# Isoforms of Protein Kinase C and Their Distribution in Human Adrenal Cortex and Tumors

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The cytosol and microsomal fractions of human adrenal cortex contain 3 isoforms of protein kinase C: α, ζ, and ε. The latter fraction is present in trace amounts. No isoforms $\beta_1$, $\beta_2$, γ and δ were found in these cell fractions. The distribution of α-isoform between the cytosol and microsomal fraction is determined by tissue origin: in normal tissue its content differs by no more than 10%, while in most tumors this isoform is translocated into the microsomal fraction. The distribution of ζ-isoform did not depend on tissue origin.

**Key Words:** *adrenal cortex; protein kinase C; proteinase C isoforms*

The role of protein kinase C (PKC) in the regulation of adrenocortical function is little studied. It was found that corticotropin can induce activation and translocation of PKC in the adrenals [5,9], but the major role in corticotropin signal transduction is played by cAMP-dependent protein kinase [14]. Activation of aldosterone synthesis in high-potassium incubation medium also leads to PKC translocation to the membrane fraction [10]. Angiotensin II activated and ACTH suppressed phosphorylation of endogenous MARCKS (myristoylated, alanine-rich C kinase substrate) in aldosterone-synthesizing adrenocortical granular cells [3]. However, possible involvement of MARCKS in the regulation of aldosterone synthesis was never investigated. PKC phosphorylates cytochrome $P\text{-}450_{SCC}$ catalyzing the most important reaction of steroidogenesis in the adrenal cortex, but this does not change the rate of steroidogenesis [13].

Activity $Ca^{2+}$-dependent PKC was evaluated for human adrenal tissue [8]. No differences in the PKC activities in the tumor, hyperplastic and normal tissue were found. PKC-α, -β, and -γ isozymes in human adrenal tissue were measured using monoclonal antibodies [12]. In all samples PKC-α was found in the cytosol fraction; no translocation of the enzyme from the cytosol into the membrane fraction was detected in adrenocortical tumors [12]. In medullary tumors (pheochromocytoma and neuroblastoma) PKC was present mainly in the membrane fraction.

Here we evaluated PKC isoforms in human adrenal tissue and the relationship between the disease and enzyme distribution.

## MATERIALS AND METHODS

Adrenal samples were obtained from 8 patients operated at the Surgery Department (Institute of Endocrinology): 6 tumor samples and 2 samples of hyperplastic tissue (Itsenko—Cushing disease). Fragments of apparently unchanged tissue removed with the tumors were studied as normal tissue.

Monoclonal antibodies to PKC-α, -$\beta_1$, -$\beta_2$, -γ, -ε, -δ and -ζ (Sigma) were used in Western blot analysis. Second peroxidase-labeled antibodies and reagents for chemiluminescent imaging were from ECL kits (Amersham Life Science). Homogenate of rat brain containing all PKC isoforms was used as the positive control. The tissues were homogenized and fractionated as described previously [1]. The cytosol and microsomal fractions obtained after 1-h centrifugation at 105,000*g* were used. Electrophoresis was carried out according to Laemmli. Proteins were transferred onto

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nitrocellulose membranes. The membranes were hybridized with first antibodies to PKC isoforms, and visualized using ECL kits. Densitometry was carried out with photographs of processed nitrocellulose replicas and the total intensity of protein staining with Coumassi G250 in PAAG was measured for each preparation without replica transfer. The results were processed using Scion Image software (Scion Corporation).

## RESULTS

Of 7 studied PKC isoforms, only 3 isozymes (α, ζ, and ε) were detected in human adrenal cortex (Fig. 1). Immunoblotting revealed no PKC-$\beta_1$, -$\beta_2$, -γ, and -δ in 11 samples of the adrenal cortex and tumors, although some scientists reported the presence of these isoforms [4]. The expression of α- and ζ-isoenzymes were similar, while PKC-ε was weakly expressed (Fig. 1).

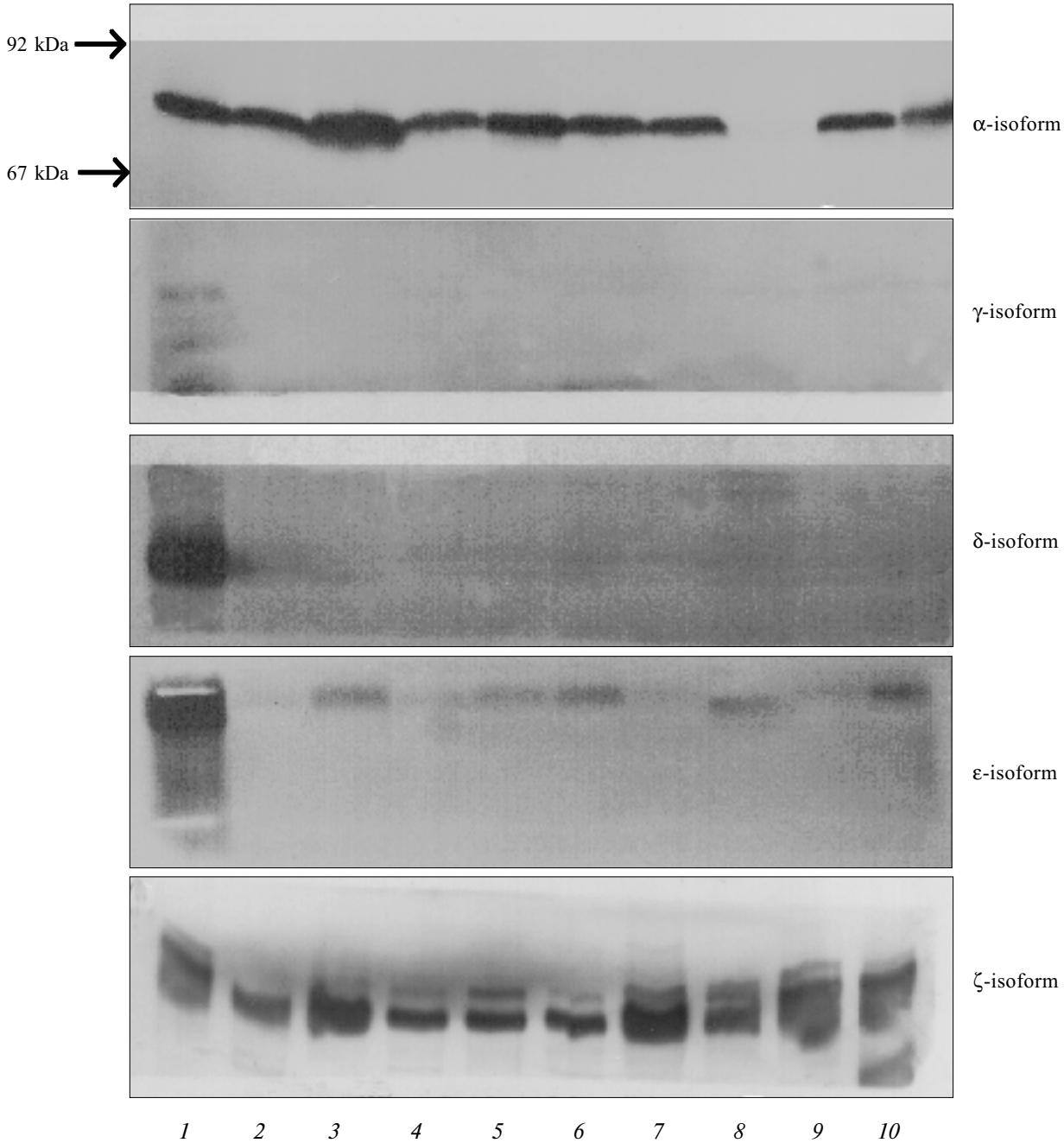


**Fig. 1.** Immunoblots of some protein kinase C isoforms in the cytosol fraction of human adrenal cortex and tumors. *1*) homogenate of rat brain, positive control; *2, 6*) Itsenko—Cushing disease; *3*) malignant paraganglioma; *4*) corticosteroma; *5, 7, 9*) normal human adrenal cortex; *8, 10*) aldosteroma (aldosteroma cytosol fraction was erroneously not added into well No. 8 for detection of α-isoform). Each well contains about 50 μg protein.

**TABLE 1.** Relative Content of PKC-α and PKC-ζ Isoforms (in %) in Subcellular Fractions of Human Adrenal Cortex and Tumors

| Sample No. | Tissue | PKC-α | | PKC-ζ | |
|---|---|---|---|---|---|
| | | cytosol | microsomes | cytosol | microsomes |
| 1 | Normal | 50.56 | 49.44 | 70.60 | 29.40 |
| 2 | Normal | 45.20 | 54.80 | 51.97 | 48.03 |
| 3 | Normal | 45.44 | 54.56 | 73.33 | 26.67 |
| 4 | Adrenal cortex, Icenko—Cushing disease | 54.58 | 45.42 | | |
| 5 | Adrenal cortex, Icenko—Cushing disease | 32.84 | 67.16 | 68.94 | 31.06 |
| 6 | Aldosteroma | 48.79 | 51.21 | 45.71 | 54.29 |
| 7 | Paraganglioma | 24.69 | 75.31 | 23.83 | 76.17 |
| 8 | Corticosteroma | 33.95 | 66.05 | 53.88 | 46.12 |
| 9 | Corticosteroma | 35.48 | 64.52 | 57.81 | 42.19 |
| 10 | Corticosteroma | 12.90 | 87.10 | 70.41 | 29.59 |
| 11 | Corticoblastoma | 17.32 | 82.68 | 79.35 | 20.65 |

In 3 samples of normal adrenal cortex, PKC-α was almost equally distributed between the cytosol and microsomal fractions and the content of this isoform differed by no more than 10% (Table 1). In 5 various tumors we observed pronounced translocation of PKC-α into the microsomal fraction (Table 1). The absence of translocation in Icenko—Cushing disease (sample 4) is not surprising, because adrenocortical hypertrophy is caused by corticotropin hyperproduction, but not cell degeneration. The presence of visually indiscernible tumor fragments can be hypothesized in sample 5. It is interesting to compare samples 1 and 8 obtained from the same adrenal: in apparently normal tissue PKC-α was evenly expressed in the cytosol and microsomal fractions, while in tumor tissue this isoform was translocated into the microsomal fraction.

Three variants of PKC-ζ distribution were distinguished: even distribution, translocation into the membrane fraction, and translocation into the cytosol (never detected for α-isoform). No relationship between PKC-ζ distribution and tumor characteristics was detected.

PKC-α and -ζ are expressed in human ovarian cancer cells [7]. *In vitro* treatment of these cells with 12-O-tetradecanoylphorbol-13-acetate reduced the expression of PKC-α (but not PKC-ζ) and increased cell sensitivity to platinum drugs. The authors emphasized the role of α-isoforms in the modulation of cell sensitivity. However, the inhibition of PKC-α in cells originating from malignant glioblastomas had no effect on cell proliferation, while inhibition of PKC-ζ decreased it [6]. Interestingly that prolactin, acting as a mitogen in the adrenal cortex, activated PKC in the cytosol and microsomal fractions long before accumulation of diacylglycerol in cells [11]. *In vitro* activation of PKC with prolactin, but not with ACTH was demonstrated in porcine adrenal cell nuclei [2]. The role of individual PKC isoforms and their translocation in the pathogenesis of tumors was analyzed for a long time [4], but the problem is still unclear.

These data indicate that adrenocortical tumors are characterized by pronounced translocation of PKC-α into the microsomal fraction. No relationship between tissue characteristics and enzyme distribution was detected for PKC-ζ.

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