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Short communication

Effect of experimental diabetes on rat prostate endothelin receptors

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Abstract

We studied the properties of endothelin receptors in the prostate of 8-week streptozotocin-diabetic and control rats. The density of endothelin receptors, as determined by saturation experiments with [^{125}I]endothelin-1, were 95.8 ± 5.4 and 171.3 ± 16.7 fmol/mg of protein in control and diabetic rat prostates, respectively. The pharmacological profile of the endothelin receptors was similar in both groups and was consistent with the predominance of the endothelin ET_A receptor subtype in the prostate. Thus, the induction of diabetes upregulates the expression of endothelin receptors in the rat prostate, but does not alter the pharmacological profile of the receptors in these tissues.

Keywords: Endothelin receptor; Diabetes; Prostate; (Rat)

1. Introduction

Diabetes mellitus-induced dysfunctions in the genitourinary tract are manifested by impaired bladder sensation, detrusor hypotonicity, incontinence, impotence and retrograde ejaculation (Ellenberg, 1980). Experimentally induced diabetes has been shown to result in decreased fertility and reduced spermatogenesis, which is accompanied by decreases in the weights of reproductive organs such as the prostate, seminal vesicle, vas deferens and testis, and decreases in serum testosterone levels (Seethalakshmi et al., 1987).

There is evidence implicating possible alterations in endothelin receptor-effector systems in diabetes mellitus such as the modulatory effects of glucose and insulin on endothelin-1 release from endothelial cells (Hu et al., 1993) and from mesenteric arteries in streptozotocin-induced diabetic rats (Takeda et al., 1991), and elevated levels of endothelin-1 in blood and urine of diabetic patients and streptozotocin-induced diabetic rats (Takeda et al., 1991; Morabito et al., 1994). Furthermore, a decrease in cardiac endothelin receptors (Nayler et al., 1989) and in aortic contractile responses to endothelin has been observed in the streptozotocin-induced diabetic rat (Fulton et al., 1991). Utilizing radioligand receptor binding techniques, we investigated the possible changes in the expression and subtype specificity of endothelin receptors in the diabetic and age-matched control rat prostate.

2. Materials and methods

2.1. Animals

Diabetes was induced in male Sprague-Dawley rats (55–56-day-old, 250–300 g) with a single injection of 65 mg/kg streptozotocin as previously described (Latifpour et al., 1989). Eight weeks after the injection of streptozotocin, rats were killed by decapitation, blood samples were collected, and prostates were dissected, trimmed, frozen in liquid nitrogen and stored at −80°C until assayed.

Glucose concentration was measured by the hexokinase method (Glucose H.K., Sigma Chemical Co., St. Louis, MO, USA). Serum insulin and serum testosterone levels were determined by radioimmunoassay kits (Diagnostic Systems Laboratories, Webster, TX, USA).

2.2. Binding experiments

Prostatic membrane particulates were prepared in ice-cold 20 mM HEPES buffer (pH 7.4) containing 100 mM NaCl, 3 mM EDTA, 1 mM EGTA and the following protease inhibitors: 0.1 mM phenylmethylsulfonyl fluoride

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and 10 μg/ml each of aprotinine, leupeptine, pepstatin A and soybean trypsin inhibitor as previously described (Latifpour et al., 1995).

In the saturation experiments, aliquots of prostatic membrane particulates were incubated in assay buffer (50 mM Tris-HCl, containing 154 mM NaCl, 25 mM $MnCl_2$, 1 mM EDTA, 1 mM *N*-acetyl-DL-methione, 0.25% bovine serum albumin and 0.14% bacitracin, pH 7.4) with increasing concentrations of [^{125}I]endothelin-1 (4–120 pM) for 120 min at 23°C. After the incubation, the reaction mixtures were filtered rapidly under a vacuum through Whatman GF/B glass fiber filters, which had been previously treated with a 1% solution of bovine serum albumin to reduce nonspecific bindings to the filter papers. Filters were washed intensively with ice-cold 50 mM Tris-HCl buffer (pH 8.0) and the radioactivity was counted with a gamma counter. Nonspecific binding was determined in the presence of 100 nM of unlabeled endothelin-1.

In inhibition binding studies, aliquots of membrane particulates were incubated with a fixed concentration of [^{125}I]endothelin-1 (approximately 30–40 pM) in the presence of increasing concentrations of the following unlabeled peptides: endothelin-1 (nonselective), endothelin-3 (endothelin ET_B receptor-selective), BQ 610, *N*,*N*-hexamethylene)carbamoyl-Leu-D-Trp(CHO)-D-Trp (endothelin ET_A receptor-selective) and IRL 1620, Suc-[Glu^9,Ala^{11,15}]endothelin-1-(8–21) (endothelin ET_B receptor-selective) (Ishikawa et al., 1993; Takai et al., 1992). The remainder of the procedure was the same as that of the saturation studies.

Saturation and inhibition data were analyzed as previously described (Latifpour et al., 1995) using a computer-assisted program (GraphPad Prism, GraphPad Software, San Diego, CA, USA).

2.3. Drugs and chemicals

[^{125}I]Endothelin-1 (2200 Ci/mM) was purchased from New England Nuclear (Boston, MA, USA). Endothelin-1, endothelin-3, BQ 610 and IRL 1620 were obtained from Peninsula Laboratory (Belmont, CA, USA). Phenylmethylsulfonyl fluoride, aprotinine, leupeptine, pepstatin A, *N*-acetyl-DL-methionine, bovine serum albumin and bacitracin were obtained from Sigma Chemical Co. Soybean trypsin inhibitor was obtained from Worthington Biochemical Co. (Freeholt, NJ, USA).

3. Results

At the time of sacrifice, body and prostate weights of the diabetic group were significantly lower than that of the control group. Furthermore, the diabetic rats had significantly greater serum glucose levels and lower serum insulin and testosterone levels than the control animals (Table 1).

Table 1
General features and saturation of [^{125}I]endothelin-1 binding to prostatic tissues of experimental animals

	Control	Diabetic
Body weight (g)		
Initial	285 ± 3	280 ± 3
Final (8 weeks)	559 ± 11	321 ± 14 [a]
Prostate weight (mg)	1584 ± 96	509 ± 79 [a]
S. glucose (mM)	8.6 ± 0.3	30.7 ± 1.5 [a]
S. insulin (μU/ml)	19.6 ± 2.6	2.2 ± 0.5 [a]
S. testosterone (ng/ml)	2.64 ± 0.37	0.77 ± 0.36 [a]
B_{max} (fmol/mg protein)	95.8 ± 5.4	171.3 ± 16.7 [a]
K_D (pM)	35.7 ± 3.9	39.0 ± 2.6

B_{max}, maximum number of binding sites; K_D, equilibrium dissociation constant. Data are shown as mean ± S.E.M. of 6 separate determinations in each group. [a] Significantly different from control group. $P \leq 0.05$ is level of significance.

In saturation experiments with [^{125}I]endothelin-1, the presence of a single class of specific, saturable, high-affinity binding sites was identified in rat prostates from diabetic and control groups. The densities (B_{max} values) of endothelin receptors in diabetic rat prostate were significantly higher than that of the control group (Table 1). No significant differences were found in the affinity constants (K_D values) for [^{125}I]endothelin-1 binding sites between the two groups.

Computer-assisted analyses of inhibition data with a nonlinear curve-fitting program (GraphPad Prism, GraphPad Software) confirmed that all binding curves for unlabeled endothelin-1 (nonselective) were better fitted to a one-site than to a two-site model, whereas the data for BQ 610 (endothelin ET_A receptor-selective) and endothelin-3 and IRL 1620 (endothelin ET_B receptor-selective) were fitted to a two-site model better than to a one-site model, suggesting the presence of multiple endothelin receptor

Table 2
Inhibition of [^{125}I]endothelin-1 binding to rat prostate by various compounds

	Control	Diabetic
Endothelin-1 K_i (pM)	50 ± 2	62 ± 7
Endothelin-3		
K_{iH} (pM)	418 ± 201	105 ± 14
K_{iL} (nM)	81 ± 9	225 ± 69
% R_H / R_T	25.6 ± 2.9	23.4 ± 1.7
BQ 610		
K_{iH} (pM)	22.1 ± 16.0	26.3 ± 11.1
K_{iL} (nM)	4422 ± 3536	3612 ± 2898
% R_H / R_T	78.3 ± 4.4	77.5 ± 3.5
IRL 1620		
K_{iH} (pM)	207 ± 195	374 ± 226
K_{iL} (nM)	3418 ± 1313	5114 ± 1517
% R_H / R_T	19.8 ± 2.9	11.0 ± 3.2

K_i, inhibition constant; K_{iH} and K_{iL}, inhibition constants for high- and low-affinity binding sites, respectively. % R_H / R_T is the proportion of high affinity to total binding sites. Values are mean ± S.E.M. of 3–4 separate experiments.

subtypes in the rat prostate. The inhibition constants (K_i values) and the proportions of high- and low-affinity binding sites for endothelin ET_A and endothelin ET_B receptors are shown in Table 2. The inhibition constants and proportions of endothelin ET_A and endothelin ET_B receptors are similar in the diabetic and control prostates.

4. Discussion

The impact of diabetes mellitus on biochemical, physiological and pharmacological aspects of mammalian prostate is poorly understood, due in part to the lack of adequate information regarding regulatory mechanisms involved in prostate function. Studies have demonstrated the presence of high concentrations of α-adrenergic (Hedlund et al., 1985), β-adrenergic (Fukumoto et al., 1993) and muscarinic acetylcholine (Fukumoto et al., 1993) receptors in the prostate of various species. Experimental diabetes has been shown to alter the biochemical properties of β-adrenergic and muscarinic acetylcholine receptors in 8- and 16-week rat prostate without affecting the subtype specificity of these receptors in these tissues (Fukumoto et al., 1993). The downregulation in the expression of autonomic receptors and the reduction in the prostate weight observed with diabetes can be prevented and reversed by early and late insulin treatment, respectively (Fukumoto et al., 1993). The presence of endothelins in human prostate and endothelin-1-induced contractile responses in this tissue suggest a possible functional role for endothelin receptors in this accessory organ (Langenstroer et al., 1993). Kobayashi et al. (1994) reported that human prostate contains a significant density of endothelin receptors that predominantly are of the endothelin ET_A receptor subtype (approximately endothelin ET_A : endothelin $ET_B = 2:1$), and that both endothelin ET_A and endothelin ET_B receptor subtypes mediate contraction in human prostate. Furthermore, using light microscopic autoradiography, Kobayashi et al. (1994) demonstrated that stroma and glandular epithelium in human prostate contain predominantly endothelin ET_A and endothelin ET_B receptor subtypes, respectively.

The present saturation data demonstrate that unlike autonomic receptors, endothelin receptors in the rat prostate are upregulated by the induction of experimental diabetes. This indicates that expression of endothelin receptors in the rat prostate is not directly correlated with serum testosterone levels whose low concentration has been implicated as a cause for lower prostate growth and downregulation of prostatic muscarinic receptors in streptozotocin-diabetic animals (Fukumoto et al., 1993). These data, however, do not rule out the possibility of an inverse relationship between serum testosterone levels and endothelin receptor expression in the rat prostate.

Using subtype-selective compounds, our inhibition studies demonstrated that the induction of diabetes did not significantly affect the subtype specificity and the composition of endothelin receptor subtypes in the rat prostate as the proportion of endothelin ET_A and endothelin ET_B receptor subtypes is shown to be similar in control and diabetic prostates. These findings suggest that diabetes may affect the quantitative rather than qualitative properties of endothelin receptors in the rat prostate.

In summary, the present investigation is the first to demonstrate that streptozotocin-induced diabetes increases the density of endothelin receptors in rat prostate without affecting endothelin subtype specificity in this tissue.

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