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RESEARCH ARTICLE

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Immunohistochemical localisation of two phosphatidylinositol 4-kinase isoforms, PI4K230 and PI4K92, in the central nervous system of rats

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Abstract The distribution and cellular localisation of the phosphatidylinositol 4-kinase isoforms, PI4K230 and PI4K92, that are believed to play important roles in the intracellular signalling mechanisms were studied in the rat brain (cortex, cerebellum, hippocampus and spinal cord) using immunocytochemistry with light and electron microscopy. PI4K230 was detected with a specific antibody purified by affinity chromatography from the egg yolk of chicken immunised with a 33-kDa fragment of bovine PI4K230, comprising amino acids 873–1175 of the native protein. PI4K92 was immunostained with a commercially available antibody raised in rabbit against amino acid residues 410–537 of human PI4K92. At the light microscopic level, the immunostaining of PI4K230 and PI4K92 showed a very similar distribution throughout the neurons and appeared as dense punctate labelling in the cytoplasm of perikarya and stem dendrites of various neurons. In addition to neurons, a strongly stained cell population was observed in the molecular layer of the cerebellar cortex that resembled Bergmann glia cells. Electron microscopy of neurons in the ventral horn of the spinal cord showed dense granular immunoprecipitates for both PI4K230 and PI4K92, mostly associated with the outer membrane of mitochondria and membranes of the rough endoplasmic reticulum. In addition, immunostaining of PI4K92 was also frequently found on the outer surface of cisterns and vesicles of Golgi complexes, whereas PI4K230 immunoreactivity was colocalised with some multivesicular bodies. Neither nuclear localisation nor a regular attachment to the cell membrane of these enzymes were observed. Our findings indicate that PI4K230 and PI4K92 are not involved directly in the ligand-stimulated turnover of phosphoinositides at the plasma membrane of neurons. However, they may provide regulatory phosphoinositides for intracellular vesicular traffic being associated with various organelles.

Key words Phosphoinositide signalling · Immunocytochemistry · Ultrastructural localisation

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Introduction

Polyphosphoinositides play important roles in the intracellular signal transduction in response to a variety of hormones, neurotransmitters and growth factors (Berridge 1984; Divecha and Irvine 1995). In the classic signalling pathway, phosphatidylinositol 4-kinase (PI4K) catalyses the first committed step that leads to the phosphorylation of plasma membrane-bound phosphatidylinositol (PtdIns) at the D4 position of the inositol ring. Phosphatidylinositol 4-phosphate (PtdIns(4)*P*) can be further phosphorylated by PtdIns 4P 5-kinase to yield PtdIns(4,5)P_2, the precursor of the second messengers inositol(1,4,5)P_3 and diacylglycerol. PtdIns(4)*P* and PtdIns(4,5)P_2 are also substrates of phosphoinositide 3-kinase, giving rise to a wide variety of lipid messengers (for review see Carpenter and Cantley 1996). Polyphosphoinositides are formed not only in the plasma membranes but also in the cell nucleus (Boronenkov et al. 1998; D'Santos et al. 1998) and intracellular membranes like the sarcoplasmic reticulum, endoplasmic reticulum, Golgi complexes, lysosomal membranes and secretory vesicles (for review see Gehrman and Heilmeyer 1998). Some of them, like PtdIns(4,5)P_2 regulate the actin-modifying effect of various actin-binding proteins, thereby modulating structural changes in the actin cytoskeleton (Hinchliffe et al. 1998; Toker 1998). Phosphoinositides are also involved in the control of intracellular vesicular traffic (Schu et al. 1993; De Camilli et al. 1996; Martin 1997; Odorizzi et al. 1998).

Since the precursor for the most polyphosphoinositides is PtdIns(4)*P* (Martin 1998), PI4K is essential for these processes.

Many phosphatidylinositol kinases have been purified, characterised and cloned (Gehrmann and Heilmeyer 1998; Rao et al. 1998). PI4Ks with known amino acid sequences belong to two subfamilies, best represented by PI4K92 and PI4K230. A third form, PI4K55, has not yet been cloned. PI4K55 is associated with plasma membranes of several cells (for review see Gehrmann and Heilmeyer 1998), liver microsomes (Olsson et al. 1995), chromaffin granules (Husebye et al. 1990; Wiedemann et al. 1996) and lysosomes (Arneson et al. 1999). Soluble PI4K92 and PI4K230 were detected in the adrenal cortex (Downing et al. 1996). Most membrane-associated PI4Ks have not been differentiated on a molecular basis till now. In transfected cells, antibodies detected overexpressed PI4K92 and PI4K230 associated with the Golgi apparatus and/or endoplasmic reticulum (Nakagawa et al. 1996a,b; Wong et al. 1997).

The high level of mRNA for PI4K92 (Nakagawa et al. 1996b; Balla et al. 1997) and PI4K230 (Nakagawa et al. 1996a; Balla et al. 1997; Gehrmann et al. 1999) in the brain is in accordance with earlier observations that indicate the preferential occurrence of these enzymes in nervous tissue (Carpenter and Cantley 1990). More recent findings suggest that PI4Ks are involved in neuron-specific functions such as neurotransmitter release (Wiedemann et al. 1996, 1998; Khvotchev and Sudhof 1998) and endocytosis of some acetylcholine receptors (Sorensen et al. 1998). Additionally, PI4Ks take part in the regulation of ion channels (Zeng et al. 1994; Baukrowitz et al. 1998; Shyng and Nichols 1998; Kim and Bang 1999; Zhainazarov and Ache 1999). While PI4Ks have been isolated predominantly from the brain, their intracellular localisation and function in nerve cells are still unknown. In the present paper we provide data on the cellular and subcellular localisation of endogenous native lipid kinases, PI4K92 and PI4K230, in the central nervous system of rats.

Materials and methods

Materials

Anti-rabbit immunoglobulin peroxidase conjugate, anti-chicken immunoglobulin peroxidase conjugate and Tween 20 were from Sigma, Phenyl-Sepharose from Pharmacia and biotinylated anti-rabbit immunoglobulin, biotinylated anti-chicken immunoglobulin, normal goat serum and avidin-biotinylated horseradish peroxidase complex (ABC) system were from Vector Laboratories. Polyclonal antibodies against PI4K92 raised in rabbit against the polypeptide comprising amino acids 410–537 of PI4K92 (Meyers and Cantley 1997) were from Upstate Biotechnology (anti-PtdIns 4-K 110β). Enhanced chemiluminescence (ECL) reagents were from Amersham and Pierce. All other chemicals used were obtained from Sigma or Reanal (Budapest).

Preparation of antibodies against PI4K230

Monospecific antibodies to the N-terminal part of bovine PI4K230 comprising amino acids 873–1175 (Balla et al. 1997) were prepared and affinity purified from chicken egg yolk as described by Gehrmann et al. (1999). Protein concentration of the purified preparation was 50 µg/ml. For control experiments, chicken IgY was purified from egg yolk of non-immunised chickens on Phenyl-Sepharose according to Hassl and Aspöck (1988), obtaining a protein concentration of 6.4 mg/ml. Treatment of the experimental animals was carried out according to established protocols consistent with the "Principles of Laboratory Animal Care" (NIH publication number 86–23, revised 1985) and following the recommendations of the Hungarian Law on Animal Protection.

Sodium dodecyl sulphate-polyacrylamide gel electrophoresis (SDS-PAGE) and western blotting

SDS-PAGE was performed according to Laemmli (1970) using a mini-slab gel apparatus (Bio-Rad). The separated proteins were then electrophoretically transferred onto a polyvinylidene fluoride (PVDF) membrane (Immobilon-P; Millipore). After electrotransfer, the unspecific binding sites of the PVDF membrane were blocked with 5% (w/v) skimmed milk in 10 mM potassium phosphate, 120 mM NaCl, 2.7 mM KCl, pH 7.4 (phosphate-buffered saline) containing 0.1% (v/v) Tween 20 for 1 h at room temperature. The PVDF membranes were incubated with the primary antibody either at 4°C overnight or at room temperature for 1 h and then with secondary antibody at room temperature for 1 h. Usually, the secondary antibodies were labelled with peroxidase. ABC system was used when the secondary antibodies were labelled with biotin. Immunoreactive proteins were detected using an ECL kit and recorded using Fuji medical X-ray or ECL Hyperfilm (Amersham).

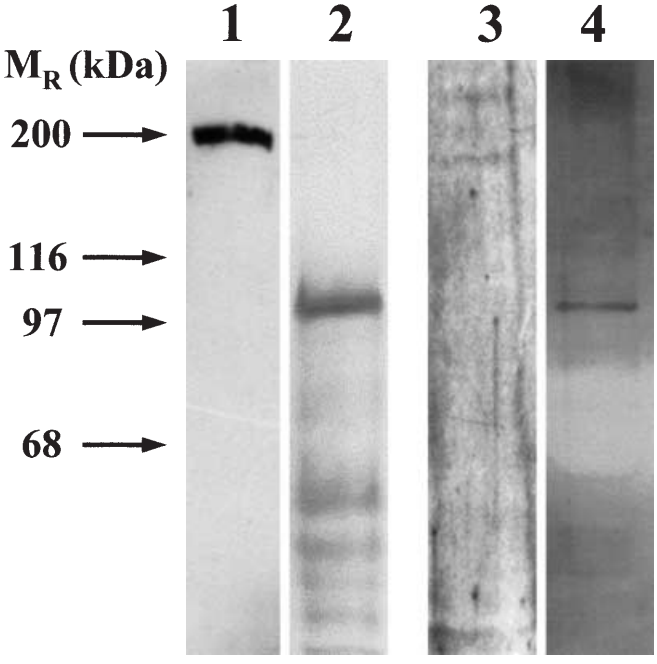

Fig. 1 Isoenzyme specificity of antibodies and detection of phosphatidylinositol 4-kinase isoforms, PI4K230 and PI4K92, in bovine brain preparation and rat brain homogenate using immunoblots. Samples from bovine brain preparation were separated by sodium dodecyl sulphate-polyacrylamide gel electrophoresis and immunoblotted with anti-PI4K230 (*lane 1*) or anti-PI4K92 (*lane 2*). Rat brain homogenates were immunoblotted also with either anti-PI4K230 (*lane 3*) or anti-PI4K92 (*lane 4*). *Arrows* represent the molecular mass standard

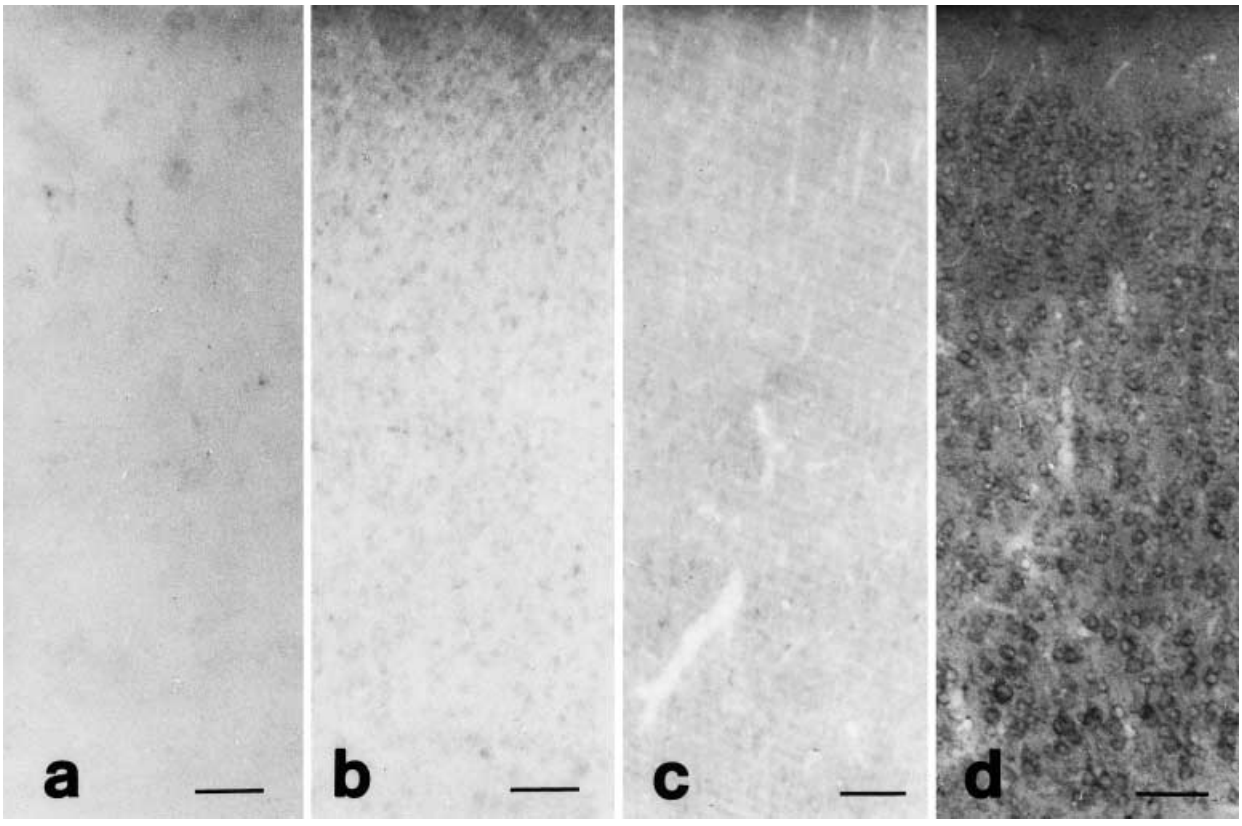

Fig. 2a–d Specificity of immunohistochemical detection of PI4K230. Photomicrographs from sections of the cerebral cortex of rats: **a** The primary antibodies were replaced with normal goat serum. **b** The primary antibodies were replaced with non-specific chicken IgY. **c** The primary antibodies against PI4K230 were adsorbed by PI4K230 preparation. **d** Section treated with anti-PI4K230 as the primary antibody. *Bars* 50 µm

Purification of PI4K230 from bovine brain

PI4K230 was prepared from the grey matter of bovine brain as described previously (Gehrmann et al. 1996). The solubilised membrane fraction after the first hydroxylapatite chromatography was used for western blot analysis and a highly purified preparation (3000 U/mg protein) was used for adsorbing the specific antibodies against PI4K230 in control immunostainings. Protein concentration was determined according to Bradford (1976) with bovine serum albumin as a standard.

Specificity of the detection of PI4K92 and PI4K230 with antibodies using immunoblots

Since the different PI4Ks have partially similar sequences (Gehrmann and Heilmeyer 1998), it is important to verify that the antibodies against the PI4K230 and PI4K92 react only with the appropriate enzyme species without any crossreaction. The specificity of anti-PI4K230 and anti-PI4K92 was tested by western blotting either a solubilised bovine brain preparation enriched in PI4K230 and PI4K92 or rat brain homogenate. Western blot analysis of solubilised bovine brain preparation showed a single band of ca 200 kDa with the anti-PI4K230 corresponding to the purified PI4K230 (Fig. 1 *lane 1*), while anti-PI4K92 reacted specifically with a ca 100-kDa protein (Fig. 1 *lane 2*). We also tested the specificity of anti-PI4K230 and anti-PI4K92 western blotting rat brain homogenate. The anti-PI4K230 (Fig. 1 *lane 3*) revealed two faintly stained bands (190 kDa and 230 kDa), while the anti-PI4K92 (Fig. 1 *lane 4*) reacted with a single immunoreactive band of about 100 kDa. The 190-kDa band could be the result of a splice alternative or of proteolysis. A PI4K species with similar molecular weight was stained with the anti-PI4K230 in extracts of CHO cells as well (Wong et al. 1997). The biotin-labelled secondary antibodies and the ABC system used in immunocytochemistry were also tested and yielded western blots identical to those presented (data not shown).

Immunocytochemistry

Wistar rats were deeply anaesthetised with chloral hydrate (35 mg/kg i.p.) and perfused through the heart first with Tyrode's solution, followed by a fixative containing 4% paraformaldehyde or 4% paraformaldehyde and 0.1% glutaraldehyde in 0.1 M phosphate buffer pH 7.4 (PB). At higher concentrations of glutaraldehyde the immunoreactivity was lost. The brain and the spinal cord were removed and fixed in the same fixative for 1–3 h. Blocks of the cerebral cortex, hippocampus, cerebellum and the lumbar segments of the spinal cord were dissected and immersed sequentially into 10 and 20% sucrose dissolved in 0.1 M PB until they sank. Tissue blocks were frozen in liquid nitrogen and sectioned at 60 µm on a vibratome. Following extensive washes in 0.1 M PB, the free-floating sections were first incubated with the antibody against PI4K230 (dilution 1:100 or 1:200) or PI4K92 (dilution 1:100 or 1:200) for 2 days. After several washes they were transferred into biotinylated goat anti-chicken (1:200) or biotinylated goat anti-rabbit immunoglobulin (1:200) solution for 5–6 h. Thereafter, they were treated with a solution of ABC (1:100), and the immunoreaction was completed with a diaminobenzidine (Sigma) chromogen reaction. All incubation steps were performed under continuous gentle agitation and in the presence of normal goat serum (dilution 1:100) in 50 mM TRIS-HCl, 150 mM NaCl, pH 7.4. Immunostained sections were mounted on chrome alum–gelatine-coated slides, air dried, dehydrated in ethanol, cleared in xylene and mounted with DePex neutral medium.

For electron microscopy, sections were treated with 1% OsO_4 for 45 min after the chromogen reaction, dehydrated and embed-

ded into Durcupan ACM resin (Fluka). Ultrathin sections were collected onto Formvar-coated grids and counterstained with lead citrate and examined with a Jeol 1010 electron microscope.

Specificity of the immunohistochemical detection of PI4K230

Consecutive sections of the cerebral cortex were treated as described above with the following modifications: in negative controls the primary antibodies were replaced with normal goat serum (dilution 1:100; Fig. 2a), with non-specific chicken IgY purified from egg yolk on Phenyl-Sepharose (dilution 1:10; Fig. 2b) or with specific antibodies adsorbed with highly purified bovine brain PI4K230 preparation (1 µg enzyme protein per ml antibody preparation diluted to 1:100; Fig. 2c). Under these conditions, no specific peroxidase reaction was observed. In the positive control (Fig. 2d), the use of specific antisera against PI4K230 (dilution 1:200) yielded a clearly distinguishable immunostaining.

Results

Distribution of PI4K230 immunoreactivity in the rat brain

In the cerebellum, the most prominent immunostaining was observed in the molecular layer of the cerebellar cortex (Fig. 3a). The intense staining was mainly due to a large number of immunoreactive filaments that were oriented perpendicular to the outer surface and traversed the entire thickness of the molecular layer (Fig. 3c). The immunostained filaments showed a triangular enlargement at their inner end at or just above the level of the Purkinje cells. In some cases, the triangular enlargement was completed into a circular ring that surrounded an unstained central spheroid area that made the immunoreactive structure similar to the cell body and process of Bergmann glia cells (Fig. 3a). In addition to the strong immunoreactivity of the molecular layer, some neurons in the cerebellar nuclei showed a punctate labelling in their cytoplasm (Fig. 3b). No further analysis of the cerebellar nuclei has been performed.

A large number of immunoreactive neurons were revealed in all layers of the cerebral cortex (Fig. 4a). The immunoreactivity appeared as a dense granular staining in the cytoplasm of the soma and stem dendrites (Fig. 4c,d,g).

Principal cells were heavily stained in the hippocampus (Fig. 4b). Similar to the cerebral cortex, the labelling appeared as a granular staining and was confined to the cytoplasm of somata and stem dendrites of the pyramidal cells. Neurons in the stratum oriens, stratum radiatum and lacunosum-moleculare were stained only occasionally.

At the level of the lumbar spinal cord, immunoreactive neurons with cytoplasmic granular staining were revealed both in the dorsal and ventral horns. In addition to neurons in lamina VII (Fig. 4f), some motoneurons were also labelled in the lateral motor column (Fig. 4e).

To associate the granular staining with specific cytoplasmic organelles, an ultrastructural study has been done on immunostained neurons in the ventral horn of the spinal cord. Most of the electron-dense immunoprecipitates with diameters varying in the range of

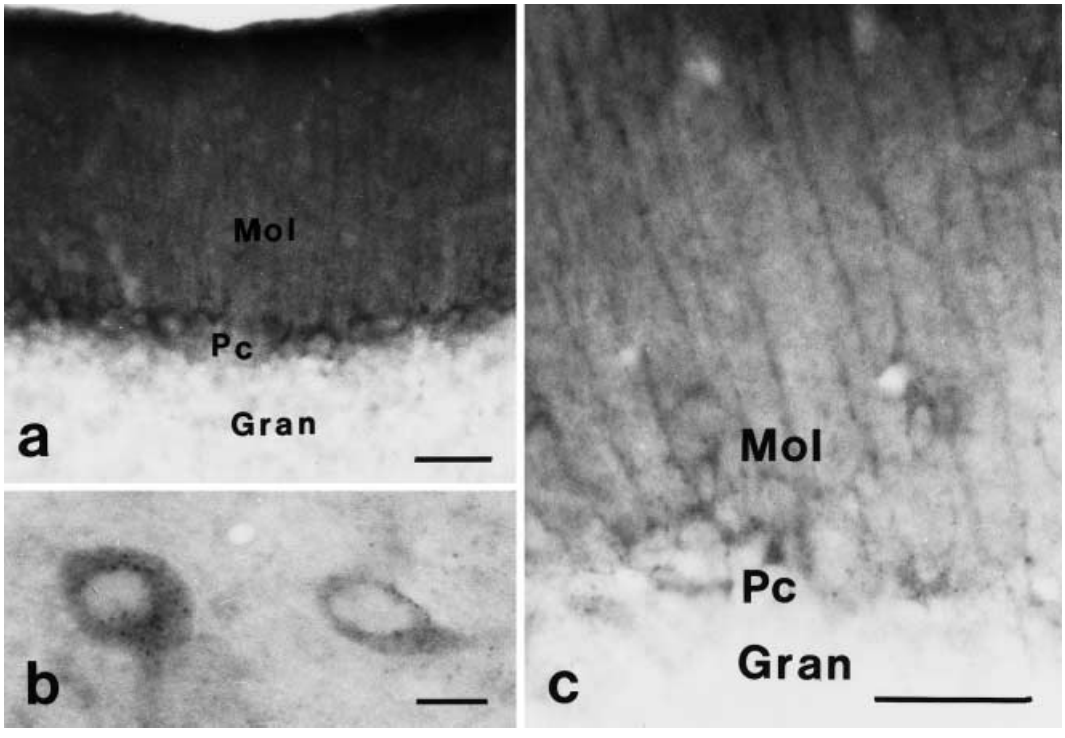

Fig. 3a–c PI4K230-immunoreactive structures in the cerebellum of rats. Photomicrographs from rat cerebellum show: (**a**) strong immunoreactivity in the molecular layer of the cerebellar cortex, (**b**) immunoreactive neurons in a cerebellar nucleus and (**c**) immunoreactive cell bodies and processes in the molecular layer of the cerebellar cortex. *Pc* layer of Purkinje cells, *Mol* molecular layer, *Gran* granular layer. *Bars* 50 µm (**a**); 10 µm (**b,c**)

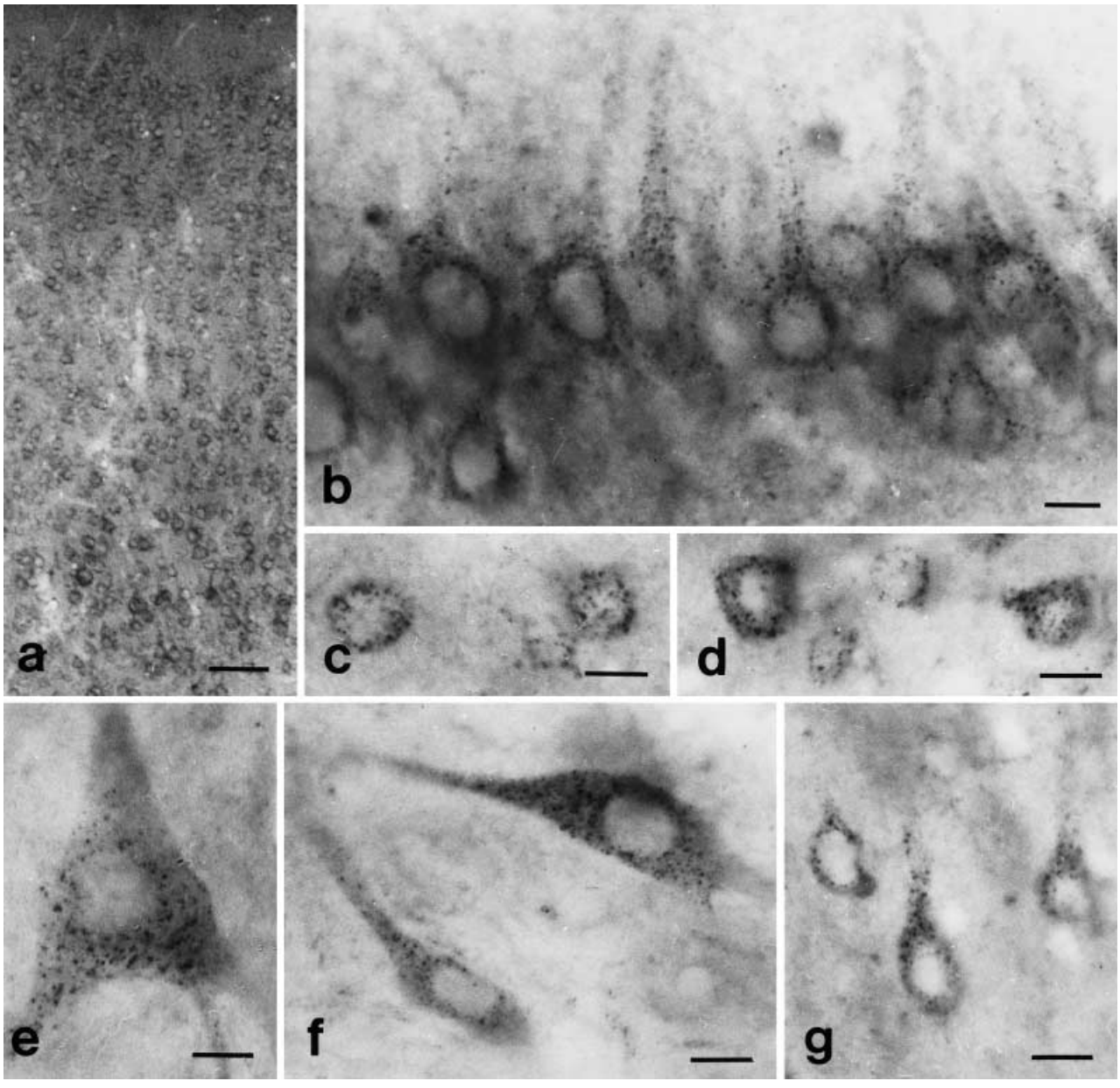

Fig. 4a–g Light microscopy of PI4K230-immunoreactive neurons in the brain and spinal cord of rats. **a** Cerebral cortex. **b** Stratum pyramidale of the hippocampus. **c,d** Layer IV of the cerebral cortex. **e** Lateral motor column of the spinal cord. **f** Lamina VII of the spinal grey matter. **g** Lamina III of the cerebral cortex. *Bars* 50 µm (**a**); 10 µm (**b–g**)

300–400 nm were scattered close to the cisterns of the rough endoplasmic reticulum (Fig. 5b,c) and/or covering the outer membrane of mitochondria (Fig. 5c). Some of them were associated with multivesicular bodies (Fig. 5d) or infrequently attached to the cell membrane in the vicinity of synaptic specialisations (Fig. 5a).

Distribution of PI4K92 immunoreactivity

To compare the distribution and appearance of PI4K230 with those of PI4K92, identical parts of the brain were immunostained under identical conditions. At the light microscopic level, the distribution of immunoreactivity for PI4K92 (Fig. 6b,d,f) was similar to that obtained for PI4K230 (Fig. 6a,c,e). The intensity of the staining, however, showed marked differences at certain locations. For instance, the immunostaining in the molecular layer of the cerebellar cortex was far more intense for PI4K92 (Fig. 6b) than for PI4K230 (Fig. 6a). In contrast to this, the density of immunostaining for PI4K230 in neurons within the cerebral cortex and spinal cord surpassed that obtained for PI4K92 (Fig. 6c–f). In addition, the granular immunoprecipitates were smaller in neurons stained for PI4K92 than in neurons stained for PI4K230 (Fig. 6c–f).

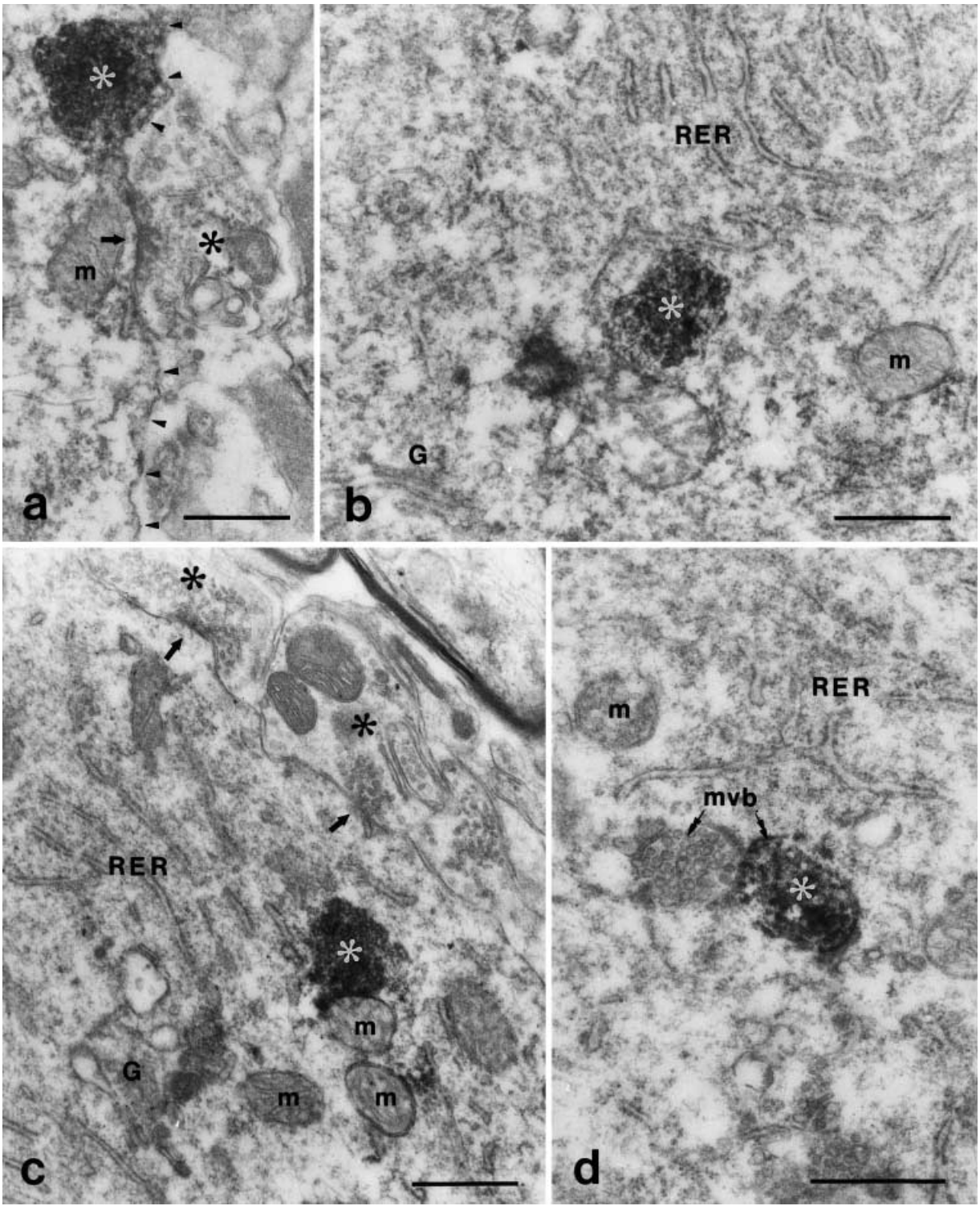

Fig. 5a–d Electron micrographs of PI4K230-immunoreactive granules in the cytoplasm of neurons in the ventral horn of the spinal cord of rats. The immunoprecipitates: (**a**) are attached to the cell membrane in close vicinity of a synaptic specialisation, (**b**) are located close to the cisterns of the rough endoplasmic reticulum, (**c**) are attached to the outer membrane of a mitochondrion and (**d**) cover a multivesicular body. *White asterisks* label the immunoprecipitates that represent areas immunoreactive for PI4K230. *Black asterisks* label non-immunoreactive axon terminals. *Arrows* on **a** and **c** point to synaptic contacts. *Arrowheads* on **a** point to the plasma membrane. *G* Golgi apparatus, *RER* rough endoplasmic reticulum, *m* mitochondrion, *mvb* multivesicular body. *Bars* 0.5 µm

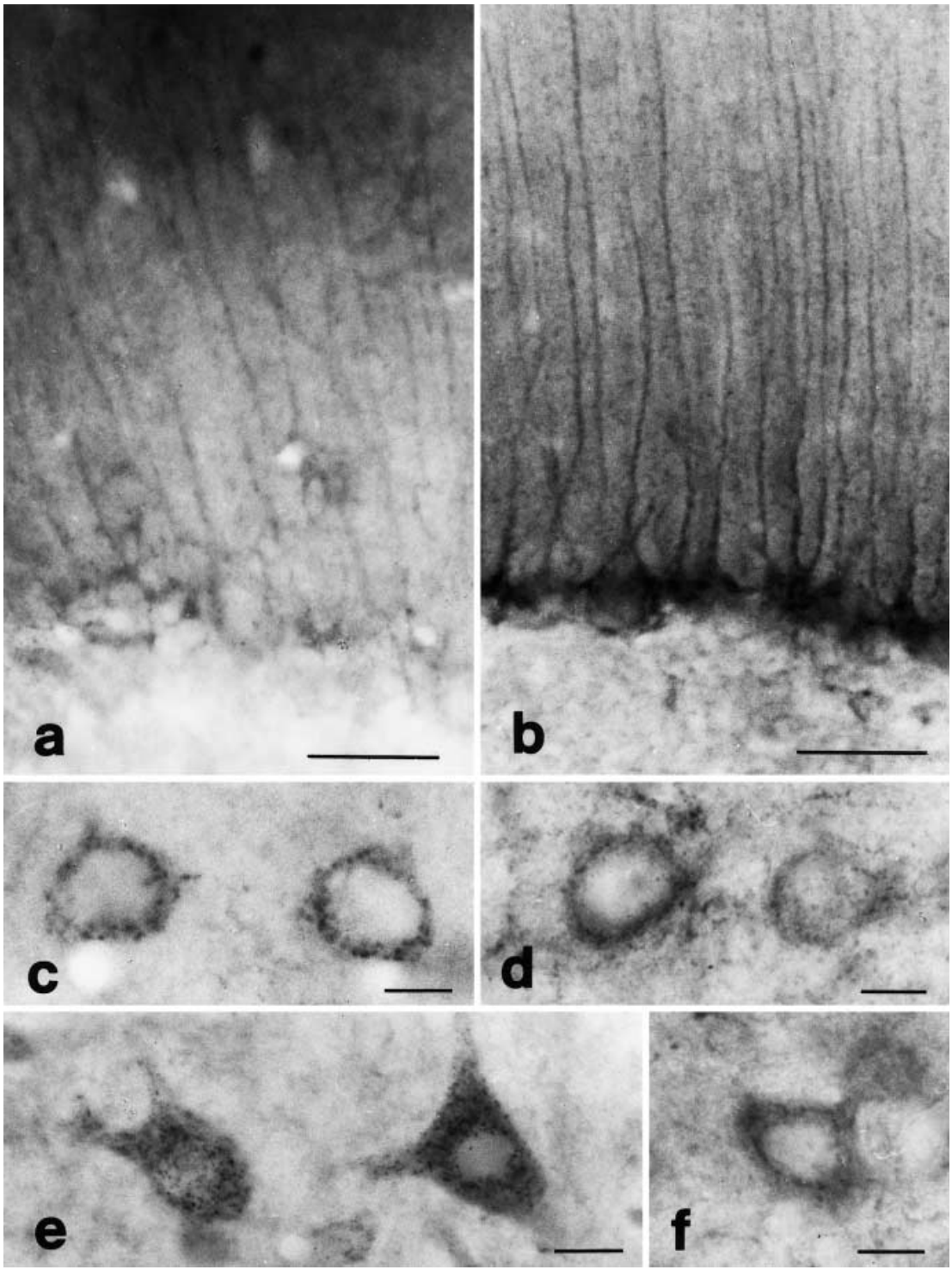

Fig. 6a–f Immunolocalisation of PI4K92 in comparison with that of PI4K230. Micrographs show cells immunostained for PI4K92 (**b,d,f**) and PI4K230 (**a,c,e**). Immunoreactive cells are: (**a,b**) in the molecular layer of the cerebellar cortex, (**c,d**) in layer IV of the cerebral cortex and (**e,f**) in lamina VII of the spinal cord. *Bars* 50 µm (**a,b**); 10 µm (**c–f**)

This difference in the size of granules was also observed in electron microscopic studies (see below).

Electron microscopy of the neurons immunostained for PI4K92 in the ventral horn of the spinal cord showed that the electron-dense immunoprecipitates covered areas in the cytoplasm that were not larger than 100–200 nm in diameter (Fig. 7). Most of these small granules were attached to the outer membrane of mitochondria (Fig. 7b) or to the membranes of the rough en-

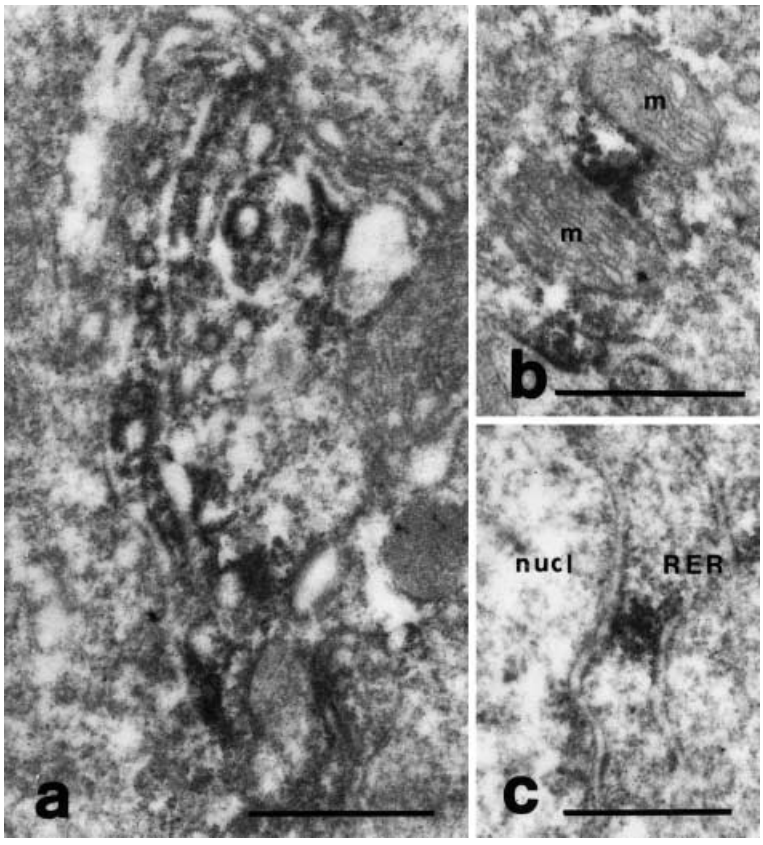

Fig. 7a–c Electron micrographs of PI4K92-immunoreactive granules in the cytoplasm of interneurons in the ventral horn of the spinal cord of rats. The immunoprecipitates are attached to: (**a**) the outer surface of cisterns and vesicles of Golgi complexes, (**b**) outer cell membrane of a mitochondrion and (**c**) membranes of rough-surfaced endoplasmic reticulum and cytoplasmic surface of the nuclear envelope. *RER* rough-surfaced endoplasmic reticulum, *m* mitochondrion, *nucl* nucleus. *Bars* 0.5 µm

doplasmic reticulum (Fig. 7c). They were also frequently seen on the outer surface of cisterns and vesicles of Golgi complexes (Fig. 7a). Immunoprecipitates were occasionally also attached to the cytoplasmic surface of the nuclear envelope (Fig. 7c).

Discussion

It is well established that PI4K is involved in the production of the plasma membrane-bound PtdIns(4,5)P_2. The regulated hydrolysis of PtdIns(4,5)P_2 in response to extracellular signals provides the second messengers inositol(1,4,5)P_3 and diacylglycerol. A variety of studies (for review see Gehrmann and Heilmeyer 1998) lead to the hypothesis that plasma membranes contain PI4K55, the PI4K species responsible for agonist-stimulated PtdIns(4)*P* formation in these membranes. The localisation studies reported here are consistent with this suggestion. Our results show that both PI4K92 and PI4K230 are present in various neurons of the central nervous system, including the cerebellum, cerebral cortex, hippocampus and the spinal cord. Cell types stained for one isoform are usually also positive for the other. However, both isoforms appear solely in the cytoplasm of neurons, indicating that neither PI4K92 nor PI4K230 are involved directly in the ligand-stimulated turnover of phosphoinositides at the plasma membrane.

The absence of immunostaining for PI4K230 in the nucleus (at least at the level of sensitivity of our methods) is somewhat surprising since PI4K230 contains nuclear localisation signals (NLS; Gehrmann et al. 1996, 1999). However, recent results indicate that phosphorylation of the NLS itself or other areas of the transported protein causing its export from the nucleus (Hopper 1999) often regulate the NLS-dependent nuclear transport. Other proteins located in the cytosol are imported into the nucleus after dephosphorylation (Heist et al. 1998). One NLS of PI4K230 contains a putative phosphorylation site (T1417) for protein kinase C (PKC) and this NLS is flanked by putative phosphorylation sites for PKC (S1414), and cAMP- or cGMP-dependent protein kinases (T1432) (Gehrmann et al. 1999) making plausible its regulation by phosphorylation. In addition, some proteins with NLS show reduced or no nuclear occurrence in non-mitotic cells (Carlock et al. 1996) and in myoblasts following the differentiation into myotubes (Brown et al. 1999). Moreover, there are proteins with NLS sequence that are exclusively located in the cytoplasm (Tolerico et al. 1999). Even the cytoplasmic function of some proteins with NLS was described, for example, the regulation of the association of importin alpha with microtubules and microfilaments (Smith and Raikhel 1998) or binding to RNA (Eister et al. 1997).

The subcellular localisation of PI4K92 and PI4K230 is in accordance with the concept that phosphoinositides are involved in the regulation of the intracellular mem-

brane traffic. A growing amount of evidence indicates that, in addition to the protein machinery organising and controlling the intracellular vesicular transport via clathrin-coat, COP-coatomers and SNARE proteins (Rothman 1994; Schekman and Orci 1996), phosphoinositides also play key roles in various aspects of vesicle formation, exocytosis and endocytosis (De Camilli et al. 1996). The regulation of ARF cycling, phospholipase-D and dynamin by PtdIns(4,5)P_2 observed during vesicle formation requires spatially localised synthesis of phosphoinositides (Martin 1997). The association of PI4K with subcellular particles can provide for such focal synthesis of phosphoinositides. Indeed, endogenous or overexpressed PI4K92 was localised at the Golgi region in several cell lines (Nakagawa et al. 1996b; Wong et al. 1997). However, the subcellular localisation of overexpressed PI4K230 remained unclear. In COS-7 cells this enzyme has been found at the cytoplasmic surface of the membranes of Golgi vesicles (Nakagawa et al. 1996a), whereas a cloned variant of PI4K230, PI4K97 (Wong and Cantley 1994), was colocalised with the endoplasmic reticulum in HeLa cells (Wong et al. 1997). Our results (Figs. 5, 7) clearly show that in the native neural cells both PI4K230 and PI4K92 are generally associated with the endoplasmic reticulum and the outer membrane of some mitochondria, while only PI4K92 is associated with the Golgi membranes. The enrichment of PI4K92 in the perinuclear region and, moreover, its association with the outer surface of vesicles attached to the nuclear envelope, ergastoplasmic membranes and Golgi apparatus may indicate a possible involvement in the transfer of newly synthesised membranes and/or proteins. The appearance of PI4K230 close to the synaptic membranes and its association with some multivesicular bodies suggests a possible involvement in retrograde transport processes (Schmid and Ambron 1997), endocytosis, receptor recycling (Felder et al. 1990) and in the multivesicular body sorting pathway (Odorizzi et al. 1998). Our present findings, showing dense patches of immunoreactivity at the outer membrane of mitochondria and the immunostained cerebellar filaments suggest further functions of PI4K230 and PI4K92, as well.

Several observations suggest that the participation of PI4K isoforms in the vesicular transport, exocytosis and endocytosis of neural cells may be very complex. Experimental data show the involvement of PI4K92 in the endocytosis of muscarinic cholinergic receptors in the brain (Sorensen et al. 1998). PI4K55 seems also to be involved in the vesicular traffic playing a role in the fusion of chromaffin granules with the cell membrane (Wiedemann et al. 1996). The glutamate release from isolated small synaptic vesicles (Wiedemann et al. 1998) is also associated with the function of PI4K, although its type has not been identified.

Our results show that PI4K92 and PI4K230 are associated selectively with membranes of distinct subcellular organelles. This finding suggests that the investigated two isoforms of PI4K may be involved differently in the localised synthesis of regulatory phosphoinositides. A spatially and temporally transient appearance of PI4Ks might explain that only a part of the neurons or subcellular particles show positive staining at the same time. Consistent with this hypothesis, a higher expression level of PI4Ks was observed in the brain of embryonal rats than in adults (Nakagawa et al. 1996a,b).

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