Volume 290, number 1,2, 115–118 FEBS 10178 September 1991

ADONIS 0014579391008893

Immunological demonstration of εPKC

Murine tissue distribution, ontogeny, cellular localization and translocation

Michael Gschwendt, Hanno Leibersperger, Gabriele Rincke and Friedrich Marks

Institute of Biochemistry, German Cancer Research Center, Im Neuenheimer Feld 280, D-6900 Heidelberg, Germany

Received 3 July 1991

An antiserum raised against an εPKC-specific peptide recognizes εPKC with an apparent molecular weight of 97 kDa in cytosol of mouse brain. No cross-reaction with α,β,γPKC or the δPKC-like p76-kinase is observed. εPKC is mainly present in brain. Just traces of this PKC isoenzyme can be detected in some other murine tissues. Ontogenetic studies indicate that the amount of εPKC in murine brain increases constantly and reaches a maximal level at day 7 after birth. Upon TPA activation εPKC is translocated from the cytosol to the particulate fraction in a brain homogenate.

ε-Protein kinase C; Antiserum; Mouse brain; Ontogeny; Translocation

1. INTRODUCTION

Many proteins involved in signal transduction belong to families of closely related enzymes. Each individual protein within such a family might be involved in a specific pathway.

Protein kinase C (PKC), one of the key enzymes of phospholipase-C-mediated signal transduction, also consists of a family of several related enzymes [1]. Molecular cloning analysis has revealed that the PKC family can be subdivided into two major groups. Conventional α,β and γPKC contain a putative Ca-binding domain C2 [1], whereas δ,ε and ζPKC are lacking this domain [2,3]. The latter finding was in accord with our earlier report on a Ca^{2+}-unresponsive PKC-like kinase in murine epidermis and spleen [4,5]. Recently, we were able to purify this kinase from porcine spleen. The novel enzyme does indeed not respond to Ca^{2+} and appears to be a PKC of the δ-type [6,7]. Very recently, the cDNA of ηPKC, a new member of the Ca^{2+}-independent subgroup of the PKC family, was cloned and sequenced [8]. The novel enzyme exhibits an about 60% sequence homology to εPKC. Another PKC cDNA (PKC-L) described by Bacher et al. [9] appears to be identical with ηPKC. εPKC was expressed in transfected COS cells [2,3] as well as in transfected insect cells [10] and was purified from these cells. εPKC mRNA was found to be expressed predominantly in brain [2,1].

Here we show by means of an εPKC-specific antiserum that murine εPKC is present above all in brain, and there mainly in the cytosol, and that the enzyme is translocated from the cytosol to the particulate fraction upon TPA activation.

Abbreviations: PKC, protein kinase C; TPA, 12-*O*-tetradecanoylphorbol-13-acetate; SDS-PAGE, SDS-polyacrylamide gel electrophoresis; ELISA, enzyme-linked immunosorbent assay.

Correspondence address: M. Gschwendt, Institute of Biochemistry, German Cancer Research Center, Im Neuenheimer Feld 280, 6900 Heidelberg, Germany.

2. MATERIALS AND METHODS

2.1. *Materials*

12-*O*-Tetradecanoylphorbol-13-acetate (TPA), Freund's adjuvans (Sigma, Munich, Germany); the oligopeptide DEAIVKQINQEEFK was synthesized by Novabiochem (Laufingen, Switzerland).

2.2. *Antiserum*

An antiserum was raised against the oligopeptide DEAIVKQINQEEFK in rabbits. The amino acid sequence of the oligopeptide corresponded to the sequence 712–725 of εPKC [2,3]. The oligopeptide was coupled to thyroglobulin using carbodiimide as described by Harlow and Lane [12]. Female rabbits (Chincilla-Bastard, 2.5–3 kg) were used for immunization as described previously [7].

2.3. *Preparation of cytosol from various murine tissues*

Female NMRI mice (age 7–8 weeks) were used in all experiments. After sacrifice of the animals, tissues were dissected, immediately frozen in liquid nitrogen and stored at −70°C. The tissues were homogenized in Tris-EDTA buffer (50 mM Tris, pH 7.5, 1 mM EDTA) and the homogenate was centrifuged at 100 000 × *g* for 30 min. The supernatant was termed cytosol.

2.4. *Translocation of εPKC in a mouse brain homogenate*

One mouse brain was homogenized in 4 ml Tris-EDTA buffer with an Ultraturax homogenizer. 2 ml each of the homogenate were incubated with 20 μl of acetone and 20 μl of 100 μM TPA in acetone, respectively, at 0–2°C for 10 min. Then the homogenate was separated by centrifugation at 100 000 × *g* for 30 min into cytosol and particulate fraction. The particulate fraction was extracted with 2 ml of sample buffer (10 mM $NaKHPO_4$, pH 7.2, 5% SDS, 10% β-mercaptoethanol,

10% glycerol) and both the cytosol and the particulate extract were applied to SDS-polyacrylamide gel electrophoresis (SDS-PAGE).

The following procedures were performed as described previously: purification of α,β,γPKC from mouse brain [5], purification of p76-kinase (δ-type PKC) from porcine spleen [7], enzyme-linked immunosorbent assay (ELISA) [7], immunoblot [5], phosphorylation of cytosol proteins [7], SDS-PAGE [13], determination of protein [14].

3. RESULTS AND DISCUSSION

εPKC belongs to a recently discovered subgroup of related PKC isoenzymes designated δ,ε,ζ [2,3] and η [8]. Recently, we described the purification and characterization of a δ-type PKC from porcine spleen (p76-kinase; [7]). This was the first enzyme of these PKC subspecies purified to homogeneity from a tissue. The δ-type PKC was found to be almost ubiquitous in different species and murine tissues [15]. Particularly high concentrations were observed in epithelial tissues, such as epidermis. The enzyme was barely detectable in the cytosol but appeared to be restricted to the particulate fraction.

The aim of this study was to compare the εPKC with the δ-type PKC regarding tissue distribution and cellular localization. For this purpose an antiserum was raised in rabbits against a synthetic peptide with a sequence corresponding to the COOH-terminal residues 712–725 of εPKC (εpeptide). The specificity of the anti-εpeptide antiserum is demonstrated in Figs. 1 and 2A. In an enzyme-linked immunosorbent assay (ELISA) the anti-εpeptide antiserum reacts in a dose-dependent manner with the εpeptide, but not with α,β,γPKC from

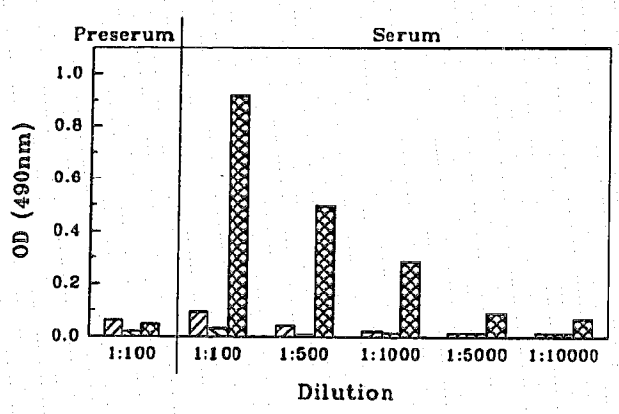

Fig. 1. Enzyme-linked immunosorbent assay (ELISA) of the antiserum raised against a peptide of εPKC. The ELISA was performed as described previously [7]. Purified PKC (type α,β,γ) from mouse brain (left, 0.15 μg protein/well), purified δ-type PKC from porcine spleen (center, 0.15 μg protein/well) and the εpeptide (right, 6.25 μg/well) were used to coat the microtiter plates. Pre-immune serum (preserum) served as a control.

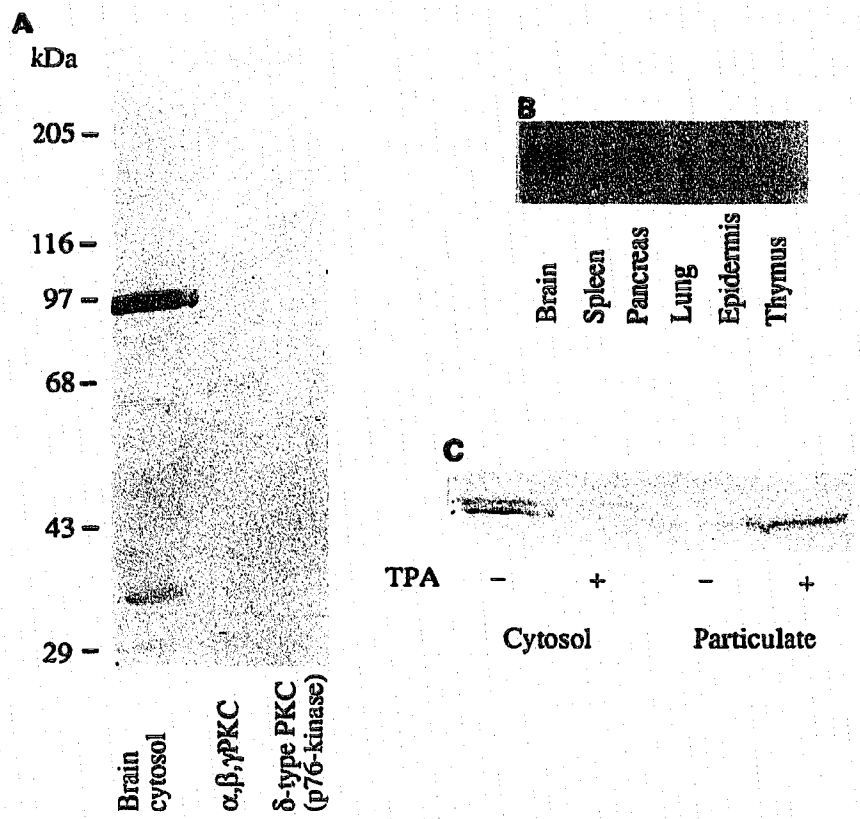

Fig. 2. Immunoblots with the ε-peptide antiserum. A. Murine brain cytosol, purified α,β,γPKC from mouse brain and purified δ-type PKC from porcine spleen. B. Cytosols of various murine tissues. C. Cytosol and particulate fraction of a mouse brain homogenate after incubation with or without 1 μM TPA at 0–2°C for 10 min (see section 2). Cytosols and particulate fractions (100 μg protein each) as well as the purified enzymes (0.15 μg protein each) were applied to SDS-PAGE and the immunoblots were performed as described in section 2. Molecular masses were determined from standard proteins: myosin (205 kDa), β-galactosidase (116 kDa), phosphorylase b (97 kDa), bovine serum albumin (68 kDa), ovalbumin (43 kDa), and carbonic anhydrase (29 kDa).

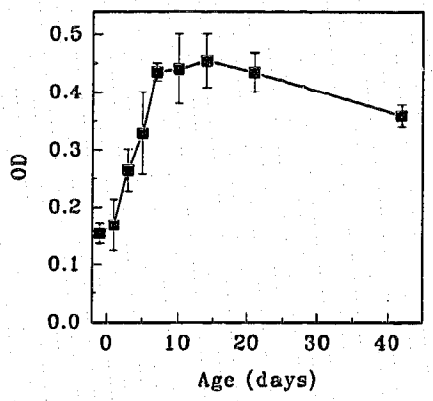

Fig. 3. Ontogeny of the εPKC in mouse brain. Brain was taken from mice on various days after birth (see abscissa). Cytosols (100 μg of protein) were applied to SDS-PAGE and immunoblots were performed using the anti-εpeptide antiserum and an ^{35}S-labelled anti-rabbit-IgG instead of the peroxidase-conjugated antibody, as described in section 2. The autoradiograms of the immunoblots were quantitated by densitometry.

mouse brain or the δPKC-like p76-kinase from porcine spleen (Fig. 1). The pre-immune serum does not react with any of the presented antigens (α,β,γPKC; p76-kinase, εpeptide). Immunoblots of murine brain cytosol using this antiserum showed a protein doublet around 97 kDa, co-migrating with the marker protein phosphorylase b (Fig. 2A). The immunostained doublet appears to be characteristic for εPKC, as reported previously by Strulovici et al. [16]. The evidently higher molecular weight of εPKC as compared to the other PKC isoenzymes (around 80 kDa or lower) facilitates an unequivocal identification of the immunostained band with εPKC. Upon immunoblotting neither α,β,γPKC nor the δPKC-like p76-kinase are recognized by the antiserum. After phosphorylation of brain cytosol proteins with [γ-^{32}P]ATP in the presence of phosphatidyl serine and TPA, the 97-kDa-protein is labelled, indicating an autophosphorylation of εPKC (data not shown).

When we examined the cytosol (100 μg of protein) of several murine tissues, εPKC was found to be present predominantly in brain (Fig. 2B). This is in agreement with the almost exclusive expression of mRNA for εPKC in brain [2,8,11]. Traces of εPKC can be detected in lung and pancreas, especially when freshly prepared total tissue extracts are examined (data not shown). Other tissues, such as spleen, epidermis and thymus (7-day-old mice) do not contain εPKC in amounts demonstrable by this method (Fig. 2B). εPKC can still be detected by immunoblotting in a 10-fold diluted brain cytosol (10 μg of protein; data not shown). Brain of newborn mice also contains εPKC. The enzyme concentration increases after birth and reaches a maximal level at around day 7 after birth (Fig. 3). Conversely, the amount of the δ-type PKC in mouse brain [15] and similarly of γPKC in rat brain [17] does not change significantly up to day 7 after birth and then increases dramatically. After treatment of a mouse brain homogenate with 1 μM TPA at 0–2°C for 10 min with subsequent separation of the homogenate into a cytosolic and a particulate fraction, the concentration of εPKC is reduced in the cytosol and increases in the particulate fraction (Fig. 2C). While in the cytosol two protein bands are immunostained in the 97 kDa range (doublet), apparently only the lower band becomes visible in the particulate fraction after immunostaining. In untreated samples εPKC is located predominantly in the cytosol. This is in contrast to the δ-type PKC [7,15] which is located almost exclusively in the particulate fraction. Our observation of the TPA-induced in vitro translocation of εPKC is in agreement with data of Akita et al. [18] on the phorbol ester-induced translocation of εPKC in transfected COS cells and of Kiley et al. [19] on the phorbol ester-induced translocation of εPKC in rat pituitary cells. On the other hand, Heidenreich et al. [20] were unable to show translocation of εPKC upon treatment of cultured neurons with TPA or insulin.

Taken together, δ and εPKC, both Ca^{2+}-unresponsive subspecies of the PKC family, differ significantly with respect to their murine tissue distribution, cellular localization and ontogeny. The special roles of these isoenzymes in the various tissues and cell compartments remain to be elucidated. Since PKC is thought to play an essential role in the phorbol ester-induced tumor promotion in mouse skin, it is intriguing that concerning the Ca^{2+}-unresponsive PKC subspecies mouse epidermis lacks εPKC and instead contains the δPKC-like p82-kinase which resembles the p-76-kinase of porcine spleen [7,15].

REFERENCES

[1] Nishizuka, Y. (1988) Nature 334, 661–665.
[2] Ono, Y., Fujii, T., Ogita, K., Kikkawa, U., Igarashi, K. and Nishizuka, Y. (1988) J. Biol. Chem. 263, 6927–6932.
[3] Ohno, S., Akita, Y., Konno, Y., Imajoh, S. and Suzuki, K. (1988) Cell 53, 731–741.
[4] Gschwendt, M., Kittstein, W. and Marks, F. (1986) Biochem. Biophys. Res. Commun. 137, 766–774.
[5] Gschwendt, M., Kittstein, W., Horn, F., Leibersperger, H. and Marks, F. (1989) J. Cell. Biochem. 40, 295–307.
[6] Gschwendt, M., Leibersperger, H. and Marks, F. (1989) Biochem. Biophys. Res. Commun. 164, 974–982.
[7] Leibersperger, H., Gschwendt, M. and Marks, F. (1990) J. Biol. Chem. 265, 16108–16115.
[8] Osada, S., Mizuno, K., Saido, T.C., Akita, Y., Suzuki, K., Kuroki, T. and Ohno, S. (1990) J. Biol. Chem. 265, 22434–22440.
[9] Bacher, N., Zisman, Y., Berent, E. and Livneh, E. (1991) Mol. Cell. Biol. 11, 126–135.
[10] Schaap, D. and Parker, P.J. (1990) J. Biol. Chem. 265, 7301–7307.
[11] Schaap, D., Hsuan, J., Totty, N. and Parker, P.J. (1990) Eur. J. Biochem. 191, 431–435.
[12] Harlow, E. and Lane, D. (1988) Antibodies: a Laboratory Manual, pp. 84–85, Cold Spring Harbor Laboratory, Cold Spring Harbor, NY.
[13] Horn, F., Gschwendt, M. and Marks, F. (1985) Eur. J. Biochem. 148, 533–538.

[14] Lowry, O.H., Rosebrough, N.J., Farr, A.L. and Randall, R.J. (1951) J. Biol. Chem. 193, 265–275.
[15] Leibersperger, H., Gschwendt, M., Gernold, M. and Marks, F. (1991) J. Biol., Chem. in press.
[16] Strulovici, B., Daniel-Issakani, S., Baxter, G., Knopf, J., Sultzman, L., Cherwinski, H., Nestor Jr., J., Webb, D.R. and Ranson, J. (1991) J. Biol. Chem. 266, 168–173.
[17] Yoshida, Y., Huang, F.L., Nakabayashi, H. and Huang, K.P. (1988) J. Biol. Chem. 263, 9868–9873.
[18] Akita, Y., Ohno, S., Konno, Y., Yano, A. and Suzuki, K. (1990) J. Biol. Chem. 265, 354–362.
[19] Kiley, S., Schaap, D., Parker, P., Hsieh, L.-L. and Jaken, S. (1990) J. Biol. Chem. 265, 15704–15712.
[20] Heidenreich, K.A., Toledo, S.P., Brunton, L.L., Watson, M.J., Daniel-Issakani, S. and Strulovici, B. (1990) J. Biol. Chem. 265, 15076–15082.