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Receptor Subtypes for Endothelin in the Kidney of the Freshwater Turtle (*Amyda japonica*)

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The distribution of receptor subtypes for endothelin (ET) in the kidney of the freshwater turtle, *Amyda japonica*, was examined by quantitative *in vitro* receptor autoradiography using iodinated mammalian type ET-1 (^{125}I-ET-1) as a radiolabeled ligand. Specific ^{125}I-ET-1 bindings were localized to renal tubules, renal arteries and ureter with binding densities of 111.21±19.14, 182.13±10.57 and 219.46±12.83 amol/mm^2, respectively. Binding dissociation constants in renal tubules, renal arteries and ureter were 1.05±0.63, 2.03±0.56 and 1.70±0.47 nM, respectively. Receptor subtypes for ET in the kidney were characterized by competition with BQ 123 and BQ 788 as specific antagonists for ET receptors, type A (ET_A), and type B (ET_B) subtypes, respectively. Specific ^{125}I-ET-1 bindings in renal arteries and ureter were potently inhibited by BQ 123 in a dose-dependent manner, whereas BQ 788 was not in competing for specific ^{125}I-ET-1 bindings in this structure. However, specific ^{125}I-ET-1 bindings in renal tubules were inhibited more potently by BQ 788. Therefore, these results indicate that specific ET receptors are localized in renal tubules, renal arteries and the ureter of the freshwater turtle. Results also suggest that the predominant ET receptor subtypes are like the ET_A receptor in renal arteries and ureter, and like the ET_B receptor in the renal tubule.

Endothelin (ET) was initially identified as a 21-amino acid peptide isolated from the cultured porcine aortic endothelial cells on the basis of vasoconstrictor activity (Yanagisawa et al., 1988). The existence of three isopeptides (ET-1, ET-2, and ET-3) belong to the ET family with structural similarity characterized by analysis of human genomic DNA (Inoue et al., 1989). Recently, the diverse biological actions of ET isopeptides have been extensively studied in various peripheral organs including the vascular and extravascular systems (Luscher et al., 1991; Stojikovic et al., 1992). These diverse actions of ETs have been shown to be mediated through at least two receptor subtypes, ET_A and ET_B receptors (Arai et al., 1990; Sakurai et al., 1992; Sokolovsky, 1995).

In mammals, kidney is a primary target organ for the biological actions of ETs including the modulation of renal blood flow, glomerular filtration and renal tubular transepithelial transport (Miller et al., 1989; Hirata et al., 1989; Goetz et al., 1989; Edwards et al., 1990; Eiam-Ong et al., 1992). In the kidney, messenger RNAs encoding ETs were reported to be expressed in glomerular and tubular epithelial cells (Uchida et al., 1992; Chen et al., 1993), and cultured mesangial and tubule cells also produce ETs (Sakamoto et al., 1990; Kohan, 1991). Furthermore, the presence of abundant ET receptors with high affinity was demonstrated in the kidney membrane fractions using macro- and microreceptor autoradiography. Specific high affinity binding sites for ET in renal structures have been localized to intrarenal arteries, glomeruli, inner medulla, vasa recta bundles, and proximal convoluted tubules (Kohzuki et al., 1989; Koseki et al., 1989; Martin et al., 1989; Orita et al., 1989; Furuya et al., 1992; Dean et al., 1994). Therefore, these findings suggest that ETs may function as an autocrine and/or paracrine system in the kidney in addition to a possible circulating endocrine system.

Previously, the presence of ET-related peptides has been examined in nonmammalian vertebrates and invertebrates (Kasuya et al., 1990, 1991; Hasegawa and Kobayashi, 1991). It was also reported that ET-1 potently constricts blood vessels from fish, amphibian, and reptile, suggesting that ET may be a peptide hormone which was conserved during evolution (Poder and Silberberg, 1991). However, the distribution and properties of ET receptors in nonmammalian species still remain unknown. Recently we have found that specific ^{125}I-ET binding sites with ET_A-like receptor

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subtype were localized to the epididymal smooth muscle layer of the freshwater turtle, *Amyda japonica* (Kim et al., 2000). This finding strongly suggests the presence of an ET receptor subtype for its action in the peripheral tissues of nonmammals. Although the kidney is a primary organ for the biological actions of ETs in mammals, there is no information on the renal functions of ET-like peptides in nonmammalian species.

Therefore, we have performed experiments to elucidate the distribution of receptors for ET, and to characterize the binding properties and receptor subtypes in the kidney of the freshwater turtle, *A. japonica*, by quantitative *in vitro* receptor autoradiography using ^{125}I-ET as a radiolabeled ligand.

Materials and Methods

Animals

Male freshwater turtles (*Amyda japonica*), weighing 500-1,000 g, were obtained from O-Am Freshwater Turtle Farm (Jeonju, Korea). They were allowed to acclimate in tanks for several days with access to aerated and filtered fresh-water (20℃) under a simulated natural photoperiod condition.

Preparation of radiolabeled ET-1

^{125}I-ET-1 was prepared as described previously (Cho et al., 1995; Kim et al., 2000). Since reptilian ET was not available, mammalian ET-1 was used for iodination. In brief, 5 μg of synthetic ET-1 (Bachem) were introduced into the vial containing 25 μl of 0.5 M phosphate buffered saline (pH 7.4) followed by an addition of 0.5 mCi of ^{125}I-Na (Amersham International plc). Chloramine-T (10 μg/10 μl) was added to the reaction vial, mixed gently, and the reaction was terminated 30 sec later by addition of 200 μl of 30% bovine serum albumin (BSA) solution. The reaction mixture was immediately applied to a Sephadex G-25 column (1 × 20 cm) and eluted with 0.1 M acetic acid containing 0.3% BSA, 0.3% lysozyme, and 0.1% glycine. The iodinated ET-1 was divided and stored at -70℃ until used. Immediately before use, ^{125}I-ET-1 was repurified by high performance liquid chromatography on a reversed phase μ Bondapak column (0.4 × 30 cm, Waters Associates) with (20% to 60% acetonitrile) linear gradient elution. The specific activity of ^{125}I-ET-1, measured by the radioimmunoassay (RIA) (Joseph et al., 1988), was approximately 2,000 Ci/mmol. For RIA of ET-1, antibody was obtained from Sigma Co.

In vitro receptor autoradiography

For ET binding sites, the binding conditions of ^{125}I-ET-1 to the kidney sections of the freshwater turtle were prepared according to the methods described previously for ET (Kim et al., 2000) and natriuretic peptide binding (Kim et al., 1997, 1998). The turtles were killed by decapitation and rapid exsanguination. The kidney was quickly removed, immediately snap frozen in liquid nitrogen, and stored in sealed boxes at -70℃ until sectioned. Serial 20 μm sections were cut on a cryostat at -20℃, thaw-mounted onto gelatin-chrome-alum coated slides, and dried in a desiccator at 4℃ overnight. The sections were preincubated with 30 mM phosphate buffer (pH 7.2) containing 100 mM NaCl and 1 mM phenanthroline at room temperature for 10 min. They were then incubated with 250 pM ^{125}I-ET-1 in fresh preincubation buffer containing 40 μg/ml bacitracin, 100 μg/ml phenylmethylsulfonyl fluoride, and 0.5% BSA at room temperature for 60 min. To determine nonspecific binding, 1 μM unlabeled ET-1 was added into the incubation buffer. To characterize ET receptor subtypes, the competitive inhibition of ^{125}I-ET-1 binding to the kidney was examined in consecutive sections from six freshwater turtles by coincubation with various concentrations of unlabeled ET-1, ET-A antagonist BQ 123, [Cyclo (D-α-aspartyl-L-prolyl-D-valyl-L-leucyl-D-tryptophyl)] or ET-B antagonist BQ 788, {N-[N-[N-[(2,6-Dimethyl-1-piperidinyl)carbonyl]-4-methyl-L-leucyl]-1-(methoxycarbonyl)-D-tryptophyl]-D-norleucine monosodium} (RBI).

After incubation with ^{125}I-ET-1, the sections were rinsed and washed with fresh preincubation buffer for 5 min at 4℃. Subsequently, they were rinsed three times in cold distilled water at 4℃ and quickly dried under a stream of cold air.

Quantitative analysis of autoradiograms

Autoradiographic images were generated by exposing the slides with dried ^{125}I-ET-1 labeled kidney sections to Hyperfilm-^{3}H (Amersham International) at room temperature for 7 d. Autoradiograms were developed in a Kodak D-19 developer (Eastman Kodak Co.) for 3 min and fixed in Kodak rapid fixer for 5 min at room temperature. After exposure, sections were fixed in formaldehyde and then counterstained with hematoxylin and eosin for precise tissue localization (Kim et al., 1999, 2000).

Autoradiographic images were viewed with a Leica Wild M420 Macroscope, and captured using a Sony video camera with a charge-coupled device iris and a Hamamatsu AC adaptor connected to a Power Macintosh 8100/80AV computer. Regional bindings of ^{125}I-ET-1 in the kidney were analyzed for a mean grayscale value using the PRISM image program (Version 3.6-1, Improve Vision). Optical densities were measured as disintegrations per minute (dpm) per square millimeter, based on the comparison with a calibration curve from the autoradiograms of a ^{125}I standard microscale included in each X-ray cassette. These data were converted into molar concentrations of ^{125}I-ET-1 per square millimeter, as described elsewhere (Benfenati et al., 1986).

Analysis of binding data

The number of ligand binding sites of different affinities

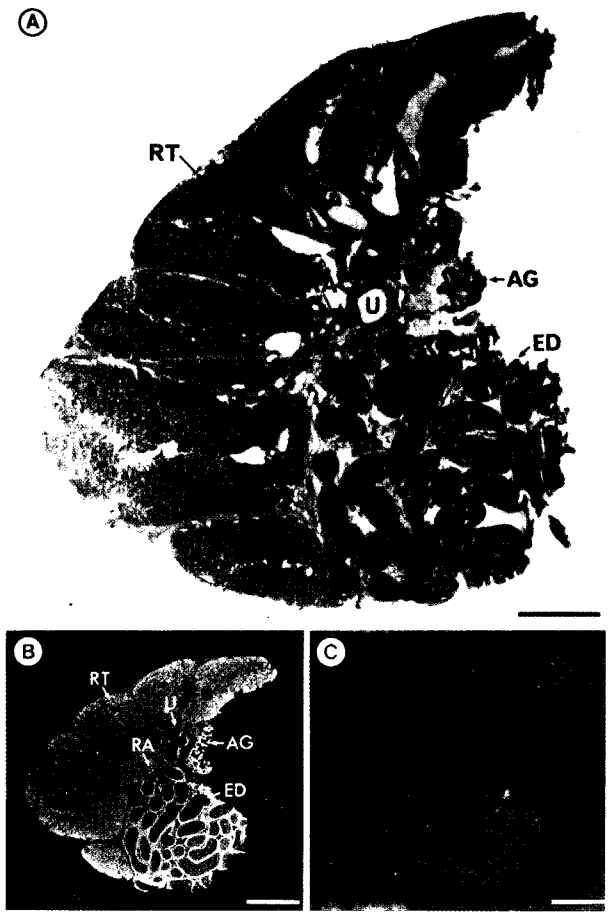

Fig. 1. Comparison of a hematoxylin-eosin stained transverse section of the kidney and adjacent structures (AG, adrenal gland; ED, epididymis) of the freshwater turtle, *Amyda japonica* (A) and a dark-field photomicrograph of autoradiogram for ^{125}I-ET-1 bindings to these structures. Tissue section was incubated with 250 pM ^{125}I-ET-1 alone (B), and its adjacent section was incubated with 250 pM ^{125}I-ET-1 plus 1 M unlabeled ET-1 to define nonspecific bindings (C). Specific ^{125}I-ET-1 binding sites in the freshwater turtle kidney appear as white silver grains in renal tubules (RT), renal arteries (RA) and ureter (U). Scale bars=2 mm (A) and 3 mm (B, C).

for a given radioligand, its apparent dissociation constants (K_d) and its maximal binding capacities (B_{max}) on particular structures were derived separately in each individual by Scatchard analysis using the LIGAND iterative model-fitting computer program (Munson and Rodbard, 1980). The inhibitory constant (K_i) and B_{max} for the binding of a non-homologous unlabeled ligand that inhibited radioligand binding were also determined separately for each individual by LIGAND. Comparison of the results were performed by paired Student's *t*-tests and ANOVA with a Duncan multiple range test, accepting $P<0.05$ as the criterion of significance.

Results

Comparison of the autoradiographic films with their adjacent hematoxylin-eosin stained section (Fig. 1A) of the freshwater turtle kidney revealed renal tubules,

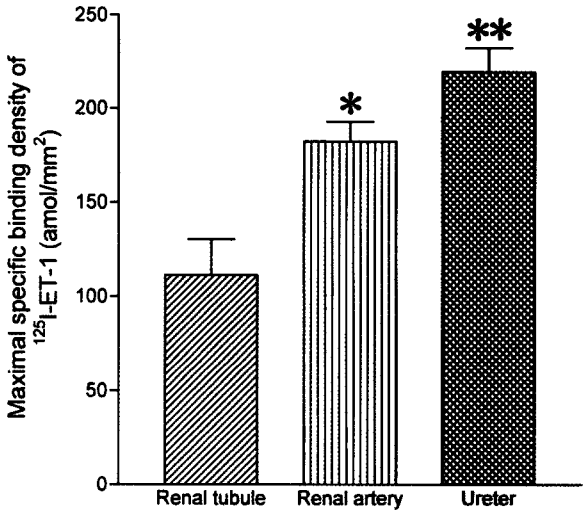

Fig. 2. Comparison of specific binding densities of ^{125}I-ET-1 in the renal tubule, renal arteries and ureter of the freshwater turtle, *Amyda japonica*. *P<0.05 and **P<0.01 for the renal tubule vs the renal artery or ureter.

renal arteries, and ureter bound ^{125}I-ET-1 (Fig. 1B). In the presence of 1 μM unlabeled ET-1, the bindings to these structures were completely displaced, but the diffuse background bindings were not significantly affected (Fig. 1C). Specific bindings of ^{125}I-ET-1 to renal tubules, renal arteries, and ureter were 87.28±1.53, 94.04±0.94, and 95.33±0.65% of total bindings, respectively, and were not altered by unrelated peptides, angiotensin II and arginine vasopressin (all 10 μM, data not shown). As shown in Fig. 2, the specific binding densities of renal tubules, renal arteries, and ureter were 111.21±19.14, 182.13±10.57, and 219.46±12.83 amol/mm^2 (n=6), respectively. Two hundred and fifty picomolar concentration of ^{125}I-ET-1 bound specifically to renal structures of the freshwater turtle, and reached equilibrium at approximately 60 min in room temperature (data not shown). In subsequent experiments, 60 min incubation time was used.

As shown in Fig. 3A, specific ^{125}I-ET-1 binding sites were found in the renal tubules of the freshwater turtle kidney. However, dense bindings for ET in the glomeruli were not observed as shown in previous investigations for specific bindings of atrial natriuretic peptide and angiotensin II (Kim et al., 1996, 1997). The displacement of ^{125}I-ET-1 binding to renal tubules by selective ET_A subtype antagonist BQ 123 and ET_B subtype antagonist BQ 788 was also investigated. BQ 123 and BQ 788 (all 1 μM) inhibited 27.67± 9.94 and 51.34±4.48% of specific ^{125}I-ET-1 bindings to renal tubules, respectively (Fig. 4). From analysis of the competitive inhibition of the binding of ^{125}I-ET-1 by unlabeled ET-1 on renal tubules, K_d and B_{max} were 1.05±0.63 nM and 1.32±0.55 fmol/mm^2, respectively. Increasing concentrations of BQ 788 also progressively inhibited the bindings of ^{125}I-ET-1 to these structures with K_i of 445.69±96.47 nM and B_{max} of 2.85±1.03 fmol/mm^2 (Table 1). Therefore, BQ 788 competed with

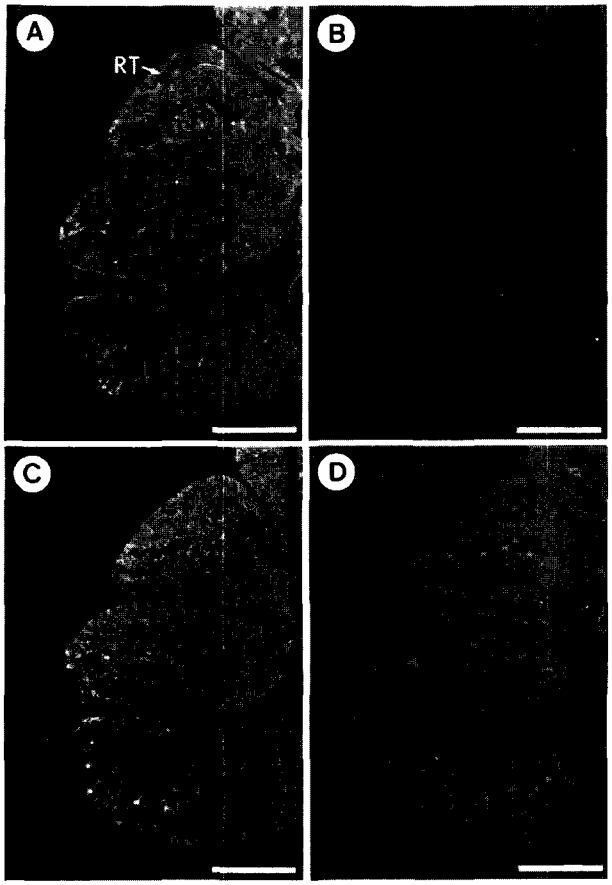

Fig. 3. Dark-field photomicrographs of autoradiograms of ^{125}I-ET-1 bindings to renal tubules (RT) of the freshwater turtle, *Amyda japonica*. Tissue sections were incubated with 250 pM ^{125}I-ET-1 alone (A), and their adjacent sections were incubated with 250 pM ^{125}I-ET-1 plus 1 μM unlabeled ET-1 to define nonspecific bindings (B), or 1 M BQ 123, a specific ET_A subtype antagonist (C), or 1 μM BQ 788, a specific ET_B subtype antagonist (D). Bindings of ^{125}I-ET-1 were shown by white silver grains on a dark background. Scale bars=2 mm.

much less affinity than ET-1 for specific ^{125}I-ET-1 bindings on renal tubules ($P<0.001$).

Specific ^{125}I-ET-1 binding sites were also found in renal arteries and the ureter of the freshwater turtle kidney (Fig. 5). BQ 123 (1 μM) inhibited by 96.75±1.14 and 77.20±3.14% of specific ^{125}I-ET-1 bindings to renal arteries and ureter, respectively, whereas BQ 788 (1 μM) had very little inhibitory effect with <15% of specific ^{125}I-ET-1 bindings to these tissues (Fig. 6). Competitive inhibition of the binding of ^{125}I-ET-1 by unlabeled ET-1 on renal arteries and ureter was consistent with reversible binding sites for ET-1 of uniform affinity on each structure. The K_d and B_{max} of these sites were 2.03±0.56 nM and 3.96±0.79 fmol/mm^2 on renal arteries, respectively, and 1.70±0.47 nM and 4.02±1.14 fmol/mm^2 on ureter, respectively. Increasing concentrations of BQ 123 also progressively inhibited the bindings of ^{125}I-ET-1 to these structures. This inhibition in renal arteries and ureter was consistent with sites of a single affinity for BQ 123 characterized by K_i of 132.13±25.74 and 181.50±40.63 nM, respectively,

Table 1. Mean values of binding constants for specifically reversible bindings of ^{125}I-ET-1 by unlabeled ligands in the kidney of the freshwater turtle, *Amyda japonica*

Tissue	Ligand	K_d (nM)	K_i (nM)	B_{max} (fmol/mm^2)
Renal tubule	ET-1	1.05 ± 0.63		1.32 ± 0.55
	BQ 123		NC	NC
	BQ 788		445.69 ± 96.47$^{\#\#}$	2.85 ± 1.03
Renal artery	ET-1	2.03 ± 0.56		3.96 ± 0.79*
	BQ 123		132.13 ± 25.74$^{\#\#}$	2.55 ± 0.89
	BQ 788		NC	NC
Ureter	ET-1	1.70 ± 0.47		4.02 ± 1.14*
	BQ 123		181.50 ± 40.63$^{\#\#}$	2.08 ± 1.11
	BQ 788		NC	NC

Values are means ± S.E. (n=6 turtles). Apparent dissociation constants (K_d), inhibitory constants (K_i) and maximum binding capacities (B_{max}) were assessed from competitive inhibition of 250 pM ^{125}I-ET-1 binding by various concentrations of unlabeled ET-1, BQ 123 or BQ 788. *$P<0.05$ for comparison of B_{max} values of renal tubule vs. the renal artery or ureter. $^{\#\#}P<0.001$ for comparison of binding affinities of ET-1 vs. BQ 123 or BQ 788 in each tissue.

and B_{max} of 2.55±0.89 and 2.08±1.11 fmol/mm^2, respectively (Table 1). BQ 123 competed with much less affinity than ET-1 for the binding sites of ^{125}I-ET-1 on these renal structures ($P<0.001$).

Discussion

The present study shows for the first time the localization of the specific receptor for ET, and its receptor subtypes in the kidney of the freshwater turtle, *Amyda japonica*.

The autoradiograms of the kidney sections indicated that specific ^{125}I-ET-1 bindings were localized to renal tubules, renal arteries and the ureter (Fig. 1). Through analysis of the competitive inhibition of the binding of ^{125}I-ET-1 by unlabeled ET-1 on these renal structures

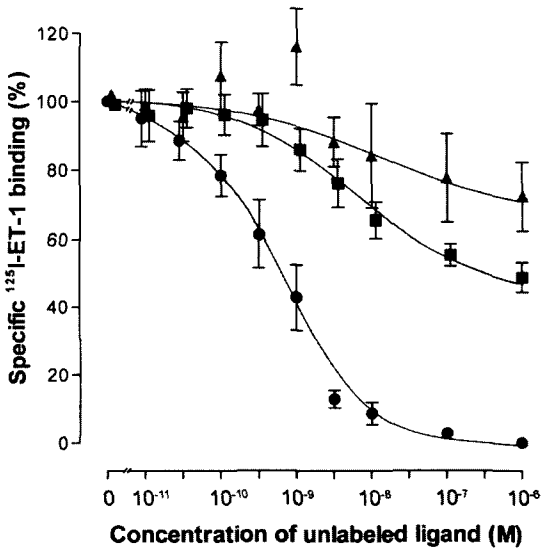

Fig. 4. Competitive inhibition curves of specific 250 pM ^{125}I-ET-1 bindings to renal tubules of the freshwater turtle, *Amyda japonica*. Mean values from 6 individuals were plotted for competitive binding of 250 pM ^{125}I-ET-1 by increasing concentrations of unlabeled ET-1 (●), BQ 123 (▲) or BQ 788 (■).

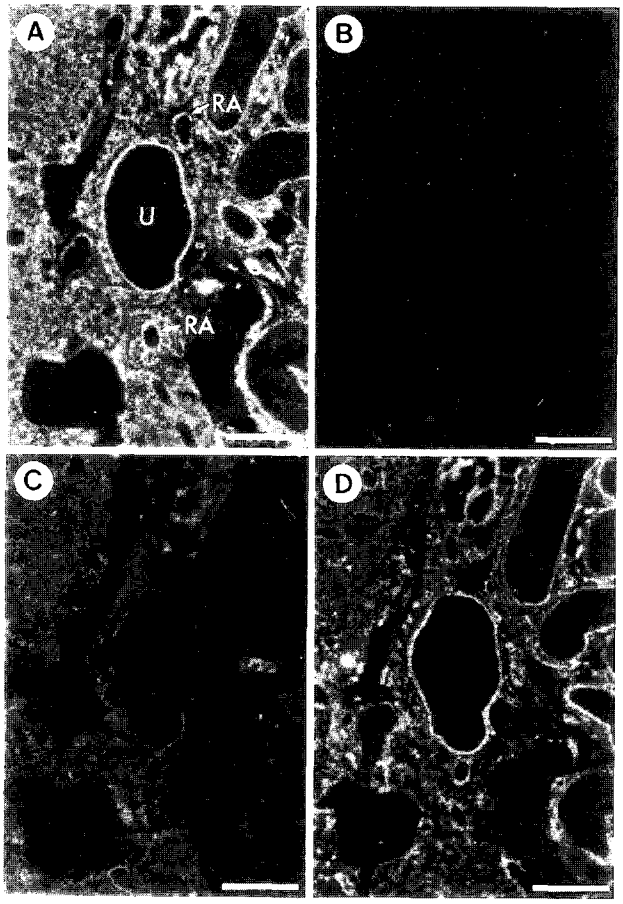

Fig. 5. Dark-field photomicrographs of autoradiograms of ^{125}I-ET-1 bindings to renal arteries (RA) and ureter (U) of the freshwater turtle, *Amyda japonica*. Tissue sections were incubated with 250 pM ^{125}I-ET-1 alone (A), and their adjacent sections were incubated with 250 pM ^{125}I-ET-1 plus 1 μM unlabeled ET-1 to define nonspecific bindings (B), or 1 μM BQ 123 (C), or 1 μM BQ 788 (D). Bindings of ^{125}I-ET-1 were shown by white silver grains on a dark background. Scale bars=1 mm.

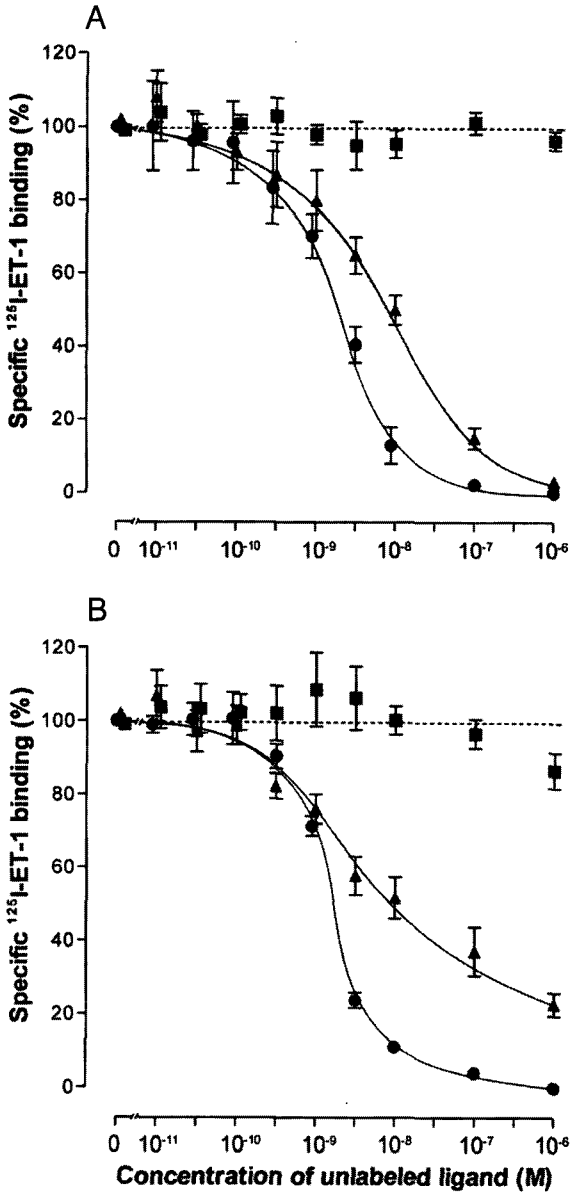

Fig. 6. Competitive inhibition curves of specific 250 pM ^{125}I-ET-1 bindings to renal arteries (A) and ureter (B) of the freshwater turtle, *Amyda japonica*. Mean values from 6 individuals were plotted for competitive of binding of 250 pM ^{125}I-ET-1 by increasing concentrations of unlabeled ET-1 (●), BQ 123 (▲) or BQ 788 (■).

of the freshwater turtle, the displacement curve was characterized to be the specific receptor that has a single binding site with high affinity. As shown in Fig. 2, however, a difference in the specific binding densities of ^{125}I-ET-1 on these renal structures were noted. It seems that this difference in binding densities was derived from the difference in receptor populations because the binding affinities among these renal structures were very similar, whereas B_{max} values were different as shown in Table 1.

Previously, two distinct ET receptor subtypes for ET isopeptides in mammalian species have been classified as ET_A and ET_B, which were G-protein-coupled membrane receptors, and exhibited different binding affinities for ET isopeptides (Arai et al., 1990; Sakurai et al., 1992; Sokolovsky, 1995). The ET_A receptor subtype exhibits high affinity for ET-1 and ET-2 than for ET-3 (Arai et al., 1990), whereas ET_B binds to three ET isopeptides with equal affinity (Sakurai et al., 1990). Functionally, the ET_A receptor subtype mediates most of the vasoconstriction induced by ET-1 (Ihara et al., 1992). The ET_B receptor subtypes have been subclassified as ET_{B1} and ET_{B2}, which mediates endothelium-dependent vasodilation (Takayanagi et al., 1991) and vasoconstriction (Clozel et al., 1992; Moreland et al., 1992), respectively. Additionally, the existence of a third type of ET receptor, designated ET_C, were confirmed by the isolation of a cDNA clone from *Xenopus laevis* (Karne et al., 1993), although the presence of the ET_C subtype in mammalian species remains unclear. The recent development of ET receptor antagonists has been employed to distinguish ET receptor subtypes in diverse tissues of mammals: BQ 123 and BQ

788 which are selective for ET_A and ET_B receptor subtypes, respectively (Ihara et al., 1991; Karaki et al., 1994).

In renal tubules of the freshwater turtle, ^{125}I-ET-1 binding was specifically inhibited by BQ 788, whereas BQ 123 was very weak in competing for the specific binding of ^{125}I-ET-1 in this structure (Figs. 3 and 4). These results clearly demonstrate that the majority of ET receptors localized in renal tubules belongs to the ET_B-like subtype rather than ET_A-like subtype. In the mammalian kidney, specific binding sites for ET were localized in the epithelial cells of various renal tubules including proximal convoluted tubules, the limbs of the loop of Henle, and medullary collecting ducts (Martin et al., 1990; Jones et al., 1991; Cernacek et al., 1992; Hori et al., 1992). Membrane binding studies with selective ET_A and ET_B receptor ligands have demonstrated that a main population of the ET-1 binding sites in the inner medullary collecting duct of the rat kidney were of the ET_B subtype (Edwards et al., 1993; Uchida et al., 1993). Moreover, Edwards et al. (1993) reported that the selective ET_B receptor agonist sarafotoxin 6c inhibited arginine vasopressin (AVP)-induced increases of osmotic water permeability and cyclic adenosine monophosphate (cAMP) accumulation in the inner medullary collecting duct of the rat kidney. However, BQ 123 had no effects on ET-1-induced inhibition of AVP-dependent osmotic water permeability and cAMP formation. On the basis of these findings, our results suggest that the ET-like peptide in the freshwater turtle can modulate renal tubular function *via* the putative ET_B-like subtype.

On the other hand, BQ 123 specifically inhibited ^{125}I-ET-1 binding to renal arteries and ureter of the freshwater turtle, whereas BQ 788 was very weak in competing for specific binding of ^{125}I-ET-1 in these structures (Figs. 5 and 6). The present autoradiographic results clearly demonstrate that the majority of ET receptors localized in renal arteries and ureter belongs to the ET_A-like subtype. These properties were similar to those found in previous results from ^{125}I-ET-1 bindings to the epididymal smooth muscle layer of the freshwater turtle, suggesting that the ET-like peptide may be involved in the modulation of smooth muscle tone *via* ET_A-like subtype for transport of sperm (Kim et al., 2000). Although ET_{B2} subclassified subtype of ET_B can mediate vasoconstrictor response (Clozel et al., 1992; Moreland et al., 1992), it was initially thought that ET_A receptors mediate the vasoconstrictor effects of the ET in the mammalian vasculature, while ET_B receptors mediate their vasodilator effects. Previously, Maguire et al. (1994) reported that messenger RNA encoding both ET_A and ET_B receptors was identified in the smooth muscle layer of the human renal artery and vein by reverse transcriptase-polymerase chain reaction. However, autoradiographic studies indicated that the majority of ET receptors expressed are of the ET_A subtype in the human and rat renal arteries (Maguire et al., 1994; Devadason and Henry, 1997). Functionally, ET caused concentration-dependent increases in renal arterial contractions, and these response were displaced in a parallel rightward fashion by BQ 123 (Maguire et al., 1994; Clark and Pierre, 1995; Devadason and Henry, 1997). These results indicated that the ET_A receptor mediated the contractile effects of ET in renal arteries of mammals. Considering that ET-1 potently contracts blood vessels of the nonmammalian species including the reptile (Poder and Silberberg, 1991), the present results suggest that the ET-like peptide may induce the vasoconstriction *via* the putative ET_A-like subtype in the freshwater turtle kidney.

In addition to the effects on blood vessels in mammals, ETs caused contractile responses in various non-vascular organs with smooth muscles (Ninomiya et al., 1992; Sakata and Karaki, 1992). In particular, the presence of specific receptors for ET were found in the urinary tract by autoradiographic and radioligand binding studies (Garcia-Pascual et al., 1990; Eguchi et al., 1991). ET-1 increased in a dose-dependent manner the contractile activities of the smooth muscle in the urinary tracts, and these activities were dose-dependently blunted by the ET_A-selective antagonist, FR 139317 (Donoso et al., 1994; Okamoto-Koizumi et al., 1999). These findings indicate that the ET-1-induced contractile effects of urinary tract smooth muscle seem to be mediated mainly by the ET_A receptor, not by the ET_B receptor. Therefore, our findings suggest that the putative ET_A-like subtype for ET-like peptides in the ureter of the freshwater turtle may exert a contractile effect on the ureteral smooth muscle fiber for urine transport.

In conclusion, we have provided autoradiographic evidence for the localization of specific ET receptors with high affinities in the renal tubules, renal arteries, and ureter of the freshwater turtle, *A. japonica*. Our results also indicate that the ET_A-like receptor is the predominant subtype for ET-like peptides in renal arteries and ureter, whereas the ET_B-like receptor is the predominant subtype in renal tubules. Considering the fact that ET_A- and ET_B-receptor subtypes in mammals are well correlated with their own physiological functions, these findings suggest possible functional implications of ET-like peptides *via* ET-like receptor subtypes in the freshwater turtle kidney.

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