Phosphoramidon-Sensitive Effects of Big Endothelins in the Perfused Rabbit Kidney

Sabine Télémaque, David Lemaire, Audrey Claing, and Pedro D’Orléans-Juste

The enzymatic conversion of human big endothelins (1, 2, and 3) to their respective active metabolites (endothelin-1, -2, and -3) was investigated in the perfused rabbit kidney through the pressor- and eicosanoid-releasing properties of these peptides. Intra-arterial bolus injections of endothelin-1 and -2 (5–50 pmol), endothelin-3 (100–250 pmol), and big endothelin-1 and -2 (100–250 pmol) into the kidney produced dose-dependent increases of perfusion pressure, whereas big endothelin-3 was inactive at doses up to 1,000 pmol. Endothelin-1 and -2 (10 nM), endothelin-3 (100 nM), and big endothelin-1 and -2 (100 nM) are potent enhancers of prostacyclin release without inducing any release of thromboxane B2 in the perfused kidney. In contrast, big endothelin-3 did not trigger the release of eicosanoids. A metalloprotease inhibitor, phosphoramidon (100 µM, 60 minutes), reduced the prostanoic release and pressor responses induced by big endothelin-1 and -2 without affecting the response induced by endothelin-1, -2, and -3. These results suggest the presence of a phosphoramidon-sensitive endothelin converting enzyme that converts the precursors of endothelin-1 and -2, but not of endothelin-3, to the renal vasculature of the rabbit. (Hypertension 1992;20:518–523)

KEY WORDS • endothelins • epoprostenol • enzyme inhibitors • kidney • rabbit studies

Human big endothelin-1 (big ET-1) is the 38-amino acid intermediate precursor of endothelin-1 (ET-1).1 We and others have suggested that big ET-1 is converted to its active metabolite (ET-1) by a putative endothelin converting enzyme (ECE)2,3 before inducing some of its pharmacological effects (i.e., pressor response and prostanoic release). More recently, it has been reported in the guinea pig and the rat in vivo4,5 and in vitro in perfused lungs6,7 that the ECE was sensitive to phosphoramidon, because the pressor- and prostanoic-releasing properties of porcine or human big ET-1 were markedly reduced by the metalloprotease inhibitor.5-7 The release of prostanoïds (predominantly prostacyclin [PGI2]) by ET-1 was previously illustrated in the perfused rabbit kidney4 and in the rat in vivo.3 Indomethacin significantly potentiated the pressor response of ET-1 in the perfused kidney and abolished the ET-1-induced inhibition of ex vivo platelet aggregation.9 We had subsequently shown that intravascular administration of big ET-1 increased the circulating levels of ET-1 and PGI2 and that indomethacin enhanced the big ET-1-induced increase of the left ventricular systolic pressure in anesthetized rabbits.3

One of the aims of the present study was to investigate the activity of a phosphoramidon-sensitive ECE in the perfused rabbit kidney model8 by comparing the pressor- and prostanoic-releasing properties of big ET-1 and ET-1. Furthermore, the conversion of the precursor of endothelin-3 (ET-3), big ET-3,9 was analyzed by comparing the pharmacological profile of these two peptides in the kidney. We had previously suggested that the ECE activity was specific for big ET-1 because big ET-3 was shown to be inactive in vivo in anesthetized guinea pigs as well as in the guinea pig pulmonary vasculature and the rat urogenital tract7,11.

In addition, there has been a recent report on the presence of endothelin-2 (ET-2) messenger RNA (mRNA) in the medullary part of the human kidney.12 Because ET-2 has been suggested to derive from a 37-amino acid precursor, big ET-2,12 we have investigated the pressor and prostanoic properties of these two peptides and assessed the effect of phosphoramidon on their responses in the perfused rabbit kidney model.

Our results show that big ET-1 and -2, but not big ET-3, are pharmacologically active and that their responses are markedly reduced by phosphoramidon in the renal vasculature of the rabbit. Thus, in the rabbit, although we have shown earlier the activity of the ECE in the pulmonary vasculature4,6 the organs involved in the production of ET-1 and -2 from their exogenously administered precursors, big ET-1 and -2, may include the renal vasculature as well.

Methods

Tissue Preparation

Experiments were performed on kidneys taken from rabbits (New Zealand White, 1.5–2.0 kg, Cuniper St-Vallérian, Canada) of either sex. Briefly, the rabbits were anesthetized with sodium pentobarbital (Sagatal, 20–30 mg/kg i.v.), and their spinal cords were sec-
tioned. The right renal artery was cannulated, and the kidney was excised while continuously perfused (5 ml/min) with heparinized (10 units/ml) Krebs' solution. The kidney was then rapidly placed in a heated chamber (37°C) and perfused (5 ml/min) with oxygenated (95% O2:5% CO2) protein-free Krebs' solution.

Experimental Protocols

After an equilibration period of 60 minutes, bolus injections of big ET-1, -2, or -3 (50-1,000 pmol) or ET-1, -2, or -3 (5-250 pmol) were administered intra-arterially (i.a.) in the kidney, and the perfusion pressure was measured with a pressure transducer (Statham P23AC, Statham Division, Gould Inc., Oxnard, Calif.) in a lateral port closed arterially and recorded on a Grass physiograph (model 7D, Grass Instrument Co., Quincy, Mass.). The prostanoid-releasing properties of the peptides were evaluated while big ET-1, -2, and -3 (10-100 nM) or ET-1, -2 and -3 (10-100 nM) were infused intra-arterially for 5 minutes via the renal artery. In some experiments, phosphoramidon (100 μM, 60 minutes) was added to the Krebs' solution before and during the administration of peptides. The venous effluent from the kidney (1-minute sample) was collected before, during, and after infusion of peptides. The samples were stored (−20°C) and later analyzed for the stable, hydrolytic metabolites of prostacyclin (6-keto-prostaglandin-F1α, 6-keto-PGF1α) and of thromboxane A2 (thromboxane B2 [TXB2]) by radioimmunoassay.13

To monitor the edema formation, the kidneys were weighed before being placed in a heated chamber and subsequently at the end of each experiment; experiments were pursued for a maximal time period of 180 minutes (weight of kidneys before the experiments, 11.3±0.5 g; at the end of the experiments, 12.7±0.5 g; n=51, p<0.05).

Drugs and Solutions; Statistical Analysis

Synthetic human big ET-1, ET-1, and ET-2 were purchased from Hukabel (Longueuil, Canada); synthetic human big ET-2, big ET-3, and ET-3 were purchased from IAF Biochem International, Inc. (Montréal, Canada); and phosphoramidon was obtained from Peninsula Laboratories (Belmont, Calif.). Heparin, 6-keto-PGF1α, 6-keto-PGF1α antiserum, thromboxane B2 (TXB2), and TXB2 antiserum were purchased from Sigma Chemical Co., St. Louis, Mo. The tracers [3H]-6-keto-PGF1α and [3H]TXB2 were purchased from Amersham (Oakville, Canada). Sodium pentobarbital (Sagatal) was obtained from May & Baker, Dagenham, UK.

The 6-keto-PGF1α antiserum has a 100% cross-reactivity with 6-keto-PGF1α, less than 10% with PGE1, PGE2, PGD2, and PGF2α, less than 0.1% with TXB2, and did not cross-react with big ET-1, -2, and -3 or ET-1, -2, and -3,13

All agents were dissolved either in phosphate-buffered saline (PBS) (pH 7.4; Sigma) or distilled water except for indomethacin, which was dissolved in Trizma base (Sigma) (0.2 M; pH 7.4). All values in the text and figures are expressed as mean±SEM of n observations. Statistical comparisons between groups were made by unpaired Student's t test. Values of p≤0.05 were considered statistically significant.

Results

Effect of Big ET-1 and ET-1 on Perfusion Pressure

The mean basal perfusion pressure in the rabbit kidney was 51±2 mm Hg (n=51) before the administration of peptides. Intra-arterial bolus injections of ET-1 (5 and 10 pmol) in the rabbit kidney induced a dose-dependent pressor response (ΔPP=14±3 and 41±6 mm Hg, respectively; n=14; Figure 1) that lasted 10-15 minutes. Although big ET-1 at 50 pmol was found to be inactive (n=4), higher doses (100 and 250 pmol) caused a dose-dependent increase in perfusion pressure (by 11±2 and 16±2 mm Hg, respectively; n=10; Figure 1).

The pressor responses to ET-1 (5 and 10 pmol) were unaffected in kidney pretreated with phosphoramidon (100 μM i.a.) given 60 minutes before the administration of ET-1 (n=8; Figure 1). In contrast, the increase of perfusion pressure induced by big ET-1 (100 and 250 pmol) was markedly reduced in the presence of phosphoramidon (n=4-9, p<0.05; Figure 1).

Effect of ET-2 and ET-3 and Their Respect Precursors on the Perfusion Pressure in the Isolated Kidney

Table 1 illustrates the comparative pressor effects of ET-2 and -3 and their respective precursors in the perfused rabbit kidney. ET-2 at doses ranging from 10 to 50 pmol induced a dose-dependent increase in perfusion pressure (n=4-5). Similarly, big ET-2 and ET-3 also induced the same type of pressor effects; however, the doses necessary to induce similar increases were about 10 times higher than for ET-2. In contrast, big ET-3 at concentrations up to 1,000 pmol was inactive in that system (n=4). Pretreatment of the kidney with phosphoramidon (100 μM for 60 minutes) abolished the pressor responses induced by big ET-2 (100-250 pmol) without significantly affecting the responses to ET-2 (10-50 pmol) and ET-3 (100-250 pmol) (n=4-6, p<0.05; Table 1).

Prostanoid-Releasing Properties of Endothelins and Their Precursors in the Perfused Rabbit Kidney

The infusion of ET-1 (10 nM) through the perfused rabbit kidney significantly increased the release of PGI2 (from 0.5±0.2 to 4.1±0.5 ng/ml; n=8, p<0.05). The maximal release was reached 5-6 minutes after the start

Figure 1. Bar graph shows pressure effects of endothelin-1 (ET-1) (5 and 10 pmol) and big ET-1 (100 and 250 pmol) on the rabbit renal vasculature, in the absence (open bars) or presence (solid bars) of phosphoramidon (100 μM, 60 minutes). Each bar was the mean±SEM of four to nine experiments; *p<0.05.
TABLE 1. Pressor Effects of ET-2, Big ET-2, ET-3, and Big ET-3 in the Perfused Rabbit Kidney

<table>
<thead>
<tr>
<th>Peptide</th>
<th>Dose (pmol)</th>
<th>Increase in perfusion pressure (mm Hg)</th>
<th>In presence of phosphoramidon (100 μM)</th>
</tr>
</thead>
<tbody>
<tr>
<td>ET-2</td>
<td>5</td>
<td>0</td>
<td>Control</td>
</tr>
<tr>
<td></td>
<td>10</td>
<td>25±7</td>
<td>22±5</td>
</tr>
<tr>
<td></td>
<td>50</td>
<td>43±8</td>
<td>56±11</td>
</tr>
<tr>
<td>Big ET-2</td>
<td>50</td>
<td>0</td>
<td>Control</td>
</tr>
<tr>
<td></td>
<td>100</td>
<td>12±3</td>
<td>3±2*</td>
</tr>
<tr>
<td></td>
<td>250</td>
<td>24±7</td>
<td>1±1*</td>
</tr>
<tr>
<td>ET-3</td>
<td>50</td>
<td>0</td>
<td>Control</td>
</tr>
<tr>
<td></td>
<td>100</td>
<td>5±2</td>
<td>8±1</td>
</tr>
<tr>
<td></td>
<td>250</td>
<td>22±5</td>
<td>25±4</td>
</tr>
<tr>
<td>Big ET-3</td>
<td>50</td>
<td>0</td>
<td>Control</td>
</tr>
<tr>
<td></td>
<td>100</td>
<td>2±2</td>
<td>2±2</td>
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<tr>
<td></td>
<td>250</td>
<td>2±2</td>
<td>2±2</td>
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</tbody>
</table>

ET-2, endothelin-2; big ET-2, big endothelin-2; ET-3, endothelin-3; big ET-3, big endothelin-3. Each value represents the mean±SEM of four to six determinations.

*p<0.05.

of the infusion (Figure 2). Big ET-1 at the same concentration failed to enhance the release of PGI$_2$ (from 0.3±0.1 to 0.4±0.2 ng/ml; n=4) from the perfused rabbit kidney, yet a concentration 10 times higher (100 nM) of big ET-1 significantly increased the basal release of PGI$_2$ (from 0.6±0.3 to 5.1±1.4 ng/ml; n=5, p<0.05; Figure 2). Big ET-1 (100 nM) and ET-1 (10 nM i.a.) infused for 5 minutes did not induce a significant release of TxB$_2$ (from 1.3±0.2 to 1.4±0.3 ng/ml; n=8).

As for ET-1 and big ET-1, a similar prostanoid-releasing profile was observed with ET-2 and big ET-2 (10 and 100 nM, respectively), although the maximum

releases of PGI$_2$ induced by the latter peptides are lower (2.5±0.2 and 3.2±0.8 ng/ml, respectively; n=4–6; Figure 3) than the release triggered by ET-1 and big ET-1 (Figure 2). Infusion of both peptides through the arterial renal vasculature induced a marked release of PGI$_2$, which was sustained even after the interruption of the infusion. ET-3 at 10 nM, in contrast to ET-1 and ET-2 (10 nM) and big ET-2 (100 nM) from the perfused rabbit kidney in the absence (open bars) or in the presence (solid bars) of phosphoramidon (100 μM). Prostacyclin was monitored for 10 minutes after the interruption of a 5-minute infusion of either peptide through the kidney arterial vasculature (i.a.). B, basal levels of prostacyclin measured before the administration of peptides. Each bar was the mean±SEM of at least four determinations.

**Figure 2.** Profile of prostacyclin release (measured as 6-ketoprostaglandin F$_{1\alpha}$ [6-keto-PGF$_{1\alpha}$] in nanograms per milliliter) induced by endothelin-1 (ET-1) (10 nM) and big ET-1 (•, 10 nM and ○, 100 nM) from the perfused rabbit kidney. Prostacyclin was monitored for 10 minutes after the interruption of a 5-minute infusion of either peptide through the kidney arterial vasculature (i.a.). B, basal levels of prostacyclin measured before the administration of peptides. Each point was the mean±SEM of at least four determinations.

**Figure 3.** Bar graphs show profile of prostacyclin release (measured as 6-ketoprostaglandin F$_{1\alpha}$ [6-keto-PGF$_{1\alpha}$] in nanograms per milliliter) induced by endothelin-2 (panel A) and big ET-2 (panel B) from the perfused rabbit kidney in the absence (open bars) or in the presence (solid bars) of phosphoramidon (100 μM). Prostacyclin was monitored for 10 minutes after the interruption of a 5-minute infusion of either peptide through the kidney arterial vasculature (i.a.). B, basal levels of prostacyclin measured before the administration of peptides. Each bar was the mean±SEM of at least four determinations.

**Effect of Phosphoramidon on Big ET-1, Big ET-2, ET-1, and ET-2 Release of Prostacyclin**

Pretreatment of the kidney with phosphoramidon (100 μM i.a.) for 60 minutes before and during the infusion of peptides did not significantly affect the PGI$_2$ release induced by ET-1 (10 nM) but markedly reduced
the release of PGI$_2$ induced by big ET-1 (100 nM) ($n$=5-6, $p<0.05$; Figure 5). Indeed, the PGI$_2$ level measured in phosphoramidon-treated kidney did not increase significantly compared with basal level after a 5-minute infusion of big ET-1 (from 1.0±0.3 to 1.8±0.6 ng/ml; $n$=5).

In another series of experiments, the release of PGI$_2$ induced by big ET-2 was inhibited by phosphoramidon (100 nM, 60 minutes; maximal release of PGI$_2$: control, 3.2±0.8 ng/ml; in presence of phosphoramidon, 1.2±0.2 ng/ml; $n$=5, $p<0.05$). In contrast, the release of PGI$_2$ induced by ET-2 (10 nM) was not significantly reduced by phosphoramidon (Figure 3).

**Discussion**

Our results show that big ET-1 and ET-1 induce an increase of perfusion pressure and significantly enhance the release of PGI$_2$ from the perfused rabbit kidney. We had initially reported that big ET-1 and ET-1 induced similar pressor responses in the anesthetized rabbit because of a gradual conversion of big ET-1 to ET-1 by an ECE. In the present study, we observed that big ET-1 markedly increases the perfusion pressure in the kidney, but at a concentration 20–25 times higher than ET-1. Furthermore, in kidneys pretreated with phosphoramidon, the pressor effect of big ET-1, but not ET-1, was significantly reduced, suggesting the presence of a phosphoramidon-sensitive ECE in the renal vasculature of the rabbit.

It has been reported that injections of ET-1 into the perfused rabbit kidney stimulated the release of PGI$_2$. In addition, in the anesthetized rabbit, ET-1 induced an indomethacin-sensitive platelet aggregation, suggesting that ET-1 in vivo releases PGI$_2$ into the circulation in sufficient amounts to inhibit platelet function. We subsequently reported that big ET-1 increased the amount of immunoreactive ET-1 in the circulation and caused the inhibition of ex vivo platelet aggregation. Big ET-1 also enhances the release of PGI$_2$ in perfused rabbit lungs at concentrations 10 times higher than those required for ET-1.

In the present study, we were able to significantly reduce the pressor response and prostanooid release induced by big ET-1 in the rabbit renal vasculature by the use of phosphoramidon without affecting the responses to ET-1. These results are in accordance with previous observations showing that phosphoramidon abolished the pressor responses of big ET-1 in vivo in the rat and guinea pig and its prostanooid-releasing properties from rat and guinea pig perfused lungs.

Prostacyclin has been shown to modulate the renal vascular effects of ET-1 in the dog in vivo. Because big ET-1 and ET-1 markedly increase the release of PGI$_2$ from the perfused rabbit kidney, we suggest that pressor effects of both peptides on the renal renal vascular system are masked by the release of vasodilatory prostanooids, as is the canine renal vasculature. This is in accord with the potentietating effects of indomethacin on the pressor responses of ET-1 and big ET-1 in anesthetized rats and rabbits. Although ET-1 predominantly releases PGI$_2$ from the perfused rabbit kidney, it also enhances the release of PGE$_2$, albeit in much smaller amounts than PGI$_2$. Hence, one cannot exclude a modulatory effect of PGE$_2$ in the ET-1 and big ET-1-induced renal vasoconstriction. Furthermore, big ET-1 was 10 times less potent than ET-1 as a releaser of PGI$_2$ from the perfused rabbit kidney. It is worthy of mention that the protein-free Krebs' solution used in our study may alter renal responses to endothelins. Nonetheless, a prostanooid-releasing profile of big ET-1 similar to that of ET-1 was also found in the perfused rabbit lung, where the precursor was found to be 10 times less potent than ET-1. While this report was under revision, a study showed similar results in the perfused rat kidney, in which model it was reported that the pressor
effects induced by big ET-1 were phosphoramidon sensitive. In addition, we show for the first time that big ET-2 induces phosphoramidon-sensitive pharmacological effects. Indeed, although the intermediate precursors of the three endothelins have been described, their pharmacological profile is yet to be fully studied. In the perfused kidney, big ET-2 has a profile similar to that of big ET-1 in terms of phosphoramidon-sensitive pressor effects and prostanoid-releasing properties. This is of interest, considering that the highest concentration of ET-2 mRNA has been found in the medullary part of the kidney in humans.12

Big ET-3 is inactive at concentrations 10 times higher than those of big ET-1 or -2 in the perfused rabbit kidney. The lack of effect of big ET-3 may be explained by the fact that its active metabolite, ET-3, is relatively weak both in terms of pressor agent and as a releaser of prostanoids in the perfused kidney. Indeed, ET-3 was found to be 10–25 times less potent than ET-1 and -2 as a pressor agent and as a releaser of PGI2. Thus, the biotransformation of exogenously administered big ET-3 in the perfused rabbit kidney might result in the release of concentrations of ET-3 that are insufficient to induce any pharmacological effects in that system. Alternatively, since we have shown in vivo and in vitro in the guinea pig and also in the rat vas deferens in vitro11 that big ET-3 may not be converted to its active metabolite, the inactivity of big ET-3, as opposed to ET-3, suggests a lack of converting activity of the ECE for the former peptide in the perfused rabbit kidney as in other experimental models.6,11,19 Our results are in accordance with the observations made by Okada et al,17 who reported a very weak conversion of big ET-3 to ET-3 by the phosphoramidon-sensitive ECE in cultured bovine endothelial cells.

It is also worthy of mention that when comparing the potencies of ET-1 and ET-3 in terms of both pressor and prostanoid-releasing properties, we have shown that the latter peptide was much less potent than ET-1 in the perfused kidney. This suggests that the receptor for endothelins, the activation of which induces renal vasoconstriction and prostanoid release, is of the ETA type18 as opposed to ETB receptors,19 on which the three endothelin isoforms are equipotent. In a recent study, we have shown that ET-1–induced release of PGI2 was mediated by the activation of ETB receptors in the rat pulmonary vasculature.20

In conclusion, we have shown that big ET-1 and big ET-2, to induce their pressor effect as well as the release of prostanoids in the rabbit kidney, require the activity of a phosphoramidon-sensitive ECE that is present and active in the renal vasculature. Thus, the activity of the ECE that has been initially identified in the pulmonary vasculature6 is also present in the renal vasculature in the rabbit. Because the ECE has not yet been identified as a unique single enzyme species, further biochemical investigations will be required to assess whether intravascularly administered big ET-1 and -2 are processed by a single or alternatively by two distinct phosphoramidon-sensitive enzymes in vivo and in vitro.

Because the renal vasculature in many animal species has been shown to be exquisitely sensitive to ET-1,12–23 the study of the endogenous generation of the three potent endothelin isoforms may yield some essential information on their respective roles in the renal vasculature homeostasis as well as their implications in renal physiopathology.

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References


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