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ALTERATION OF PROTEIN KINASE C ACTIVITY IN DIABETIC RAT PROSTATE

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SUMMARY: Protein kinase C activity is present in rat prostatic epithelial cells in both cytosolic and membrane subcellular fractions. Partial purification by ion-exchange chromatography and characterization of cofactor requirements showed its behavior as a classical Ca^{2+}- and phospholipid-dependent enzyme activated by 1,2-diacylglycerol (or by mimicking agents such as tumor-promoting phorbol esters). Streptozotocin-induced diabetes resulted in an increase of the membrane/cytosolic enzyme ratio suggesting a redistribution of protein kinase C from the cytosolic to the membrane fraction (an index of enzyme activation) that could be reversed toward control conditions by insulin treatment. Differences observed in cofactor requirements for maximal enzyme activation argue for a some distinct expression of protein kinase C isozymes in control and diabetic conditions. These results are a new aspect of the complex set of alterations exhibited by the diabetic prostate in the signal transduction mechanisms that mediate cell functions and proliferation in this gland which could be related to the prostate atrophy and impaired fertility characteristic of this disease.

Protein kinase C is a family of phospholipid/Ca^{2+}-dependent phosphorylating enzymes that play a central role as a ubiquitous cellular mediator of signal transduction involving receptor-dependent hydrolysis of inositol phospholipids, which result in the regulation of cell metabolism, gene expression, proliferation and tumor promotion (1,2).

A limited number of reports have regarded the characterization of protein kinase C activity in the prostate. The basic properties of the enzyme have been studied in rat ventral prostate (3,4) as well as its enhancing after castration (4) and its possible role as receptor for tumor-promoting phorbol esters (5). These tumor-promoting agents attenuate the stimulating activity of vasoactive intestinal peptide (VIP) on cyclic AMP levels in rat prostatic epithelial cells (6)

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The abbreviations used are: STZ, streptozotocin; VIP, vasoactive intestinal peptide; PMSF, phenylmethylsulfonyl fluoride; TPA, 12-O-tetradecanoylphorbol 13-acetate; DG, 1,2-dioleoylglycerol; β-ME, β-mercaptoethanol; PS, phosphatidylserine.

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suggesting a link between the adenylyl cyclase and the prospholipid metabolizing pathways of signal transduction in this gland.

The impact of diabetes mellitus on prostatic function has received a limited attention in spite that there are many studies relating this important disease to reproductive dysfunction (7). Streptozotocin (STZ)-diabetic rats present decreased fertility with prostate alterations including atrophy (8), low androgen receptor content (9), high density of both neuropeptide Y and VIP nerve fibers (10), and underexpression of the components of the adenylyl cyclase system of signal transduction (guanine nucleotide regulatory proteins Gs and Gi, and adenylyl cyclase catalytic subunit) as studied through VIP receptor activation (11). The present investigation considered the other important arm of signal transduction and it was therefore undertaken to know whether protein kinase C activity is altered in prostate from STZ-diabetic rats and if so, whether it is normalized by insulin treatment.

MATERIALS AND METHODS

[γ-^{32}P] ATP (25 Ci/mmol) was purchased from New England Nuclear, and diethylaminoethyl cellulose (DE-52) from Whatman. Histone III-S, phosphatidylserine (PS), 1,2--dioleoylglycerol (DG), 12-O-tetradecanoilphorbol 13-acetate (TPA), bovine serum albumin and other biochemical reagents were supplied by Sigma.

Four-month-old male Wistar rats were made diabetic by ip injection of STZ (45 mg/kg b.w.) in 0.1 M citrate buffer (pH 4.5) after 24 hours of fasting. Control rats received the vehicle alone. Two weeks after, the animals were divided into three groups: control, diabetic and insulin-treated diabetic (this group was injected daily with two units insulin during the two following weeks). Four weeks after diabetes induction, the rats were killed, whole blood was collected for measurement of glucose levels by a glucose-oxidase method, and the ventral prostate was immediately taken.

Rat prostatic epithelial cells were isolated after suspension of the gland in a medium consisting of 2.5 mM EDTA, 1.4 % NaCl, and 1% bovine serum albumin, pH 7.5 (12). The cells were homogenized in 20 mM Tris-HCl buffer (pH 7.5) containing 2mM EDTA, 10 mM EGTA, 0.4 mM phenylmethylsulfonyl fluoride (PMSF), 10 mM β-mercaptoethanol (β-ME), and 0.01% trypsin inhibitor. The homogenate was centrifuged at 100,000 x g for 60 min for separating the cytosolic and the particulate fractions. Particulate material was solubilized in the same buffer containing 1% Triton X-100, separated from insoluble elements by a new centrifugation step, and taken as the membrane fraction.

Protein kinase C was purified from cytosolic and membrane fractions of rat prostatic epithelial cells by chromatography (13) in a DE-52 column (6x1cm) equilibrated with 20 mM Tris-HCl buffer (pH 7.5) containing 0.5 mM EDTA, 0.5 mM EGTA, 10 mM β-ME, 0.4 mM PMSF, and 0.01% trypsin inhibitor. After washing, enzyme elution was performed by applying a 0-0.4 M NaCl linear gradient in the same buffer at a flow rate of 0.65 ml/min. In some experiments, the enzyme purification was achieved in a stepwise manner employing 0.15 M NaCl in the equilibrating buffer. The active enzyme fractions were finally pooled and used.

Protein kinase C activity was assayed by measuring ^{32}P incorporation from [γ-^{32}P]ATP into histone (13). Briefly, the reaction mixture (50 µl) consisted of 56 mM Tris-HCl buffer (pH 7.5) containing 10 mM magnesium acetate, 5 mM NaF, 0.2% bovine serum albumin, 0.5 mg/ml histone III-S, 0.1 mM EGTA, 0.1 mM EDTA, 1.5 mM $CaCl_2$, 2 mM β-ME, 0.3 mg/ml PS, 14 µg/ml DG, 0.1 mM [γ-^{32}P]ATP (80-120 cpm/pmol), and the enzyme preparation (added in 10 µl containing 2-10 µg protein at a degree of purification of 5- to 10-fold). After

5 min at 30°C, the reaction was stopped by the addition of 0.2 ml of 30% trichloroacetic acid. After filtering through Whatman P-81 filter paper and washing, acid-precipitable material was counted for radioactivity. Specific protein kinase C activity was expressed as pmol Pi/mg protein/min. Protein concentration was determined (14) using bovine serum albumin as a standard. Results are expressed as mean ± S.E.M. Statistics were carried out using the Student's *t* test. The level of significance was set at $p<0.05$.

RESULTS

STZ-treated rats exhibited the characteristics of the diabetic state (Table 1). With time, control rats showed weight gain whereas STZ injection reduced body weight although diabetic rats exhibited hyperfagia and polydipsia. Diabetes was also accompanied by a decreased prostate weight and severely increased blood glucose levels. The interval chosen for insulin treatment of diabetic rats was not enough for a total normalization of these parameters.

Fig.1 shows the chromatographic elution profiles of protein kinase C activity from either cytosolic and membrane fractions of control rat prostatic epithelial cells. The application of a linear gradient of NaCl to the DE-52 cellulose column gave a main peak of enzyme activity around 0.1 M NaCl in both subcellular fractions that was stimulated by the addition of Ca^{2+}, PS and DG. Protein kinase C activity eluting at high salt concentrations (0.20-0.25 M NaCl) was insensitive to Ca^{2+} and PS and it could correspond to proteolytic fragments that result irreversibly activated, as observed in other systems. As indicated in Methods, protein kinase C activity was routinely eluted from the column with 0.15 M NaCl.

Table 2 shows the relative distribution of protein kinase C activity in the cytosolic and membrane fractions from rat prostatic epithelial cells in the three experimental groups studied. The specific protein kinase C activity, as measured by phosphorylation of histone III-S, decreased by 56% in the cytosolic fraction of diabetic rats and returned partially to control values after insulin treatment.

The cofactor requirements of protein kinase C activity in control, diabetic, and insulin-treated diabetic prostatic epithelial fractions are shown in Fig.2. The data were normalized to the

Table 1. Characteristics of experimental animals

	Control (n = 13)	Diabetic (n = 12)	Insulin-treated diabetic (n = 13)
Initial BW (g)	484±16	442±5	444±6
Final BW (g)	530±19	378±12	409±9
Prostate wt (g)	0.71±0.02	0.49±0.02	0.50±0.03
Blood glucose (mM)	5.5±0.2	34.3±3.2	14.6±2.9

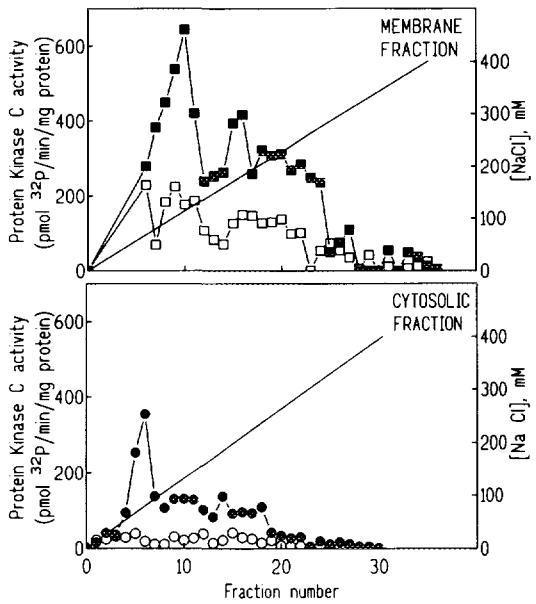

Figure 1. Chromatographic elution profile of protein kinase C activity from rat prostatic epithelial cells. The cytosolic and membrane subcellular fractions were partially purified in a DE-52 cellulose column by applying a linear gradient of NaCl, as described in Methods. The fraction volume was 1 ml. Aliquots of 0.01 ml were assayed for protein kinase C activity in the absence (open symbols) and presence (solid symbols) of Ca^{2+}, PS and DG. The data correspond to control rats but experiments performed with diabetic and insulin-diabetic animals gave qualitatively similar results.

results obtained without Ca^{2+}, PS and DG. The simultaneous presence of the three agents was needed for optimal stimulation of the enzyme activity in all subcellular fractions considered. A similarly maximal activation of protein kinase C was achieved when using the tumor-promoter TPA instead of DG. However, some differences in the relative values obtained in the various subcellular fractions could be seen: a)cytosolic and membrane fractions showed similar Ca^{2+}/PS/DG requirements only in control conditions; b)no activation of protein kinase C could be obtained without DG or TPA in the membrane fraction of diabetic rats; and c)TPA

Table 2. Subcellular distribution of protein kinase C activity. Specific activity values in either membrane or cytosolic fractions from prostatic epithelial cells of control, diabetic and insulin-treated diabetic rats were normalized for protein concentration of the DE-52 cellulose fraction and expressed as a percentage of total (membrane + cytosolic fractions) activity. Twelve rats were used in each group.

Specific activity (%)	Control	Diabetic	Insulin-treated diabetic
Cytosolic fraction	64.8±5.0	28.5±2.7	55.2±1.6
Membrane fraction	35.2±5.1	71.5±2.6	44.8±1.6

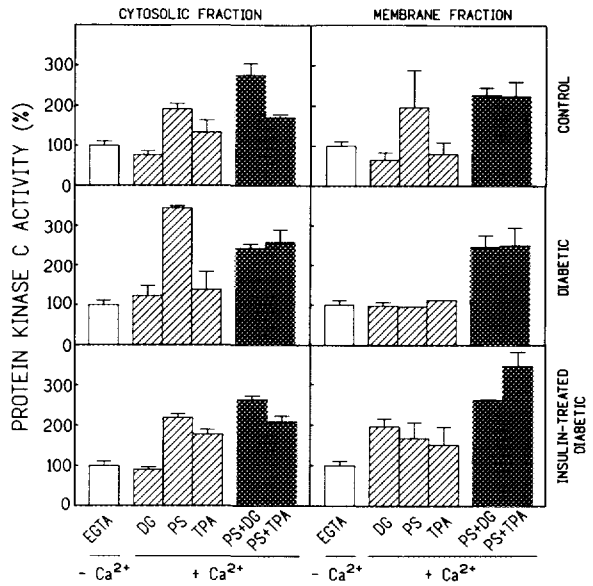

Figure 2. Cofactor requirements for protein kinase C activation in rat prostatic epithelial cells. After purification of cytosolic and membrane fractions by DE-52 cellulose chromatography, protein kinase C activity was determined in the absence or presence of 1.5 mM Ca^{2+}, 0.3 mg/ml PS, 14 µg/ml DG or 1µM TPA as indicated. The corresponding basal enzyme activities in the absence of Ca^{2+} or any other cofactor were taken as 100% to normalize the results. Data are the mean±S.E.M. of 3-6 experiments performed in triplicate.

was maximally effective, even more than DG, in the membrane fraction from insulin-treated diabetic rats.

DISCUSSION

The present study confirms the presence of Ca^{2+}/PS-dependent protein kinase C activity in rat ventral prostate, and shows particularly that the epithelial cells of the gland express this enzyme. Furthermore, it proves that streptozotocin-induced diabetes leads to a higher protein kinase C activity relative level in the membrane fraction and a lower one in the cytosolic fraction as compared with those in the controls, whereas chronic insulin treatment of diabetic rats results in an intermediate pattern. Finally, some differences in the cofactors required for maximal activation of the enzyme were evidenced when comparing the three groups of animals considered.

Protein kinase C activity represents at least nine related isozymes requiring different cofactors and/or exhibiting characteristic kinetic patterns and tissue distribution (1,2). A previous study performed on extracts of whole rat ventral prostate partially purified on a hydroxyapatite column suggested that PKC is mainly present at this level as α and β (probably β_1 and β_2) isoforms which behave as typically Ca^{2+}-dependent enzymes. Present results obtained in

cytosolic and membrane fractions from isolated rat prostatic epithelial cells confirm and extend that observation that is common to classical studies on the characterization of protein kinase C subtypes in rat brain and their dependence on the simultaneous presence of Ca^{2+}, PS and DG for maximal activation (15). The use of histone III-S as phosphorylating substrate in the present study is a good condition for determining this group of protein kinase C isozymes but not other new members of the family (15,16) which could represent a significant fraction of protein kinase C activity in rat prostatic epithelial cells. Furthermore, studies as the present one involving chromatography on DEAE-cellulose columns use cytosolic fractions containing substantial amounts of DG and other lipids that could modify protein kinase C activity (17). With respect to the enzyme activation by the tumor-promoting phorbol ester TPA, it is a well-known feature consistent with other reports on the activation of protein kinase C by this exogenous DG analog (18).

The main observation of the present study is that diabetes appears to result in a redistribution of protein kinase C from the cytosolic to the membrane fraction, by that increasing the membrane/cytosolic enzyme ratio, whereas insulin treatment could reverse this tendency toward the restoration of control conditions. Similar patterns of protein kinase C redistribution have been observed in liver (19), heart (20) and renal glomeruli (21) from STZ-diabetic rats. However, the significance of this finding remains unclear at this time and it needs further study. The relative increase in membrane-bound protein kinase C activity in the diabetic prostatic epithelial cell could be related to increases of either cytosolic Ca^{2+} that binds to the cell membrane, or PS and/or DG levels in the membrane which lead the enzyme to the final active conformation (1,2). There are various observations on such changes in some diabetic tissues that could contribute to an increase in membrane protein kinase C activity (22,23). In this regard, preliminary results from our laboratory on the *in vitro* [1-C^{14}]acetate incorporation into different lipid subclasses by rat prostatic epithelial membranes show similar diacylglycerol levels in membranes from either control, diabetic and insulin-treated diabetic rats (data not shown).

Alternate interpretations of present findings must also be considered besides the translocation of the enzyme from the cytosolic to the membrane fraction. Diabetes could result in an altered pattern of protein kinase C isozyme expression in prostatic epithelium, which is according to the observed differences in cofactor requirements between subcellular fractions from control, diabetic and insulin-treated diabetic preparations. Besides the present phosphorylation assays, further studies using specific antisera in Western blot analysis are in progress in our laboratory to determine the protein mass for each protein kinase C isozyme. Whatever the mechanisms, the present finding on the increase in the membrane/cytosolic protein kinase C ratio (an index of enzyme activation) in the prostatic epithelium from diabetic rats is

interesting due to the involvement of the enzyme in transducing extracellular signals from many hormones, neurotransmitters, growth factors and drugs, which regulate cellular functions and proliferation (1,2). This result together previous reports on the decrease of Gs/Gi/adenylyl cyclase proteins (11) and androgen receptors (9) in prostate from STZ-diabetic rats show a complex array of changes in the signal transduction machinery at this level that may be related to the atrophy of the prostate gland and the impairment of the fertility in this disease.

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