Diuretic-Natriuretic Actions and Pressor Effects of Big-Endothelin (1–39) in Phosphoramidon-Treated Rats (43693)

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Abstract. **The effects of phosphoramidon, a metalloproteinase inhibitor, on the pressor and renal actions of big-endothelin (BET), the precursor of porcine Endothelin-1 (ET), was studied in rats. In control rats, BET (0.3, 1.0, and 3.0 nmol/kg) elicited a marked increase in mean arterial blood pressure (from 110 ± 7 to 105 ± 7, 120 ± 8, 147 ± 6 mm Hg, respectively), and a prominent, dose-dependent, diuretic and natriuretic response (fractional sodium excretion (FENa) increased from 0.4 ± 0.2 to 0.8 ± 0.2, 3.1 ± 0.1, and 8.5 ± 1.7%, respectively). Pretreatment with phosphoramidon (10 mg/kg + 0.25 mg/kg/min) completely abolished the increase in blood pressure induced by BET, but the diuretic-natriuretic effects were only partially inhibited (FENa increased from 2.0 ± 0.9 to 3.7 ± 1.5, 3.9 ± 1.3, and 4.3 ± 1.2%, respectively, $P < 0.05$). Rats treated with phosphoramidon only had no natriuresis over time (FENa changed from 1.9 ± 0.5 to 2.3 ± 0.3, 1.6 ± 0.4, 1.7 ± 0.6 respectively, *P*—NS). The data suggest that, unlike the vascular type of the enzyme, the renal *endothelin converting enzyme* is relatively insensitive to phosphoramidon. Further, diuresis and natriuresis can be induced by BET in the absence of any pressor effect.** [P.S.E.B.M. 1994, Vol 205]

The endothelins are a family of potent biologically active peptides with diverse effects on various vascular and extravascular organs and systems (1, 2). The putative precursor of porcine endothelin-1 (ET-1), big-endothelin (1–39) (BET), is purported to be relatively inactive by itself and needs to be converted to the active, 21 amino-acid residue peptide to exhibit any significant biological activities (3).

ET-1 has marked antidiuretic and antinatriuretic properties (4), whereas BET has surprisingly potent diuretic and natriuretic actions (5). The mechanisms mediating the diuretic-natriuretic actions of BET have not been elucidated at present. However, these effects of BET may be related to the observed inhibition of ET-1 on sodium transport (6) and on osmotic water permeability in the collecting duct (7). Alternatively, because of the pressor effect of BET, the observed increase in sodium and water excretion may be secondary to the rise in renal perfusion pressure (8). In contrast, the reduced urinary excretion of sodium and water following ET-1 may be due to the intense prerenal vasoconstriction, with its associated decrease in renal plasma flow (RPF) and glomerular filtration rate (GFR) (5).

The enzyme responsible for the conversion of BET to the active ET-1 has been termed *endothelin converting enzyme* (1). Phosphoramidon (PA), a metalloproteinase inhibitor, has been recently reported to block the conversion of BET to ET-1 by endothelial cells *in vitro* (9, 10) as well as to inhibit the pressor response to BET *in vivo* (11). PA, therefore, seems to be an effective inhibitor of the endothelin converting enzyme, at least of the isoform present in the vascular endothelium.

The aim of the present study was to assess the effect of pretreatment with phosphoramidon on the di-

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uretic-natriuretic actions of BET and thus to evaluate whether the diuretic-natriuretic effect of BET depends on the pressor action of the peptide.

Materials and Methods

Chemicals. Porcine big-endothelin (1–39) and phosphoramidon were purchased from Peninsula Laboratories (Belmont, CA). Para-aminohippurate (PAH) was purchased from Sigma Chemical Co. (St. Louis, MO). Inulin was purchased from BDH Chemicals (Poole, UK). Inactin (5-sec-butyl-5-ethyl-2-thiobarbituric acid) was purchased from BYK, Guldens (Konstanz, FRG).

Animals. Male Sprague-Dawley rats, 250–300 g, were anesthetized with Inactin (100 mg/kg ip), placed on a thermoregulated operating table and prepared for clearance studies. Catheters (PE 50) were inserted into the right jugular vein for infusion of fluids and drugs, into the right carotid artery for measurement of blood pressure and for blood sampling, and into the left femoral vein for administration of PA. A PE 200 catheter was inserted into the trachea to allow clearing of the airway when necessary. Urine was collected through a catheter (PE 60) inserted into the urinary bladder. Immediately following surgery an infusion of 0.9% NaCl containing Inulin (20 mg/ml) and PAH (5 mg/ml) was started at a rate of 1.5 ml/hr.

Experimental Protocol. After an equilibration period of 1 hr a baseline clearance period of 30 min was obtained, and then an infusion of PA was started with a bolus injection of 10 mg/kg followed by a sustained infusion at 0.25 mg/kg/min ($n = 7$). Bolus injections of increasing doses of BET at 0.3, 1, and 3 nmol/kg in 0.3 ml saline were given 30 min later, and urine was collected at 30-min intervals. Blood samples were obtained at the midpoint of each collection period. The control rats ($n = 6$) were handled in a similar manner with the exception that they received 0.9% NaCl instead of PA. A third group of rats ($n = 5$) served as time control. Those rats were prepared identically but received PA only. The total volume of fluids given was equivalent to 2% body wt/hr in all groups.

Analytical Methods. Blood pressure was measured with a pressure transducer (Gould, Statham, P23Db) and the mean arterial pressure (MAP) was recorded on a paper chart. Urine flow rate was determined gravimetrically. Urinary and plasma sodium and potassium concentrations were determined by a flame photometer (Instrumentation Laboratories, model 943). Concentrations of Inulin and PAH were determined colorimetrically, and their clearance values were equated to the glomerular filtration rate (GFR) and renal plasma flow (RPF), respectively. Renal Vascular Resistance (RVR) was calculated from MAP and calculated renal blood flow using RPF and blood hematocrit. Data are expressed as mean ± SEM. Statistical analysis was performed using Student's t-test, or analysis of variance (ANOVA) as appropriate. Differences were considered statistically significant at $P < 0.05$.

Results

In control rats bolus injections of BET consistently induced within minutes a characteristic increase in MAP, which unlike ET-1 injections, were not preceded by any vasodepressor effect (5). The hypertensive response was dose-dependent and prolonged, usually lasting throughout the entire 30-min collection period. The maximal increase in MAP was obtained with the highest dose of 3 nmol/kg BET, and ranged between +23 mm Hg and +45 mm Hg, with a mean of +36 ± 6 mm Hg.

Pretreatment with PA, in the experimental group, did not significantly reduce basal MAP (115 ± 4 vs 118 ± 4 mm Hg, P—NS), but completely abolished the pressor responses to BET; the MAP after the highest dose of BET was actually lower than baseline (−9 mm Hg). In the time control group, treatment with PA only induced a similar decrease in MAP (Fig. 1A).

Renal function parameters are detailed in Table I. Renal hemodynamics were not significantly affected by incremental doses of BET. PAH clearance remained constant (Fig. 1B), and RVR increased only with the highest dose of BET (Fig. 1C). Likewise, GFR did not change significantly throughout the experiment (Fig. 2A). However, urine flow and urinary sodium excretion increased dramatically (Fig. 2B) and FENa reached 8.6 ± 1.7% at the highest dose of 3.0 nmol/kg (Fig. 2C).

In contrast to the complete inhibitory effect of PA pretreatment on MAP and on RVR changes induced by BET (Fig. 1A and C), statistically significant diuresis and natriuresis persisted during PA administration (Fig. 2). In fact, at a dose of 1.0 nmol/kg of BET, diuretic and natriuretic effects of similar magnitude were observed in both experimental and control rats (Fig. 2B and C). PA pretreatment significantly attenuated only the effects of the highest dose of 3.0 nmol/kg of BET, nevertheless, a substantial increase in urine output, and particularly a 2-fold increase in sodium excretion was still evident.

Time control rats treated with PA only had initial diuresis and natriuresis but these did not increase significantly over time (FENa changed from 1.9 ± 0.5 to 2.3 ± 0.3, 1.6 ± 0.4, 1.7 ± 0.6 respectively, P—NS) (Fig. 2). In addition their MAP as well as RPF and urine flow tended to decrease during the experiment (Figs. 1 and 2).

Discussion

The major finding of this study is that PA pretreatment has disparate effects on the pressor and on the

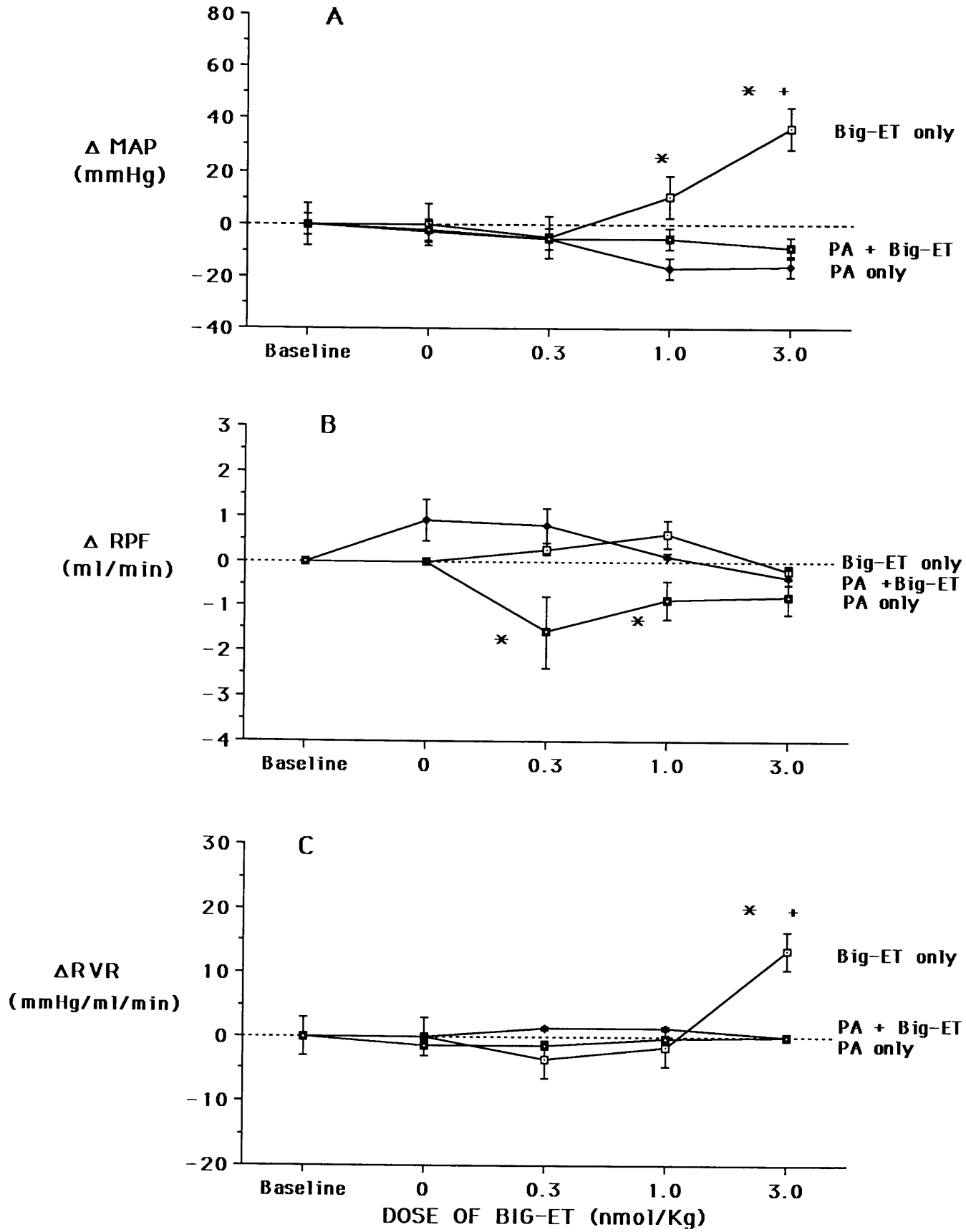


Figure 1. Changes in the systemic and renal hemodynamics following incremental doses of big-endothelin (BET) in the presence (n = 7), or in the absence of phosphoramidon (PA) (n = 6), and in time control rats treated with PA only (n = 5). MAP = Mean arterial pressure, RPF = Renal plasma flow, RVR = Renal vascular resistance. $^{*}P < 0.05$ vs baseline. $^{+}P < 0.05$ for differences between groups.

Table I. Renal Function Parameters in Three Groups of Rats Treated with BET only (n = 6), PA + BET (n = 7), or PA only (n = 5), Following Bolus Injections of Incremental Doses of BET (0.3, 1, and 3 nmol/kg)

BET (nmol/kg)	BET only				PA + BET				PA only			
	0	0.3	1	3	0	0.3	1	3	0	0	0	0
V	6.5	12.7	43.1*	114.8*	36.2	49.1	44.8	43.9*,+	52.3	48.3	30.6	21.2*,+
(μl/min)	± 0.7	± 1.7	± 6.5	± 19.5	± 14.9	± 17.6	± 12.5	± 10	± 4.8	± 8.7	± 12.5	± 7
UNaV	0.7	1.9	7.0*	16.6*	4.2	7.6	8.1*	7.8*,+	4.98	6.2	4.4	3.3*,+
(μEq/min)	± 0.3	± 0.4	± 1.2	± 2.9	± 1.9	± 2.7	± 2.3	± 1.7	± 0.9	± 0.8	± 1.3	± 1
UKV	1.1	1.8	2.9*	2.6*	2.2	2.5	2.4	2.3	3.02	2.69	2.43	2.32
(μEq/min)	± 0.1	± 0.1	± 0.6	± 0.3	± 0.3	± 0.2	± 0.3	± 0.3	± 0.3	± 0.3	± 0.5	± 0.3
%ENa (%)	0.4	0.8	3.1*	8.6*	2.0	3.7	3.9*	4.3*,+	1.9	2.3	1.6+	1.7+
	± 0.2	± 0.2	± 0.5	± 1.7	± 0.9	± 1.5	± 1.3	± 1.2	± 0.5	± 0.3	± 0.4	± 0.6
%EK (%)	25.8	33.1	38.1	43.7*	46.3	48.0	39.5	50.4	37.5	41.3	34.6	34.6
	± 3.4	± 2.6	± 1.9	± 4.6	± 4.7	± 2.9	± 1.9	± 4.6	± 10.6	± 5.7	± 4.3	± 6.7
GFR	1.3	1.5	1.6*	1.4	1.5	1.4	1.5	1.3	1.9	1.8	1.7	1.7
(ml/min)	± 0.1	± 0.1	± 0.1	± 0.2	± 0.2	± 0.1	± 0.2	± 0.1	± 0.1	± 0.1	± 0.2	± 0.3

BET, big-endothelin; V, urine flow rate; UNaV, sodium excretion rate; UKV, potassium excretion rate; %ENa, fractional excretion of sodium; %EK, fractional excretion of potassium; GFR, glomerular filtration rate.
* $P < 0.05$ vs baseline, $^{+}P < 0.05$ vs BET only.

renal actions induced by intravenous bolus injection of BET. Whereas PA effectively abolishes the increase in MAP and RVR induced by BET, it does not completely inhibit the diuretic and natriuretic effects of the peptide. During PA infusion, increases in sodium excretion are evident at all doses of BET. Only the excessive diuretic-natriuretic response induced by the highest dose of 3.0 nmol/kg of BET is significantly attenuated by PA.

Several possible explanations can be forwarded for the persistent diuretic-natriuretic effects of BET in the presence of effective inhibitory doses of PA. First, the possibility that BET has direct renal effects independent of its conversion to the active ET-1 seems unlikely, since such a mechanism has not been demonstrated in any other organ system (3, 12). Moreover, the kidney has been demonstrated to produce and contain both ET-1 (13–15), as well as abundant receptors for ET-1 (16), suggesting that conversion of BET to ET-1 is necessary for full activity of BET.

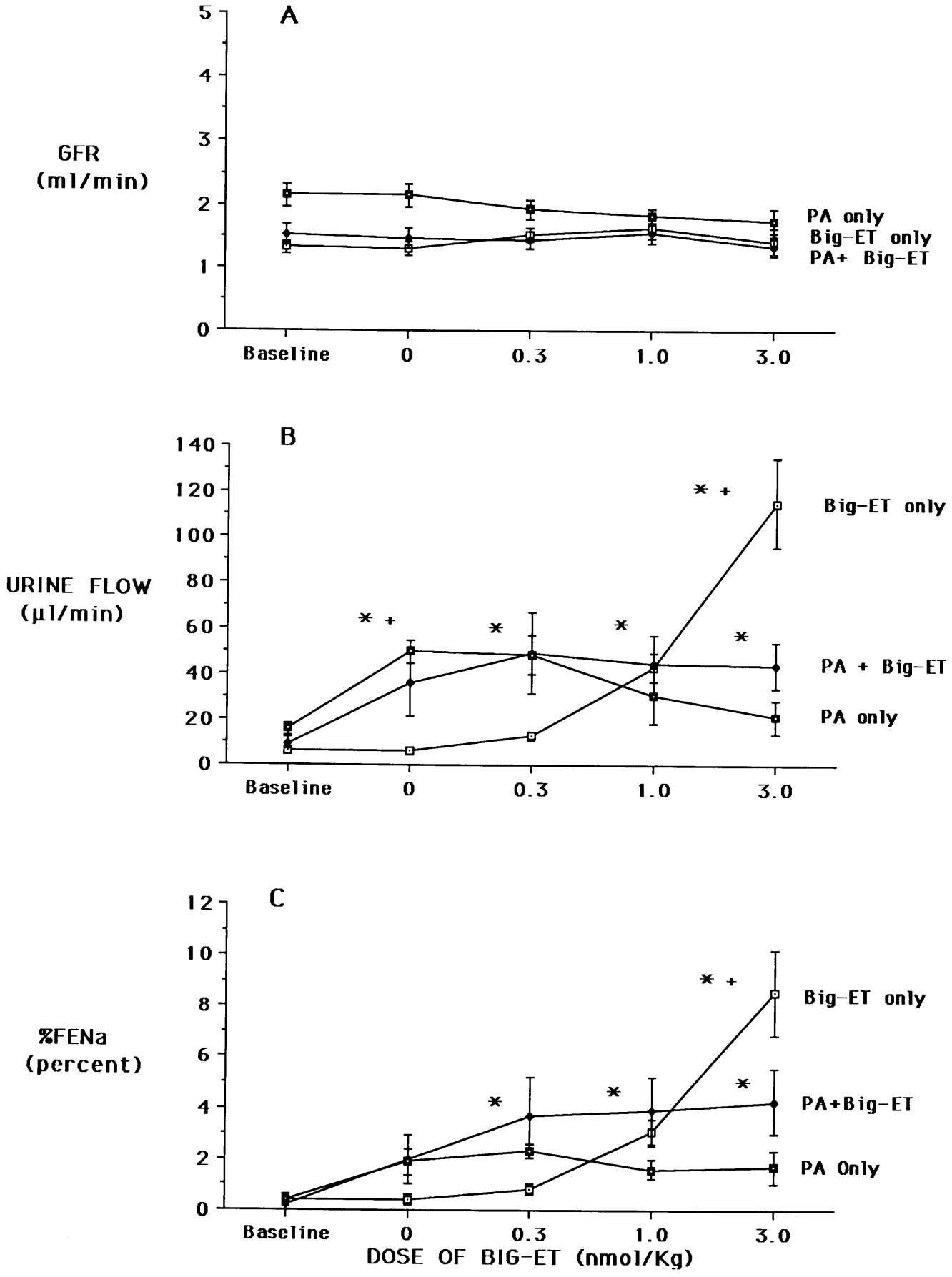
Another possibility is that PA has a limited access into renal cells containing endothelin converting enzyme, which could explain why some renal effects of BET were preserved whereas vascular effects were abolished. Such a mechanism, although not excluded, is unlikely because endothelin converting enzyme activity is probably localized on the cell membrane (10).

The most plausible explanation then is that the renal endothelin converting enzyme, which is responsible for the diuresis-natriuresis induced by exogenous BET, is not sensitive to PA. It has been shown *in vitro* that vascular endothelial cells contain a PA sensitive enzyme (9), but other tissues, *i.e.*, bovine adrenal medulla (17) and rat lung (18) contain endothelin converting enzymes that are not inhibitable by PA. Thus, although the properties of the rat renal endothelin converting enzyme have not been elucidated *in vitro*, it is possible that this enzyme is less sensitive to inhibition by PA. The differences between the endothelial form and the tubular form of the enzyme could possibly account for the disparate vascular and renal effects of BET in the presence of PA. This difference may have importance in the development of synthetic endothelin converting enzyme inhibitors for both experimental and possible clinical use.

The results of this study indicate that at least part of the diuretic-natriuretic effect of BET is not dependent on the increase in blood pressure, because a marked diuresis and natriuresis was observed at low doses of 0.3 and 1.0 nmol/kg of BET, when the changes in renal perfusion pressure were minimal. Furthermore, a significant increase in sodium and water excretion was seen when all pressure changes were abolished by PA. When the increase in systemic arterial pressure is large, as with the high dose of 3.0 nmol/kg, an additional component of pressure-natriuresis is observed. This suggests that the mechanism of the diuresis-natriuresis induced by BET is multifactorial.

Recently, a study by Pollock and Opgenorth using continuous infusion of BET reached contrasting conclusions regarding the renal effects of BET based on inhibition of the natriuretic action of the peptide by PA (20). The reason for the differences between the studies is unclear, but may have been due to the different methods used. However, a careful analysis of the data presented in their study shows that the inhibition by PA was incomplete. For example, urine flow, total sodium excretion and FENa in the group that received

Figure 2. Renal effects of incremental doses of big-endothelin (BET) in the presence (n = 7), or in the absence of phosphoramidon (PA) (n = 6), and in time control rats treated with PA only (n = 5). GFR = Glomerular filtration rate, FENa = Fractional excretion of sodium. *P <0.05 vs baseline, ^{+}P <0.05 for differences between groups.


A
GFR
(ml/min)
5
4
3
2
1
0
Baseline
0
0.3
1.0
3.0
PA only
Big-ET only
PA+ Big-ET
B
URINE FLOW
(µl/min)
140
120
100
80
60
40
20
0
Baseline
0
0.3
1.0
3.0
Big-ET only
PA + Big-ET
PA only
C
%FENa
(percent)
12
10
8
6
4
2
0
Baseline
0
0.3
1.0
3.0
DOSE OF BIG-ET (nmol/Kg)
Big-ET only
PA+Big-ET
PA Only

BET + PA was two to three times higher than in the time control group, even though blood pressure was less than 10% higher. Therefore, our view of the findings of both studies is that BET has significant residual renal effects, even in the presence of PA at levels which effectively inhibit the vascular actions of BET.

The data also support the hypothesis that endogenous ET has a significant role in renal transport processes (5), possibly by autocrine or paracrine mechanisms. The fact that diuresis and natriuresis were induced in the absence of significant effects on either renal plasma flow or GFR, suggests a direct tubular site of action for ET. These tubular effects on water and sodium transport may be induced either by inhibition of Na/K ATPase activity by ET-1 (6), or by other mechanisms like inhibition of some of the effects of vasopressin on collecting ducts (7, 19).

In conclusion, this study demonstrates that big-endothelin (1–39) has marked diuretic-natriuretic properties in rats that are not pressure dependent. These actions are not abolished by pretreatment of the rats with PA at a dose which effectively inhibits the vascular effects of the peptide. The renal effects of BET appear to be due to direct tubular action of mature ET-1 on sodium transport and water permeability. It is possible that the renal isoform of the putative endothelin converting enzyme is relatively insensitive to inhibition by PA. Future specific inhibitors of endothelin converting enzyme are expected to possess disparate effects on different target organs with potentially important therapeutic implications.

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