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A role for endothelin ET_A receptors in regulation of renal function in spontaneously hypertensive rats

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Abstract

While there is evidence to suggest that endothelin-1 is involved in regulation of kidney function and blood pressure, the importance of endothelin ET_A receptors in this area has not been clearly defined. The novel, non-peptide endothelin ET_A receptor antagonist, BMS-182874, (5-(dimethylamino)-*N*-(3,4-dimethyl-5-isoxazolyl)-1-naphthalene sulfonamide) was used to examine effects of endothelin ET_A receptor blockade on renal function in spontaneously hypertensive rats. Preliminary studies were conducted to determine an effective dose of BMS-182874. Infusion of BMS-182874 (10 μmol/kg/min, i.v.) inhibited effects of exogenous endothelin-1 on glomerular filtration rate, renal blood flow, and mean arterial pressure in Sprague-Dawley rats. Administration of BMS-182874 (10 μmol/kg/min, i.v.) to anesthetized, male, spontaneously hypertensive rats decreased renal blood flow by ~50% (1.2 ± 0.11 ml/min/100 g body weight) compared to vehicle (2.7 ± 0.23). There was no effect of BMS-182874 on glomerular filtration rate (0.5 ± 0.05 ml/min/100 g body weight; vehicle: 0.7 ± 0.06). Mean arterial pressure decreased significantly after BMS-182874 (123 ± 3.8 mm Hg; vehicle: 162 ± 4.8). Urine flow and renal vascular resistance were unchanged by BMS-182874. Endothelin ET_A receptor density was increased ~50% in spontaneously hypertensive rat kidneys compared to normotensive kidneys, with no change in equilibrium dissociation constant. Endothelin ET_B receptor density and equilibrium dissociation constant were similar in the two rat strains. Plasma immunoreactive endothelin was higher in hypertensive (5.9 ± 0.31 fmol/ml) than normotensive rats (2.8 ± 0.15). The results suggest endothelin ET_A receptors may play a role in the regulation of renal function in this model of hypertension.

Keywords: Endothelin receptor antagonist; Renal blood flow; Hypertension

1. Introduction

It has been proposed that endothelin acts as an autocrine or paracrine hormone involved in the regulation of renal function (Simonson and Dunn, 1992). Endothelin-1 is produced by numerous cell types, including glomerular endothelial cells (Zoja et al., 1991), and has multiple effects on the kidney. Administration of endothelin-1 to normotensive rats decreased glomerular filtration rate and renal blood flow (Badr et al., 1989; Miller et al., 1989). Diuresis and natriuresis were induced by infusion of endothelin in normotensive rats (Perico et al., 1991). Renal vasoconstriction induced by endothelin-1 in isolated perfused kidneys from normotensive animals appears to be mediated by endothelin ET_A and endothelin ET_B receptors (Warner et al., 1993).

The contribution of the two receptor subtypes in mediation of the renal effects of endothelin in hypertensive animals is less well defined. Administration of endothelin-specific antibodies increased glomerular filtration rate by ~50% in spontaneously hypertensive rats (Ohno et al., 1992), while treatment with the peptide endothelin ET_A receptor antagonist, BQ-123, decreased glomerular filtration rate by ~15% in this model (Gellai et al., 1994). The differences in the effects on glomerular filtration rate in the two studies may be due to blockade of endothelin ET_B receptors by the endothelin-specific antibodies, but not by BQ-

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123. We used the selective endothelin ET_A receptor antagonist, BMS-182874 to further define the role of endothelin ET_A receptors in regulation of renal function in spontaneously hypertensive rats.

BMS-182874, (5-(dimethylamino)-*N*-(3,4-dimethyl-5-isoxazolyl)-1-naphthalene sulfonamide), is a non-peptide, endothelin ET_A receptor antagonist (Stein et al., 1994), that was discovered by screening of Bristol-Myers Squibb compounds by radioligand binding at the endothelin ET_A receptor, and optimization of the structure-activity relationships of these compounds. The selectivity of BMS-182874 was described previously (Webb et al., 1995). BMS-182874 inhibited binding of [^{125}I]endothelin-1 to vascular smooth muscle A10 cell membranes (endothelin ET_A $K_i = 66 \pm 12$ nM), with minimal effects on the binding of [^{125}I]endothelin-3 to rat cerebellar membranes (endothelin ET_B $K_i > 200$ μM), suggesting that it is a selective endothelin ET_A receptor antagonist.

The multiple renal effects of endothelin may be mediated by endothelin ET_A and endothelin ET_B receptors, as both subtypes have been identified in the rat and human kidney. Using subtype selective radioligands, normotensive rat kidney cortex was found to contain endothelin ET_A and endothelin ET_B receptors in a 50:50 ratio (Nambi et al., 1992a). In the human kidney, the endothelin ET_A to endothelin ET_B receptor ratio was 30:70 (Nambi et al., 1992b). In aortas from spontaneously hypertensive rats, [^{125}I]endothelin-1 binding site density was increased 50% compared to normotensive controls; however, differences in endothelin ET_A and endothelin ET_B receptor subtypes were not examined (Tomobe et al., 1991). The endothelin ET_A to endothelin ET_B receptor ratio in the renal cortex of spontaneously hypertensive rats was decreased compared to normotensive rats (Gellai et al., 1994). In the current study, we measured binding of subtype selective radioligands in kidneys from spontaneously hypertensive and normotensive rats, to evaluate changes in the ratio of endothelin ET_A and endothelin ET_B receptors in this genetic model of hypertension.

2. Materials and methods

2.1. Normotensive rats

All procedures involving animals were in accordance with the Bristol-Myers Squibb Pharmaceutical Research Institute Animal Care and Use Committee. Preliminary studies were performed in order to determine a dose of BMS-182874 that inhibited changes in renal function induced by exogenous endothelin-1. Fasted male Sprague-Dawley rats (205–295 g) were anesthetized with 100 mg/kg i.p. inactin (Byk-Gulden Pharmaceuticals, Konstanz, Germany). Catheters were placed in the jugular vein, femoral artery, bladder, and trachea, and the urethra was ligated. Body temperature was maintained at 36–38°C. [^{14}C]Inulin (0.1 μCi/ml) and [^{3}H]*p*-aminohippuric acid (2.0 μCi/ml) were infused (30 μl/min, i.v.) for a 1 h equilibration period along with donor plasma (5.0 ml/kg/h, i.v.). For the remainder of the study, plasma was infused at 1.0 ml/kg/h. The arterial catheter was used to obtain blood samples and to monitor mean arterial pressure using a Statham pressure transducer and a Gould RS 3200 recorder.

Three groups were evaluated: 5% $NaHCO_3$ + saline, 5% $NaHCO_3$ + endothelin-1, or BMS-182874 + endothelin-1 ($n = 4$–5/group). Urine and midpoint arterial samples were collected during each 20 min clearance period. There were two baseline clearance periods, two infusion clearance periods, and two post-infusion clearance periods in each experiment. The values for the two baseline, two infusion, and two post-infusion clearance periods were averaged, and are presented as periods 1, 2, and 3. After period 1, vehicle (5% $NaHCO_3$) or BMS-182874 (10 μmol/kg/min, i.v.) was infused for 10 min prior to and during period 2 (a total of 50 min). Vehicle (0.9% saline) or endothelin-1 (75 ng/kg/min, i.v.) was also administered during period 2. Mean values for the three groups were compared by analysis of variance and Tukey's test.

Glomerular filtration rate was estimated from clearance of [^{14}C]inulin, and renal plasma flow from clearance of [^{3}H]*p*-aminohippuric acid. Radioactivity in urine and plasma samples was quantitated by liquid scintillation counting. Duplicate samples were counted in a Beckman Model LS 5801 scintillation counter using a dual-label DPM program. Background counts were averaged and subtracted from the counts in urine and plasma for each collection period. The counts for [^{3}H]*p*-aminohippuric acid and [^{14}C]inulin of the duplicate samples were averaged. Glomerular filtration rate and renal plasma flow were determined from standard formulas. Renal blood flow was calculated by dividing renal plasma flow by 1 minus hematocrit. To correct for variations in body weight, glomerular filtration rate and renal plasma flow are expressed as ml/min/100 g body weight. Renal vascular resistance was calculated by dividing mean arterial pressure by renal blood flow.

2.2. Hypertensive rats

Fasted male spontaneously hypertensive rats (239–270 g) were prepared for renal clearance studies as described above. Rats were treated with either vehicle (5% $NaHCO_3$) or BMS-182874 ($n = 5$–6/group). After the baseline clearance periods, vehicle or BMS-182874 (10 μmol/kg/min) was infused i.v. for 10 min prior to and during the two 20 min infusion periods, for a total

of 50 min. The infusion of vehicle or BMS-182874 was stopped during the two post-infusion clearance periods. The values for the two baseline, two infusion, and two post-infusion clearance periods were averaged, and are presented as periods 1, 2, and 3. Mean values from vehicle-treated and BMS-182874-treated rats were compared using unpaired Student's *t*-test.

2.3. Radioligand binding

Kidneys from normotensive Sprague-Dawley rats and spontaneously hypertensive rats were homogenized in 50 mM Tris-HCl pH 7.4, 1 mM EDTA, 0.23 U/ml aprotinin and centrifuged at $800 \times g$ for 10 min at 4°C. The supernatants were centrifuged at $100000 \times g$ for 1 h at 4°C. Membrane pellets were resuspended in homogenization buffer and homogenized again. Aliquots were stored at −80°C.

Endothelin receptor binding assays were conducted as described previously (Webb et al., 1993). Since endothelin-1 binds to endothelin ET_A and endothelin ET_B receptors with equal affinity, [^{125}I]endothelin-1 (3–400 pM) saturation binding should reflect the sum of endothelin ET_A and endothelin ET_B receptors. In contrast, [^{125}I]IRL-1620 is 120000-fold selective for endothelin ET_B receptors (Takai et al., 1992), and concentrations of [^{125}I]IRL-1620 up to 400 pM are estimated to occupy $< 1\%$ of the endothelin ET_A receptors. For saturation binding, membranes (10–30 μg) were incubated with 3–400 pM of [^{125}I]endothelin-1 or [^{125}I]IRL-1620 in the absence and presence of 100 nM endothelin-1 or endothelin-3, respectively, in a final volume of 0.25 ml assay buffer (50 mM Tris-HCl pH 7.4, 0.1% bovine serum albumin, 2 μM phosphoramidon) at 37°C for 2 h. Binding reactions were terminated by rapid filtration in a Tomtec cell harvester over a Filtermat B (Pharmacia LKB, Uppsala, Sweden) pre-soaked for 1 h in assay buffer. Filtermats were rinsed with 150 mM NaCl, 5 mM Tris-HCl, pH 7.4 at 4°C, microwaved, and counted in a Betaplate liquid scintillation counter (Pharmacia LKB) in the presence of a Meltilex solid scintillant wax (Pharmacia LKB). Counting efficiency was 65%. Analysis of saturation binding data was performed using non-linear least-square curve fitting to the non-transformed data. Linear transformation of data was conducted as described by Scatchard (1949). Radioligand binding data was analyzed using unpaired Student's *t*-test.

2.4. Plasma endothelin analysis

Plasma immunoreactive endothelin levels (fmol/ml) were measured in Sprague-Dawley rats and spontaneously hypertensive rats ($n = 6$/group). Samples were collected from decapitated rats in chilled tubes containing EDTA (1 mg/ml blood); aprotinin and sodium azide were added to final concentrations of 1000 KIU/ml and 0.2%, respectively. Samples were centrifuged at 4°C for 20 min at 2800 rpm, and plasma was collected, and stored at −80°C. For extraction of endothelin-1, thawed plasma samples were acidified with trifluoroacetic acid and applied to octyl (C-8) solid phase extraction columns preconditioned with methanol then water. Column beds were washed following sample application with saline followed by water and briefly air-dried. Peptides were eluted with 90% methanol-1% trifluoroacetic acid. Eluants were dried and stored at −80°C. Radioimmunoassay was performed with an Amersham endothelin 1–21 specific ^{125}I radioimmunoassay system, which does not significantly cross-react with big endothelin but cannot discriminate endothelin-1 from endothelin-2 or endothelin-3. Sample extracts were assayed against a standard curve constructed of synthetic endothelin-1 Mean val-

Table 1
Effects of infusion of BMS-182874 on endothelin-1-induced changes in glomerular filtration rate (GFR), renal blood flow (RBF), and mean arterial pressure (MAP) in normotensive rats

	GFR (ml/min/100 g body weight)	RBF	MAP (mm Hg)
Vehicle			
Period 1	1.1 ± 0.06	6.3 ± 0.39	115 ± 8.9
Period 2	1.1 ± 0.10	5.8 ± 0.41	110 ± 9.1
Period 3	1.1 ± 0.06	4.9 ± 0.62	104 ± 4.5
Endothelin-1			
Period 1	1.1 ± 0.08	5.8 ± 0.34	109 ± 5.5
Period 2	0.8 ± 0.04	3.5 ± 0.13 [b]	127 ± 5.4
Period 3	0.[illegible] ± 0.11 [a]	1.5 ± 0.44 [b]	127 ± 6.5 [a]
BMS-182874 + endothelin-1			
Period 1	1.0 ± 0.03	5.6 ± 0.07	122 ± 5.9
Period 2	0.9 ± 0.13	4.5 ± 0.67	121 ± 8.5
Period 3	0.7 ± 0.08	2.7 ± 0.26 [a]	108 ± 5.7

Rats were treated with vehicle, endothelin-1 (75 ng/kg/min), or BMS-182874 (10 μmol/kg/min) and endothelin-1 ($n = 4$–5/group) for 10 min prior to and during period 2. Data are expressed as means ± S.E.M. [a] $P < 0.05$; [b] $P < 0.01$ versus vehicle-treated rats.

ues for plasma immunoreactive endothelin levels were analyzed using unpaired Student's *t*-test.

2.5. Materials

[^{125}I]Endothelin-1 (2200 Ci/mmol) and [^{125}I]IRL-1620 (2200 Ci/mmol) were obtained from New England Nuclear, Boston, MA); endothelin-1 and endothelin-3 from Peninsula Labs (Belmont, CA). [^{14}C]Inulin and [^{3}H]*p*-aminohippuric acid were obtained from American Radiolabeled Chemicals, (St. Louis, MO). BMS-182874 was synthesized by the Department of Chemistry, Bristol-Myers Squibb.

3. Results

3.1. Normotensive rats

Preliminary studies were performed in order to determine a dose of BMS-182874 that inhibited changes in renal function induced by exogenous endothelin-1. Values for glomerular filtration rate, renal blood flow, and mean arterial pressure were consistent in vehicle-treated rats throughout the clearance experiments (Table 1).

Administration of endothelin-1 significantly decreased glomerular filtration rate and renal blood flow compared to vehicle-treated rats. These results are presented in Table 1. Urine flow did not change significantly in endothelin-treated rats (period 2: 8.4 ± 2.87 μl/min; period 3: 4.5 ± 1.19 μl/min) compared to vehicle-treated rats (period 2: 17.3 ± 8.94 μl/min; period 3: 13.8 ± 9.2 μl/min). Systemic endothelin-1 administration induced a mean pressor response of 18 mm Hg (Table 1).

There was no significant change in glomerular filtration rate induced by endothelin-1 in rats treated with BMS-182874 (Table 1). Infusion of BMS-182874 partially blocked the pronounced change in renal blood flow due to endothelin-1 administration. Urine flow did not change significantly in rats given BMS-182874 and endothelin-1 (period 2: 8.4 ± 1.91 μl/min; period 3: 6.1 ± 0.43 μl/min). Pressor effects of endothelin-1 administration were not evident during i.v. infusion of BMS-182874 and endothelin-1 (Table 1).

3.2. Hypertensive rats

The effect of endothelin ET_A receptor antagonism by infusion of BMS-182874 on renal function in anesthetized, spontaneously hypertensive rats was evaluated. Pretreatment values for renal function and mean arterial pressure were similar for drug- and vehicle-treated groups. There was no significant effect of BMS-182874 on glomerular filtration rate compared to

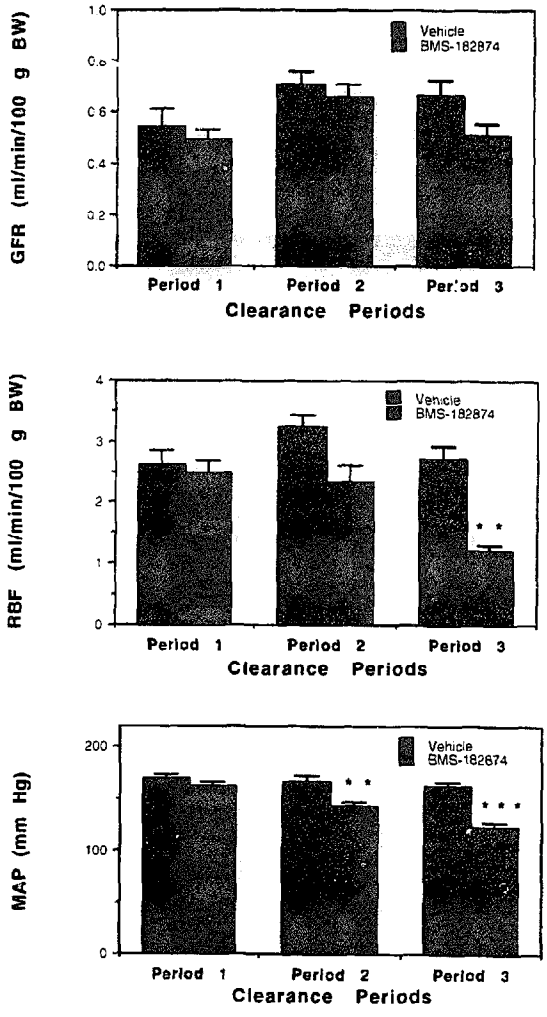

Fig. 1. The effects of i.v. infusion of vehicle or BMS-182874 (10 μmol/kg/min) for 10 min prior to and during period 2, on glomerular filtration rate (GFR), renal blood flow (RBF), and mean arterial pressure (MAP) in anesthetized, spontaneously hypertensive rats, are summarized below. Means ± S.E.M. are given. * * $P < 0.01$; * * * $P < 0.001$ versus vehicle-treated rats.

effects of vehicle infusion (Fig. 1). Renal blood flow was significantly reduced by 60% during period 3 in BMS-182874-treated rats compared to vehicle (Fig. 1). Urine flow rate was not significantly changed by infusion of BMS-182874 (period 2: 7.8 ± 0.93; period 3: 5.7 ± 0.28 μl/min) compared to vehicle (period 2: 8.7 ± 1.12; period 3: 7.0 ± 0.67 μl/min). Although numerically increased, renal vascular resistance was not changed significantly by infusion of BMS-182874 (period 2: 25.0 ± 2.23; period 3: 42.0 ± 4.4 mm Hg min/ml) compared to vehicle (period 2: 20.2 ± 1.42; period 3: 24.9 ± 2.8 mm Hg min/ml), suggesting that the magnitude of effects of BMS-182874 on renal blood flow and on the systemic circulation was similar.

Infusion of BMS-182874 significantly lowered blood pressure in anesthetized, spontaneously hypertensive rats. Mean arterial pressure decreased by 21 mm Hg during infusion of BMS-182874, and decreased by 40

mm Hg after infusion (Fig. 1). In vehicle-treated spontaneously hypertensive rats, mean arterial pressure remained stable (167 ± 5 mm Hg; 162 ± 5 mm Hg).

3.3. Radioligand binding

In order to determine the quantity of specific binding sites for the endothelin ET_A receptor antagonist used in this study, saturation binding of [^{125}I]endothelin-1 and [^{125}I]IRL-1620 to rat kidney membranes from normotensive and hypertensive rats was compared under equilibrium binding conditions, reached after 2 h at 37°C (data not shown). For each radioligand, specific binding was defined in the presence of 100 nM unlabeled ligand. Total and non-specific binding increased in a radioligand concentration-dependent fashion and resulted in specific binding isotherms consistent with a single population of receptor binding sites over the radioligand concentration range.

[^{125}I]Endothelin-1 and [^{125}I]IRL-1620 binding to endothelin receptors in rat kidney membranes was specific and saturable. In normotensive controls, the equilibrium dissociation constants for [^{125}I]endothelin-1 and [^{125}I]IRL-1620 were 85 ± 7 and 180 ± 87 pM, respectively. The receptor density for [^{125}I]endothelin-1 (201 ± 29 fmol/mg protein) was greater than that for [^{125}I]IRL-1620 (135 ± 16 fmol/mg protein) in normotensive rat kidney membranes. These data are consistent with the suggestion that binding sites for [^{125}I]IRL-1620 comprise approximately 67% of the total [^{125}I]endothelin-1 receptor sites in normotensive rat kidneys.

Comparison of [^{125}I]endothelin-1 binding in kidney membranes obtained from normotensive and spontaneously hypertensive rats revealed similar equilibrium dissociation constants for this radioligand in both groups (Table 2). However, the [^{125}I]ET-1 receptor density was significantly greater in membranes from spontaneously hypertensive rat kidneys than kidneys from normotensive rats (Table 2), with the receptor density approximately 50% greater in the hypertensive group. Comparison of [^{125}I]IRL-1620 binding in normotensive and spontaneously hypertensive rat kidney membranes revealed no significant increase in specific binding sites in spontaneously hypertensive rats (Table 2).

Table 2
Equilibrium dissociation constants (K_d in pM) and binding site maxima (B_{max} in fmol/mg protein) from [^{125}I]endothelin-1 and [^{125}I]IRL-1620 saturation binding in membranes from normotensive Sprague-Dawley and spontaneously hypertensive rat kidneys

	[^{125}I]ET-1		[^{125}I]IRL-1620	
	K_d	B_{max}	K_d	B_{max}
Normotensive	85 ± 7	201 ± 29	180 ± 31	135 ± 16
Hypertensive	90 ± 9	305 ± 23 [a]	104 ± 22	158 ± 16

Values are means ± S.E.M. from eight determinations. [a] $P < 0.05$ versus normotensive.

3.4. Plasma immunoreactive endothelin

Plasma immunoreactive endothelin levels were compared in normotensive and hypertensive rats. Plasma endothelin levels were significantly higher in spontaneously hypertensive rats (5.9 ± 0.31 fmol/ml) than in normotensive rats (2.8 ± 0.15 fmol/ml).

4. Discussion

The potent effects of endothelin-1 on the renal circulatory bed, the production of endothelin-1 in the kidney, and the presence of renal endothelin receptors, suggest that endothelin plays a role in regulation of renal function. The results of the current study suggest that a portion of the renal effects of endogenous endothelin are mediated through endothelin ET_A receptors. Blockade of endothelin ET_A receptors with the novel, non-peptide, naphthalene sulfonamide, BMS-182874, decreased renal blood flow in anesthetized, spontaneously hypertensive rats. This finding is similar to that of others who reported a significant fall in renal blood flow of ~25% after infusion of the peptide endothelin ET_A receptor antagonist, BQ-123, in conscious, spontaneously hypertensive rats (Gellai et al., 1994). These results suggest that endothelin ET_A receptors are important in regulation of renal vascular tone in spontaneously hypertensive rats.

Administration of BMS-182874 induced a significant decrease in mean arterial pressure in spontaneously hypertensive rats, which may have contributed to the fall in renal blood flow. However, it is unlikely that the changes in renal blood flow were caused solely by the antihypertensive effects of the drug, as the decrease in blood pressure did not reach the threshold (< 90 mm Hg) of autoregulation of renal blood flow in spontaneously hypertensive rats described by Roman and Cowley (1985).

It is somewhat surprising that blockade of endothelin ET_A receptors produced a fall in renal blood flow, as endothelin ET_A and endothelin ET_B receptors are considered to mediate renal vasoconstriction (Warner et al., 1993). While others have reported a similar effect on renal blood flow after administration of BQ-123 (Gellai et al., 1994), the exact mechanism is unclear. A highly speculative explanation would involve different distribution of endothelin ET_A and endothelin ET_B receptors on the afferent and efferent arterioles. Endothelin ET_A and endothelin ET_B receptor mRNA have been localized in rat glomeruli (Terada et al., 1992), but the distribution of the two receptor

subtypes on afferent and efferent arterioles has not been defined. It is possible that endothelin ET_A receptor antagonism might inhibit endothelin-1-induced constriction at afferent arterioles, revealing endothelin ET_B receptor-mediated vasoconstrictive effects primarily at efferent arterioles. The divergent changes in renal plasma flow and glomerular filtration rate in the current study may be due to a greater increase in efferent arteriolar resistance than in afferent arteriolar resistance.

Glomerular filtration rate did not change significantly in anesthetized rats treated with BMS-182874. A decrease of ~15% in glomerular filtration rate has been described in conscious, spontaneously hypertensive rats after administration of BQ-123 (Gellai et al., 1994). Endothelin-specific antibodies increased glomerular filtration rate by ~50% in anesthetized, spontaneously hypertensive rats (Ohno et al., 1992). The difference in the findings of these studies is likely due to the blockade of endothelin ET_B receptors by the antibodies.

Administration of BMS-182874 had no significant effect on urine flow in spontaneously hypertensive rats, suggesting that endothelin ET_A receptors are not important in mediating the effects of endogenous endothelin on basal urine output in spontaneously hypertensive rats. Natriuresis and diuresis were induced by infusion of endothelin in normotensive animals (Perico et al., 1991), but these effects are likely mediated through endothelin ET_B receptors (Edwards et al., 1993; Kamphuis et al., 1994), at least partially via inhibition of arginine vasopressin responsiveness in the inner medullary collecting duct (Kohan, 1993).

Using subtype selective radioligands, we have demonstrated that the endothelin ET_A to endothelin ET_B receptor distribution in kidneys from normotensive rats is 33:67. This proportion of endothelin ET_A to endothelin ET_B subtypes differs slightly from the 50:50 ratio previously reported in the renal cortex from normotensive rats (Nambi et al., 1992a). The reason for the difference is likely due to the region of the kidney evaluated. This result suggests a higher proportion of endothelin ET_B receptors in medulla from normotensive rats than in the cortex, which has been observed by other investigators (Gellai et al., 1994).

Consistent with a role for endothelin ET_A receptor regulation of renal function in this model of genetic hypertension was the 50% increase in endothelin ET_A receptor density observed in spontaneously hypertensive rats. The [^{125}I]endothelin-1 equilibrium dissociation constant in spontaneously hypertensive rat kidney (90 pM) indicates that the increased endothelin ET_A receptor population has retained high affinity binding to endothelin-1. Tomobe et al. (1991) also found a 50% increase in [^{125}I]endothelin-1 binding sites in aorta of spontaneously hypertensive rats with no change in equilibrium dissociation constant values. This radioligand is non-selective for endothelin receptor subtypes, precluding evaluation of effects on endothelin ET_A or endothelin ET_B receptor populations.

Using the endothelin ET_B selective agonist, [^{125}I]IRL 1620, there was no increase in endothelin ET_B receptor density in kidneys from spontaneously hypertensive rats. This indicates that the increased [^{125}I]endothelin-1 binding in spontaneously hypertensive rats compared to normotensive controls was due to an increase in endothelin ET_A receptor subtype. Thus, using both [^{125}I]endothelin-1 and [^{125}I]IRL-1620, the data indicate that the endothelin ET_A to endothelin ET_B receptor ratio is ~30:70 in kidneys from normotensive rats, and 50:50 in spontaneously hypertensive rat kidneys.

We observed significantly higher plasma immunoreactive endothelin levels in spontaneously hypertensive rats than in normotensive rats, using a radioimmunoassay that cannot discriminate endothelin-1 from endothelin-2 or endothelin-3. The results reported by Khraibi et al. (1993) were similar to those described above. However, endothelin-1 and endothelin-3 levels were comparable in another study in spontaneously hypertensive rats and normotensive rats (Vemulapalli et al., 1991), and lower levels of immunoreactive endothelin were reported in spontaneously hypertensive rats than Wistar-Kyoto rats (Suzuki et al., 1990). The differences in these studies may depend on the age and substrain of rats used. The rise in plasma immunoreactive endothelin and the increased endothelin-A receptor expression observed in the current study may indicate an aberration in hormonal feedback in spontaneously hypertensive rats, or local tissue levels of endothelin may affect receptor expression to a greater extent than plasma levels.

The role of endothelin in the pathogenesis of hypertension remains open to question (Schiffrin, 1995). We have shown that blockade of endothelin ET_A receptors with BMS-182874 decreased blood pressure by 40 mm Hg in anesthetized, spontaneously hypertensive rats. These results agree with the findings of other investigators. Ohlstein et al. (1993) reported antihypertensive effects of BQ-123, infused during a 6 h period in conscious, spontaneously hypertensive rats. McMahon et al. (1993) found a decrease in mean arterial pressure of 25 mm Hg in conscious, spontaneously hypertensive rats infused with BQ-123 (50 mg/kg/h) for 5 h, but lower doses of BQ-123 (10 and 30 mg/kg/h), that inhibited an endothelin-1-induced pressor response, failed to reduce blood pressure. While we observed an acute effect of BMS-182874 in anesthetized, spontaneously hypertensive rats, consistent antihypertensive effects were not present after 3 days of administration in conscious, spontaneously hypertensive rats (Bird et al., 1995). Additional long-term testing of selective and

non-selective endothelin receptor antagonists is necessary to verify a role for endothelin in hypertension.

In summary, administration of the novel, non-peptide, selective endothelin ET_A receptor antagonist, BMS-182874, decreased renal blood flow and mean arterial pressure in anesthetized, spontaneously hypertensive rats. Endothelin ET_A, but not endothelin ET_B, receptor density was increased in kidneys from spontaneously hypertensive rats. Taken together, the renal effects of BMS-182874, the increased endothelin ET_A receptor expression, and the elevated plasma concentration of endothelin in spontaneously hypertensive rats suggest a role for endothelin ET_A receptors in the regulation of renal function in this genetic model of hypertension.

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