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Binding and Functional Properties of Endothelin Receptor Subtypes in the Human Prostate

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SUMMARY

The objective of the present study was to characterize the binding and functional properties of endothelin (ET) receptor subtypes in the human prostate. Human prostatic tissue was obtained from male subjects undergoing radical prostatectomy for low-volume prostate cancer. The optimal assay conditions for characterizing human prostatic ET-1 binding sites on slide-mounted tissue sections were defined. Maximal specific ^{125}I-ET-1 binding was achieved after a 10-min preincubation, a 120-min incubation, and a washing procedure that consisted of a brief rinse and a 1-min wash. The mean equilibrium dissociation constant (K_d) and density (B_{max}) of ET-1 binding sites determined from six saturation studies were 0.72 ± 0.13 nM and 40.4 ± 6.9 fmol/mg of wet weight, respectively. The mean Hill coefficient was 0.99 ± 0.01, indicating that ^{125}I-ET-1 identifies a single population of binding sites. The pharmacology of ^{125}I-ET-1 binding sites was characterized using competitive binding experiments. The competition plots for ET-1 were best fit by a one-binding site model, whereas the plots for sarafotoxin 6C (S6C) and BQ123 were consistently best fit by a two-site model. The mean K_i value of ET-1 was 0.34 ± 0.12 nM. The mean K_i values for the high and low affinity S6C binding sites were 0.50 ± 0.09 nM and 0.84 ± 0.28 μM, respectively. The mean K_i values for the high and low affinity BQ123 binding sites were 5.51 ± 1.05 nM and 24.9 ± 6.5 μM, respectively. The ratio of ET_A to ET_B binding sites was approximately 2:1. The ET receptor subtype mediating prostatic smooth muscle tension was investigated using agonist-antagonist competition studies. ET-1, a nonselective ET agonist, elicited a potent contraction of prostate smooth muscle. The pA_2 of BQ123 for inhibiting ET-1-mediated contraction was 6.84. S6C, a selective ET_B agonist, also elicited a potent contraction of prostate smooth muscle. BQ123 at concentrations between 0.1 and 10 μM did not shift the S6C dose-response curve. These functional studies suggest that both ET_A and ET_B receptors mediate the tension of prostate smooth muscle. Endogenous ETs may be involved in the pathophysiology of bladder outlet obstruction in men with benign prostatic hyperplasia. If this is the case, then ET antagonists may represent effective treatment for benign prostatic hyperplasia.

ET, a potent vasoconstrictive peptide, comprises 21 amino acid residues and was originally isolated from cultured porcine vascular endothelial cells (1). Yanagisawa and co-workers (2, 3) subsequently demonstrated the existence of three genes for ET. These genes encode three very structurally similar 21-amino acid residue peptides (ET-1, ET-2, and ET-3). In addition to its potent vasoconstrictor properties, ET has been reported to possess diverse biological activities in a wide range of tissues, including cardiac (4), bronchial (5), renal (6), neural (7), gastrointestinal (8), and genitourinary (9, 10) tissues. The physiological and pharmacological properties of ETs are currently under investigation. We have recently reported the presence of ET-1 in the human prostate, using a radioimmunoassay and immunohistochemistry (11). Although ET-1 immunoreactivity was localized exclusively to the epithelial component of the prostate, ET-1 elicited a potent contractile response in human prostate. The role of ETs in normal and pathological prostatic function has not been investigated.

High affinity cell surface ET-1 receptors have recently been characterized in a variety of tissues by using the ligand ^{125}I-ET-1 (12–16). At least two cDNA clones for ET receptors have recently been isolated from cDNA libraries (17, 18). The ET receptors were classified based on different molecular weights and binding affinities for ET-3. The existence of two ET binding sites ET_A and ET_B has been demonstrated using sodium dodecyl sulfate-polyacrylamide gel electrophoresis and competitive binding studies (12, 14–16, 19–21). Recently, subtypes of the ET receptor have been further characterized based upon the different binding affinities of S6C and the cyclic pentopeptide BQ123 for ^{125}I-ET-1 binding sites (14, 16, 22). ET_A receptors are characterized by a relatively higher affinity for BQ123 and lower affinity for S6C, whereas ET_B receptors are characterized by relatively higher affinity for S6C and lower

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ABBREVIATIONS: ET, endothelin; S6C, sarafotoxin 6C; BPH, benign prostatic hyperplasia.

affinity for BQ123. ET receptors have not been previously characterized in the human prostate. The present study was designed to establish the optimal conditions for characterizing human prostatic ET-1 binding sites. Saturation binding studies were performed directly on 20-μm-thick slide-mounted human prostatic tissue sections. The relative proportions of ET_A and ET_B receptor subtypes were determined from ^{125}I-ET-1 competitive binding studies utilizing ET-1, S6C, and BQ123. The ET receptor subtypes mediating the contraction of prostate smooth muscle were characterized using agonist-antagonist competition experiments.

Materials and Methods

Tissue Specimens

Human prostatic tissues were obtained from male subjects with low-volume prostate cancer who were undergoing radical prostatectomies. The whole surgical specimens were transferred immediately to the surgical pathology laboratory. Prostatic tissues for the binding studies were excised from the benign-appearing elements of the transition zone and were stored at −80°. The prostate tissues were embedded in Tissue Tek and 20-μm-thick tissue sections were cut using a cryostat set at −20°. The slide-mounted tissue sections were stored at −80° until the binding assays were performed. The weight of a single tissue section was estimated by the following equation: (cross-sectional area) × (thickness) × (tissue density). Cross-sectional area of the tissue was determined using a computed image-analyzing system (MCID Image Analyzer; Imaging Research, St. Catherine, Ontario, Canada). Random tissue sections were stained with hematoxylin and eosin and inspected to ensure that the selected specimens did not contain prostatic carcinoma. The tissues for the isometric tension studies were placed in iced Krebs solution (130 mM NaCl, 15 mM $NaHCO_3$, 5 mM KCl, 1.2 mM NaH_2PO_4, 1.2 mM $MgSO_4$, 2.5 mM $CaCl_2$, 11.4 mM dextrose) before experimentation. The tissues were cut into strips with cross-sectional areas varying between 11 and 16 mm^2.

Assay Conditions

Preincubation time. The requirement for preincubation is ligand dependent (23). To determine the requirement for preincubation, total and nonspecific ^{125}I-ET-1 binding was determined at several preincubation intervals. The tissue sections were dried in room air for 30 min. The assays were performed in triplicate. Tissue sections were preincubated in Tris buffer (50 mM Tris·HCl, 10 mM $MgCl_2$, pH 7.4) for 0, 5, 10, 20, or 40 min. The tissue sections were immersed in the incubating solution by pipetting 70 μl of the appropriate stock solution directly over the tissue section. Stock solutions were made in Tris buffer containing 0.5% bovine serum albumin. Total binding was determined by immersing the prostatic tissue sections in the stock solution of 0.3 nM ^{125}I-ET-1, and nonspecific binding was determined in parallel experiments using the stock solution containing 0.3 nM ^{125}I-ET-1 and 1 μM ET-1. Tissue sections were incubated for 60 min at room temperature. Immediately after incubation, the tissue sections were rinsed and then washed once in buffer for 1 min. The tissue sections were then wiped off the slides using Q-tip swabs and the dpm values of the Q-tip swabs were counted with a γ counter.

Incubation time. The optimal incubation interval was determined by measuring total and nonspecific ^{125}I-ET-1 binding at varying incubation intervals between 10 and 180 min. Total binding and nonspecific binding were determined at 0.3 nM ^{125}I-ET-1.

Washing time. The optimal washing time was determined by measuring total and nonspecific ^{125}I-ET-1 binding at varying washing intervals. The washing procedure consisted of a brief rinse, a 1-min wash, and varying intervals of a second wash in the buffer at room temperature.

Saturation Studies

Saturation studies were performed at six different concentrations (0.0625–2.0 nM) of ^{125}I-ET-1 (constant specific activity, 1800–2000 Ci/mmol). Nonspecific binding was performed in parallel assays in the presence of a final concentration of 1 μM nonradioactive ET-1. Total and nonspecific ^{125}I-ET-1 binding was determined in triplicate for each ^{125}I-ET-1 concentration. The tissue slides were preincubated in Tris buffer for 10 min. Incubation was performed by immersing the slide-mounted tissue sections in 70 μl of solution for 120 min at room temperature. Total binding and nonspecific binding were determined as described previously for the preincubation studies. Immediately after the incubation, the tissue sections were briefly rinsed and washed once in the buffer for 1 min. The tissue sections were removed from the slides with Q-tip swabs and the dpm values were measured as described above.

Competition Binding Studies

Competitive binding experiments were performed on the slide-mounted tissue sections in the presence of a constant final concentration of ^{125}I-ET-1 (0.1 nM) and varying concentrations of unlabeled ET-1 (10^{-12} to 10^{-6} M), S6C (10^{-11} to 10^{-5} M), and BQ123 (10^{-11} to 10^{-5} M). The assays were performed in triplicate for each concentration of the unlabeled ligand. Four separate experiments were performed for each ligand. The assays were terminated as described previously. Nonspecific binding represented ^{125}I-ET-1 bound in the presence of 1 μM nonradioactive ET-1. Specific binding was determined by subtracting the nonspecific binding component from total ^{125}I-ET-1 binding at the various concentrations of ET-1, S6C, and BQ123. Maximum binding was determined in the presence of ^{125}I-ET-1 only. The best two-site fit for a binding curve was calculated by minimizing the sum of the squares of the errors, using nonlinear regression analysis (24). Two-site models were compared with one-site models by using a partial F test to determine whether inclusion of additional parameters in a model significantly increased the "goodness of fit" for the model more than would be expected on the basis of chance alone; p values of <0.05 were considered significant and indicated that the more complicated model (additional parameters) was the better choice.

Isometric Tension Studies

Human prostatic tissue strips were suspended in 5-ml tissue chambers at 2 g resting tension (as determined by prior length tension studies). The tissue chambers contained Krebs buffer aerated with 95% O_2/5% CO_2 and thermoregulated to 37°. The tension was measured with Grass FT03C force displacement transducers and recorded with a computer-based oscillograph and data acquisition system (CODAS; DATAQ Instruments, Inc., Akron, OH).

After a 1-hr resting tension equilibration, the tissues were challenged with 150 mM KCl and washed until baseline values were obtained. Prostatic tissue strips were then exposed to cumulative concentrations of either ET-1 (10^{-10} to 10^{-6} M) or S6C (10^{-10} to 10^{-6} M). ET-1 and S6C dose-response experiments were performed in the presence of four different concentrations of the antagonist BQ123 (10^{-4} to 10^{-7} M). The pA_2 values were calculated from the Schild plots.

Statistical Analysis

The saturation studies were analyzed using the EBDA computer program. The competition displacement studies were analyzed with the ReceptorFit Competition computer program.

Materials

^{125}I-ET-1 (1800–2000 Ci/mmol) was obtained from Amersham (Arlington Heights, IL). ET-1, ET-2, S6C, and BQ123 were obtained from American Peptide (Sunnyvale, CA).

Results

Assay conditions. The optimal preincubation interval was determined from two separate experiments. Specific binding

was determined for 0.3 nM ^{125}I-ET-1 after preincubation intervals ranging between 0 and 40 min. These preliminary experiments demonstrated that maximal specific ET-1 binding was achieved after a 10-min preincubation (data not shown).

The optimal incubation interval was also determined from two separate experiments. Specific binding was determined with 0.3 nM ^{125}I-ET-1 after incubation intervals ranging between 10 and 180 min. Specific binding reached a plateau at 120 min (Fig. 1).

The optimal washing time was also determined from two separate experiments. Nonspecific binding was reduced remarkably by a brief rinse and a 1-min wash. Total binding and nonspecific binding were not significantly reduced by longer washing intervals (data not shown). A 1-min wash after a brief rinse was considered optimal because the ratio of specific binding to nonspecific binding was maximal under these washing conditions.

Saturation studies. Saturation studies were performed on slide-mounted tissue sections derived from the transition zone of radical prostatectomy specimens uninvolved in the malignant process. Specific ^{125}I-ET-1 binding was determined at six different concentrations of ^{125}I-ET-1, ranging from 0.0625 nM to 2.0 nM. Representative saturation and Scatchard plots are shown in Fig. 2. The binding of ^{125}I-ET-1 was consistently saturable and of high affinity. The equilibrium dissociation constants, receptor densities (B_{max}), and Hill coefficients were determined from Scatchard analyses of the saturation experiments. The mean (± standard error) values of K_d, B_{max}, and Hill coefficient determined from six saturation studies are summarized in Table 1. The mean Hill coefficient was approximately unity, indicating that ^{125}I-ET-1 identifies a single population of binding sites.

Competition binding experiments. The pharmacology of prostatic ^{125}I-ET-1 binding sites was characterized using competitive binding experiments. All of the individual studies were performed at a constant ^{125}I-ET-1 concentration, ranging from 0.09 nM to 0.14 nM, and varying concentrations of unlabeled ET-1, S6C, and BQ123. A total of four individual competitive displacement experiments were performed using ET-1, S6C, and BQ123. The composite competitive binding plots are shown in Fig. 3. The corrected IC_{50} (K_i) was determined by analyzing the individual competition experiments using the ReceptorFit Competition computer program. The competition plots for ET-1 were consistently best fit by a one-binding site model. The K_i value of ET-1 was 0.34 ± 0.12 nM. The individual displacement plots for S6C and BQ123 were consistently best fit by a two-binding site model. The mean K_i values for the high and low affinity binding sites are summarized in Table 2. By convention, the higher affinity S6C and BQ123 binding sites correspond to the ET_B and ET_A receptors, respectively, and the lower affinity S6C and BQ123 binding sites correspond to the ET_A and ET_B receptors, respectively. S6C and BQ123 were found to be highly selective for ET_B and ET_A binding sites in the human prostate.

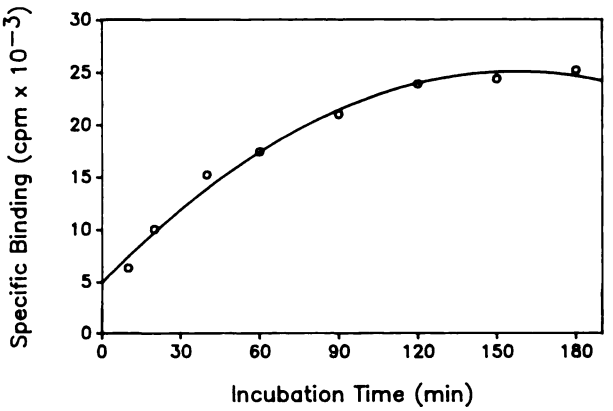

Fig. 1. Specific ^{125}I-ET-1 binding was determined at eight different time points between 10 and 180 min. The composite plot for two separate experiments is shown.

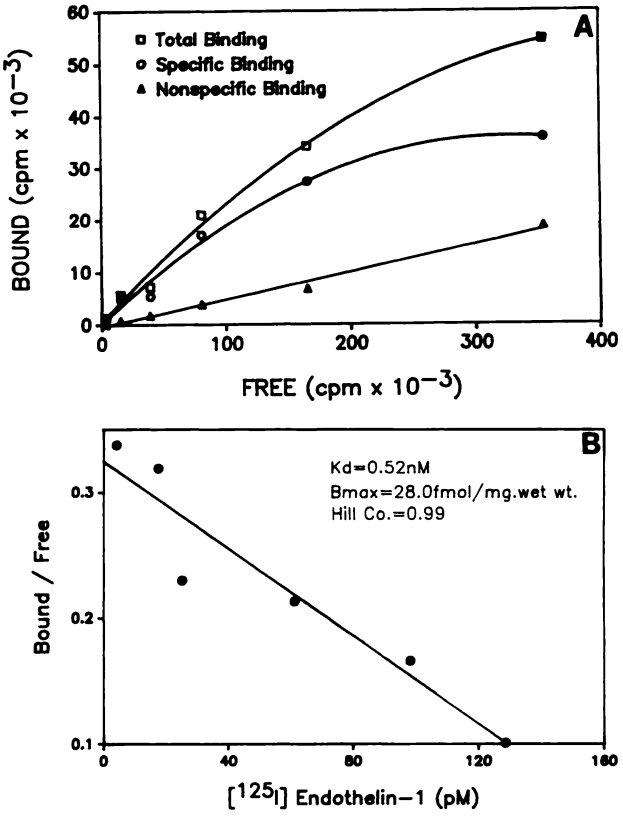

Fig. 2. Saturation studies were performed at six different concentrations of ^{125}I-ET-1 (0.0625–2.0 nM). Representative saturation (A) and Scatchard (B) plots are shown.

TABLE 1

^{125}I-ET-1 saturation studies in human prostate

Saturation studies were performed on slide-mounted human prostatic tissue sections, with six different concentrations (0.0625–2.0 nM) of ^{125}I-ET. Total binding and nonspecific binding were determined in triplicate. The equilibrium dissociation constants (K_d), receptor densities (B_{max}), and Hill coefficients were determined from Scatchard analysis of six individual saturation experiments.

Assay	K_d	B_{max}	Hill coefficient
	nM	fmol/mg of wet weight	
1	0.36	43.7	0.98
2	1.04	45.0	0.99
3	0.75	70.3	0.99
4	0.47	23.4	0.98
5	1.16	31.9	0.99
6	0.53	28.0	0.99
Mean ± standard error	0.72 ± 0.13	40.4 ± 6.9	0.99 ± 0.005

Isometric tension studies. A total of five ET-1 and S6C dose-response experiments were performed using human prostatic tissue. The contractions in response to ET-1 and S6C

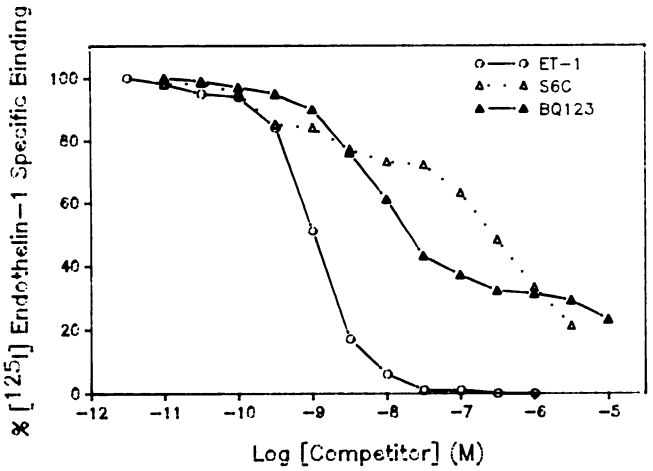

Fig. 3. Competitive binding experiments were performed on slide-mounted tissue sections, in the presence of a constant final concentration of ^{125}I-ET-1 (0.1 nM) and varying concentrations of unlabeled ET-1, S6C, and BQ-123. The assays were performed in triplicate for each concentration of unlabeled ligand. Four separate binding experiments were performed for each inhibitor. The composite data are shown.

TABLE 2

Competition by S6C and BQ123 for specific ^{125}I-ET-1 binding

Competition binding experiments were performed on slide-mounted prostatic tissue specimens, with a constant concentration of ^{125}I-ET-1 (0.1 nM) and varying concentrations of unlabeled S6C (10^{-11} to 10^{-6} M) and BQ123 (10^{-11} to 10^{-5} M). Four separate binding experiments were performed for each inhibitor. The binding curves were consistently best fit by a two-site model. The inhibitory dissociation constants (K_i) and the relative proportions (percentage of B_{max}) of the higher and lower affinity S6C and BQ123 binding sites were determined from the individual experiments using the computer program ReceptorFit Competition. The values are reported as mean ± standard error.

Inhibitor	Higher affinity site		Lower affinity site	
	K_i	Proportion	K_i	Proportion
	nM	% of B_{max}	μM	% of B_{max}
S6C	0.50 ± 0.09	36 ± 1.0	0.84 ± 0.28	64 ± 1.0
BQ123	5.51 ± 1.05	69 ± 5.2	24.9 ± 6.5	31 ± 5.2

TABLE 3

Summary of ET-1 and S6C dose-response studies

Five ET-1 and S6C dose-response experiments were performed on tissue strips of human prostate. The magnitudes of the contractile responses reached plateaus within the dose range investigated (10^{-10} to 10^{-6} M). The maximal contractile response (E_{max}) and the concentration of agonist eliciting half-maximal response (EC_{50}) were determined from the individual dose-response experiments. The values are expressed as mean ± standard error.

Agonist	No. of assays	E_{max}	EC_{50}
		g of force/mm^2	nM
ET-1	5	0.16 ± 0.03	0.53 ± 0.12
S6C	5	0.21 ± 0.10	0.14 ± 0.06

were of slow onset. The magnitude of the contractile response to ET-1 and S6C was dose dependent and saturable. The mean E_{max} and EC_{50} values for ET-1- and S6C-mediated contractions are presented in Table 3.

ET-1 and S6C dose-response experiments were performed in the presence of four different concentrations of the antagonist BQ123. The antagonist studies are presented in Fig. 4. ET-1 is a nonselective agonist for ET_A and ET_B receptors. All concentrations of BQ123 shifted the ET-1 dose-response curve. The pA_2 values were determined from the Schild plots. The pA_2 value of BQ123 for ET-1-mediated contracton was 6.84. S6C is

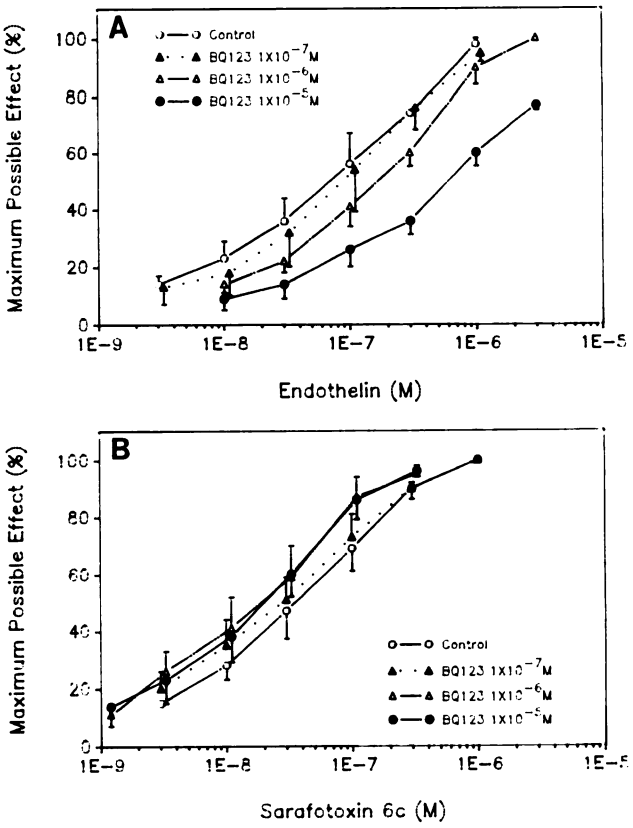

Fig. 4. ET-1 and S6C agonist-antagonist competition experiments were performed in the presence of three different concentrations of BQ123. A total of four agonist-antagonist competition experiments were performed at each concentration of BQ123 for the agonists ET-1 (A) and S6C (B). The composite agonist-antagonist competition plots are shown. Tension (g of force/mm^2 cross-sectional area) was expressed as the percentage of the maximal contraction elicited by 3 μM ET (maximum possible effect).

a selective ET_B agonist. The only concentration of BQ123 that shifted the S6C dose-response curve was 10^{-4} M. Therefore, the pA_2 value of BQ123 for S6C-mediated contraction was indeterminant. The K_B of BQ123 was calculated from the Furchgott equation (25), as follows: K_B = [antagonist]/(dose ratio −1). The K_B determined at a BQ123 concentration of 100 μM was 5.01×10^{-5} M.

Discussion

The present study represents the first characterization of the physiology and pharmacology of human prostatic ET-1 binding sites using radioligand receptor binding studies and isometric tension studies. The binding studies were performed directly on 20-μm-thick slide-mounted tissue sections. The optimal binding assay conditions were defined. Specifically, the present study demonstrated that maximal specific ^{125}I-ET-1 binding was achieved after a 10-min preincubation, a 120-min incubation, and a 1-min wash after a brief rinse. Saturation studies were performed at concentrations of ^{125}I-ET-1 between 0.063 and 2.0 nM. The binding of ET-1 to prostatic tissue sections was consistently saturable and of high affinity. The mean K_d and B_{max} values for ET-1 binding sites in prostatic tissue were 0.72 ± 0.13 nM and 40.4 ± 6.9 fmol/mg of wet weight, respectively. The K_d for prostatic ET-1 binding sites is in agreement with previously reported values for other tissues (12–16). Lepor and associates previously characterized the muscarinic cholin-

ergic, α_1-adrenergic, α_2-adrenergic, and dihydropyridine calcium channel receptors in the human prostate (26–29). The density of ET-1 binding sites was 20-, 35-, 130-, and 100-fold greater than that of muscarinic cholinergic, α_1-adrenergic, α_2-adrenergic, and dihydropyridine binding sites, respectively. The mean Hill coefficient was 0.99, indicating that ^{125}I-ET-1 identifies a single population of prostatic binding sites. The saturation studies were performed directly on slide-mounted tissue sections. We previously reported that the pharmacology of [^{3}H] prazosin binding sites is not affected by the source of prostatic tissue (prostatic tissue homogenates versus slide-mounted tissue sections) (30). The primary advantages of using slide-mounted tissue sections, compared with tissue homogenates, are lower cost and the need for a smaller amount of tissue. The cost of performing a single ^{125}I-ET-1 saturation assay using slide-mounted tissue sections versus tissue homogenates is approximately 4-fold less, using commercially available reagents. Another advantage of using slide-mounted tissue sections is that malignancy is excluded by staining and inspecting the random tissue sections.

Competition binding experiments were performed using ^{125}I-ET-1 and unlabeled ET-1, S6C, and BQ123. S6C and BQ123 discriminate two ET-1 binding sites in several tissues (14, 16, 22). According to convention, the higher and lower affinity S6C binding sites correspond to the ET_B and ET_A receptor subtypes, respectively, whereas the higher and lower affinity BQ123 binding sites correspond to the ET_A and ET_B receptor subtypes, respectively. The mean K_i values for the higher (ET_B) and lower (ET_A) affinity prostatic ET-1 binding sites discriminated by S6C were 0.50 ± 0.09 nM and 0.84 ± 0.28 μM, respectively (16). The K_i values of the higher and lower affinity S6C binding sites reported in lung, stomach, liver, and kidney range from 0.06 to 0.9 nM and from 0.046 to 0.93 μM, respectively (14, 16). The K_i values for the high and low affinity S6C binding sites in the present study are similar to values previously reported in other tissues. The mean K_i values for the higher (ET_A) and lower (ET_B) affinity binding sites discriminated by BQ123 were 5.51 ± 1.05 nM and 24.9 ± 6.5 μM, respectively. Nambi *et al.* reported that the K_i values of the higher and lower affinity BQ123 binding sites in the kidney were 9.3 nM and $>10^{-6}$ M, respectively. The K_i values for the higher and lower affinity BQ123 binding sites in the present study are similar to values reported for the kidney. The relative proportion of ET_A and ET_B receptors varies among different tissues. For example, the relative percentages of ET_A binding sites in the lung, stomach, liver, and kidney are 82%, 78%, 37%, and 56%, respectively (14, 16). The studies performed with S6C demonstrated that the relative percentages of ET_A and ET_B prostatic binding sites were 64% and 36%, respectively. The competitive binding studies with BQ123 demonstrated that the relative percentages of ET_A and ET_B binding sites were 69% and 31%, respectively. The binding studies in the human prostate using S6C and BQ123 are consistent and suggest that approximately two thirds of the ET-1 binding sites are of the ET_A subtype.

The prostate gland is composed primarily of smooth muscle, connective tissue, and secretory epithelium (31). Approximately 40% of the human prostate is smooth muscle (32). We previously reported that ET-1 elicits a potent contractile response in the human prostate (11). The magnitudes of the contractile responses elicited by ET-1 and phenylephrine are similar. The present study was designed to determine which ET receptor mediates prostate smooth muscle contraction. ET-1 is a nonselective ET receptor agonist, whereas S6C is a selective ET_B receptor agonist. Both of these agonists elicited a potent contractile response in human prostate smooth muscle. BQ123 represents one of the few well characterized selective ET_A antagonists. The ET_A receptor is dominant in porcine aortic smooth muscle cells. Ihara *et al.* (22) reported that the pA_2 of BQ123 for inhibiting ET-1-mediated contraction in isolated porcine coronary artery was 7.4. BQ123 at concentrations between 10^{-7} and 10^{-5} M shifted the ET-1 dose-response curve to the right in a dose-dependent manner, indicating that the ET_A receptor mediates prostatic smooth muscle contraction. The pA_2 value of BQ123 for ET-1-mediated prostate smooth muscle contraction was 6.84. The similar pA_2 values in the prostate, compared with the porcine coronary artery, provide additional evidence that prostatic smooth muscle contraction is mediated by the ET_A receptor. BQ123 at concentrations between 10^{-7} and 10^{-5} M did not shift the S6C dose-response curve, indicating that S6C-induced contraction is mediated by the ET_B receptor. The similar values observed in the present study for the K_B of BQ123 for S6C-mediated contraction (5.01×10^{-5} M) and the K_i of BQ123 for the lower affinity ET-1 binding site (2.49×10^{-5} M) further suggest that prostate smooth muscle responses are also mediated by the ET_B receptor. The relative functional significance of ET_A and ET_B-mediated prostate smooth muscle tension has yet to be determined.

The prostate is composed of glandular and stromal elements. The functional studies provide compelling evidence that both ET_A and ET_B receptors mediate prostate smooth muscle function. We recently reported a methodology for localizing prostatic α_1-adrenoceptors, using autoradiography and computer-assisted image analysis (30). Studies are presently underway in our laboratory to determine the cellular localization of the ET receptor subtypes in the human prostate.

Approximately 70% of men develop urinary symptoms before the seventh decade of life (33). The pathophysiology of these urinary symptoms is felt to be secondary to the development of BPH. It has been proposed that the pathophysiology for the development of urinary symptoms in men with BPH is attributed to increased bladder outflow resistance (34). Approximately 400,000 prostatectomies are performed in the United States on men with BPH, at a cost of $4 billion dollars (35). Prostatectomy represents the second most costly surgical procedure reimbursed under Medicare. Approximately 15 years ago, Caine *et al.* (36) reported that the human prostate contracts in the presence of epinephrine. High affinity α_1- and α_2-adrenoceptors were subsequently characterized in the human prostate (27, 28). Isometric tension studies demonstrated that the contractile responses to the α agonists were mediated via the α_1-adrenoceptor (37, 38). Based upon these observations, α blockers were evaluated for the treatment of symptomatic BPH. Several multicenter randomized placebo-controlled studies have unequivocally demonstrated the safety and efficacy of α blockers for the treatment of BPH (39).

ET has recently been shown to elicit a potent contractile response in the human prostate (11). The novel conclusions of the present study are that human prostate tissues derived from patients with BPH contain an abundance of ET receptors that mediate a specific physiological function. Both ET_A and ET_B receptors exist in the prostate, with the ET_A receptor being the dominant subtype. Both ET_A and ET_B receptors mediate pros-

tate smooth muscle contraction. Because the clinical manifestations associated with BPH are related to bladder outlet obstruction, it is conceivable that the endogenous ETs are involved in the pathophysiology of BPH. If this is the case, then ET antagonists may represent an effective treatment for BPH. Because the ET response in the prostate is not mediated via α-adrenoceptors (11), ET antagonists may ultimately prove to be a novel therapy for the treatment of BPH.

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