Renal sympathetic nerves modulate erythropoietin plasma levels after transient hemorrhage in rats

Tilmann Ditting, Karl F. Hilgers, Alexander Stetter, Peter Linz, Christina Schönweiss, and Roland Veelken

1Department of Nephrology, Friedrich-Alexander-University, Erlangen; and 2Boehringer, Mannheim, Germany

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Ditting T, Hilgers KF, Stetter A, Linz P, Schönweiss C, Veelken R. Renal sympathetic nerves modulate erythropoietin plasma levels after transient hemorrhage in rats, Am J Physiol Renal Physiol 293: F1099–F1106, 2007. First published July 25, 2007; doi:10.1152/ajprenal.00267.2007.—In contrast to other sympathetic outflow tracts, renal sympathetic nerve activity (RSNA) decreases in response to hypotensive hemorrhage. The functional significance of this “paradox” is not known. We tested the hypothesis that RSNA modulates renal perfusion and thus erythropoietin (EPO) release after transient hypotensive hemorrhage in anesthetized rats. Plasma EPO was measured before and after 30 min of transient hypotensive hemorrhage (i.e., 40 mmHg from mean baseline blood pressure, followed by reinfusion of shed blood) and 120 min thereafter in sham-denervated rats, and after renal denervation (DNX) or bilateral cervical vagotomy (VX) to abolish/blunt the RSNA decrease mediated by a cardiopulmonary reflex. RSNA, renal Doppler flow, renal vascular resistance (RVR), resistance index, and oxygen delivery/uptake (DO2/VO2) were measured. RSNA decreased in intact animals (−40 ± 5% from baseline, P < 0.05). This was blunted by VX. With intact nerves, EPO level did not increase. In DNX rats, EPO was increased at minute 120 (49 ± 3 vs. 74 ± 2 ml/mi; P < 0.05), in VX rats this (47 ± 2 vs. 62 ± 4 ml/mi; P < 0.05) was less pronounced. DO2 in DNX rats was lower compared with intact and VX rats (0.25 ± 0.04 vs. 0.51 ± 0.06 and 0.54 ± 0.05 ml O2/min; P < 0.05) due to lower Doppler flow and increased RVR. RVR and DO2 were similar in intact and VX rats, but resistance index differed between all groups (0.70 ± 0.02 vs. 0.78 ± 0.02 vs. 0.85 ± 0.02; P < 0.05, intact vs. VX vs. DNX), indicating differential reactivity of renal vasculature. VO2 was unaffected by VX and DNX. Renal sympathoinhibition during hypotensive hemorrhage might help to preserve sufficient oxygenation of renal tissue by modulation of hemodynamic mechanisms that act to adapt renal oxygen availability to demand.

renal nerve; oxygen delivery

In contrast to other sympathetic outflow tracts (e.g., adrenal sympathetic nerve activity), renal sympathetic nerve activity (RSNA), after a short-lived increase, is persistently decreased in the setting of hypotensive hemorrhage. The functional significance of this seemingly paradox reflex regulation, which is mediated by a cardiopulmonary reflex (6, 39), is as yet poorly understood.

The intense sympathetic innervation of the kidney has been shown to provide a tonic level of vasoconstriction in the renal cortex, and increasing RSNA further augments cortical vasoconstriction, whereas medullary perfusion seems to be unaffected (24, 26). Interestingly, in these studies, among other stimuli, hypoxia was used to increase RSNA, which in turn is a major stimulus for erythropoietin (EPO) formation.

EPO is a glycoprotein that plays a crucial role in formation and maturation of erythrocytes, and the major site of EPO production is the kidney (14). The production of EPO by peritubular cortical fibroblasts seems to be directly related to renal oxygen (1, 27). The major control of EPO formation operates at the level of its mRNA (2, 9), and oxygen-dependent regulation of EPO gene expression is controlled by a hypoxia-inducible factor (14, 34, 40). It is generally accepted that EPO is regulated by renal oxygen sensing (32); however, extrarenal factors might play a role as well (9).

Among possible pathways by which extrarenal signals might affect EPO formation in the kidneys, the intense sympathetic renal innervation was considered. However, earlier studies investigating such a role of renal nerves showed conflicting results: in rabbits exposed to hypobaric hypoxia, renal denervation (DNX) decreased EPO levels (13). Others found that DNX increased EPO in rats exposed to normobaric hypoxia (3). In a more recent study, unilaterally denervated rats were exposed to different hypoxic stimuli, but no differences in EPO mRNA levels were found when innervated and denervated kidneys were compared. It was concluded that renal nerves play no role in oxygen-dependent control of EPO mRNA levels (9). All of these studies investigated mainly clear-cut hypoxic conditions, which continued for several hours; however, RSNA was not measured directly.

In the present study, we investigated a putative role of renal nerves in EPO formation in the setting of transient hypotensive hemorrhage (tr-hHem). tr-hHem uniquely combines a short-acting “hypoxic” stimulus [due to reduction of oxygen delivery (DO2)] with a seemingly paradox decrease in RSNA. Although it is well established that sympathetic nerve activity is regulated differentially in terms of target organs and tissue (28), the physiological significance of hemorrhage-induced renal sympathoinhibition remains ill defined.

We wanted to test the hypothesis that renal nerves modulate EPO formation in the setting of hypotensive hemorrhage. To this end, the influence of renal nerves and the above-mentioned cardiopulmonary reflex on plasma EPO levels in the setting of hypotensive hemorrhage were investigated under three different conditions: 1) afferent (i.e., vagus nerve) and efferent limb (i.e., renal nerves) intact; 2) afferent limb interrupted [bilateral cervical vagotomy (VX)]; and 3) efferent limb interrupted (bilateral DNX). Plasma EPO levels, RSNA, renal Doppler flow (DOP), DO2, and oxygen consumption (VO2), as well as renal vascular resistance (RVR), were measured. Furthermore,
resistance index (RI) was assessed as an indirect method to provide insight into putative perturbations of the renal vascular bed. This approach might possibly give some clue to our understanding of the effects of hemorrhage-induced renal sympathoinhibition.

**MATERIALS AND METHODS**

**Animals.** Male Sprague-Dawley rats (Charles River, Sulzfeld, Germany) weighing 281 ± 15 g were used for the experiments. They were kept at 24 ± 2°C with a light-dark cycle of 12:12 h and fed standard rat diet (no. C-1000, Altromin, Lage, Germany) containing 0.2% NaCl by weight with free access to tap water. All procedures performed in animals were done in accordance with the guidelines of the American Physiological Society and with the National Institutes of Health Guide for the Care and Use of Laboratory Animals and were approved by the Experimental Animal Care Committee of the local government agency (Regierung von Mittelfranken, Ansbach, Germany).

**Anesthesia.** Rats spontaneously breathing room air were anesthetized with thiobarbital sodium (Trapanal; Byk Gulden, Konstanz, Germany; 40 mg/kg intraperitoneally), and supplemental doses were given when experiments lasted longer than 6 h. For DNX or sham DNX (see below), which was done 1 wk before the experiments, inhalant anesthesia via face mask was used (mixture of oxygen with ~50% N₂O and ~2% 1-chlor-2,2,2-trifluorethyl-difluormethyl-ether: Isofluran; Abbot). Depth and adequacy of anesthesia were confirmed by the lack of cardiovascular responses and/or movement to mildly noxious stimuli, e.g., absence of pedal withdrawal reflex (41).

**Vascular catheters: blood pressure and heart rate measurement.** Two catheters were inserted in the femoral arteries. One was connected to a strain-gauge transducer (Statham P23Db) to record mean arterial blood pressure (MAP) and heart rate (HR) using a pressure processor amplifier (type 13-4615-52; Gould Instrument Systems, Valley View, OH). The other catheter was used for blood withdrawal (i.e., hemorrhage) and for blood-gas analysis samples. Two femoral venous lines were inserted for continuous infusion of normal saline (1.8 ml·kg⁻¹·h⁻¹) to compensate fluid loss due to surgery, reinfusion of the withdrawn blood, administration of substances, and to collect blood samples for plasma EPO measurement. To take renal arterial and venous blood samples for blood-gas analysis, a subset of rats was additionally instrumented with a curved tip catheter (PE-10), which was advanced to the left renal artery orifice via the femoral artery. Proper placement of the “aortic” catheter tip in the immediate vicinity of the left renal arterial orifice was controlled postmortem. Direct placement of catheters in the renal artery was not possible without obstructing the vessel. Renal veins could be cannulated directly without obstruction using very short PE-10 curved tip catheters (intravascular tip length 1.5 mm) through a left lateral flank incision. The venous catheter was glued to the vessel with octylcyanacrylat glue (World Precision Instruments, Berlin, Germany). This catheter was also used to record renal venous pressure.

**RSNA recording.** Renal sympathetic nerve recording was performed as described previously (7). Through a left lateral approach, a renal nerve bundle was dissected free from connective tissue and placed on a bipolar electrode (0.2-mm stainless steel wire, Science Products, Frankfurt, Germany). RSNA was amplified 10,000–50,000 times and filtered (low pass 1 kHz; high pass 100 Hz) using a band-pass amplifier (Bioelectric amplifier (7DA, Grass-Telefactor). The signal was recorded as mV × s. Individual amplifier settings were documented for recalculation of actual baseline activity (in µV × s) to allow for comparison of baseline activities between groups.

**Renal blood flow, RVR, oxygen supply, and Vo₂.** Measurement of renal blood flow (RBF) was adapted to a method previously described by our laboratory (36, 38). A 20-MHz piezo Doppler-crystal (0.8-mm diameter; Iowa Doppler Products, Iowa City, IA) was placed on the left renal artery using the same surgical approach that was used for the renal nerve preparation. It was connected to a directional pulsed Doppler flowmeter (University of Iowa model 545C-4, Bioengineering, Iowa City, IA) to measure mean and phasic Doppler shift frequency (kHz). The crystal was mounted on a small silastic tubing, cut at a 45° angle, which was filled with normal saline. This probe was glued to the renal artery with octylcyanacrylat glue when an optimal flow signal could be obtained while care was taken to preserve all visible nervous structures. Before the probe was glued to the artery, the vessel diameter was measured using a Castroviejo surgical caliper (Fine Science Tools, Heidelberg, Germany) combined with a micrometer caliper. Furthermore, focus depth of the Doppler signal was documented. Based on these parameters, the effective vessel diameter was estimated for the calculation of instantaneous volume flow. Arterial (CaO₂) and venous oxygen content (CvO₂) were calculated based on blood-gas analysis samples from the “aortic” and renal venous catheters, which were also used to measure the effective renal arterial and venous blood pressure. These values were used to calculate renal Do₂ and Vo₂, RVR, and RI. The following equations were used.

For RBF velocity
\[ V = \frac{(F_d \times C)}{(2 \times F_0 \times \cos \alpha)} \]  
(1)

where \( V \) is blood flow velocity (mm/s); \( F_d \) is Doppler shift frequency (kHz); \( C \) is velocity of sound in blood (1.565,000 mm/s); \( F_0 \) is transmitter frequency (kHz) (20 MHz with 545C-4); \( \alpha \) is angle of “sound-beam” and blood flow vector, 45°.

For RBF
\[ Q = V \times \Pi \times 0.06 \times (D/2)^2 \]  
(2)

where \( Q \) is instantaneous volume flow (ml/min); \( \Pi \) is 3,14159. . . ; and \( D \) is lumen diameter (mm).

For RI
\[ RI = \left( \text{sys} \frac{kHz_{max} - \text{dia} kHz_{min}}{sys kHz_{max}} \right) \]  
(3)

where sys is systole; dia is diastole; and kHz_{max} and kHz_{min} are maximum and minimum kHz, respectively.

For RVR
\[ RVR = \frac{(RAP - RVP)/Q}{(mmHg \cdot min \cdot ml^{-1})}; \]  
(4)

where RAP is mean renal arterial pressure (mmHg); RVP is mean renal venous pressure (mmHg); and \( Q \) is RBF (ml/min).

For CaO₂
\[ CaO₂ = (1.34 \times Hb) \times SaO₂ + (a)PO₂ \times 0.0031 \]  
(5a)

where 1.34 is Hufner’s constant, giving oxygen-carrying capacity of Hb (ml O₂/g Hb); Hb is hemoglobin concentration (g/dl); SaO₂ is arterial O₂ saturation (%); a is arterial; and PO₂ is partial pressure of O₂ (Torr).
For $C_{vO_2}$

$$C_{vO_2} = \frac{(1.34 \times Hb) \times SV_{O_2}}{2}$$

where $SV_{O_2}$ is venous oxygen saturation (%); and $v$ is venous.

For $DO_2$

$$DO_2 = \frac{Q \times Ca_{O_2}}{2} \times 100 \text{ (ml O}_2/\text{min)}$$

For $VO_2$

$$VO_2 = \frac{Q \times (Ca_{O_2} - Cv_{O_2})}{2} \times 100 \text{ (ml O}_2/\text{min)}$$

For $O_2$ extraction rate (ER)

$$O_2-ER = \frac{VO_2}{DO_2} \times 100 \text{ (8)}$$

Data acquisition. Integrated RSNA, MAP, HR, as well as mean and phasic DOP, were recorded on a polygraph (RS 3400, Gould Instrument Systems) and additionally stored on a computer using an analog-to-digital converter board (DT 2821-F-SE; Data Translation, Marlborough, MA) and appropriate software (PSW 6.0; Data Wave Technologies, Longmont, CO) for evaluation. Sampling rate was 200 Hz.

Blood sample analyses. Analysis of blood gases (pH, $P_{O_2}$, oxygen saturation) and Hb content were done from 50-μl arterial samples using a blood-gas analyzer (Radiometer Copenhagen, Willich-Schief- bahn, Germany). Plasma EPO was measured from 150-μl venous samples, which were immediately centrifuged at room temperature (20°C). Plasma was rapidly frozen and stored at −20°C. EPO was measured by use of a commercially available RIA kit (EPO-Trac RIA Kit 23200; Sorin Biomedica, Puchheim, Germany). Coefficient of variation was <6%. Values are given as means ± SE in milliliters per milliliter (23). The RIA kit used was evaluated for the use in rats by means of renal sympathetic nerve traffic control, VX and DNX rats ($n = 6$) underwent the same transient hemorrhage protocol (tr-Hem) as in protocol 1. MAP was continuously recorded until 120 minutes. Blood samples for the measurement of baseline EPO levels and blood gases were taken 10 min before hemorrhage started. Further samples were taken at minute 30 (just before reinfusion of the shed blood), at minute 60, and at minute 120. Rats ($n = 6$ per group) were then euthanized with an overdose of thiopental.

Protocol 2. Transient hemorrhage: influence of VX and DNX on plasma EPO levels. To investigate the effect of transient hemorrhage on plasma EPO levels and to elucidate a putative regulatory role of renal sympathetic nerves control, VX and DNX rats ($n = 6$) underwent the same transient hemorrhage protocol (tr-Hem) as in protocol 1. MAP was continuously recorded until minute 120. Blood samples for the measurement of baseline EPO levels and blood gases were taken 10 min before hemorrhage started. Further samples were taken at minute 30 (just before reinfusion of the shed blood), at minute 60, and at minute 120. Rats ($n = 6$ per group) were then euthanized with an overdose of thiopental.

Protocol 3. Transient hemorrhage: influence of VX and DNX on RBF, RVR, RI, Do2, and VO2. Another subset of rats underwent the same denervation or sham procedures ($n = 7$ each), as described above, and was equipped with renal arterial DOP probes and an additional “aortic” and renal venous catheter to investigate the influence of renal sympathetic nerve traffic on RBF (calculated on the basis of renal DOP), RVR, RI, and renal Do2 and VO2. Again, MAP was recorded over 120 min, as well as mean and phasic DOP. Aortic and renal venous blood-gas samples were taken at the same time points as in protocol 2.

Data analysis. RSNA (full-wave rectified, integrated over 1 s) was recorded as (mV × s). To compare baseline RSNA between groups (i.e., control vs. VX, protocol 1), individually measured RSNA was recalculated in consideration of individual amplifier settings and after subtraction of background noise. This was the minimum activity after ganglionic blockade (trimetaphan-camsylate, Arfonad) and/or post-mortem activity (30-min average). Baseline values of RSNA ($μV \times s$), MAP (mmHg), HR (beats/min), mean DOP (kHz), and RI, as well as calculated parameters, were averaged from 10-min periods before hemorrhage. Changes in RSNA are expressed as percent changes from baseline (ARSNA %). Baseline values for EPO, pH, $P_{O_2}$, $S_aO_2$, and Hb were obtained from blood samples taken 10 min before hemorrhage. All data to be analyzed were tested for normality using Kolmogorov-Smirnov (KS) test to decide for parametric or nonparametric tests. Baseline parameters were analyzed using one-way ANOVA with Student Newman-Keuls post hoc “all pairwise comparison,” according to KS results. Changes of parameters over time within groups were tested with one-way repeated-measures ANOVA or Friedman’s test (according to KS), with Dunn’s post hoc “multiple comparisons control” (i.e., baseline). Differences among groups (controls vs. VX vs. DNX) at particular points in time were tested with ANOVA or ANOVA on ranks with post hoc Student Newman-Keuls or Dunn’s test (all pairwise), according to KS. Statistical significance was defined as $P < 0.05$. Data are given as group means ± SE. SigmaStat 2.03 software package (Systat Software) was used for statistical analysis.

RESULTS

Baseline parameters. Analysis of MAP, HR, RSNA, EPO levels, and mean DOP at baseline did not show significant...
differences between groups (controls vs. VX vs. DNX). Averaged over all groups, MAP was 103 ± 4 mmHg, and HR was 353 ± 23 beats/min. Recalculated mean baseline RSNA (protocol 1) was 92.2 ± 2.7 μV × s. Mean EPO level (protocol 2) was 46.2 ± 2.1 mU/ml. Mean DOP (protocol 3) was 5.87 ± 0.24 kHz. Furthermore, pH, PO2, and Hb levels did not differ between groups (Table 1). Thus none of these parameters was affected by VX, DNX, or sham procedures.

Protocol 1. Results from protocol 1 are shown in Fig. 1. In controls, hypotensive hemorrhage leads to biphasic response of RSNA and HR. RSNA showed a short-lived increase (~2 min) followed by sustained suppression (~39 ± 5% from baseline; *P < 0.05). HR increased, but slowly returned toward baseline level within 6 min. VX abolished these second-phase responses. After the initial increase, suppression of RSNA was nearly completely abolished. HR increased but failed to return to baseline. At minute 60, well after reinfusion of the shed blood, MAP, HR, and RSNA in both groups had returned to baseline levels and remained stable until the end of the experiment (minute 120).

Table 1. Parameters that determine arterial oxygen content and pH

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Baseline</th>
<th>30 min</th>
<th>120 min</th>
</tr>
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<tbody>
<tr>
<td></td>
<td>C</td>
<td>VX</td>
<td>DNX</td>
</tr>
<tr>
<td>Po2</td>
<td>99 ± 3.8</td>
<td>98 ± 3.6</td>
<td>100 ± 3.2</td>
</tr>
<tr>
<td>Hb</td>
<td>14.2 ± 0.6</td>
<td>13.8 ± 0.8</td>
<td>14.1 ± 0.7</td>
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<tr>
<td>O2 sat</td>
<td>97.1 ± 0.6</td>
<td>97.4 ± 0.8</td>
<td>98.3 ± 0.5</td>
</tr>
<tr>
<td>pH</td>
<td>7.371 ± 0.041</td>
<td>7.373 ± 0.039</td>
<td>7.368 ± 0.037</td>
</tr>
</tbody>
</table>

Values are group means ± SE. Po2, partial pressure of oxygen (Torr); Hb, hemoglobin content (g/dl); O2 sat, oxygen saturation of hemoglobin (%); C, controls with intact nerve traffic; VX, vagotomized rats; DNX, renal-denervated rats. Data are pooled from protocols 2 and 3. Parameters determining arterial oxygen content differed neither between groups nor during time course in protocols 2 and 3. Only pH decreased in all groups, but this was not different between groups. *P < 0.05, 30 min vs. baseline.

Protocol 2. The main results from protocol 2 are shown in Fig. 2. As in protocol 1, in all three groups (control, VX, DNX), the tr-hHem protocol decreased MAP within 4–6 min. Again, withdrawal rate was adjusted by the observed decrease
in MAP, and, after reinfusion of the shed blood, MAP returned to baseline in all groups and stabilized until the end of the experiment (minute 120) as in protocol 1. During hemorrhage and the first hour after reinfusion, plasma EPO levels in all groups just tended to increase very slightly. However, at minute 120, in DNX rats, EPO was significantly increased ~1.5-fold to 75 ± 3 mU/ml (P < 0.05; DNX vs. control and VX), and in VX rats ~1.3-fold to 64 ± 4 mU/ml (P < 0.05; VX vs. control and DNX), whereas, in controls, EPO remained unchanged.

In none of the groups was there a statistically significant change in Hb content, PO2, or oxygen saturation, which determined the CaO2, nor was there any difference between the groups in these parameters (Table 1). Only pH was decreased at the end of hemorrhage, possibly due to increased anaerobic metabolism (Table 2).

Protocol 3. Results from protocol 3 are shown in Figs. 3 and 4 and Table 2. Again, the same hemorrhage protocol was used in controls, VX, and DNX rats. Figure 3 shows that renal DO2 and VO2 did not differ between groups at baseline conditions. As expected, a significant decrease in DO2 at the end of hemorrhage (minute 30) was found in all groups, but this was significantly more pronounced in DNX rats. However, there was no difference in DO2 between controls and VX. VO2 did not change significantly throughout the experiment; it only tended to be increased in controls and VX at the end of hemorrhage, but this was not significant. Oxygen ER increased in all groups and tended to be higher in DNX; however, it was not significant (see Table 2).

The fall in DO2 was not due to changes in CaO2 (see Table 2), but to renal perfusion, as shown in Fig. 4B. Renal DOP and thus blood flow decreased, along with reduced blood pressure in all groups, whereas this was even less pronounced in VX rats compared with controls and DNX during the first 10 min of hemorrhage. In controls, blood flow partially recovered, whereas in DNX it further decreased until the end of hemorrhage.

RVR (Fig. 4C) did not differ between groups at baseline. RVR decreased to similar values (minute 7) in all groups; however, in DNX rats, RVR then increased to significantly higher values compared with controls and VX.

RI (Fig. 4D) in controls and VX rats did not differ at baseline but was significantly higher in DNX. In all groups, a steep increase in RI was found. In VX and DNX, RI remained constantly high, but in controls RI decreased progressively until the end of hemorrhage. RI curves from DNX and VX rats showed a similar shape, whereas the “DNX curve” was significantly shifted upward compared with VX. At the end of hemorrhage, there was a statistically significant difference among all three groups.

DISCUSSION

Our study was done to test the hypothesis that renal nerves modulate EPO formation in the setting of tr-hHem, where RSNA is suppressed and, if so, to elucidate putative underlying mechanisms.

Hemorrhage-induced RSNA inhibition is thought to be mediated by a cardiopulmonary reflex, whereas the adrenal sympathetic nerve activity augmentation observed in this setting rather seems to be mediated by arterial baroreceptor pathways (5, 39). Neither the intrinsic trigger (6), nor the physiological significance of this “paradox” RSNA inhibition are as yet clearly understood.

We measured plasma EPO before, during, and after tr-hHem under conditions in which RSNA was differentially manipulated. With intact renal nerve traffic (controls), hemorrhage-induced sympathoinhibition of approximately ~40% from baseline was observed, but there was no significant increase in EPO level (at best, a subtle trend was seen; approximately +7–12%). This might indicate that the transient hemorrhage protocol used was just subthreshold “hypoxic”. It is noteworthy that the hemorrhage protocol used did not lead to significant changes in “systemic oxygenation parameters,” such as PO2, SaO2, or Hb, which determine CaO2. Furthermore, systemic MAP, renal DOP, RVR, as well as renal DO2 and VO2 were unaffected by acute VX and chronic DNX.

However, with VX, which attenuated RSNA suppression (RNSA just beneath baseline), we could observe a significant, albeit small, increase (~1.3-fold) in EPO levels. Moreover, with bilateral DNX, i.e., discontinuation of any reflex RSNA influence, surprisingly, an even more pronounced increase in EPO levels was observed (~1.5-fold).

Keeping in mind that EPO formation is regulated on its mRNA level, it is not surprising that these changes could only be observed with delay, well after cessation to the hemorrhagic stimulus (42). The EPO effects we observed in our experiments are quite small compared with earlier studies investigating the

<table>
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<th>Parameter</th>
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<td></td>
<td>C</td>
<td>VX</td>
<td>DNX</td>
</tr>
<tr>
<td>CaO2</td>
<td>19.1±0.20</td>
<td>19.2±0.49</td>
<td>18.6±0.59</td>
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<td>16.3±0.58</td>
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<td>a-vCO2</td>
<td>2.56±0.24</td>
<td>2.88±0.34</td>
<td>2.42±0.14</td>
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<tr>
<td>O2-ER</td>
<td>13.4±1.34</td>
<td>15.1±1.77</td>
<td>13.0±1.2</td>
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<tr>
<td>Lactate</td>
<td>5.1±0.23†</td>
<td>9.1±1.3</td>
<td>10.6±1.3</td>
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</table>

Values are group-means ± SE. CaO2, arterial oxygen content; CV02, venous oxygen content; a-vCO2, arteriovenous oxygen difference; O2-ER, oxygen extraction rate. CaO2 differed neither between groups nor during time course. CV02 was decreased at the end of hemorrhage; thus a-vCO2 was increased, but there was no difference between the groups. O2-ER was increased in all groups significantly and tended to be higher in DNX rats, but this difference did not reach statistical significance. Lactate was increased in all groups at the end of hemorrhage compared with baseline, and this increase was significantly higher in VX rats. Baseline lactate in controls was lower in controls than in VX and DNX. *P < 0.05, 30 min vs. baseline; †P < 0.05, C vs. VX and DNX; ‡P < 0.05, VX vs. C and DNX.
role of renal nerves in EPO formation (3, 9, 13), which used clear-cut hypoxic stimuli lasting for hours. This discrepancy is probably due to stimulus intensity and duration. These earlier studies, which showed conflicting results concerning RSNA impact on EPO formation (see Introduction), used DNX techniques, but RSNA response due to hypoxic stimulation was not measured in these studies. Indeed, hypoxia is known to be a reliable stimulus to increase RSNA, which in turn reduces renal cortical blood flow without affecting medullary blood flow, or renal or systemic arterial blood pressure (24, 26).

Therefore, the tr-hHem protocol used in our experiments is completely different from these earlier studies in that the kidneys are exposed to a rather short-acting “hypoxic stimulus,” i.e., reduced DO$_2$ (as shown in protocol 3), however, along with reduced RSNA in animals with intact renal sympathetic nerve traffic. In accordance with previous studies (6, 39), data from protocol 1 clearly show hemorrhage-induced RSNA inhibition, which was nearly completely abolished by VX.

Thus, in VX rats exposed to reduced DO$_2$, nearly normal RSNA obviously lead to increased EPO formation. Moreover, with DNX, i.e., discontinuation of any RSNA control, EPO formation was even pronounced. What might be the underlying mechanism?

Since there is no clear-cut indication in the literature in favor of a direct RSNA impact on EPO formation (9), changes in EPO levels in our experimental setting should rather be interpreted as a surrogate of RSNA-derived modulation of RBF or flow redistribution and/or changes in renal $\dot{V}O_2$.

Although there is evidence in the literature that EPO formation is related not only to DO$_2$ but also to $\dot{V}O_2$ (8), in our experiments (protocol 3), renal $\dot{V}O_2$ did not change significantly throughout the experiments, nor was there a significant difference between the groups (see Fig. 3). Only a subtle increase in $\dot{V}O_2$ was seen in control and VX rats, not in DNX rats at the end of hemorrhage; however, this was not statistically significant.

If at all, based on our findings, $\dot{V}O_2$ seems to play a subordinate role for the increase in EPO, which was found in VX and DNX rats at the end of the transient hemorrhage protocols.

However, the only rather weak indication for some renal metabolic effects of denervation procedures was the finding that the increase in lactate at the end of hemorrhage was significantly more pronounced in VX rats than in controls and

It is important to note that the Fontana et al. (1907) study, which aimed to investigate the role of renal nerves in EPO formation, used clear-cut hypoxic stimuli lasting for hours. This discrepancy is probably due to stimulus intensity and duration. These earlier studies, which showed conflicting results concerning RSNA impact on EPO formation (see Introduction), used DNX techniques, but RSNA response due to hypoxic stimulation was not measured in these studies. Indeed, hypoxia is known to be a reliable stimulus to increase RSNA, which in turn reduces renal cortical blood flow without affecting medullary blood flow, or renal or systemic arterial blood pressure (24, 26).

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If at all, based on our findings, $\dot{V}O_2$ seems to play a subordinate role for the increase in EPO, which was found in VX and DNX rats at the end of the transient hemorrhage protocols.

However, the only rather weak indication for some renal metabolic effects of denervation procedures was the finding that the increase in lactate at the end of hemorrhage was significantly more pronounced in VX rats than in controls and
DNX. This might reflect increased anaerobic metabolism also in the kidneys; however, there was no difference between systemic and renal venous lactate values. Since vagotomy is a practicable, yet completely unspecific, method to interrupt cardiopulmonary reflex afferents, the increased lactate in VX rats might rather be derived from other organ systems with vagal innervation and the ability of anaerobic metabolism (guts, liver).

In contrast to $V_O^2$, $D_O^2$ was found to decrease during hemorrhage in all groups, whereas this decrease was much more pronounced in DNX rats. Therefore, at least in DNX rats, the increase in EPO level might be due to critical reduction of $D_O^2$, while $V_O^2$ remained unchanged.

Since $C_aO_2$ was not changed significantly, this stronger decrease of $D_O^2$ is due to a more severe reduction in RBF due to increased RVR (Fig. 4, B and C).

The finding that EPO production is even pronounced after DNX might seem surprising on first sight, because it has been shown that acute DNX might lead to cortical vasodilatation (16). However, there is a body of evidence in favor of hypersensitivity of the chronic denervated kidney to circulating catecholamines, which presumably are increased in hemorrhage (39). For example, severe hemorrhage in rabbits produced a greater degree of vasoconstriction in the denervated kidney compared with the contralateral innervated kidney (20), a finding that seems to be confirmed by our data. Furthermore, it has been shown in many studies that renal vasculature becomes supersensitive to norepinephrine after chronic denervation (19, 21, 22). One possible mechanism might be an upregulation of $\alpha_1$-receptors following denervation (43). Furthermore, renal arteries from denervated rat kidneys were shown to be supersensitive to vasoconstrictors (17).

Even though some controversy exists about hypersensitivity and chronic changes in renal function (31), denervation hypersensitivity could at least have transient effects (25), especially when renal perfusion is reduced (21, 22). Thus the observed increase of EPO levels in DNX rats might reflect inadequate vasoconstriction due to hypersensitivity of the kidney to catecholamines released from the adrenal gland. However, acute denervation protocols were not done in our study to prove the concept of denervation hypersensitivity.

Surprisingly, when VX rats were compared with intact controls, there was no difference in $D_O^2$ at the end of hemorrhage or RVR. Furthermore, reduction of renal DOP was even less pronounced in VX rats compared with controls and DNX rats during the first 10 min of hemorrhage. In controls, blood flow partially recovered, whereas in DNX it further decreased until the end of hemorrhage. These data cannot explain the increase in EPO levels found in VX rats.

However, RI in controls and VX rats was similar at baseline but significantly higher in DNX. In all groups, a steep increase in RI was found. In VX and DNX, RI remained high, but in controls RI decreased slowly until the end of hemorrhage, possibly due to some dynamic regulation due to RSNA suppression in these rats. The RI curves in DNX and VX showed a similar shape, whereas the “DNX curve” was shifted upwards compared with VX. It seems that the dynamic “regulation” of RI seen in intact controls was likewise abolished in VX and DNX rats, whereas vascular compliance was more reduced in DNX rats, possibly due to increased catecholamine susceptibility.

At the end of hemorrhage, there was a statistically significant difference among all three groups.

According to recent literature, RI is rather influenced by downstream cross-sectional area of the arterial bed and vascular compliance than by vascular resistance (4, 35). Therefore, the obvious lack of correlation between RI and RVR results is not surprising. However, our RI results might indicate, albeit indirectly, some disturbance of the renal vascular bed due to differential interruption of renal sympathetic nerve traffic.

Since it is the renal cortical circulation that is strictly controlled by RSNA, whereas medullary circulation seems to be rather resistant to such an influence (10, 12), and EPO-producing cells as well as the “oxygen sensors” are located within the renal cortex (8, 32), one might speculate that the differential RI curve progressions might reflect denervation-related changes of renal (cortical) vascular reactivity. It has been shown previously that increase in RSNA due to direct electrical stimulation (29, 33) or by reflex action (24) leads to cortical vasodilatation, whereas RSNA attenuation or blockade leads to vasodilatation (16), effects that seem to be mediated by $\alpha_1$-receptors (11). Moreover, there is some indication that cortical circulation is even more sensitive to RSNA input when renal perfusion pressure is decreased (15), which might be of relevance in the setting of hypotensive hemorrhage.

Thus it seems reasonable that the increase in EPO levels seen in VX rats, in which the kidneys were exposed to reduced $D_O^2$ but “normal” RSNA, might be due to a failure of compensatory cortical vasodilatation, which might normally be prevented by RSNA suppression.

Further studies using more sophisticated techniques able to measure renal cortical and medullary perfusion and oxygenation simultaneously (30) are needed to further emphasize the concept that renal sympathoinhibition might act to preserve renal tissue oxygenation in the setting of tr-hHem.

REFERENCES


