0026-895X/92/020336-04$03.00/0

MOLECULAR PHARMACOLOGY, 42:336-339

# Identification of Endothelin Receptor Subtypes in Rat Kidney Cortex using Subtype-Selective Ligands

PONNAL NAMBI, HSIAO LING WU, MARK PULLEN, NAMBI AIYAR, HEIDEMARIE BRYAN, and JOHN ELLIOTT

*Departments of Pharmacology (P.N., H.L.W., M.P., N.A.), Peptidomimetic Research (H.B.), and Medicinal Chemistry (J.E.), SmithKline Beecham Pharmaceuticals, King of Prussia, Pennsylvania 19406-0939*

Received February 6, 1992; Accepted May 7, 1992

## SUMMARY

$^{125}$I-Endothelin (ET)-1 and $^{125}$I-ET-3 displayed specific, saturable, and high affinity binding to membranes prepared from rat kidney cortex. Saturation binding experiments using $^{125}$I-ET-1 and $^{125}$I-ET-3 revealed that $^{125}$I-ET-3 binding sites were 40–50% less abundant than $^{125}$I-ET-1 binding sites. The dissociation constants ($K_d$) and maximum binding ($B_{max}$) for $^{125}$I-ET-1 and $^{125}$I-ET-3 with these membranes were 218 ± 23 pM and 275 ± 20 fmol/mg of protein and 207 ± 19 pM and 113 ± 17 fmol/mg of protein, respectively. In the presence of 10 nM sarafotoxin 6c, a selective agonist for $ET_B$ receptors, $^{125}$I-ET-1 binding was decreased by 45–50% and $^{125}$I-ET-3 binding was totally abolished, suggesting that approximately 40–50% of kidney cortex ET receptors are of the $ET_B$ subtype and that $^{125}$I-ET-1 binds to both $ET_A$ and $ET_B$ receptors with the same high affinity, whereas $^{125}$I-ET-3 binds to only $ET_B$ receptors with high affinity. In addition, in the presence of BQ123 [cyclo(D-Trp,D-Asp,L-Pro,D-Val,L-Leu)], a selective antagonist for $ET_A$ receptors, $^{125}$I-ET-1 binding was decreased by 50%, whereas $^{125}$I-ET-3 binding was unaffected. Our results strongly suggest that rat kidney cortex contains $ET_A$ and $ET_B$ receptors in a 50:50 ratio and that sarafotoxin 6c and BQ123 are valuable tools in identifying the subtypes of ET receptors in various tissues.

ET-1, a 21-amino acid peptide isolated from the culture medium of endothelial cells, has been shown to have potent vasoactive properties (1). Following its discovery, two related peptides, ET-2 and ET-3, and a family of 21-amino acid peptide toxins (sarafotoxins) that show high degrees of homology to ET-1 were identified (2). The physiological actions of the ETs have been shown to be mediated by the interaction of ET with specific cell surface receptors, and ET receptors have been identified in a variety of tissues and cell lines (for review, see Refs. 3 and 4). ET-1 acts as a potent vasoconstrictor, especially in kidney, when administered *in vivo*. It has been shown to decrease glomerular filtration rate, renal plasma flow, and ultrafiltration coefficient (5–7). It also appears to play an important role in renal failure and cyclosporine nephrotoxicity (8–10). Based on radioligand binding studies, the existence of subtypes of ET receptors has been proposed (for review, see Ref. 11). Recently, ET receptors have been cloned from bovine (12) and rat lung (13) and designated $ET_A$ and $ET_B$, respectively, based upon the relative binding affinity of ET-1 and ET-3. $ET_A$ receptors are selective for ET-1, with ET-3 showing a 100-fold diminished binding affinity. $ET_B$ receptors, termed "nonselective," display similar binding properties for ET-1 and ET-3. Williams *et al.* (14) have demonstrated that S6c, isolated from snake venom, binds selectively to $ET_B$ receptors with high affinity and stimulates phosphatidylinositol turnover only in tissue slices possessing $ET_B$ receptors (15). Recently, Ihara *et al.* (16) have described a cyclic pentapeptide (BQ123) that showed >1000-fold selectivity for $ET_A$ receptors. We have used S6c and BQ123 as tools to characterize the subtypes of ET receptors present in rat kidney cortex.

## Materials and Methods

$^{125}$I-ET-1 (specific activity, 2200 Ci/mmol) and $^{125}$I-ET-3 (specific activity, 2200 Ci/mmol) were obtained from New England Nuclear (Boston, MA). Unlabeled ET-1 and ET-3 were from American Peptides (Santa Clara, CA). S6c was from Bachem Biosciences (Philadelphia, PA) and American Peptides. BQ123 was synthesized in the Department of Peptidomimetic Research, SmithKline Beecham Pharmaceuticals (King of Prussia, PA). All other chemicals were of the highest grade available.

**Membrane preparation.** Whole kidneys of male Sprague-Dawley rats (175–200 g) were removed, and the cortex was dissected out. This cortical tissue was homogenized (1 g/10 ml) in 20 mM Tris·HCl, pH 7.4, 5 mM EDTA, 0.25 M sucrose, 100 μg/ml phenylmethylsulfonyl fluoride, 10 μg/ml aprotinin, 10 μg/ml leupeptin (buffer A), using a tissue homogenizer (15 strokes). The homogenate was centrifuged for 15 min at 4° at 1000 × *g*. The supernatant was decanted, filtered through cheesecloth, and centrifuged for 30 min at 4° at 40,000 × *g*. The resulting pellets were resuspended in buffer B (50 mM Tris·HCl, pH 7.5, 20 mM $MgCl_2$).

**$^{125}$I-ET-1 binding.** $^{125}$I-ET-1 binding to membranes from rat kidney cortex was performed in buffer containing 50 mM Tris·HCl, pH 7.5, 10 mM $MgCl_2$, and 0.05% bovine serum albumin, as described (10). Membrane protein (2–6 μg/tube) was added to tubes containing either

**ABBREVIATIONS:** ET, endothelin; S6c, sarafotoxin 6c; BQ123, [cyclo(D-Trp,D-Asp,L-Pro,D-Val,L-Leu)].

buffer (total binding) or 100 nM unlabeled ET-1 (nonspecific binding). The reaction was started by the addition of 0.3 nM $^{125}$I-ET-1 and continued for 60 min at 30°. After the incubation, the reactions were stopped with 3 ml of cold buffer containing 50 mM Tris·HCl, pH 7.5, and 10 mM $MgCl_2$. Membrane-bound radioactivity was separated from free ligand by filtering through Whatman GF/C filter paper that had been presoaked in 0.1% bovine serum albumin. The filters were washed five times with 3 ml of buffer, using a Brandel cell harvester, and the filter papers were counted in a $\gamma$ counter with an efficiency of 75%. Saturation binding experiments were performed using increasing concentrations of $^{125}$I-ET-1 (30–500 pM), in the absence and presence of 100 nM unlabeled ET-1. Competition binding experiments were performed using 0.3 nM $^{125}$I-ET-1, in the absence and presence of increasing concentrations of the indicated compounds. $^{125}$I-ET-3 binding was performed in a fashion similar to that described above. Each experiment was performed three or four times; the data shown are from one experiment and are representative of those obtained in similar experiments.

**Data analysis.** Saturation binding experiments were analyzed by nonlinear regression analysis of untransformed data, using the Lundon 1 program (Lundon Software Inc., Cleveland, OH). Competition curves were analyzed using the Lundon 2 program. Single- and multiple-site models were statistically compared to determine the best fit, and differences among models were tested by comparing the residual variance, using an $F$ test and a significance level of $p < 0.05$.

## Results

$^{125}$I-ET-1 and $^{125}$I-ET-3 bound to rat kidney cortex membranes in a specific and saturable manner, as shown in Fig. 1, A and B. Scatchard transformation of the specific binding from the saturation binding experiments revealed linear Scatchard plots, suggesting a single class of high affinity binding sites, with a dissociation constant ($K_d$) of 218 ± 23 pM and maximum binding ($B_{max}$) of 275 ± 20 fmol/mg of protein for $^{125}$I-ET-1 and a $K_d$ of 207 ± 19 pM and a $B_{max}$ of 113 ± 17 fmol/mg of protein for $^{125}$I-ET-3 (Fig. 1C). The membrane protein concentrations chosen were in the linear range of the protein concentration curve. Time course experiments suggested that both $^{125}$I-ET-1 and $^{125}$I-ET-3 binding reached steady state by 60 min at 30°, at the ligand and protein concentrations used (data not shown).

Competition of $^{125}$I-ET-1 binding by unlabeled ET-1, ET-3, S6c, and BQ123 is shown in Fig. 2A. Unlabeled ET-1, ET-3, S6c, and BQ123 displaced $^{125}$I-ET-1 binding in a concentration-dependent manner. ET-1 gave a monophasic curve, with $K_d$ and $B_{max}$ values of 0.3 ± 0.04 nM and 260 fmol/mg of protein (represented as 100% in Table 1), respectively. ET-3 and S6c displayed shallow competition curves (Fig. 2A). ET-3 blocked 48% of $^{125}$I-ET-1 binding sites with high affinity (0.7 ± 0.06 nM) and 52% with low affinity (540 ± 260 nM). Similarly, S6c blocked 34% of $^{125}$I-ET-1 binding with high affinity (0.9 ± 0.1 nM) and 66% with low affinity (930 ± 240 nM) (Table 1A; Fig. 2A). In addition, BQ123, an $ET_A$ subtype-selective antagonist, displaced only 50% of $^{125}$I-ET-1 binding, even at concentrations as high as 1 $\mu$M (Fig. 2A), suggesting the presence of subtypes of ET receptors.

Next, we attempted to quantitate the proportion of these subtypes by performing $^{125}$I-ET-1 competition binding experiments in the presence of the $ET_B$-selective ligand S6c (to block all $ET_B$ receptors) or the $ET_A$-selective antagonist BQ123 (to block all $ET_A$ receptors). Fig. 2B shows the competition curves for ET-1, ET-3, and S6c performed in the presence of 1 $\mu$M BQ123. In the presence of 1 $\mu$M BQ123, $^{125}$I-ET-1 binding was

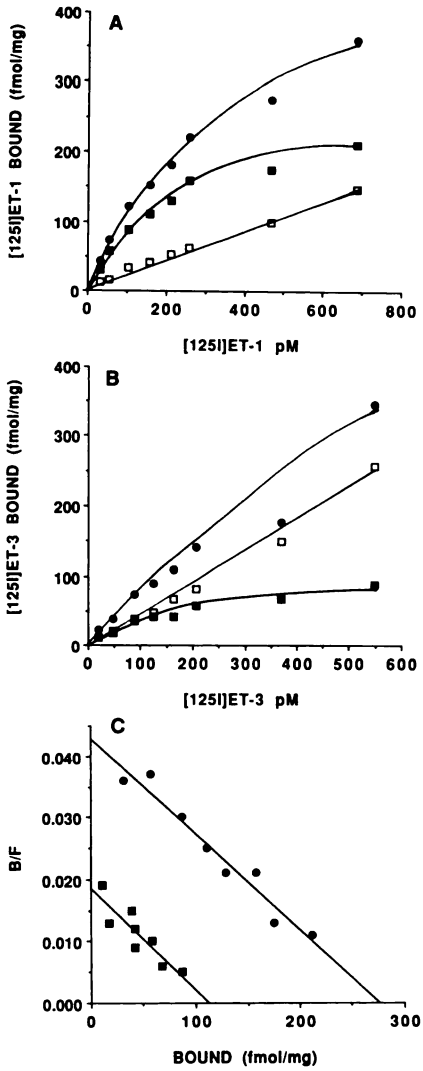


**Fig. 1.** A and B, Saturation binding of $^{125}$I-ET-1 (A) and $^{125}$I-ET-3 (B) to membranes prepared from rat kidney cortex. Assays were performed in duplicate at 30° for 60 min. Increasing concentrations of $^{125}$I-ET-1 or $^{125}$I-ET-3 were added to membranes in the absence (total binding) (●) or presence (nonspecific binding) (□) or 100 nM unlabeled ligand. Specific binding (■) was the difference between total and nonspecific binding. Bound and free ligands were separated as described in Materials and Methods. C, Scatchard transformations of the specific binding of $^{125}$I-ET-1 (●) and $^{125}$I-ET-3 (■) from A and B.

decreased by 48% (135 fmol/mg of protein), compared with control (260 fmol/mg of protein); the competition curves for ET-3 and S6c were monophasic, like that for ET-1, with $K_d$ values of 0.2 ± 0.03, 3.5 ± 0.3, and 0.3 ± 0.04 nM, respectively (Fig. 2B; Table 2B). These data further indicated that 48% of $^{125}$I-ET-1 binding sites were $ET_A$ receptors. Similar competition experiments were performed in the presence of 100 nM S6c, and the data are shown in Fig. 2C and Table 1C. Total binding in the presence of S6c was decreased by 56% (115 fmol/mg of protein), compared with control. Whereas the ET-1 curve did not change, compared with control (Fig. 2A), the ET-3 curve was shifted to the right and predominantly displayed low affinity binding, with a $K_d$ of 308 ± 160 nM, suggesting that, when $ET_B$ receptors were blocked by S6c, ET-3 bound to $ET_A$ receptors with low affinity. On the other hand, the BQ123 curve was shifted to the left (Fig. 2C), compared with control (Fig. 2A), and was monophasic, with a $K_d$ of 0.4 ± 0.06 nM. In addition,

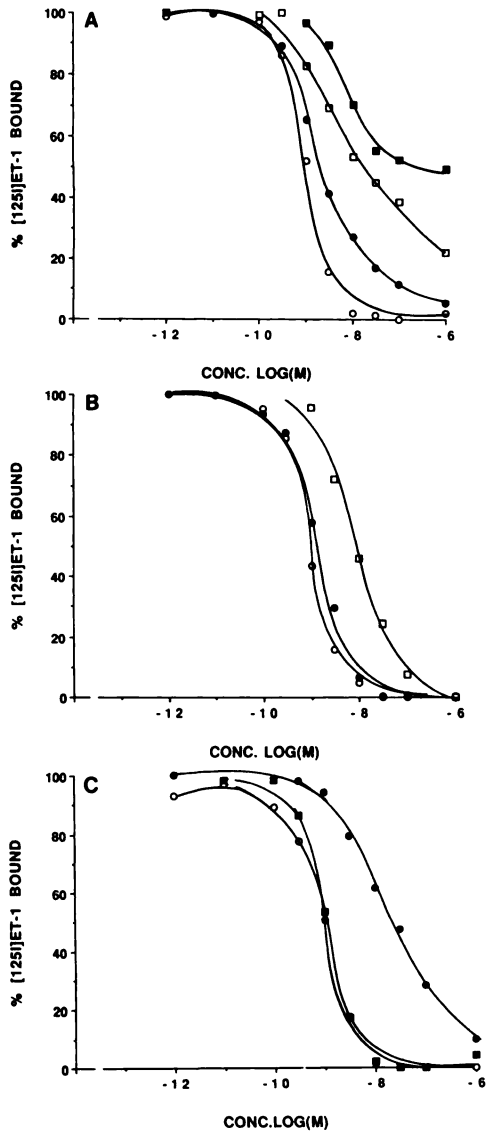


**Fig. 2.** A, Competition of $^{125}$I-ET-1 binding to membranes prepared from rat kidney cortex, by unlabeled ET-1 (○), ET-3 (●), S6c (□), and BQ123 (■). Membranes were incubated in triplicate with 0.2 nM $^{125}$I-ET-1 and increasing concentrations of unlabeled ligands, for 60 min, at 30°. Bound and free ligands were separated as described. Data obtained in the absence of competitor were normalized to 100% and those obtained in the presence of 1 μM ET-1 were 0%; data are expressed as the percentage of $^{125}$I-ET-1 binding. B, Competition of $^{125}$I-ET-1 binding to cortex membranes by unlabeled ET-1 (○), ET-3 (●), and S6c (□) in the presence of 1 μM BQ123. The experiment was performed as described for A. C, Competition of $^{125}$I-ET-1 binding to cortex membranes by unlabeled ET-1 (○), ET-3 (●), and BQ123 (■) in the presence of 100 nM S6c. The experiment was performed as described for A.

BQ123 displaced >90% of $^{125}$I-ET-1 binding (Fig. 2C), compared with control, where the maximum displacement was 50% (Fig. 2A).

The percentage distribution of $ET_A$ and $ET_B$ receptors in this tissue was further quantitated by the binding of $^{125}$I-ET-1 and $^{125}$I-ET-3, in the presence and absence of S6c and BQ123, and the data are presented in Fig. 3. $^{125}$I-ET-1 binding was 193 ± 5 fmol/mg of protein (100%) and $^{125}$I-ET-3 binding was 92 ± 17 fmol/mg of protein (48%), suggesting that 48% of $^{125}$I-ET-1 binding was to the $ET_B$ receptor subtype. In the presence of 10 nM S6c, $^{125}$I-ET-1 binding was inhibited by 52%, to 90.0 ± 2

TABLE 1

**Percentage distribution and dissociation constants for ET-1, ET-3, S6c, and BQ123 in competing for $^{125}$IET-1 binding to membranes from rat kidney cortex**

$^{125}$IET-1 competition binding experiments using unlabeled ET-1, ET-3, S6c, and BQ123 were performed as described in Materials and Methods and the Fig. 2 legend. The data presented are mean ± standard error of three determinations and were analyzed using the Lundon 2 program. $R_H$ and $R_L$ represent high and low affinity sites for the respective ligands. $K_H$ and $K_L$ represent dissociation constants for high and low affinity sites for the respective ligands. For A, B, and C, 100% represents 260 ± 15,135 ± 10, and 115 ± 9 fmol/mg of protein, respectively.

| | $R_H$ | $K_H$ | $R_L$ | $K_L$ |
|---|---|---|---|---|
| | % | nM | % | nM |
| A. Control | | | | |
| ET-1 | 100 | 0.3 ± 0.04 | | |
| ET-3 | 48 ± 4 | 0.7 ± 0.06 | 52 ± 5 | 540 ± 260 |
| S6c | 34 ± 3 | 0.9 ± 0.10 | 66 ± 4 | 930 ± 240 |
| BQ123 | 50 ± 8 | 9.3 ± 2.0 | 50 ± 12 | |
| B. In the presence of 1 μM BQ123 | | | | |
| ET-1 | 100 | 0.3 ± 0.04 | | |
| ET-3 | 100 | 0.2 ± 0.03 | | |
| S6c | 100 | 3.5 ± 0.30 | | |
| C. In the presence of 100 nM S6c | | | | |
| ET-1 | 100 | 0.4 ± 0.04 | | |
| ET-3 | 10 ± 2 | 3.0 ± 0.70 | 90 ± 6 | 308 ± 160 |
| BQ123 | 100 | 0.4 ± 0.06 | | |

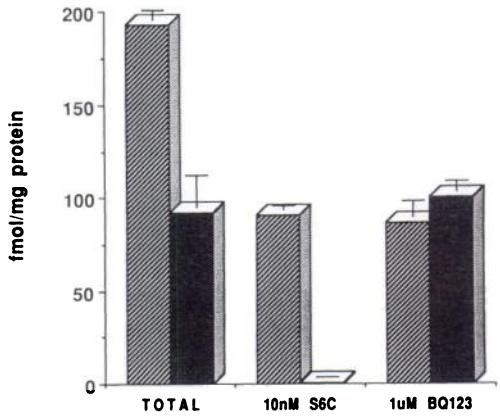


**Fig. 3.** $^{125}$I-ET-1 and $^{125}$I-ET-3 binding to rat kidney cortex membranes in the absence and presence of 10 nM S6c or 1 μM BQ123. $^{125}$I-ET-1 (▨) or $^{125}$I-ET-3 (■) (0.3 nM) was added to membranes in the absence of S6c or BQ123 (total) or the presence of 10 nM S6c or 1 μM BQ123, and membranes were incubated for 60 min at 30°. Bound and free ligands were separated as described in Materials and Methods. Nonspecific binding was measured in the presence of 100 nM unlabeled ET-1 or ET-3 and was 10 and 20% of total binding for $^{125}$I-ET-1 and $^{125}$I-ET-3, respectively. The data shown are mean ± standard error of three determinations.

fmol/mg of protein, whereas $^{125}$I-ET-3 binding was totally abolished, further confirming that 50–55% of $^{125}$I-ET-1 binding was to $ET_B$ receptors and that, at the concentration used (0.3 nM), $^{125}$I-ET-3 bound only to $ET_B$ receptors (Fig. 3). Addition of 1 μM BQ123 did not have any effect on $^{125}$I-ET-3 binding (100 ± 5.7 fmol/mg of protein), whereas it inhibited $^{125}$I-ET-1 binding by 53% (86.7 ± 8.3 fmol/mg of protein), further confirming that 50% of $^{125}$I-ET-1 binding was to $ET_A$ receptors. In the presence of BQ123 and S6c, $^{125}$I-ET-1 binding was totally abolished.

## Discussion

There are many reports in the literature suggesting the presence of subtypes of ET receptors in various tissues (17–

21), and the recent cloning of $ET_A$ and $ET_B$ receptors from bovine and rat lung (12, 13) confirmed these suggestions. The present study demonstrates the presence of high affinity $ET_A$ and $ET_B$ receptors in membranes prepared from rat kidney cortex. Whereas $^{125}$I-ET-1 bound to both $ET_A$ and $ET_B$ receptors with the same high affinity, $^{125}$I-ET-3 bound to only $ET_B$ receptors with high affinity. Analysis of $^{125}$I-ET-1 competition binding experiments with unlabeled ET-1, ET-3, S6c, and BQ123 suggested that the ET-1 competition curve was monophasic, with high affinity, in the absence or presence of subtype-selective ligands. On the other hand, the ET-3 competition curve was shallow in the absence of any subtype-selective ligands and was monophasic and shifted to the left (high affinity) in the presence of BQ123 or to the right (low affinity) in the presence of S6c. Similar results were obtained for S6c and BQ123. In the presence of BQ123, the S6c competition curve was shifted to the left and was monophasic, whereas in the absence of BQ123 the S6c competition curve was shallow and of low affinity. In the presence of S6c the BQ123 competition curve was monophasic and shifted to the left, whereas in its absence BQ123 inhibited only 50% of $^{125}$I-ET-1 binding, even at 1 $\mu$M. Also, in the presence of S6c or BQ123 $^{125}$I-ET-1 binding was decreased by ~50%, and in the presence of both together $^{125}$I-ET-1 binding was totally abolished, suggesting that kidney cortex displayed only $ET_A$ and $ET_B$ receptors. In addition, $^{125}$I-ET-3 binding was totally abolished by S6c and was unaffected by BQ123, further confirming that BQ123 is selective for $ET_A$ receptors and that $^{125}$I-ET-3 bound to only $ET_B$ receptors with high affinity.

At present, we do not know the physiological functions of these two subtypes of receptors, although both $ET_A$ and $ET_B$ receptors, when transiently expressed in COS cells or *Xenopus* oocytes, mediated calcium release when challenged with the agonists (12, 13, 22). Whereas ET-1 binds to both $ET_A$ and $ET_B$ receptors with the same affinity, ET-3 is more selective for $ET_B$ receptors. The physiological function of ET-3 is not known at this time. Both ETs and sarafotoxins display prolonged vasoconstrictor actions in many vascular beds (23). In addition to the vasopressor effects, ETs produce a characteristic short-lived vasodepressor effect before the pressor effect (24). Warner *et al.* (25) and Douglas and Hiley (26) reported endothelium-dependent relaxation in response to ET-3, ET-2, ET-1, and S6b. The order of potency of these peptides for relaxation was different from that for contraction. ET-3 was the most potent relaxant but the least potent constrictor (26), suggesting that different receptors may be mediating these two responses. Recently, MacCumber *et al.* (27) and Matsumoto *et al.* (28) have reported the tissue distribution of various ET-related peptides in rat. Their data showed that both ET-1 and ET-3 were identified in kidney. This distribution of ET-1 and ET-3 is consistent with the ET receptor subtypes identified in this study and might suggest a role for ET-1 and ET-3 as local hormones in renal physiology.

**Acknowledgments**

We would like to thank Drs. W. Huffman and M. Moore for helpful suggestions and Miss Sue Tirri for expert secretarial assistance.

**References**

1. Yanagisawa, M., H. Kurihara, S. Kimura, Y. Tomobe, M. Kobayashi, Y. Mitsui, Y. Yazaki, K. Goto, and T. Masaki. A novel potent vasoconstrictor peptide produced by vascular endothelial cells. *Nature* (*Lond.*). **332**:1111–1115 (1988).
2. Kloog, Y., and M. Sokolovsky. Similarities in mode and sites of action of sarafotoxins and endothelins. *Trends Pharmacol. Sci.* **10**:212–214 (1989).
3. Simonson, M. S., and M. J. Dunn. Cellular signalling by peptides of the endothelin gene family. *FASEB J.* **4**:2989–3000 (1990).
4. Rubanyi, G. M., and L. H. Parker Bothelo. Endothelins. *FASEB J.* **5**:2713–2720 (1991).
5. Goetz, K. L., B. C. Wang, J. B. Madwed, J. L. Zhu, and R. J. Leadly. Cardiovascular, renal and endocrine responses to intravenous endothelin in conscious dogs. *Am. J. Physiol.* **255**:R1064–R1068 (1988).
6. King, A. J., B. M. Brenner, and S. Anderson. Endothelin: a potent renal and systemic vasoconstrictor peptide. *Am. J. Physiol.* **256**:F1051–F1058 (1989).
7. Miller, W. L., M. M. Redfield, and J. C. Burnett. Integrated cardiac, renal and endocrine actions of endothelin. *J. Clin. Invest.* **83**:317–320 (1989).
8. Firth, J. D., A. E. Raine, P. J. Ratcliffe, and J. G. Ledingham. Endothelin: an important factor in acute renal failure. *Lancet.* **2**:1179–1181 (1988).
9. Kon, V., T. Yoshioka, A. Fogo, and I. Ichikawa. Glomerular actions of endothelin *in vivo*. *J. Clin. Invest.* **83**:1762–1767 (1989).
10. Nambi, P., M. Pullen, L. C. Contino, and D. P. Brooks. Up-regulation of renal endothelin receptors in rats with cyclosporine A-induced nephrotoxicity. *Eur. J. Pharmacol.* **187**:113–116 (1990).
11. Simonson, M. S., and M. J. Dunn. Endothelins: a family of regulatory peptides. *Hypertension* (*Dallas*). **17**:856–863 (1991).
12. Arai, H., S. Hori, I. Aramori, H. Ohkubo, and S. Nakanishi. Cloning and expression of a cDNA encoding an endothelin receptor. *Nature* (*Lond.*). **348**:730–732 (1990).
13. Sakurai, T., M. Yanagisawa, Y. Takuwa, H. Miyazaki, S. Kimura, K. Goto, and T. Masaki. Cloning of a cDNA encoding a nonisopeptide-selective subtype of the endothelin receptor. *Nature* (*Lond.*). **348**:732–735 (1990).
14. Williams, D. L., K. L. Jones, D. J. Pettibone, E. V. Lis, and B. V. Clinschmidt. Sarafotoxin S6c: an agonist which distinguishes between endothelin receptor subtypes. *Biochem. Biophys. Res. Commun.* **175**:556–561 (1991).
15. Williams, D. L., K. L. Jones, C. D. Colton, and R. F. Nutt. Identification of high affinity endothelin-1 receptor subtypes in human tissues. *Biochem. Biophys. Res. Commun.* **180**:475–480 (1991).
16. Ihara, M., Noguchi, K., Sacki, T., Fukuroda, T., Tsuchida, S., Kimura, S., Fukami, T., Ishikawa, K., Nishikibe, M., and Yano, M. Biological profiles of highly potent novel endothelin antagonists selective for the ETA receptor. *Life Sci.* **50**: 247–255 (1992).
17. Watanabe, H., H. Miyazaki, M. Kondoh, Y. Masuda, S. Kimura, M. Yanagisawa, T. Masaki, and K. Murakami. Two distinct types of endothelin receptors are present in chick cardiac membranes. *Biochem. Biophys. Res. Commun.* **161**:1252–1259 (1989).
18. Bolger, G. T., F. Liard, R. Krogsrud, D. Thibeault, and J. Jaramillo. Tissue specificity of endothelin binding sites. *J. Cardiovasc. Pharmacol.* **16**:367–375 (1990).
19. Kloog, Y., D. Bousso-Mittler, A. Bdolah, and M. Sokolovsky. Three apparent receptor subtypes for endothelin/sarafotoxin family. *FEBS Lett.* **253**:199–202 (1989).
20. Jones, C. R., C. R. Hiley, J. T. Pelton, and R. C. Miller. Endothelin receptor heterogeneity: structure activity, autoradiographic and functional studies. *J. Receptor Res.* **11**:299–310 (1991).
21. Takayanagi, R., K. Ohhaka, C. Takasaki, M. Ohashi, and H. Nawata. Multiple subtypes of endothelin receptors in porcine tissues: characterization by ligand binding, affinity labelling and regional distribution. *Regul. Peptides.* **32**:23–37 (1991).
22. Elshourbagy, N. A., J. A. Lee, D. R. Korman, P. Nuthulaganti, A. G. DiLella, D. R. Sylvester, J. A. Sutiphong, and C. S. Kumar. Expression cloning of porcine and human endothelin receptor. *Circulation* **84** (Suppl. II):1610 (1991).
23. Lovenberg, W., and R. C. Miller. Endothelin: a review of its effects and possible mechanism of action. *Neurochem. Res.* **15**:407–411 (1990).
24. Wright, C. E., and J. R. Fozard. Regional vasodilation is a prominent feature of the hemodynamic response to endothelin in anaesthetized, spontaneously hypertensive rats. *Eur. J. Pharmacol.* **155**:201–203 (1988).
25. Warner, J. D., G. DeNucci, and J. R. Vane. Rat endothelin is a vasodilator in the isolated perfused mesentery of the rat. *Eur. J. Pharmacol.* **159**:325–326 (1989).
26. Douglas, S. A., and C. R. Hiley. Endothelin-dependent vascular activities of endothelin-like peptides in isolated superior mesenteric arterial bed of the rat. *Br. J. Pharmacol.* **101**:81–88 (1990).
27. MacCumber, M. W., C. A. Ross, B. M. Glaser, and S. H. Snyder. Endothelin: visualization of mRNAs by *in situ* hybridization provides evidence for local action. *Proc. Natl. Acad. Sci. USA* **86**:7285–7289 (1989).
28. Matsumoto, H., N. Suzuki, H. Onda, and M. Fujino. Abundance of endothelin-3 in rat intestine, pituitary gland and brain. *Biochem. Biophys. Res. Commun.* **164**:74–80 (1989).

**Send reprint requests to:** Ponnal Nambi, Ph.D., SmithKline Beecham Pharmaceuticals, Department of Pharmacology, L521, P.O. Box 1539, King of Prussia, PA 19406–0939.