ELSEVIER

European Journal of Pharmacology 451 (2002) 177–184

www.elsevier.com/locate/ejphar

Role of endothelin ET_B receptors in the renal hemodynamic and excretory responses to big endothelin-1

Fumiko Konishi [a], Yuka Okada [a], Masanori Takaoka [a], Cheryl E. Gariepy [b], Masashi Yanagisawa [c], Yasuo Matsumura [a,*]

[a] *Department of Pharmacology, Osaka University of Pharmaceutical Sciences, 4-20-1 Nasahara, Takatsuki, Osaka 569-1094, Japan*
[b] *Department of Pediatrics, University of Michigan, Ann Arbor, MI, USA*
[c] *Department of Molecular Genetics, Howard Hughes Medical Institute, University of Texas Southwestern Medical Center, Dallas, TX, USA*

Received 22 April 2002; received in revised form 26 July 2002; accepted 2 August 2002

Abstract

We determined the role of endothelin ET_B receptor in the renal hemodynamic and excretory responses to big endothelin-1, using A-192621, a selective endothelin ET_B receptor antagonist and the spotting-lethal (sl) rat, which carries a naturally occurring deletion in the endothelin ET_B receptor gene. An intravenous injection of big endothelin-1 produced a hypertensive effect, which is greater in wild-type (+/+) rats pretreated with A-192621 and in homozygous (*sl*/*sl*) rats. Big endothelin-1 markedly increased urine flow, urinary excretion of sodium and fractional excretion of sodium in wild-type rats treated with the vehicle. These excretory responses to big endothelin-1 were markedly reduced by pharmacological endothelin ET_B receptor blockade. On the other hand, big endothelin-1 injection to the endothelin ET_B receptor-deficient homozygous animals resulted in a small diuretic effect. When renal perfusion pressure was protected from big endothelin-1-induced hypertension by an aortic clamp, the excretory responses in vehicle-treated wild-type rats were markedly attenuated. In homozygous or A-192621-treated wild-type rats, there was a small but significant decreasing effect in urine flow. In addition, big endothelin-1 significantly elevated nitric oxide (NO) metabolite production in the kidney of wild-type rats but not in the homozygous rats. We suggest that the diuretic and natriuretic responses to big endothelin-1 consist of pressure-dependent and pressure-independent effects and that the increased NO production via the activation of endothelin ET_B receptors in the kidney is closely related to the big endothelin-1-induced excretory responses.

Keywords: Endothelin-1, big; Endothelin ET_B receptor; Diuresis; Hypertension; Nitric oxide (NO)

1. Introduction

Endothelin-1, which was originally discovered as an endothelium-derived factor with powerful and lasting vasoconstricting activity (Yanagisawa et al., 1988), has a wide variety of potent physiological effects in various tissues such as blood vessels, heart, brain, lung, and kidney (Rubanyi and Polokoff, 1994). Endothelin-1 activates two subtypes of receptors, endothelin ET_A and ET_B (Arai et al., 1990; Sakurai et al., 1990). The activation of either subtype on vascular smooth muscle cells leads to prolonged vasoconstriction (Cristol et al., 1993; Fukuroda et al., 1992), whereas endothelin ET_B receptors on endothelial cells are functionally linked to vasodilation, through the release of nitric oxide (NO) and prostaglandin I_2 (De Nucci et al., 1988; Takayanagi et al., 1991). Moreover, it should be emphasized that the kidney contains both endothelin ET_A and ET_B receptors, which mediate the renal hemodynamic and excretory responses to endothelin-1 (Kohan, 1997). In addition, it has been reported that the renal medulla is very rich in endothelin ET_B receptors (Karet et al., 1993). Endothelin-1 is produced from an inactive intermediate form, termed big endothelin-1, through a proteolytic processing at the Trp^{21}–Val^{22} bond by a phosphoramidon-sensitive endothelin-converting enzyme (Matsumura et al., 1990a,b; Pollock and Opgenorth, 1991). An important site of this proteolytic processing from big endothelin-1 in the kidney seems to be the medullary tissue, in which high immunoreactive levels of endothelin-converting enzyme are known to exist (Kohan, 1997).

It has been reported that the intrarenal infusion of selective endothelin ET_B agonists such as IRL1620 and sarafotoxin

* Corresponding author. Tel.: +81-726-90-1050; fax: +81-726-90-1051.
E-mail address: matumrh@gly.oups.ac.jp (Y. Matsumura).

0014-2999/02/$ - see front matter © 2002 Elsevier Science B.V. All rights reserved.
PII: S0014-2999(02)02228-8

S6c in anesthetized dogs produces diuretic and natriuretic responses without affecting glomerular filtration rate (GFR) (Brooks et al., 1994; Matsuo et al., 1997; Yukimura et al., 1994). Big endothelin-1 is also known to exert a similar effect, probably via an intrarenal conversion to endothelin-1. However, several studies demonstrated that the diuretic and natriuretic effects of big endothelin-1 were much greater than that observed by endothelin-1 (Hoffman et al., 1990, 1994; Pollock and Opgenorth, 1994). Recently, Hoffman et al. (2000) noted that the diuretic and natriuretic responses to an intravenous injection of big endothelin-1 were markedly attenuated in rats pretreated with a selective endothelin ET_B receptor antagonist or NO synthase inhibitor, thereby suggesting that the big endothelin-1-induced actions are related to the stimulation of NO production coupled to endothelin ET_B receptor activation. Pollock (2001) also indicated an importance of functional ET_B receptors in the big endothelin-1-induced diuretic action, by using an endothelin ET_B receptor antagonist and ET_B-deficient rats. However, the mechanisms underlying the renal excretory responses to big endothelin-1 have not been fully elucidated.

In the present study, to further evaluate a role of endothelin ET_B receptors in big endothelin-1-induced renal actions, we used the selective endothelin ET_B receptor antagonist and the spotting-lethal (sl) rat, which carries a naturally occurring deletion in the endothelin ET_B receptor gene (Gariepy et al., 1996). Since homozygous (*sl*/*sl*) rats do not live beyond 1 month because of intestinal aganglionosis and resulting intestinal obstruction, dopamine β-hydroxylase promoter was used to direct endothelin ET_B transgene expression in *sl*/*sl* rats to support normal enteric nervous system development (Gariepy et al., 1998). These transgenic *sl*/*sl* rats live into adulthood and are healthy, expressing endothelin ET_B receptors in adrenal glands and other adrenergic neurons. They are endothelin ET_B-deficient in other tissues, but most important is the deficiency in the kidney, vascular endothelium, and vascular smooth muscle (Gariepy et al., 2000).

2. Materials and methods

2.1. Animals

The creation of transgenic *sl*/*sl* rats has been described previously (Gariepy et al., 1998). Homozygous (*sl*/*sl*) rats have dark eyes and pigmented coats only in small spots on their heads. Wild-type rats have pigmented heads, backs, and tails. To definitively differentiate these rats, polymerase chain reaction was performed on DNA isolated from tail biopsy specimens, as described (Gariepy et al., 1998).

2.2. Surgical procedures

Homozygous (*sl*/*sl*) and wild-type (+/+) rats (12–14 weeks of age), all of which were dopamine β-hydroxylase-endothelin ET_B transgenic. The rats were anesthetized with sodium thiobutabarbital (Inactin, 100 mg/kg, i.p.) and placed on a heated surgical tray that maintained rectal temperature between 37 and 38 °C. After tracheotomy, the right femoral vein was cannulated for infusion of 0.9% saline containing 0.5% inulin (6 ml/h). The right and left femoral arteries were also cannulated to measure mean arterial pressure and for blood sampling, respectively. After abdominal midline incision was made, the left kidney was exposed, and the renal artery was carefully stripped connective tissue, followed by the application of 0.5% phenol in 70% ethanol to exclude the influence of renal sympathetic nerves. An electromagnetic flow probe (1.0 mm in diameter, Nihon Kohden, Tokyo, Japan) connected to a square-wave flowmeter (MFV-2100, Nihon Kohden) was positioned on the renal artery to measure renal blood flow. A polyethylene cannula was inserted into the left ureter for urine collection. Mean arterial pressure and renal blood flow were continuously recorded on a polygraph (RM 6000, Nihon Kohden) throughout the experiment. A 60–90-min period was allowed for stabilization of mean arterial pressure, renal blood flow, and urine flow.

2.3. Experimental protocol

In the first part of the experiment, we examined the hemodynamic and excretory responses to big endothelin-1 with or without pharmacological blockade of endothelin ET_B receptor, using the wild-type rats and the responses were compared with those observed in the endothelin ET_B receptor-deficient homozygous animals. After equilibration period, an endothelin ET_B-selective receptor antagonist A-192621 (Von Geldern et al., 1999) (3 mg/kg) or vehicle was administered intravenously by slow bolus injection (volume 1 ml/kg; duration 2 min). This dose of A-192621 has previously been shown to almost abolish sarafotoxin S6c-induced depressor and pressor effects (Matsumura et al., 1999). Urine samples were collected during two 15-min control clearance periods. Results for the second control period served as the basal values for renal hemodynamics and function. Following the control periods, big endothelin-1 (1 nmol/kg) was administered intravenously by slow bolus injection. This dose of big endothelin-1 has been reported to produce both hypertensive and diuretic responses (Hoffman et al., 2000). During the first 5 min after injection, urine was not collected in order to take into account the dead space in the collection system. Following this, urine samples were collected during four consecutive 15-min periods (Experimental period 1–4: E1–E4). Blood samples (0.2 ml each) were obtained at 15 min before the big endothelin-1 injection, and 20 and 50 min after the injection, respectively. The blood loss was replaced by injecting an equal volume of blood from donor rats. Plasma was immediately separated by centrifugation.

In the second part, we examined the hemodynamic and excretory responses to big endothelin-1 under the constant

renal perfusion pressure. A Blalock clamp was placed around the aorta just above the origin of the left renal artery and renal perfusion pressure was protected from big endothelin-1-induced hypertension. In this case, mean arterial pressure above the clamp was measured from a catheter inserted into the right carotid artery, and this served as an index of left renal perfusion pressure. Renal clearance study was performed in the same manner as the first part of the experiment.

2.4. Analytical procedures

Urine and plasma inulin levels were measured by spectrofluorometry (Hitachi 650-50, Hitachinaka, Japan), as described by Vurek and Pegram (1966). GFR was calculated from the inulin clearance. Urine and plasma sodium concentrations were determined using a flame photometer (Hitachi 205D). Fractional excretion of sodium (FE_{Na}) (%) was calculated from the formula $FE_{Na} = U_{Na}V/(P_{Na} \times GFR)100$, where $U_{Na}V$ is urinary excretion of sodium and P_{Na} is plasma sodium concentration.

2.5. Measurement of nitrite (NO_2^-) and nitrate (NO_3^-)

Animals were anesthetized with sodium thiobutabarbital and placed on a heated surgical tray that maintained rectal temperature between 37 and 38 °C. After tracheotomy, the right femoral vein was cannulated for injection of big ET-1. After abdominal midline incision was made, the left kidney was exposed and a microdialysis tube (EICOM, Kyoto, Japan) was inserted into kidney from the renal surface. The microdialysis tube was perfused with Ringer's solution at 1 μl/min. A 60–90-min stabilization period was allowed before the experiments. The baseline dialysate sample (10 μl) was collected, and then big endothelin-1 (1 nmol/kg) was administered intravenously by slow bolus injection. After that, six dialysate samples were collected during 60-min period.

NO_2^- and NO_3^- in the sample were separated and quantitated by the use of the High Performance Liquid Chromatography-Griess system (ENO-20, EICOM). The method consists of separation and reduction columns, and has been previously described in detail (Yamada and Nabeshima, 1997). In brief, 10 μl of the sample was injected into the system, in which NO_2^- and NO_3^- were separated by a reverse-phase separation column packed with polystyrene polymer, and NO_3^- was successively reduced to NO_2^- in the next (downstream) reduction column packed with copper-plated cadmium fillings. NO_2^- (the original NO_2^- and reduced NO_2^- from NO_3^-) was mixed with the Griess reagent to form a purple azo dye in the reaction coil. The separation and reduction columns and the reaction coil were placed in a column over that was kept at 35 °C. Absorbance of the color of the product dye at 540 nm was measured by a flowthrough spectrophotometer. $NaNO_2$ and $NaNO_3$ were dissolved in the mobile phase to prepare standard solutions for quantification on the basis of area under curve of their spectra using an analysis system (Power Chrom System, AD Instruments, Japan). This device allows the detection and discrimination of 100 pmol (10 μl of 10 μM solution) of both NO_2^- and NO_3^-.

2.6. Drugs

Big endothelin-1 was purchased from Peptide Institute (Osaka, Japan). The peptide was dissolved in saline containing 0.1% bovine serum albumin. A-192621 (Von Geldern et al., 1999) was provided by Abbott Laboratories (Abbot Park, IL). The drug was dissolved in 0.02 N NaOH. Other chemicals were purchased from Nacalai Tesque (Kyoto, Japan) and Wako (Osaka, Japan).

2.7. Statistical analysis

All values were expressed as mean ± S.E.M. For statistical analysis, we used the unpaired Student's *t*-test for two-sample comparisons and one-way analysis of variance combined with Dunnett's multiple range test for multiple comparisons. Differences were considered significant at $P < 0.05$.

3. Results

3.1. Systemic and renal hemodynamic responses to big endothelin-1

Fig. 1 shows changes in systemic and renal hemodynamic responses after the intravenous injection of big endothelin-1 at a dose of 1 nmol/kg. In wild-type rats (Fig. 1A), big endothelin-1 significantly elevated the mean arterial pressure (from the basal value of 103 ± 3 to 111 ± 5 and 109 ± 4 mm Hg in E1 and E2 period, respectively). Renal blood flow and renal vascular resistance tended to decrease and to increase, respectively, although there were no significant alterations. In A-192621-treated animals, basal mean arterial pressure was significantly increased and big endothelin-1-induced hypertensive effect was also augmented (from the basal value of 111 ± 7 to 130 ± 4 mm Hg at E1 period). There were decreased basal renal blood flow and increased basal renal vascular resistance, and big endothelin-1 produced marked renal vasoconstrictive effects. Qualitatively, similar results were obtained by using endothelin ET_B receptor-deficient homozygous animals. There were no significant changes in basal GFR and its responses to big endothelin-1 in three groups.

3.2. Renal excretory responses to big endothelin-1

As shown in Fig. 2, big endothelin-1 produced significant diuretic and natriuretic effects in vehicle-treated wild-

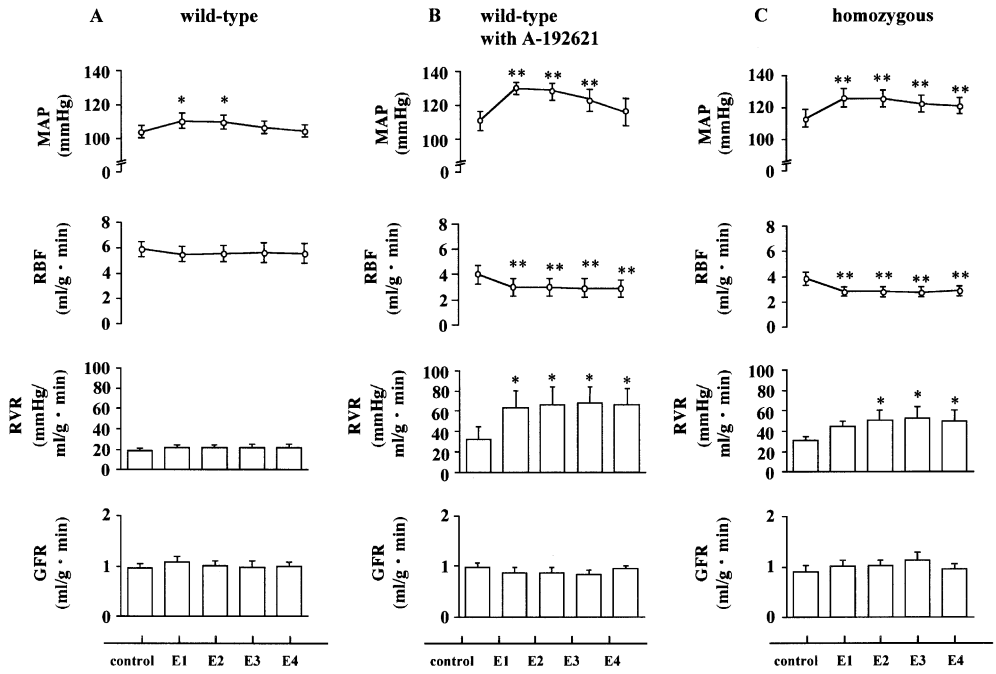

Fig. 1. Effects of intravenous injection of big ET-1 on systemic and renal hemodynamics in (A) wild-type rats with vehicle, (B) wild-type rats with A-192621 and (C) homozygous rats. Values are means ± S.E.M. of six rats. * $P<0.05$ and * * $P<0.01$ vs. values in control period. MAP, mean arterial pressure; RBF, renal blood flow; RVR, renal vascular resistance; GFR, glomerular filtration rate; E1–E4, Experimental period 1–4.

type rats. Urine flow, $U_{Na}V$ and FE_{Na} were increased by 40–50%, 40–60%, and 30–80% compared with each control value, respectively. These excretory responses were markedly attenuated in homozygous and A-192621-treated wild-type rats, although slight but significant diuretic responses were observed.

3.3. Systemic and renal hemodynamic responses to big endothelin-1 under the constant renal perfusion pressure

In order to exclude the influence of big endothelin-1-induced hypertension from its excretory responses, we examined the hemodynamic and excretory responses to

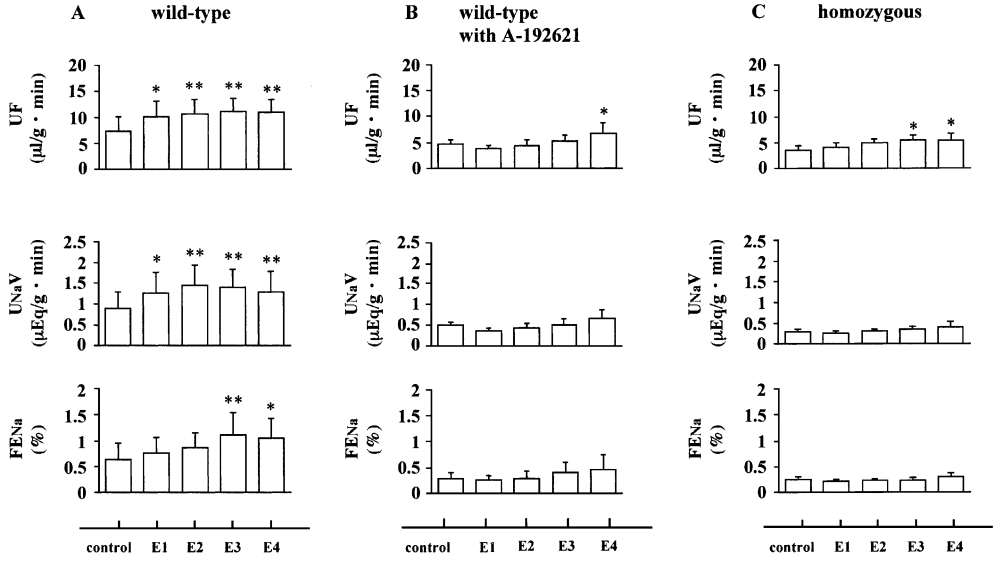

Fig. 2. Excretory responses to intravenous injection of big ET-1 in (A) wild-type rats with vehicle, (B) wild-type rats with A-192621 and (C) homozygous rats. Values are means ± S.E.M. of six rats. * $P<0.05$ and * * $P<0.01$ vs. values in control period. UF, urine flow; $U_{Na}V$, urinary excretion of sodium; FE_{Na}, fractional excretion of sodium; E1–E4, Experimental period 1–4.

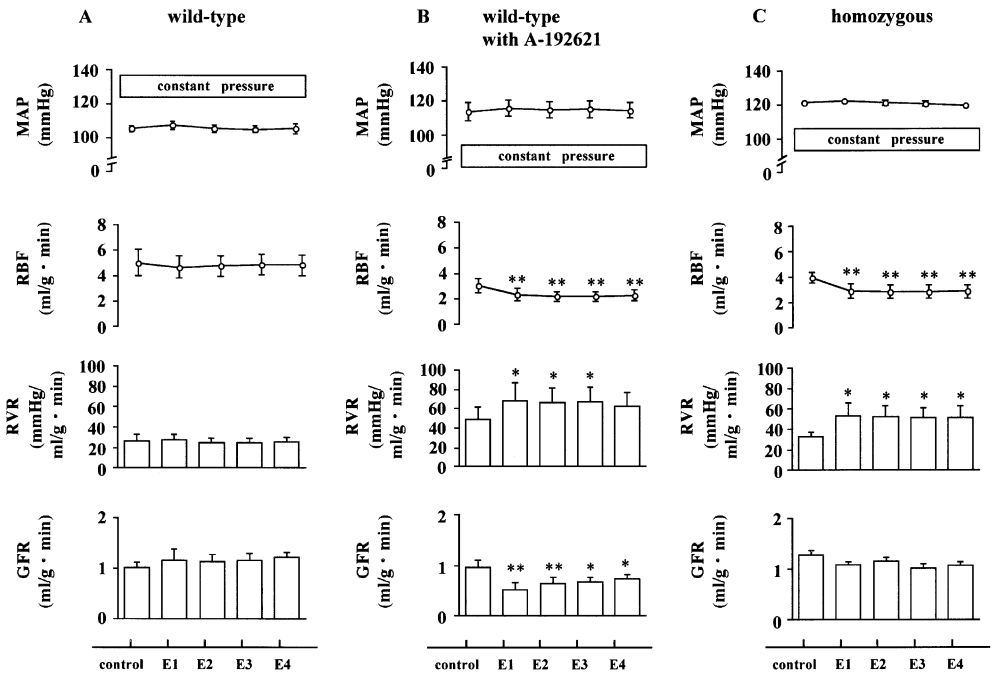

Fig. 3. Effects of intravenous injection of big ET-1 on systemic and renal hemodynamics in (A) wild-type rats with vehicle, (B) wild-type rats with A-192621 and (C) homozygous rats, under the constant renal perfusion pressure. Values are means ± S.E.M. of six rats. * $P<0.05$ and ** $P<0.01$ vs. values in control period. MAP, mean arterial pressure; RBF, renal blood flow; RVR, renal vascular resistance; GFR, glomerular filtration rate; E1–E4, Experimental period 1–4.

big endothelin-1 under the constant renal perfusion pressure. To attain this, a Blalock clamp was placed around the aorta just above the origin of the left renal artery and renal perfusion pressure was protected from big endothelin-1-induced pressor action. In this condition, the changes in renal blood flow and renal vascular resistance observed after the big endothelin-1 injection were similar to those observed without the clamp in all groups. On the other hand, there was a significant decrease in GFR in A-192621-treated wild-type animals (Fig. 3).

3.4. Renal excretory responses to big endothelin-1 under the constant renal perfusion pressure

As shown in Fig. 4, diuretic responses to big endothelin-1 in wild-type rats were markedly attenuated under the

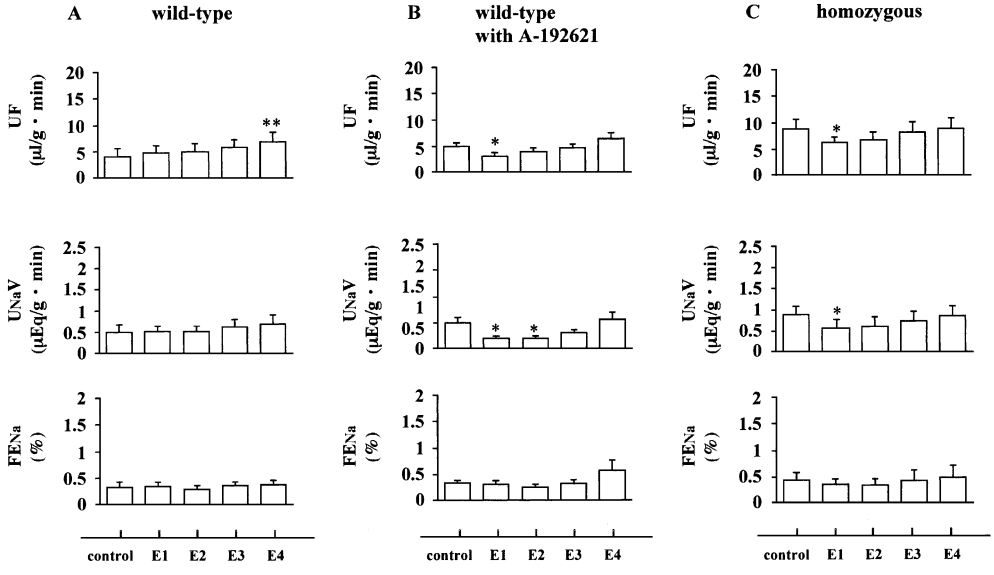

Fig. 4. Excretory responses to intravenous injection of big ET-1 in (A) wild-type rats with vehicle, (B) wild-type rats with A-192621 and (C) homozygous rats, under the constant renal perfusion pressure. Values are means ± S.E.M. of six rats. * $P<0.05$ and ** $P<0.01$ vs. values in control period. UF, urine flow; $U_{Na}V$, urinary excretion of sodium; FE_{Na}, fractional excretion of sodium; E1–E4, Experimental period 1–4.

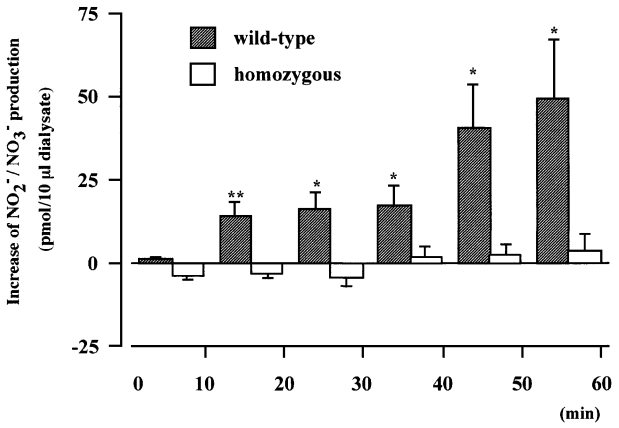

Fig. 5. Increases in renal NO_3^-/NO_2^- production in response to the big ET-1 injection in wild-type and homozygous rats. Values are means ± S.E.M. of five rats. $*P<0.05$ and $**P<0.01$ vs. values before the injection (wild-type, 130 ± 25; homozygous, 129 ± 25 pmol/10 ml dialysate, respectively).

constant renal perfusion pressure. Slight but significant increase in urine flow was observed only in E4 period. In contrast, in wild-type rats with A-192621 and in homozygous rats, big endothelin-1-induced increase in urine flow was not observed, rather, there were significant antidiuretic and antinatriuretic responses to big endothelin-1 injection.

3.5. Total NO_2^- and NO_3^- (NO_x) concentrations in the kidney

In wild-type rats, big endothelin-1 injection produced notable and gradual increases of total NO_x (NO_3^-/NO_2^-) production. In contrast, there was no increase of NO_x production in homozygous rats (Fig. 5). In addition, the injection of big endothelin-1 to A-192621-treated wild-type animals did not increase the NO_x production.

4. Discussion

Big endothelin-1 is thought to produce similar effects to endothelin-1 as a result of its conversion to the latter peptide by phosphoramidon-sensitive endothelin-converting enzyme (Matsumura et al., 1990a). However, big endothelin-1 is a less potent renal vasoconstrictor than endothelin-1, despite the fact that both peptides produce comparable increases in systemic arterial pressure (Pollock and Opgenorth, 1991). Moreover, several groups have previously shown that big endothelin-1, but not endothelin-1, provokes remarkable diuresis and natriuresis (Hoffman et al., 1994; Pollock and Opgenorth, 1994). In agreement with a previous study by Pollock et al. (1993), we observed that the pretreatment of phosphoramidon, an endothelin-converting enzyme inhibitor, almost completely blocked the big endothelin-1-induced renal vasoconstrictor, diuretic, and natriuretic effects, as well as systemic hypertension, thereby suggesting that substantial conversion of big endothelin-1 to endothelin-1 is necessary to exhibit the above biological activities.

In the present study, big endothelin-1 significantly increased the blood pressure in both wild-type and "rescued" endothelin ET_B-deficient homozygous rats, but the effect was somewhat greater in the latter animals. This homozygous animal is known to exhibit an exaggerated hypertensive response to acute treatment with exogenous endothelin-1, probably via activation of vascular endothelin ET_A receptors (Gariepy et al., 2000). Moreover, in A-192621-pretreated wild-type rats, big endothelin-1 produced a greater hypertensive action, compared with that seen in the vehicle-pretreated animals. Qualitatively, similar results were observed in a recent study by Pollock (2001). These enhanced vasoconstrictor responses are most likely due to enhanced endothelin ET_A receptor activation, as well as the absence of endothelial endothelin ET_B receptor-mediated vasodilatation, since the big endothelin-1-induced hypertension was completely suppressed by endothelin ET_A receptor blockade. In our study, enhanced vasoconstrictor effects of big endothelin-1 in wild-type rats pretreated with A-192621 and in homozygous rats were also observed in renal hemodynamic responses, i.e., there were significant decreases in renal blood flow and increases in renal vascular resistance, which are independent of the elevation of systemic blood pressure.

Although the diuretic and natriuretic actions of big endothelin-1 are well documented (Hoffman et al., 2000), the mechanisms underlying these effects have not been fully elucidated. Most recently, Pollock (2001) have investigated systemic and renal responses to big endothelin-1 using the same endothelin ET_B-deficient homozygous rats, and the responses were compared with those in the heterozygous animals. In contrast to our results, he did not observe an exaggerated hypertensive action of big endothelin-1 in the homozygous animals, but clearly indicated the important role of a functional endothelin ET_B receptor in the renal responses to big endothelin-1. In addition, they noted that big endothelin-1-induced increasing effect on urine flow rate in Sprague–Dawley rats was opposed by the pretreatment with ET_B receptor blockade, thereby indicating that the diuretic actions of big endothelin-1 require a functional ET_B receptor. Also in the present study, big endothelin-1-induced diuresis and natriuresis were efficiently suppressed by pharmacological blockade of endothelin ET_B receptor or by genetic deficiency for this receptor subtype, although blood pressure increased more markedly. However, the suppressive effects were incomplete particularly in the latter case. Since big endothelin-1 injection significantly elevated blood pressure both in wild-type and homozygous animals, we evaluated a possible involvement of the elevation of blood pressure in the peptide-induced diuresis and natriuresis. Hence, we performed the next studies using the aortic clamp to control renal perfusion pressure. When big endothelin-1 was administered to vehicle-treated wild-type rats,

under the constant renal perfusion pressure, slight increasing effect on urine flow was still observed. On the other hand, small but significant antinatriuretic and antidiuretic responses were observed in A-192621-treated wild-type rats and in homozygous rats, respectively. Thus, it seems likely that the diuretic and natriuretic responses to big endothelin-1 consist of pressure-dependent and pressure-independent effects, both of which are closely related to endothelin ET_B receptor-mediated events.

Under the constant renal perfusion pressure, big endothelin-1 produced a significant decrease in GFR in A-192621-treated wild-type rats, but not in the homozygous rats. In addition, there was a marked antinatriuresis in response to the big endothelin-1 injection, in the former animals. It seems reasonable to consider that endothelin ET_A receptor-mediated actions are involved in the observed changes, but the reason for the differences between homozygous and A-192621-treated wild-type animals is unclear.

One possible candidate involving in diuretic and natriuretic effects via activation of the endothelin ET_B receptor, may be NO, based on findings that the stimulation of endothelin ET_B receptors in vascular endothelium leads to increased NO production/release and that a NO synthase inhibitor can suppress the big endothelin-1-induced diuresis and natriuresis (Hoffman et al., 2000). In our microdialysis study, big endothelin-1 enhanced NO_2^- and NO_3^- productions in the kidney of wild-type but not of homozygous rats, thereby supporting the above view. It is well documented that NO plays a pivotal role in the regulation of renal hemodynamics and excretory function (Majid and Navar, 2001). Selective endothelin ET_B receptor agonists are known to produce renal vasodilator effects, diuretic responses and increases in urinary NO metabolite excretion (Matsuo et al., 1997; Yukimura et al., 1994). Moreover, the pretreatment with a NO synthase inhibitor could suppress the endothelin ET_B-mediated renal actions (Yukimura et al., 1994). Taken together, big endothelin-1-induced renal hemodynamic and excretory effects seem to be closely related to the increased NO production, via activation of endothelin ET_B receptors in the kidney. In fact, inhibition of intrarenal NO production has been reported to impair the pressure-natriuresis response (Majid and Navar, 2001).

Intravenous administration of endothelin-1 to anesthetized animals at hypertensive doses significantly diminishes renal hemodynamics and urine formation by potent renal vasoconstriction (Miller et al., 1989). In contrast, endothelin-1 at lower doses has been known to produce diuretic responses (Clavell et al., 1995). Several studies have demonstrated that endothelin-1 inhibits arginine vasopressin-induced water permeability and arginine vasopressin-stimulated cyclic AMP accumulation in rat inner medullary-collecting duct (Oishi et al., 1991; Tomita et al., 1990). Within the kidney, the endothelin ET_B receptor is known to be present abundantly in the inner medullary-collecting duct (Terada et al., 1992). Furthermore, Edwards et al. (1993) have reported that arginine vasopressin-induced increases in water permeability and cyclic AMP accumulation in isolated inner medullary-collecting duct were inhibited by a selective endothelin ET_B receptor agonist and that BQ123 (selective endothelin ET_A receptor antagonist) had no effect on endothelin-1-induced inhibition of hydro-osmotic responses to arginine vasopressin. Taken together, it seems likely that endothelin-1 antagonizes the antidiuretic effect of arginine vasopressin through the activation of endothelin ET_B receptors. Further studies are required to determine whether the above mechanisms are involved in the big endothelin-1-induced diuretic action.

In summary, our findings clearly indicated that systemic and renal vasoconstrictor responses to exogenous big endothelin-1, through the endothelin ET_A receptor activation, were enhanced by selective endothelin ET_B receptor blockade or by genetic deficiency of this receptor subtype, thereby suggesting that endothelin ET_B-mediated vasodilatory actions are functionally opposed to endothelin ET_A-mediated vasoconstrictor activity. We also suggest that the diuretic and natriuretic responses to big endothelin-1 consist of pressure-dependent and pressure-independent effects and that the increased NO production via the activation of endothelin ET_B receptors in the kidney is closely related to the big endothelin-1-induced excretory responses.

Acknowledgements

This study was supported in part by a Grant-in-Aid for Scientific Research from the Ministry of Education, Science, Sports, and Culture of Japan. Dr. Yanagisawa is an investigator of the Howard Hughes Medical Institute. The authors are grateful to Dr. Terry J. Opgenorth (Abbott Laboratories) for supporting this work.

References

Arai, H., Hori, S., Aramori, I., Ohkubo, S., Nakanishi, S., 1990. Cloning and expression of a cDNA encoding an endothelin receptor. Nature 348, 730–732.

Brooks, D.P., DePalma, P.D., Pullen, M., Nambi, P., 1994. Characterization of canine renal endothelin receptor subtypes and their function. J. Pharmacol. Exp. Ther. 268, 1091–1097.

Clavell, A.L., Stingo, A.J., Margulies, K.B., Brandt, R.R., Burnett, J.C., 1995. Role of endothelin receptor subtypes in the in vivo regulation of renal function. Am. J. Physiol. 268, F455–F460.

Cristol, J.P., Warner, T.D., Thiemermann, C., Vane, J.R., 1993. Mediation via different reports of vasoconstrictor effects of endothelins and sarafotoxins in the systemic circulation and vasculature of anaesthetized rat. Br. J. Pharmacol. 108, 776–779.

De Nucci, G., Thomas, R., D'Orlèans-Juste, P., Antunes, E., Walder, C., Warner, T.D., Vane, J.R., 1988. The pressor effects of circulating endothelin are limited by its removal in the pulmonary circulation and release of prostacyclin and endothelium-derived relaxing factor. Proc. Natl. Acad. Sci. U. S. A. 85, 9797–9800.

Edwards, R.M., Stack, E.J., Pullen, M., Nambi, P., 1993. Endothelin inhibits vasopressin action in rat inner medullary collecting duct via the ET_B receptor. J. Pharmacol. Exp. Ther. 267, 1028–1033.

Fukuroda, T., Nishikibe, M., Ohta, Y., Ihara, M., Yano, M., Ishikawa, K., Fukami, T., Ikemoto, F., 1992. Analysis of responses to endothelins in isolated porcine blood vessels by using a novel endothelin antagonist BQ-153. Life Sci. 50, PL107–PL112.

Gariepy, C.E., Williams, S.C., Cass, D.T., Yanagisawa, M., 1996. Null mutation of endothelin receptor type B gene in spotting lethal rats causes aganglionic megacolon and white coat color. Proc. Natl. Acad. Sci. U. S. A. 93, 867–872.

Gariepy, C.E., Williams, S.C., Richardson, J.A., Hammer, R.E., Yanagisawa, M., 1998. Transgenic expression of the endothelin-B receptor prevents congenital intestinal aganglionosis in rat model of Hirschsprung disease. J. Clin. Invest. 102, 1092–1101.

Gariepy, C.E., Ohuchi, T., Williams, S.C., Richardson, J.A., Yanagisawa, M., 2000. Salt-sensitive hypertension in endothelin-B receptor-deficient rats. J. Clin. Invest. 105, 925–933.

Hoffman, A., Grossman, E., Keiser, H.R., 1990. Opposite effects of endothelin-1 and big-endothelin-(1–39) on renal function in rats. Eur. J. Pharmacol. 182, 603–606.

Hoffman, A., Haramati, A., Dalal, I., Shuranyi, E., Winaver, J., 1994. Diuretic–natriuretic actions and pressor effects of big-endothelin (1–39) in phosphoramidon-treated rats. Proc. Soc. Exp. Biol. Med. 205, 168–173.

Hoffman, A., Abassi, Z.A., Brodsky, S., Ramadan, R., Winaver, J., 2000. Mechanisms of big endothelin-1-induced diuresis and natriuresis. Role of ET_B receptors. Hypertension 35, 732–739.

Karet, F.E., Kuc, R.E., Davenport, A.P., 1993. Novel ligands BQ123 and BQ3020 characterize endothelin receptor subtypes ET_A and ET_B in human kidney. Kidney Int. 44, 36–42.

Kohan, D.E., 1997. Endothelins in the normal and diseased kidney. Am. J. Kidney Dis. 29, 2–26.

Majid, D.S.A., Navar, L.G., 2001. Nitric oxide in the control of renal hemodynamics and excretory function. Am. J. Hypertens. 14, 74S–82S.

Matsumura, Y., Hisaki, K., Takaoka, M., Morimoto, S., 1990a. Phosphoramidon, a metalloproteinase inhibitor, suppresses the hypertensive effect of big endothelin-1. Eur. J. Pharmacol. 185, 103–106.

Matsumura, Y., Ikegawa, R., Tsukahara, Y., Takaoka, M., Morimoto, S., 1990b. Conversion of big endothelin-1 to endothelin-1 by two types of metalloproteinases derived from porcine aortic endothelial cells. FEBS Lett. 272, 166–170.

Matsumura, Y., Taira, S., Kitano, R., Hashimoto, N., Kuro, T., 1999. Selective antagonism of endothelin ET_A or ET_B receptor in renal hemodynamics and function of deoxycorticosterone acetate-salt-induced hypertensive rats. Biol. Pharm. Bull. 22, 858–862.

Matsuo, G., Matsumura, Y., Tadano, K., Hashimoto, T., Morimoto, S., 1997. Effects of sarafotoxin S6c on renal haemodynamics and urine formation in anaesthetized dogs. Clin. Exp. Pharmacol. Physiol. 24, 487–491.

Miller, W.L., Redfield, M.M., Burnett, J.C., 1989. Integrated cardiac, renal, and endocrine actions of endothelin. J. Clin. Invest. 83, 317–320.

Oishi, R., Nonoguchi, H., Tomita, K., Marumo, F., 1991. Endothelin-1 inhibits AVP-stimulated osmotic water permeability in rat inner medullary collecting duct. Am. J. Physiol. 261, F951–F956.

Pollock, D.M., 2001. Contrasting pharmacological ET_B receptor blockade with genetic ET_B deficiency in renal responses to big ET-1. Physiol. Genomics 6, 39–43.

Pollock, D.M., Opgenorth, T.J., 1991. Evidence for metaroprotease involvement in vivo effects of big endothelin-1. Am. J. Physiol. 261, R257–R263.

Pollock, D.M., Opgenorth, T.J., 1994. ET_A receptor-mediated responses to endothelin-1 and big ET-1 in the rat kidney. Br. J. Pharmacol. 111, 729–732.

Pollock, D.M., Divish, B.J., Novosad, E.I., Burres, N.S., Opgenorth, T.J., 1993. In vivo characterization of a phosphoramodon-sensitive endothelin converting enzyme in the rat. Eur. J. Pharmacol. 231, 459–464.

Rubanyi, G.M., Polokoff, M.A., 1994. Endothelins: molecular biology, biochemistry, pharmacology, physiology and pathophysiology. Pharmacol. Rev. 46, 325–415.

Sakurai, T., Yanagisawa, M., Takakuwa, Y., Miyazaki, H., Kimura, S., Goto, K., Masaki, T., 1990. Cloning of a cDNA encoding anon-Isopeptide-selective subtype of the endothelin receptor. Nature 348, 732–735.

Takayanagi, R., Kitazumi, K., Takasaki, C., Ohnaka, K., Aimoto, S., Tasaka, K., Ohashi, M., Nawata, H., 1991. Presence of non-selective type of endothelin receptor on vascular endothelium and its linkage to vasodilation. FEBS Lett. 282, 103–106.

Terada, Y., Tomita, K., Nonoguchi, H., Marumo, F., 1992. Different localization of two types of endothelin receptor mRNA in microdissected rat nephron segments using reverse transcription and polymerase chain reaction assay. J. Clin. Invest. 90, 107–112.

Tomita, K., Nonoguchi, H., Marumo, F., 1990. Effects of endothelin on peptide-dependent cyclic adenosine monophosphate accumulation along the nephron segments of the rat. J. Clin. Invest 85, 2014–2018.

Von Geldern, T.W., Tasker, A.S., Aorensen, B.K., Winn, M., Szczepankiewicz, B.G., Dixon, D.B., Chiou, W.J., Wang, L., Wessale, J.L., Adler, A., Marsh, K.C., Nguyen, B., Opgenorth, T.J., 1999. Pyrrolidine-3-carboxylic acids as endothelin antagonists: 4. Side chain conformational restriction leads to ET (B) selectivity. J. Med. Chem. 42, 3668–3678.

Vurek, G.G., Pegram, S.E., 1966. Fluorometric method for the determination of nanogram quantities of inulin. Anal. Biochem. 16, 409–419.

Yamada, K., Nabeshima, T., 1997. Simultaneous measurement of nitrite and nitrate levels as indices of nitric oxide release in cerebellum of conscious rats. J. Neurochem. 68, 1234–1243.

Yanagisawa, M., Kurihara, H., Kimira, S., Tomobe, Y., Kobayashi, M., Mitsui, Y., Yazaki, Y., Goto, K., Masaki, T., 1988. A novel potent vasoconstrictor peptide produced by endothelial cells. Nature 332, 411–415.

Yukimura, T., Yamashita, Y., Miura, K., Kim, S., Iwao, H., Takai, M., Okada, T., 1994. Renal vasodilating and diuretic actions of a selective endothelin ET_B receptor agonist, IRL1620. Eur. J. Pharmacol. 264, 399–405.