Endothelin Receptors in Testosterone-Induced Prostatic Hypertrophy in Rats

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ABSTRACT—Endothelin receptors were characterized in rat prostate and potential modification of these receptors was investigated in prostatic hypertrophy induced by testosterone. Both ET$_{A}$ and ET$_{B}$ endothelin receptor mRNA were detected in rat prostate, whereas binding experiments show the presence of only ET$_{A}$ receptors. Testosterone administration produced a 75% increase in prostate weight. Although the density of prostatic endothelin receptors was decreased from 348±75.0 fmol/mg protein in control rats to 252±39.9 fmol/mg protein in testosterone-treated animals, the total amount of receptors per prostate was unchanged. The steady-state level of ET$_{A}$- and ET$_{B}$-receptor mRNA was not altered by testosterone treatment. These results suggest that endothelin receptors are not affected in prostatic hypertrophy induced by testosterone.

Keywords: Prostate, Endothelin receptor, Testosterone

Endothelin represents a family of three isopeptides ET-1, ET-2 and ET-3 that possess via specific receptors potent constrictive properties on smooth muscle and exerts a variety of pharmacological actions in vascular and non-vascular tissues including the heart, lung, kidney (1) and urogenital tracts (2). In the lower urinary tract, endothelin modulates bladder contraction and is a potent contractile agent of prostatic smooth muscle (3). Both endothelin receptors, ET$_{A}$ and ET$_{B}$, are present in the human prostate (4).

Two components of benign prostatic hypertrophy (BPH) could be determined: a static component due to compression induced by the increased prostate volume and a dynamic component related to the contraction of urethra and prostatic smooth muscle (5). The contraction of the smooth muscle is mainly mediated by $\alpha$-adrenoceptors. As a potent constrictor of smooth muscle, it is conceivable that endothelin may be also involved in the pathophysiology of BPH (6).

In the present study, characteristics of endothelin receptors in the rat prostate were investigated using binding experiments and ET$_{A}$- and ET$_{B}$-receptor mRNA levels were determined by using the polymerase chain reaction coupled to reverse transcription (RT-PCR). We used the experimental model of prostatic hypertrophy induced by testosterone to examine eventual alterations of endothelin receptors in the prostate.

Prostatic hypertrophy was induced by subcutaneous injections of testosterone (3 mg/kg) for 15 days (daily injection for 5 days/week) to male Sprague Dawley rats weighing 240−250 g (Charles River, Cléon, France) according to the method described by Maggi et al. (7). Rats were killed 72 hr after the final injection, and prostates were collected and pooled by groups for binding experiments in 50 mM Tris-HCl, pH 7.7 at 4°C and homogenized with a polytron. Homogenates were filtered and centrifuged at 52,000×g for 10 min at 4°C. The pellets were resuspended and centrifuged twice at 52,000×g for 10 min. Prostatic membranes (120 µg protein) were incubated with $^{125}$IET-1 (2200 Ci/mmol), $^{[25]}$IRL1620 (2200 Ci/mmol; New-England-Nuclear, Les Ulis, France), $[^3]$H]BQ-123 (30.0 Ci/mmol) or $[^{25}]$IBQ 3020 (2000 Ci/mmol; Amersham, Les Ulis, France) for 30 min at 25°C in 50 mM Tris-HCl, pH 7.4 containing 10 mM MgCl$_2$, 20 mg/l bacitracin, 20 mg/l phenylmethyl-sulfonylfluoride, 100,000 U/l trasylol and 0.05% (w/v) bovine serum albumin. Nonspecific binding was determined in the presence of 0.1 µM unlabelled ligand, except for BQ-123 (1 µM). Bound from free radiolabeled ligands were separated by filtration through Whatman GF/C filters presoaked with 0.05% (w/v) bovine serum albumin, using a Brandel cell harvester. Filters were washed and assayed for radioactivity. Binding data were analyzed by computer-assisted nonlinear regression analysis using
the LIGAND program (Biosoft, Cambridge, UK).

To determine endothelin receptor mRNA levels, RNA was isolated from frozen prostate, using a guanidinium thiocyanate-phenol-chloroform extraction (RNA B; Bioprobe-Systems, Montreuil/Bois, France). Primers specific for ET\textsubscript{A} and ET\textsubscript{B} receptors were previously described by Wang et al. (8) and synthesized by GeneSet (Paris, France). RT-PCR was performed using recombinant *Thermus thermophilus* (rTth) DNA polymerase (Perkin Elmer Cetus, Saint-Quentin-Yveline, France). Total RNA (100 ng) was reverse transcribed for 15 min at 60°C for ET\textsubscript{A} and ET\textsubscript{B} receptors with 24 pmol of primers. The lengths of the resulting cDNA fragment to be amplified were predicted to be 546 and 475 bp for ET\textsubscript{A} and ET\textsubscript{B} receptors, respectively. Template-specific cDNA was then amplified for 32 and 40 cycles for ET\textsubscript{A} and ET\textsubscript{B} receptors, respectively. An initial cycle of 3 min at 94°C was followed by 1 min at 54°C, 3 min at 72°C, and subsequent cycles of 15 sec at 94°C, 20 sec at 54°C and 1 min at 72°C. Aliquots of the PCR products were resolved by electrophoresis on 2.5% agarose gel. The gel was placed under ultraviolet light, and a process image of the nucleic acid was taken by a densitometer (Vilber Lourmat, Marne-la Vallée, France).

The binding of \[^{125}I\]ET-1 to prostate endothelin receptors was specific, saturable and of high affinity. It showed an apparent single class of binding sites with a dissociation constant (K\textsubscript{d}) of 0.32 ± 0.068 nM and a binding capacity (B\textsubscript{max}) of 348 ± 75.0 fmol/mg protein (n = 3). ET-1 and the selective ET\textsubscript{A}-receptor ligand BQ-123 fully inhibited \[^{125}I\]ET-1 specific binding according to a one-site model, whereas no inhibition was observed with the selective ET\textsubscript{B}-receptor ligand IRL1620 (Fig. 1A). Inhibition constants (K\textsubscript{i}) were 0.25 ± 0.035 and 3.71 ± 0.544 nM for ET-1 and BQ-123, respectively (n = 3). Neither \[^{125}I\]IRL1620 (0.01 to 0.5 nM) nor \[^{125}I\]BQ 3020 (0.1 to 2 nM), another ET\textsubscript{B}-receptor ligand, were capable of specific binding to prostate membrane. As shown in Fig. 1B, ET-1 and BQ-123 also inhibited \[^{3}H\]BQ-123 specific binding with a Ki of 0.32 ± 0.038 and 4.23 ± 0.471 nM, respective-

![Fig. 1. Competitive inhibition of specific \[^{125}I\]ET-1 and \[^{3}H\]BQ-123 binding to rat prostate. Competitive inhibition of 0.3 nM \[^{125}I\]ET-1 (A) and 2 nM \[^{3}H\]BQ-123 (B) binding to membranes prepared from rat prostate was performed as described in "Materials and Methods" with unlabeled ET-1 ( ), BQ-123 ( ), and IRL1620 ( ). Total binding is presented as a percent of control binding (in absence of unlabeled ligand). Binding data were analyzed by the LIGAND program and inhibition constants (K\textsubscript{i}) were determined. The assay was performed in triplicate and results are expressed as means ± S.E.M. of three separate experiments.](image1)

![Fig. 2. Effect of testosterone administration on \[^{125}I\]ET-1 binding to rat prostatic endothelin receptors. Crude rat prostate membranes from control ( ) and testosterone-treated animals ( ) were incubated with increasing concentrations of \[^{125}I\]ET-1. Data represent the Scatchard analysis of a typical experiment, which was replicated three times. B\textsubscript{max} and K\textsubscript{i} values were 294 ± 17.9 fmol/mg protein and 0.27 ± 0.032 pM, respectively, for control rat prostate and 211 ± 8.4 fmol/mg protein and 0.20 ± 0.016 pM, respectively, for testosterone-treated animals. Each point is a mean of triplicate determinations.](image2)
ly, IRL1620 being inactive (K_i > 1 μM). These results suggest the presence of only ET_A receptors in the prostate, the ET_B receptor subtype being undetectable in the binding experiments.

Testosterone administration increased the prostate weight from 231.4 ± 8.00 mg/100 g body weight in vehicle-treated controls to 397.1 ± 5.05 mg/100 g body weight in testosterone-treated rats. The binding capacity of [3H]ET-1 to crude prostate membrane preparation was significantly decreased by 28% in testosterone-treated animals (P = 0.04). Scatchard analysis of the saturation curves gave a B_max of 252 ± 39.9 fmol/mg protein with a K_d of 0.25 ± 0.041 nM (n = 3) in testosterone-treated rats (Fig. 2). Relating to the whole prostate and considering that 10.5 and 16.3 mg protein per prostate were obtained from the control and treated animals, respectively, the total amount of endothelin receptors was 3.65 ± 0.788 and 4.11 ± 0.650 pmol per prostate from the control and testosterone-treated rats, respectively. The binding values were not significantly modified (P = 0.20).

Total RNA from prostate was isolated and the relative levels of endothelin receptor subtype mRNA were measured by RT-PCR (Fig. 3). RT-PCR using ET_A- and ET_B-receptor primers resulted in a single band at the expected molecular length: 546 bp for ET_A receptors and 475 bp for ET_B receptors. The signal of ET_A receptors became visible at 30 cycles of PCR amplification and 36 cycles were required for ET_B receptors. The lack of signals in unmatched lanes (where the RT step was not performed) as well as without RNA demonstrated the absence of DNA contamination. These results indicate that both receptor mRNAs are expressed in the prostate.

In our conditions, i.e., not quantitative RT-PCR, the relative proportion of ET_A- and ET_B-receptor mRNA could not investigated. However, possible alteration of each receptor expression in treated animals could be qualitatively observed. The signals measured by RT-PCR for ET_A, ET_B receptors and for positive control β-actin were not modified by testosterone treatment (Fig. 3). This suggests that endothelin receptor mRNA expression is not changed by testosterone treatment of the animals.

Endothelin is synthesized in the prostate of various species including humans and is primarily localized in the secretory glandular epithelium (3). In human prostate,

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Fig. 3. Effect of testosterone administration on prostatic endothelin receptor mRNA expression in rat prostate. The expression of α_1-adrrenergic receptor mRNA was evaluated by RT-PCR in hypertrophic prostate from testosterone-treated rats (T) and control animals (C). RT-PCR assay was performed on total RNA using specific primers for ET_A and ET_B receptors as described in "Materials and Methods". The resulting products were resolved by electrophoresis on a 2.5% agarose gel in Tris borate EDTA buffer and visualized by ethidium bromide staining. A phi-X-174-RF DNA hinc II digest (m1) and phi-X-174RF DNA hae III digest (m2) (Pharmacia Biotech, Orsay, France) were used as DNA size markers. Arrows indicate expected PCR product sizes. RT-PCR using specific primers for β-actin was used as a positive control. Negative controls were performed in the absence of RNA [RNA(−)] and without RT [RT(−)]. Data shown are from a typical experiment, which was replicated three times with several prostates.
ET-1 induces a contractile response that is mediated by both ET\(_A\) and ET\(_B\) receptors (3, 4). Human prostate predominantly contains the ET\(_A\)-receptor subtype (65% to 85%) that is principally located on the prostatic stroma whereas glandular epithelium mainly contains the ET\(_B\) receptors (4, 9–12).

The present report shows the presence of mRNA for ET\(_A\) and ET\(_B\) receptors in rat prostate. However, only ET\(_A\) receptors are observed either by competitive inhibition binding experiments or by the lack of specific binding obtained with \[^{[25]}\text{I}]IRLI1620 and \[^{[125]}\text{I}]BQ 3020. A low rate of transcription leading to a low level of receptor protein may explain why ET\(_B\) receptors are not detected in binding experiments. Another hypothesis might be an inhibited translation of ET\(_B\)-receptor mRNA. Detection of ET\(_B\)-receptor mRNA with no ET\(_B\) binding demonstrated was previously observed in human prostate cancer cell lines (9). On the other hand, using competitive binding experiments, Saito et al. (13) found 80% ET\(_A\) and 20% ET\(_B\) receptors in the prostate of control and streptozotocin-induced diabetic rats. The discrepancy between our results and those of Saito et al. (13) are difficult to explain. A low proportion of receptor subtype might be difficult to measure with accuracy by competitive binding. Nevertheless, our study shows that most if not all of the endothelin receptors in the prostate are of the ET\(_A\) subtype.

Testosterone administration represents an experimental model of prostatic hypertrophy that induces urodynamic alterations comparable to those observed in patients with BPH (7). In the present study, injection of testosterone for 15 days produces a 75% increase of prostate weight. RT-PCR data show that the steady-state level of mRNA for ET\(_A\) and ET\(_B\) receptors is not modified by testosterone treatment. Subsequently, binding experiments show that the endothelin receptor density is decreased in the prostate of testosterone-treated animals, whereas the total amount of receptors per prostate is not modified. Furthermore, histological studies have shown that ET\(_A\) receptors are mainly located in the fibromuscular stroma of the prostate (9, 11). The observed decrease of receptor density is likely due to the increase of prostatic glandular epithelium induced by testosterone and reflects a reduced proportion of smooth muscular tissue rather than down regulation of endothelin receptors.

Conflicting results have been obtained in humans. Endothelin receptor density is increased in BPH (14), whereas no modification was noted by Le Brun et al. (10). Moriyama et al. (15) have shown that responsiveness to ET-1 decreased, although endothelin receptors increase in hyperplastic prostate. Our findings in rats show that endothelin receptors are not affected in the experimental model of prostatic hypertrophy induced by testosterone.

REFERENCES