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Developmental and Regional Studies of the Metabolism of Inositol 1,4,5-Trisphosphate in Rat Brain

Anne M. Heacock, Edward B. Seguin, and Bernard W. Agranoff

Neuroscience Laboratory, University of Michigan, Ann Arbor, Michigan, U.S.A.

Abstract: Coupling of CNS receptors to phosphoinositide turnover has previously been found to vary with both age and brain region. To determine whether the metabolism of the second messenger inositol 1,4,5-trisphosphate also displays such variations, activities of inositol 1,4,5-trisphosphate 5'-phosphatase and 3'-kinase were measured in developing rat cerebral cortex and adult rat brain regions. The 5'-phosphatase activity was relatively high at birth (~50% of adult values) and increased to adult levels by 2 weeks postnatal. In contrast, the 3'-kinase activity was low at birth and reached ~50% of adult levels by 2 weeks postnatal. In the adult rat, activities of the 3'-kinase were comparable in the cerebral cortex, hippocampus, and cerebellum, whereas much lower activities were found in hypothalamus and pons/medulla. The 5'-phosphatase activities were similar in cerebral cortex, hippocampus, hypothalamus, and pons/medulla, whereas 5- to 10-fold higher activity was present in the cerebellum. The cerebellum is estimated to contain 50–60% of the total inositol 1,4,5-trisphosphate 5'-phosphatase activity present in whole adult rat brain. The localization of the enriched 5'-phosphatase activity within the cerebellum was examined. Application of a histochemical lead-trapping technique for phosphatase indicated a concentration of inositol 1,4,5-trisphosphate 5'-phosphatase activity in the cerebellar molecular layer. Further support for this conclusion was obtained from studies of Purkinje cell-deficient mutant mice, in which a marked decrement of cerebellar 5'-phosphatase was observed. These results suggest that the metabolic fate of inositol 1,4,5-trisphosphate depends on both brain region and stage of development. **Key Words:** Inositol phosphate metabolism—Development—Cerebellum—Inositol 1,4,5-trisphosphate receptor. **Heacock A. M. et al.** Developmental and regional studies of the metabolism of inositol 1,4,5-trisphosphate in rat brain. *J. Neurochem.* **54,** 1405–1411 (1990).

The CNS contains more pharmacologically distinct receptors coupled to phosphoinositide turnover than has been demonstrated for any other tissue (for review, see Fisher and Agranoff, 1987). Activation of these receptors results in the formation of the second messengers, diacylglycerol, which stimulates protein kinase C, and inositol 1,4,5-trisphosphate (IP_3), which mobilizes intracellular Ca^{2+}. Regulation of the IP_3 signal transduction pathway presumably occurs not only at the level of its production via stimulation of phospholipase C, but also at the level of its subsequent metabolism. IP_3 degradation occurs via the action of IP_3 5'-phosphatase with production of inositol 1,4-bisphosphate (IP_2; Downes et al., 1982; Connolly et al., 1985). An alternative route of IP_3 metabolism is phosphorylation via a 3'-kinase to inositol 1,3,4,5-tetrakisphosphate (IP_4; Irvine et al., 1986), a compound that may also play a role in intracellular Ca^{2+} homeostasis (Irvine, 1989). Both of these IP_3 metabolizing enzymes are present at relatively high concentrations in brain (Erneaux et al., 1986; Irvine et al., 1986), as is the IP_3 receptor (Worley et al., 1987). Variations in the concentrations of these components would be expected to have effects on the second messenger functions of IP_3. Whereas the coupling of CNS receptors to phosphoinositide turnover has been found to vary as a function both of developmental age (Heacock et al., 1987) and of brain region (Fisher and Agranoff, 1987), less information is available regarding such alterations in individual components of the pathway. In an autoradiographic study of IP_3 binding to rat brain sections, Worley et al. (1987) noted a heterogeneous distribution of IP_3 receptors throughout the brain, with particular enrichment in the molecular layer of the cerebellum. A more recent study (Worley et al., 1989) described IP_3 binding in developing brain. Here we examine the regional localization of IP_3 5'-phosphatase and 3'-kinase activities in rat brain and the ontogeny of these enzymes in rat

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Address correspondence and reprint requests to Dr. A. M. Heacock at Neuroscience Laboratory, University of Michigan, 1103 East Huron, Ann Arbor, MI 48104-1687, U.S.A.

Abbreviations used: IP_2, inositol 1,4-bisphosphate; IP_3, inositol 1,4,5-trisphosphate; IP_4, inositol 1,3,4,5-tetrakisphosphate.

cerebral cortex. Our results, taken together with those of Worley et al. (1987*a*, 1989), suggest that both the extent and route of metabolism of IP_3 and its ability to mobilize intracellular Ca^{2+} vary as a function of brain region and stage of development. Preliminary reports of this study have appeared in abstract form (Heacock et al., 1988, 1989).

MATERIALS AND METHODS

[4,5-^{32}P]IP_3 (110 Ci/mmol), [1-^{3}H]IP_3 (20 Ci/mmol), and [2-^{3}H]IP_4 (5 Ci/mmol) were purchased from New England Nuclear (Boston, MA, U.S.A.). Unlabeled IP_3 and IP_4 were purchased from Calbiochem (San Diego, CA, U.S.A.). IP_2, 2,3-diphosphoglyceric acid, and low-molecular-weight heparin (porcine, 4,000–6,000) were purchased from Sigma Chemical (St. Louis, MO, U.S.A.). The fluorescent Ca^{2+} indicator, fluo-3, was a product of Molecular Probes (Eugene, OR, U.S.A.). Phytic acid (40% solution) was purchased from Aldrich Chemical (Milwaukee, WI, U.S.A.). For preparation of phytic acid hydrolysate, 12.5 ml of phytic acid solution was added to 100 ml of 0.2 *M* sodium acetate buffer, pH 4.0, heated at 100°C for 4–8 h, desalted by treatment with Dowex 50 × 8 resin (H^+ form, BioRad, Rockville Centre, NY, U.S.A.), lyophilized, then dissolved in 10 ml of deionized water and stored at 4°C.

Wistar rats of the indicated ages were killed by decapitation, and brain tissue was dissected on ice and either assayed immediately for IP_3 5′-phosphatase and 3′-kinase or frozen at −70°C for subsequent determination of IP_3 binding. Female Purkinje cell-deficient mutant mice (nervous) and control littermates were obtained from Jackson Labs (Bar Harbor, ME, U.S.A.).

Measurement of IP_3 5′-phosphatase and 3′-kinase activity

Brain tissue was homogenized in ice-cold 20 m*M* HEPES buffer, pH 7.0, containing 1 m*M* EGTA, 0.5 m*M* $CaCl_2$, and 10 m*M* LiCl. Homogenates were either assayed directly or centrifuged at 100,000 *g* for 30 min to yield total particulate and soluble fractions. Assays were carried out at 37°C in a total volume of 200 μl with 10 μ*M* [^{32}P]IP_3 (20,000–30,000 cpm/tube) as substrate. A subsaturating concentration of IP_3 was routinely used to conserve the substrate. For determination of K_m and V_{max} values, additional unlabeled IP_3 (up to 300 μ*M*) was included. In addition to the above buffer components, the reaction mixture for the 5′-phosphatase contained 4 m*M* $MgCl_2$, and that for the 3′-kinase contained 10 m*M* $MgCl_2$, 5 m*M* ATP, and 5 m*M* 2,3-diphosphoglyceric acid. The latter, an inhibitor of IP_3 5′-phosphatase activity (Downes et al., 1982), had no effect on 3′-kinase activity in adult cortex. It was nevertheless included routinely to protect against IP_3 and IP_4 hydrolysis in samples in which 5′-phosphatase activity may have been high relative to 3′-kinase activity. After preincubation at 37°C for 2 min, reactions (in duplicate) were initiated by the addition of prewarmed tissue samples (25–500 μg of protein). The amount of protein added was adjusted to ensure that <25% of the substrate was metabolized. Reactions were terminated after 2 min by addition of 1.5 ml of chloroform/methanol (1:2). Inositol phosphates were extracted and desalted by the sequential addition of 1 ml of chloroform, 0.5 ml of 1.2 *M* HCl, 25 μl of phytic acid hydrolysate (50 mg/ml), and 1 ml of Dowex 50 × 8 (H^+ form, 50% slurry). After vortex-mixing and low-speed centrifugation, the upper layer was transferred to a 12 × 75 mm tube, the lower layer was washed with 1 ml of deionized water, and the combined upper layers were evaporated to dryness in a Savant vacuum centrifuge. Dried samples were reconstituted in 60 μl of water and one-half of each sample was subjected to high-voltage electrophoresis at 4,000 V for 20 min in sodium oxalate buffer, pH 1.5 (Seiffert and Agranoff, 1965). Electrophoretograms were exposed to x-ray film overnight (with or without intensifying screens). Spots corresponding to IP_3, IP_4, and P_i were cut out (Agranoff et al., 1983) and radioactivity was determined by scintillation counting. Recovery of initial radioactivity through the extraction and electrophoresis procedures was 85–90%. Enzyme blanks, to which chloroform/methanol was added before the tissue samples, were run for each assay. Electrophoresis of these enzyme blanks indicated that the [^{32}P]IP_3 substrate was >98% pure, with only trace amounts of radioactivity comigrating with IP_4 and P_i. IP_3 5′-phosphatase activity was calculated from the loss of IP_3 in the sample compared with the enzyme blank. Only a small fraction of the starting radioactivity remained in the IP_2 produced, because 80–90% of the radioactivity in the [^{32}P]IP_3 was in the 5′ position. For assay of 3′-kinase activity, the amount of IP_4 produced was determined. Results are expressed as picomoles per minute per milligram of protein. In addition to high-voltage electrophoresis, product identity was verified by comigration of authentic ^{3}H-labeled standards on a Partisil-Sax HPLC column (0.01–1.0 *M* ammonium phosphate gradient; Dean and Moyer, 1987), monitored by a Beckman on-line radioisotope detector. *p*-Nitrophenyl phosphatase activity was determined in adult cortical or cerebellar homogenates by measurement of absorbance at 405 nm on incubation with 6 m*M* *p*-nitrophenyl phosphate at 20°C, in a reaction mixture of the same composition as that used for IP_3 5′-phosphatase. Protein was measured by the method of Geiger and Bessman (1972).

Measurement of IP_3 binding activity

Assays were carried out essentially as described by Worley et al. (1987*a*). Tissue was thawed, homogenized in 50 m*M* Tris-HCl, 1 m*M* EDTA, pH 8.3, then centrifuged at 27,000 *g* for 15 min. The pellet was resuspended in the same buffer to a protein concentration of approximately 2 mg/ml for cerebral cortex and 0.4 mg/ml for cerebellum. Tissue samples (0.5 ml) were then incubated at 4°C in a total volume of 1 ml with 1 n*M* [^{3}H]IP_3 (15,000–20,000 dpm/tube) for 15 min. Protein was then pelleted in a microfuge, the supernatant was aspirated, and the pellet was resuspended in 0.5 ml of 1% Triton X-100, then transferred to a vial for scintillation counting. Results are expressed as dpm bound per milligram of protein, after subtraction of nonspecific binding, determined in the presence of 1 μ*M* IP_3.

IP_3 5′-phosphatase histochemistry

The procedure was a modification of a histochemical phosphatase detection method (Wachstein and Meisel, 1957; Schoen et al., 1988). Unfixed frozen cross-sections of adult rat brain (20 μm thick) were incubated at 37°C for 60 min with 1 m*M* IP_3 in a reaction mixture containing 0.25 *M* sucrose, 2 m*M* lead nitrate, 10 m*M* magnesium chloride, 10 m*M* β-mercaptoethanol, and 20 m*M* HEPES buffer, pH 7.0. Sections were then rinsed in 0.25 *M* sucrose, 10 m*M* HEPES buffer, pH 7.0 (3 × 5 min at room temperature), fixed in 2% buffered paraformaldehyde for 10 min, rinsed in distilled water, then developed in sodium sulfide (diluted 1:30 in distilled water) for 1 min. Controls, which were incubated either in

the absence of substrate or in the absence of Mg^{2+}, showed no reaction product.

RESULTS

Ontogeny of IP_3 5'-phosphatase and 3'-kinase

Activities of IP_3 5'-phosphatase and 3'-kinase were measured in cerebral cortex from adult rats and from developing rats of the following ages: 18-day embryos (E18), and 1-, 7-, 14-, and 29-day postnatal (Fig. 1). Preliminary experiments established the amount of tissue homogenate required, for each age tested, to ensure linearity of the reactions throughout the 2-min incubation period. Cerebral cortical IP_3 5'-phosphatase activity increased from 46% to 89% of adult values between birth and 2 weeks postnatal. IP_3 3'-kinase developed more slowly, reaching 46% and 84% of adult levels at 14 days and 29 days postnatal, respectively. The changes in activities of both these enzymes during development reflect differences in V_{max} because the affinities of each enzyme for IP_3 did not vary significantly as a function of age: the K_m values for IP_3 5'-phosphatase were 27 μM and 45 μM and for IP_3 3'-kinase they were 3.6 μM and 1.5 μM in adult and 7-day postnatal homogenates, respectively. The V_{max} values in 7-day postnatal and adult cerebral cortex homogenates, respectively, for the 5'-phosphatase were 4.7 and 11.6 nmol/min/mg protein and for the 3'-kinase they were 0.5 and 2.5 nmol/min/mg protein. IP_3 5'-phosphatase activity was Mg^{2+} dependent and insensitive to NaF (2 mM). Addition of 5 mM 2,3-diphosphoglyceric acid inhibited 5'-phosphatase activity by 94%. The inclusion of Li^+ had no effect on the activity of either enzyme. Neither were there changes in subcellular distribution

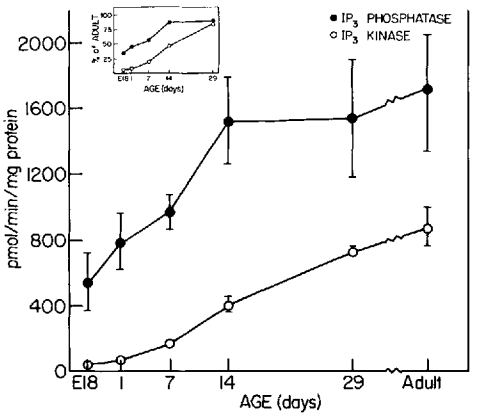

FIG. 1. Developmental changes in IP_3 phosphatase and kinase activities in rat cerebral cortex. Tissue from 18-day embryos (E18), and 1-, 7-, 14-, and 29-day postnatal or adult rats was homogenized, then aliquots were incubated with 10 μM [^{32}P]IP_3 for 2 min at 37°C. Reaction products were separated by high-voltage electrophoresis. IP_3 phosphatase and kinase activities are expressed as picomoles of IP_3 hydrolyzed or picomoles of IP_4 formed per minute per milligram of protein, respectively. Values are means ± SEM for three to six experiments. **Inset** shows the same data calculated as percent of adult values.

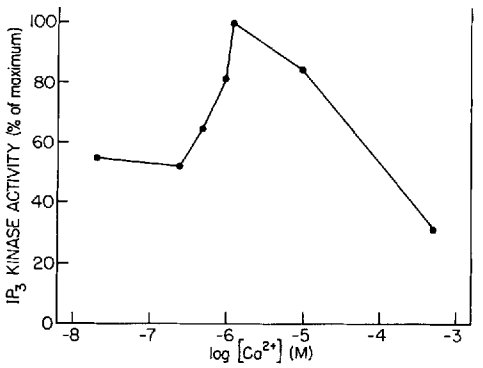

FIG. 2. Sensitivity of IP_3 3'-kinase activity to the concentration of free Ca^{2+}. Aliquots of adult rat cerebral cortex supernatant (66 μg of protein) were incubated for 2 min with 10 μM [^{32}P]IP_3 in the presence of 1 mM EGTA and the following concentrations of added $CaCl_2$: 0, 0.15, 0.25, 0.4, 0.5, 1.0, or 1.5 mM. $[Ca^{2+}]_f$ was determined fluorometrically with fluo-3 as the indicator. The standard reaction mixture containing 0.5 mM added Ca^{2+} corresponded to a $[Ca^{2+}]_f$ of 1.2 μM, which supported maximal kinase activity (3,020 pmol/min/mg protein). Values are the means of duplicate determinations which agreed within 13%. Data shown are from one of two experiments which gave similar results.

of the enzymes during development. In both 7-day postnatal and adult cerebral cortex, IP_3 3'-kinase activity was >95% soluble whereas 5'-phosphatase activity was 60–70% particulate. [^{3}H]IP_3 binding in 7-day, 14-day, and 29-day cortex was 20%, 45%, and 90% of that in adult cortex (1,980 ± 150 dpm/mg protein; mean ± SEM, n = 4). The possibility of age-related alterations in the affinity of IP_3 for the receptor was not assessed.

Effect of $[Ca^{2+}]_f$ on IP_3 3'-kinase activity

There have been conflicting reports in the literature as to whether the IP_3 3'-kinase activity in CNS tissue is (Hansen et al., 1987; Ryu et al., 1987) or is not (Irvine et al., 1986; Morris et al., 1988) stimulated by physiological concentrations of Ca^{2+}. In an attempt to clarify this issue further, the Ca^{2+} sensitivity of IP_3 kinase in adult cerebral cortical supernatant was determined (Fig. 2). The Ca^{2+} concentration in the kinase reaction mixture buffer (which contained 1 mM EGTA) was varied from zero added Ca^{2+} to 1.5 mM added Ca^{2+} and the $[Ca^{2+}]_f$ was directly determined fluorometrically, using the calcium indicator, fluo-3 (Minta et al., 1987). On increasing $[Ca^{2+}]_f$ from 0.3 to 1.2 μM, IP_3 3'-kinase activity increased twofold. Inhibition of the enzyme was apparent at 1.5 mM added Ca^{2+} ($[Ca^{2+}]_f$ ~0.5 mM). IP_3 5'-phosphatase activity was insensitive to physiological levels of $[Ca^{2+}]_f$, although inclusion of 2 mM $CaCl_2$ caused 40–42% (n = 2) inhibition.

Regional distribution of IP_3 5'-phosphatase and 3'-kinase

Activity for both enzymes in five adult rat brain regions was heterogeneously distributed (Fig. 3). Of particular note were the very low levels of IP_3 3'-kinase in hypothalamus and pons/medulla. The possibility that

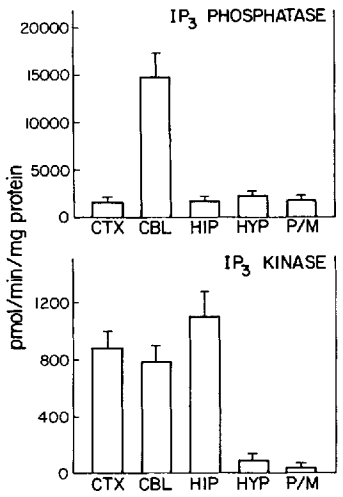

FIG. 3. Regional distribution of IP_3 5′-phosphatase and 3′-kinase in adult rat brain. Homogenates of cerebral cortex (CTX), cerebellum (CBL), hippocampus (HIP), hypothalamus (HYP), or pons/medulla (P/M) were incubated for 2 min with 10 μM [^{32}P]IP_3 in the absence or presence of ATP. The loss of IP_3 (for 5′-phosphatase activity) or formation of IP_4 (for 3′-kinase activity) was determined and expressed as picomoles per minute per milligram of protein. Values shown are means ± SEM for four to six experiments.

an inhibitor of the enzyme in these tissues could account for this low activity was ruled out by assay of a mixture of pons/medulla and hippocampal homogenates, which resulted in additive activity. In addition, there was a marked enrichment of IP_3 5′-phosphatase in the cerebellum. The latter was not due to differences in substrate affinity, as the K_m for IP_3 in cerebellar homogenates was, at 27 μM, equivalent to that of adult cerebral cortex. The V_{max} for the 5′-phosphatase in cerebellum was 50 nmol/min/mg protein. The issue of whether nonspecific phosphatase activity might be contributing to this enrichment in the cerebellum was addressed by determination of *p*-nitrophenyl phosphatase activity. Homogenates of adult rat cortex and cerebellum displayed similar *p*-nitrophenyl phosphatase activity, 5.89 and 7.1 nmol/min/mg protein, respectively. A greater proportion (>90%) of the IP_3 5′-phosphatase activity was membrane bound in the cerebellum than in the cerebral cortex. Enzyme activity in cerebellum supernatant fractions was equivalent to that in cerebral cortex supernatant. Because IP_4 may also be a substrate for the same 5′-phosphatase (Erneaux et al., 1987; Hansen et al., 1987; Shears, 1989), activities against [^{3}H]IP_4 were also determined. When assayed on the same tissue samples with each substrate at 10 μM, 5′-phosphatase activities for IP_3 were 1,670 and 14,900 pmol/min/mg protein and for IP_4 they were 150 and 900 pmol/min/mg protein in adult cerebral cortex and cerebellum homogenates, respectively. HPLC was used to verify that the product of IP_4 hydrolysis was inositol 1,3,4-trisphosphate. Under the assay conditions employed, no 3′-phosphatase activity (Hoer et al., 1988) was detected. Because the cerebellum represents 15–20% of the total wet weight of the adult rat brain, it can be estimated that 50–60% of total brain IP_3 5′-phosphatase activity resides in the cerebellum.

Localization of IP_3 5′-phosphatase within the cerebellum

The enrichment of IP_3 receptor in the cerebellum is selectively localized to the Purkinje cells (Worley et al., 1987*a*, 1989). Two approaches were undertaken to determine whether the IP_3 5′-phosphatase is similarly localized. The first approach was the application of a histochemical method based on the ability of Pb^{2+} to trap released P_i in situ. Figure 4 shows staining of frozen sections of adult rat cerebellum incubated in the presence or absence of 1 mM IP_3. Heavy staining of the cerebellar molecular layer was observed following incubation with IP_3, whereas no reaction product was

FIG. 4. Histochemical localization of IP_3 5′-phosphatase activity in adult rat cerebellum. Frozen sections were incubated at 37°C for 60 min in the presence (**top**) or absence (**bottom**) of 1 mM IP_3. The P_i released was trapped, in situ, as $Pb_3(PO_4)_2$, then visualized by conversion to PbS.

observed if IP_3 was omitted. Only faint staining was observed following incubations of IP_3 with sections from other brain regions. Prior denaturation of the cerebellar tissue sections by heating at 70°C for 15 min blocked the staining reaction, as did omission of Mg^{2+} from the incubation mixture. No reaction product was apparent when either IP_2 or IP_4 was used as substrate. Staining produced with IP_3 as substrate was unaltered in the presence of heparin (100 μg/ml), an inhibitor of the binding of IP_3 to its receptor (Worley et al., 1987*b*; Ghosh et al., 1988), but was completely inhibited by the inclusion of 5 m*M* 2,3-diphosphoglyceric acid. However, the latter effect was nonspecific inasmuch as staining for 5′-nucleotidase activity with 5′-AMP as substrate was also blocked. The second approach undertaken was determination of IP_3 5′-phosphatase activity in cerebellum and cortex of Purkinje cell-deficient (nervous) mice. [^{3}H]IP_3 binding, which was shown by an autoradiographic method (Worley et al., 1989) to be absent in the cerebellum of a different strain of Purkinje cell-deficient mice (lurcher), was also determined (Fig. 5). Both the enzyme activity and the receptor density were markedly reduced in the mutant mouse cerebellum, whereas the cerebral cortex was unaffected. The IP_3 receptor density in the cerebellum was more affected (−87%) by the loss of Purkinje cells than was IP_3 5′-phosphatase activity (−61%). If the activities in the cortex are used as baselines and the enrichment above these values is calculated, then the loss of the IP_3 5′-phosphatase activity in mutant mouse cerebellum was 81% whereas that of the IP_3 receptor was 94%. Thus both the histochemical and the mutant mouse data support a selective localization of cerebellar IP_3 5′-phosphatase to the Purkinje cells.

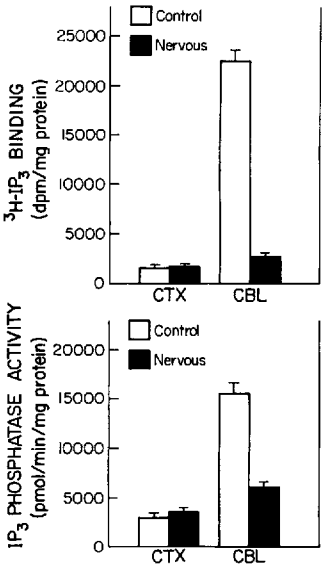

FIG. 5. Determination of IP_3 receptor density and IP_3 5′-phosphatase activity in cerebella of Purkinje cell-deficient mice. Aliquots of homogenates of freshly dissected cerebral cortex (CTX) or cerebellum (CBL) from individual nervous mutant mice or control littermates (age, 53 days) were incubated with 10 μ*M* [^{32}P]IP_3 at 37°C for 2 min and reaction products were analyzed by high-voltage electrophoresis. Activity is expressed as picomoles of IP_3 hydrolyzed per minute per milligram of protein. A total particulate fraction was prepared from each homogenate and IP_3 receptor density was determined by a centrifugation assay following incubation with 1 n*M* [^{3}H]IP_3. Data are expressed as dpm bound per milligram of protein, following subtraction of nonspecific binding obtained in the presence of 1 μ*M* IP_3. Values shown are means ± SEM of three determinations from one of two experiments which gave similar results.

DISCUSSION

An initial step in signal transduction at phosphoinositide-linked cell surface receptors is the phosphodiesteratic cleavage of phosphatidylinositol 4,5-bisphosphate. The IP_3 thus formed functions to release Ca^{2+} from intracellular stores, most likely via the mediation of an IP_3 receptor. Support for such a role for the latter in the CNS was recently obtained from studies of Ca^{2+} release in cerebellum microsomes (Stauderman et al., 1988; Joseph et al., 1989). The ability of and extent to which IP_3 may function to modulate intracellular [Ca^{2+}] is dependent not only on the relative density of IP_3 receptors, but also on the rate and route of IP_3 metabolism. Dephosphorylation via the 5′-phosphatase appears to be an essentially deactivating route, because there is no known physiological action for IP_2. In contrast, action of the 3′-kinase on IP_3 results in the formation of IP_4, which may itself modulate Ca^{2+} homeostasis (Irvine, 1989). The activities of these two enzymes and the density of IP_3 receptor sites are determinants of the second messenger function of the IP_3 limb of the phosphoinositide-signal transduction pathway. The efficiency of coupling of CNS receptors to phosphoinositide turnover has previously been shown to differ within a given brain region as a function of the stage of development (Nicoletti et al., 1986; Heacock et al., 1987; Rooney and Nahorski, 1987) and among adult brain regions (Fisher and Agranoff, 1987 and references therein). The results of the present study demonstrate that the enzymes of IP_3 metabolism also vary as a function of age and location within the rat brain, further contributing to ontogenetic and regional differences in signal transduction.

The ontogeny of IP_3 3′-kinase in the rat cerebral cortex was found to lag behind that of the 5′-phosphatase such that the ratio of phosphatase to kinase activity decreased as a function of age. Although the enzyme activities were determined at subsaturating substrate concentrations, the similarities of the K_m values when adult and 7-day cortex were compared indicate that the data in Fig. 1 accurately reflect age-related changes in the tissue activities of each enzyme. The IP_3 affinities obtained for both enzymes in crude adult cerebral cortex homogenates were somewhat higher than those reported for highly purified soluble brain IP_3 5′-phosphatase (3 or 18 μ*M*; Hansen et al., 1987) or 3′-kinase (0.2 μ*M*; Johanson et al., 1988). Variations in assay conditions and/or the presence of additional factors in

the crude homogenates may account for these differences. The properties of the IP_3 5′-phosphatase observed in crude homogenates, i.e., requirement for Mg^{2+}, sensitivity to 2,3-diphosphoglyceric acid, and insensitivity to Li^+ and F^- are the same as those described for both soluble and membrane-bound forms of this enzyme in brain and other tissues (Downes et al., 1982; Seyfred et al., 1984; Hansen et al., 1987). The activity of the IP_3 3′-kinase was found to be sensitive to physiological concentrations of free Ca^{2+}, in contrast to the conclusions of Irvine et al. (1986) and Morris et al. (1988), but in agreement with Ryu et al. (1987) and Johansen et al. (1988), who also provide evidence for a role for calmodulin in mediating this Ca^{2+} sensitivity. A similar enhancement of IP_3 3′-kinase by Ca^{2+} has been described in other systems (Biden et al., 1987; Morris et al., 1987; Daniel et al., 1988; Yamaguchi et al., 1988). Thus, the rise in intracellular Ca^{2+} that results from increased IP_3 formation may serve to accelerate the rate of its phosphorylation.

In regional studies of adult rat brain, the most striking findings were that IP_3 3′-kinase activity was very low in hypothalamus and pons/medulla compared to activities in cerebral cortex, hippocampus, and cerebellum, whereas IP_3 5′-phosphatase activity was highly enriched in cerebellum. Although it is tempting to speculate that the observed differences in relative amounts of these two IP_3-metabolizing enzymes during development and within adult brain regions may be reflected in differences in the metabolic fate of IP_3, this conclusion would be an oversimplification, because additional factors that would affect these reactions must be taken into account, not least of which is the substrate concentration. Estimates of resting concentrations of IP_3 in brains of rats killed by microwave irradiation of 1.6 μM (Meek, 1986) or 16 μM (Bredt et al., 1989) are of the same order as that used in the enzyme assays described here. However, it is not possible to extrapolate directly from the relative rates of 3′-kinase and 5′-phosphatase activities assayed in vitro to the in vivo situation, because in intact tissue the extent of substrate and/or enzyme compartmentation and the possible effects of endogenous regulatory factors would all serve to affect the metabolic fate of IP_3.

The brain presents an additional layer of complexity relative to other tissues due to the multiplicity of cell types within each brain region, as is evident from our finding that the enrichment of IP_3 5′-phosphatase activity observed in cerebellum homogenates is predominantly a property of cerebellar Purkinje cells. The histochemical assay used to visualize IP_3 5′-phosphatase activity in frozen sections of rat brain revealed heavy staining of the cerebellar molecular layer. Although it is likely that the observed staining with IP_3 as substrate was due to specific 5′-phosphatase activity, this cannot be definitively established without identification of IP_2 as the other product. Therefore this finding was extended by determination of IP_3 5′-phosphatase activity in mutant mice lacking Purkinje cells. The 81% loss of the enrichment in cerebellar IP_3 5′-phosphatase in nervous mutant mice verifies the conclusion arising from the results of the histochemical assay. Measurements of [^{3}H]IP_3 binding by a centrifugation assay also demonstrated a marked loss of cerebellar IP_3 receptor in Purkinje cell-deficient mice, a finding in agreement with results of a recent autoradiographic study (Worley et al., 1989). Cerebellar Purkinje cells are also reported to possess high concentrations of certain species of phospholipase C (Gerfen et al., 1988) and protein kinase C (Nishizuka, 1988). The results reported here extend the list of components of the phosphoinositide second messenger system that are enriched in these cells to include IP_3 5′-phosphatase. There is no obligatory link between levels of IP_3 5′-phosphatase and IP_3 receptor density, however, because hypothalamus and pons/medulla, which are devoid of IP_3 receptors (Worley et al., 1989), express normal IP_3 5′-phosphatase activities (i.e., similar to cortex and hippocampus), but very low activities of the 3′-kinase.

We have previously shown that the efficiency of coupling of muscarinic receptors to phosphoinositide turnover in cerebral cortex varies with postnatal age (Heacock et al., 1987). The present study, indicating that the metabolic fate of IP_3 may vary according to brain region, cell type, and stage of development, introduces a further level of complexity to be superimposed upon the observed ontogenetic and regional differences in the coupling of CNS receptors to phosphoinositide turnover.

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