Involvement of Endothelin in Renal Processes
Sylvie Cornet, Alain Braillon, Christine Guilraard, Pierre-Etienne Chabrier,
Eduardo Pirotzky, and Pierre Braquet

This study examined the effect of various doses of endothelin (from 0.2 to 2 nmol/kg body wt) on regional hemodynamics in conscious unrestrained rats. Normal rats were instrumented chronically with femoral artery and vein catheters and pulsed Doppler flow probes simultaneously on the renal and superior mesenteric arteries and the abdominal aorta. Endothelin induced a biphasic response of mean arterial pressure. First, endothelin provoked a sharp hypotension with tachycardia, vasodilation of the hindquarter, and a pronounced decrease in renal and mesenteric blood flows. After this initial response, endothelin induced a dose-dependent increase of mean arterial pressure. Changes in the hindquarter vascular resistance were less pronounced than those in renal and mesenteric vascular resistances. Endothelin (2 nmol/kg) reduced renal flow (−86%) resulting from a vasoconstriction (+1,818%) significantly more pronounced than for the mesenteric vascular bed. In another set of experiments, endothelin (2 nmol/kg) induced an increase in proteinuria, characterized by an increase in excreted albumin and by the appearance of proteins with molecular weights of 20,000–280,000. Renal vascular bed exhibited a pronounced sensitivity to the vasoconstrictive effect of endothelin associated with changes in renal function. (Hypertension 1990;15:724–728)

Endothelin (ET-1) is a 21-amino peptide that has recently been isolated from porcine aortic endothelial cells. ET-1 produces systemic hypertension in several species and induces a pronounced long-lasting constriction in isolated vessels. In anesthetized spontaneously hypertensive rats, hemodynamic responses to ET-1 revealed a complex situation with a vasodilatation of hindquarter and carotid arteries but a vasoconstriction in mesenteric and renal arteries. Several studies also underlined an important impact of ET-1 on kidney. [125I]ET-1 injection in the rat showed an important ET-1 binding mainly localized to the glomeruli. Low concentrations of ET-1 caused an intense long-lasting renal vasoconstriction and a reduction in the glomerular filtration rate. Finally, ET-1 inhibits renin release from isolated rat glomeruli. No study, however, has investigated the effect of ET-1 in normal pretrained rats. This last fact is a major concern because anesthesia and surgical stress can alter cardiovascular control, and ET-1 modulates adrenergic neuroeffector transmission.

The present study investigated the effects of ET-1 simultaneously in different vascular beds on unrestrained rats by using the micropulsed Doppler flowmeter method. This study indicated that the effect of ET-1 varied from territory to territory, and the predominant effect was on the renal vasculature. This organ was the focus of a second set of experiments analyzing the proteinuria and renal morphology of rats treated with ET-1.

Methods
This study was performed in male Sprague-Dawley rats weighing 350–400 g (Charles River Labs., St. Aubin, France) kept in standard conditions.

Hemodynamic Study
Regional blood flows were measured with miniaturized pulsed Doppler flow probes connected to a Doppler flowmeter (545C directional pulsed Doppler, University of Iowa, Iowa City, Iowa) as previously described. Briefly, the rats were anesthetized with 100 mg/kg ketamine hydrochloride (Ketalar, Parke-Davis, Courbevoie, France) and acepromazine maleate 3 mg/kg (Abbott Labs., Rungis, France) by intraperitoneal injection. After a laparotomy, the superior mesenteric and left renal arteries and the abdominal aorta were carefully dissected to avoid damage to the nearby nerves. A miniaturized flow probe was sutured around each vessel under a microscope (magnification, ×6). The wire leads were tunneled subcutaneously, exited, and soldered to a connector plug fixed to the rat's skull with dental cement. The abdominal incision was closed with 4-0 silk. For mean arterial pressure (MAP) measurement and

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Mean Arterial Pressure (mmHg)

Heart Rate (b/min)

Aortic Flow (KHz)

Renal Flow (KHz)

Mesenteric Flow (KHz)

FIGURE 1. Typical recordings of hemodynamic responses in a conscious rat after intravenous bolus injection of 2 nmol/kg endothelin (↑). b/min, beats per minute.

Hemodynamic Studies

A typical response to bolus intravenous injection of ET-1 in conscious rats is shown in Figure 1. The ET-1

Analysis of Proteinuria

Forty rats were placed in individual metabolic cages. At the beginning of the experiment, the rats received an oral administration of 25 ml saline. Urine was collected for 6 hours after drug injection. Rats were divided into four groups (C, E1, E2, and Ang II) receiving a bolus intravenous injection of 0.5 ml saline (control [C] group), ET-1 1 nmol/kg body wt (E1 group), ET-1 2 nmol/kg body wt (E2 group), or angiotensin II 2 nmol/kg body wt (Ang II group).

Urinary protein concentration was determined by using the Bradford method. The composition of urinary proteins was analyzed by electrophoretic migration on 4/30 percent gradient polyacrylamide gel (PAA 4/30, Pharmacia, St. Quentin en Yullines, France) in the presence of standard proteins (known molecular weight) and by using a buffer composed of 90 mM Tris-HCl, 2.7 mM EDTA, and 89 mM boric acid (pH 8.4). A better separation of proteins with molecular weights of less than 100,000 was obtained by migration on 12% polyacrylamide gel.

Drugs

ET-1 (human or porcine, Peninsula Labs., Inc., Belmont, California) was dissolved in saline and stored at −20°C. The doses were 0.2, 0.4, 1, and 2 nmol/kg body wt Angiotensin II (human, Sigma Labs., l’Isle d’Abeau Chesnes, France) was dissolved in saline.

Statistical Analysis

Results are expressed as mean±1 SEM. The statistical comparisons included analysis of variance (ANOVA) (repeated measurements and factorial) and Student’s t test for paired data. A p value of less than 0.05 was considered statistically significant.

Results

Hemodynamic Studies

A typical response to bolus intravenous injection of ET-1 in conscious rats is shown in Figure 1. The ET-1
The hypotensive phase was rapidly followed by a gradual and long-lasting increase in MAP that was dose dependent (Table 1). This hypotension was associated with a decrease in heart rate and aortic flow at 10 minutes. The renal and mesenteric flow velocities remained reduced. Thus, ET-1-induced blood flow changes varied with the type of vascular bed studied (Table 1). In the aorta, ET-1 induced a slight decrease in flow that was not significant. In contrast, in renal and mesenteric arteries, ET-1 caused a pronounced decrease in flow. The maximum reduction of renal artery flow (~86%) was higher than the reduction in flow in the mesenteric bed (~65%). The blood flows progressively returned to normal after a 20-120-minute period for ET-1 0.2 and 2 nmol/kg body wt, respectively.

ET-1 induced a dose-dependent increase in renal and mesenteric vascular resistances (Table 2). For ET-1 0.2-1 nmol/kg body wt, this increase in vascular resistance was similar in the renal and mesenteric beds; however, with ET-1 2 nmol/kg body wt, only the renal vascular bed exhibited further vasoconstriction, +1,818.6% and +274.8%, respectively. The hindquarter appeared to be much less sensitive than the other arteries, and its resistance was increased significantly only with ET-1 2 nmol/kg body wt.

### Analysis of Proteinuria

Six hours after drug injection, an increase of proteinuria was only observed in rats receiving ET-1 2 nmol/kg body wt, and no proteinuria was induced by angiotensin II (C, 0.55±0.073; E1, 4.97±1.220 NS versus C; E2, 21.09±0.402, p<0.001; Ang II, 0.69±0.115 NS).

The electrophoretic migration of urinary proteins in gradient gel revealed that increase of proteinuria in rats in the E2 group was characterized by an increase in the excretion of albumin and smaller proteins and the appearance of proteins with molecular weights of 110,000 and 280,000.

The migration on 12% polyacrylamide gel showed that the increase of.excreted proteins with molecular weights less than that of albumin was caused by an increase of usually excreted proteins and an appearance of proteins with molecular weights of approximately 21,000, 40,000, and 50,000.

### Discussion

The present study described the acute hemodynamic effects of increasing doses of ET-1 in MAP and regional vascular resistances in chronically instrumented normal rats. As previously demonstrated in anesthetized rats,1 ET-1 induced a biphasic effect on arterial pressure that was dose dependent.

#### TABLE 1. Effect of Endothelin-1 on Mean Arterial Pressure and the Regional Blood Flow Velocities

<table>
<thead>
<tr>
<th>Endothelin (nmol/kg)</th>
<th>Changes in MAP (mm Hg)</th>
<th>% Blood flow velocity change</th>
<th>Hindquarter</th>
<th>Renal artery</th>
<th>Mesenteric artery</th>
</tr>
</thead>
<tbody>
<tr>
<td>0.2</td>
<td>9.8±1.38</td>
<td>−7.4±1.38</td>
<td>−20.5±3.30</td>
<td>−37.7±3.75</td>
<td></td>
</tr>
<tr>
<td>0.4</td>
<td>19±2.09§</td>
<td>−6.4±6.63*</td>
<td>−32.0±2.82‡</td>
<td>−50.2±1.47§</td>
<td></td>
</tr>
<tr>
<td>1</td>
<td>33.3±5.73‡</td>
<td>−9.2±8.99*</td>
<td>−49.0±4.25‡</td>
<td>−59.0±3.02§</td>
<td></td>
</tr>
<tr>
<td>2</td>
<td>50.0±3.92‡</td>
<td>−15.6±27.80*</td>
<td>−86.0±6.03†</td>
<td>−65.2±5.34*</td>
<td></td>
</tr>
</tbody>
</table>

Values are mean±SEM. n=5–8. MAP, mean arterial pressure. Statistical significance is considered from the previous dose.

* p=NS.
† p<0.001.
‡ p<0.01.
§ p<0.05.

The hypotension was followed by a gradual and long-lasting increase in MAP that was dose dependent (Table 1). This hypotension was associated with a decrease in heart rate and hindquarter flow, a pronounced decrease in renal flow velocity, and a slight decrease in mesenteric flow. Then, ET-1 acted immediately on the vascular beds by vasoconstriction in renal (+990% for 2 nmol/kg body wt) and mesenteric arteries (+83%) but vasodilation in the hindquarter (~60%).

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#### TABLE 2. Hindquarter, Renal, and Mesenteric Resistance Changes Induced by Endothelin-1

<table>
<thead>
<tr>
<th>% Resistance change</th>
<th>Endothelin (nmol/kg)</th>
<th>0.2</th>
<th>0.4</th>
<th>1</th>
<th>2</th>
</tr>
</thead>
<tbody>
<tr>
<td>Hindquarter</td>
<td>10.6±7.08</td>
<td>28.6±10.34*</td>
<td>47.5±4.90*</td>
<td>156.0±66.62†</td>
<td></td>
</tr>
<tr>
<td>Renal artery</td>
<td>39.9±6.15</td>
<td>68.2±9.08*</td>
<td>151.2±29.07‡</td>
<td>1,818.6±473.23‡</td>
<td></td>
</tr>
<tr>
<td>Mesenteric artery</td>
<td>75.8±7.73</td>
<td>132.9±9.65§</td>
<td>194.3±25.5$</td>
<td>274.8±49.34§</td>
<td></td>
</tr>
</tbody>
</table>

Values are mean±SEM. The data reported in this table represents the resistance changes (expressed in %) induced by different doses of endothelin-1 in comparison with the basal value. The increase in resistance induced by a dose of endothelin-1 is compared with the increase induced by the previous dose.

* p=NS.
† p<0.001.
‡ p<0.01.
§ p<0.05.
Initially, ET-1 involved a sharp and short-lasting hypotension associated with tachycardia and an increase in hindquarter flow, contrasting with decreases in mesenteric and renal flows. Subsequently, a progressive and long-lasting increase in MAP appeared, associated with bradycardia and decreases in the three regional flows studied. Accordingly, a pronounced increase in vascular resistances was induced by ET-1 in the three vascular beds; however, this vasoconstrictive effect was not uniform. The renal and to a lesser extent mesenteric vascular beds exhibited a greater reactivity to ET-1 than the hindquarter, and the time course of the effect also depended on the vascular bed studied, as shown in Figure 1. The general pattern of ET-1 contrasted with the effect of angiotensin II, another potent vasoconstrictor that is short lasting.2

The initial hypotension associated with a pronounced vasodilation of the hindquarter probably depends on the release of vasoactive substances. Indeed, in isolated preparations, ET-1 induced a release of prostaglandin I$_2$ and endothelium-derived relaxing factor, which can reverse contraction of the mesenteric artery induced by ET-1.16 In anesthetized cats, however, ET-1 injections of 0.03 and 0.1 nmol/kg body wt involve only a decrease in MAP and renal and mesenteric blood flows, whereas a 0.3 nmol/kg body wt dose induced biphasic responses.17,18 This opposite ET-1 effect could reflect a balance between vasoconstrictive and relaxing factors.

In isolated heart preparations, ET-1 exhibited inotropic and chronotropic actions.19,20 The precise mechanisms that mediated the vasoconstrictive action of ET-1 are uncertain. Previous studies conducted in vitro21-22 suggested a Ca$^{2+}$-mediated mechanism.

These different experiments confirmed that the investigation of ET-1 action is complex and reflects not only differences in regional sensitivity to ET-1 but also regional reflex mechanisms that oppose the constrictor action of ET-1. Such a nonuniform response was also observed with angiotensin II23 but was not so pronounced.

The greater reactivity of renal artery to ET-1 (2 nmol/kg body wt) is surprising. A putative explanation can be a greater number of ET-1 receptors on the renal artery as compared with other vessels, the release of a dilator factor such as endothelium-derived relaxing factor, which is less important in the renal vascular bed, or in contrast, a potentiation of the vasoconstrictive effect by other mediators.

ET-1 exhibited a pronounced effect on renal circulation; renal vasoconstriction was more pronounced and abrupt than in other vascular beds. Therefore, we investigated whether hemodynamic changes induced by ET-1 could have consequences on renal function or structure. ET-1 induced an increase in proteinuria, characterized by an increase of proteins usually excreted and by the appearance of proteins with molecular weights in the range of 20,000-280,000, which suggests an alteration in permselectivity of the glomerular basement membrane, tubular functions, or both. The observations of kidney under light and electronic microscopy did not reveal structural alterations or cellular death (data not shown). Because hemodynamic factors also influence the glomerular permeability,24 it is more likely that ET-1-induced hemodynamic changes are responsible for this proteinuria. Indeed, ET-1 can also contract the efferent and afferent arterioles and mesangial cells2,10 and $K_t$ (glomerular capillary ultrafiltration coefficient).7,22 The diminution of these two factors seems inconsistent with the increase of proteinuria, but the diminution of renal blood flow might involve a loosening of the glomerular basement membrane, which allows the passage of a greater quantity of proteins and larger proteins.22 Moreover, the intensive and long-lasting effect of ET-1 (2 nmol/kg body wt) on the kidney could be a determinant factor because no proteinuria was induced with the bolus intravenous injection of angiotensin II (2 nmol/kg body wt). Additionally, the hypertension induced by angiotensin II is more intense than that induced by ET-1, and the decrease of renal blood flow is similar; however, the basal values are recovered after 10 minutes for angiotensin II and 90 minutes for ET-1. The identification of new proteins excreted in the urine of rats in the ET-1 group could lead to understanding the origin of proteinuria and evaluating the impact of ET-1 on renal functions.

ET-1 can cause pronounced and long-lasting vasoconstriction that predominates in renal and mesenteric vascular beds and is associated with an increase of proteinuria. In contrast, hindquarter vessels exhibit a low sensitivity to this action of ET-1. Although a direct pathophysiological implication of ET-1 remains to be demonstrated, our results suggest that ET-1 is a new endogenous substance that can modulate and alter renal function.

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References


**KEY WORDS** • glomerular filtration rate • flowmeters, Doppler • blood pressure • vascular resistance • proteinuria • endothelin
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