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# Differential regulation of prostatic protein kinase C isozymes by androgens

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Multiple isozymes of $Ca^{2+}$/phospholipid-dependent protein kinase (PKC) were isolated from the rat ventral prostate. The enzyme exists mainly as type II ($\beta$), and type III ($\alpha$) forms, and it is possible that type II isozyme may comprise the subspecies $\beta_1$ and $\beta_2$. The total and specific activities of prostatic PKC isoforms were reduced in castrated animals; this decrease was specific since administration of androgens to castrated animals reversed such a decline. Also, there was a differential response to androgen deprivation so that type III isozyme declined at a faster rate than that of type II. Thus, our studies show for the first time that PKC of the rat ventral prostate comprises multiple isozymes, and that the activity of these various forms are differentially regulated by androgens.

$Ca^{2+}$/phospholipid-dependent protein kinase; $Ca^{2+}$/phospholipid-dependent protein kinase isozyme; Ventral prostate; Androgen

## 1. INTRODUCTION

It is well known that the growth and function of the rat ventral prostate is under strict androgenic control, and therefore it is considered an excellent model for investigating the mechanism of androgen action [1]. The prostate gland produces several growth factors such as prostate-derived growth factor, basic fibroblast growth factor, etc. [2–5] and their production is also reported to be under androgenic controls [6,7]. Several of these growth factors elicit their physiological responses via receptor-mediated hydrolysis of inositol phospholipids which is essential for the activation of $Ca^{+}$/phospholipid-dependent protein kinase (PKC) [8,9]. More recently, other steroid hormones such as estrogen [10], and vitamin $D_3$ [11] were found to activate PKC directly. Thus, it was hypothesized that PKC may also be under androgenic regulation in the prostate. To this end, the present work was undertaken to investigate the androgenic regulation of PKC isozymes in the rat ventral prostate. Our results indicate that the rat ventral prostatic PKC exists in multiple forms ($\alpha$, $\beta_1$, and $\beta_2$). The total and specific activities of prostatic PKC isozymes were decreased in castrated animals. Furthermore, the various forms of PKC were found to be differentially regulated by androgens.

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## 2. MATERIALS AND METHODS

### 2.1. *Partial purification and separation of PKC isozymes*

#### 2.1.1. Buffers

Buffer A = 20 mM Tris/HCl, pH 7.5, 2 mM EDTA, 10 mM EGTA, and 10 mM $\beta$-mercaptoethanol ($\beta$ME), buffer B = 20 mM Tris/HCl, pH 7.5, 0.5 mM EDTA, 0.5 mM EGTA, protease inhibitors, and 10 mM $\beta$ME; and buffer C = 20 mM potassium phosphate, 0.5 mM EGTA, 10 mM $\beta$ME, and 10% glycerol. All buffer solutions contained the following protease inhibitors: leupeptin (5.0 $\mu$g/ml); benzamidine (0.5 mM); and phenylmethylsulfonyl fluoride (0.5 mM).

The ventral prostates from fifty 3-month-old sham-operated (control) or orchiectomized rats (20–25 g) were pooled, and a 20% homogenate was prepared in buffer A containing 0.32 M sucrose. Nuclei were removed by low-speed centrifugation and the supernatant was centrifuged at $105\,000 \times g$ for 60 min. The pellet (membranous fraction) was extracted with buffer A containing 0.1% NP-40 detergent on ice for 30 min and centrifuged for 60 min at $105\,000 \times g$. The two supernatants were pooled and applied to a DEAE-52 column (1.6 × 10 cm) pre-equilibrated in buffer B. After washing the column thoroughly with buffer B, a 300-ml gradient of NaCl (0–400 mM) in buffer B was applied to the column. Fractions containing PKC activity were pooled, and applied directly to a DNA-grade hydroxyapatite (Bio-Rad) column (0.9 × 10 cm) pre-equilibrated in buffer C. The column was washed with buffer C and a 200-ml gradient of potassium phosphate (20–250 mM) in buffer C was applied to the column. Fractions were assayed for PKC activity. Isozymes were well-separated on this column and the active fractions under each peak were pooled and assayed for protein, DNA, and PKC activity.

### 2.2. *Determination of protein kinase C activity:*

Measurement of enzyme activity was carried out at 30°C for 0, 3, and 6 min after addition of the enzyme according to published procedure [2]. The reaction mixture consisted of 20 mM Tris/HCl buf-

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fer, pH 7.5, 10 mM magnesium acetate, 0.1 mM [$\gamma$-$^{32}$P]ATP (spec. act. = 100–500 dpm/pmol), 1 mM EGTA, 1.2 mM $CaCl_2$, 0.04% NP-40, 50 $\mu$g/ml PtdSer, 10 $\mu$g/ml Diolein, 1 mg/ml histone H1, and protein kinase C. PKC activity was expressed as nmol $^{32}$P incorporated in histone/h per mg of enzyme protein in the presence of $Ca^{2+}$/phospholipid minus that in the absence of $Ca^{2+}$/phospholipid.

2.3. *Other methods*

Rats were orchiectomized as described by us [12] and examined 1, 2, 3, and 7 days post-orchiectomy. Sham-operated animals served as normal intact controls. Protein and DNA were assayed as described by us earlier [13,14].

## 3. RESULTS AND DISCUSSION

A profile of PKC isozymes from prostatic extract (cytosol + membrane) fractionated on hydroxyapatite column is shown in Fig.1. It appears that multiple forms of PKC are present in the rat ventral prostate. Two major peaks of PKC with almost equal activities were present, and both peaks were stimulated up to 20-fold in the presence of $Ca^{2+}$/phospholipids. Type II (isozyme $\beta$), and Type III (isozyme $\alpha$) forms of PKC were eluted at 100 mM and 150 mM potassium phosphate, respectively. There was also indication of an additional isozyme located between peaks I and II (which is eluted at 125 mM potassium phosphate). Also, the absence of Type I or isozyme $\gamma$ (which is eluted from the column at 70 mM potassium phosphate) is consistent with previous reports on its specific association with the central nervous system [1]. The effect of androgen withdrawal for periods of 1, 2, 3, and 7 days on the distribution and activity of the various PKC isozymes is shown in Fig. 1A–D and Table I. A modest decline (10–15%) in total and specific activities in both peaks I and II of PKC was observed 24 h after castration (Table I, and Fig.1A). However, the total as well as specific activities of PKC isozymes were significantly reduced in the prostatic tissue from rats 2 days or longer after castrated, when compared with sham-operated animals. For example, in the prostatic tissue from rats 2 days after castration, the total and specific activities of peak II declined by 54% while that of peak I decreased by 30% as a result of castration (Table I and Fig. 1B). Thus, it appears that there was a differential effect of androgen withdrawal on the activities of PKC isozymes so that type III isozyme is more sensitive to androgen deprivation than type II. At 3 and 7 days post-orchiectomy, the total and specific activities of both peaks I and II were further reduced as compared with controls (Table I and Fig.1C, D). For example, the specific activity of peaks I and II declined

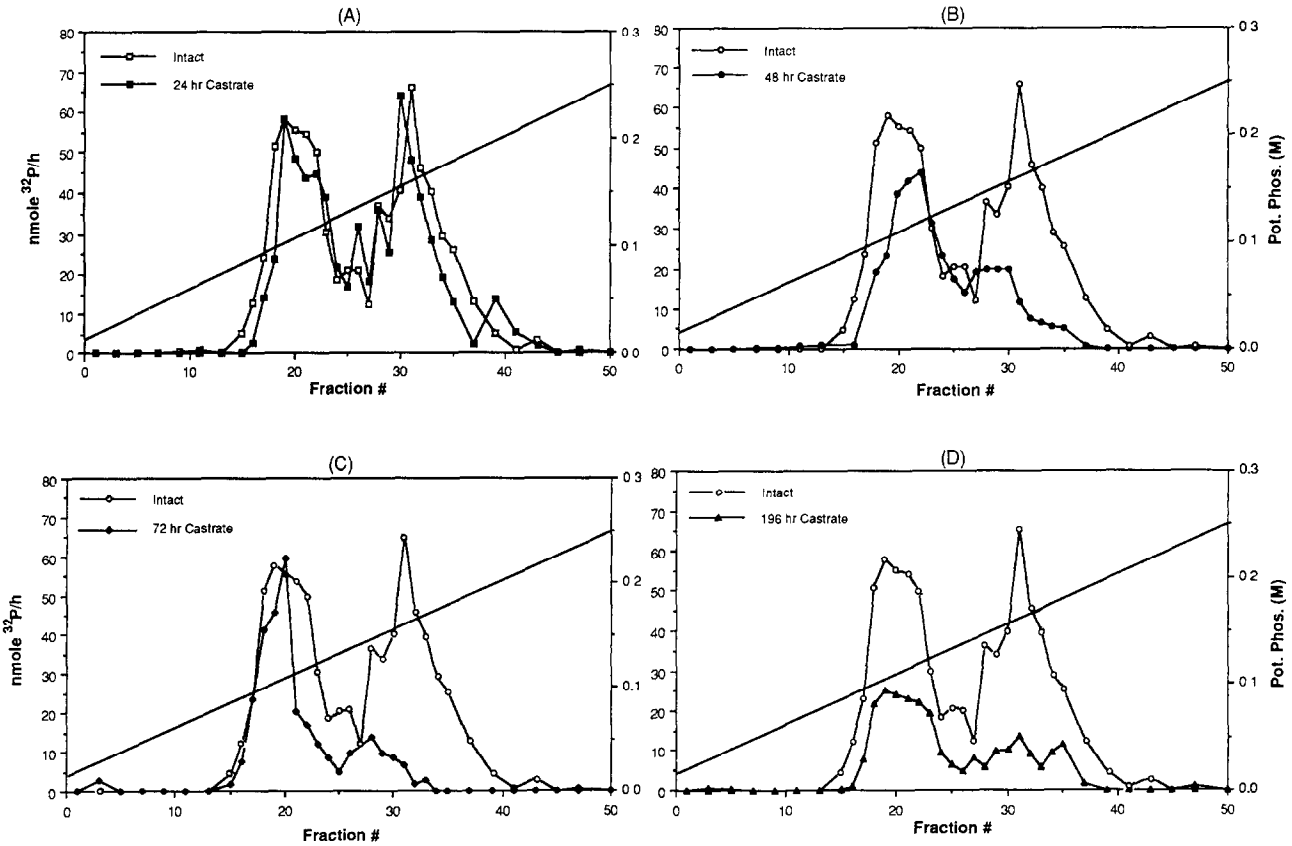


Fig.1. Hydroxyapatite column chromatographic profile of PKC isolated from rat ventral prostate. Prostatic tissue from sham-operated or castrated rats was used to isolate PKC isozymes. Column chromatographic profiles of PKC isozymes from prostates of rats 24 h (A), 48 h (B), 72 h (C), and 196 h (D) post-castration are shown. PKC isolated from sham-operated rats served as the control in each panel.

Table I

Total and specific activity of PKC isozymes of rat ventral prostate: effects of castration

| Days after castration | DNA (mg) | Total activity ($\mu$mol$^{32}$P/h) | | Spec. act. ($\mu$mol$^{32}$P/mg DNA per h) | |
|---|---|---|---|---|---|
| | | PK I | PK II | PK I | PK II |
| 0 | 16.3 (100)* | 418 (100) | 683 (100) | 25.6 (100) | 41.9 (100) |
| 1 | 16.2 (99) | 345 (83) | 596 (87) | 21.3 (83) | 36.8 (88) |
| 2 | 14.7 (90) | 254 (61) | 308 (45) | 17.3 (68) | 21.0 (50) |
| 3 | 13.1 (80) | 158 (38) | 174 (26) | 12.1 (47) | 13.3 (32) |
| 7 | 6.5 (40) | 65 (16) | 54 (8) | 10.0 (40) | 8.3 (20) |

* Values in parentheses are expressed as % of the corresponding control. Data are representative of three different experiments. 50 rats were used for each time point. Controls are sham-operated rats. Total PKC activity is activity per 50 prostates. Injection of 5$\alpha$-DHT dissolved in sesame oil (1 mg/100 g b. wt.) into castrated rats maintained the total and specific activity of peaks I and II of PKC at the normal levels

by 60% and 70%, respectively, at 3 days. The total activity for both peaks declined to 30–40% of that of control. The administration of 1 mg of 5$\alpha$-dihydrotestosterone per 100 g of body weight to previously castrated rats reversed the decline in these activities, suggesting that the aforementioned changes in prostatic PKCs are functions of alteration in the androgenic status in the animals. These results document for the first time the regulation of PKC isozymic pattern by androgens. In this regard, it was reported recently that other steroids also regulate PKC activity [10,11]. For example, chronic treatment of ovarectomized rats or normal pituitary cells with estrogens significantly increased the total PKC activity. The amounts of both soluble and membrane-bound forms of the enzyme were equally increased by the active but not by the inactive estrogen analogue [10]. Also, it was reported that 1,25-(OH)$_2$ D$_3$ induced differential induction of PKC isozymes in HL-60 cells where the steroid enhanced the transcriptional activity of the gene for PKC$\beta$ and to a lesser extent the gene for PKC$\alpha$ [11]. It is noteworthy that this induction was independent of phospholipid hydrolysis and diacylglycerol generation. This is important since steroid hormone receptors are not localized at the cell surface and therefore are not coupled to phospholipid turnover. Whether androgens regulate the PKC isozyme pattern in a similar manner to vitamin D$_3$ and estrogens as described above, or that androgens differentially activate existing PKC isozymes awaits further investigation.

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