Hemodynamic Characterization of Hypertension Induced by Chronic Intrarenal or Intravenous Infusion of Norepinephrine in Conscious Rats

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SUMMARY The present study was designed to determine the hemodynamic changes underlying the hypertension induced by chronic intrarenal infusion of norepinephrine (NE) in conscious rats. NE was infused for a 5-day period intrarenally with osmotic minipumps via a chronic catheter in the right suprarenal artery at rates of 4 and 36 µg·kg⁻¹·hr⁻¹ or intravenously at a rate of 36 µg·kg⁻¹·hr⁻¹. Control rats received a 1 µl·hr⁻¹ intrarenal infusion of pyrogen-free 0.9% NaCl. In separate experiments, short-term effects were measured continuously during a 22- to 24-hour intrarenal infusion of 4 and 36 µg NE·kg⁻¹·hr⁻¹ or intravenous infusion of 36 µg NE·kg⁻¹·hr⁻¹. Intrarenal infusion of NE produced a more pronounced long-term hypertensive effect than infusion of the same dose intravenously. This hypertension was characterized by a rapid and sustained increase in total peripheral resistance index (TPRI). Despite of the initial renal vasoconstriction, specifically produced during the first 24 hours of intrarenal NE application, cardiac index (CI) in parallel to stroke volume index (SVI) decreased significantly during intrarenal as well as during intravenous NE infusion. Furthermore, no signs of sodium retention were observed. Both rates of intrarenal NE infusion have been shown previously to produce a significant long-term increase in plasma potassium concentration, and the present study indicates that this is presumably the result of decreased urinary potassium output. It is concluded that chronic hypertension produced by intrarenal or intravenous infusion of NE is not volume-dependent. The relatively greater increase in TPRI during intrarenal NE infusion is attributed to vascular wall receptor sensitization by increased plasma potassium levels resulting from effects of intrarenally present NE on tubular cation exchange mechanisms.

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KEY WORDS cardiac output • total peripheral resistance • plasma volume • plasma electrolytes • intrarenal norepinephrine-induced hypertension • experimental hypertension model • intrarenal infusions

The basic role of the kidney in chronic arterial pressure regulation is widely accepted. The fundamental renal control mechanism is the pressure-diuresis/natriuresis system that counterbalances small alterations in arterial pressure by large changes in renal water and salt excretion. The level at which the renal hydraulic system controls arterial pressure is influenced by hormonal and neurogenic factors; any change in renal vascular resistance and tubular handling of fluid and electrolytes can result in a shift of this renal function curve setpoint to other levels of arterial pressure.

In recent years, the impact of the sympathetic nervous system on renal function in relation to cardiovascular regulation has been recognized. Electrical stimulation of renal efferent sympathetic nerves causes renal vasoconstriction and increases renin secretion. Reabsorption of water and salt is enhanced at various tubular levels during low-level sympathetic stimulation.

Chronic hypertension that presumably only persists by means of a shift of the renal function curve to higher arterial pressure levels could be initiated or maintained by renal sympathetic hyperactivity. Direct recording of renal efferent nerve activity shows increases of sympathetic discharge frequency in anesthetized and conscious spontaneously hypertensive rats. Renal denervation, disrupting both efferent and afferent nerve fibers, delays the development of genetic hypertension and DOCA-salt hypertension in rats and decreases arterial pressure in established Goldblatt hypertensive rats.
In a previous study, we reported that hypertension was induced in conscious uninephrectomized rats by chronic intrarenal adrenergic hyperactivity. We mimicked the effects of increased sympathetic discharge frequency by means of long-term intrarenal infusion of norepinephrine (NE). Hypertension was produced at an intrarenal dose of 4 μg NE·kg⁻¹·hr⁻¹, but was not produced when the same dose of NE was infused intravenously (i.v.). Furthermore, any elevation of plasma NE levels caused by intrarenal NE infusion resulted in greater increases in arterial pressure compared with i.v. infusion. A second series of experiments in these uninephrectomized rats indicated that long-term intrarenal infusion of NE reduced plasma volume while plasma potassium levels were dose-dependently increased and plasma sodium concentrations were fallen below levels measured during saline infusion.

Earlier experiments in conscious dogs have shown that intrarenal NE infusion is also more effective in elevating arterial pressure than intravenous infusion of the same dose of NE. Hypertension is characterized in the dog model by a positive sodium balance and, after several days of NE infusion, increased total peripheral resistance.

In rats, the elucidation of the additional pressor response observed during intrarenal NE infusion may come from measurements of the hemodynamic effects of chronic stimulation of intrarenal adrenergic mechanisms on a continuous basis. In this study, we sought to determine whether a possible initial renal vasoconstriction during intrarenal NE infusion raises the extracellular fluid volume. This may lead to increased cardiac output in the primary phase of hypertension and, after whole-body autoregulation, to sustained increases in total peripheral resistance that could be superimposed on the direct vasoconstrictory effects of elevated plasma NE levels.

The data indicate that, despite specific renal vasoconstriction, no such increase in cardiac output occurs, in agreement with earlier findings on plasma volume responses. Instead, increased total peripheral resistance is apparent immediately, which suggests that this hypertension model is not volume-dependent.

**Methods**

**Animals**

Male Wistar rats (TNO, Zeist, The Netherlands) aged 16 to 20 weeks underwent left-sided nephrectomy under light ether anesthesia 4 weeks before the NE-infusion experiments. During this recovery period, right kidney function was fully restored when normalized for right kidney weight, as was shown previously.

**Chronic Infusion System**

Long-term intrarenal infusion in conscious rats has not been achieved so far. In this study, we applied a technique that was developed in our laboratory. The right suprarenal artery originating from the right renal artery and ascending toward the right adrenal gland was cannulated in rats under ether anesthesia. We used a PE 10 catheter (volume 12 μl) with its tip stretched over hot iron so that the ultimate diameter became 0.3 mm. We advanced this catheter in a retrograde fashion until its tip reached the bifurcation with the renal artery. Thus, substances could be infused intrarenally via the renal blood flow without disturbing the kidney’s blood supply. This type of catheterization provides a reliable tool for intrarenal application of drugs in conscious rats; no interference with renal function has been observed so far. Although right adrenal ischemia may result from this catheterization method, adrenal insufficiency does not occur due to hypertrophy of the contralateral adrenal gland. Intravenous infusion was performed via a Silastic catheter (volume 17 μl) inserted into the right jugular vein.

Catheters were connected to subcutaneously implanted osmotic minipumps (Alzet TM, Type 2001 (Alzo, Palo Alto, California); pumping rate: approximately 1 μl/hr) and perfused with 0.9% pyrogen-free saline. Upon completion of this and possibly other surgery, rats were housed individually with free access to food and water.

After a 2-day control period, the rats were anesthetized with ether and the saline minipumps were replaced by pumps containing saline or NE solutions ((−)-arterenol bitartrate, Sigma Chemical Company, St. Louis, Missouri) in concentrations that achieved intrarenal infusion rates of 4 and 36 μg NE·kg⁻¹·hr⁻¹. Only the 36 μg NE·kg⁻¹·hr⁻¹ infusion rate was applied i.v., since in a previous study we showed that i.v. infusion of 4 μg NE·kg⁻¹·hr⁻¹ does not elevate arterial pressure and plasma NE concentrations. Ascorbic acid (1 mg/ml) was added to the solutions to prevent oxidation of NE.

**Renal Function Measurement**

We used 61 rats in the study of renal function during intrarenal and i.v. NE infusion. While the rats were under ether anesthesia, in addition to the NE infusion catheters we cannulated the left femoral artery with a PE 10 catheter for measurements of mean arterial pressure (MAP) according to the protocol described below, and for blood sampling. A PE 10 catheter in the left femoral vein was used for rapid i.v. bolus injections of ⁵¹Cr-EDTA and ¹²⁵I-para hippuric acid (PAH). MAP was measured before the start of the renal function experiments. Glomerular filtration rate (GFR) and effective renal plasma flow (ERPF) were determined, as described. Loss of red blood cells was compensated for by the administration of 0.2 ml donor blood. Radioactivity in plasma was measured and log plasma concentration-time curves were fitted according to open two-compartment kinetics. GFR was calculated as the clearance of ⁵¹Cr-EDTA, and ERPF was calculated as the clearance of ¹²⁵I-PAH. We also calculated renal vascular resistance (RVR). Renal function parameters were normalized for (right) kidney weight. Described measurements were not repeated in the same animals over the NE infusion period, but seven experimental groups were created: renal function parameters were determined in one group on the control
day, and in separate groups of rats on the 1st and 4th day of intravenous infusion of 36 \( \mu \)g NE-kg\(^{-1}\)-hr\(^{-1}\) and on the 1st and 4th day of intrarenal infusion of 4 and 36 \( \mu \)g NE-kg\(^{-1}\)-hr\(^{-1}\).

Hemodynamic Measurements

In 37 rats under Nembutal anesthesia we inserted an electromagnetic flowprobe (Skalar Medical, Delft, The Netherlands) around the ascending aorta to measure cardiac output according to methods previously used in our laboratory. The rats were allowed to recover for 1 week, and then we cannulated the right suprarenal artery or jugular vein for NE infusion and the right femoral artery for measurements of MAP. We recorded MAP using a CTC CP-01 strain gauge and a Grass 7D-polygraph (Grass Instruments, Quincy, Massachusetts).

Cardiac output was monitored by means of a Skalar 506 electromagnetic blood flow meter and a Grass amplifier and then normalized for body weight, which gave the cardiac index (CI). Total peripheral resistance index (TPRI) was calculated from the MAP and CI data. Heart rate (HR) was derived from the cardiac output signal with a Grass tachograph, and stroke volume index (SVI) was calculated from the CI and HR data.

Measurements were performed on the control day and then daily for 5 consecutive days after starting the intrarenal infusion of saline of 4 and 36 \( \mu \)g NE-kg\(^{-1}\)-hr\(^{-1}\) and intravenous infusion of 36 \( \mu \)g NE-kg\(^{-1}\)-hr\(^{-1}\). Measurements were made between 1400 and 1700 hours during a 2-hour recording session.

Analog data were converted to digital data by a MINC RT-11 minicomputer (Digital Equipment, Maynard, Massachusetts) and sampled every 15 seconds; mean values of the last 45 minutes of the recording session were calculated.

Arterial blood samples (0.5 ml) were taken on the control day as well as on the 1st and 4th day of NE or saline infusion. Plasma sodium and potassium concentrations were determined with a flame photometer.

Acute Cardiac Output Measurements

To obtain information on the acute hemodynamic effects of NE infusion, 12 rats were prepared with an electromagnetic flowprobe as described above, and a catheter was inserted into the suprarenal artery or jugular vein as well as into the left femoral artery. NE infusion was not performed by means of osmotic mini-pumps but by a Precidor infusion pump (Infors AG, Basal, Switzerland; pumping rate 100 \( \mu \)l-hr\(^{-1}\)). Catheters and wires were protected with a light steel spring and connected to a Tech Serv swivel system (Tech Serv, Inc., Beltsville, Maryland). A continuous flow of pyrogen-free saline (0.5 ml-hr\(^{-1}\)) was maintained via the femoral artery catheter by using a PVB F 100 continuous flush device and a Harvard 975 infusion pump (Harvard Apparatus, Millis, Massachusetts). The MAP, HR, and cardiac output were recorded on a Grass 7D-polygraph.

Generally, long-term continuous measurements of cardiac output are not possible due to zero-level variations caused by off-set fluctuations in the electromagnetic flowprobe. Therefore, a microprocessor unit was constructed by our electronics department. This consisted of an analog system that generated MAP and mean cardiac output as well as HR and compensated for zero-level aberrations in the flow signal as indicated below, and of a digital system (6800-micro) that digitized and sampled the corrected analog data on a minute-to-minute basis. Thereupon, data were presented to a MINC RT-11 minicomputer for storage and further calculations.

In the analog part of the measuring device, mean cardiac output containing the zero-level variations was calculated from the unfiltered flow signal. A peak detector determined the minimal value of each flow complex. A sample-hold circuit combined with an integrator calculated the mean zero level over a 1-minute period which was subtracted from the mean flow values determined earlier and which resulted in mean cardiac output data compensated for off-set fluctuations.

Acute hemodynamic measurements were performed over 22 hours while the animals were in their own cages with free access to food and water. After an equilibration period of 1 hour, the suprarenal artery or jugular vein catheters were filled with the particular NE solutions, and after a 20-minute control period, the intrarenal or intravenous NE infusions were started. The MAP, CI, TPRI, HR, and SVI were measured every minute and averaged from hour to hour.

Cumulative Water and Salt Intake and Excretion

After 23 rats were habituated for 4 days in metabolic cages with free access to food and water, we measured food intake, water intake (WI), and urine output (UO) daily during long-term intrarenal infusions of saline or NE and intravenous infusion of NE. Sodium and potassium concentrations in tap water and rat chow and in urine were measured by means of a flame photometer. Cumulative WI and UO as well as cumulative intake and urinary excretion of sodium (I\(_{\text{IN}}\) vs. U\(_{\text{K+}}\)) and potassium (I\(_{\text{K+}}\) vs. U\(_{\text{K+}}\)) were calculated.

Statistics

All data are expressed as means ± SEM. Data on kidney function and central hemodynamics were statistically analyzed by means of a Student's \( t \) test for unpaired or, when necessary, paired values. Data on intake and excretion of water and salt were analyzed by means of a one-way nonparametric analysis of variance (ANOVA).

Results

Effects on Renal Function

Changes in renal function parameters normalized for kidney weight during intrarenal infusion of 4 and 36 \( \mu \)g NE-kg\(^{-1}\)-hr\(^{-1}\) and during intravenous infusion of 36 \( \mu \)g NE-kg\(^{-1}\)-hr\(^{-1}\) are summarized in Table 1.
The GFR varied between 0.76 and 1.30 ml·min⁻¹·g⁻¹·kw⁻¹ on control day and stayed within this range during intrarenal infusion of 4 μg NE-kg⁻¹·hr⁻¹ and intravenous infusion of 36 μg NE-kg⁻¹·hr⁻¹ for 5 days. A slight but insignificant decrease to 0.88 ± 0.13 ml·min⁻¹·g⁻¹·kw⁻¹ was found on the 4th day of intrarenal infusion of 36 μg NE-kg⁻¹·hr⁻¹.

Control values of ERPF ranged from 2.36 to 4.84 ml·min⁻¹·g⁻¹·kw⁻¹, and intravenous infusion of 36 μg NE-kg⁻¹·hr⁻¹ did not produce any change in ERPF throughout the 5-day infusion period. Intrarenal infusion of both doses of NE caused significant decreases (see p values in Table 1) in ERPF to 2.78 ± 0.27 ml·min⁻¹·g⁻¹·kw⁻¹ on the 1st day of NE application. The ERPF returned to control levels in the later phase of infusion of 4 μg NE-kg⁻¹·hr⁻¹, but stayed at the decreased level during intrarenal infusion of 36 μg NE-kg⁻¹·hr⁻¹.

The RVR on control day was 29.8 ± 3.5 mmHg·min·g·kw·ml⁻¹, and an insignificant increase was observed during intravenous infusion of 36 μg NE-kg⁻¹·hr⁻¹. Intrarenal NE application at both infusion rates produced significant increases in RVR on the 1st day. Although RVR had returned toward control levels on the 4th day of intrarenal NE infusion of 4 μg·kg⁻¹·hr⁻¹, it had increased further to 66.0 ± 13.0 mm Hg·min·g·kw·ml⁻¹ at the 36 μg·kg⁻¹·hr⁻¹ rate. Furthermore, the extracellular fluid volume measured in the course of the renal function experiments as the volume of distribution of ⁵¹Cr-EDTA showed no response to long-term intrarenal or intravenous NE infusion (data not shown).

Chronic Effects of Hemodynamics

Before the NE infusions were started, MAP varied between 96 and 126 mm Hg (Figure 1) and stayed within this range during chronic intrarenal infusion of saline (n = 9). Intrarenal infusion of 4 μg·kg⁻¹·hr⁻¹ for 5 consecutive days (n = 9) raised MAP to 133 ± 8 mm Hg; increases were immediately statistically significant when compared with saline data. When the 36 μg·kg⁻¹·hr⁻¹ infusion rate was applied intrarenally (n = 10), increases in MAP up to 150 ± 5 mm Hg were produced on Day 5; statistical significance of the pressor response was obtained over the complete infusion period. The same dose of intravenous NE (n = 9) induced relatively smaller increases in MAP, although they were also significant from the 2nd day; ultimate levels of MAP were 145 ± 3 mm Hg.

Figure 2 shows the effects on normalized cardiac output. Control values were between 19.6 and 35.8 ml·min⁻¹·100 g·bw⁻¹. During intrarenal infusion of saline, CI rose to levels of 28.8 ± 2.6 ml·min⁻¹·100 g·bw⁻¹. During intrarenal infusion of both doses of NE, CI decreased significantly to approximately 22.0 ml·min⁻¹·100 g·bw⁻¹. However, significant decreases were obtained earlier in the infusion period of 36 μg NE·kg⁻¹·hr⁻¹. Although statistical significance was not reached for the entire infusion period, CI decreased to the same extent on the 5th day of intravenous infusion of 36 μg NE·kg⁻¹·hr⁻¹.

Figure 3 indicates that the decreases in CI during intrarenal NE infusion were associated with decreases...
in both determinants of CI: HR (Figure 3A) and SVI (Figure 3B). Whereas changes in HR were rather marginal, significant decreases in SVI paralleled significant decreases in CI exactly. During intravenous NE infusion, HR rose slightly so that the low CI level on Day 5 was caused exclusively by a relatively greater decrease in SVI.

Effects on TPRI are shown in Figure 4. On control day, TPRI ranged between 3.02 and 5.83 mm Hg·min⁻¹·100 g bw⁻¹. During chronic intrarenal infusion of saline, TPRI decreased somewhat along with the increases of CI. Intrarenal infusion of 4 µg NE·kg⁻¹·hr⁻¹ elevated TPRI significantly over the

whole infusion period to levels of 5.93 ± 0.45 mm Hg·min⁻¹·100 g bw⁻¹ on Day 5. The 36 µg NE·kg⁻¹·hr⁻¹ infusion rate produced immediately significant increases to final levels of 7.49 ± 0.35 mm Hg·min⁻¹·100 g bw⁻¹. Intravenous infusion of 36 µg NE·kg⁻¹·hr⁻¹ raised TPRI significantly from Day 2 to
Acute Effects on Hemodynamics

Control levels of MAP over a 20-minute period before the start of the acute intrarenal infusion of 4 \( \mu \)g NE·kg\(^{-1}\)·hr\(^{-1}\) (\( n = 4 \)) were 119 ± 5 mm Hg (Figure 5). No significant increase in MAP vs control values were observed during the 22 hours of intrarenal NE application, except for the 4th hour when MAP reached levels of 128 ± 1 mm Hg. A transient significant decrease in CI below starting values of 32.8 ± 4.1 ml·min\(^{-1}\)·100 g bw\(^{-1}\) was observed during the first 10 hours of NE infusion, with a minimum level of 26.6 ± 3.5 ml·min\(^{-1}\)·100 g bw\(^{-1}\) in the 9th hour. Decreases in CI were caused by decreases in SVI (minimum value 76.7 ± 12.8 ml·100 g bw\(^{-1}\) during the 9th hour), whereas no effects on HR were observed. Control values of TPRI were 3.9 ± 0.6 mm Hg·min·100 g bw·ml\(^{-1}\). Both CI and TPRI returned to control values in the latter half of the 22-hour infusion period of 4 \( \mu \)g NE·kg\(^{-1}\)·hr\(^{-1}\).

Acute intrarenal infusion of 36 \( \mu \)g NE·kg\(^{-1}\)·hr\(^{-1}\) (\( n = 4 \)) produced changes in hemodynamic parameters which were sustained throughout the 22-hour infusion period (Figure 6). The MAP was 122 ± 1 mm Hg before the start of the NE administration and rose immediately to levels of approximately 145 mm Hg. Statistical significance vs control values were gained over the first 15 hours. After that, MAP decreased slightly but never reached initial levels. Control values of CI were 26.1 ± 1.4 ml·min\(^{-1}\)·100 g bw\(^{-1}\), and intrarenal infusion of 36 \( \mu \)g NE·kg\(^{-1}\)·hr\(^{-1}\) caused immediate decreases to a minimum value of 16.1 ± 1.6 ml·min\(^{-1}\)·100 g bw\(^{-1}\) after 7 hours. Although a small increase was observed in the latter period of NE infusion, CI stayed below the starting levels for these 22 hours. Obvious decreases were seen again in SVI, which reached a minimum of 49.6 ± 6.9 ml·100 g bw\(^{-1}\) in the 9th hour. No effects on HR were produced. TPRI was elevated immediately by intrarenal NE infusion at the 36 \( \mu \)g·kg\(^{-1}\)·hr\(^{-1}\) rate; from a level of 5.1 ± 0.4 mm Hg·min·100 g bw·ml\(^{-1}\), values of 8.9 ± 0.9

![Figure 5](http://hyper.ahajournals.org/)

![Figure 6](http://hyper.ahajournals.org/)
mm Hg·min·100 g bw·ml⁻¹ were obtained in the 6th hour. Statistically significant differences vs control levels in MAP and CI. In the later phase of NE infusion, TPRI decreased slightly but did not return to starting values.

During 22 hours of intravenous infusion of 36 μg NE·kg⁻¹·hr⁻¹ (n = 4, data not shown), irregular patterns of increases in MAP and TPRI, interspersed with normotensive periods were observed. Throughout the whole 22 hours, CI decreased slightly.

**Effects on Cumulative Water and Salt Intake and Excretion**

Figure 7 shows the changes in cumulative WI and UO produced by long-term NE infusion. Both intravenous and intrarenal infusion of 36 μg NE·kg⁻¹·hr⁻¹ decreased cumulative WI significantly over the whole infusion period as compared with saline data. The 4 μg NE·kg⁻¹·hr⁻¹ infusion remained ineffective in decreasing the WI, but it caused a statistically significant increase in cumulative UO, as did intravenous infusion of 36 μg NE·kg⁻¹·hr⁻¹.

Chronic intrarenal infusion of 36 μg NE·kg⁻¹·hr⁻¹ produced significant decreases in the cumulative intake of Na⁺ and K⁺ over the complete infusion period, as compared to saline infusion. Intrarenal application of 4 μg NE·kg⁻¹·hr⁻¹ and intravenous infusion of 36 μg NE·kg⁻¹·hr⁻¹ did not affect the cumulative salt intake (Figures 8 and 9). Intrarenal infusion of the lower dose of NE increased cumulative urinary Na⁺ excretion during 5 days of application (Figure 8). Intrarenal infusion of 4 and 36 μg NE·kg⁻¹·hr⁻¹ reduced the cumulative urinary excretion of K⁺ significantly, as shown in Figure 9. Decreases in urinary K⁺ excretion were not statistically significant during intravenous infusion of 36 μg NE·kg⁻¹·hr⁻¹.

![Figure 7](image_url)

**Figure 7.** Changes in cumulative water intake (WI, upper graph) and urine output (UO, lower graph) induced by long-term intrarenal and intravenous norepinephrine (NE) infusion compared with saline data by analysis of variance. Significant differences in WI with saline infusion were found during intrarenal and intravenous application of 36 μg NE·kg⁻¹·hr⁻¹ (p < 0.05). Significant differences in UO were found during intrarenal and intravenous application of 36 μg NE·kg⁻¹·hr⁻¹ (p < 0.005) and during intravenous infusion of 36 μg NE·kg⁻¹·hr⁻¹ (p < 0.05).

![Figure 8](image_url)

**Figure 8.** Changes in cumulative intake of sodium (I₉⁺) and urinary excretion of sodium (U₉⁺) induced by long-term intrarenal and intravenous infusion of norepinephrine (NE) compared with saline data by analysis of variance. Significant differences in I₉⁺ vs intrarenal saline infusion were found during intrarenal infusion of 36 μg norepinephrine (NE)·kg⁻¹·hr⁻¹ (p < 0.005). Significant differences in U₉⁺ were found during intrarenal infusion of 36 μg norepinephrine (NE)·kg⁻¹·hr⁻¹ (p < 0.005). Significant differences in U₉⁺ were found during intrarenal infusion of 4 and 36 μg NE·kg⁻¹·hr⁻¹ (p < 0.05).
Discussion

In a previous study, we showed that chronic low-dose intrarenal infusion of NE maintained via the suprarenal artery catheterization technique caused hypertension in conscious rats. Sustained hypertension was induced at intrarenal infusion rates that were non-elevating when applied intravenously. Doses of NE that did raise MAP via the intravenous route caused considerably larger increases in MAP upon intrarenal infusion, as was shown in Figure 1 in this study by comparison of intrarenal and intravenous infusions of 36 \( \mu g \) NE kg\(^{-1}\) hr\(^{-1}\) (see also our previous results\(^6\)). In addition to the effects of relatively higher levels of circulating NE, this extra pressor response could be related to an altered function of the two basic renal mechanisms for control of MAP, namely, the renin-angiotensin system and the renal hydraulic system.\(^2\)

We have previously reported a contribution of circulating angiotensin II to MAP levels during intrarenal NE infusion.\(^2\) But chronic intraperitoneal infusion of the converting-enzyme blocker captopril (400 \( \mu g \cdot kg^{-1} \cdot hr^{-1} \)) along with the NE infusions was as effective in lowering MAP during intrarenal as during intravenous infusion of 36 \( \mu g \) NE kg\(^{-1}\) hr\(^{-1}\). Furthermore, plasma renin activity is basically the same in the established stage of hypertension caused by intravenous as well as intrarenal infusion of 36 \( \mu g \) NE kg\(^{-1}\) hr\(^{-1}\) (unpublished data).

Therefore, in this study we aimed to investigate whether intrarenal NE infusion interferes primarily with the relationship between arterial pressure and urine output. If so, changes in renal function could lead to volume retention and increases in cardiac output, so that additional elevations in TPRI would be induced reflexively by the whole body autoregulation mechanism. This chain of events could thus be responsible for the relatively higher MAP levels. Any factor influencing RVR, GFR, and tubular sodium and water reabsorption can alter the setpoint of the renal hydraulic system.

Neurogenic stimuli may influence renal function. Direct or indirect increases in renal efferent nerve activity\(^4\)-\(^8\) cause renal vasconstriction and possibly decreases in GFR.\(^4\) Tubular adrenergic innervation has been histochemically verified in the rat\(^29\) and the dog.\(^7\) Direct or baroreceptor-reflex-mediated stimulation of renal efferent nerves has been shown to decrease urinary sodium excretion in dogs, without affecting renal blood flow or GFR.\(^7\)\(^\text{x}\) Applying Henle's loop microperfusion technique in rats, DiBona and Sawin\(^8\) showed that low-frequency renal nerve stimulation increases reabsorption of sodium and chloride at this level. Rather high concentrations of NE enhance reabsorption of water in isolated rabbit proximal tubules.\(^31\) Hyperactivity of renal sympathetic nerves has been found to contribute decisively to the development and maintenance of elevated arterial pressure in various animal hypertension models\(^12\)-\(^19\) and has been suggested to be related to the shift of the renal function curve setpoint to higher levels of MAP, as observed in spontaneously hypertensive rats and in one-kidney, one clip hypertensive rats.\(^32\)

In this study, we found that renal adrenergic hyperactivity as caused by intrarenal NE infusion interfered primarily with renal function. The RVR was increased by approximately 60% on the 1st day of intrarenal infusion of both doses of NE. Since TPRI was elevated by 30% or less compared with control values by that time, intrarenal NE infusion produced initially selective renal vasoconstriction. Intrarenal NE infusion in anesthetized rats and dogs has been developed as a
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model for human acute renal failure.33,34 Severe renal insufficiency as characterized in these studies by decreases of GFR to practically zero levels during intrarenal NE application may cause hypertension. However, in our model GFR declined slightly only on Day 4 of intrarenal infusion of 36 μg NE-kg⁻¹-hr⁻¹ in conscious rats. It can therefore be excluded that the increases in MAP during intrarenal NE infusion in this study were evoked by induction of acute renal failure.

Despite the renal vasoconstriction and the plausibly high tubular and peritubular load of NE, no indication of volume retention was discovered during long-term intrarenal NE infusion in conscious rats. Chronic measurements of hemodynamic parameters revealed that CI decreased, mostly by reductions in SVI, slowly and secondarily to increases in MAP caused by intrarenal as well as intravenous NE infusion. The observed increases in CI during intrarenal saline infusion were probably due to hypovolemia during the first days after laparotomy necessary for suprarenal artery catheterization. Such an early hypovolemia was already indicated by the plasma volume data as reported previously.21 TPRI was already elevated significantly on the 1st day of intrarenal infusion of 4 and 36 μg NE-kg⁻¹-hr⁻¹ compared with saline infusion and the 2nd day of intravenous infusion of 36 μg NE-kg⁻¹-hr⁻¹. Increased TPRI was sustained over the whole NE infusion period and differences in MAP levels during intrarenal NE infusion vs intravenous infusion correlated well with differences in elevations in TPR.

The earliest evaluation of cardiovascular parameters in our study of chronically NE-infused rats was at 24 hours after the saline-containing osmotic minipumps were replaced. Since it remained possible that volume retention occurred within this period of latency, we studied hemodynamic changes during 22 hours after starting intrarenal and intravenous NE infusion. Application of 4 NE-kg⁻¹-hr⁻¹ via the suprarenal artery caused transient early decreases of CI and elevations in TPRI. The higher infusion rate produced changes of CI and TPRI in the same direction, which were maintained throughout the infusion period. During intravenous infusion of 36 μg NE-kg⁻¹-hr⁻¹, only minor transient decreases in CI were observed. Thus, no evidence of volume retention related increases in CI were found in the acute or chronic phase of intrarenal adrenergic stimulation. This was confirmed by the fact that the extracellular fluid volume did not respond to intrarenal or intravenous NE application.

Previously published measurements of plasma volume21 showed decreases parallel to the present reductions in CI. In the established stage of intrarenally NE-induced hypertension, plasma volume was decreased by 18%, which was consolidated in the present study by findings that chronic intrarenal infusion of 4 μg NE-kg⁻¹-hr⁻¹ increased the cumulative UO but did not affect WI and that the 36 μg NE-kg⁻¹-hr⁻¹ infusion rate decreased the cumulative WI while UO remained normal. Only intrarenal infusion of 36 μg NE-kg⁻¹-hr⁻¹ was able to produce a significant 7% reduction of plasma sodium concentrations at the 4th day of infusion.21 In the present study, it is shown that this was probably caused by a decline of cumulative sodium intake. Cumulative urinary excretion of sodium was slightly increased during intrarenal infusion of 4 μg NE-kg⁻¹-hr⁻¹, but decreased during intrarenal infusion of 36 μg NE-kg⁻¹-hr⁻¹, in contradiction to the report22 that elevated renal perfusion pressure induced by NE application in isolated rat kidneys is followed by decreased tubular sodium reabsorption.

To a certain extent, our data are comparable with results obtained with chronic intrarenal vs intravenous infusion of NE in conscious dogs. During intrarenal infusion of 17 μg NE-kg⁻¹-hr⁻¹ over an 11-day period in dogs,22 renal blood flow was chronically decreased while GFR was unaffected; RVR was increased continuously during the NE-infusion period. During this renal vasoconstriction, MAP rose by 25 mm Hg; cardiac output measured by means of the dye-dilution technique was found to be decreased, and hypertension was correlated with elevations of TPR. The most striking difference between rats and dogs during intrarenal NE infusion is that in dogs a positive sodium balance and an increase in plasma sodium concentration are produced, even when sodium intake is raised in stepwise fashion,22,23 and a shift of the renal function curve to higher MAP levels occurs.21 In our experiments in rats, however, the plasma sodium concentrations were decreased mainly because the rats were eating less sodium-containing food. Maybe, this difference occurred because our rats were not on a fixed sodium diet but had free access to water and food during the time of NE infusion.

Also on the basis of other methods, elevation of renal vascular resistance does not necessarily lead to volume retention and increase in cardiac output. In one-kidney, one clip rats and dogs, hypertension is characterized by immediately apparent and sustained increases in peripheral resistance; volume and cardiac output changes were not essential for the development of chronic hypertension.30 In another experimental renal hypertension model that used bilateral renal celophane wrapping in rabbits, elevation of MAP was also due to increases in TPR from the earliest stage.37 Evidence has been reported that volume factors influencing cardiac output and vasoconstriction factors influencing TPR can produce chronic hypertension independent of each other.36,37 Our study of chronic intrarenal NE infusion in conscious rats has confirmed that renovascular hypertension can develop without a body-fluid volume-dependent stage.

Alternative mechanisms that may raise MAP during intrarenal infusion additionally to raising plasma NE levels must therefore be implied. One expects that stimulation of tubular sodium reabsorption by NE must be quite dominant in our model. However, as already mentioned, tubular transmembrane transport of water and salt is dependent of renal hemodynamics.35 Tubular NE handling is furthermore featured by active secretion of NE. Electrical stimulation of renal nerves in rabbits causes increases in urinary NE excretion when compensated for changes in GFR38 and acute denerva-
tions decrease NE excretion in rats.\textsuperscript{39} The salient detail is that low-sodium intake reduces baroreflex-stimulated urinary excretion,\textsuperscript{39} which could indicate that normal body sodium load is a necessary prerequisite for tubular NE secretion. Active secretion of NE is inhibited by cyanine 863, which suggests a tubular transmembrane exchange of NE via a cation transport mechanism.\textsuperscript{40}

In our study, steeply increased plasma potassium concentrations could be explained by the fact that peritubular unbound NE, which is probably highly concentrated during chronic intrarenal NE infusion due to plasma protein saturation, was actively exchanged for potassium, since cumulative urinary potassium excretion was reduced during intrarenal application of 4 and 36 $\mu$g NE·kg$^{-1}$·hr$^{-1}$. In turn, these increased plasma potassium concentrations could result in exaggerated vascular tension, since depolarization of arterial vascular muscle cells is facilitated and arterial responsiveness to NE is elevated by increased extracellular potassium concentrations.\textsuperscript{41-43}

Lastly, as an alternative mechanism, stimulation of renal afferent nerves during intrarenal NE infusion may account for the relatively higher pressor response when intravenous application of the same doses of NE is compared. Interruption of renal afferent nerves has been proposed to account for the blood-pressure-lowering effect of renal denervation in several rat hypertension models.\textsuperscript{44-46} Increasing afferent renal nerve activity leads to overall sympathetic hyperactivity, as indicated by higher plasma NE levels.\textsuperscript{18,19} During intrarenal NE infusion in conscious rats, a pressor response additional to the direct effect of elevation of plasma NE concentrations has been observed.\textsuperscript{20} Stimulation of afferent renal nerves directly\textsuperscript{47} or indirectly via stimulation of secretion of other hormonal agents\textsuperscript{48,49} during intrarenal NE infusion may involve reflexively the synthesis or release of an unknown vasoactive substance, or reversely, inhibit the contribution of endogenous vasodepressor agents.

In summary, in our study chronic hypertension produced by intrarenal infusion of NE was associated with an immediate elevation of TPRI and was not volume-dependent at any developmental stage. Despite the primary renal vasoconstriction, changes in body water and sodium load did not initiate increases in MAP. It is possible that the relatively higher TPRI during intrarenal NE infusion compared with intravenous NE application is attributed to secondary effects of intrarenal NE on the electrolyte balance, such as on the plasma potassium concentration or on activation of vasopressor mechanisms through stimulation of renal afferent nerves. Further work is in progress in our laboratory to investigate these alternatives.

References


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