

Short Review

Inositol Lipids and Signal Transduction in the Nervous System: An Update

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The role that inositol lipids play in cellular signaling events in eukaryotic cells remains one of the most intensively investigated areas of cell biology. In this respect, phosphoinositide-mediated signal transduction in the CNS is no exception; major advances have been made since a previous review on this subject (Fisher and Agranoff, 1987). Not only have stimulated phosphoinositide turnover and its physiological sequelae been demonstrated repeatedly in a variety of neural preparations, but, in addition, the detailed molecular mechanisms underlying these events continue to unfold. Here we review the progress that has occurred in selected aspects of this topic since 1987. In the first two sections of this article, emphasis is placed on novel functional roles for the inositol lipids and on recent insights into the molecular characteristics and regulation of three key components of the phosphoinositide signal transduction system, namely, the inositol lipid kinases, phospholipases C (PLCs), and the inositol 1,4,5-trisphosphate [I(1,4,5)P₃] receptor. The metabolic fate of I(1,4,5)P₃ in neural tissues, as well as its control, is also detailed. Later we focus on identification of the multiple receptor subtypes that are coupled to inositol lipid turnover and discuss possible strategies for intervention into phosphoinositide-mediated signal transduction. Due to space limitations, an extensive evaluation of the diacylglycerol/protein kinase C (DAG/PKC) limb of the signal transduction pathway is not included (for reviews, see Nishizuka, 1988; Kanoh et al., 1990).

INOSITOL LIPIDS AND ENZYMES OF THEIR METABOLISM

Phosphoinositides and cell function: new roles

It is by now well-established that the major inositol-containing lipids that serve as precursors of intracellular second messenger molecules in both neural and non-neural tissues are phosphatidylinositol (PI), phosphatidylinositol 4-phosphate (PIP), and phosphatidylinositol 4,5-bisphosphate (PIP₂). However, recent studies indicate the additional presence of a number of quantitatively minor inositol lipids that are characterized by the presence of a phosphate group at the D-3 position of the inositol ring. Although much of the evidence accumulated for the existence of these lipids comes from nonneural tissues (for review, see Carpenter and Cantley, 1990), PI(3)P has been identified in 1321NI astrocytoma cells (Stephens et al., 1989) and in NG 115-401L-C3 neuroblastoma cells (Poyner et al., 1990), and phosphatidylinositol 3,4,5-trisphosphate (PIP₃) in cerebral cortex (Vadnal and Parthasarathy, 1989). Because the 3-phosphoinositides are only poorly separated from their quantitatively major counterparts by present TLC methods, definitive identification of the lipid structure requires an initial removal of the glycerol backbone to yield the inositol phosphate, followed by periodate oxidation, reduction, and dephosphorylation to the corresponding polyol. Although such rigorous analysis has been used for identification of PI(3)P in astrocytoma cells (Stephens et al., 1989), in

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Abbreviations used: ACPD, 1-aminocyclopentyl-1,3-dicarboxylic acid; [Ca²⁺]_i, concentration of intracellular Ca²⁺; CDP-DAG, cytidine diphosphodiacylglycerol; DAG, diacylglycerol; ET, endothelin; G protein, guanine nucleotide binding protein; G_p, putative G protein that regulates PLC activity; GTPγS, guanosine 5'-O-(3-thiotriphosphate); IP₁, *myo*-inositol monophosphate; IP₂, *myo*-inositol bisphosphate; IP₃, *myo*-inositol trisphosphate; IP₄, *myo*-inositol tetra-

kisphosphate; IP₅, *myo*-inositol pentakisphosphate; IP₆, *myo*-inositol hexakisphosphate; (numbering of phosphate groups is related to the 1-D structure of PI); mAChR, muscarinic acetylcholine receptor; PI, phosphatidylinositol; PIP, phosphatidylinositol 4-phosphate; PIP₂, phosphatidylinositol 4,5-bisphosphate; PIP₃, phosphatidylinositol 3,4,5-trisphosphate; (positions of phosphate groups are designated when different from those indicated); PKA, protein kinase A; PKC, protein kinase C; PLA₂, phospholipase A₂; PLC, phospholipase C; SDS-PAGE, sodium dodecylsulfate-polyacrylamide gel electrophoresis; SRTX, sarafotoxin.

most instances, structures of the 3-phosphoinositides have been inferred from a comparison of HPLC elution profiles of deacylated lipid products with authentic standards. The physiological role of these novel phosphoinositides is not yet known. Upon the addition of carbachol to astrocytoma cells, a slow but marked decrease in PI(3)P radiolabel was noted (Stephens et al., 1989). In contrast, labeling of the same lipid was unaltered following the addition of either mitogenic or nonmitogenic stimuli to NG 115-401L-C3 cells (Poyner et al., 1990). Although the 3-phosphoinositides were first identified in cells that undergo rapid cell division, PI(3)P has now been identified in both proliferative and nonproliferative tissues (Downes and MacPhee, 1990). Furthermore, because these phosphoinositides are present only in trace concentrations and do not serve as substrates for PLC activity (Lips et al., 1989; Serunian et al., 1989), their role as precursors of second messenger molecules appears unlikely. This raises the possibility that the 3-phosphoinositides undergo further metabolism, e.g., dephosphorylation (see Lips and Majerus, 1989).

A separate role for inositol lipids in the maintenance of the cytoskeleton has also been proposed based upon the known ability of PIP₂ to bind to actin-binding proteins, the best documented of which is gelsolin. Bound PIP₂ facilitates the release of gelsolin from the actin filament, thereby regulating new filament formation (for review, see Majerus et al., 1990). PIP₂ also binds with high affinity ($K_D < 0.1 \mu M$) to profilin, a protein which inhibits actin polymerization (Goldschmidt-Clermont et al., 1990). It is possible that PIP₂ thus promotes actin polymerization by regulation of the gelsolin-actin and profilin-actin complexes. Recently, a direct role for phosphoinositides in exocytosis from chromaffin cells was proposed (Eberhard et al., 1990). It is conceivable that, in these cells, cytoskeletal elements involved in exocytosis are regulated by the inositol lipids.

The presence of a PI glycan in brain was first indicated some 30 years ago by Klenk and Hendricks (1961), who demonstrated that human brain contained an inositol lipid with glucosamine, mannose, and ethanolamine. In both neural and nonneural cells, PI glycans may anchor proteins to the outer membrane leaflet (thereby conferring increased lateral mobility). The common structural features of this attachment are an ethanolamine residue with an amide linkage to the terminal carboxyl group of the protein, a mannose-containing glycan, and a nonacetylated glucosamine residue linked to the D-6 position of *myo*-inositol via a glycosidic linkage (for reviews, see Low, 1989; Lisanti et al., 1990). In brain, Thy-1 and a number of other cell-surface glycoproteins, the expression of which may be regulated during development, are anchored to the membrane via glycosyl PI linkages (Low and Kincade, 1985; Margolis et al., 1988). Moreover, brain contains at least two PLC activities which appear specific for the glycosylated form of PI (Fouchier et al., 1990). Un-

like the bulk of cellular PI in which stearate and arachidonate predominate, these substituents are no longer prominent in PI anchors, being replaced in various tissues and species by myristate, octadecanol (as a 1-alkyl substituent), or docosanoate. In PC12 pheochromocytoma cells, the addition of nerve growth factor stimulates both the production of a [³H]myristate-labeled species of DAG (but not that of [³H]arachidonyl-DAG) and an inositol phosphate glycan (Chan et al., 1989). The latter may serve as an intracellular second messenger, as has been proposed for insulin action (Lisanti et al., 1990). The possibility that *D-chiro*-inositol-anchored proteins occur (Ferguson and Williams, 1988; Kennington et al., 1990) must also be considered.

Phosphoinositide kinases and their regulation

Although the concept that multiple forms of PI kinase might exist in tissues was first raised over 20 years ago (Harwood and Hawthorne, 1969), only recently have distinct forms of the enzyme been isolated and purified from brain. At least three forms of PI kinase can be distinguished on the basis of the product formed, M_r , ATP requirement, effects of detergents, and inhibition by adenosine (Table 1A). A type I or PI 3-kinase, originally purified from fibroblasts (Whitman et al., 1987), has been purified to near homogeneity from bovine brain cytosol (Morgan et al., 1990). This kinase specifically phosphorylates PI at the D-3 (rather than the D-4) position of *myo*-inositol, is strongly inhibited by detergents, and exhibits a high affinity for ATP. This enzyme will also phosphorylate added PIP and PIP₂ to a comparable extent with the presumed formation of PI(3,4)P₂ and PIP₃, respectively (Morgan et al., 1990). Two additional 4-kinase activities have been described by Endemann et al. (1987), both of which are specific for PI. One (type II PI kinase) can be distinguished from PI 3-kinase in that it is an integral membrane protein, phosphorylates only at the D-4 position, is of a lower M_r , and is activated by detergents. It is also potentially inhibited by adenosine. A type III PI kinase is also present in brain and shows the same phosphorylation pattern as type II PI kinase, but exhibits a larger M_r and a lower affinity for ATP. Recently, a monoclonal antibody has been developed which is highly specific for the type II kinase (Endemann et al., 1991). Saltiel et al. (1987) have also isolated a 4-kinase activity from myelin which is distinct from that described as type I, but phosphorylates both PI and PIP. Little is known of the regulation of PI kinase activities in brain. In contrast, in nonneural cells, inhibition and activation of enzyme activity by adenosine and polyamines, respectively, have been proposed (Endemann et al., 1987; Downes and MacPhee, 1990).

PIP 5-kinases are present in both the cytosol and brain membranes, although they predominate in the former (Table 1B). These enzymes phosphorylate PIP, but not PI, and have been purified from both the cytosol and membranes. There is some suggestion that PIP

TABLE 1. Biochemical characteristics of phosphoinositide kinases in brain

	Source	M _r	Substrate	Product	K _m (ATP) (μ M)	Detergent	K _i (adenosine) (μ M)	Reference
A. PI kinases								
Type I	Bovine brain (whole brain)	85K	PI PIP PIP ₂	PI(3)P PIP ₂ PIP ₃	67	Inhibition	ND	Morgan et al. (1990)
Type II	Bovine brain (membranes)	55K	PI	PIP	54	Activation	18	Endemann et al. (1987)
Type III	Bovine brain (membranes)	230K	PI	PIP	742	Activation	1,520	Endemann et al. (1987)
	Rat brain (membranes)	76K	PI	PIP	150	Activation	200	Yamakawa and Takenawa (1988)
?	Bovine brain (myelin)	45K	PI PIP	PIP PIP ₂	150	ND	ND	Saltiel et al. (1987)
B. PIP 5-kinase								
	Rat brain (cytosol)	45K	PIP	PIP ₂	25			Cochet and Chambaz (1986)
	Rat brain (cytosol)	45K	PIP	PIP ₂	ND			Van Dongen et al. (1984)
	Bovine brain (membranes)	110K	PIP	PIP ₂	ND			Mortiz et al. (1990)

ND, not determined.

kinase in brain membranes may be activated by GTP and its nonhydrolyzable analogues, an effect apparently not mediated via an inhibition of phosphomonoesterase or PLC activities (Smith and Chang, 1989; Strosznajder and Strosznajder, 1989). A further means of regulation of PIP kinase may be through a brain-specific protein B₅₀, which upon phosphorylation by PKC, inhibits PIP kinase (Van Hooff et al., 1988).

PLC and its regulation

Multiple forms of PLC are now known to exist in both neural and nonneural tissues. The five isozymes thus far identified (designated as α , β , γ , δ , and ϵ , based upon the chronological order of their purification) are immunologically distinct entities and the products of separate genes (Rhee et al., 1989). In addition, a number of isoforms presumed to reflect proteolytic cleavage of the parent enzymes have been identified and purified. Amino acid sequence data indicate that only a very limited homology exists between the β , γ , and δ isozymes, whereas none is observed for PLC- α . The two regions of homology include one of approximately 150 and a second of 120 amino acid residues which are 54% and 42% identical between the isozymes, respectively, but are differentially localized within each isozyme. PLC- γ also exhibits some amino acid sequences that are related to those found in nonreceptor tyrosine kinases of the *src* family, GTPase-activating protein, and α -spectrin. Deletion mutant forms of PLC that lack portions of these sequences retain enzyme activity, thereby indicating that they serve a regulatory, rather than catalytic function (Emori et al., 1989).

Of the five known isozymes, PLC- β , - γ , and - δ predominate in brain and have been extensively purified from a variety of sources (Table 2). The cDNA se-

quences of the β , γ , and δ forms of brain PLC have also been elucidated and antibodies to these isozymes raised. Although PLC has been purified routinely from cytosol, enzyme activity is also present in membrane fractions, the proportions of PLC activity present in each cellular compartment being isozyme-specific (Lee et al., 1987). Confirmation that the same enzyme activity may reside in both cytosol and membrane fractions was obtained by Lee et al. (1987), who demonstrated that PLC- β activities derived either from KCl-washed membranes or from cytosol had similar M_r values, were recognized by the same monoclonal antibodies, and exhibited a similar elution profile of tryptic peptides. Although the bimodal subcellular distribution of PLC has raised the suggestion that the enzyme undergoes translocation, there is little direct evidence for this at present. The availability of monoclonal antibodies to PLC- β , - γ , and - δ has permitted studies of the regional and cellular distribution of the isozymes

TABLE 2. PLC isozymes isolated from brain

PLC isozyme	Source	M _r	Reference
β -1	Bovine brain	150K	Ryu et al. (1986, 1987a,b)
	Bovine brain	154K	Katan and Parker (1987)
	Rabbit brain	155K	Carter et al. (1990b)
β -2	Bovine brain	140K	Ryu et al. (1987a,b)
β -3	Bovine brain	100K	Ryu et al. (1986, 1987a,b)
γ	Bovine brain	145K	Ryu et al. (1986, 1987a,b)
δ	Bovine brain	85K	Ryu et al. (1987a,b)
	Rat brain	85K	Homma et al. (1988)
	Bovine brain	88K	Rebecchi and Rosen (1987)
(ϵ)	Rat brain	85K	Homma et al. (1988)

within the CNS. Whereas PLC- γ is distributed relatively uniformly within neurons in all brain regions, PLC- β immunoreactivity is most concentrated in neurons present in the globus pallidus, substantia nigra, cerebral cortex, hippocampus, and thalamic nuclei (Gerfen et al., 1988). In contrast, PLC- δ immunoreactivity appears preferentially localized to glial cells in all brain areas examined (Choi et al., 1989).

The activity of PLC is strongly dependent upon the assay conditions chosen. However, when conditions comparable to those of the intracellular ionic environment are used (pH 7, high K^+ , $[Ca^{2+}] \leq 1 \mu M$), two key properties of PLC become evident. The first is that for all isozymes (in particular, PLC- β ; see Rhee et al., 1989), PIP_2 and PIP are the preferred substrates. Inositol lipids containing a 3'-phosphate do not appear to serve as substrates for PLC (Lips et al., 1989; Serunian et al., 1989). The specificity with which the polyphosphoinositides are hydrolyzed in vitro by PLC is consistent with studies of their receptor-stimulated turnover in brain and neuroblastomas, in which evidence is obtained for the breakdown of PIP_2 and/or PIP , but not of PI (Batty and Nahorski, 1989; Fisher et al., 1990). The second property of note is that the concentration of Ca^{2+} required for full activation of PIP_2 hydrolysis is within the physiological range (0.1–1.0 μM), whereas much higher concentrations of the cation are required for PI hydrolysis. Thus, PLC is regulated by physiologically relevant concentrations of Ca^{2+} , an observation consistent with the ability of agents which promote a rise in the concentration of intracellular Ca^{2+} ($[Ca^{2+}]_i$), such as K^+ depolarization, Ca^{2+} ionophores, or maitotoxin, to elicit an increased phosphoinositide hydrolysis in neural tissues (Eberhard and Holz, 1988; Gusovsky et al., 1989; Baird and Nahorski, 1990a). In digitonin-permeabilized neuroblastoma cells, PLC activity could be regulated by alterations in $[Ca^{2+}]_i$ (Fisher et al., 1989).

It has been calculated that the activity of brain PLC, if unregulated, would be sufficient to elicit complete hydrolysis of PIP_2 within 2–20 s, even in the absence of receptor activation (Rhee et al., 1989). Although such calculations ignore PIP_2 resynthesis, it is probable that PLC activity in vivo is subject to stringent control. A further consideration is that the breakdown and resynthesis of PIP_2 is metabolically expensive (3 mol of ATP and 1 mol of CTP consumed per mol of PIP_2 hydrolyzed). These observations have led to the (as yet unconfirmed) suggestion that a regulatory protein exists which represses PLC in vivo (Fain et al., 1988; Rhee et al., 1989). More definite evidence exists for the ability of guanine nucleotide binding protein(s) [G protein(s)] to regulate PLC activity. The activation of PLC by guanine nucleotides (Gonzales and Crews, 1985) is presumably mediated through G protein (G_p) which in neural, as in nonneural tissues, is usually insensitive to either pertussis or cholera toxins. A novel pertussis toxin-insensitive G protein, G_q , has been shown recently to activate specifically the β isozyme of PLC

from bovine brain (Smrcka et al., 1991; Taylor et al., 1991). More elusive has been the demonstration of GTP-dependent agonist-stimulated PLC activity in brain membranes, but such has been observed recently (Chiu et al., 1988; Claró et al., 1989; Carter et al., 1990a). Whereas carbachol alone elicits little or no activation of PLC, a potentiative interaction occurs in the presence of guanosine 5'-*O*-(3-thiotriphosphate) ($GTP\gamma S$). Inhibitory G proteins may also regulate PLC. Nanomolar concentrations of nonhydrolyzable GTP analogues inhibit basal PLC activity, whereas at higher concentrations of the guanine nucleotide ($>1 \mu M$), the predicted stimulation of enzyme activity occurs (Litosch, 1989). The inhibitory (but not the stimulatory) effect on PLC activity can be prevented by preincubation of the membranes with pertussis toxin. Addition of F^- is also able to activate phosphoinositide hydrolysis in membranes, an effect usually attributed to activation of G_p (Litosch, 1987; Godfrey and Watson 1988; Gonzales and Crews, 1988; Jope, 1988). However, some caution is warranted in this interpretation, because the F^- effect on inositol phosphate release is not blocked by guanosine 5'-*O*-(2-thiodiphosphate) (P. P. Li et al., 1990). Furthermore, F^- has been demonstrated to activate purified PLC- β directly (Carter et al., 1990b). The mechanism whereby G_p activation regulates PLC activity is still uncertain, but may be a lowering of the enzyme's requirement for Ca^{2+} . Thus, in the absence of guanine nucleotides, supraphysiological concentrations of Ca^{2+} (1–10 μM) are needed for enzyme activation in permeabilized neuroblastoma cells, whereas in the presence of $GTP\gamma S$, concentrations of Ca^{2+} found in the cytosol of these cells (30–150 nM) suffice (Fisher et al., 1989).

PLC may also be regulated through phosphorylation. Numerous reports exist to indicate that activation of PKC by phorbol esters results in an inhibition of receptor-activated phosphoinositide hydrolysis (see Fisher and Agranoff, 1987, and references therein; Orellana et al., 1987; Pearce et al., 1988), and studies with permeabilized cells indicate that this inhibition resides at, or distal to, the G_p -PLC interaction site (Orellana et al., 1987; Cioffi and Fisher, 1990). Direct phosphorylation of PLC- α , $-\beta$, $-\gamma$, and $-\delta$ has been demonstrated in vitro upon the addition of purified PKC (Crooke and Bennett, 1989). However, no change in the catalytic activity of PLC occurs under such conditions. This raises the possibilities that PLC itself is not the target for PKC-mediated phosphorylation or, alternatively, that phosphorylated PLC loses its ability to interact with G_p . Phosphorylation of PLC by protein kinase A (PKA) also remains a possibility. Increases in tissue cyclic AMP are accompanied by an inhibition of both receptor- and $GTP\gamma S$ -stimulated phosphoinositide turnover in neuroblastoma and glioma cells, primary glial cultures, and isolated membranes (Akil and Fisher, 1989; Kim et al., 1989; Campbell et al., 1990; McAtee and Dawson, 1990; Robertson et al., 1990). It is suggested that the site of inhibition is at, or is distal

to, the G_p -PLC interaction (Akil and Fisher, 1989; McAtee and Dawson, 1990; Robertson et al., 1990), in a manner analogous to that observed for PKC inhibition. There is also evidence from nonneural cells that PLC is phosphorylated in response to the addition of growth factors. The latter bind to receptors which possess intrinsic tyrosine kinase activity, which can then phosphorylate a tyrosine residue on the PLC- γ (Rhee et al., 1989, and references therein). This means of receptor-activated PLC activity can be distinguished from the aforementioned types in that an intervening G protein is *not* involved (Boyer et al., 1989). Rhee et al. (1989) note that the molecular masses of PLC are significantly larger than those of related enzymes [e.g., phospholipase A_2 (PLA $_2$)], lending support to their suggestion that large portions of the PLC molecule are devoted to the enzyme's regulation.

Few specific inhibitors of PLC are currently available. Manoalide, which causes the irreversible inactivation of PLC- α , also has other sites of action, such as the blockade of Ca^{2+} channels and inhibition of PLA $_2$ (Crooke and Bennett, 1989). Mastoparan, a wasp venom undecapeptide, blocks GTP γ S-stimulated phosphoinositide turnover in neuroblastoma and astrocytoma cells (Wojcikiewicz and Nahorski, 1989; Nakahata et al., 1990). This compound does not penetrate intact cells uniformly, it is not specific for G_p -linked receptors, and its mode of inhibition (i.e., through G_p or direct interaction with PIP $_2$) is uncertain. To date, the most promising agent is the aminosteroid U-73122. This agent readily gains access to intact cells and blocks stimulated inositol lipid turnover, Ca^{2+} signaling, and related events (Bleasdale et al., 1990; Smith et al., 1990; Thompson et al., 1991). It is particularly effective at blocking GTP γ S-stimulated phosphoinositide turnover, whereas that induced by the addition of Ca^{2+} is less affected.

INOSITOL PHOSPHATE ISOMER FORMATION, METABOLISM, AND FUNCTION

Metabolism of I(1,4,5)P $_3$

The complexity of the metabolism of the inositol phosphates has become increasingly evident with the identification in tissues of more than 20 of the 63 possible isomers. Much effort has been expended in the elucidation of both their metabolic interrelationships and the characteristics of the relevant enzymes involved (for review, see Shears, 1989). It is now generally accepted that in brain, as in other tissues, the initial product of receptor-stimulated PLC activity is I(1,4,5)P $_3$ (and possibly its cyclic 1,2 derivative). Because it has been established that IP $_3$ acts as a second messenger in the mobilization of intracellular Ca^{2+} , the routes of its enzymatic metabolism and their regulation assume considerable importance (see Fig. 1). I(1,4,5)P $_3$ can be metabolized by either 5-phosphatase or 3-kinase activities to yield inositol 1,4-bisphosphate [I(1,4)P $_2$] or

inositol 1,3,4,5-tetrakisphosphate [I(1,3,4,5)P $_4$], respectively, either of which could be regarded as "off" signals. The 5-phosphatase is particularly enriched in cerebellum (50–60% of total brain activity) and has been localized histochemically to the cerebellar molecular layer (Heacock et al., 1990). Although primarily particulate (Erneux et al., 1986), two forms of the 5-phosphatase have been purified from brain cytosol (Hansen et al., 1987). Both type I (60 kDa) and type II (160 kDa) attack I(1,4,5)P $_3$ and I(1,3,4,5)P $_4$, although the type II enzyme is relatively weak against the latter substrate. With I(1,4,5)P $_3$ as substrate, the I(1,4)P $_2$ that results from 5-phosphatase action is dephosphorylated further to inositol 4-monophosphate [I(4)P $_1$] via the action of a Mg^{2+} -dependent inositol polyphosphate 1-phosphatase (Inhorn and Majerus, 1987, 1988; Gee et al., 1988a). This enzyme has been purified from brain cytosol, has an M_r of 40K–44K, and is inhibited by Ca^{2+} , and uncompetitively by Li^+ . 4-Phosphatase activity against I(1,4)P $_2$ in various tissue homogenates, including brain (Ackermann et al., 1987; Ragan et al., 1988) and neuroblastoma (Fisher et al., 1990), is either absent or low.

Alternatively, I(1,4,5)P $_3$ may be converted to I(1,3,4,5)P $_4$ via IP $_3$ kinase. In contrast to the 5-phosphatase, the 3-kinase is largely cytosolic and is particularly enriched in cortex, cerebellum, and hippocampus (Heacock et al., 1990). Recent *in situ* hybridization studies indicate a preponderance of the enzyme's mRNA in CA1 pyramidal neurons, granule cells of the dentate gyrus, and cerebellar Purkinje cells (Mailleux et al., 1991). The kinase has been purified from brain (Johanson et al., 1988; Lee et al., 1990; Takazawa et al., 1990a), and its cDNA has been cloned and sequenced (Choi et al., 1990; Takazawa et al., 1990b). The predicted molecular mass (50 kDa) is in close agreement with values obtained for the protein on sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE). Ca^{2+} and calmodulin increase the V_{max} of the enzyme at IP $_3$ concentrations greater than the K_m (Johanson et al., 1988; Takazawa et al., 1988; Heacock et al., 1990). A rise in [Ca^{2+}], may thus promote the formation of I(1,3,4,5)P $_4$, which is itself implicated in Ca^{2+} homeostasis. In addition to possessing a calmodulin-binding site, IP $_3$ kinase also exhibits six regions enriched in sequences that contain five amino acids (proline, glutamate, aspartate, serine, and threonine) which render it susceptible to hydrolysis by the protease calpain (Choi et al., 1990). A further potential means of regulation is via phosphorylation of the enzyme. Sim et al. (1990) have demonstrated that PKA- and PKC-induced phosphorylations of serine residues on brain IP $_3$ kinase increase and decrease, respectively, the V_{max} of enzyme activity.

Once formed, the major route of I(1,3,4,5)P $_4$ metabolism is that of dephosphorylation to I(1,3,4)P $_3$, catalyzed by the same 5-phosphatase that acts on I(1,4,5)P $_3$. In addition, an I(1,3,4,5)P $_4$ 3-phosphatase activity has been found in brain cytosol, which results

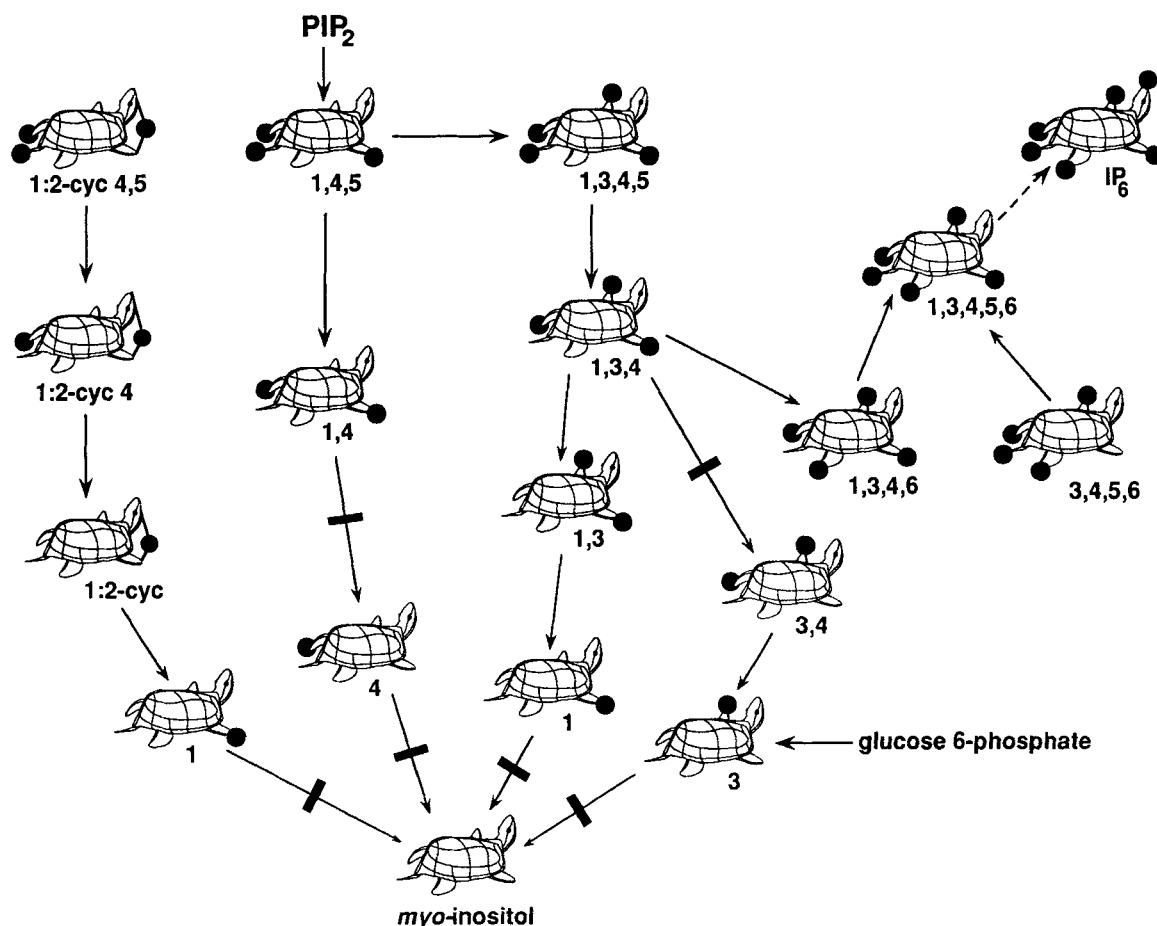


FIG. 1. Pathways of I(1,4,5)P₃ metabolism in neural tissues. *myo*-Inositol is visualized as a turtle in which the axial (2) position is the head and the five equatorial hydroxyls constitute the four legs and tail (Agranoff, 1978). The solid spheres indicate the positions of the phosphate groups. See text for details of enzymatic conversions. The dashed line indicates a pathway that is yet to be demonstrated in CNS. It is also possible that an initial product of PIP₂ hydrolysis is the cyclic 1,2 derivative of IP₃. This inositol phosphate is presumed to undergo sequential dephosphorylations to (cyclic 1,2)IP₁, then I(1)P₁, and thence to *myo*-inositol. The dephosphorylation of I(1,3)P₂ to I(3)P₁ has yet to be described. Also not shown is an alternative pathway for IP₆ formation (see text). The solid bars indicate enzymatic steps subject to Li⁺ inhibition.

in the reformation of I(1,4,5)P₃ (Höer et al., 1988, 1990). The physiological significance of a 3-kinase/3-phosphatase futile cycle has been questioned (Downes and MacPhee, 1990). I(1,3,4)P₃ is acted upon either by inositol polyphosphate 1-phosphatase (Inhorn and Majerus, 1987, 1988; Gee et al., 1988a) to produce I(3,4)P₂ or, alternatively, by a Li⁺-insensitive 4-phosphatase to yield I(1,3)P₂. These two compounds are then dephosphorylated further by 4- or 3-phosphatases to yield I(3)P₁ and I(1)P₁, respectively. Whereas the 4-phosphatase is insensitive to both Li⁺ and Mg²⁺, two separate 3-phosphatase activities can be distinguished by their Mg²⁺ requirements (Howell et al., 1989). As was originally proposed (Ackermann et al., 1987), a single enzyme, inositol monophosphatase, exhibits similar affinities for the dephosphorylation of I(1)P₁ and I(4)P₁ and, most likely, for other IP₁ isomers (Shears, 1989). The enzyme, which has been purified to homogeneity (Gee et al., 1988b; Meek et al., 1988),

exists as a dimer of subunit M_r 29K and is inhibited by Li⁺ in an uncompetitive manner with a K_i of <1 mM. The cDNA for the enzyme, which has been cloned, sequenced, and expressed, encodes a protein of 277 amino acids with an M_r of ~30K (Diehl et al., 1990). Unlike many other tissues, brain can synthesize inositol de novo (Eisenberg, 1967). The key enzyme, IP₁ synthase, which catalyzes the cyclization of glucose 6-phosphate, has been localized immunohistochemically to the brain vasculature (Wong et al., 1987).

As yet, relatively little is known of the pathways of synthesis for inositol pentakisphosphate (IP₅) and inositol hexakisphosphate (IP₆) in neural tissues. These assume importance in view of the recent observation that the addition of either high K⁺ or agonists elicits an increase in IP₅ formation in chromaffin cells (Sasakawa et al., 1990). In homogenates of both brain (Stephens et al., 1988) and chromaffin cells (Sasakawa et al., 1990), added [³H]I(1,4,5)P₃ can be converted to

[³H]IP₅. In brain, the I(1,3,4,5,6)P₅ isomer has been shown to be synthesized from I(1,3,4,6)P₄, which in turn is formed from kinase action on I(1,3,4)P₃ (Stephens et al., 1988). In a preliminary study, evidence was provided for the presence in brain cytosol of an additional kinase which preferentially phosphorylates IP₅ (Stanley et al., 1990). However, the enzyme appears to be specific for the 1,2,4,5,6 isomer, rather than I(1,3,4,5,6)P₅.

Although a principal function of the complex series of metabolic reactions that inactivates IP₃ seems to be to replenish the intracellular inositol pool, the possibility must also be considered that some of the intermediates formed may be physiologically relevant. In this context, I(1,4)P₂ has been reported to activate DNA polymerase (Sylvia et al., 1988). Although roles in cellular functions have been proposed for the higher inositol phosphates (IP₄, IP₅, and IP₆), at present, the only inositol phosphate that has been linked definitively to cellular signaling events is I(1,4,5)P₃.

I(1,4,5)P₃ receptors and Ca²⁺ homeostasis

The direct injection of I(1,4,5)P₃ into cells, or its addition to permeabilized cells or membrane fractions, elicits an increase in Ca²⁺ release from nonmitochondrial stores in both neural and nonneural tissues. The release is specific for the D isomer, is unaffected by known Ca²⁺-channel blockers, requires the presence of K⁺, and can occur at low temperatures (for reviews, see Berridge and Irvine, 1989; Joseph and Williamson, 1989; Nahorski and Potter, 1989). That these effects of I(1,4,5)P₃ on Ca²⁺ release are likely mediated through the action of specific intracellular receptor sites was recognized some time ago. The first direct demonstration of such sites (albeit in low density) was made in liver and neutrophils by Spät et al. (1986). A detailed distribution of I(1,4,5)P₃ binding sites in brain has been obtained by means of both receptor autoradiography (Worley et al., 1987a, 1989) and radioligand binding assays (Worley et al., 1987b). Moderate levels of binding sites are present in the hippocampus, cerebral cortex, caudate nucleus, and substantia nigra, whereas the highest concentrations by far are found in the cerebellum (Purkinje cell layer). Their enrichment in the cerebellum has greatly facilitated progress in the elucidation of the molecular characteristics of the I(1,4,5)P₃ receptor. When assayed at 4°C and in the absence of Mg²⁺ (to avoid ligand degradation), the binding of [³H]I(1,4,5)P₃ to cerebellar membranes is saturable ($K_D = 80\text{--}100\text{ nM}$) and highly stereospecific. Because intracellular concentrations of I(1,4,5)P₃ in brain are reportedly at least 10-fold higher than this K_D value (Challis et al., 1988), the concept of compartmentation of I(1,4,5)P₃ has been invoked (Challis et al., 1990). Heparin is a potent antagonist, and inclusion of Ca²⁺ at physiological concentrations (1 μM) inhibits binding. The ability of heparin to bind the IP₃ receptor has been used to advantage in purification of the IP₃ receptor (Supattapone et al., 1988). The receptor

has an M_r of 260K on SDS-PAGE and is highly selective for I(1,4,5)P₃. Ligand binding to the purified receptor is insensitive to Ca²⁺, but Ca²⁺ sensitivity is restored by the addition of detergent-solubilized cerebellar membranes. Danoff et al. (1988) subsequently established the presence in these extracts of a protein named "calmedin," which exhibited an M_r of 300K on gel filtration and which conferred Ca²⁺ sensitivity on the purified receptor. Calmedin is abundant in neural tissues, in keeping with the observation that Ca²⁺ sensitivity of I(1,4,5)P₃ binding is prevalent in CNS. Although differences in Ca²⁺ sensitivity and in K_D values of I(1,4,5)P₃ binding might indicate the existence of multiple receptor subtypes, studies which directly address the issue have not previously supported this proposition (Nunn et al., 1990; Varney et al., 1990). However, Danoff et al. (1991) have recently identified distinct neuronal and nonneuronal forms of the I(1,4,5)P₃ receptor that are formed by alternative splicing. The longer transcript corresponds to the neuronal form of the receptor which contains a 120-nucleotide insert (absent from the nonneuronal receptor) located between two PKA phosphorylation consensus sequences.

In retrospect, the IP₃ receptor had been first encountered, in fact, some 12 years earlier by Mallet et al. (1976) who, in a study of cerebellar ataxic mice, observed that a membrane glycoprotein (P₄₀₀) was enriched in Purkinje cells of normal mice, but was reduced in Purkinje cell-deficient mutants. As noted for the IP₃ receptor, P₄₀₀ is a glycoprotein of M_r 250K on SDS-PAGE and is highly enriched in the cerebellum. The availability of monoclonal antibodies permitted the cloning of cDNA for P₄₀₀ and determination of its amino acid sequence (Furuichi et al., 1989). On the basis of its cDNA sequence, P₄₀₀ is comprised of 2,749 amino acids with an M_r of 313K, a value greater than that obtained on SDS-PAGE. This discrepancy may be due to either an aberrant electrophoretic migration of the protein or posttranslational proteolytic processing. Mignery et al. (1990) independently obtained the complete primary structure for the IP₃ receptor in rat cerebellum using a series of overlapping cDNA clones that encode for proteins which contain either 2,734 or 2,749 amino acids. Only 21 amino acid substitutions were observed when the rat receptor sequence was compared with that of murine P₄₀₀. Furthermore, purified P₄₀₀ bound [³H]I(1,4,5)P₃ in a saturable and specific manner analogous to that observed for the purified IP₃ receptor (Maeda et al., 1990). Confirmation that P₄₀₀ and the IP₃ receptor are one and the same was obtained from experiments in which transfection of P₄₀₀ cDNA into NG108-15 cells resulted in the expression of IP₃-binding sites (Furuichi et al., 1989). The IP₃ receptor is not identical to the ryanodine receptor, but shares considerable homology with it (Mignery et al., 1989).

There is less agreement regarding the subcellular localization of the receptor. Using monoclonal antibodies

to P_{400} , Maeda et al. (1989) observed that the receptor was localized to the endoplasmic reticulum, plasma membrane, and postsynaptic density. In contrast, using polyclonal antibodies, Ross et al. (1989) localized the IP_3 receptor to the endoplasmic reticulum, the subplasmalemmal cisternae, and nuclear membrane, but *not* to the plasma membrane. Two further studies in which an immunogold labeling technique was employed also failed to detect IP_3 receptors at the plasma membrane (Mignery et al., 1989; Satoh et al., 1990). It appears then that IP_3 receptors are associated predominantly, if not exclusively, with the smooth endoplasmic reticulum.

IP_3 receptors may be regulated *in vivo* by both ATP and cyclic AMP, in addition to Ca^{2+} . When reconstituted into either lipid vesicles or a planar lipid bilayer, the IP_3 receptor mediates an increase in Ca^{2+} flux (Ferris et al., 1989; Maeda et al., 1991). Submillimolar concentrations of ATP increase this flux following interaction of the nucleotide with an ATP-binding site (Ferris et al., 1990; Maeda et al., 1991), whereas higher concentrations (millimolar) inhibit Ca^{2+} flux, consistent with the reported inhibitory effects of ATP on [3H]I(1,4,5) P_3 binding (Willcocks et al., 1987). The IP_3 receptor also contains three consensus amino acid sequences that fulfill the criteria for PKA action (Mignery et al., 1990). Addition of PKA catalytic subunits to cerebellar microsomes markedly reduces the potency with which IP_3 enhances $^{45}Ca^{2+}$ release (Snyder and Supattapone, 1989).

The native IP_3 receptor is thought to be a tetramer, composed of four noncovalently bound identical subunits of $M_r \sim 300K$, each subunit possessing an independent ligand binding site (Mignery et al., 1990; Maeda et al., 1991). It is proposed that the tetramer forms a single central transmembrane pore. Upon the binding of three to four molecules of IP_3 (Meyer et al., 1988), a conformational change in the receptor occurs and an open ion channel forms (Maeda et al., 1991). The Ca^{2+} signal so generated is frequently found to oscillate in single cells. Whether such oscillations reflect cyclical changes in IP_3 mass or, alternatively, that a constant concentration of IP_3 drives intracellular Ca^{2+} oscillations is currently under investigation (Wakui et al., 1989; Harootunian et al., 1991).

Higher inositol phosphates

Although there has been much speculation over the possible involvement of IP_4 in the maintenance of a prolonged receptor-mediated Ca^{2+} signal in nonneural tissues, there is little direct evidence for a similar role in the CNS. However, it should be stressed that I(1,3,4,5) P_4 is formed readily upon receptor activation in brain slices, during which time its concentration may rise 20-fold (Challis and Nahorski, 1990). Furthermore, specific I(1,3,4,5) P_4 binding sites in cerebellum have been purified 1,000-fold from homogenates (Donié et al., 1990). In oocytes, I(1,3,4,5) P_4 and I(1,3,4,6) P_4 (both of which can be formed in brain) are effective in altering

Ca^{2+} homeostasis (Ivorra et al., 1991). For further discussion of the possible role of IP_4 , the reader is referred to reviews by Joseph and Williamson (1989), Nahorski and Potter (1989), and Downes and MacPhee (1990).

In contrast to the intracellular roles envisioned for IP_3 and IP_4 , an extracellular role has been proposed for IP_5 and IP_6 by Vallejo et al. (1987) based upon the ability of these inositol phosphates to elicit changes in heart rate and blood pressure when injected into the nucleus tractus solitarius. Bath application of IP_6 to slices of rat brainstem failed, however, to alter the excitability, membrane potential, or resistance at concentrations of 44 μM (Brooks and Spyer, 1989). Nonetheless, demonstration of specific IP_6 binding sites in both brain and pituitary, and their linkage to $^{45}Ca^{2+}$ flux in the latter tissue, leave open the possibility of an extracellular role for these inositol polyphosphates (Hawkins et al., 1990; Nicoletti et al., 1990; Sortino et al., 1990).

RECEPTOR SUBTYPES COUPLED TO PHOSPHOINOSITIDE TURNOVER

More than 25 pharmacologically distinct receptors present on neurons and/or glia have been linked thus far to phosphoinositide turnover. Whereas an impressive number of receptors are now purported to couple to inositol lipid hydrolysis, two groups can still be clearly distinguished. Activation of category I receptors elicits a robust increase in inositol lipid turnover in tissue preparations obtained from both the CNS (brain slices, primary neuronal and glial cultures) and neurotumor cells. For a second group (category II), receptor activation elicits only small increases in inositol lipid turnover in CNS-derived preparations, such that most of the evidence accumulated in favor of their involvement has been obtained not from brain, but from the use of neurotumor cells or other neural-related tissues. A comprehensive list of receptor subtype(s) known to be linked to phosphoinositide turnover is shown in Table 3. Discussion below is restricted to newly identified receptors (or subtypes) for which substantive information exists and to previously unrecognized aspects of receptor function.

Category I

Muscarinic cholinergic. The previous division of muscarinic acetylcholine receptors (mAChRs) into two groups (M_1 and M_2) based on pirenzepine sensitivity is now superseded by molecular cloning studies. At least five biochemically and pharmacologically distinct subtypes of mAChRs are known (Bonner et al., 1987, 1988). Transfection studies with cDNAs encoding individual mAChR subtypes have indicated that M_1 , M_3 , and M_5 receptors couple primarily to phosphoinositide turnover, whereas the M_2 and M_4 subtypes are linked to inhibition of adenylate cyclase (Bonner et al., 1988; Ashkenazi et al., 1989). M_1 and M_3 mAChRs can be

TABLE 3. Receptor activation of phosphoinositide turnover in neural tissues

CATEGORY I	
Muscarinic cholinergic (M ₁ and M ₃)	
Brain slices—	Hynie et al. (1989); Forray and El-Fakahany (1990)
Primary neuronal cultures—	Akins et al. (1990); Ellis et al. (1990)
Neuroblastoma, SK-N-SH—	Fisher and Heacock (1988); Ashkenazi et al. (1989); Baumgold and White (1989)
Neuroblastoma, SH-SY-5Y—	Serra et al. (1988); Lambert et al. (1989); Cioffi and Fisher. (1990)
Neuroepithelioma, SK-N-MC—	Fisher and Landon (1991)
Astrocytoma, 1321N1—	Ashkenazi et al. (1989); Kunysz et al. (1989)
Pheochromocytoma, PC12—	Horowitz (1989); Takashima and Kenimer (1989)
Retina—	Moroi-Fetters et al. (1988)
Cochlea—	Guiramand et al. (1990)
Peripheral nerve—	Day et al. (1991)
Glioma, C ₆ —	Ananth et al. (1987)
Adrenergic (α _{1A} and α _{1B})	
Brain slices—	Michel et al. (1990)
Primary neuronal cultures—	Xu and Chuang (1987 <i>a,b</i>); Wilson and Minneman (1990); Wilson et al. (1990)
Primary glial cultures—	Wilson et al. (1990)
Neuroepithelioma, SK-N-MC—	Fisher and Landon (1991)
Glioma, C ₆ —	Ananth et al. (1987)
Histaminergic (H ₁)	
Primary glial cultures—	Arbonés et al. (1988)
Neuroblastoma, NIE-115—	Oakes et al. (1988)
Glioma, C ₆ —	Ananth et al. (1987)
Serotonergic (5-HT ₂ and 5-HT _{1c})	
Brain slices—	Claustre et al. (1988 <i>a</i>); Godfrey et al. (1988); Pierce and Peroutka (1988); Sanders-Bush et al. (1988)
Brain (in vivo)—	Hide et al. (1989)
Primary neuronal cultures—	Xu and Chuang (1987 <i>b</i>); Malhotra et al. (1990)
Pituitary tumor, P11—	Ivins and Molinoff (1990)
Glioma, C ₆ —	Ananth et al. (1987)
Glutamatergic (metabotropic)	
Brain slices—	Blackstone et al. (1989); Palmer et al. (1989); Alexander et al. (1990); Schoepp et al. (1990 <i>a</i>)
Synaptoneuroosomes—	Dudek et al. (1989); Hynie et al. (1989); Guiramand et al. (1990)
Primary glial cultures—	Pearce et al. (1990)
Retina—	Osborne (1990)
Endothelin	
Brain slices—	Kloog et al. (1988, 1989); Crawford et al. (1990); MacCumber et al. (1990)
Primary neuronal cultures—	Lin et al. (1990)
Primary glial cultures—	Lin et al. (1990)
Gliomas, C ₆ and A ₁₇₂ —	Lin et al. (1990); Zhang et al. (1990)
Neuroepithelioma, SK-N-MC—	Fisher and Landon (1991)
CATEGORY II	
Purinergic (P ₂)	
Primary glial cultures—	Pearce et al. (1990)
Neuroblastoma, NIE-115—	Ehrlich et al. (1988)
Neuroepithelioma, SK-N-MC—	Fisher and Landon (1991)
Adrenal medulla—	Sasakawa et al. (1989); Allsup and Boarder (1990)
Pituitary—	Van der Merwe et al. (1989); Davidson et al. (1990)
Thromboxane (A ₂)	
Astrocytoma, 1321N1—	Nakahata et al. (1989)
Nerve growth factor	
Pheochromocytoma, PC12—	Volonté and Racker (1988); Volonté et al. (1988); Van Calker et al. (1989)
Prostaglandin (E ₂)	
Adrenal medulla—	Negishi et al. (1989)
Bradykinin (B ₂)	
Primary glial cultures—	Cholewinski et al. (1988)
Dorsal root ganglion—	Burgess et al. (1989); Gammon et al. (1989); Perney and Miller (1989);
Neuroblastoma-glioma NG108-15—	Chiang and Hauser (1989); Imaizumi et al. (1989)
Pheochromocytoma, PC12—	Volonté et al. (1988)
Neurohybridoma, NCB-20—	Chuang and Dillon-Carter (1988)
Pituitary tumor, Flow 9000—	Sharif et al. (1988)
Anterior pituitary—	T. H. Jones et al. (1989)
Vasopressin (V ₁)	
Brain slices—	Moratalla et al. (1988); Shewey and Dorsa (1988)
Primary glial cultures—	Cholewinski et al. (1988)

TABLE 3—Continued

Cholecystokinin
Neuroblastoma, CHP 212—Barrett et al. (1989)
Pituitary tumor, Flow 9000—Lo and Hughes (1988)
Neuropeptide Y
Dorsal root ganglion—Perney and Miller (1989)
Neurotensin
Neuroblastoma-glioma, NG108-15—Imaizumi et al. (1989)
Gastrin-releasing peptide
Brain slices—Hollingsworth (1989)
Bombesin
Brain slices—Hollingsworth (1989); Prasad and Moody (1989)
Substance P
Primary glial cultures—Cholewinski et al. (1988)
Anterior pituitary—Mau et al. (1990)
Retina, superior colliculus—Osborne and Ghazi (1989)
Oxytocin
Primary glial cultures—Cholewinski et al. (1988)
Eleoisin
Anterior pituitary—Mau et al. (1990)
Retina, superior colliculus—Osborne and Ghazi (1989)
Neurokinin
Primary glial cultures—Cholewinski et al. (1988); Torrens et al. (1989)
Anterior pituitary—Mau et al. (1990)
Retina, superior colliculus—Osborne and Ghazi (1989)
Vasointestinal peptide
Primary neuronal cultures—Malhotra et al. (1990)
Angiotensin
Neuroblastoma-glioma, NG108-15—Carrithers et al. (1990)
Gonadotropin-releasing hormone
Anterior pituitary—Sortino et al. (1988)
Platelet-activating factor
Primary glial cultures—Murphy and Welk (1990)
Pituitary cells—Grandison (1990)
Thyrotropin-releasing hormone
Pituitary—Desrues et al. (1990)
Pituitary tumor, GH ₃ —Sharif et al. (1989); Wood and Schofield (1989); Cubitt et al. (1990)

distinguished on the basis of their pirenzepine sensitivities ($M_1 > M_3$) and their M_r values ($M_3 > M_1$). Whereas the involvement of the M_1 subtype in phosphoinositide hydrolysis had been firmly established previously, it has since been shown that the M_3 receptor is also functionally linked. mAChRs present on both SK-N-SH neuroblastoma and 1321N1 astrocytoma cells exhibit a higher M_r (90K–100K) than that associated with the M_1 subtype (60K–70K), and pirenzepine only weakly inhibits the ability of these M_3 receptors to activate inositol lipid hydrolysis (Liang et al., 1987; Fisher and Heacock, 1988; Baumgold and White, 1989; Kunysz et al., 1989). For the SH-SY-5Y cell line, a neuroblast subclone of the SK-N-SH cell, there is conflicting evidence as to the predominant subtype present. From both pharmacological and biochemical considerations (Lambert et al., 1989; Cioffi and Fisher, 1990), it has been concluded that these cells also express the M_3 subtype. In contrast, the results of Serra et al. (1988), obtained with the same cells, are more consistent with the presence of a functionally linked M_1 receptor. Additional pharmacological evidence for the involvement of M_3 receptors in phosphoinositide hydrolysis has been obtained recently for

both primary neuronal cultures (Ellis et al., 1990) and brain slices (Forray and El-Fakahany, 1990).

A characteristic of mAChR-stimulated phosphoinositide hydrolysis in a variety of neural preparations is its slow rate of desensitization (Nakahata and Harden, 1987; Xu and Chuang, 1987a; Lenox et al., 1988; Thompson and Fisher, 1990; but see also Eva et al., 1990). In SK-N-SH neuroblastoma cells, inositol lipid hydrolysis proceeds at a constant rate during a period in which mAChRs are sequestered from the cell surface (Thompson and Fisher, 1990). Because only cell surface mAChRs can activate PLC, the ability of mAChRs to sustain phosphoinositide hydrolysis may reflect their ability to recycle continuously to the cell surface (Thompson and Fisher, 1991).

Adrenergic. Two subtypes (α_{1A} and α_{1B}) of the α_1 -adrenergic receptor have been defined, on the basis of their susceptibility to alkylation by chloroethylclonidine ($\alpha_{1B} > \alpha_{1A}$) and the 10–70-fold greater affinities of two competitive antagonists (WB-4101 or 5-methylurapidil) for the α_{1A} subtype (Minneman, 1988; Hanft and Gross, 1989). Although it has been suggested that the α_{1B} subtype couples to inositol lipid turnover, whereas the α_{1A} receptor is linked to Ca^{2+} influx

(Minneman, 1988), such a clear distinction now appears unlikely. In brain slices, norepinephrine-stimulated inositol phosphate formation is inactivated by chloroethylclonidine and is inhibited relatively weakly by WB-4101, thereby implicating the involvement of an α_{1B} subtype (Michel et al., 1990). In contrast, in primary cultures of neurons and glia, alkylation has little effect on norepinephrine-stimulated inositol phosphate release, whereas WB-4101 is a potent inhibitor. Furthermore, unlike the case in brain slices, phosphoinositide turnover in primary cultures is blocked by pertussis toxin (Wilson and Minneman, 1990). In SK-N-MC neuroepithelioma cells, the α_{1A} receptor appears to be the major subtype linked to inositol lipid turnover (Fisher and Landon, 1991). These findings suggest that both α_{1A} and α_{1B} subtypes have the potential to activate phosphoinositide hydrolysis.

Glutamate. Progress in the pharmacological characterization of the metabotropic glutamate receptor has been aided by the recent introduction of the relatively selective agonist, 1-aminocyclopentyl-1,3-dicarboxylic acid (ACPD)¹ (Palmer et al., 1989; Desai and Conn, 1990; Manzoni et al., 1990). The ability of this rigid analogue of glutamate to activate effectively the metabotropic, but not the ionotropic, excitatory amino acid receptor has been demonstrated in both brain slices and cultured neurons and is in contrast to the relative nonselectivity of quisqualic and ibotenic acids. The availability of a more potent, but noncompetitive, antagonist, 2-amino-3-phosphonopropionic acid, may also facilitate characterization and functional analysis of this receptor (Schoepp et al., 1990a). In some preparations, a portion of the phosphoinositide turnover induced by quisqualate may be due to activation of ionotropic receptors, perhaps as a result of activation of PLC by elevated $[Ca^{2+}]_i$ (Alexander et al., 1990; Baird and Nahorski, 1990b). A G-protein link with the metabotropic glutamate receptor is evidenced by the reported sensitivity of its phosphoinositide response to pretreatment with pertussis toxin, a property which distinguishes this receptor from most other CNS phosphoinositide-linked receptors thus far examined (Nicoletti et al., 1988; Ambrosini and Meldolesi, 1989). Earlier studies demonstrating enhanced coupling in neonatal rat brain suggested a role for metabotropic glutamate receptors in neuronal plasticity. Further support for this concept is provided by reports that signal transduction at this receptor is enhanced following hypoxic-ischemic brain injury (Chen et al., 1988; Seren et al., 1989) and following lesions in the hippocampus, striatum, and amygdala (Nicoletti et al., 1987; Akiyama et al., 1989). In addition, in developing cat visual cortex, the critical period for synaptic modification coincides with a transient rise in the ability of glutamate to stimulate phosphoinositide turnover, a

phenomenon which is absent in kittens raised in the dark (Dudek and Bear, 1989). Further details regarding the properties of the metabotropic glutamate receptor may be found in a recent review by Schoepp et al. (1990b). Further characterization of this receptor should be facilitated by its recent cloning from rat brain (Houamed et al., 1991; Masu et al., 1991). The nucleotide sequence of the metabotropic glutamate receptor encodes a protein with a molecular mass of 133 kDa and no apparent homology to other known members of the G protein-coupled receptor family.

Endothelin. In addition to its potent vasoconstrictive effects on vascular smooth muscle, endothelin (ET) is also synthesized in the CNS where it may exert a neuroregulatory role. The ETs exhibit an unusually high degree of sequence homology with the sarafotoxins (SRTXs), venoms derived from the Israeli snake *Atractapsis eingadensis*. High-affinity SRTX/ET binding sites exist within the CNS and exhibit a distinctive regional distribution (Kloog et al., 1988; C. R. Jones et al., 1989). Moreover, the addition of either SRTX or ET elicits an increased phosphoinositide turnover in several brain regions, in cultured C₆ glioma cells, and in primary cultures of both neurons and glia. All three forms of ET (ET-1, ET-2, and ET-3) have been shown to activate inositol lipid hydrolysis (but with different potencies). Although the precise role of ET-stimulated phosphoinositide turnover in neural function is not known, a role in mitogenesis has been suggested (MacCumber et al., 1990).

Category II

Purinergic. There is now a considerable body of evidence to suggest that ATP serves as a neuromodulator in both the CNS and PNS. The addition of ATP to a variety of neural preparations can elicit an increase in phosphoinositide turnover, whereas adenosine and AMP are relatively ineffective. The receptor involved can thus be classified as P₂ purinergic rather than P₁ (Burnstock and Kennedy, 1985). However, given the ability of pyrimidines (CTP and UTP) to enhance inositol phosphate release in adrenal (Sasakawa et al., 1989) and other nonneural tissues, the term "nucleotideceptor" may be most appropriate (Pfeilschifter, 1990). Although P₂ receptors can be subdivided pharmacologically into P_{2y} or P_{2x} subtypes (Burnstock and Kennedy, 1985), it is not yet possible to ascribe phosphoinositide turnover to either of these receptors in neural tissues. In the adrenal medulla, in which the pharmacology of the response has been carefully examined, the characteristics fit neither of the known profiles (Allsup and Boarder, 1990).

Neuropeptides. The list of neuropeptide receptors implicated in phosphoinositide hydrolysis continues to grow. Those for neuropeptide Y, gastrin-releasing peptide, oxytocin, and gonadotropin-releasing hormone have been identified recently. Many of these neuropeptide receptors are to be found in primary cultures of glia. Cholewinski et al. (1988) examined the ability

¹ Although previously designated as *trans*-ACPD, it is in fact *cis*-ACPD (see Schoepp et al., 1990b).

of 12 neuropeptides to enhance inositol lipid turnover in astrocytes obtained from cerebral cortex, cerebellum, and spinal cord. Each culture displayed a unique pattern of neuropeptide stimulation. Thus, whereas all three tissues responded to bradykinin, eledoisin, and neurokinin β , the addition of oxytocin and vasopressin elicited a response only in cortical and cerebellar cultures. Only spinal cord cultures responded to substance P and neurokinin α . The regional responsiveness of astrocytes to peptides approaches that displayed by neurons.

Other CNS receptors

Dopamine. Conflicting reports exist regarding the ability of dopamine to enhance phosphoinositide turnover. No effects of dopamine on either basal or stimulated inositol phosphate release were observed by Kelly et al. (1988) and Rubinstein and Hitzemann (1990), whereas the stimulatory effect reported by Dyck (1990) could be blocked by the α_1 antagonist, prazosin. Wallace and Claró (1990) reported a negative effect on carbachol-stimulated release in membranes following D_1 -receptor activation. In contrast, Undie and Friedman (1990) have provided compelling evidence for the involvement of D_1 receptors in rat striatum. In this context, it may be relevant that injection of mRNA from rat striatum into oocytes results in the expression of a D_1 receptor linked to both IP_3 production and Ca^{2+} efflux (Mahan et al., 1990).

Opioid. Opiates have been reported either to have no effect on, to stimulate, or to inhibit inositol lipid hydrolysis (Bunn et al., 1988; Misawa et al., 1990; Periyasamy and Hoss, 1990).

Modulation of receptor-stimulated phosphoinositide hydrolysis

Given the complexity of neural tissue, it is not surprising that activation of inositol lipid-linked receptors is itself subject to modulation following agonist occupancy of certain pharmacologically distinct receptors. Both positive and negative modulations (and species dependence) have been reported (Table 4). The underlying molecular mechanisms of such regulation often remain obscure, due perhaps either to the absence of selective antagonist data, to difficulty in excluding the involvement of secondary effects, such as inter-neuronal communication or depolarization, or to the concurrent rise or fall in intracellular concentrations of other second messengers (e.g., cyclic AMP).

STRATEGIES FOR INTERVENTION OF PHOSPHOINOSITIDE SIGNAL TRANSDUCTION

Within the series of events that link receptor occupancy to PLC activation, a number of sites might be amenable to pharmacological disruption. From the viewpoint of drug development, the receptor is an obvious target, because it is extracellular and thus accessible. The expression of individual receptor molecules in cells following transfection with appropriate cDNA clones and demonstration of their functional coupling to phosphoinositide turnover may allow for the identification of "tailored subtype-specific" ligands (Lester, 1988), which may be useful in drug development. Intervention at the level of the G_p -PLC interaction is more problematic due to the uncertainty of G_p 's iden-

TABLE 4. Modulation of receptor-stimulated phosphoinositide hydrolysis in brain

Stimulus	Modulator	Antagonist	Tissue	Reference
A. Positive				
Norepinephrine	γ -Aminobutyric acid, nipecotic acid		Rat cerebral cortex	Crawford and Young (1990a,b); X. Li et al. (1990)
Glutamate	Serotonin	Ketanserin/prazosin	Cerebellar granule cells	Yu and Chuang (1988)
Acetylcholine	Glycine		Primary glial cultures	Hansson et al. (1990)
Histamine	Vasointestinal peptide		Cerebellar granule cells	Nicoletti and Canonico (1989)
	2-Chloroadenosine	8- <i>O</i> -Cyclopentyl-1,3- dipropylxanthine	Rat cerebral cortex	Raiteri et al. (1987)
			Guinea pig cerebral cortex	Alexander et al. (1989)
B. Negative				
Carbachol	<i>N</i> -Methyl-D-aspartate	MK-801	Rat cerebral cortex	Gonzales and Moerschbaeher (1989)
		2-Amino-5-phosphopentanoic acid		Noble et al. (1989); Morrisett et al. (1990)
	8-Hydroxy-2-(di- <i>n</i> - dipropylamino)tetralin	Cyanopindolol	Rat hippocampus	Claustre et al. (1988b)
	Dopamine, SKF-38393	SKF-83566 SCH-23390	Rat cerebral cortex	Wallace and Claró (1990)
Norepinephrine	Glutamate		Rat cerebral cortex	Jope and Li (1989); X. Li et al. (1990)
Histamine	Adenosine	Cyclopentyladenosine	Mouse cerebral cortex	Kendall and Hill (1988)
	Adenosine	Theophylline	Human cerebral cortex	Kendall and Firth (1990)

tity, its insensitivity to either cholera or pertussis toxins, and the existence of multiple isozymes of PLC. Nonetheless, assuming G_p is a heterotrimer, one potential approach would be the use of synthetic peptides corresponding to known regions of the α subunit of the G protein, the 40 carboxyl-terminal amino acids of which are highly conserved (Hamm et al., 1988). As previously discussed, inhibitors of specific PLC-isozyme activation are not as yet identified. For investigation of the interrelationship between the product of PLC action, i.e., $I(1,4,5)P_3$, its receptor, and Ca^{2+} mobilization, the recent development of synthetic IP_3 analogues that are resistant to hydrolysis offers promise (Willcocks et al., 1988).

At present, the strategy of limiting the availability of lipid substrates has attracted most attention. Previously, inositol analogues have been shown to be effective inhibitors of brain cytidine diphosphodiacylglycerol (CDP-DAG) inositol transferase, the enzyme responsible for PI synthesis (Benjamins and Agranoff, 1969; see also Agranoff and Fisher, 1991). In addition, Moyer et al. (1988) have demonstrated that two analogues, 5-deoxy-*myo*-inositol and 5-deoxy-5-fluoro-*myo*-inositol, can permeate intact cells and be incorporated into cellular inositol phospholipid. This "fraudulent" lipid, however, can only be phosphorylated at the 4-position of the inositol ring, and hence PIP_2 cannot be synthesized. Interference with inositol lipid synthesis is considered to be an explanation for the antimanic effect of Li^+ in humans (Berridge et al., 1982). Largely as a result of studies by Sherman and colleagues, it is now established that "therapeutically" relevant concentrations of Li^+ in the rat result in the accumulation of inositol phosphates both in vivo and in vitro, the result of selective inhibition of inositol monophosphatase (Allison et al., 1976; Hallcher and Sherman, 1980). The net effect is to reduce the availability of inositol, which could theoretically reduce PI synthesis, given the limited permeability of the blood-brain barrier to inositol. Because the mode of inhibition by Li^+ is uncompetitive (i.e., Li^+ binds to the E·S complex), lipid resynthesis is anticipated to be most compromised in hyperactive neurons which should produce correspondingly larger amounts of IP_1 . Whereas experimental evidence has been obtained in favor of a link between Li^+ and phosphoinositide turnover (Casebolt and Jope, 1987; Kendall and Nahorski, 1987; Godfrey et al., 1989), questions still remain. For example, although the addition of Li^+ to tissue incubations results in the accumulation of CDP-DAG (due to inositol depletion), a reduction of agonist-stimulated PIP_2 breakdown invariably is not found, suggesting the existence of a pool of lipid that is spared the effects of Li^+ (Drummond and Raeburn, 1984; Downes and Stone, 1986). Furthermore, chronic administration (27–39 days) of Li^+ to rats failed to alter significantly the concentration of brain PI, PIP, or PIP_2 (Honchar et al., 1989), nor did this treatment influence the extent of $I(1)P_1$ accumulation observed in the presence of

muscarinic agonists (Honchar et al., 1990). A deficit in the phosphoinositide pathway has also been proposed in the PNS in diabetic neuropathy (Greene and Lattimer, 1985).

CONCLUDING REMARKS

In the 4 years that have elapsed since we last reviewed phosphoinositides in the nervous system, there have been more publications than in the entire preexisting literature. It is daunting from the standpoint of writing a minireview that the rate of publication continues to accelerate. Among novel findings since 1987 is the presence in many tissues, including brain, of PI-linked proteins. The recent findings of intracellular PIP_2 and IP_3 binding proteins and of 3-phosphoinositides certainly will have implications eventually for brain function. It is likely that there will be yet other revelations in the years to come.

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