Abstract  We previously have shown that experimental diabetes in rats causes prostatic involution, reduces serum testosterone levels, and causes an upregulation in prostatic endothelin (ET) receptors. Furthermore, insulin treatment normalizes these changes (Saito et al., Mol Cell Biochem 210:1–12, 2000). Since experimental diabetes-induced reduction in serum testosterone may be a factor in the alteration of the ET receptors and of prostatic growth, we investigated the effect of castration, another means of involuting the prostate and decreasing serum testosterone levels, on the expression of ET receptors in ventral and dorsolateral regions of the rat prostate.

Three-month-old Sprague-Dawley rats were surgically castrated or sham operated, and then killed on the 7th post-operative day. Biochemical and pharmacological properties, and localization of ET receptors in the rat prostate, were determined by performing a series of binding experiments with $[^{125}\text{I}]$ET-1 and by light microscopy autoradiography, respectively. The expression levels of ET-1, ET-3, ET receptor subtypes and endothelin converting enzyme-1 (ECE-1) mRNAs were assessed by relative multiplex reverse transcription polymerase chain reaction (RT-PCR).

The total density of ET receptors increases 3.7-fold in the ventral and 2.1-fold in the dorsolateral regions of the castrated rat prostate compared to sham operated animals. Castration causes a 2.4-fold increase in the density of $\alpha_1$-adrenoceptors ($\alpha_1$-ARs) in the ventral region of the prostate, but no change in the density of $\alpha_1$-ARs in the dorsolateral region of the rat prostate. The predominant ET receptor subtype in the rat prostate is the ETA subtype, which is mainly located in the prostatic stroma. In addition, RT-PCR data show an upregulation in the expression of ETB receptor subtype, ET-1 and ECE-1 mRNA in both regions, and a downregulation in the expression of ETA receptor subtype mRNA in the dorsolateral region of the castrated rat prostate. There is no change in the expression of ET-3 mRNA in either region. Castration does not cause significant changes in the pharmacological properties of prostatic ET receptors, i.e., the predominance of ETA receptors in either region of the prostate, or the expression of ETA receptor subtype mRNA in the ventral region of the castrated rat prostate. These results suggest the existence of a region/lobe-specific regulatory role for testosterone in the expression of the ET receptor system in the rat prostate.

Keywords  Endothelin · Rat · Prostate · Castration · RT-PCR

Introduction

Alterations in testosterone production influence the contractile responses of a variety of smooth muscles, including genitourinary tract smooth muscle, to autonomic agonists and cell growth of the mammalian prostate. The latter is related to control of proliferation and apoptotic processes (Isaacs 1984; Collins et al. 1988; Colombel and Buttyan 1995; Homma et al. 2000). Testosterone deprivation causes a dramatic reduction in prostate size by the process of apoptosis (Kyprianou and Isaacs 1988; Colombel and Buttyan 1995; Lacey et al. 1996; Homma et al. 2000), decreases $\alpha_1$-adrenoceptor-induced contractility by a down-regulation of prostatic $\alpha_1$-adrenoceptor density (Homma et al. 2000), and decreases muscarinic receptor-induced prostatic secretion by a downregulation of muscarinic receptors (Shapiro et al. 1985). On the other hand, testosterone administration to intact or castrated rats results in benign and/or atypical prostatic hyperplasia (Colombel and Buttyan 1995). Testosterone regulates the expression of various growth factors and their receptors, including epidermal growth factor (EGF) and EGF receptor, transforming growth factors (TGFs), TGF-α, TGF-β1, TGF-β2,
and TGF-β3 and TGF-β type II receptor, basic fibroblast growth factor (bFGF) and FGF receptor 1, hepatocyte growth factor (HGF), and HGF receptor, keratinocyte growth factor (KGF) and vascular endothelial growth factor (VEGF) in the rat prostate (Nishi et al. 1996; Häggsström et al. 1999; Desai and Kondaiah 2000).

Endothelin-1 (ET-1), a 21-amino-acid peptide, isolated from the conditioned medium of cultured porcine aortic endothelial cells (Yanagisawa et al. 1988), is not only a potent vasoconstrictor, but also mediates contraction in a variety of smooth muscle preparations including urinary tract smooth muscle (Maggi et al. 1989; Eguchi et al. 1991; Saenz de Tejada et al. 1991; Tschirhart et al. 1991; Sakata and Karaki 1992; Traish et al. 1992; Warner et al. 1993; Kobayashi et al. 1994a). The ET family consists of three isopeptides: ET-1, ET-2 and ET-3 (Inoue et al. 1989) which are cleaved by ECEs from low-activity precursors, big ETs. Three isoymes of ECE (ECE-1, ECE-2 and ECE-3) have been identified and cloned (Shimada et al. 1994; Emoto and Yanagisawa 1995; Hasegawa et al. 1998). The mRNA for ECE-1 and ET-1 along with the functional ET receptor subtypes, ETA and ETB, have been identified in the prostate of mammals including the central and peripheral zones of that of the human (Lanenstroer et al. 1993; Prayer-Galetti et al. 1997; Ishizuka et al. 1999). ET-1 and ET-3 cause contractile responses in prostatic smooth muscle, and/or mediate mitogenic activity in human prostatic smooth muscle cells (Saiga et al. 1998) and in canine prostatic epithelial cells (Ishizuka et al. 1999).

We have previously shown that 8 weeks of experimental diabetes in rats causes prostatic involution, reduces serum testosterone levels, and causes an upregulation in prostatic ET receptors at both protein and gene transcript levels (Saito et al. 1996, 2000). Insulin treatment started 8 weeks after the induction of diabetes normalizes these changes (Saito et al. 2000). Therefore, it has been proposed that experimental diabetes-induced reduction in serum testosterone may be a factor in the alteration of the ET receptors and of prostatic growth. The present study, using radioligand receptor binding, autoradiography and relative multiplex RT-PCR techniques, was undertaken to examine the regulatory effects of castration on ET receptors and on the expression of ET isopeptides, ET receptor subtypes and ET converting enzyme-1 (ECE-1) mRNA in rat prostate. In order to determine whether castration has a specific effect on ET receptors, we also determined the effects of castration on the expression level of α1a-adrenoceptors (α1a-AR) in the same regions of the rat prostate.

**Materials and methods**

**Animals and tissue preparation**

Three-month-old castrated or sham operated Sprague-Dawley rats weighing 300–400 g were purchased from Charles River Laboratories (Wilmington, Mass., USA). On 7th post-operative day, they were killed by decapitation, and their prostates were rapidly dissected, trimmed from peripheral fat, urethra and seminal vesicles, separated to ventral and dorsolateral regions, frozen in liquid nitrogen, and stored at −80°C until used for radioligand binding and RT-PCR assays. For autoradiographic studies, the whole prostate including urethra and seminal vesicles was embedded in Tissue Tek OCT compound (Sakura Finetek USA, Torrance, Calif., USA), frozen on dry ice and stored at −80°C until used.

**Radioligand receptor binding assay**

**ET receptor binding experiment.** The ET binding experiments were performed as previously described (Latippour et al. 1995; Saito et al. 2000). The rat prostates were homogenized in an ice-cold HEPES buffer (pH 7.4 at 25°C) containing 100 mM NaCl, 3 mM EDTA, 1 mM EGTA, 0.1 mM phenylmethylsulfonyl fluoride (PMSF) and 10 µg/ml aprotinin, leupeptine, pepstatin A and soybean trypsin inhibitor, using a power homogenizer. The homogenate was spun at 4°C for 15 min at 49,000 g. The supernatant was discarded and the resultant pellet was rehomogenized, filtered through a 250-µm-pore-size metal mesh and recentrifuged at 4°C for 15 min at 49,000 g. The final pellets containing the membrane particulates were dispersed in the same buffer, and the protein content was determined using bovine serum albumin as standard.

In saturation studies, the rat prostate membrane particulates were incubated in 0.1 ml buffer containing 50 mM Tris-HCl, 154 mM NaCl, 25 mM MnCl2, 1 mM EDTA, 1 mM N-acetyl-dl-tryptophan amide, 0.25% BSA and 0.14% bacitracin (pH 7.4 at 25°C) with increasing concentrations of [125I]ET-1 (4–120 pM) at room temperature for 2 h. Non-specific binding was determined in the presence of 100 nM unlabeled ET-1. In the inhibition studies, the membrane particulates were incubated in the presence of a fixed concentration of [125I]ET-1 (approximately 60 pM) and varying concentrations of unlabeled BQ 234 (10–10–10–3 M), an ETA receptor antagonist. After 2 h of incubation, the mixtures were filtered through Whatman GF/B glass fiber filters, pre-soaked in 1% BSA, and then the filters were washed extensively with 80 ml ice-cold 50 mM Tris-HCl buffer (pH 8.0 at 25°C). The radioactivity, trapped on the glass fiber filters, was counted using a gamma counter (Packard Multi-Prias Analyzer; Downers Grove, Ill., USA) at an efficiency of 70%–75%.

**α1a-AR binding experiment.** The α1a-AR binding experiments were performed, with some modifications, as previously described (Lacey et al. 1996). In brief, the rat prostates were homogenized and spun multiple times in an ice-cold 50 mM Tris-HCl buffer (pH 7.4 at 25°C) containing 10 mM MgCl2 and 150 mM NaCl. In saturation studies for α1a-AR, the rat prostate membrane particulates were incubated in 0.5 ml of binding buffer containing 10 mM glycylglycine, 10 mM EDTA, 0.1% ascorbic acid, 0.1% BSA (pH 7.4 at 25°C) with increasing concentrations of [125I]iodo-[2-[β-(4-hydroxyphenyl)-ethylaminomethyl]tetralone ([125I]HEAT, 4–90 PM) at room temperature for 1 h. Non-specific binding for α1a-ARs was determined in the presence of 100 µM norepinephrine. After incubation, the mixtures were filtered through Whatman GF/B glass fiber filters, pre-soaked in 0.05% polyethyleneimine, washed with 15 ml ice-cold 50 mM Tris-HCl buffer (pH 8.0 at 25°C), and radioactivity, trapped on the glass fiber filters, was counted as described for ET receptors.

**Autoradiography**

The tissue sections (10 µm) were cut with a cryostat (2800 FRIGOCUT-N; Leica, Pike Malvern, Pa., USA) at −20°C, mounted on aminoalkylsilane-coated slides, air-dried and stored at −80°C until used. The serial slide-mounted tissue sections were preincubated in 0.25 ml incubation buffer (20 mM HEPES, pH 7.4 at 25°C, containing 140 mM NaCl, 4 mM KCl, 1 mM KH2PO4, 1 mM MgCl2, 1 mM CaCl2, 10 mM D-glucose, 2.5 mM NaHCO3, 100 µg/ml bacitracin) at room temperature for 30 min. After the pre-incubation, an additional 0.25 ml of incubation buffer containing increasing increasing concentrations of [125I]ET-1 (12–48 pM, final concentration) was added, and the incubation was continued at room