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Localization of PDK-1 mRNA in the Brain of Developing and Adult Rats

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YOSHIDA, S., SAKAGAMI, H., OWADA, Y., KOKUBUN, S. and KONDO, H. *Localization of PDK-1 mRNA in the Brain of Developing and Adult Rats.* Tohoku J. Exp. Med., 1999, **187** (3), 249-255 —— Gene expression for 3-phosphoinositide-dependent protein kinase-1 (PDK-1) in developing and adult rat brains was examined by in situ hybridization histochemistry. In embryonic days, the mRNA was evident throughout the entire neuraxis. The expression remained evident throughout the entire gray matters until postnatal day 7, and thereafter it decreased overall in the mantle and ventricular zones except for the cerebellar Purkinje and granule cell layers, the olfactory and hippocampal neuronal layers. The pattern of this gene expression is similar to those of for protein kinase B and class I phosphoinositide 3-kinases. ———— 3-phosphoinositide-dependent protein kinase-1; Rat; Brain; Development; in situ hybridization © 1999 Tohoku University Medical Press

It is known that phosphoinositide 3-kinases (PI3K) are a diverse family of enzymes capable of phosphorylating inositol phospholipids at the D3 position and that their products are involved in activation of several signal transducers such as protein kinase B (PKB) and protein kinase C (PKC) in processes controlling cell proliferation, cell survival and metabolic changes (Nakanishi et al. 1993; Toker et al. 1994; Akimoto et al. 1996). PKB, also known as Akt, was originally identified as a cellular homologue of the viral oncogene V-act (Coffer and Woodgett 1991; Cheng et al. 1992) and contains not only a catalytic domain closely related to that of PKC family, but also an Akt homology domain, part of which is related to pleckstrin homology (PH) domain (Bellacosa et al. 1991). The regulation of PKB is not accounted for by binding of the D3-phosphoinositides to its PH domain alone, but it is made possible by phosphorylation of PKB residues Thr-308 and Ser-473 in a PI3K-dependent manner by upstream PKB kinases, termed 3-phosphoinositide-dependent protein kinase (PDK) (Hemmings 1997;

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Klarlund et al. 1997; Downward 1998). Recent studies by purification and cDNA cloning have identified one of PDKs termed PDK-1, which phosphorylates Thr-308 only when PKB is bound to phosphatidylinositol (PtdIns) (3, 4, 5) P_3 or PtdIns (3, 4) P_2 (Alessi et al. 1997; Stephens et al. 1998). PDK-1 has a predicted molecular size of 63 kDa and contains an amino-terminal protein kinase domain and a carboxyl-terminal PH domain.

In general, it is informative to determine the localization of given molecules for understanding their functional significance. We have so far reported the localization of mRNA for PKBs as well as PI3Ks in the brain (Ito et al.1995; Owada et al. 1997), but no information is available yet about the localization of PDK-1. The present study addressed this point and described the detailed localization of mRNA for PDK-1 in developing and mature brain of rats by in situ hybidization histochemistry in comparison with those for PKBs and PI3Ks.

Materials and Methods

An antisense 45-mer oligonucleotide probe was complementary to the nucleotide residues 1811-1855 of the 3′ noncoding region of the rat PDK-1 cDNA (Alessi et al. 1997; Stephens et al. 1998). The oligonucleotide was labeled using terminal deoxynucleotidyl transferase (GIBCO BRL, Life Technologies, Inc., Rockville, MD, USA) with α-^{35}S dATP for in situ hybridization histochemistry. The sections, 20 μm thick, were made on a cryostat from fresh frozen whole bodies of Wistar rats on the embryonic day 15 (E15), E18, E20, and brains on the postnatal day 0 (P0), P7, P14, P21 and P49. After fixation in 4% paraformaldehyde/0.1M sodium phosphate buffer (PB, pH7.2), the sections were acetylated with 0.25% acetic anhydride in 0.1 M triethanolamine (pH8.0) and prehybridized for one hour in a buffer containing 50% deionized formamide, 4×SSC, 1× Denhardt's solution, 1% sodium N-lauroyl sarcosinate (Sarkosyl), 0.1 M PB and 250 μg/ml denatured salmon sperm DNA. After prehybridization, the sections were hybridized overnight at 42°C with α-^{35}S labeled oligonucleotides (1.5×10^7 cpm/ml) diluted in the solution, which consisted of the prehybridization solution with the addition of 10% dextran sulfate and 100 mM dithiothreitol. The sections were washed with 0.1×SSC/0.1% Sarkosyl at 50°C four times for 30 minutes. They were exposed to Hyperfilm β-max (Amersham, Arlington Heights, IL, USA) for 2 weeks at room temperature. They were subsequently autoradiographed using NTB2 nuclear track emulsion (Kodak, Rochester, NY, USA) for 4 weeks at 4°C. To examine the influence on the gene expression in the hypoglossal neurons of its nerve transection, we also used the same preparations as previously described (Owada et al. 1997).

Results

During E15-E20, the gene expression for PDK-1 was evident in the ventricular germinal zone and mantle zone without regional difference in the

intensity throughout the entire neuraxis (Fig. 1a). Outside the brain, the expression signals of varying intensities were observed in almost all tissues and organs. Among them, more intense signals were detected in anlagen of the thymus and liver, whereas substantial levels of expression signals were seen in the olfactory epithelium, hair follicles, kidney, gut anlagen, and dorsal root ganglia (Figs. 1a and 2). On P0-P7, the expression was evident throughout the gray matter of the entire brain and it was slightly more intense in olfactory mitral cell layer, cerebral cortex, subiculum and hippocampal anlagen, and thalamic and pontine nuclei (Fig. 1b). The expression in the ventricular germinal zones was much less evident than that at the prenatal stages. No significant expression was seen in the white matters including the corpus callosum. In the cerebellum, the expression was evident in both external and internal granule cell layers (Fig. 4). On P14 and thereafter, the expression decreased progressively in forebrain and brain stem and no significant expression was discerned in the diencephalon and brain stem on P21, although it remained evident in the olfactory mitral and granule cells, the hippocampal pyramidal and dentate granule cells, the neurons of pontine nuclei and the cerebellar Purkinje and granule cells (Fig. 5). Weak expression was seen in the neocortical layers II-VI on P14 and P21.

In the hypoglossal neurons after the transection of the hypoglossal nerve, no significant change in the expression intensity was discerned on the operated side as compared with the contra-lateral non-operated side during 3 weeks after the operation.

Discussion

The gene expression pattern for PDK-1 in the brain was basically similar to those for PKBs and PI3Ks as expected because PKB is the main substrate for PDK-1 and the upstream kinase phosphorylates only PKB bound with PtdIns(3, 4, 5)P_3, a product of PI3K (Toker et al. 1994; Downward 1998). The expression is evident throughout the gray matters in the embryonic and early postnatal brain and decreased afterwards throughout the brain except for the cerebellar granule cell layer. Although the expression for PKBα in the cerebellar granule cell layer was reported to be as weak as in the remaining gray matter in the adult brain unlike that for PDK-1 (Owada et al. 1997), this discrepancy is simply due to the isoform variety because the expression for PKBγ in the cerebellar granular cell layer is revealed in the present study to be similar to that for PDK-1 (unpublished). In the ventricular zone of embryonic brain and the cerebellar external granule cell layer, both of which are the neurogenic zones, the gene expression is evident for PDK-1 as well as PKBα, β and class I PI3Ks (Ito et al. 1996; Owada et al. 1997). This finding is compatible with the possibility that activated PKBs may be involved in mitogenic signal pathways and DNA synthesis in normal development (Staal 1987; Coffer and Woodgett 1991; Cheng et al. 1992; Franke et al. 1995). However, the expression for PDK-1 as well as PKBs and PI3Ks were

also evident throughout the mantle zones, or immature gray matter, of developing brain. This expression feature seems to be ascribed to postmitotic neurons settled in the final loci after migration from their germinal zones because no significant expression was detected in the white matter composed of glial cells. This suggests that activated PKBα is involved in some physiological processes unrelated to mitogenic signaling exerted by neurons.

One of the possible physiological processes may be the involvement in axonal elongation. In support of this possibility, the transient enhancement of the expression for PKBα and PI3Ks was reported in the hypoglossal neurons after hypoglossal nerve transection (Ito et al. 1996; Owada et al. 1997). However, no expression enhancement was detected for PDK-1 in the present study. This discrepancy in the post-axotomy expression between PKB and PDK may be ascribed to the isoform variety because of the following two facts: The expression for PKBβ or γ was not enhanced after the axotomy (Owada et al. 1997; and unpublished data); PDK seems to be composed of multiple isoforms, one of which is cloned as PDK-1, although PDK-2 is supposed to phosphorylate a residue Ser-473 different from the phosphorylation of Thr-308 by PDK-1 (Alessi et al. 1996; Downward 1998). There is thus a possibility that the axotomy enhances the gene expression for PDK-2 instead of PDK-1. The cDNA cloning of PDK-2 remains to be elucidated to examine this possibility.

It has recently been shown that PDK-1 regulates multiple kinases including various PKC isoforms and p70 ribosomal protein S6 kinase in addition to PKB, and that PKC is controlled through a PI3K pathway, operating through PDK-1 dependent phosphorylation of the activation loop site in the PKC isoforms (Le Good et al. 1998). The present finding on localization of PDK-1 mRNA in the brain gives us a basis to understand more clearly the functional relation of these lipid kinases in further experiments.

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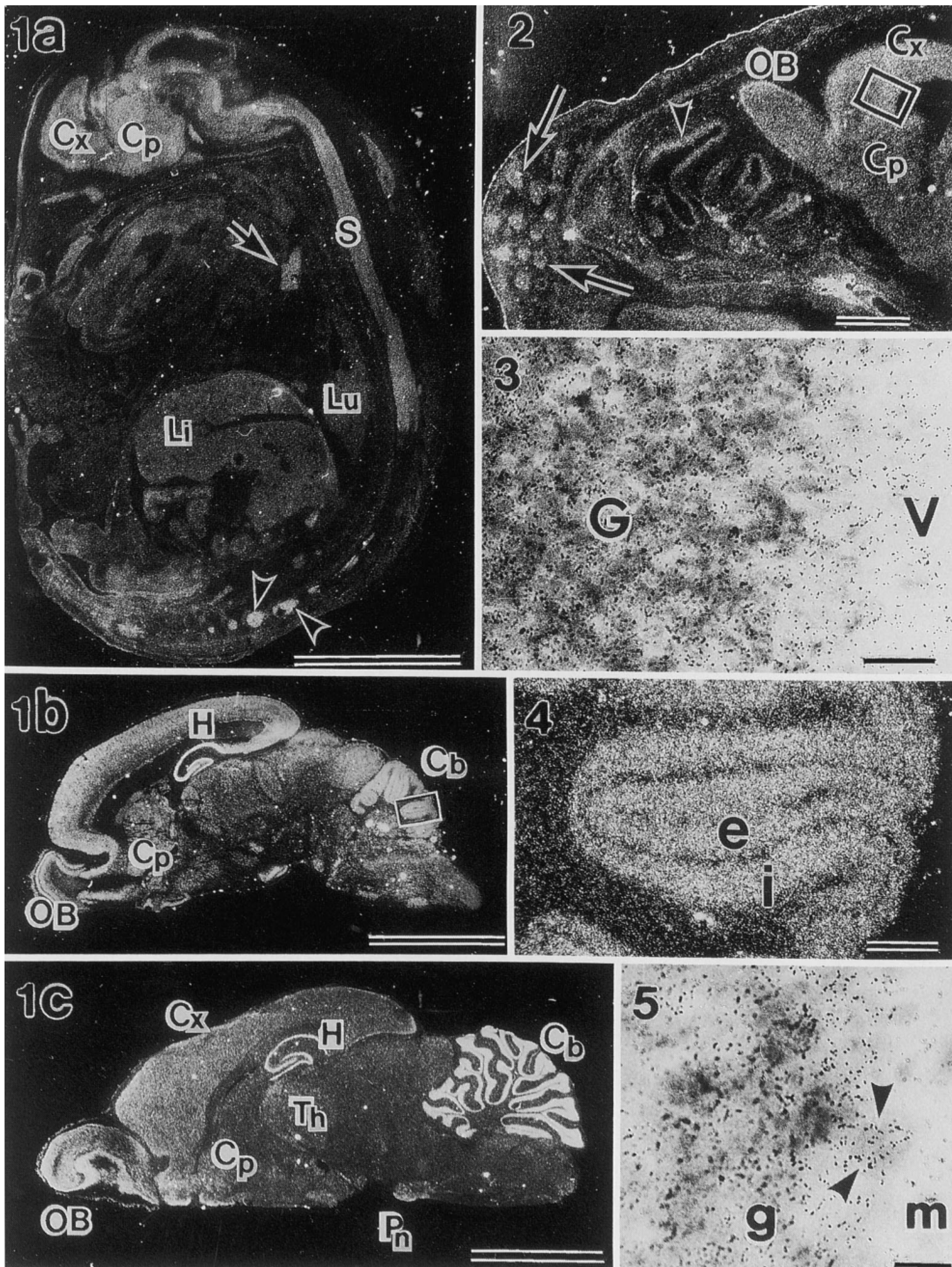
Fig. 1. Overview of the developmental changes in the gene expression for PDK-1 in whole embryo on E18 (Fig. 1a), and in the brains on P7 (Fig. 1b) and P14 (Fig. 1c) in dark-field microscopy. Note the marked decrease in the expression in the diencephalon and brain stem on P14 as compared with the proceeding stages. Cb, cerebellum; Cp, caudate putamen; Cx, cerebral cortex; H, hippocampus; Li, liver; Lu, lung; S, spinal cord; Th, thalamus; OB, olfactory bulb; Pn, pontine nuclei. Note also intense expression in the thymus (arrow) and dorsal root ganglia (arrow heads). Bars = 5 mm.

Fig. 2. Sagittal section of the head region of E20 rat embryo in dark-field microscopy. Note the positive expression in olfactory epithelium (arrow head) and hair follicles (arrows). Cp, caudate putamen; Cx, cerebral cortex; OB, olfactory bulb; Bar = 1 mm.

Fig. 3. Bright-field photomicrograph at high magnification of the E20 neocortical area enclosed by a rectangle in Fig. 2. Note intense levels of the gene expression in the ventricular germinal zone (G). V, ventricle. Bar = 50 μm.

Fig. 4. Dark-field photomicrograph at higher magnification of the area of P7 cerebellar cortex enclosed by a rectangle in Fig. 1b. Note high levels of the gene expression in the external (e) and internal (i) granule cell layers. Bar = 200 μm.

Fig. 5. Bright-field photomicrograph at higher magnification of the cerebellar cortex in P21. Note the positive gene expression on both Purkinje (arrow heads) and granule cells(g). m, molecular layer. Bar = 50 μm.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