increased the excretion of sodium ($U_{Na}V$) by six-fold (Fig. 3) and urine flow (V) by two-fold, in DNX, as well as in sham rats without measurable changes in GFR or potassium excretion (Table 1). RPF increased progressively, and the increase reached significance in the 2nd h of the hypertonic infusion (Fig. 4). Both in DNX and sham rats, the salt infusion induced a statistically significant ($P < 0.01$) increase in plasma sodium concentration, by 4 ± 2 mmol/l and 5 ± 1 mmol/l, respectively (Table 1). Substantial changes in plasma potassium were not observed in any series (Table 1).

The constant, basal slow infusion of isotonic saline in the control experiments did not induce any changes in the measured parameters, apart from a modest decrease in PRC observed in DNX rats (Table 1, Fig. 2).

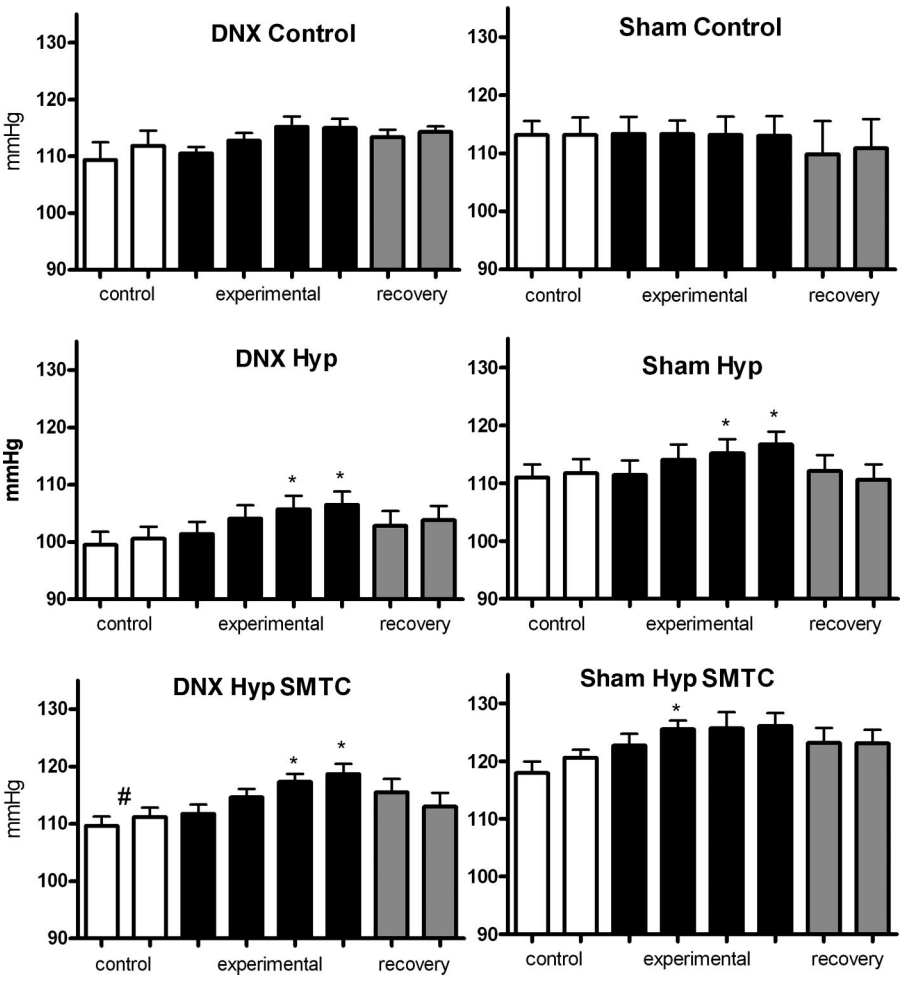

Fig. 1. The responses of mean arterial blood pressure (MABP) to control conditions (Control) and sodium loading [hypoxia (Hyp), 50 μmol·kg^{-1}·min^{-1}] conditions in denervated (DNX) and sham-denervated rats, untreated or pretreated with nNOS inhibitor S-methyl-thiocitrulline (SMTC) (20 μg·kg^{-1}·min^{-1}). Bars represent mean values ± SE in 30-min collecting periods. *Significantly different from two control periods at $P < 0.05$. #Significantly different from mean value of two control periods in rats untreated with SMTC, at $P < 0.05$.

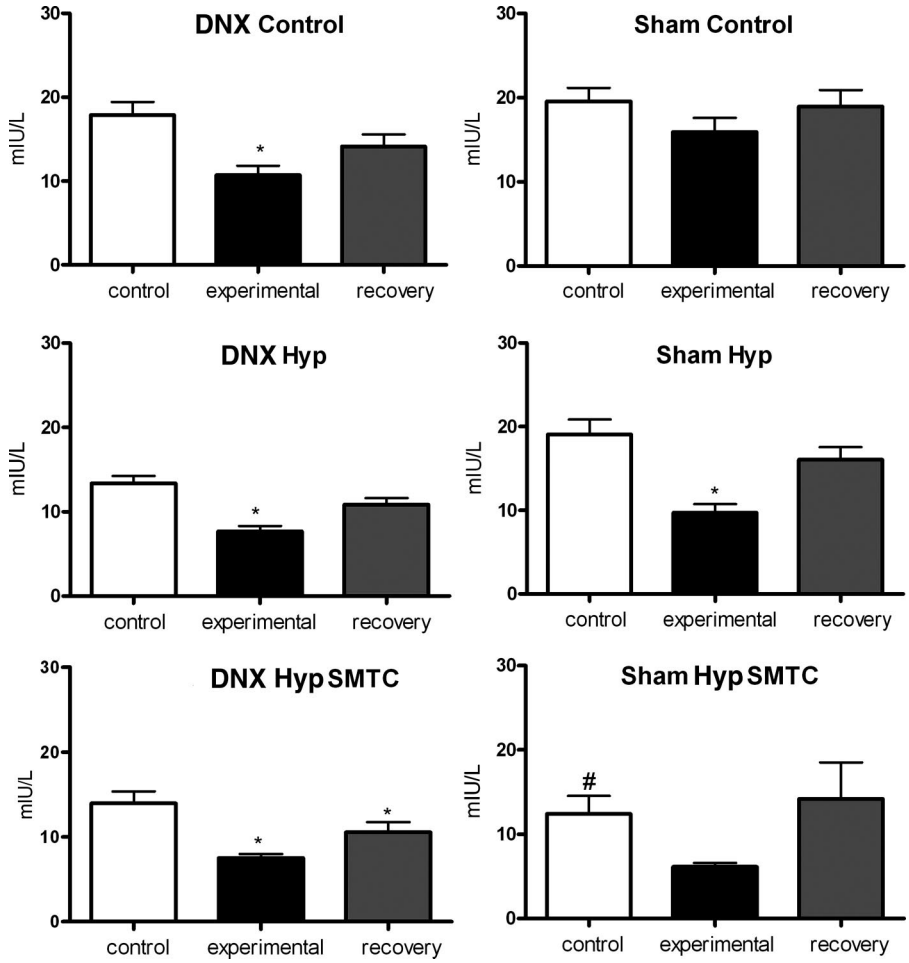

Fig. 2. Plasma renin concentration during control and sodium loading conditions (Hyp, 50 μmol·kg^{-1}·min^{-1}) in DNX or sham-denervated conscious rats, untreated or pretreated with nNOS inhibitor SMTC (20 μg·kg^{-1}·min^{-1}). Bars represent values ± SE from last control, experimental, and recovery period. *Significantly different from control period at $P < 0.05$. #Significantly different from control period in rats untreated with SMTC, at $P < 0.05$.

Effect of Salt Loading After Inhibition of nNOS

After acute blockade of nNOS with SMTC, the basal MABP was higher ($P < 0.02$) by almost 10 mmHg in both DNX and sham rats (both $n = 6$, Fig. 1); that is, SMTC increased MABP irrespective of the presence of renal nerves. On the other hand, only in sham rats did such pretreatment result in lower basal PRC (12 ± 2 mIU/l compared with 19 ± 2 mIU/l, $P < 0.005$, Fig. 2); that is, in the DNX rats, PRC was not decreased further by SMTC treatment.

Irrespective of renal denervation, the salt loading in SMTC-treated rats induced an increase in MABP that was not different from that observed in control rats (Fig. 1). However, in DNX rats, this increase in MABP was accompanied by a significant decrease in HR (Table 1). The sham-operated SMTC rats showed a similar, but insignificant trend, toward HR reduction.

Plasma renin concentration invariably decreased during salt loading. In the sham-operated, SMTC-treated rats, the decrease did not reach statistical significance by standard ANOVA (Fig. 2). However, this is due to the large variation in the rebound of PRC after the infusion; the 51% decrease elicited by the salt loading is highly significant (paired *t*-test, $P = 0.013$). In the SMTC-treated DNX rats, plasma renin concentration fell by 46%.

In SMTC pretreated rats, the salt loading significantly increased $U_{Na}V$ and urine flow (V), both in DNX and sham-operated animals (Fig. 3, Table 1) There were no differences in $U_{Na}V$ and V between individual groups of rats, neither in absolute values nor in the areas under the curve. Sodium excretion decreased during the 1-h recovery period but was still significantly different from control values in each group (Fig. 3). The increase in MABP induced by SMTC was not associated with increased renal sodium excretion.

Intriguingly, the increases in plasma sodium level and RPF accompanying the salt loading in control animals were not present during nNOS inhibition (Table 1, Fig. 4), similarly in renal denervated and sham-operated rats.

In summary, chronic renal denervation caused simultaneous decreases in MABP and plasma renin concentration; however, the acute decrease in plasma renin and the natriuretic response to sodium loading were independent of the renal nerves and pretreatment with SMTC.

Early Effects of Chronic Renal Denervation on Changes in Water and Sodium Balance

Normal rats show positive sodium balance also in the days immediately before and after surgery (cf. Fig. 5). In DNX rats, this sodium retention was severely impaired during the first two days (significant on *day 2*, $P < 0.001$) (Fig. 5). The impairment was transient; on *day 3*, results from DNX and sham rats were very similar with regard to levels and patterns of sodium retention. Matching the changes in sodium metabolism, water retention during the two first postsurgical days

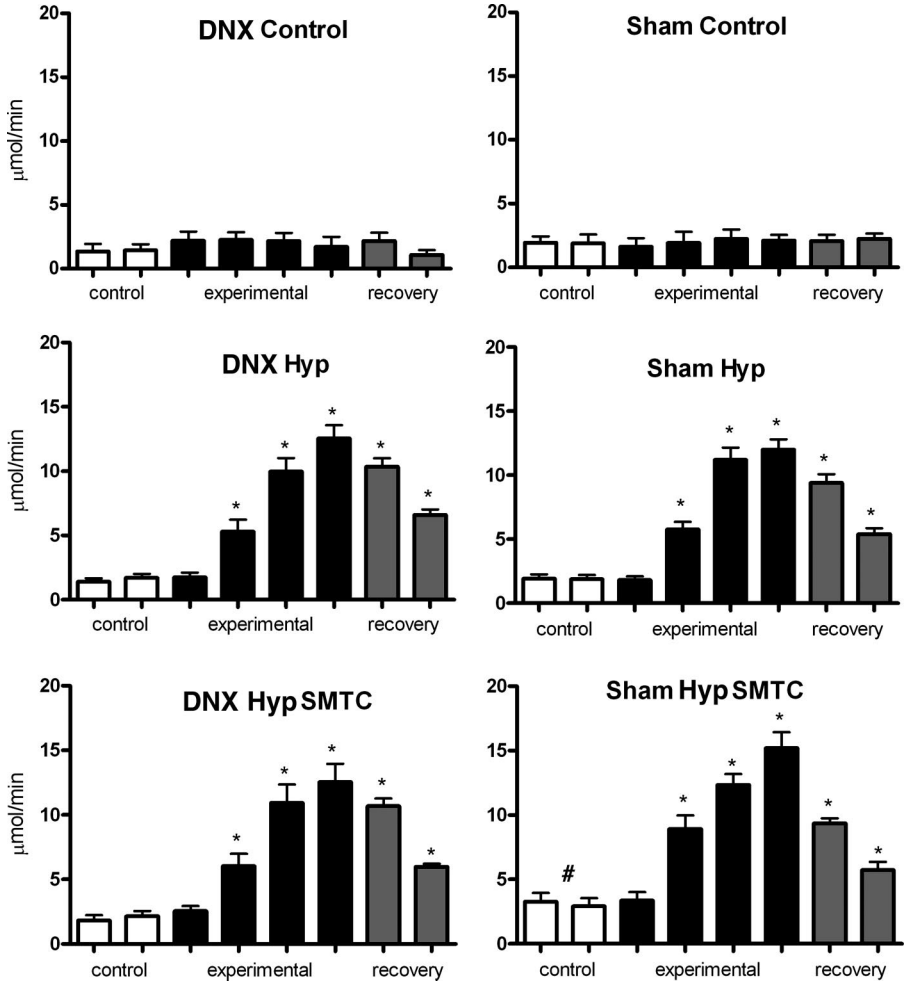

Fig. 3. Sodium excretion ($U_{Na}V$) during control and sodium loading conditions (Hyp, 50 μmol/kg/min) in DNX and sham-denervated conscious rats, untreated or pretreated with nNOS inhibitor SMTC (20 $\mu g \cdot kg^{-1} \cdot min^{-1}$). Bars represent mean values ± SE in 30-min collecting periods. *Significantly different from two control periods at $P < 0.05$. #Significantly different from mean value of two control periods in rats untreated with SMTC, at $P < 0.05$.

was ~5 $ml \cdot rat^{-1} \cdot day^{-1}$ less in denervated than in innervated rats (data not shown).

Acute Effect of Renal Denervation on MABP

In the anesthetized rats, MABP decreased from 128 ± 4 to 118 ± 5 mmHg ($P < 0.05$) within the first 30 min after one kidney denervation and to 108 ± 7 mmHg ($P < 0.001$) within next 30 min after second kidney denervation (Fig. 6) without any measurable changes in HR. The decrease in MABP was accompanied by significant increases in cortical and medullary blood perfusion (Fig. 6), supporting the effectiveness of renal denervation (14, 36). In anesthetized rats exposed to sham surgery, neither MABP nor local renal blood flow indices changed significantly. After sham surgery, the cortical, outer medullary, and inner medullary flow indices were changed by 3.2 ± 2.7%, −5.6 ± 3.1%, and 2.8 ± 2.7% compared with control, respectively. Following denervation, the corresponding changes were 32 ± 10%, 62 ± 6%, and 50 ± 6%, respectively. For each region, the difference was highly significant ($P < 0.01$).

DISCUSSION

The experiments were designed to elucidate the participation of the renal nerves and nNOS in the deactivation of the renin system and the natriuresis elicited by an acute, but modest, sodium load. The main result is that, under the present circumstances, neither renal nerve activity nor nNOS seems to be indispensable either with regard to the relative inhibition of renin secretion or to the natriuretic response. However, the results also confirm that the renal nerves do participate in the regulation of sodium balance and arterial blood pressure. Formally, it cannot be excluded that renal nerve activity and/or nNOS-derived NO normally mediate part of the natriuretic response and that after renal denervation, other mechanisms are upregulated to an extent that makes the natriuretic responses before and after denervation indistinguishable. However, the present protocol was not designed to address this issue, and in any case, the results indicate that renal nerve activity and nNOS-derived NO are not necessary for the natriuretic response.

The results obtained in the control situations before the sodium loading illustrate the importance of renal nerves in the context of the long-term regulation of arterial blood pressure and plasma renin concentration. In these conscious, renally denervated rats, both MABP and PRC were significantly less than in the sham-operated controls; however, GFR and sodium and water excretion were very similar. The reduction in MABP was modest (<10%) and evident only when data from all animals were pooled. These findings are concordant with previous studies. In a similar setting, Jacob et al. (13) showed

Table 1. *Heart rate, plasma, and renal variables during control conditions and sodium loading (Hyp, 50 μmol·kg^{-1}·min^{-1}) in DNX and sham denervated conscious rats, untreated or pretreated with nNOS inhibitor SMTC*

		Experimental Series					
	Time, min	Sham Control	DNX Control	Sham Hyp	DNX Hyp	Sham Hyp SMTC	DNX Hyp SMTC
HR, beats/min	0–30	441±9	415±16	422±9	402±20	412±14	410±11
	30–60	435±7	420±15	425±20	397±15	409±10	407±12
	60–90	433±8	408±14	411±8	408±25	413±18	408±13
	90–120	418±9	399±16	416±9	403±14	399±16	381±9*
	120–150	423±8	404±25	403±5	400±13	389±10	366±13*
	150–180	421±9	401±20	412±10	387±11	385±13	368±14*
	180–210	414±16	406±17	422±6	387±8	396±7	387±12*
	210–240	417±14	407±22	423±5	398±8	404±11	387±11*
P(Na$^+$), mmol/l	0–60	142.8±2.0	145.8±1.7	136.9±1.5	136.8±1.5	139.1±1.4	142.1±3.3
	60–180	139.4±2.9	145.1±1.4	141.9±1.8*	141.0±1.3*	136.0±2.8	143.8±2.6
	180–240	138.4±3.3	144.4±2.1	137.1±2.5	138.7±1.5	138.8±3.0	145.2±1.8
P(K$^+$), mmol/l	0–60	4.3±0.2	4.5±0.1	4.2±0.1	4.0±0.1	4.1±0.1	4.2±0.1
	60–180	4.5±0.2	4.2±0.1	4.0±0.1	3.8±0.1	3.8±0.1	4.3±0.3
	180–240	4.2±0.2	4.1±0.1	4.0±0.1	3.9±0.1	3.9±0.2	4.1±0.1
P(Osm), mOsm/kg	0–60	292.7±0.7	293.8±3.3	294.1±2.8	291.7±1.6	295.0±1.1	294.7±0.4
	60–180	290.7±0.8	289.8±2.3	297.0±2.5	291.6±3.0	293.0±1.4	298.0±0.7*
	180–240	291.2±1.2	291.8±2.8	294.5±1.5	292.6±3.3	295.0±1.6	295.1±1.5
GFR, ml/min	0–30	2.5±0.2	2.1±0.3	2.4±0.2	2.2±0.3	2.7±0.1	2.2±0.3
	30–60	2.4±0.2	2.6±0.3	2.8±0.2	2.5±0.3	2.8±0.2	2.6±0.2
	60–90	2.2±0.2	2.3±0.4	2.6±0.1	2.5±0.3	2.9±0.1	2.9±0.3
	90–120	2.4±0.1	2.4±0.4	3.0±0.2	2.7±0.3	3.3±0.2	2.9±0.3
	120–150	2.8±0.1	2.5±0.4	3.1±0.1	2.8±0.3	3.1±0.1	3.4±0.6
	150–180	2.5±0.4	2.4±0.4	2.8±0.1	2.9±0.3	3.1±0.1	3.3±0.6
	180–210	2.8±0.4	2.4±0.5	2.7±0.1	2.6±0.4	2.7±0.2	2.7±0.2
	210–240	2.1±0.4	2.3±0.5	2.5±0.2	2.6±0.4	2.5±0.2	2.4±0.1
Diuresis, μl/min	0–30	19±4	14±2	17±2	14±2	28±5	18±3
	30–60	16±4	16±3	17±2	16±2	24±4	17±2
	60–90	14±5	16±6	14±2	12±2	19±3	16±3
	90–120	11±2	16±4	25±4*	20±2	31±4	19±4
	120–150	12±2	15±2	38±3*	33±3*	41±2*	36±5*
	150–180	13±2	15±2	39±3*	39±3*	50±4*	42±5*
	180–210	13±2	11±3	28±2*	30±1*	27±1	33±2*
	210–240	9±1	11±4	15±1	19±2	15±2	17±2
K$^+$-exc, μmol/min	0–30	2.5±0.6	2.4±0.2	2.6±0.3	2.5±0.3	3.0±0.3	2.8±0.4
	30–60	1.9±0.4	2.6±0.2	2.7±0.3	2.8±0.2	2.6±0.2	2.9±0.2
	60–90	1.5±0.3	2.0±0.2	2.4±0.2	2.2±0.2	2.2±0.2	2.4±0.1
	90–120	1.5±0.4	2.1±0.1	3.0±0.3	2.7±0.2	2.6±0.2	2.2±0.2
	120–150	1.6±0.5	2.0±0.1	3.2±0.2	3.0±0.2	2.6±0.1	2.4±0.2
	150–180	1.2±0.4	1.8±0.2	2.8±0.2	2.8±0.1	2.6±0.1	2.4±0.2
	180–210	1.2±0.3	1.7±0.1	2.3±0.1	2.3±0.1	2.0±0.1*	2.2±0.2
	210–240	1.3±0.4	1.6±0.1*	1.8±0.1*	1.9±0.1*	1.7±0.1*	1.7±0.2*

Values are given as means ± SE for 30-min periods. DNX, denervated; HR, heart rate; P(Na$^+$), plasma sodium concentration; P(K$^+$), plasma potassium concentration; P(osm), plasma osmolality; GFR, glomerular filtration rate; K$^+$-exc, potassium excretion. *Significantly different from both control periods, $P < 0.05$.

that MABP was less than control from *day 1* after denervation. Other results have shown a modest decrease in systolic blood pressure (by tail cuff) after renal denervation (8). However, the time course of the development of hypotension after renal denervation has been unclear. In our acute studies in anesthetized rats, MABP was reduced within 60 min; it is possible that the well-known renal-denervation diuresis and natriuresis (14, 32) play a role in the hypotension, but this issue was not addressed by the present study. Neither do our study designs allow identification of the mechanisms involved in the permanent change in MABP, except that the constancy of both renal blood flow and filtration rate makes it less likely that renal hemodynamics are involved. The measured acute increase in renal blood flow associated with renal denervation could contribute to the short-term deficit in sodium balance. However, as the deviation in sodium balance was transient, this is unlikely to explain the chronic decrease in MABP. In the absence of lasting changes in sodium balance (compared to the steady positive balance of appropriate control animals), the present 20% decrease in PRC, confirming previous results of renal denervation (8, 18), does not provide a convincing explanation of the relative hypotension. Only further studies, including measurements of sympathetic tone and vasoactive hormones, may disclose these mechanisms.

Our results clearly show that the control levels of PRC are dependent on the presence of both renal sympathetic nerves and nNOS. PRC was decreased in both denervated and in SMTC-pretreated rats (Fig. 2), suggesting either that the renal nerves stimulate renin release via NO derived from neuronal isoform or that both renal nerve activity and nNOS activity are necessary for effective renin stimulation by other mediators. The latter possibility fits the notion that renin secretion is orchestrated through a number of mediators (30).

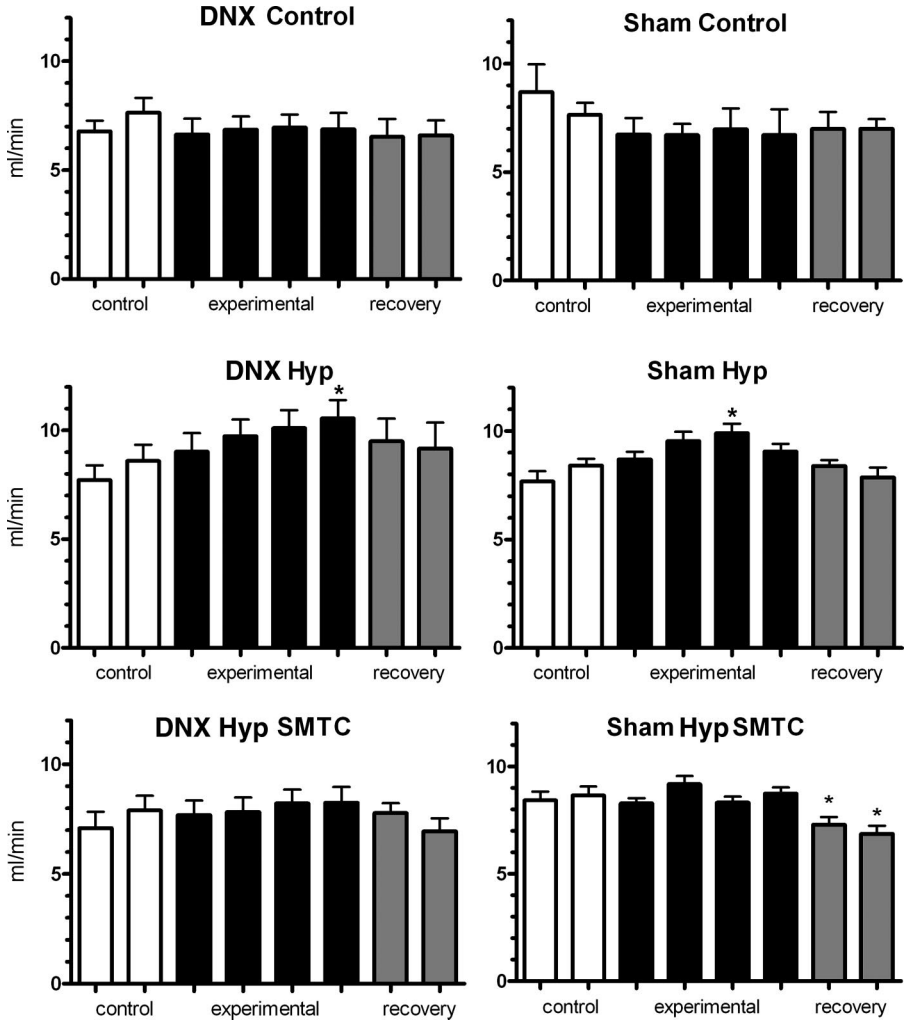

Fig. 4. Renal plasma flow (RPF) during control and sodium loading conditions (Hyp, 50 μmol·kg^{-1}·min^{-1}) in DNX and sham-denervated conscious rats, untreated or pretreated with nNOS inhibitor SMTC (20 μg·kg^{-1}·min^{-1}). Bars represent mean values ± SE in 30-min collecting periods. *Significantly different from two control periods at $P < 0.05$.

The magnitude of the increase in MABP and sodium and water excretion in response to slow sodium loading was independent of renal innervation and nNOS activity. There is little doubt that the increase in MABP, and the simultaneous enhancement in renal plasma flow observed during the sodium loading, could contribute to the augmented sodium and water excretion, probably through both pressure natriuresis and medullary concentration gradient washout mechanisms. Rates of

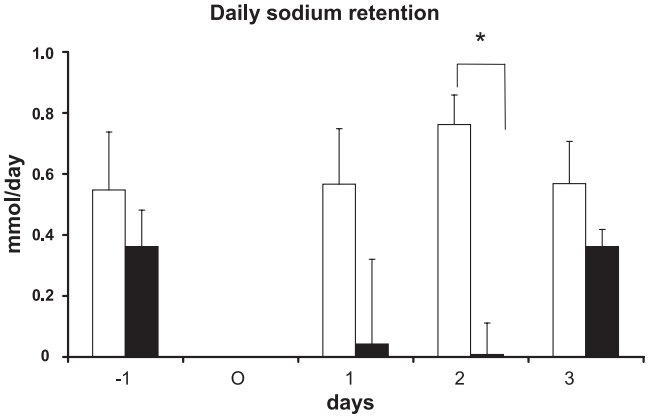

Fig. 5. Effect of renal sympathetic denervation on changes in sodium retention measured in control (sham operated, open bars) and denervated (solid bars) conscious rats. Bars represent mean values ± SE for daily sodium retention before (−1 day, control period) and during 3 consecutive days after surgery (O, day of surgery). *Significant difference between innervated and denervated rats at $P < 0.001$.

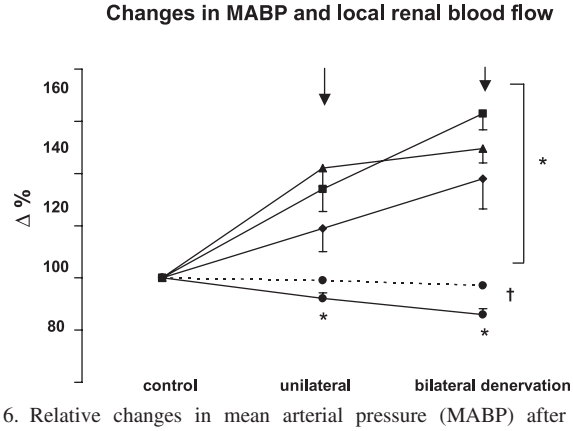

Fig. 6. Relative changes in mean arterial pressure (MABP) after acute, consecutive kidney denervation (●, solid line) and sham (●, dotted line) denervation. The effect of denervation on cortical (CBF, ◆), outer- (OMBF, ■) and inner-medullary blood flow (IMBF, ▲) of the left kidney. Arrows: values seen 30 min later after first and second denervation. *Significantly different from predenervation control at $P < 0.05$. †Change from control significantly different from that seen after sham denervation ($P < 0.01$).

sodium loading smaller than those presently applied are necessary to avoid direct activation of the pressure natriuresis mechanism. In preliminary experiments, we have found a decrease in RSNA without changes in MABP in rats receiving a smaller load of hypertonic saline (20 μmol NaCl·kg^{-1}·min^{-1}).

Our results indicate that NO produced by nNOS is not an important factor mediating the decrease in PRC during sodium loading, at least under the present conditions, including an increase in MABP. This decrease in PRC occurring irrespective of renal nerve activity and nNOS, might well be the most important determinant of the magnitude of the excretory responses. The role of NO on renin release is still controversial. Many investigators have reported that NO stimulates renin secretion, both in vivo (21, 25, 29) and in the perfused kidney (15, 16), while early work reported an inhibitory NO effect on renin secretion (39). Some authors suggest that NO from the macula densa cells may stimulate renin release, while NO derived from the endothelial cells in the afferent arteriole may inhibit renin secretion (2, 4, 12). Current concepts are seemingly rather complex (30). However, the results of Castrop et al. (6) in nNOS-deficient mice seem interesting in the current context. These mice show significantly reduced basal PRC, while their macula densa-mediated control of renin secretion seems dependent on a source of NO other than nNOS. The decrease in basal PRC with SMTC treatment in the present experiments was not proven to be due to changes in nNOS activity (see below); however, the finding that in our denervated animals, nNOS inhibition did not change PRC, despite an increase in MABP, indicates that the small pressure changes in the present study are not per se sufficient to change PRC significantly. Therefore, the decrease in PRC seen in the innervated animals after SMTC treatment can be assumed to be due to nNOS inhibition and thus compatible with the results obtained in the nNOS-deficient mice (6). Our finding that the intact homeostatic regulation of PRC occurs independent of SMTC treatment, seems more directly concordant with the findings of Castrop et al. (6). It is remarkable that our data show that the acute sodium load inhibited PRC very similarly (by 43% to 51%), irrespective of renal denervation and/or SMTC treatment.

In our study, the decrease in renin concentration was tightly linked to the increase of MABP during sodium loading. Because the increase in MABP and the associated change in shear stress would raise eNOS activity, renin concentration might be decreased even in SMTC-pretreated animals where nNOS, but not eNOS, would be blocked. However, the change in MABP will also affect renin secretion directly. This complexity emphasizes the need for further studies of subtle salt loading unable to generate changes in MABP, renal blood flow, or glomerular filtration rate.

We hypothesized that an augmented NO production by nNOS could be a link between hypertonic loading and the ensuing natriuresis. A diminished effect of RSNA, directly by reducing the neurally mediated tubular transport and/or indirectly by other mechanisms, would evoke a more efficient excretion of sodium and water in innervated rats with intact nNOS. However, this hypothesis was not supported by our results, as we found very similar responses in renal excretion due to the sodium loading in both groups. It is difficult to predict the final excretory effect in a situation of systemic NO blockade, as nNOS is involved at many levels in regulatory mechanisms, also extrarenally. For instance, a lack of presynaptic inhibitory influence of NO in SMTC-pretreated rats, would augment the neurogenic vascular tone, could result in a more pronounced increase in MABP, and accelerate sodium excretion during hypertonic saline loading. Alternatively, elevated baroreflex sensitivity would more effectively limit further increases in MABP during hypertonic infusion and the potentially higher sodium and water excretion in SMTC-pretreated rats. Additional experiments, including measurement of sympathetic nerve activity in rats during nNOS inhibition, are required to explore these mechanisms.

In summary, the present results support the notion that the renal nerves play an important role in the maintenance of arterial blood pressure and renin secretion; in contrast, data indicate that neither renal nerve activity nor nNOS is essential to the regulatory response to a modest sodium load, at least not under the present conditions, including a slight increase in MABP. The increase in MABP and the decrease in plasma renin concentration, but not changes in renal sympathetic nerve and nNOS activity, seem to be the crucial factors responsible for the regulatory changes in sodium and water excretion. Except for excessive intakes of salt, sodium balance is achieved without changes in arterial blood pressure. Therefore, sodium loading should preferably be studied in the absence of blood pressure changes. This is possible either by using smaller salt loads or by active control of arterial pressure. Clearly, direct measurements of renal sympathetic nerve activity under such conditions would provide interesting results.

Perspectives and Significance

The present data indicate that long-term regulation of renin secretion and blood pressure is dependent on renal innervation. However, the importance of the renal nerves and nNOS activity for the acute homeostatic responses remains essentially unresolved. Integrative studies, such as the present, expose the redundancy of the regulatory systems; maintenance of efficient regulation despite the exclusion of a system indicates that its function is replaced by another system to an extent that prevents identification of the excluded system by the present tools. Our data confirm that even modest blood pressure increases are important to the acute regulation of the renal sodium excretion in the rat. In the absence of blood pressure changes, other mediators, such as RSNA and nNOS-derived NO, are operative and functionally important; studies in dogs and humans (3, 27, 28) have shown that marked natriuresis may result from acute salt loading, which does not affect mean arterial blood pressure at all. One of the conclusions of the present investigation, therefore, is that the neurohumoral control of renal excretion in the conscious rat should be studied by procedures that do not generate changes in renal arterial blood pressure, i.e., under conditions emulating the normal homeostatic regulation.

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REFERENCES

1. **Andersen LJ, Jensen TU, Bestle MH, Bie P.** Gastrointestinal osmoreceptors and renal sodium excretion in humans. *Am J Physiol Regul Integr Comp Physiol* 278: R287–R294, 2000.
2. **Beierwaltes WH.** Selective neuronal nitric oxide synthase inhibition blocks furosemide-stimulated renin secretion in vivo. *Am J Physiol Renal Fluid Electrolyte Physiol* 269: F134–F139, 1995.
3. **Bie P, Wamberg S, Kjolby M.** Volume natriuresis vs. pressure natriuresis. *Acta Physiol Scand* 181: 495–503, 2004.
4. **Braam B.** Renal endothelial, and macula densa NOS: integrated response to changes in extracellular fluid volume. *Am J Physiol Regul Integr Comp Physiol* 276: R1551–R1561, 1999.
5. **Brubacher ES, Vander AJ.** Sodium deprivation and renin secretion in unanesthetized dogs. *Am J Physiol* 214: 15–21, 1968.
6. **Castrop H, Schweda F, Mizel D, Huang Y.** Permissive role of nitric oxide in macula densa control of renin secretion. *Am J Physiol Renal Physiol* 286: F848–F857, 2004.
7. **DiBona GF.** Physiology in perspective: The wisdom of the body. Neural control of the kidney The Walter B Cannon Memorial Award Lecture. *Am J Physiol Regul Integr Comp Physiol* 289: R633–R641, 2005.
8. **Fernandez-Repollet E, Silva-Netto CR, Colindres RE, Gottschalk CW.** Role of renal nerves in maintaining sodium balance in unrestrained conscious rats. *Am J Physiol Renal Fluid Electrolyte Physiol* 249: F819–F826, 1985.
9. **Gabel RA, Ranaei RA, Kivlighn SD.** A new method of measuring renal function in conscious rats without the use of radioisotopes. *J Pharmacol Toxicol Methods* 36: 189–197, 1996.
10. **Gramsbergen JB, Sandberg M, Dall AM, Kornblit B, Zimmer J.** Glutathione depletion in nigrostriatal slice cultures: GABA loss, dopamine resistance and protection by the tetrahydrobiopterin precursor sepiapterin. *Brain Res* 935: 47–58, 2002.
11. **Grassi G.** Counteracting the sympathetic nervous system in essential hypertension. *Curr Opin Nephrol Hypertens* 13: 513–519, 2004.
12. **He XR, Greenberg SG, Briggs JP, Schnermann JB.** Effect of nitric oxide on renin secretion. II. Studies in the perfused juxtaglomerular apparatus. *Am J Physiol Renal Fluid Electrolyte Physiol* 268: F953–F959, 1995.
13. **Jacob F, Ariza P, Osborn JW.** Renal denervation chronically lowers arterial pressure independent of dietary sodium intake in normal rats. *Am J Physiol Heart Circ Physiol* 284: H2302–H2310, 2003.
14. **Kompanowska-Jezierska E, Walkowska A, Johns EJ, Sadowski J.** Early effects of renal denervation in the anaesthetised rat: natriuresis and increased cortical blood flow. *J Physiol* 531: 527–534, 2001.
15. **Kurtz A, Gotz KH, Hamann M, Wagner C.** Stimulation of renin secretion by nitric oxide is mediated by phosphodiesterase 3. *Proc Natl Acad Sci USA* 95: 4743–4747, 1998.
16. **Kurtz A, Wagner C.** Role of nitric oxide in the control of renin secretion. *Am J Physiol Renal Physiol* 275: F849–F862, 1998.
17. **Malpas SC, Leonard BL.** Neural regulation of renal blood flow: a re-examination. *Clin Exp Pharmacol Physiol* 27: 956–964, 2000.
18. **Mizelle HL, Hall JE, Woods LL, Montani JP, Dzielak DJ, Pan YJ.** Role of renal nerves in compensatory adaptation to chronic reductions in sodium intake. *Am J Physiol Renal Fluid Electrolyte Physiol* 252: F291–F298, 1987.
19. **Morita H, Vatner SF.** Effects of volume expansion and renal nerve activity, renal blood flow, and sodium and water excretion in conscious dogs. *Am J Physiol Renal Fluid Electrolyte Physiol* 249: F680–F687, 1985.
20. **Mu JY, Hansson GC, Lundgren O.** The intestinal tract and the pathophysiology of arterial hypertension: an experimental study on Dahl rats. *Acta Physiol Scand* 155: 137–146, 1985.
21. **Naess PA, Christensen G, Kirkboen KA, Kiil F.** Effect on renin release of inhibiting renal nitric oxide synthesis in anaesthetized dogs. *Acta Physiol Scand* 148: 137–142, 1993.
22. **Nakamura A, Johns EJ.** Effect of renal nerves on expression of renin and angiotensinogen genes in rat kidneys. *Am J Physiol Endocrinol Metab* 266: E230–E241, 1994.
23. **Petersen JS, Shalmi M, Lam HR, Christensen S.** Renal response to furosemide in conscious rats: effects of acute instrumentation and peripheral sympathectomy. *J Pharmacol Exp Ther* 258: 1–7, 1991.
24. **Peterson TV, Emmeluth C, Bie P.** Renal effects of nitric oxide synthase inhibition in conscious water-loaded dogs. *Am J Physiol Regul Integr Comp Physiol* 281: R584–R590, 2001.
25. **Persson PB, Baumann JE, Ehmke H, Hackenthal E, Kirchheim HR, Nafz B.** Endothelium-derived NO stimulates pressure-dependant renin release in conscious dogs. *Am J Physiol Renal Fluid Electrolyte Physiol* 264: F943–F947, 1993.
26. **Poulsen K, Jorgensen J.** An easy radioimmunological microassay of renin activity, concentration and substrate in human and animal plasma and tissues based on angiotensin I trapping by antibody. *J Clin Endocrinol Metab* 39: 816–825, 1974.
27. **Rasmussen MS, Simonsen JA, Sandgaard NCF, Hoilund-Carlsen PF, Bie P.** Mechanisms of acute natriuresis in normal humans on low-sodium diet. *J Physiol* 546: 591–603, 2003.
28. **Sandgaard NCF, Andersen JL, Bie P.** Hormonal regulation of renal sodium and water excreation during acute, non-hypertensive sodium loading in conscious dogs. *Am J Physiol Regul Integr Comp Physiol* 278: R11–R18, 2000.
29. **Schricker K, Hamann M, Kurtz A.** Nitric oxide and prostaglandins are involved in macula densa control of the renin system. *Am J Physiol Renal Fluid Electrolyte Physiol* 269: F825–F830, 1995.
30. **Schweda F, Friis U, Skott O, Kurtz A.** Renin release. *Physiology* 22: 310–319, 2007.
31. **Thiesson HC, Jensen BL, Bistrup C, Ottosen PD, McNeilly AD, Andrew R, Seckl J, Skott O.** Renal sodium retention in cirrhotic rats depends on glucocorticoid-mediated activation of mineralocorticoid receptor due to decreased renal 11β-HSD-2 activity. *Am J Physiol Regul Integr Comp Physiol* 292: R625–R636, 2007.
32. **Thorup C, Kurkus J, Morsing P, Persson AE.** Acute renal denervation causes time-dependent resetting of the tubuloglomerular feedback mechanism. *Acta Physiol Scand* 153: 43–49, 1995.
33. **Toda N, Okamura T.** The pharmacology of nitric oxide in the peripheral nervous system of blood vessels. *Pharmacol Rev* 55: 271–324, 2003.
34. **Trostel KA, Osborn JW.** Do renal nerves chronically influence renal function and arterial pressure in spinal rats? *Am J Physiol Regul Integr Comp Physiol* 263: R1265–R1270, 1992.
35. **Wallenstein S, Zucker CL, Fleiss JL.** Some statistical methods useful in circulation research. *Circ Res* 47: 1–9, 1980.
36. **Walkowska A, Kompanowska-Jezierska E, Sadowski J.** Nitric oxide and renal nerves: Comparison of effects on renal circulation and sodium excretion in anesthetized rats. *Kidney Int* 66: 705–712, 2004.
37. **Wangensteen R, Rodriguez-Gomez I, Moreno JM, Chamorro V, Osuna A, Vargas F.** Role of neuronal nitric oxide synthase in response to hypertonic saline loading in rats. *Acta Physiol Scand* 182: 389–395, 2004.
38. **Wilcox C.** Role of macula densa NOS in tubuloglomerular feedback. *Curr Opin Nephrol Hypertens* 7: 443–449, 1998.
39. **Vidal MJ, Romero JC, Vanhoutte PM.** Endothelium-derived relaxing factor inhibits renin release. *Eur J Pharmacol* 149: 401–402, 1988.