0026-895X/94/020182-07$03.00/0

MOLECULAR PHARMACOLOGY, 45:182-188

Endothelin Receptor Subtypes A and B Are Up-regulated in an Experimental Model of Acute Renal Failure

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Received May 27, 1993; Accepted November 4, 1993

SUMMARY

The two endothelin (ET) receptor subtypes (ET_A and ET_B) have been characterized in rat kidney from normal rats and rats with acute renal failure induced by hypertonic glycerol administration. In control rats, the total number of ET receptors in kidney cortex and medulla was 155 and 386 fmol/mg of protein, respectively. The ratio of ET_A to ET_B receptors was 54:46 in renal cortex and 35:65 in renal medulla. Treatment of rats with 10 ml/kg glycerol (50%, w/v) intramuscularly resulted in severe renal dysfunction; the serum urea concentration increased from 0.46 to 2.65 g/liter and the creatinine clearance decreased from 1.06 to 0.30 ml/min. Ligand binding studies showed that glycerol-induced acute renal failure was associated with a marked up-regulation of ET_A and ET_B receptor subtypes in both cortex and medulla. In glycerol-treated rats, the total ET receptor density in kidney cortex and medulla was increased to 294 and 1172 fmol/mg of protein, with ET_A/ET_B ratios of 52:48 and 31:69, respectively. The up-regulatory effect of glycerol treatment was significantly more pronounced in renal medulla than renal cortex and affected ET_B receptors preferentially, compared with ET_A receptors. Subsequently, ET_A and ET_B receptor mRNA levels were markedly increased by glycerol administration in both kidney cortex and medulla, as assessed by polymerase chain reaction coupled to reverse transcription. These results suggest that up-regulation of renal ET receptors, particularly ET_B receptors in kidney medulla, may account for or contribute to renal function impairment induced by glycerol, and they support a pathophysiological role for ET in acute renal failure.

ETs represent a family of three isopeptides (ET-1, ET-2, and ET-3) with potent vasoactive properties. ET-1 was initially discovered as a vasoconstrictive peptide secreted and released by the vascular endothelium (1), and subsequent analysis of the ET genes proved the existence of the two other members of the ET family, ET-2 and ET-3 (2). By interacting with specific receptors, ETs exert a variety of pharmacological actions in vascular and nonvascular tissues including heart, lung, and kidney (for review, see Ref. 3). Two ET receptor subtypes, termed ET_A and ET_B, have been cloned and shown to belong to the G protein-coupled superfamily of receptors with seven transmembrane domains (4, 5). ET_A receptors recognize preferentially ET-1 and ET-2, whereas the ET_B subtype appears to bind the three ET isoforms with similar affinities. Although ET_A receptors are typically located on vascular smooth muscle cells and ET_B receptors in endothelial cells, both are widely distributed in the organism (6, 7). However, the respective physiological and/or pathophysiological roles of these two ET receptor subtypes still remain to be determined.

Considering its potent vasoconstrictive and pressor activities and the large number of its receptors in the kidney (8), ET-1 has been proposed to be an important factor implicated in acute renal failure (9). Indeed, low concentrations of ET-1 cause potent long-lasting renal vasoconstriction and a decrease of renal blood flow, glomerular filtration rate, urine volume, and sodium and potassium excretion, with increased renal vascular resistance and proteinuria (9–13). Furthermore, the severity of postischemic renal failure in rats is reduced by anti-ET-1 antibody (14, 15), and an increased plasma level of ET-1 is found in patients with acute renal failure (16).

In the present study, the distribution and the regulation of ET_A and ET_B receptors have been investigated in kidney from normal rats and from rats subjected to acute renal failure. For this purpose, we examined whether hypertonic glycerol-induced myoglobinuric acute renal failure (17) affects renal ET receptors, and we determined which of the two ET receptor subtypes may be modified, because both ET_A and ET_B receptors are present in the kidney (5, 18). We compared the competitive inhibition by unlabeled ET-1, ET-3, BQ-123 (a selective ET_A receptor ligand) (19), and sarafotoxin S6c (a selective ET_B receptor ligand) (20) of radiolabeled ^{125}I-ET-1 binding in membrane preparations from kidney cortex and medulla of hypertonic glycerol-treated rats and control animals. Concomitantly, specific ET_A and ET_B receptor mRNA levels were determined using RT-PCR.

ABBREVIATIONS: ET, endothelin; BSA, bovine serum albumin; RT, reverse transcription; PCR, polymerase chain reaction; TBE, Tris-borate-EDTA.

Materials and Methods

Glycerol-induced acute renal failure. Male Wistar rats (200 g) were housed individually in plastic metabolic cages and deprived of drinking water for 24 hr, while food was allowed *ad libitum*. Acute renal failure was produced by intramuscular injection of 50% (w/v) glycerol in 0.9% NaCl (10 ml/kg), in divided doses, into two sites of each hindlimb. Glycerol was administered to rats 48 hr before ET binding studies. Control rats were similarly dehydrated and received 0.9% NaCl injections (10 ml/kg), instead of glycerol, into the hindlimbs. Drinking water was immediately restored after either glycerol or NaCl administration.

To evaluate renal function impairment, serum and urine samples were assayed for creatinine and urea concentrations by standard spectrophotometric methods with an Abbott ABA-200 auto-analyzer, i.e., creatinine by reaction with picrate in alkaline solution (21) and urea by measurement of urease activity (22). Creatinine clearance was determined for a 24-hr period by dividing the amount excreted in urine by the serum concentration.

Renal membrane preparation. Rats were anesthetized with 60 mg/kg pentobarbital and kidneys were removed, excised from their renal capsules, weighed, placed in 50 mM Tris·HCl, pH 7.4 at 4°, and dissected to separate renal cortex from renal medulla. Both were then cut into small pieces and homogenized for 30 sec with a Thomas homogenizer (setting 10). Homogenates were filtered through two layers of gauze and centrifuged at 500 × *g* for 10 min at 4°. Supernatants were centrifuged at 52,000 × *g* for 30 min at 4° and pellets were resuspended in the same buffer for another centrifugation at 52,000 × *g* for 30 min at 4°. Resulting pellets were stored at −80°. An aliquot was taken for the assessment of protein content by the method of Bradford (23), with BSA as a standard.

Renal membrane binding assay. Renal cortex or medulla membrane preparations (35 μg of protein/tube) were incubated in triplicate for 1 hr at 25° in 50 mM Tris·HCl, pH 7.4, containing 10 mM $MgCl_2$, 20 mg/liter bacitracin, 20 mg/liter phenylmethylsulfonyl fluoride, 100,000 units/liter trasylol, and 0.05% BSA. Competitive inhibition of 0.1 nM ^{125}I-ET-1 binding was performed with unlabeled ET-1, ET-3, BQ-123, and S6c at concentrations ranging from 1 pM to 100 μM. Bound ^{125}I-ET-1 was separated from free ^{125}I-ET-1 by immediate filtration through Whatman GF/C glass fiber filters that had been presoaked in 0.05% BSA, using a Brandel cell harvester. Filters were washed four times with the same buffer at 0–4° and were assayed for radioactivity in a γ counter (Pharmacia) with 70% efficiency. Specific binding was obtained by subtracting nonspecific binding (determined in the presence of 1 μM unlabeled ET-1) from total binding.

Determination of ET receptor mRNA by RT-PCR. Total RNA was isolated from renal cortex and medulla by a modification of the method of Chomczynski and Sacchi (24), using guanidinium thiocyanate/phenol chloroform extraction (RNA B kit; Bioprobe Systems). Relative levels of specific ET_A and ET_B receptor mRNA were determined by RT-PCR using recombinant *Thermus thermophilus* DNA polymerase (GeneAmp thermostable r*Tth* reverse transcriptase RNA PCR kit; Perkin Elmer Cetus) and specific ET receptor primers (Bio-Inova, Plaisir, France). Primers used for the ET_A receptor were the "upstream" primer 5′-CAGACAAAAATCACGACGGC-3′, starting at nucleotide 213 of the rat sequence (18), and the "downstream" primer 5′-GTGACAACAGCAACAGAGGC-3′, starting at nucleotide 1147. Primers used for the ET_B receptor were the upstream primer 5′-GTGGTCTCTGTGGTTCTGGC-3′, starting at nucleotide 675 of the rat sequence (5), and the downstream primer 5′-GTTTTCCG-TTTGGGTTTGGC-3′, starting at nucleotide 1397. One microgram of total RNA from either kidney cortex or medulla was reverse transcribed for 15 min at 55° on a thermal cycler (TRIO-Thermoblock; Biometra), and template-specific cDNA was then amplified for 28 cycles of denaturation at 94° for 1 min, annealing at 57° for 1 min, and polymerization at 72° for 0.5 min (cycles 1–16) or 1 min (cycles 17–28). The PCR mixture consisted of 1.5 mM $MgCl_2$ and 0.075 μM ET_A primers or 1 mM $MgCl_2$ and 0.03 μM ET_B primers, all four deoxynucleoside triphosphates (each at 200 μM), and 5 units of r*Tth* DNA polymerase. The resulting amplified cDNA lengths were predicted to be 954 and 742 base pairs for ET_A and ET_B receptors, respectively. RT-PCR (using the same procedure as described above) of β-actin mRNA, which did not vary significantly with glycerol administration, served as a positive control. Upstream and downstream primers for β-actin (0.03 μM) were 5′-ATCGTGGGCCGCCCTAGGCA-3′ and 5′-GGGGAGACTTGGGAT-TCCGGT-3′, respectively. Aliquots of the amplified cDNA samples were electrophoresed in a 1.5% agarose gel in TBE buffer (89 mM Tris, 2 mM EDTA, 0.0896 mM boric acid pH 8.3) containing ethidium bromide. The gel was photographed under UV light, and the staining intensity was determined with a laser densitometer (Pharmacia, Sint Quentin en Yvelines, France).

Data analysis. Binding data were analyzed by computer-assisted nonlinear regression analysis using the LIGAND program (Biosoft, Cambridge, UK) for statistical comparison (statistically significant reduction in the sum of squares of residuals) between single- and multiple-binding site models and for determination of affinity constants and binding capacities for the best fits. Results are expressed as mean ± standard error in each animal group, one-way analysis of variance was used to compare between groups, and significance was assumed if p values were <0.05.

Materials. ^{125}I-ET-1 was the 13-[^{125}I]iodotyrosyl derivative of ET-1 (2200 Ci/mmol; New England Nuclear, Les Ulis, France). ET-1 and ET-3 were purchased from Novabiochem (E.M.A., Cléry en Vexin, France). BQ-123 was from Neosystem (Strasbourg, France). Sarafotoxin S6c, glycerol, bacitracin, phenylmethylsulfonyl fluoride, BSA, and ethidium bromide were purchased from Sigma Chemical Co. (St. Louis, MO). Trasylol was from Bayer (Sens, France), pentobarbital from Sanofi (Libourne, France), agarose from Promega (Madison, WI), and TBE buffer from Bioprobe Systems (Montreuil sous Bois, France).

Results

Glycerol-treated rats developed severe renal dysfunction, as indicated by alterations of creatinine clearance and serum urea levels, which were maximal 48 hr after glycerol injection (Table 1). Creatinine clearance was decreased by 72% and serum urea levels were increased by 6-fold, compared with saline-injected control animals. Furthermore, kidney weight was significantly increased, although protein contents in membrane preparations from kidney cortex and medulla were unchanged 48 hr after glycerol administration (Table 1).

Monophasic competitive inhibition curves for ET-1 binding revealed that ET-1 bound to an apparent single class of high affinity binding sites on membrane preparations from kidney cortex (Fig. 1A) and medulla (Fig. 1B). Renal medulla appeared to possess more ET binding sites (B_{max} = 386 ± 23 fmol/mg of protein, $p < 0.01$ versus renal cortex) than did renal cortex (B_{max} = 155 ± 4 fmol/mg of protein). The apparent dissociation constant (K_d) was not significantly lower in renal cortex (0.177 ± 0.050 nM) than in renal medulla (0.369 ± 0.087 nM). After treatment with glycerol, a large increase of ET receptor density occurred in both kidney cortex (Fig. 1A) and medulla (Fig. 1B). B_{max} values were 294 ± 32 fmol/mg of protein ($p < 0.05$ versus control) in renal cortex and 1172 ± 81 fmol/mg of protein ($p < 0.001$) in renal medulla. Glycerol-induced acute renal failure did not significantly affect ET-1 binding affinity, with K_d values being 0.234 ± 0.058 nM and 0.339 ± 0.090 nM for renal cortex and medulla, respectively, in glycerol-treated rats.

To determine the relative proportions of ET_A and ET_B receptor subtypes, which are both expressed in the kidney (5, 18), and to investigate whether these subtypes are distinctly affected by glycerol treatment, competition binding experiments were performed using ET-1, ET-3, BQ-123 (an ET_A-selective antag-

TABLE 1

Effects of glycerol treatment on creatinine clearance, serum urea concentration, kidney weight, and protein content in membrane preparations

Creatinine clearance, serum urea concentration, total kidney weight, and protein content in membrane preparations from kidney cortex and medulla were determined 48 hr after intramuscular injection of glycerol/saline (50%, w/v). Results are mean ± standard error of at least 10 animals.

Treatment	Creatinine clearance	Serum urea concentration	Total kidney weight	Membrane proteins	
				Kidney cortex	Kidney medulla
	ml/min/24 hr	*g/liter*	*g*	*mg*	
Control	1.06 ± 0.048	0.46 ± 0.022	0.67 ± 0.015	7.43 ± 0.519	3.27 ± 0.318
Glycerol	0.30 ± 0.097[a]	2.65 ± 0.549[a]	1.06 ± 0.044[a]	7.37 ± 1.155	3.38 ± 0.223

[a] Statistical difference from control, $p < 0.001$.

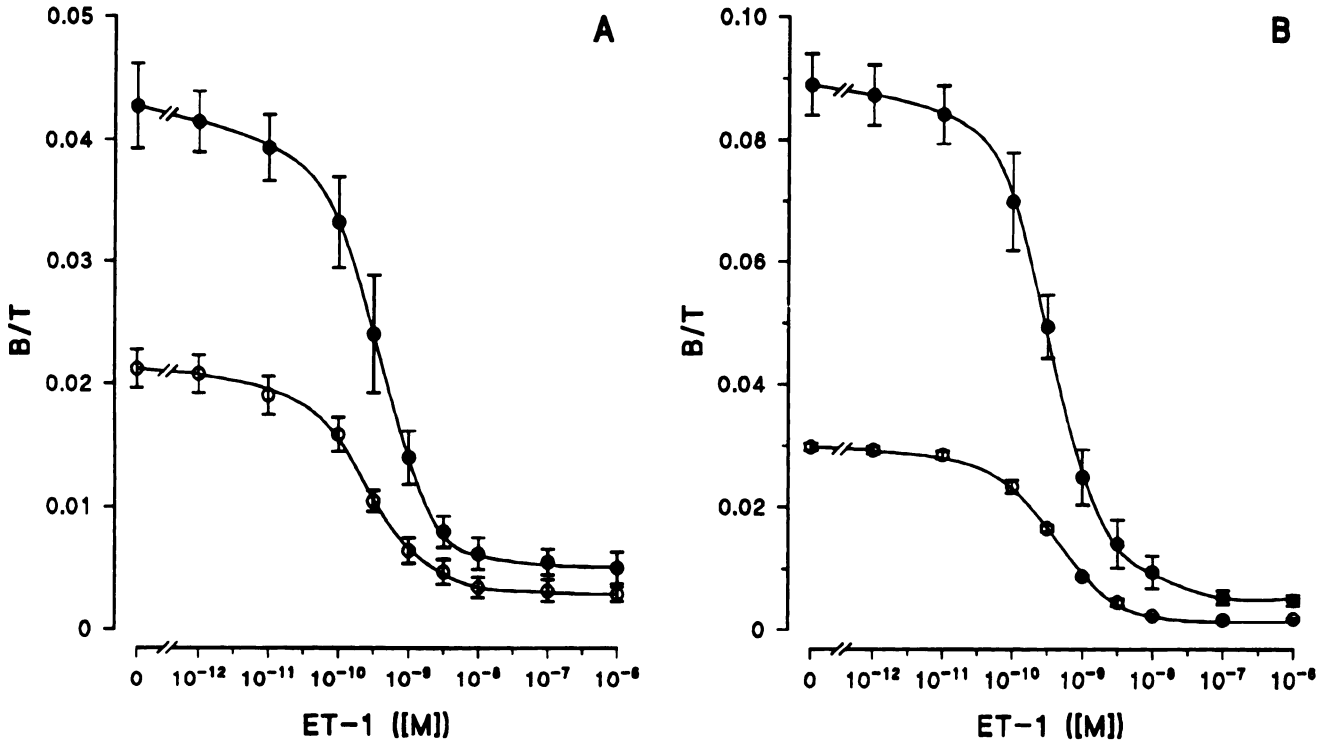

Fig. 1. Competition curves for ^{125}I-ET-1 (0.1 nM) binding to membrane proteins (35 μg/tube) from control (○) and glycerol-treated (●) rat kidney cortex (A) and medulla (B), in the presence of increasing concentrations of unlabeled ET-1. Results are expressed as bound/total (*B/T*) ratio versus unlabeled ET-1. Each *point* is the mean ± standard error of four experiments performed in triplicate.

onist) (19), and sarafotoxin S6c (an ET_B-selective agonist) (20). Whereas ET-1 recognized ET_A and ET_B receptors with identical affinities and thus inhibited ^{125}I-ET-1 binding according to a one-site model, the inhibition curves for ET-3, BQ-123, and sarafotoxin S6c were significantly better fitted using a two-site model (Fig. 2). Whichever competitor was used in inhibition experiments (i.e., ET-3, sarafotoxin S6c, or BQ-123), calculated values for the relative number of ET_A or ET_B receptors (Table 2) were similar in each group (cortex or medulla, control or glycerol-treated), and mean B_{max} values were calculated (Fig. 3). In control rats, ET_A and ET_B binding capacities were 1.7-fold and 3.6-fold higher, respectively, in the medulla than in the cortex. The ratios of ET_A to ET_B receptors were 54:46 and 35:65 in cortex and medulla, respectively. Inhibition constant (K_i) values (Table 2) conformed to the expected selectivity of the competitors; ET-3 and sarafotoxin S6c bound with highest affinity to ET_B receptors, whereas BQ-123 selectively recognized ET_A binding sites.

After glycerol administration, ET_A and ET_B receptor densities were markedly increased. This elevation was significantly higher in renal medulla (2.8- and 3.4-fold increases for ET_A and ET_B, respectively), compared with renal cortex (2.2- and 2.5-fold increases for ET_A and ET_B, respectively). The ratios of ET_A to ET_B binding sites in glycerol treated-animals were 52:48 and 31:69 for cortex and medulla, respectively. Up-regulation of renal ET receptors in glycerol-induced acute renal failure was associated with a slight but not significant decrease in the affinity of BQ-123 for ET_A and ET_B receptors in both cortex and medulla (Table 2).

Moreover, total RNA from kidney cortex and medulla in control and glycerol-treated rats was isolated, and the relative levels of ET_A and ET_B receptor mRNA were estimated by RT-PCR. As shown in Fig. 4, the choice of primers used provided single specific signals of the expected PCR product size for both ET receptor subtypes. An increase in ET_A and ET_B receptor mRNA was found in kidney cortex and medulla from glycerol-treated animals. Densitometric analysis of the ethidium bromide-stained gel estimated this increase to be about 2–

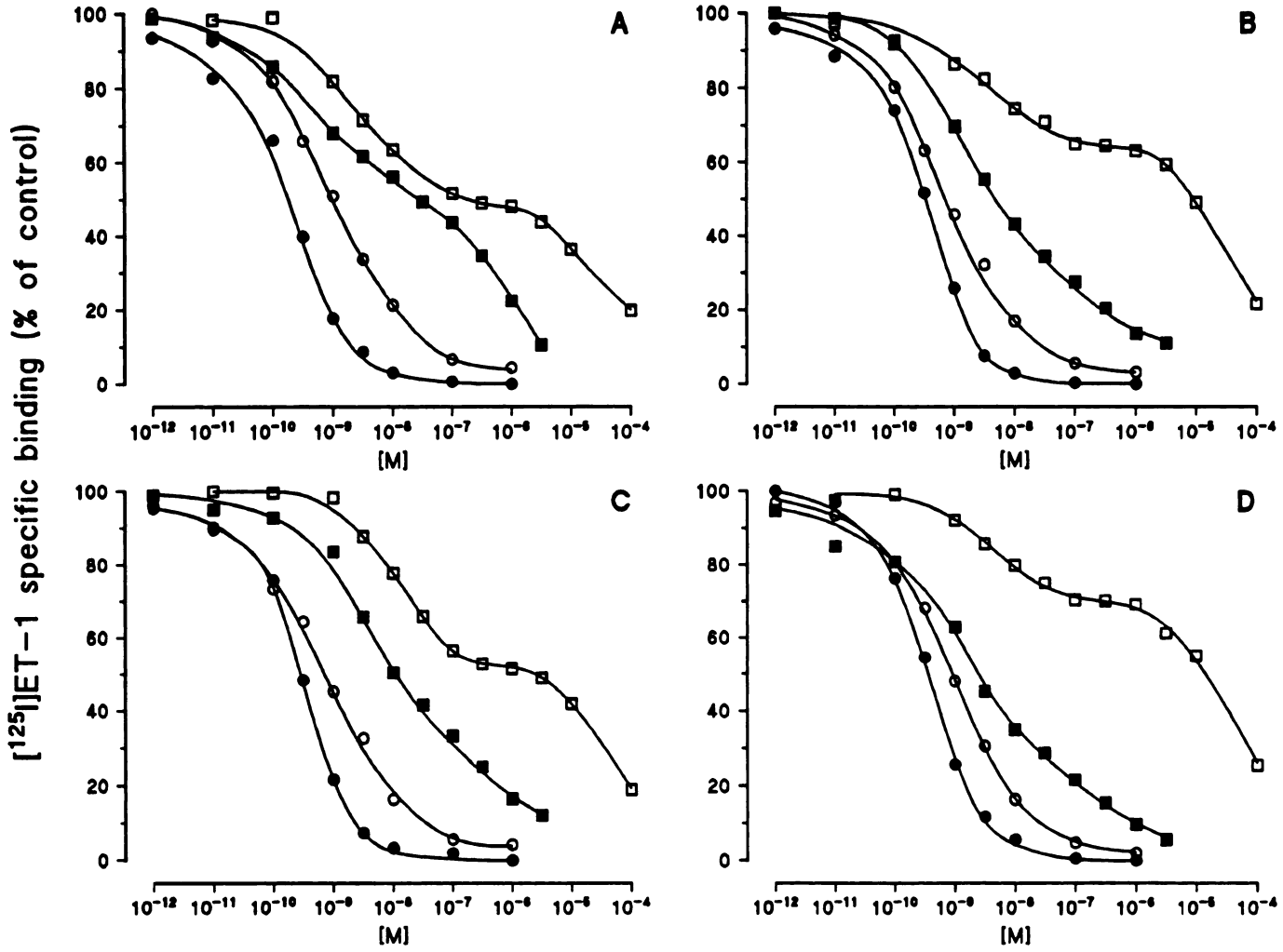

Fig. 2. Competitive inhibition of specific ^{125}I-ET-1 (0.1 nM) binding by unlabeled ET-1 (●), ET-3 (○), sarafotoxin S6c (■), and BQ-123 (□). Mean results are shown for renal cortex (A) and medulla (B) from control rats and for renal cortex (C) and medulla (D) from glycerol-treated animals. Data were corrected for nonspecific binding and are presented as percentage of control bound (in the absence of any unlabeled ligand). Affinity constants and receptor densities are reported in Table 2.

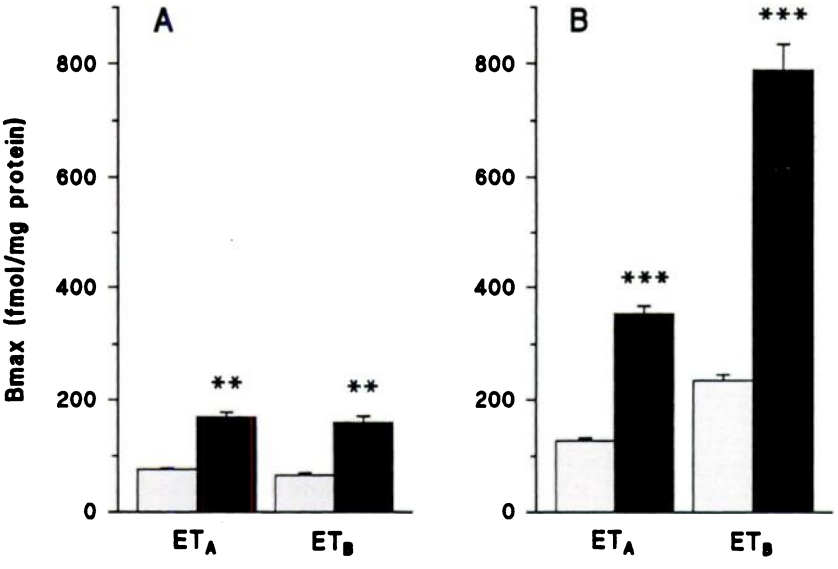

Fig. 3. Up-regulation of renal ET receptor subtypes in glycerol-induced acute renal failure. Data represent ET_A and ET_B receptor densities in kidney cortex (A) and medulla (B) from control (□) and glycerol-treated (■) rats. Results are expressed as mean ± standard error of the values obtained for each structure (cortex and medulla, control and glycerol-treated) using ET-3, S6c, and BQ-123 as competitors and are reported in Table 2. Statistical difference from control: **, $p < 0.01$; ***, $p < 0.001$.

TABLE 2

Renal ET receptors in glycerol-induced acute renal failure

Characteristics of competitive inhibition by ET-3, sarafotoxin S6c (S6c), and BQ-123 of ^{125}I-ET-1 binding to ET_A and ET_B receptor subtypes are given.

	K_i		B_{max}				n^a
	ET_A	ET_B	ET_A		ET_B		
	nM		fmol/mg of protein	%	fmol/mg of protein	%	
Renal cortex							
Control							
ET-3	3.21 ± 0.90	0.154 ± 0.048	78 ± 13	52	72 ± 15	48	4
S6c	147 ± 52	0.230 ± 0.084	73 ± 13	53	64 ± 2	47	3
BQ-123	3.12 ± 1.50	20,200 ± 5,300	78 ± 5	56	61 ± 6	44	5
Glycerol-treated							
ET-3	1.94 ± 0.49	0.080 ± 0.041	184 ± 39[b]	55	153 ± 49	45	4
S6c	521 ± 210	2.12 ± 0.64	157 ± 36	47	180 ± 39[b]	53	4
BQ-123	7.43 ± 1.76	27,400 ± 5,900	169 ± 25[b]	53	148 ± 22[b]	49	6
Renal medulla							
Control							
ET-3	7.57 ± 2.58	0.210 ± 0.078	130 ± 28	35	245 ± 45	65	4
S6c	145 ± 47	0.891 ± 0.190	118 ± 29	33	244 ± 19	67	3
BQ-123	5.09 ± 1.86	23,900 ± 7,100	133 ± 11	38	215 ± 15	62	6
Glycerol-treated							
ET-3	10.0 ± 3.9	0.280 ± 0.052	328 ± 75[c]	27	878 ± 66[d]	73	4
S6c	254 ± 117	0.853 ± 0.296	367 ± 49[d]	33	749 ± 33[d]	67	3
BQ-123	5.15 ± 0.88	39,900 ± 8,400	367 ± 37[d]	33	742 ± 70[d]	67	6

[a] *n*, number of experiments.
[b] Statistical difference from control, $p < 0.05$.
[c] $p < 0.01$.
[d] $p < 0.001$.

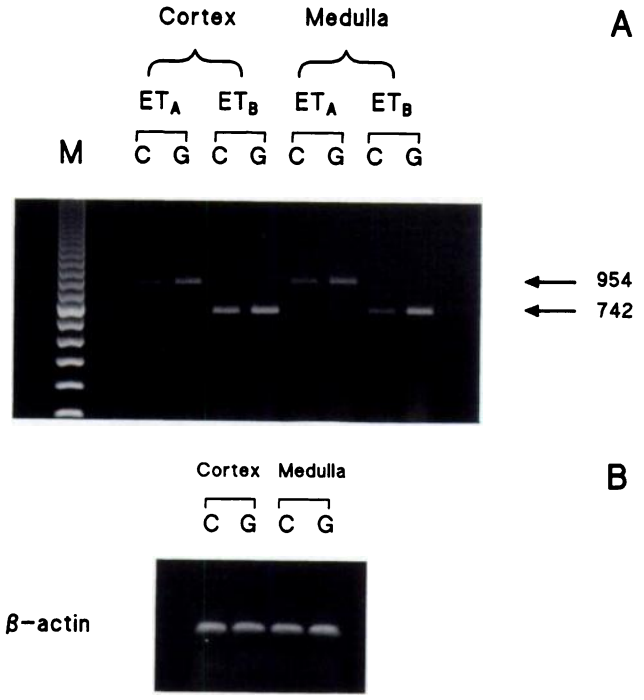

Fig. 4. Up-regulation of renal ET receptor mRNA expression in glycerol-induced acute renal failure. A, RT-PCR was performed on total RNA from control (*C*) and glycerol-treated (*G*) rat kidney, using specific primers for ET_A and ET_B receptors, as described in Materials and Methods. The resulting products were resolved on a 1.5% agarose gel in TBE buffer and visualized by ethidium bromide straining. A 100-base pair ladder (Pharmacia) was used as DNA size markers (*M*). *Arrows*, expected PCR product sizes. B, RT-PCR using specific primers for β-actin is shown as a positive control. Data shown are from a typical experiment, which was replicated three times.

3-fold. Amplification of the same samples with β-actin primers confirmed that equal amounts of RNA were reverse transcribed.

Discussion

The present study demonstrates, using two complementary approaches, i.e., ligand binding studies and RT-PCR assay, the distribution of ET receptor subtypes in rat kidney and their regulation in an experimental model of acute renal failure induced by glycerol administration. The use of three distinct competitors selective for ET_A or ET_B receptors in inhibition binding experiments provides a valuable and reproducible way to determine the densities and the relative proportions of ET_A and ET_B receptors in the kidney. Moreover, the RT-PCR approach allows correlation of these results with ET receptor mRNA steady state levels.

ET_A and ET_B receptors coexist in rat kidney membrane preparations, with, however, significant differences in their densities and their distribution. Renal cortex displays a total ET binding capacity of 155 fmol/mg of protein, which is composed of 54% ET_A and 46% ET_B receptors. These findings agree with another report using subtype-selective ET receptor ligands, suggesting that ET_A and ET_B receptors are present in equal proportions in rat kidney cortex (25). Consistent with the high ET receptor density found in the inner medullary collecting duct of the rat (26), the total number of ET binding sites appears to be significantly higher in renal medulla (386 fmol/mg of protein) than in cortex, with a reversed relative proportion of ET receptor subtypes (35% and 65% ET_A and ET_B receptors, respectively).

The presence of ET_A and ET_B subtypes in rat kidney was confirmed in our study by measurement of the expression of ET receptor mRNA by RT-PCR, using specific probes for ET_A and ET_B. This is in accordance with recent work showing that, for both ET_A and ET_B receptors, mRNA can be detected in rat inner medullary collecting duct cells (26). Moreover, it was

shown on microdissected rat nephron segments that ET_B receptor mRNA is distributed mainly in the collecting ducts and glomeruli, with ET_A receptor mRNA in the vascular system and glomeruli (27). This suggests that the renal actions of ET-1 are mediated by both ET receptor subtypes. Whether ET_A receptors play a predominant role in renal vasculature and ET_B receptors participate in urine formation and concentration remains to be elucidaded.

Accumulating evidence has suggested that ET-1 is an important factor implicated in acute renal failure. ET-1 induces potent and long-lasting renal vasoconstriction and decreases renal blood flow and glomerular filtration rate (9–13). Furthermore, anti-ET-1 antibody (14, 15) as well as BQ-123, a selective ET_A antagonist (28), are able to ameliorate the severity of postischemic renal failure (14, 15). Animal models of experimental acute renal failure are often classified as ischemic models (caused by an interruption of renal blood flow) or nephrotoxic models (caused by various nephrotoxins). In the present study we have used the experimental model of myoglobinuric acute renal failure induced by hypertonic glycerol administration, which produces an interruption of renal blood flow leading to renal ischemia, thus representing an ischemic model (17, 29). Renal function impairment in glycerol-treated rats, as demonstrated in the present study by significant alterations in serum urea levels and creatinine clearance, is associated with a highly significant up-regulation of ET receptors. This represents an original *in vivo* example demonstrating a clear increase in the total number of ET receptors in kidney. The *in vivo* up-regulatory effect of glycerol treatment is significantly more pronounced in renal medulla than in cortex and is more evident for ET_B than ET_A receptors.

Moreover, we correlate for the first time the elevation of renal ET_A and ET_B receptor binding with an increase in mRNA levels. Indeed, in parallel with the rise in the number of ET receptors observed using subtype-selective ligands, an increase of ET_A and ET_B receptor mRNA was clearly observed, although the method does not allow this elevation to be quantitated precisely. These data may suggest that glycerol-induced acute renal failure modifies ET receptor gene expression at the transcriptional level, leading to increased steady state levels of ET_A and ET_B mRNA and thus to an increase of ET receptor density in the kidney.

Increased local production of ET-1 in the kidney (14–16, 28), as well as a modification of ET receptors, may account for or contribute to the appearance of acute renal failure. Interestingly, the alteration of renal function induced by glycerol reproduces some biochemical modifications induced by ET-1. In particular, an increase of proteinuria occured 48 hr after glycerol administration (95.4 ± 12.02 mg/24 hr versus 17.3 ± 1.78 mg/24 hr in control animals), which resembles that observed after administration of ET-1, in contrast to angiotensin II, which does not provoke any elevation of proteinuria (13). Although our study demonstrates a clear elevation of the density of renal ET receptors, particularly ET_B receptors in kidney medulla, in the model of acute renal failure induced by glycerol, other authors have reported different and conflicting results in experimental models of ischemic acute renal failure induced by occlusion of the renal artery in rats. Nambi *et al.* (30) have shown an increase in affinity of ET_A and ET_B receptors in renal cortex, whereas no modification of glomerular ET receptors was observed by Wilkes *et al.* (31). Furthermore, up-regulation of ET receptors in renal medulla of rats treated with cyclosporin has been measured (32), and it was suggested that ET-1 plays an important role in nephrotoxic acute renal failure induced by cyclosporin (33). Taking these observations together, it is probable that an enhanced effect of ET-1 on the kidney engendered by increased density and/or affinity of renal ET receptors participates in renal function impairment and may thus contribute to a pathophysiological role of ET peptides in acute renal failure.

Acknowledgments

We thank Dr. D. Demarquay for helpful advice and discussion.

References

1. Yanagisawa, M., H. Kurihara, S. Kimura, Y. Tomobe, M. Kobayashi, Y. Mitsui, Y. Yazaki, K. Goto, and T. Masaki. A potent vasoconstrictor peptide produced by vascular endothelial cells. *Nature (Lond.)* **332**:411–415 (1988).
2. Inoue, A., M. Yanagisawa, S. Kimura, Y. Kasuya, T. Miyauchi, K. Goto, and T. Masaki. The human endothelin family: three structurally and pharmacologically distinct isopeptides predicted by three separate genes. *Proc. Natl. Acad. Sci. USA* **86**:2863–2867 (1989).
3. Chabrier, P. E., and P. Braquet. Endothelin. *Horm. Res.* **34**:169–174 (1990).
4. 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).
5. 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).
6. Takayanagi, R., K. Ohnaka, C. Takasaki, M. Ohashi, and H. Nawata. Multiple subtypes of endothelin receptors in porcine tissues: characterization by ligand binding, affinity labeling, and regional distribution. *Regul. Peptides* **32**:23–37 (1991).
7. Hori, S., Y. Komatsu, R. Shigemoto, N. Mizuno, and S. Nakanishi. Distinct tissue distribution and cellular localization of two messenger ribonucleic acids encoding different subtypes of rat endothelin receptors. *Endocrinology* **130**:1885–1895 (1992).
8. Kohzuki, M., C. I. Johnston, S. Y. Chai, D. J. Casley, and F. A. O. Mendelsohn. Localization of endothelin receptors in rat kidney. *Eur. J. Pharmacol.* **160**:193–194 (1989).
9. Firth, J. D., P. J. Ratcliffe, A. E. G. Raine, and J. G. G. Ledingham. Endothelin: an important factor in acute renal failure. *Lancet* **2**:1179–1181 (1988).
10. Miller, W. L., M. M. Redfield, and J. C. Burnett, Jr. Integrated cardiac, renal, and endocrine actions of endothelin. *J. Clin. Invest.* **83**:317–320 (1989).
11. Badr, K. F., J. J. Murray, M. D. Breyer, K. Takahashi, T. Inagami, and R. C. Harris. Mesangial cell, glomerular and renal vascular responses to endothelin in the rat kidney. *J. Clin. Invest.* **83**:336–342 (1989).
12. Lopez-Farré, A., I. Montanes, I. Millas, and J. Lopez-Novoa. Effect of endothelin on renal function in rat. *Eur. J. Pharmacol.* **163**:187–189 (1989).
13. Cornet, S., A. Braillon, C. Guilmard, P. E. Chabrier, E. Pirotzky, and P. Braquet. Involvement of endothelin in renal processes. *Hypertension (Dallas)* **15**:724–728 (1990).
14. Kon, V., T. Yoshioka, A. Fogo, and I. Ichikawa. Glomerular actions of endothelin *in vivo*. *J. Clin. Invest.* **83**:1762–1767 (1989).
15. Shibouta, Y., N. Suzuki, A. Shino, H. Matsumoto, Z. I. Terashita, K. Kondo, and K. Nishikawa. Pathophysiological role of endothelin in acute renal failure. *Life Sci.* **46**:1611–1618 (1990).
16. Tomita, K., K. Ujiie, T. Nakanishi, S. Tomura, O. Matsuda, K. Ando, M. Schichiri, Y. Hirata, and F. Marumo. Plasma endothelin levels in patients with acute renal failure. *N. Engl. J. Med.* **324**:1127 (1989).
17. Oken, D. E., M. L. Arce, and D. R. Wilson. Glycerol-induced hemoglobinuric acute renal failure in the rat. I. Micropuncture study of the development of oliguria. *J. Clin. Invest.* **45**:724–735 (1966).
18. Lin, H. Y., E. H. Haji, G. K. Winkel, H. E. Ives, and H. F. Lodish. Cloning and functional expression of a vascular smooth muscle endothelin 1 receptor. *Proc. Natl. Acad. Sci. USA* **88**:3185–3189 (1991).
19. Ihara, M., K. Noguchi, T. Sacki, T. Fukuroda, S. Tsuchida, S. Kimura, T. Fukami, K. Ishikawa, M. Nishikibe, and M. Yano. Biological profiles of highly potent novel endothelin antagonists selective for the ET_A receptor. *Life Sci.* **50**:247–255 (1992).
20. 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).
21. Fabiny, D. L., and G. Ertingshausen. Automated reaction-rate method for determination of serum creatinine with the CentrifiChem. *Clin. Chem.* **17**:696–700 (1971).
22. Talke, H., and G. E. Schubert. Enzymatische Harnstoffbestimmung in Blut und Serum im optischen Test nach Warburg. *Klin. Wochenschr.* **43**:174–175 (1965).
23. Bradford, M. M. A rapid and sensitive method for the quantitation of

microgram quantities of protein utilizing the principle of protein-dye binding. *Anal. Biochem.* **72**:248–254 (1976).
24. Chomczynski, P., and N. Sacchi. Single-step method of RNA isolation by acid guanidinium thiocyanate-phenol-chloroform extraction. *Anal. Biochem.* **162**:156–159 (1987).
25. Nambi, P., H. L. Wu, M. Pullen, N. Aiyar, H. Bryan, and J. Elliott. Identification of endothelin receptor subtypes in rat kidney cortex using subtype-selective ligands. *Mol. Pharmacol.* **42**:336–339 (1992).
26. Kohan, D. E., A. K. Hughes, and S. L. Perkins. Characterization of endothelin receptors in the inner medullary collecting duct of the rat. *J. Biol. Chem.* **267**:12336–12340 (1992).
27. Terada, Y., K. Tomita, H. Nonoguchi, and F. Marumo. 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 (1992).
28. Mino, N., M. Kobayashi, A. Nakajima, H. Amano, K. Shimamoto, K. Ishikawa, K. Watanabe, M. Nishikibe, M. Yano, and F. Ikemoto. Protective effect of a selective endothelin receptor antagonist, BQ-123, in ischemic acute renal failure in rats. *Eur. J. Pharmacol.* **221**:77–83 (1992).
29. Stein, J. H., M. D. Lifschitz, and L. D. Barnes. Current concepts of the pathophysiology of acute renal failure. *Am. J. Physiol.* **234**:F171–F181 (1978).
30. Nambi, P., M. Pullen, M. Jugus, and M. Gellai. Rat kidney endothelin receptors in ischemia-induced acute renal failure. *J. Pharmacol. Exp. Ther.* **264**:345–348 (1993).
31. Wilkes, B. M., A. R. Pearl, P. F. Mento, M. E. Maita, C. M. Macica, and E. P. Girardi. Glomerular endothelin receptors during initiation and maintenance of ischemic acute renal failure in rats. *Am. J. Physiol.* **260**:F110–F118 (1991).
32. 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).
33. Kon, V., M. Sugiura, T. Inagami, B. R. Harvie, I. Ichikawa, and R. L. Hoover. Role of endothelin in cyclosporine-induced glomerular dysfunction. *Kidney Int.* **37**:1487–1491 (1990).

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