Mechanisms of Big Endothelin-1–Induced Diuresis and Natriuresis
Role of ET B Receptors

Aaron Hoffman, Zaid A. Abassi, Sergey Brodsky, Rawi Ramadan, Joseph Winaver

Abstract—Endothelin-1 (ET-1) at high concentrations has marked antidiuretic and antinatriuretic activities, whereas its precursor, big endothelin-1 (big ET-1), has surprisingly potent diuretic and natriuretic actions. The mechanisms underlying the excretory effects of big ET-1 have not been fully elucidated. To explore these mechanisms, we examined the effects of a highly selective ET B antagonist (A-192621.1), a calcium channel blocker (verapamil), a nitric oxide synthase inhibitor (N-nitro-L-arginine methyl ester [L-NAME]), and a cyclooxygenase inhibitor (indomethacin) on the systemic and renal actions of big ET-1 in anesthetized rats. An intravenous bolus injection of incremental doses of big ET-1 (0.3, 1.0, and 3.0 nmol/kg) produced a significant hypertensive effect that was dose dependent and prolonged (from 113 ± 7 mm Hg to a maximum of 148 ± 6 mm Hg). The administration of big ET-1 induced marked diuretic and natriuretic responses (urinary flow rate increased from 8.5 ± 1 to 110 ± 14 μL/min, and fractional excretion of sodium increased from 0.38 ± 0.13% to 7.51 ± 1.24%). Glomerular filtration rate and renal plasma flow significantly decreased only at the highest dose of big ET-1. Pretreatment with A-192621.1 (3 mg/kg plus 3 mg · kg $^{-1}$ · h $^{-1}$) significantly abolished the diuretic (17 ± 5 μL/min to a maximum of 19 ± 3 μL/min) and natriuretic (0.29 ± 0.1% to a maximum of 1.93 ± 0.37%) responses induced by big ET-1. Moreover, A-192621.1 potentiated the decline in glomerular filtration rate and renal plasma flow and the increase in mean arterial blood pressure produced by the low doses of big ET-1. Similar to A-192621.1, pretreatment with a nitric oxide synthase inhibitor (L-NAME, 10 mg/kg plus 5 mg · kg $^{-1}$ · h $^{-1}$) significantly and comparably reduced the diuretic and natriuretic actions of big ET-1 and augmented the hypoperfusion/hypofiltration and systemic vasoconstriction induced by high doses of the peptide. Pretreatment with verapamil (2 mg · kg $^{-1}$ · h $^{-1}$) slightly inhibited the diuretic/natriuretic effects of the high-dose big ET-1 and completely prevented the increase in mean arterial blood pressure provoked by the peptide. Unlike verapamil and L-NAME, only indomethacin administration was associated with significant natriuretic/diuretic responses and did not influence the pressor effect and renal actions of big ET-1. Taken together, these results suggest that big ET-1–induced diuretic and natriuretic responses are mediated mainly by stimulation of nitric oxide production coupled to ET B receptor subtype activation.

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Key Words: endothelin receptors, endothelin nitric oxide verapamil prostaglandins diuretics

Endothelins (ETs) are a family of vasoconstrictive 21–amino acid peptides that are synthesized and released by endothelial cells that act in a paracrine/autocrine mode of action. 1 ET-1, the principal isoform of the ET family, is generated through a unique proteolytic cleavage of its precursor, big ET-1 (38 or 39 amino acids), by a specific phosphoramidon-sensitive metalloprotease called ET-converting enzyme (ECE). 1-4 The mature ET-1 activates 2 subtypes of receptors, ET A and ET B . 5,6 The activation of the ET A receptor, which is located in vascular smooth muscle cells, increases intracellular Ca$^{2+}$, leading to prolonged vasoconstriction. 7,8 In contrast, the activation of ET B receptors, first described on endothelial cells, induces a release of nitric oxide (NO) and prostaglandins (PGs), thus leading to vasodilation. 9-13 ET B receptors have also been described in certain smooth muscle cells of vascular beds and may contribute under certain circumstances to ET-1–mediated vasoconstriction. 14-16 Whether relaxation or constriction is the predominant effect of ET-1 depends on the relative density of ET receptor subtypes on the endothelial and smooth muscle cells. The kidney is both a target organ and a major source of ET-1 production. 7,17-19 The highest concentrations of immunoreactive ET-1 in the body have been detected in the renal medulla. 20 In addition, gene expression and immunoreactive

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peptides of ET-1\textsuperscript{13,22} and its receptors have been demonstrated in the renal tissue, especially in the medullary region.\textsuperscript{23–26} The renal vasculature appears to be highly sensitive to the vasoconstrictor action of ET compared with other vascular beds.\textsuperscript{7,8} Whole-organ studies in intact rats have demonstrated that a short-lasting infusion of ET into the renal artery decreases renal plasma flow (RPF), glomerular filtration rate (GFR), and urine volume.\textsuperscript{27,28} Similarly, a long-term infusion of ET into conscious dogs results in increased renal vascular resistance and decreased GFR and RPF.\textsuperscript{29} The effect of ET on sodium and water excretion is variable and depends on the dose and source of ET. A systemic infusion of ET in high doses results in a profound antinatriuretic and antidiuretic effect, apparently secondary to the decrease in GFR and RBF.\textsuperscript{7,30} However, in low doses or when produced locally in tubular epithelial cells, ET has been claimed to decrease the reabsorption of salt and water.\textsuperscript{27,31} In addition, ET has been shown to have a direct inhibitory effect on vasopressin-stimulated water permeability in the collecting duct.\textsuperscript{32} In this context, we have previously shown that in contrast to ET-1, administration of the ET precursor, big ET-1, causes diuresis and natriuresis associated with a marked increase in arterial blood pressure.\textsuperscript{33} Most likely, local conversion of big ET-1 to ET by ECE allows the mature peptide to gain access to renal sites not accessible to exogenous ET-1. Thus, the hemodynamic and excretory actions of infused big ET-1 reflect the renal effects of ET-1 locally produced de novo.

Recently, evidence has accumulated that suggests the stimulatory effects of ET-1 on water and sodium excretion are mediated through ET\textsubscript{A} receptors. First, it is well known that the renal medulla is very rich in ET\textsubscript{B} receptors,\textsuperscript{19,34,35} where their activation provokes the release of NO in large amounts.\textsuperscript{36,37} Second, several studies have demonstrated that medullary NO plays a pivotal role in the regulation of renal medullary hemodynamics and excretory function.\textsuperscript{37–41} Indeed, inhibition of intrarenal NO production can reduce sodium excretion and suppress the pressure-natriuresis response.\textsuperscript{38,39,42} Third, our previous observation that ET-1 acts through ET\textsubscript{B} to induce transient medullary vasodilatory response\textsuperscript{40} appeals to the notion that the renal excretory actions of ET are mediated through these receptors. So far, few studies have investigated the effects of systemic inhibition of each step in the cascade of big ET/ET-1 cellular action on its renal function.\textsuperscript{42,43,44} These studies do not allow for an unequivocal conclusion that ET\textsubscript{B} receptors coupled to NO synthesis are responsible for the diuretic and natriuretic actions of big ET-1 or locally produced ET-1. Therefore, the present study was carried out to test the involvement of the ET\textsubscript{B} receptor/NO axis in the renal and systemic actions of big ET-1. Furthermore, because the actions of ET-1 are known to be mediated in part by an increase in cytosolic calcium,\textsuperscript{45} as well as by the stimulation of PG synthesis,\textsuperscript{36,47} we explored the effects of verapamil and indomethacin on the excretory actions of big ET-1.

**Methods**

Male Wistar rats (270 to 330 g; Harlan Laboratories) were maintained on normal rat diet and water ad libitum. Rats were anesthetized with Inactin (thiobutabarbitral sodium salt) (100 mg/kg IP; Research Biochemicals Inc) and placed on a heated surgical table to maintain body temperature at 37°C. After tracheostomy, polyethylene catheters (PE-50) were inserted into the left carotid artery, jugular vein, and urinary bladder for measurements of mean arterial pressure (MAP), infusion of various solutions, and urine collection, respectively. A solution of 0.9% saline containing 20 mg/mL inulin and 5.0 mg/kg para-aminohippuric acid was infused intravenously throughout the experiment at a rate of 1.0% body wt. The rats were treated according to the "Guide for Animal Experimentation" of the Faculty of Medicine, Technion-Israel Institute of Technology, Haifa, Israel.

**Experimental Protocol**

After an equilibration period of 1 hour, 2 baseline clearance periods of 30 minutes were obtained, but only the second period, representing the steady state period, was used for calculations. Bolus injections of incremental doses of big ET-1 (0.3, 1.0, and 3.0 nmol/kg in 0.3 mL saline) were administered at 60-minute intervals, and urine was collected into preweighed vials under mineral oil. Two 30-minute clearance periods were obtained after each dose of big ET-1, but only the second period was used for calculations. Urine volume was measured gravimetrically. Blood samples (0.3 mL) were obtained at the midpoint of each collection period. Urinary losses were replaced with an equal volume of 0.9% NaCl solution. The following protocols were designed to examine the effects of various drugs on the renal and systemic actions of incremental doses of big ET-1, as described. After an equilibration period of 1 hour, rats (n=7) were intravenously injected with the active enantiomer of a racemate, A-192621.1 [2R-(4-propoxyphenyl)-4S-(1,3-benzodioxol-5-yl)-1-(N-(2,6-diethylphenyl)aminocarbonyl methyl)-pyrrolidine-3R-carboxylic acid; Abbott Laboratories], a highly selective ET\textsubscript{B} antagonist, 30 minutes before the injection of big ET-1 at a bolus dose of 3 mg/kg, followed by the same dose per hour throughout the experiment. This antagonist has been reported to completely block the depressor response of the ET\textsubscript{B} receptor agonist sarafotoxin S6c.\textsuperscript{48} Moreover, in preliminary experiments conducted in our laboratory, we showed that this dose of ET\textsubscript{B} antagonist inhibits the depressor action of ET-1 (1 nmol/kg) without affecting the pressor response. In a second group (n=7), we evaluated the effect of pretreatment with verapamil (2 mg·kg\textsuperscript{-1}·h\textsuperscript{-1}; Sigma Chemical Co), a calcium channel blocker, on the renal action of big ET-1. In a third group (n=7), rats were pretreated with N-nitro-L-arginine methyl ester (L-NAME; Sigma Chemical Co) as a bolus (10 mg/kg), followed by a sustained infusion at 5 mg·kg\textsuperscript{-1}·h\textsuperscript{-1}, 30 minutes before the administration of big ET-1. To examine the possible involvement of the renal PG system in the renal actions of big ET-1, in the final experimental protocol, rats (n=6) were pretreated with indomethacin (1 mg/kg; Sigma Chemical Co) 30 minutes before the administration of big ET-1.

**Analytical Methods**

Urinary sodium concentrations were determined with the use of a flame photometer (model 943; Instrumentation Laboratories). Concentrations of inulin and para-aminomhippuric acid were determined colorimetrically, and their clearance values were equated with GFR and RPF, respectively.

**Statistical Analysis**

Statistical significance was assessed with ANOVA for repeated measurements. Duncan’s test was used to evaluate the level of significance in pairwise comparisons. P<0.05 was considered to represent a statistically significant difference. Data are expressed as mean±SEM.

**Results**

**Systemic and Renal Effects of Big ET-1 Injection**

Figure 1 and the Table illustrate the effects of the bolus injection of incremental doses of big ET-1 on MAP (Table), urinary flow rate (UV) (Figure 1A), fractional sodium excre-
tion (FeNa) (Figure 1B), GFR (Figure 1C), and RPF (Figure 1D) in control rats. Big ET-1 induced within minutes a significant dose-dependent increase in MAP, which, unlike ET-1 injection, was not preceded by a vasodepressor effect. The vasoconstrictive response lasted throughout the entire monitoring period and was maximal (Δ135 mm Hg) with the injection of the highest dose of big ET-1. Big ET-1 produced significant increases in both UV and FeNa that reached 110±6±14 μL/min and 7.5±1.2%, respectively, at the highest dose of 3.0 nmol/kg. A bolus injection of big ET-1 at low doses (0.3 and 1 nmol/kg) did not affect either GFR (Figure 1C) or RPF (Figure 1D). In contrast, the injection of big ET-1 at a dose of 3.0 nmol/kg slightly decreased both GFR from a baseline value of 1.5±0.2 to 1.44±0.2 mL/min and RBF from a baseline value of 5.0±1.0 to 3.9±0.6 mL/min. Thus, the injection of big ET-1 in incremental doses induced significant hypertensive, diuretic, and natriuretic responses that were associated with small changes in GFR and RPF.

Effects of A-192621.1 on Systemic and Renal Actions Induced by Incremental Doses of Big ET-1

Pretreatment with A-192621.1 did not significantly affect basal MAP (114±5 mm Hg before the infusion of A-192621.1 compared with 118±6 mm Hg after treatment) (Table). However, A-192621.1 potentiated the hypertensive response to the lower doses of big ET-1 but not to the highest dose of 3.0 nmol/kg. Initial moderate increases in UV and FeNa were obtained with the infusion of A-192621.1 alone (Figures 1A and 1B). GFR and RPF remained unchanged 30 minutes after the infusion of this antagonist (Figure 1C and 1D). However, the diuretic and natriuretic responses to big ET-1, particularly at high doses, were significantly diminished by treatment with A-192621.1 (Figures 1A and 1B), whereas the GFR and RPF were unchanged 30 minutes after the infusion of this antagonist (Figure 1C and 1D). In contrast, A-192621.1 markedly augmented the hypofiltration and hypoperfusion rates of the highest dose of 3.0 nmol/kg. Collectively, these data demonstrate that the

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<th>Effect of Injection of Big ET-1 on MAP in Normal Rats and Rats Pretreated with A-192621.1, Verapamil, L-NAME, or Indomethacin</th>
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After surgery and equilibration, baseline values of MAP were continuously recorded with a computerized data acquisition system with Labtech Acquire software. A-192621.1, verapamil, L-NAME, and indomethacin were injected intravenously, and MAP recording was continued for an additional 30-minute period before the administration of incremental doses of big ET-1 (drug alone) and throughout the periods of the peptide injections.

*P<0.05 vs baseline.
diuretic and natriuretic, but not the vasoconstrictive, responses to big ET-1 administration are mediated by the activation of ET_2 receptor subtype.

**Effects of Verapamil on Systemic and Renal Actions Induced by Big ET-1**

Pretreatment with verapamil alone significantly reduced basal MAP from 113±7 to 88±4 mm Hg (P<0.05) (Table). In addition, the pressor effect of big ET-1 was markedly inhibited by this agent, but the diuretic and natriuretic effects, particularly at the lower doses of big ET-1, were unaffected (Figures 2A and 2B). Both GFR and RPF were not affected by verapamil pretreatment (Figures 2C and 2D). Thus, these results suggest that big ET-1–induced diuresis/natriuresis is not simply pressure diuresis. In addition, the renal, but not the vascular, actions of big ET-1 are independent of extracellular calcium influx.

**Effects of L-NAME on Systemic and Renal Action of Big ET-1**

Basal MAP values for the L-NAME group (Table) are significantly higher than those of other groups. According to our experience with Wistar rats, such values are in the normal range for this strain. We have no explanation for this particular deviation in the basal MAP of the rats that were included in the L-NAME protocol compared with the other experimental groups.

The effects of pretreatment with L-NAME on blood pressure and renal excretory and hemodynamic responses are shown in the Table and Figure 3. Treatment with L-NAME...
significantly increased basal MAP from 126±1 to 146±4 mm Hg and potentiated the pressor action of the lower doses of big ET-1 but not the highest dose of 3.0 nmol/kg (Table). Moreover, L-NAME alone provoked mild diuretic and natriuretic responses. The diuretic and natriuretic responses to the intermediate and highest doses of big ET-1 were significantly diminished by treatment with L-NAME (Figures 3A and 3B). Basal GFR and RPF were reduced by 20% and 33%, respectively, after treatment with L-NAME. The simultaneous infusion of L-NAME into rats receiving a high dose of big ET-1 significantly augmented the hypofiltration/hypoperfusion actions of the latter (Figures 3C and 3D). These findings suggest that the diuretic and natriuretic effects of big ET-1 are largely dependent on an intact NO system.

**Effects of Indomethacin on Renal Actions of Big ET-1**

The effects of big ET-1 on MAP and renal excretory and hemodynamics in rats pretreated with the cyclooxygenase inhibitor indomethacin are shown in Figure 4. Indomethacin alone did not affect basal MAP (117±4 versus 110±7 mm Hg) (Table); however, it potentiated the pressor responses to 0.3 and 1.0 nmol/kg big ET-1 (\(\Delta MAP + 25\) and +21 mm Hg, respectively) but not to the highest dose. Indomethacin alone also produced a significant increase in UV (89±7 versus 9.8±2 \(\mu L/min\)) (Figure 4A) and FE\(\text{Na}\) (4.1±0.7% versus 0.5±0.2%) (Figure 4B). GFR and RPF were not changed with the administration of indomethacin (Figures 4C and 4D). During the administration of big ET-1 and indomethacin, no significant changes in UV or FE\(\text{Na}\) beyond that obtained with indomethacin alone were observed, despite partial decreases in RBF but not in GFR (Figure 4C and 4D).

**Discussion**

The findings of the present study provide new information regarding the role of ET\(_B\) receptors in mediation of the diuretic and natriuretic actions of big ET-1 in the rat. Although the diuretic and natriuretic actions of big ET-1 are well documented,\(^{33,49}\) the mechanisms underlying these effects have not yet been completely elucidated. In the present study, we extended our previous findings by demonstrating that a novel, highly selective ET\(_B\) receptor antagonist, A-192621.1, markedly attenuated big ET-1–induced diuresis and natriuresis. In addition, the inhibitory effects of A-192621.1 on the renal excretory function of big ET-1 were associated with further declines in GFR and RPF, beyond those observed after the administration of high doses of big ET-1 alone. Finally, our data demonstrated that L-NAME was highly effective in blocking the diuretic and natriuretic actions of big ET-1, suggesting that the NO system linked to ET\(_B\) receptors is a major mediator of the renal actions induced by the intravenous bolus injection of big ET-1.

Several groups have applied either mixed ET\(_A\)/ET\(_B\) antagonists, ET\(_B\) antagonists with low selectivity, or ET\(_B\) agonists such as IRL 1620 to examine the physiological and pathophysiological significance of ET\(_B\) receptors.\(^{12,44,47}\) In the present study, we used a highly selective ET\(_B\) antagonist, A-192621.1, to characterize the contribution of this receptor subtype to the diuretic and natriuretic responses induced by big ET-1. In agreement with our findings, Yukimura et al\(^{47}\) reported that the intrarenal infusion of IRL 1620 in anesthetized dogs increased UV and RBF without an affect on GFR. Pretreatment of the dogs with either l-nitroarginine (an NO synthase inhibitor) or ibuprofen (a cyclooxygenase inhibitor) decreased the IRL 1620–induced elevation in RBF, particularly in animals receiving the NO synthase inhibitor. These findings suggest that IRL 1620 enhances the release of NO and, to a lesser extent, PGs in the kidney to promote renal vasodilation and excretory functions.

The importance of the NO system in the control of renal hemodynamics and excretory functions in animals or humans is well documented.\(^{38–41}\) Several studies have demonstrated...
that the short- and long-term inhibition of intrarenal NO production reduces sodium excretion and suppresses the pressure-natriuresis response. In contrast, administration of the NO precursor L-arginine normalized the pressure-natriuresis response in Dahl salt-sensitive rats. Moreover, a study in dogs demonstrated that L-NAME at nonvasoconstrictor doses shifted the pressure-natriuresis relationship independent of any alterations in whole-kidney hemodynamics. This effect may be attributable to direct alterations in tubular transport or to reduction in medullary blood flow with secondary effects on tubular reabsorption. Therefore, the decreases in the basal GFR and RPF in rats pretreated with L-NAME alone could indirectly contribute to the inhibitory effect of big ET-1 on the diuretic and natriuretic responses of this peptide.

The ET-1 precursor big ET-1 is thought to produce similar effects to ET-1 as a result of its conversion to the latter by ECE. However, big ET-1 is a less potent renal vasoconstrictor than ET-1, despite the fact that the 2 peptides produce comparable increases in systemic arterial pressure. Moreover, we and others have previously shown that big ET-1, but not ET-1, provokes remarkable diuresis and natriuresis. Phosphoramidon pretreatment significantly diminished the excretory effects of big ET-1 and completely blocked the big ET-1–induced increase in MAP, suggesting that substantial conversion of big ET-1 to ET-1 is necessary for full activity of the former. Nevertheless, the mechanisms responsible for the distinctive actions of big ET-1 and ET-1 at the renal vasculature and tubular levels are poorly understood. The predominant hypothesis at present is that the site of big ET-1 conversion to mature ET-1 represents a location at which the de novo produced ET-1 plays a physiological role through an autocrine/paracrine mode of action. Therefore, the administration of big ET-1 rather than ET-1 may provide insight into the relevant renal actions of ET-1, whereas the infusion of either one may be suitable for an examination of the systemic effects of this peptide. An important site of the conversion of big ET-1 to ET-1 in the kidney is the medullary tissue, where high immunoreactive levels of the key enzyme ECE are known to exist. In addition, the renal medulla contains the highest concentrations of ET in the body. ET-1 specifically inhibits arginine vasopressin–dependent cAMP production in the medullary collecting duct of the rat kidney, leading to decreased water permeability in this segment of the nephron and subsequently to enhanced UV.

Recently, Gurbanov et al demonstrated that the administration of ET-1 to anesthetized rats induced medullary vasodilation but at the same time caused a marked cortical vasoconstriction. The former has been shown to be related to the activation of ETB receptors, which are also present in high abundance in the medullary tissue and are thought to mediate NO production. Taken together, these findings may suggest that paracrine ET-1 derived from either de novo or exogenous big ET-1 in the medulla, in proximity to ETB receptors, significantly contributes to the medullary vasodilation and water and salt excretion. This notion is in line with our findings that the big ET-1–induced diuresis/natriuresis and renal vasoconstriction were exclusively abolished by A-192621.1 and L-NAME. In contrast, these effects were less sensitive to cyclooxygenase inhibition by indomethacin, indicating that activation of the PG system, which is also very abundant in the renal medulla, does not contribute to the renal actions of big ET-1. These results are in agreement with the finding that acute cyclooxygenase inhibition induces only small changes in renal vascular resistance and sodium excretion when administered acutely. However, it should be emphasized that the stimulatory effect of indomethacin alone on urine flow and sodium excretion may mask or interfere with a possible involvement of the renal PG system in the diuretic and natriuretic effects of big ET-1.

Similarly, the partial inhibitory effect of verapamil on the excretory actions of big ET-1 is attributable to the fact that activation of ETB receptor increases intracellular Ca++, most likely from intracellular storage sites and to a lesser extent from calcium of extracellular origin. The lack of inhibitory effect of verapamil on the renal actions of big ET-1 at the lowest doses (0.3 and 1.0 nmol/kg) indicates that the renal actions of big ET-1 at low concentrations are independent of extracellular Ca++ influx. The finding that the excretory actions of big ET-1 were moderately but still significantly hampered by verapamil suggests that the renal actions of big ET-1 at high doses are dependent in part on extracellular Ca++. In contrast to the renal actions, the hypertensive effect of big ET-1 was completely abolished by verapamil, suggesting that the more sustained rise in intracellular Ca++ that occurs through the opening of membrane Ca++ channels is essential for big ET-1–induced systemic vasoconstriction. Our data showing that urine flow and sodium excretion were not affected by verapamil alone, despite its hypotensive effect, suggest a shift in the pressure-natriuresis relationship and that verapamil alone inhibits sodium and water reabsorption. This possibility is further supported by our finding that verapamil did not inhibit the diuretic and natriuretic response to big ET-1 at the lowest doses and moderately diminished the excretory response to the highest dose (3.0 nmol/kg) of this peptide. Thus, the possibility that verapamil affects indirectly the excretory actions of big ET-1 cannot be excluded.

It should be emphasized that the kidney contains both ETA and ETB receptors and that both mediate the renal hemodynamic effects of ET-1. For instance, renal vasoconstriction induced by ET-1 appears to be mediated by ETB. Similarly, the hemodynamic response to big ET-1 is also blocked by the ETB antagonist BQ-123, suggesting that the activation of ETA by either ET-1 or big ET-1 is capable of producing a marked degree of renal vasoconstriction. In contrast, BQ-123 is not effective in abolishing the diuretic and natriuretic actions of big ET-1, indicating that non-ETA receptors, presumably ETB, are responsible for the excretory effects of this peptide. Theoretically, the blockade of ETB by A-192621.1 may result in exaggerated systemic vasoconstrictor response by directing the ET-1 derived from big ET-1 toward ETB receptors. Hence, a pressure-natriuresis response due to big ET-1 activation of ETA receptors during ETB blockade cannot be excluded. However, our finding that A-192621.1 significantly attenuates the excretory effects of big ET-1 (especially at high dose), despite its significant potentiation of the hypertensive response to the lower doses
of big ET-1 (but not at 3 nmol/kg), argues against this possibility. Furthermore, Pollock and Opgenorth demonstrated that ET₁₅ block by BQ-123 has no effect on the diuretic and natriuretic actions of big ET-1. Interestingly, A-192621 increased the hypertensive response to big ET-1 at low doses but not at 3 nmol/kg. Theoretically, the blockade of ET₄ receptors should potentiate the hypertensive effects of the studied doses of big ET-1, either by blocking the vasoconstrictive effects of endothelial ET₁₅ receptors or indirectly due to reduced clearance of ET₁ by this receptor subtype. In fact, the mechanisms responsible for this phenomenon are unclear. However, the lack of augmentative effect of A-192621 on the highest dose of big ET-1 may result from a possible negative inotropic effect of the large amounts of ET-1 originating from a such high dose of big ET-1 and preferentially directed toward coronary ET₄ receptors.

In summary, the present study demonstrates that the intravenous bolus administration of incremental doses of big ET-1 provokes significant diuretic and natriuretic responses. These effects are mediated through ET₄ receptors because they can be significantly attenuated by A-192621, a highly selective antagonist of this receptor subtype. Moreover, the renal effects of big ET-1 were equally blocked by L-NAME, suggesting that the potent diuretic and natriuretic activity of big ET-1 is linked to stimulation of NO production via an ET₄ receptor–coupled mechanism.

Acknowledgments

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References


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