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Endothelin-3 Modulates Glomerular Filtration Rate in the Isolated Perfused Rat Kidney

H. Schramek
C. C. Willinger
G. Gstraunthaler
W. Pfaller

Institute for Physiology, University of Innsbruck, Austria

Key Words
Isolated perfused kidney
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Abstract
The present study has been performed to evaluate hemodynamic and tubular effects of various endothelin-3 (ET-3) concentrations on the isolated perfused rat kidney. Using this experimental system we observed a profound reduction of renal perfusate flow at all ET-3 concentrations tested (50, 250 and 500 pmol/l), suggesting that the vasoconstrictive potency of ET-3 in the kidney is comparable to that described for endothelin-1 (ET-1). The effects on glomerular filtration rate (GFR) differed depending on the ET-3 dose applied. While 250 pmol/l ET-3 increased GFR by 30%, 500 pmol/l ET-3 markedly reduced GFR. 50 pmol/l ET-3 did not alter GFR although renal vascular resistance significantly increased. Infusion of 1 μmol/l N-nitro-*L*-arginine, a specific and potent inhibitor of nitric oxide synthesis in endothelial cells, abolished the GFR elevation induced by 250 pmol/l ET-3. In parallel with the changes of GFR we observed an increase in sodium reabsorption at 250 pmol/l and a decrease of this parameter at 500 pmol/l ET-3. Moreover, an ET-3 concentration (500 pmol/l), which induced a dramatic fall in tubular sodium load, led to an increase of fractional sodium excretion and to a decrease of renal oxygen consumption.

We conclude that ET-3 is a potent vasoconstrictor in the isolated perfused rat kidney. Furthermore, it modulates GFR in a differentiated mode, depending on the concentration used. The GFR increase at 250 pmol/l ET-3 seems to be mediated by endothelium-derived nitric oxide. In addition to its glomerular action, ET-3 might also affect tubular sodium transport.

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Dr. Herbert Schramek
Institute for Physiology, University of Innsbruck,
Fritz-Pregl-Strasse 3
A–6010 Innsbruck (Austria)

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Introduction

The human endothelin family consists of the following three structurally and pharmacologically distinct isopeptides: endothelin-1 (ET-1), -2 (ET-2) and -3 (ET-3) [1]. Of these, ET-2 is reported to be the most potent vasoconstrictor, followed by ET-1 and ET-3 [1, 2]. ET-1 was shown to exhibit a more potent constrictor activity than ET-3 both in vivo and in vitro, whereas ET-3 exerts more profound initial depressor responses in vivo [1].

With respect to the kidney, numerous studies have reported vasoconstricting and glomerular filtration rate (GFR)-depressing activity for ET-1 [3–9]. The increase in renal vascular resistance (RVR) observed results from both afferent and efferent arteriolar constriction [3, 7, 8, 10]. In addition, endothelin was found to increase urinary sodium excretion [5] and to inhibit Na^+-K^+-ATPase activity in inner medullary collecting duct cells [11].

In contrast to ET-1, little information is available concerning the renal effects of ET-3. Zimmerman et al. [12] reported that infusion of low doses of ET-3 (40 ng/kg/min) increases sodium excretion in rats without affecting GFR, while higher infusion rates (170 and 340 ng/kg/min) markedly decrease GFR, urine flow rate (UFR) and urinary potassium excretion. Furthermore, 5 ng/kg/min ET-3 was shown to increase both renal blood flow (RBF) and UFR in dogs, while GFR was unchanged throughout the infusion period [13]. This renal vasodilatory effect of ET-3 was diminished by pretreatment with N-nitro-*L*-arginine (NNLA) leading Yamashita et al. [13] to the suggestion that ET-3 may act as a vasodilatory peptide via release of nitric oxide (NO).

Since preliminary experiments showed that ET-3 might play a refined role in the control of GFR we have utilized the isolated perfused rat kidney preparation to investigate the effects of various ET-3 concentrations upon the renal vascular system and GFR without the influence of changes in renal nerve activity, systemic hemodynamic alterations and humoral factors.

Methods

Perfusion of Isolated Rat Kidneys

For all studies male Sprague-Dawley rats (200–300 g b.w.), were used, which had free access to water and commercial chow. According to the technique described by Schurek et al. [14, 15], kidney perfusion was performed as follows: Rats were anesthetized intraperitoneally with pentobarbital-Na (60 mg/kg b.w.), placed on a servo-controlled heating pad and tracheotomized. A catheter was inserted into the right jugular vein and 0.5 ml of Tris-Ringer solution (mmol/l: NaCl 139, KCl 5, $CaCl_2$ 2, Tris-HCl 5, pH 7.4) was administered at the beginning and at the end of kidney preparation. Following a median laparotomy, the right ureter was cannulated with a polypropylene tube (PP-10) connected to a larger polyethylene catheter (PE-50). After heparin injection (1 U/g b.w.) via the jugular vein catheter, the kidney was placed in a temperature-controlled metal chamber. The arterial branches of the abdominal aorta were ligated, and a double-barreled cannula was inserted into the abdominal aorta and placed close to the origin of the renal artery. The kidneys were perfused at an effective perfusion pressure of 100 mm Hg measured through the inner part of the double-barreled cannula (Gould pressure transducer P50). Perfusion pressure was held constant by a feedback-regulated perfusion pump. In all experiments single-path perfusion was used. The perfusion medium, which was thermostated to 37°C, consisted of (mmol/l): 138.4 Na^+, 5.05 K^+, 124.0 Cl^-, 18.1 HCO_3^-, 0.98 Mg^{2+}, 1.24 Ca^{2+}, 6.0 urea, 0.08 $H_2PO_4^-$, 0.66 HPO_4^{2-}, 8.3 glucose, 0.98 glutathione, 0.71 pyruvate, 2.1 *L*-lactate, 1.66 *L*-glutamic acid, 1.0 2-oxoglutarate, 0.84 *L*-malic acid and 1.0 oxalacetic acid. The perfusate contained 60 g/l hydroxy-ethyl-starch, 22 ml/l aminoplasmal 5% E free of carbon hydrates (B. Braun, Melsungen, FRG), 0.01 g/l neomycin sulfate and 2 g/l inulin. Perfusion solutions were gassed with prewarmed and water vapor saturated 95% O_2 and 5% CO_2. To obtain steady-state conditions, the system was allowed to equilibrate for 60 min after the onset of perfusion. Oxygen partial pressure of the perfusate was measured via an in-line Clark-type O_2 electrode (AVL,

Graz, Austria) using an O_2 microanalyzer (AVL-Type 937; AVL). Before each experiment, the O_2 electrode was calibrated with 92% NO_2/8% CO_2 and 95% O_2/5% CO_2, respectively. Perfusion pressure, flow rate and venous PO_2 in the effluent were monitored continuously by a vertical recorder (ABB-SE 400; ABB-Goerz, Vienna, Austria). Arterial PO_2 was measured at the beginning and at the end of each experiment and did not show any alteration. The on-line recording of renal perfusate flow (RPF) and perfusion pressure allowed the calculation of RVR. Admixture of the respective ET-3 concentrations was enabled via a flow-controlled perfusion pump, which ensured drug administration in rates of 0.5% of arterial perfusate flow.

Experimental Protocol

Three urine samples were taken from control kidneys after 60 min of equilibration. This procedure was repeated throughout the experiments (180 min) at the end of each 20-min period. In parallel, UFR was determined volumetrically by measuring the time urine needs for filling a standardized capillary. Because ET-3 exerted long-lasting and incompletely reversible effects, three concentrations were used in two separate experimental protocols. In one experimental protocol, after 80 min of equilibration, ET-3 was first admixed in the lowest effective dose of 50 pmol/l followed by a concentration of 500 pmol/l (20 min of duration each, with control periods of 20 min in between). 250 pmol/l ET-3 was administered as a single dose in separate experiments. The admixture was started again after an 80-min equilibration period with a duration of 20 min. The experiments with 250 pmol/l ET-3 were repeated under pretreatment with 1 μmol/l NNLA using the same experimental protocol as described above. Measurements of functional parameters were carried out as described for control kidneys.

Determination of Sodium and Potassium

Sodium and potassium were analyzed by flame photometry (FLM 3; Radiometer, Copenhagen, Denmark).

Determination of GFR

GFR was calculated as inulin clearance. Inulin was determined using a *D*-glucose/*D*-fructose kit [16], pursuant to which polyfructosan is measured after enzymatic hydrolysis with inulinase, while native glucose is oxidized with glucose oxidase and H_2O_2 [17]. After phosphorylation of *D*-fructose with hexokinase, the resulting fructose-6-phosphate is isomerized by phosphoglucose-isomerase, yielding glucose-6-phosphate. In the presence of the enzyme glucose-6-phosphate dehydrogenase, glucose-6-phosphate is oxidized by nicotinamide-adenine dinucleotide phosphate (NADP) to gluconate-6-phosphate, resulting in the formation of reduced nicotinamide-adenine dinucleotide phosphate (NADPH). The amount of NADPH formed in this reaction is equivalent to the amount of fructose and, therefore, to the amount of inulin in urine. NADPH was measured using a spectrophotometer (Double-Beam Spectrophotometer UV-180; Shimadzu, Japan).

Calculation of Oxygen Consumption

Oxygen consumption (Q_{O_2}) of the kidneys was calculated from the arteriovenous PO_2 difference of physically dissolved oxygen and the perfusion flow rate. Concentrations of dissolved oxygen were determined from PO_2 values using the Bunsen coefficient for oxygen in plasma at 37°C ($\alpha O_2 = 0.0214$).

Materials

Inulin was obtained from Sigma, Munich, FRG, the test kit for polyfructosan determination from Boehringer Mannheim, FRG, and inulinase (Novozym SP 230) from Novo Industri, A/S Copenhagen, Denmark. Neomycin sulfate, glutathione, *L*-glutamic acid, urea, 2-oxoglutarate, oxalacetic acid, pyruvate and *L*-malic acid were obtained from Sigma, and *L*-lactate as sodium salt from Fluka, Buchs, Switzerland. *L*-Amino acids (aminoplasmal 5% E, free of carbon hydrates) and hydroxy-ethyl-starch were a donation from B. Braun. ET-3 (human, rat) and NNLA were obtained from Sigma. ET-3 was dissolved in N_2-gassed saline (PBS, free of Ca^{2+} and Mg^{2+}).

Statistical Analysis

All values given are arithmetic mean±SEM. No error bars are shown where symbols are larger than SEM. Statistical analysis was performed by Student's t test. $p < 0.05$ was considered significant.

Results

Table 1 summarizes the overall kidney function of isolated perfused control kidneys in the course of single-path perfusion during 180 min. After 60 min of equilibration, GFR reached a value of 674.6±76.1 μl/min/g kidney weight. These markedly lower absolute GFR

Table 1. Functional parameters of isolated perfused control kidneys in the single-path configuration

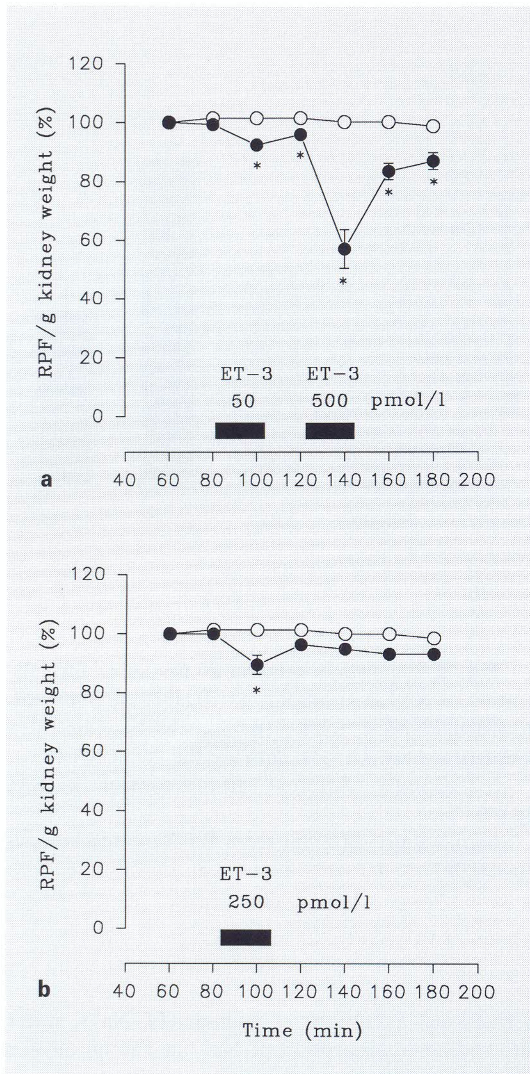
Parameter	Experimental period		
	60 min	120 min	180 min
RPF, ml min^{-1} g^{-1}	16.2±0.9	16.5±0.7	16.0±0.7
GFR, μl min^{-1} g^{-1}	674.6±76.1	652.6±71.5	531.4±43.6
UFR, μl min^{-1} g^{-1}	57.2±8.3	83.7±13.9	83.5±10.9
T-Na^+, μmol min^{-1} g^{-1}	93.4±10.5	86.6±9.9	73.6±6.0
FE-Na^+, %	4.9±0.3	8.7±0.8	11.4±1.7
FE-H_2O, %	8.5±0.3	13.3±1.2	15.8±1.8
FE-K^+, %	56.3±3.7	33.4±1.9	30.3±2.2
Q_{O_2}, μmol min^{-1} g^{-1}	4.78±0.10	4.87±0.14	4.84±0.17

RPF = Renal perfusate flow; GFR = glomerular filtration rate; UFR = urine flow rate; FE-Na^+ = fractional Na^+ excretion; FE-H_2O = fractional H_2O excretion; FE-K^+ = fractional K^+ excretion; T-Na^+ = absolute Na^+ reabsorption; Q_{O_2} = renal oxygen consumption. Each value represents the mean±SEM, n = 4.

values in isolated perfused rat kidneys (table 1) as compared with in vivo data are caused by the use of hydroxy-ethyl-starch as a colloid [18]. At the same time point, RPF amounted to 16.2±0.9 ml/min/g and UFR to 57.2±8.3 μl/min/g. For control as well as experimental kidneys the values for RPF, GFR, absolute sodium reabsorption (T-Na^+) and Q_{O_2} at 60 min were set as 100%. The admixture of vehicle alone (PBS, free of Ca^{2+} and Mg^{2+}) did not alter kidney function significantly (data not shown).

ET-3 led to a significant reduction in RPF at all concentrations tested (fig. 1). Even 50 pmol/l ET-3 (fig. 1a) significantly decreased RPF to 92.4±2.3% (n = 4). Above 50 pmol/l, ET-3 resulted in a dose-related fall of this parameter. The RPF decrease at 250 pmol/l ET-3 was even more pronounced under treatment with 1 μmol/l NNLA. Under these conditions 250 pmol/l ET-3 induced an RPF reduction from 101.5±1.4% in control to 60.8±12.0% in experimental kidneys. As seen in figure 1a, the effects of the ET-3 concentrations on RPF tested in these experiments were not completely reversible after termination of peptide admixture. Following the addition of 500 pmol/l ET-3, part of the RPF reduction persisted for up to 40 min. In parallel, the elevation of RVR at 50, 250 and 500 pmol/l ET-3 amounted to 10% (from 98.6 ± 1.4 to 108.4 ± 2.8%), 14% (from 98.6±1.4 to 112.1±4.0%) and 77% (99.9±1.3 to 177.3±17.8%), respectively (n = 4).

The effects on GFR showed a different pattern depending on the ET-3 concentrations used (fig. 2): While 500 pmol/l ET-3 led to a GFR reduction of 38% (from 90.3±4.1 to 52.2±11.0%) (fig. 2a), administration of 250 pmol/l ET-3 resulted in a 30% increase of GFR (from 97.3±5.1 to 127.4±7.8%) (fig. 2b). In comparison, 50 pmol/l ET-3 did not alter GFR significantly (fig. 2a). Pretreatment with 1 μmol/l NNLA abolished the GFR elevation seen at 250 pmol/l ET-3 (fig. 3, n = 3). NNLA alone (1 μmol/l), however, did not change GFR significantly, when compared to control kidneys (fig. 3, n = 3). In parallel, UFR increased by about 40% when 250 pmol/l ET-3 was admixed, and markedly decreased at a



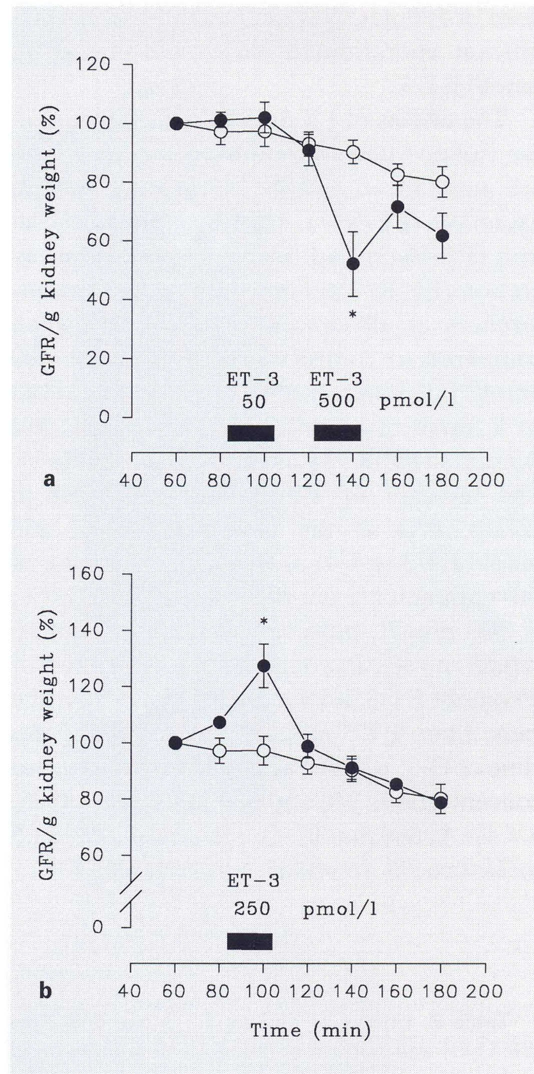


Fig. 1. Effects of various ET-3 concentrations on RPF in isolated perfused rat kidneys. In experimental kidneys (filled circles) ET-3 was added (**a**) at 50 and 500 pmol/l, respectively, or (**b**) at 250 pmol/l as indicated by filled bars above the time scales. ET-3 led to a significant decrease of RPF at all concentrations tested. Each data point represents the mean±SEM, $n = 4$. No error bars are shown where symbols are larger than SEM.
* Significantly different ($p < 0.05$) from control kidneys (open circles).

Fig. 2. Effects of various ET-3 concentrations on GFR in isolated perfused rat kidneys. In experimental kidneys (filled circles) ET-3 was added (**a**) at 50 and 500 pmol/l, respectively, or (**b**) at 250 pmol/l as indicated by filled bars above the time scales. (**a**) 500 pmol/l ET-3 led to a GFR reduction, whereas 50 pmol/l did not alter GFR significantly ($p < 0.05$). (**b**) 250 pmol/l ET-3 induced a significant increase of this parameter ($p < 0.05$). Each data point represents the mean±SEM, $n = 4$, for each condition. No error bars are shown where symbols are larger than SEM.
* Significantly different ($p < 0.05$) from control kidneys (open circles).

dose of 500 pmol/l (data not shown). No significant alteration of UFR was seen at 50 pmol/l ET-3.

The effects of ET-3 on tubular function in the isolated perfused rat kidney preparation are summarized in table 2. Fractional sodium excretion (FE-Na^+), fractional water excretion (FE-H_2O) and fractional potassium excretion (FE-K^+) did not show significant differences at all concentrations tested when compared to control kidneys, although 500 pmol/l ET-3 increased FE-Na^+ and FE-H_2O by a factor of 1.4 and 1.09, respectively, but decreased FE-K^+ by a factor of 0.95 (table 2). The increase of T-Na^+ (from 97.3±5.1 to 127.4±7.8%) at the concentration of 250 pmol/l ET-3 (n = 4) as well as the decrease of this parameter (from 90.3±4.1 to 52.3±11.0%) at 500 pmol/l, however, were significant ($p < 0.05$, n = 4). In comparison, application of 50 pmol/l ET-3 did not change T-Na^+. At the same time, ET-3 induced a dose-related decline of Q_{O_2}, which was significant for the two concentrations 250 and 500 pmol/l ($p < 0.05$, n = 4). At 500 pmol/l, Q_{O_2} fell from 100.9±1.5 to 74.0±6.3% ($p < 0.05$, n = 4) (table 2).

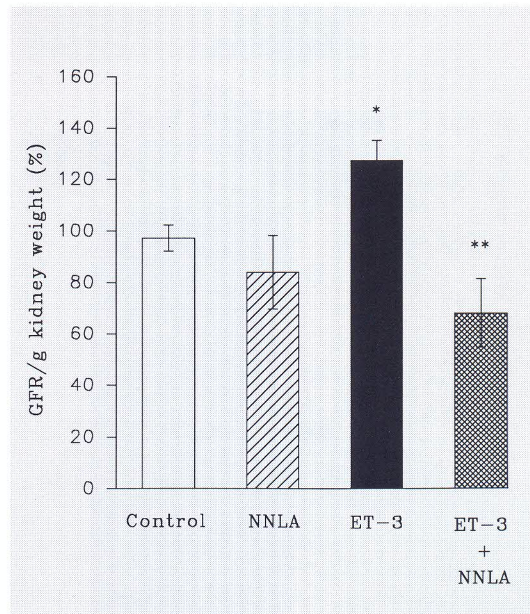


Fig. 3. Changes in GFR at 20 min after administration of NNLA (1 μmol/l, n = 3), ET-3 (250 pmol/l, n = 4) and NNLA + ET-3 (n = 3) in IPRK. Control = Control kidneys (n = 4), data are the mean±SEM.
* Significantly different from control kidneys ($p < 0.05$).
** Significantly different from ET-3-treated kidneys ($p < 0.05$).

Table 2. Effects of various ET-3 concentrations on fractional excretions of sodium (FE-Na^+), water (FE-H_2O) and potassium (FE-K^+), as well as on absolute sodium reabsorption (T-Na^+) and renal oxygen consumption (Q_{O_2}) using the same experimental protocol as described for figures 1 and 2[a]

Parameter	ET-3, pmol/l					
	control	50	control	250	control	500
T-Na^+, %	97.3±5.1	102.1±5.2	97.3±5.1	127.4±7.8*	90.3±4.1	52.3±11.0*
FE-Na^+, %	7.5±0.7	6.4±1.0	7.5±0.7	6.9±0.8	9.4±0.9	13.1±2.2
FE-H_2O, %	12.0±1.1	9.0±0.9	12.0±1.1	9.8±1.6	14.1±1.4	15.4±1.9
FE-K^+, %	37.2±2.7	31.3±3.4	37.2±2.7	32.5±3.9	31.1±1.9	29.5±3.1
Q_{O_2}, %	101.6±0.8	96.1±2.2	101.6±0.8	93.5±2.7*	100.9±1.5	74.0±6.3*

* Significantly different from control kidneys ($p < 0.05$).
[a] For control as well as experimental kidneys the values for T-Na^+ and Q_{O_2} at 60 min were set as 100%. Control = Control kidneys, data are the mean ± SEM, n = 4 for each condition.

Discussion

The current study demonstrates that ET-3 is a potent vasoconstrictor in the isolated perfused rat kidney. ET-3 dose dependently decreased RPF. Even 50 pmol/l ET-3 led to a significant fall of RPF (fig. 1a). The effect of 250 pmol/l ET-3 on RPF was more pronounced in the presence of 1 μmol/l NNLA, which is in accordance with the recently published finding of Radermacher et al. [19], who showed that NO is basally released from isolated perfused rat kidney. Since, in the present experiments, the kidneys were perfused under constant pressure, a reduced perfusate flow must correspond to an increase in RVR.

Similar results were already reported by Ferrario et al. [5] for ET-1. These authors used the isolated rat kidney, perfused at constant pressure, in a recirculating system and found a concentration-related reduction in RPF with ET-1 concentrations higher than 10 pmol/l. Recently, Yamashita et al. [13] reported that ET-3, when continuously infused in a dose of 5 ng/kg/min, causes an increase in RBF and UFR without any alteration of GFR in adult dogs. Of particular interest in this context is our present finding that GFR seems to be affected differently depending on the ET-3 concentrations used (fig. 2). No significant GFR alteration was observed when 50 pmol/l ET-3 was administered, although RVR increased. A concentration of 250 pmol/l, on the other hand, significantly increased GFR (fig. 2b), while 500 pmol/l markedly decreased this parameter (fig. 2a). Ferrario et al. [5] reported a similar and significant increase of GFR ($p < 0.05$) at ET-1 concentrations of 100 pmol/l but did not discuss this finding. In the same study, however, they observed a significant GFR reduction ($p < 0.01$) at 500 pmol/l ET-1 [5].

With respect to the ET-3 concentrations used in the present experiments it must be mentioned that endothelins are now considered as local factors rather than as circulating hormones. Although ET-3 might be a neural form of endothelins [1], the definite sites of ET-3 synthesis remain to be elucidated.

The exact sites for ET-3 action along the renal vasculature are yet unknown. In the split hydronephrotic kidney, local endothelin administration constricted the afferent as well as the efferent arterioles in segments close to the glomerulus [20]. Micropuncture studies with ET-1 in rats, measuring changes of afferent and efferent arteriolar resistances, have led to conflicting conclusions. While King et al. [7] described the constriction as predominantly postglomerular, Kon et al. [8] found a vasoconstriction of both pre- and postglomerular arterioles. At present, no such data are available for ET-3.

As shown in micropuncture studies, the GFR-depressing effects seen at higher endothelin doses can be caused by the concerted contractile action of smooth muscle-related intraglomerular mesangial cells, which seem to reduce the glomerular capillary surface area available for ultrafiltration, thereby leading to a dramatic fall in the glomerular ultrafiltration coefficient (K_f) [3, 21].

The significant GFR elevation at 250 pmol/l ET-3 described in the present study, however, could be attributed to an ET-3-induced release of NO from endothelial cells as shown in various isolated arterial vessel preparations of the rat [22–24]. Furthermore, from studies on cocultures of glomerular endothelial and mesangial cells, evidence exists that glomerular endothelial cells are able to produce a mediator with characteristics of EDRF as well as to induce the formation of guanosine 3′, 5′-cyclic monophosphate (cGMP) [25]. This elevation in mesangial cell cGMP appears to have the ability of inhibiting mesangial cell contraction [26]. To assess whether or not NO is involved in the GFR increase

seen at 250 pmol/l ET-3 we used NNLA, an arginine analogue known to be a potent inhibitor of NO synthesis by endothelial cells [13, 27]. Indeed, pretreatment with 1 μmol/l NNLA abolished the above described GFR increase observed during administration of 250 pmol/l ET-3 (fig. 3). Thus, NO at least partially accounts for the hyperfiltrative effect of ET-3. Beyond that, several lines of evidence exist that ET-1 and ET-3 act via distinct receptors, leading to different physiological effects [28–34]. Even ET-3 itself might act via two subclasses of ET-3 receptors: one with high affinity and low capacity and the other with reverse properties [29, 34].

In parallel with the above discussed changes in GFR, we observed an increased T-Na^+ at 250 pmol/l ET-3 and a marked decrease of T-Na^+ at 500 pmol/l (table 2). The increase of FE-Na^+ at 500 pmol/l ET-3 coincided with a marked fall in tubular sodium load. Furthermore, at 250 pmol/l ET-3, a decrease in the Q_{O_2} was found (table 2). This effect was enhanced at 500 pmol/l, which is in accordance with the decline in effective tubular sodium reabsorption. Several observations exist which indicate that Na^+-K^+-ATPase might be involved in these effects [11, 35]. Endothelin was shown to inhibit the Na^+-K^+-ATPase by stimulating cyclooxygenase in inner medullary collecting duct cells [11], which might explain the above mentioned Q_{O_2} reduction. On the other hand, a GFR-induced fall of Q_{O_2} due to reduced tubular sodium load must be taken into consideration. The reported marked decrease in urinary potassium excretion due to high infusion rates of ET-3 [12], however, could not be reproduced in our experimental setup.

In summary, from the experiments performed, we conclude that ET-3 has distinct hemodynamic effects in the isolated perfused rat kidney. The vasoconstrictive potency of ET-3 is comparable to that described for ET-1. Furthermore, ET-3 changes GFR in a differentiated mode depending on the concentration used. 500 pmol/l ET-3 leads to a marked GFR reduction. The GFR increase at lower ET-3 doses (250 pmol/l) is at least partially mediated by endothelium-derived NO.

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