Vol. 165, No. 1, 1989
November 30, 1989

BIOCHEMICAL AND BIOPHYSICAL RESEARCH COMMUNICATIONS
Pages 533-538

CELL TYPE-SPECIFIC EXPRESSION OF THE GENES FOR THE PROTEIN KINASE C FAMILY: DOWN REGULATION OF mRNAS FOR PKC α AND nPKC ε UPON IN VITRO DIFFERENTIATION OF A MOUSE NEUROBLASTOMA CELL LINE NEURO 2a*

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Received October 23, 1989

ABSTRACT: By the use of cloned cDNAs for protein kinase C isozymes α, β I, β II, γ, and those for novel protein kinase C, ε and ζ, the expression of the corresponding mRNA species was examined in various mouse tissues, human lymphoid cell lines, and mouse cell lines of neuronal origin. In adult brain, mRNAs for all the isozymes of PKC family are expressed. However, the expression of these mRNA species in brain is low at birth. A similar pattern of expression was also observed for β I/β II mRNAs in spleen. These expression patterns are in clear contrast to that for β I/β II mRNAs in thymus where the mRNAs are expressed at birth and the levels of expression decrease with age. Human lymphoid cell lines express large amounts of PKCβ mRNAs in addition to PKCα. Further, nPKCε mRNA is expressed in some of these cell lines. On the other hand, all the mouse cell lines of neuronal origin tested express nPKCε and ζ in addition to PKCα. In a mouse neuroblast cell line, Neuro 2a, down modulation of mRNAs for both PKCα and nPKCε was observed in association with in vitro differentiation.

Tumor-promoting phorbol esters and the naturally occurring 1,2-diacylglycerols have been shown to utilize the Ca^{2+}- and phospholipid-dependent protein kinase, protein kinase C (PKC), to mediate signal transduction in a variety of cellular processes such as cell proliferation and differentiated functions (1). Recent molecular cloning experiments in conjunction with the biochemical characterization of mammalian brain PKC have established the presence of four distinct PKC types (α, β I, β II, and γ) which are encoded by three distinct genes, α, β, and γ. The four conventional PKC types share closely related sequences and similar biochemical properties including kinase and phorbol ester-binding activities (2-7). More recently, a cDNA related to cDNAs for conventional PKCs was isolated and the encoded protein, nPKCε, has been characterized as a novel phorbol ester receptor/protein kinase distinct from the conventional PKCs (8). The presence of two additional PKC-related cDNAs, which encode proteins more related to nPKCε, have also been identified (nPKCδ and ζ) (9).

* Supported in part by a Grant-in-Aid for Cancer-Bioscience from the Ministry of Education, Science and Culture of Japan.

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0006-291X/89 $1.50

The expression of mRNAs for these PKC family was examined in several rabbit and rat tissues and shown to be tissue specific (2-5, 7,8). Immunochemical analyses using antibodies specific to each of the conventional PKCs also showed the difference in the tissue specificity of these PKC types at the protein level (10). Further, immunocytochemical analyses of brain has shown that PKCβ I, β II, and γ are expressed in different cell types (11). These observations indicate that PKC isozymes are expressed in a cell type-specific manner and that the specificity differs among PKC isozymes. In order to investigate the physiological function of each conventional PKC and nPKC, it is essential to establish a cultured cell system in which the expression of each PKC and nPKC isozymes can be characterized. It is also important to know whether the cell type-specificity of the expression is determined at the level of mRNA expression or not. Here we have found that many cell lines contain different sets of mRNAs for PKC and nPKC isozymes and that the expression alters during maturation of tissues as well as during cell differentiation in vitro.

MATERIALS AND METHODS

Cells: Cell lines used in this study were as follows: Daudi, human B-lymphoblast; MOLT4B, human T-lymphoblast; U937, human macrophage leukemia; NIE-115, NS20Y, Neuro-2a, mouse neuroblastoma; G-26, mouse glioma. For differentiation experiments, Neuro-2a cells were treated with various reagents for two days as described (4) and used for the preparation of poly(A) RNA.

RNA preparation and Northern hybridization: Extraction and purification of poly(A) RNA from mouse tissues from at least 6 bodies (Balb/c) and human and mouse cell lines were carried out according to the published procedure (4). Northern blots were made following the published procedure (4). Each lane contained poly(A) RNA equivalent to 100μ g of total cellular RNA. DNA fragments were labeled by a multi-priming procedure and used as hybridization probes. The filters were finally washed twice with 0.3x SSC, containing 0.1 % SDS at 55℃ for 20 min. DNA probes used were as follows: PKCα, nuc. no. from -204 to 2647 (4); PKC β I/β II, -221 to 1963 of PKCβ II (4); PKCγ, 239 to 2304 (5); nPKCε, 203 to 2168 (7); nPKC, sequence corresponding to the kinase domain (Kubo, K., et al. submitted for publication).

RESULTS AND DISCUSSION

Tissue and stage specific expression of PKC and nPKC mRNA species. Previous experiments on the cDNAs for PKC family of rabbit, human, bovine and rat have revealed a strong conservation of the occurrence, size and the tissue distribution of these PKC mRNA species as well as the primary sequences among these mammalian species (2-4). In order to examine whether the tissue distribution is also conserved in mouse and to examine further the oncogenic change in expression, we analyzed PKC and nPKC mRNA species in mouse tissues at various growth stages by using the corresponding rabbit cDNAs as hybridization probes. We first screened the expression in 7 mouse tissues (brain, heart, liver, lung, kidney, spleen, and thymus) obtained at various stages of growth (0, 1, 2, 3, 4, and 12 weeks) using slot-blot hybridization. RNA samples which gave positive signals were then confirmed by Northern blot analyses. The tissue distribution of mRNAs from adult tissues (12 weeks) was very similar to that previously reported for PKCs and nPKCs of rabbit or rat. Characteristic results are shown in Fig 1. Each of the rabbit cDNA probes for PKCα, β I/β II, γ, and nPKCε specifically recognized the corresponding mouse mRNA species with the same size as that of rabbit brain. On the other hand, the cDNA for nPKCζ recognized two mRNAs with different sizes from that of rabbit, in clear contrast to other members

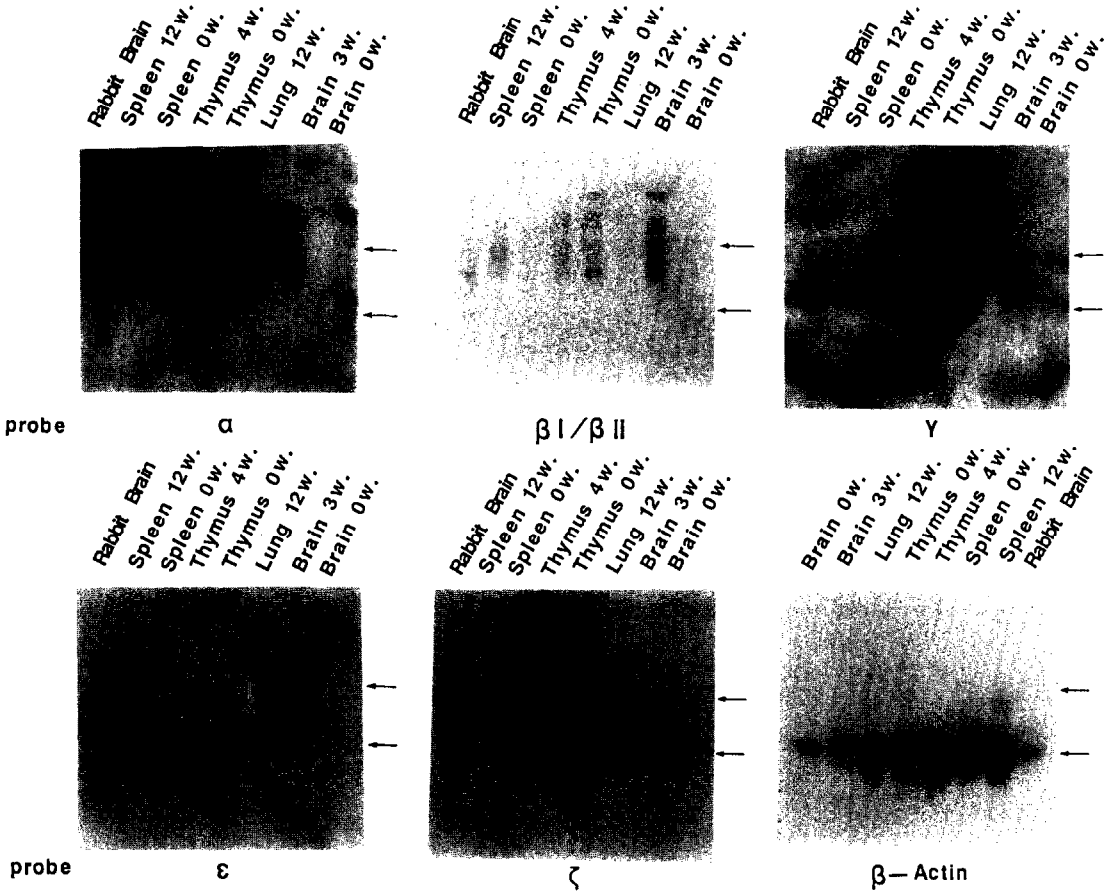

Fig 1. Northern hybridization analysis of poly(A) RNA from mouse tissues. Poly(A) RNAs were extracted from mouse tissues of 0, 3, 4, or 12 weeks as indicated. Arrows indicate 28S and 18S ribosomal RNAs. Each lane contains poly(A) RNA equivalent to 100 μ g of total RNA. As a positive control, rabbit brain total RNA (3 μ g) was used.

of the PKC family. The sizes of the mouse nPKC ζ mRNAs closely resembled those of rat brain (2.5 and 4kb). In adult (12 w) brain, mRNAs for all the PKC isozymes were highly expressed. On the other hand, no PKC mRNA species was detected in brain obtained from mice at birth. Similarly in spleen, mRNAs for PKC α and β I/β II were detectable only at 3 weeks of age; the levels of these mRNAs in spleen at birth were quite low if any at all existed. In clear contrast to these two tissues, thymus contained mRNAs for PKC α, β I/β II, and nPKC ζ at birth and the levels decreased with age. These results indicate that the expression of these mRNAs is stage-specific, with the expression level correlating with maturation of the tissue. A similar observation has been reported for rat PKC γ using type-specific antibodies (10). Taken together, these results suggest that the expression of PKC and nPKC isozymes is mainly regulated at the level of mRNA expression. It is clear that mRNAs for PKC α and β I/β II are expressed in many tissues examined, while those for PKC γ (brain only) and nPKC ε (brain, lung, and spleen) are tissue specific. Since tissues contain discrete sets of cell types, these results suggest that each cell type contains a different set of PKC and nPKC mRNA species or that different sets of cells express different sets of PKC and

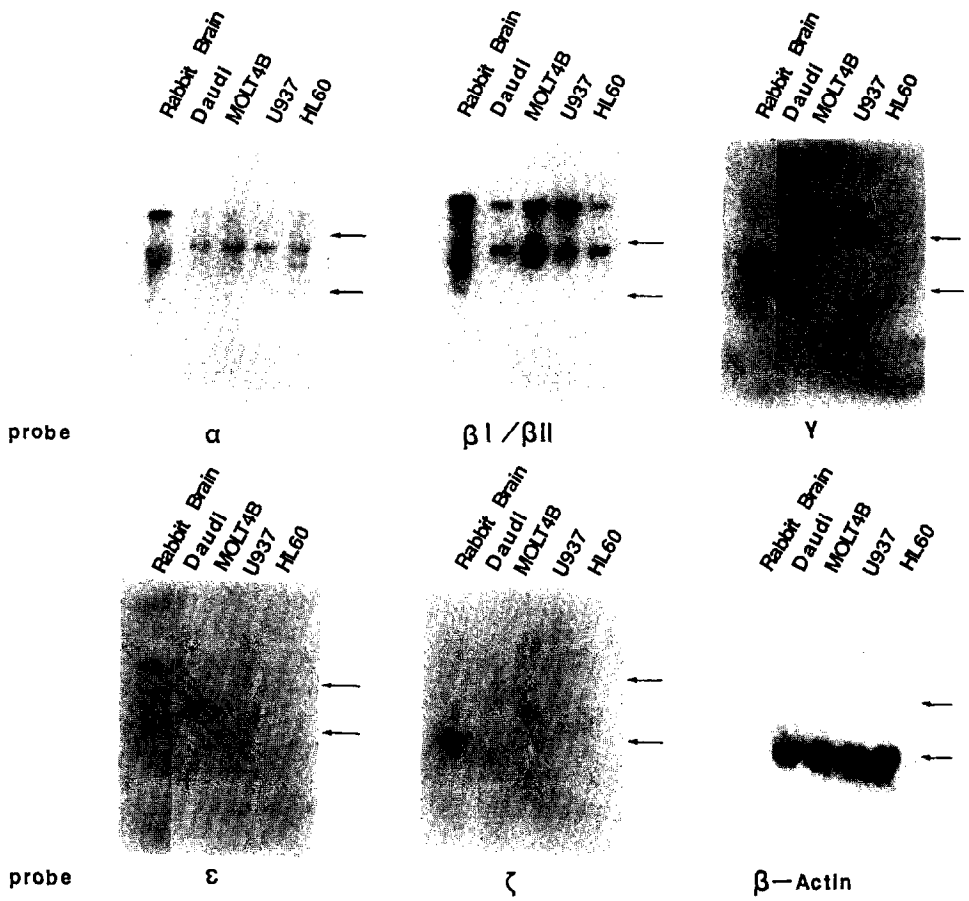

Fig 2. Northern hybridization analysis of poly(A) RNA from cloned human lymphoid cell lines. See legend to Fig.1.

nPKC mRNA species. Thus we next examined the expression of the PKC and nPKC mRNA species in cloned cell lines.

Cell type-specific expression of PKC and nPKC mRNA species. Many types of PKC and nPKC mRNAs are highly expressed in neuronal and immune tissues. Thus we analyzed expression in several human lymphoid cell lines. As shown in Fig 2, all the cell lines tested contain both β I/β II and α PKC mRNAs. Further, some of the cell lines, Daudi and U937, contain nPKCε mRNA. MOLT4 also contains trace amounts of nPKCε mRNA. On the other hand, no line contains detectable levels of either PKCγ or nPKCζ mRNAs. It is intriguing that cell lines such as U937 and Daudi contain at least three members of the PKC family, two PKCs and nPKCε. Fig 3 shows the results of similar experiments using mouse cell lines of neuronal origin. All the cell lines tested contain mRNAs for PKCα, nPKCε, and nPKCζ, while those for PKCβ I/β II and PKCγ were not detected. These results indicate that these cell lines do not cover all the cell populations of brain tissue, since mRNAs for PKCβ I/β II and PKCγ are highly expressed in brain.

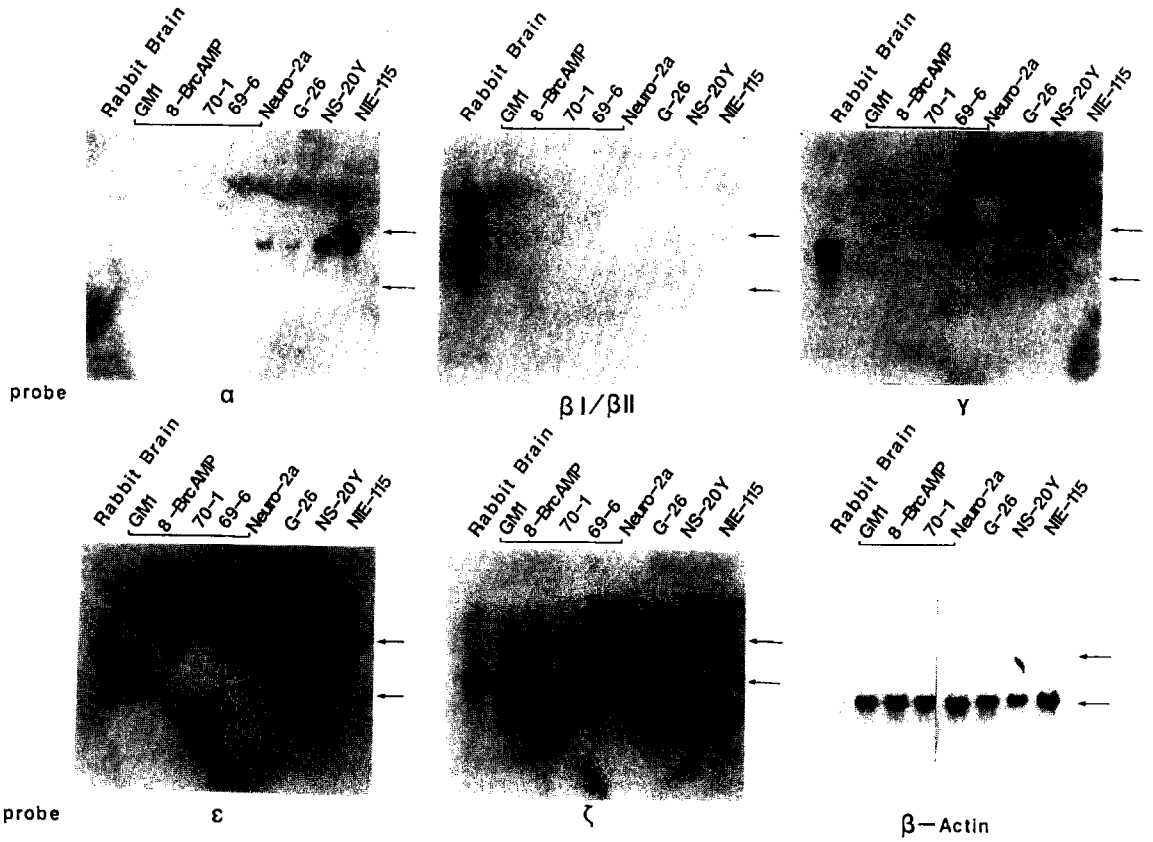

Fig 3. Northern hybridization analysis of poly(A) RNA from cloned mouse cells of neuronal origin. GM1, 8BrcAMP, 70-1, 69-6 indicate RNA samples obtained from Neuro 2a cells which were cultured 2days in the presence of GM1, 8BrcAMP, β -alkyl glycerol ether, and α -alkyl glycerol ether, respectively. See legend to Fig 1.

Down-modulation of mRNAs for PKC α and nPKC ε upon in vitro differentiation of a mouse neuroblastoma cell line Neuro 2a The addition of various compounds to a mouse neuroblastoma cell line, Neuro 2a, has been reported to cause it to differentiate in vitro (12). The extent and state of differentiation depends on the compound used. The differentiated state is characterized by neurite formation. Since PKC is suggested to be involved in neuronal functions, it is quite interesting to analyze the correlation between the expression of PKC and nPKC mRNA species and neurite outgrowth. Interestingly, the level of expression of PKC α and nPKC ε in Neuro 2a cells was down-modulated during cell differentiation (Fig.3), whereas nPKC ζ , which is not proven to possess phorbol ester binding activity, was only detected. This system might provide a clue to the function of these PKC molecules, and could also be used to analyze the regulatory mechanisms of the expression of mRNAs for PKC and nPKC species.

In the present study, we showed that the expression of the mRNAs for the five members of the PKC multi-gene family depends on the tissue and growth stage. The recent observation that the expression of mRNAs for three conventional PKCs in brain increases with development and growth is consistent with the present study (13). We have also shown that the expression levels of some of the

PKC mRNA species (β I/β II and γ) apparently correlate with the growth stage (maturation) of the tissue (brain, spleen, and thymus), suggesting that these PKCs might be involved in functions specific to these tissues. These results are consistent with the previous observation obtained using type-specific antibodies (10). This suggests that the tissue and stage-specific expression of PKC and nPKC isozymes is determined at the level of mRNA expression. The present study also shows that cloned cell lines of immune and neuronal origin contain different sets of PKC family, strongly supporting the notion that each PKC molecule is involved in specific functions. The finding that many lymphoid and neuronal cell lines contain nPKC mRNAs is important because these cells may prove useful in analyzing the function of each PKC molecule in intact cells.

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