Effect of Endothelin-3 (ET-3) on Renal Function in Rat Perfused Kidney

MASASHI WATANABE, YOICHI IZUMI, MASAYOSHI SOMA, YOSHIYASU WATANABE, NOBORU FUKUDA, YOSHINOBU ABE, MIKI ITO, HIRONOBU KAGEURA, TOMOHIRO NAKAYAMA AND MICHINOBU HATANO

The Second Department of Internal Medicine, Nihon University School of Medicine, Tokyo 173, Japan

Abstract. We examined the effect of endothelin-3 (ET-3) at a high dose (pressor dose) and a low dose (non-pressor dose) in rat perfused kidney (PK), since ET-3 has recently been reported to exert a vasodilator action especially at a low dose. Kidneys were perfused with Krebs-Henseleit buffer at a fixed flow rate (6 ml/min) in situ. After collection of the renal venous effluent and urine for 20 min, vehicle (saline; n=6), 10^{-13} M ET-3 (low dose; n=6) or 10^{-8} M ET-3 (high dose; n=6) was added to the perfusate, and sample collection was performed for the same period with each. The high dose of ET-3 significantly increased the perfusion pressure, fractional sodium excretion and synthesis of prostaglandins (PGs) consistently with a significant reduction in the glomerular filtration rate (GFR). On the other hand, the low dose of ET-3 significantly increased the GFR, urine volume and free-water clearance with no change in the perfusion pressure or synthesis of PGs. These findings suggest that a low dose of ET-3 can increase the glomerular capillary ultrafiltration coefficient and that ET-3 exerts an influence on sodium and water handling in the rat PK.

Key words: Endothelin-3, Rat perfused kidney, Renal function.

(Endocrinol Japon 38: 435-440, 1991)

ENDOTHELIN-1 (ET-1), a potent vasoconstrictor peptide [1], has been reported to play a possible role in the control of renal function through contraction of the renal vessels [2, 3] and contraction of glomerular mesangial cells [4]. The above studies demonstrated that a pressor dose of ET-1 induced a marked decrease in the glomerular filtration rate (GFR). Endothelin-3 (ET-3), which differs from ET-1, has also been reported to be a vasoconstrictor peptide [5]. Recently, both ET-1 and ET-3 have been shown to exert a vasodilator effect at low doses, and ET-3 was found to be more selective than ET-1 as a vasodilator [6]. The present study was therefore undertaken to examine the effect of ET-3 at a low dose on the renal function of the rat perfused kidney (PK).

Materials and Methods

Perfusion of the kidney

Male Wistar rats, aged 8–9 weeks and weighing 250–300 g, were used. After anesthetizing the rats with pentobarbital sodium (100 mg/kg, i.p.), an abdominal incision was made. The left kidney was exposed and the aorta was cannulated with PE 50 tubing through the superior mesenteric artery without ischemia. The kidney was then continuously perfused in situ with Krebs-Henseleit buffer containing 1 g/l glucose and 1 g/l inulin at a fixed flow rate of 6 ml/min through a filter/air eliminator (Pall Biomedical Inc., USA) with a micro-tube pump (MP-3, Tokyo Rikakikai Co., Ltd., Japan). After commencing perfusion, the
aorta above the superior mesenteric artery, the aorta below the left renal artery, the left adrenal artery, the right renal artery and the right renal vein were ligated. The left renal vein was cannulated with a 20-gauge plastic needle (Terumo Co., Japan) connected to 10 cm of tubing extension and ligated near the kidney. The left ureter was cannulated with a 23-gauge plastic needle (Terumo Co., Japan) connected to 10 cm of tubing extension. The rats were then sacrificed by bleeding. The perfusion pressure was measured via an aortic cannula with a pressure transducer (MPU-0.5, Nihon Koden Co., Japan) connected to an amplifier (AP-601G, Nihon Koden Co., Japan). The perfusate was warmed at 37°C and gassed with 5% CO₂ and 95% O₂ to maintain the pH near 7.4. The renal venous effluent was not recycled as a perfusate.

Experimental protocol

After 10–15 min as an equilibration period, the renal venous effluent and urine were collected for 20 min as a control. Subsequently, synthetic ET-3 (Peptide Institute, Inc., Japan) dissolved in saline was infused into the kidney at a final concentration of 0 M (vehicle only; n=6), 10⁻¹³ M (low dose; n=6) or 10⁻⁸ M (high dose; n=6). These doses were the same as in our previous study [7]. They were added to the perfusate with a micro-infusion pump (SP-5, Nipro, Japan) at 1.8 ml/h via the side arm of an arterial cannula. After infusion of the vehicle or ET-3, the renal effluent and urine were again collected for the same period.

The sodium concentration in the perfusate, renal venous effluent and urine was determined with a flame photometer. The inulin concentration was estimated by the anthrone method [8]. The GFR was determined from the clearance of inulin. The levels of 6-keto prostaglandin F₁α (6-keto PGF₁α), prostaglandin E₂ (PGE₂) and thromboxane B₂ (TXB₂) in the renal venous effluent were measured by a radioimmunoassay method [9]. Each renal function was expressed as per gram kidney weight employing the weight of the decapsulated unperfused right kidney.

All results in the present study are expressed as the mean±SE. Values for the various experiments were analyzed by one way analysis of variance. Changes compared to the control period were analyzed by Student's paired t-test, P values less than 0.05 were considered as significant.

Results

1. Changes in perfusion pressure (Figs. 1 and 2)

In the vehicle infused PK, there was no difference between the perfusion pressure before infusion (66.6±2.4 mmHg) and after infusion (66.6±2.5). Whereas a high dose of ET-3 caused a slight but significant (P<0.05) fall in perfusion pressure from 70.8±2.3 to 66.2±2.7 mmHg with a secondary significant (P<0.01) rise to 155.8±5.2, a low dose of ET-3 did not alter the perfusion pressure (67.5±2.8 mmHg) as compared to that before infusion (66.3±2.7). The perfusion pressure was significantly (P<0.01) higher after infusion of a high dose of ET-3 than after infusion of the vehicle or a low dose of ET-3.

![Fig. 1. Typical recordings of perfusion pressure in rat perfused kidney. Vehicle: saline. High dose ET-3: 10⁻⁸M endothelin-3. Low dose ET-3: 10⁻¹³M endothelin-3.](image-url)
2. Changes in urine volume (UV) (Fig. 2)
In the vehicle infused PK, there was no difference between UV before infusion (0.128±0.009 ml/min/g) and after infusion (0.126±0.009). A high dose of ET-3 decreased the UV from 0.110±0.006 to 0.087±0.016 ml/min/g, but no significant difference was observed. A low dose of ET-3 significantly (P<0.01) increased the UV from 0.127±0.009 to 0.190±0.021 ml/min/g. This value was significantly (P<0.01) higher than that after infusion of the vehicle or a high dose of ET-3.

3. Changes in glomerular filtration rate (GFR) (Fig. 2)
Vehicle infusion did not alter the GFR (0.220±0.016 ml/min/g) as compared to that before infusion (0.220±0.016). A high dose of ET-3 significantly (P<0.01) decreased the GFR from 0.257±0.021 to 0.119±0.021 ml/min/g. In contrast, a low dose of ET-3 significantly (P<0.01)
increased GFR from $0.223 \pm 0.008$ to $0.360 \pm 0.031$ ml/min/g. The value after the low dose of ET-3 infusion was higher than that after the vehicle or high dose ET-3 infusion.

4. Changes in sodium excretion and fractional sodium excretion (FENa) (Fig. 3)

Vehicle infusion did not alter the sodium excretion ($17.8 \pm 0.7$ μEq/min/g) as compared to that before the infusion ($18.2 \pm 1.1$). A high dose of ET-3 decreased the sodium excretion from $18.8 \pm 2.2$ to $13.2 \pm 2.5$ μEq/min/g, but no significant difference was observed. However, a low dose of ET-3 significantly ($P<0.01$) increased the sodium excretion from $19.2 \pm 1.4$ to $27.6 \pm 3.0$ μEq/min/g. There was no significant change in FENa after the vehicle infusion (from $50.1 \pm 2.2$ to $56.1 \pm 1.7$%) or a low dose of ET-3 infusion (from $51.5 \pm 2.7$ to $51.5 \pm 2.5$%). However, a high dose of ET-3 significantly ($P<0.01$) increased the FENa from $49.5 \pm 2.9$ to $74.8 \pm 2.1$%. The FENa value was significantly ($P<0.01$) higher after the high dose of ET-3 infusion than after the vehicle or low dose ET-3 infusion.

5. Changes in free-water clearance (Fig. 3)

The vehicle or high dose ET-3 infusion did not alter the free-water clearance (vehicle, $-0.78 \pm 0.80$ μl/min/g; high dose of ET-3, $0.92 \pm 0.29$) as compared to that before the infusion (vehicle, $-2.77 \pm 0.33$ μl/min/g; high dose of ET-3, $-1.65 \pm 0.78$). However, low dose ET-3 infusion slightly but significantly ($P<0.01$) increased the free-water clearance from $-2.75 \pm 1.38$ to $3.88 \pm 1.30$ μl/min/g. This value was significantly ($P<0.01$) higher than that after the vehicle or high dose of ET-3 infusion.

6. Changes in prostaglandins (PGs) in the renal venous effluent (Fig. 4)

There was no difference between the PGs in the renal venous effluent before and after vehicle infusion (6-keto PGF$_{1\alpha}$, from $0.45 \pm 0.28$ to $0.14 \pm 0.02$ ng/min/g; PGE$_2$, from $0.13 \pm 0.04$ to $0.06 \pm 0.02$ ng/min/g; and TXB$_2$, from $0.11 \pm 0.04$ to $0.10 \pm 0.03$ ng/min/g). Similarly, a low dose of ET-3 infusion did not alter the levels of PGs (6-keto PGF$_{1\alpha}$, from $0.11 \pm 0.02$ to $0.13 \pm 0.02$; PGE$_2$, from $0.11 \pm 0.02$ to $0.06 \pm 0.02$; and TXB$_2$, from $0.19 \pm 0.06$ to $0.12 \pm 0.03$). High dose ET-3 infusion significantly ($P<0.01$) increased the PGs (6-keto PGF$_{1\alpha}$, from $0.20 \pm 0.03$ to $1.44 \pm 0.56$; PGE$_2$, from $0.12 \pm 0.03$ to $0.38 \pm 0.06$; and TXB$_2$: from $0.15 \pm 0.04$ to $1.38 \pm 0.39$) as compared to the levels after the vehicle or low dose ET-3 infusion.

**Discussion**

The present results show that ET-3 at a low dose (non-pressor dose) can significantly increase the
EFFECT OF ET-3 ON RENAL FUNCTION

439

GFR, but at a high dose (pressor dose) it markedly reduced the GFR in the rat PK. Cairns et al. reported the possibility that ET-1 at a pressor dose reduced the GFR by vasoconstriction of the afferent glomerular vessels in the rabbit PK [10]. We also observed a large increase in perfusion pressure with a reduction in the GFR following infusion of a high dose of ET-3, suggesting contraction of the renal arteries. Badr et al. demonstrated a marked fall in the glomerular capillary ultrafiltration coefficient (Kf) and a contraction of mesangial cells consistently with a reduction in Kf in rats [4]. Furthermore, King et al. found that the reduction in GFR caused by ET-1 depended on a reduction in Kf rather than a vasoconstriction [11]. We were unable to clarify whether or not ET-3 at a high dose had constricted the mesangial cells in the present study. However, it seems likely that ET-3 has similar effects to ET-1 on renal function, and similar mechanisms are probably evoked by ET-3 and contribute to a reduction in GFR. It is difficult to establish why an increase in GFR occurred following ET-3 infusion at a low dose in the rat PK. It appears unlikely that a change in transcapillary oncotic pressure following perfusion with albumin-free perfusate could contribute to the increase in GFR, since we observed no difference between GFR before and after infusion of the vehicle. Changes in the perfusion pressure and flow rate can also be excluded as explanatory factors, since ET-3 did not alter the perfusion pressure at a low dose and the kidneys were perfused at a fixed flow rate in the present study. We suspect therefore that a low dose of ET-3 may increase the GFR through an increase in Kf, although it remains unclear whether an increase in Kf can be induced by an increase in the effective hydraulic permeability of the capillary wall or by an enlargement of the total surface area available for filtration, which is suspected to be caused by a relaxation of the mesangial cells. Another possibility is that a low dose of ET-3 might induce vasodilatation in favor of the afferent glomerular vessels, which may be able to increase the transcapillary hydraulic pressure gradient without a change in the perfusion pressure. Warner et al. reported that ET-3 at a low dose (0.1–3 pmol) exhibited mainly vasodilatation by release of endothelium-derived relaxing factor in the rat mesentery [6]. This discrepancy between the failure of vasodilatation after the infusion of a low dose of ET-3 in our study and the vasodilatation in their report seems unlikely to depend on differences in organ-response to ET-3, since we observed initial vasodilatation with ET-3 at a high dose. It is possible therefore that a lower dose of ET-3 may elicit intrarenal vasodilatation without affecting the overall renal vascular resistance, which results in an increase in GFR. The observed increase in FENa induced by a high dose of ET-3 might have reflected the glomerulotubular balance to some degree [12]. However, we consider that the pressure natriuresis which is thought to be caused by the increase in the hydraulic pressure in a peritubular capillary in consequence of the high perfusion pressure following the infusion of a high dose of ET-3 mainly increase the FENa, since there was no change in the perfusion pressure and FENa after infusion of the vehicle or a low dose of ET-3. We also observed marked increases in PGs in the renal venous effluent only after the infusion of a high dose of ET-3. Such findings are consistent with the reports of de Nucci et al. and Rae et al. who found that ET-1 at a pressor dose stimulated the release of PGs [13, 14]. Zeidel et al. further indicated that ET-1 inhibited Na⁺-K⁺-ATPase in the renal tubular epithelial cells by stimulating the synthesis of PGE₂ [15]. We consider therefore that tubular factors must also be involved in the increase in FENa seen after the infusion of a high dose of ET-3 by means of the natriuretic action of PGs [16, 17]. But, we could not clarify the mechanism of PGs generation or the role of TXA₂ in sodium handling after the infusion of a high dose of ET-3 in the rat PK. The high FENa values in our study might have been caused by the perfusion with albumin-free perfusate, since Firth et al. and Zamlauski-Tucker and Cohen found that a marked increase in FENa was observed when kidneys were perfused with albumin-free perfusate [18, 19]. The sodium excretion depends approximately on the GFR in the present study, suggesting that the potent effect of ET-3 on the GFR defeats the contribution of the tubulo-glomerular feedback to control of the GFR. In addition, we noted an increase in free-water clearance following infusion of ET-3 at a low dose, suggesting that ET-3 has an action on the water handling in the tubules including the collecting ducts. This might be partially supported by previous evidence indicating that ET-3 exists in the rat kidney inner medulla [20]. However, the
mechanisms involved in the disappearance of an increase in free-water clearance after infusion of a high dose of ET-3 remain obscure.

The above data suggest that a low dose of ET-3 can increase the GFR without changes in perfusion pressure or PG synthesis, suggesting an increase in Kf in the rat PK. However, the detailed mechanisms involved in the tubular action of ET-3 remain to be elucidated.

References