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THE ACTIVITY OF A BETA SUBTYPE OF PROTEIN KINASE C PURIFIED FROM NUCLEI OF HUMAN NEUTROPHILS IS ENHANCED BY TREATMENT WITH PHORBOL 12-MYRISTATE 13-ACETATE

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Two protein kinase C isoenzymes were partially purified from the nuclei of human neutrophils, and identified as beta and alpha subtypes. Treatment of neutrophils with phorbol 12-myristate 13-acetate (PMA) caused a 3.8-fold increase of nuclear βPKC activity, while a minor increase of αPKC was observed. This selective activation of βPKC could help to understand the molecular events involved in phorbol ester-induced cellular modifications.

Protein Kinase C (PKC), the cellular target of tumour promoters phorbol esters, is a family of isoenzymes which is involved in cellular physiological and pathological processes, e.g. cell growth, cell differentiation, tumour promotion. This enzyme is mostly present in the cytosolic fraction of many cell types (1); treatment with phorbol esters can cause a redistribution of the enzyme to the particulate fraction (2). Several research groups evidenced the presence of specific PKC isoforms in the nuclei of different cell types(3,4,5,6). However, the mechanism of action of this enzyme at nuclear level is still unclear. A better knowledge of the role of PKC subtypes in cell nuclei could help to explain most of the recent findings

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suggesting a linkage between mitogen stimulation, PKC activation and gene/oncogene expression (7,8,9,10,11).

The aims of the investigation presented in this paper were: (I) to partially purify PKC from the nuclei of human normal neutrophils (NTR); (II) to separate and identify the isoenzymes of nuclear PKC; (III) to assess whether the treatment of NTR with PMA could modify the pattern of the enzyme subtypes at nuclear level.

Methods

Cells and cell nuclei.
Neutrophils were isolated from buffy coats extracted from the venous blood of healthy donors by centrifugation in a differential gradient of sodium metrizoate and Dextran (Polymorphprep, Nycomed Pharma, Oslo Norway), according to Ferrante and Thong (12)
Cells obtained from the previous step were exposed for 10 minutes either to 200 nM PMA at 37°C in RPMI 1640 medium, or to RPMI 1640 alone.
NTR nuclei (from $3x10^9$ cells) were purified following the method described by Haddock Russel et al.(4)

Partial purification of PKC isoforms.
Purified nuclei, as well as intact NTR were placed in Homogenization buffer (20 mM Tris-HCl,pH 7.5, 0.25M sucrose, 10mM EGTA, 2mM EDTA, 1 mM phenylmethylsulfonyl fluoride, 20mg/ml Leupeptin, 1% NP 40), and lysed by sonication. Disrupted nuclei and cells were centrifuged at 100.000g for 60 minutes.
The supernatant obtained from the previous step was applied to a DEAE-Sepharose column (Pharmacia, Italy), previously equilibrated with Buffer A (20 mM Tris-HCl, pH 7.5, 0.5 mM EGTA, 0.5 mM EDTA, 10 mM 2-mercaptoethanol). The column was washed with 2 volumes of buffer A and 15 volumes of buffer A containing 20 mM NaCl. PKC activity was eluted by the application of 5 volumes of buffer A containing 120 mM NaCl. This eluate was applied to a hydroxylapatite (HA) column; PKC isoforms were eluted with the application of a linear gradient of potassium phosphate according to the conditions described by Shearman et al (13).

PKC assay.
80-1ml fractions were collected from HA column and tested for PKC activity as described by Kikkawa et al.(14).No PKC activity was detected when Calcium, phosphatidylserine and diacylglycerol were not present in the enzyme-assay reaction mixture.

Identification of PKC isozymes with specific antibodies.
Concentrated samples from the eluted peaks were spotted onto nitrocellulose paper. After saturation with 5% bovine serum albumin in phosphate buffered saline the blot was exposed to isozyme-specific anti PKC monoclonal antibodies. Immunogold

(Amersham, Italy) silver staining of the blot was performed according to the manufacturer's instructions.

Results and Discussion

The presence of two PKC isoforms has been detected in human neutrophils (Fig.1); the elution pattern from HA column shows that the two peaks are eluted at about 90 mM and 140 mM potassium phosphate, which correspond respectively to the elution concentrations of rat brain type II (beta subtype) and type III (alpha subtype) PKC (13).

The study performed on nuclei isolated from both untreated and PMA-treated neutrophils evidenced the presence of nuclear PKC isozymes, which were resolved with HA column chromatography into two peaks corresponding to the elution conditions of β and α PKC (Fig.2).

Immunoblot analysis, performed with specific monoclonal antibodies, confirmed that the peaks of activity either in whole

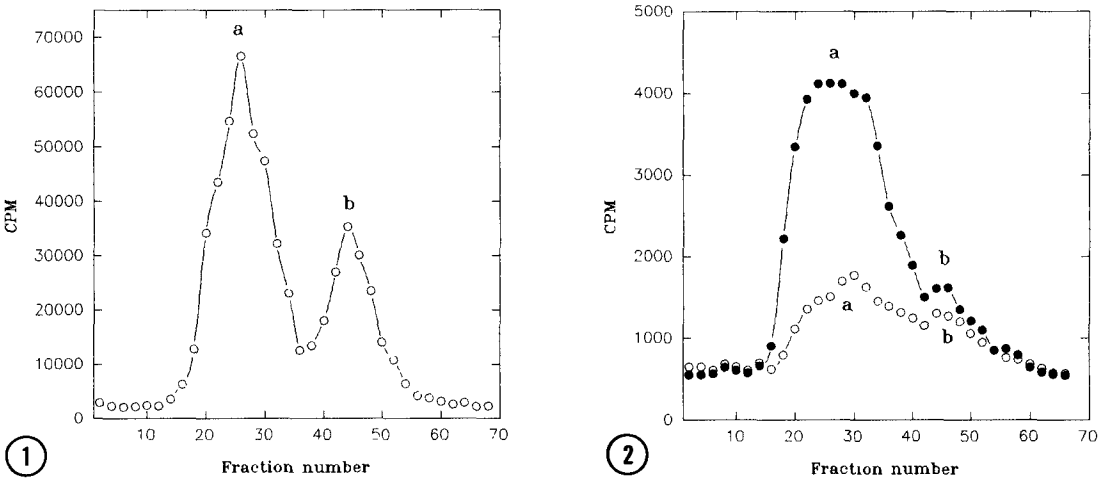

Fig.1. Hydroxylapatite chromatography of human neutrophil Protein kinase C. The two peaks of activity shown in this plot have been identified with specific antibodies to be beta (peak a) and alpha (peak b) PKC.

Fig.2. Separation of beta (peak a) and alpha (peak b) PKC subtypes from nuclei of PMA-treated (filled circles) and untreated (hollow circles) human neutrophils.

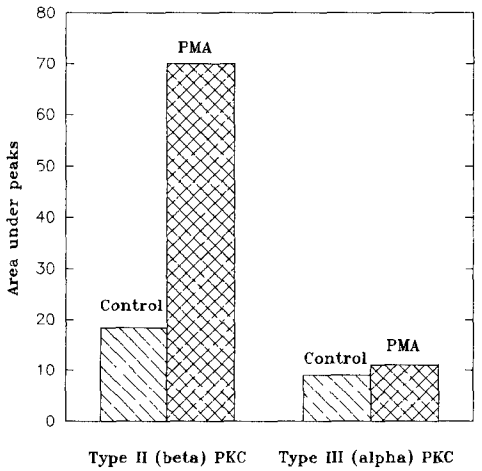

Fig.3. PMA-induced selective activation of PKC subtypes. The vertical axis range represents, in arbitrary scale, the definite integral of the single peaks of calcium activated, phospholipid dependent PKC activity.

cells or in cell nuclei correspond to β and α PKC activity (not shown in this paper). These findings are in agreement with the observations of Pontremoli et al., on whole human neutrophils (15).

The peaks of activity of PKC isoenzymes purified from cell nuclei of both untreated and PMA-stimulated cells were measured and compared (Fig.3). A marked increase in βPKC activity (3.8 fold) was observed in PMA treated cells. No major variations were observed when the activities of the alpha subtypes were compared.

Our data confirm in an experimental model consisting of PMA-treated and untreated neutrophils, recent observations of other research groups in coltures of tumour cell lines: the treatment with phorbol esters caused the association of PKC to the nuclear fraction of NIH 373 (5) and of HL 60 cells (6).

Taken together, the results of our investigations lead to postulate a major role of nuclear βPKC in the genesis of phorbol esters-induced changes in the cell environment, and a possible involvement of this isoenzyme in the regulation of gene expression.

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