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Short Review

Receptor Activation and Inositol Lipid Hydrolysis in Neural Tissues

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Recent advances in our knowledge of the biochemistry, pharmacology, and cell biology of ligand-induced, stimulated turnover of inositol lipids in such diverse tissues as insect salivary glands, platelets, lymphocytes, and a number of exocrine glands have greatly altered our understanding of intracellular events leading to secretion, contraction, chemotaxis, and other cellular responses. Much of what has been learned regarding ligand-stimulated turnover of inositol lipids has come from nonneural systems, so that the presumption that signal transduction via this mechanism mediates significant steps in neural function must still be considered inferential. This reservation has been a major theme of a recent comprehensive review (Hawthorne, 1986). Nevertheless, that the brain contains large amounts of the substrates and enzymes of inositol lipid turnover and its associated second messenger systems and, further, that it is enriched with receptors that are linked to stimulated inositol lipid turnover argue strongly that the phosphoinositides indeed play an important role in brain function. There exist several general reviews on stimulated inositol lipid turnover (Berridge, 1984; Berridge and Irvine, 1984; Nishizuka, 1984, 1986; Hokin, 1985; Abdel-Latif, 1986; Downes, 1986; Williamson, 1986), including several that emphasize the nervous system (Downes, 1982, 1983; Fisher and Agranoff, 1986; Hawthorne, 1986; Nahorski et al., 1986). The present contribution emphasizes developments of neurochemical relevance since a review on the subject in this journal 8 years ago (Hawthorne and Pickard, 1979).

CHEMISTRY AND ENZYMOLOGY OF INOSITOL LIPIDS AND INOSITOL PHOSPHATES

The inositol lipids

Phosphatidylinositol (PI), depicted in Fig. 1A, is a glycerophospholipid in which the *sn*-1 and *sn*-2 positions of glycerol are esterified to fatty acids, whereas the *sn*-3 position is phosphodiesterified to the D-1 position of *myo*-inositol. *myo*-Inositol is one of nine possible isomers of hexahydroxycyclohexane, which, in its preferred chair conformation, has one axial and five equatorial hydroxyl groups. The molecule can be likened to a turtle (Fig. 1B) in which the four legs and tail are the five equatorial hydroxyls, numbered counterclockwise from above, starting with the right foreleg as position D-1 (Fig. 1C). The turtle's head is thus the axial hydroxyl at position D-2. The phosphatidyl group in all of the phosphoinositides is linked to D-1 (the right foreleg).

PI is synthesized in brain from a liponucleotide intermediate, cytidine diphosphodiacylglycerol (CDP-DG) (Agranoff et al., 1958), and inositol in the pres-

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Abbreviations used: ACh, acetylcholine; ACTH, corticotropin (1–24)tetracosapeptide; APB, DL-2-amino-4-phosphobutyric acid; APV, DL-2-amino-5-phosphonovalerate; CDP-DG, cytidine diphosphodiacylglycerol; DG, 1,2-diacyl-*sn*-glycerol; GAP, growth-associated protein; G-protein, guanine nucleotide binding protein; 5-HT, 5-hydroxytryptamine; IP_1, D-*myo*-inositol 1-phosphate; IP_2, D-*myo*-inositol 1,4-bisphosphate; IP_3, D-*myo*-inositol 1,4,5-trisphosphate; IP_4, D-*myo*-inositol 1,3,4,5-tetrakisphosphate; mAChR, muscarinic acetylcholine receptor; NMDA, *N*-methyl D-aspartate; PA, phosphatidic acid; PI, phosphatidylinositol; PIP, phosphatidylinositol 4-phosphate; PIP_2, phosphatidylinositol 4,5-bisphosphate; PK-C, protein kinase C; PMA, 4β-phorbol 12-myristate 13-acetate; TRH, thyrotropin-releasing hormone. The 1,2 cyclic derivatives of IP_1, IP_2, and IP_3, in which there is a phosphodiester bridge between the D-1 and D-2 positions of *myo*-inositol, are designated by the prefix "c." Thus, for example, (c1,2)-IP_1 is D-1,2 (cyclic) *myo*-inositol phosphate. The abbreviations used for inositol lipids and inositol phosphates are those recommended at the Chilton Conference on Inositol and Phosphoinositides (Dallas, TX, January 1984) (see Agranoff et al., 1985). The standard IUPAC-IUB abbreviations for phosphatidylinositol, phosphatidylinositol 4-phosphate, and phosphatidylinositol 4,5-bisphosphate are PtdIns, PtdIns 4-*P*, and PtdIns 4,5-P_2.

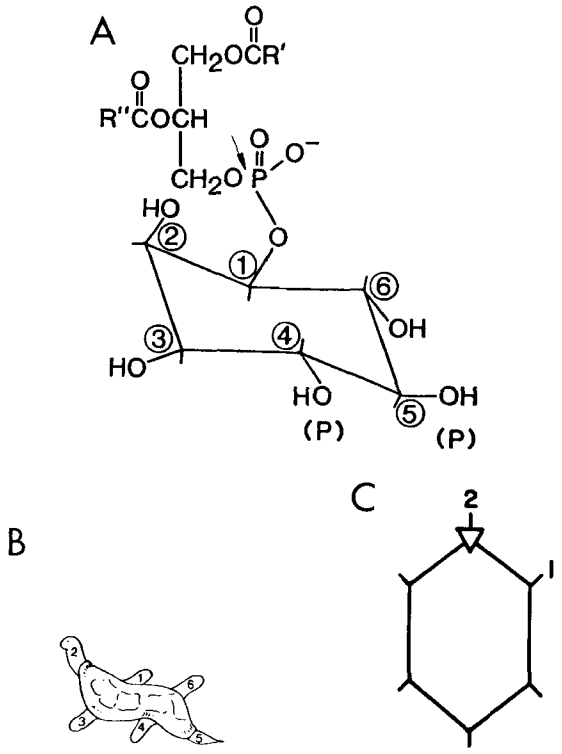

FIG. 1. The chemical structure of the inositol lipids. **A:** PI and the polyphosphoinositides. In the D numbering system, the 2 position is axial, and the phosphatidyl group is phosphodiesterified to the vicinal hydroxyl in the D-1 position. In PIP, there is an additional phosphate monoesterified in the D-4 position, and in PIP_2, a third phosphate is present on the D-5 position. The site of cleavage via phosphoinositide phosphodiesterase is shown by the arrow. *myo*-Inositol may be visualized as a turtle (**B**) in which the 2 position is the head and the five equatorial hydroxyls constitute the four legs and tail. In the inositol lipids, the phosphatidyl group is always at the right foreleg (D-1). **C:** A convenient representation of *myo*-inositol. The open triangle at the D-2 position represents the axial hydroxyl (the turtle's head) projecting out of the plane of the paper toward the reader (from Agranoff, 1987).

ence of a transferase (Benjamins and Agranoff, 1969) and can then be phosphorylated via PI kinase and ATP to form phosphatidylinositol 4-phosphate (PIP) (Colodzin and Kennedy, 1965; Kai et al., 1966). This product may be further phosphorylated via PIP kinase and ATP to form phosphatidylinositol 4,5-bisphosphate (PIP_2) (Kai et al., 1968). It had been shown by Hokin and Hokin (1955), in studies in which intact cell preparations were incubated in the presence of muscarinic ligands, that the labeling of two quantitatively minor lipids, phosphatidate (PA) and PI, was enhanced. It was suggested that the ligand stimulated the phosphodiesteratic cleavage of PI (Hokin and Hokin, 1964) to yield diacylglycerol (DG), which could then be converted to PA by DG kinase (Hokin and Hokin, 1959) and, thence, via de novo synthesis (Petzold and Agranoff, 1965, 1967; Carter and Kennedy, 1966) to PI. Phosphodiesterolysis of PI via a phospholipase C-type cleavage (Dawson, 1959; Kemp et al., 1959) was then proposed to complete a cycle (Fig. 2a). It has become increasingly clear in the last few years that the initial ligand-stimulated step is the stimulated breakdown of PIP_2 rather than that of PI (Fig. 2b), with the concomitant formation of DG and inositol 1,4,5-trisphosphate ($1,4,5\text{-}IP_3$) (Akhtar and Abdel-Latif, 1980; Agranoff et al., 1983; Berridge et al., 1983). The two products of this phosphodiesteratic cleavage are now known to have separate second messenger properties: DG stimulates protein kinase C (PK-C), a Ca^{2+}-activated protein kinase (Nishizuka, 1984), whereas IP_3 mobilizes Ca^{2+} from intracellular stores (Berridge and Irvine, 1984). The brain is particularly enriched in PK-C (Hirasawa and Nishizuka, 1985), and several brain-specific proteins have been shown to be selectively phosphorylated via this kinase (Nishizuka, 1986). In platelet membranes, the three inositol phospholipids, PI, PIP, and PIP_2, all appear to be cleaved by a common phosphodiesterase in the presence of Ca^{2+} (Wilson et al., 1984), although when Ca^{2+} is reduced by EGTA chelation, only PIP and PIP_2 are hydrolyzed. In brain membranes, Ca^{2+} is required for phospholipase C cleavage of PIP and PIP_2 (Van Rooijen et al., 1983). Although there is evidence that the initial cleavage stimulated by ligand is at the level of polyphosphoinositides, at later times the resultant elevated intracellular Ca^{2+} levels could then lead to further phosphodiesteratic breakdown of PI (Wilson et al., 1985*b*). Because the brain, like other tissues, contains much more PI than PIP or PIP_2, PI is potentially a more plentiful source of DG for activation of

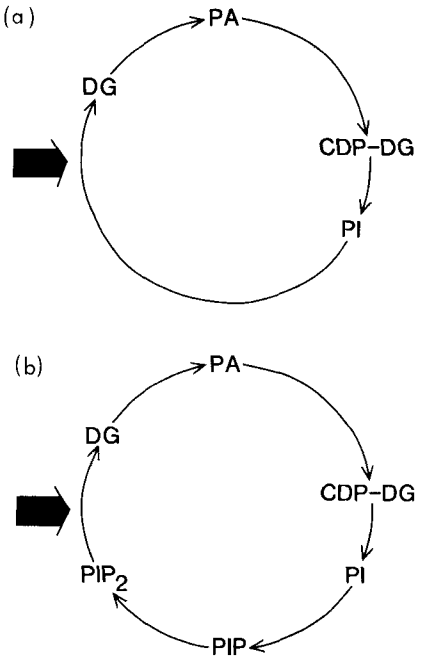

FIG. 2. Stimulated turnover of inositol lipids in response to ligand receptor activation. An earlier view of the cycle considered PI breakdown to DG to be stimulated (**a**). Evidence in the last few years indicates that the stimulated breakdown occurs at the level of PIP_2 (**b**) (from Fisher and Agranoff, 1986).

PK-C. It should be noted that lipid metabolism outside the phosphoinositide cycle can both produce and consume DG (see Daniel et al., 1986; Snider et al., 1987).

The Ca^{2+} sensitivity of stimulated phosphoinositide turnover in the CNS represents a major characteristic of neural tissues. Omission of Ca^{2+} from the incubation medium can attenuate, and inclusion of Ca^{2+} chelators can abolish, stimulated inositol lipid turnover in brain (Griffin et al., 1979; Fisher and Agranoff, 1980, 1981; Gonzales and Crews, 1984; Pearce et al., 1985). Moreover, in some circumstances, addition of the divalent cationophore A23187 can mimic the stimulatory effect of the natural ligand on phosphoinositide turnover (Griffin and Hawthorne, 1978; Fisher and Agranoff, 1981; Kendall and Nahorski, 1984). Depolarizing concentrations of K^+ can elicit an increased inositol lipid breakdown in sympathetic ganglia and brain slices (Bone and Michell, 1985; Kendall and Nahorski, 1985*a*), and the Ca^{2+} channel activator BAY-K-8644 can mimic K^+ depolarization (Kendall and Nahorski, 1985*a*). Although some of these effects may be indirect, e.g., via release of a neuropeptide, as suggested by Bone and Michell (1985), a direct effect of elevated intracellular [Ca^{2+}] on the inositide phosphodiesterase seems possible. Whether this phosphodiesterase is actually regulated by physiologically relevant changes in intracellular [Ca^{2+}] seems less certain. Nevertheless, this possibility should not be excluded, because in both permeabilized bovine chromaffin cells and GH_3 pituitary cells, PIP_2 phosphodiesterase activity is increased in the presence of Ca^{2+} concentrations that are well within physiological limits (Martin et al., 1986; Eberhard and Holz, 1987).

That it took so many years to recognize that stimulated phospholipid breakdown occurs at the level of PIP_2 rather than PI (Fig. 2) can be attributed to several technical factors. For example, conventional lipid extraction does not extract PIP or PIP_2. But even with the requisite acidified lipid extraction procedure, techniques that permit PIP and PIP_2 to migrate and separate on TLC plates were developed only in the past few years (Jolles et al., 1981; for review, see Hajra et al., 1987). Furthermore, the 4′ and 5′ positions of the polyphosphoinositides are rapidly labeled with $^{32}P_i$ via the phosphoinositide kinase and phosphatases, and these steps are insensitive to ligand stimulation. Label in these two positions tends to equilibrate quickly with that in the γ phosphate of ATP and, thus, to reflect more the amounts of PIP and PIP_2 in a preparation than their stimulated labeling. Because of this, possible stimulated labeling in the D-1 phosphodiester position is obscured.

In all three of the inositol lipids, there is an enrichment in stearate at *sn*-1 and of arachidonate at *sn*-2. One might then expect that stearoyl arachidonoyl DG would be selectively efficacious in activating PK-C. This has not yet proven to be the case. In fact, there is very little demonstrated fatty acid specificity in DG effects on PK-C (Mori et al., 1982). The synthetic DG 1-oleoyl-2-acetyl-*sn*-glycerol has been particularly useful experimentally because of its high solubility. Phorbol esters have proven to be excellent tools for studying activation of PK-C, because they can be taken up by cells and bind to the DG site. Increased intracellular Ca^{2+} levels can activate phospholipase A_2, so that phosphoinositide-related activation may release free fatty acids. Because arachidonate is a precursor of the prostanoids, there may be a link between stimulated inositide turnover and stimulation of prostanoid synthesis. It should be pointed out, however, that if the labeling cycle is to be preserved, DG must survive. This appears to be the case for nerve ending preparations, because following carbamoylcholine stimulation, 74–85% of ^{32}P-labeled PA and PI produced could be accounted for by the tetraenoic species, as detected by argentation TLC (Van Rooijen et al., 1985). Nevertheless, deacylation-reacylation reactions could exchange arachidonate for other fatty acids in DG or in any one of a number of intermediates of the cycle depicted in Fig. 2b.

Much work on stimulated inositol lipid turnover in the nervous system has used the muscarinic receptor as its model. As described below, it has been shown in hippocampus that the stimulated turnover is related to a postsynaptic neuronal site. Figure 3 attempts to integrate the presently known reactions in stimulated phosphoinositide turnover within the typical conceptualization of a muscarinic synapse.

The inositol phosphates

The brain contains several inositol phosphates (Agranoff, 1978), as well as high levels of free *myo*-inositol relative to other tissues (Sherman et al., 1968). The inositol phosphates D-*myo*-inositol 1-phosphate (D-1-IP_1), D-*myo*-inositol 1,4-bisphosphate (D-1,4-IP_2), and D-1,4,5-IP_3 (see Fig. 4) are the products of phosphodiesteratic cleavage of PI, PIP, and PIP_2, respectively. The three inositol phosphates may also exist as 1,2 cyclic derivatives, in which a five-membered phosphodiesteratic ring is formed between the D-1 equatorial and the D-2 axial positions. D-1,2 (cyclic) *myo*-inositol phosphate [(c1,2)-IP_1] is the best characterized of the cyclic inositol phosphates (Pizer and Ballou, 1959). It is produced in roughly equal amounts with D-1-IP_1 on phosphodiesteratic cleavage of PI (Dawson et al., 1971). It is tempting to speculate that the cyclic derivatives are initial products of the phosphoinositide phosphodiesterase and are eventually cleaved at the 2 position by a cyclic IP phosphodiesterase. Cyclic IP_3 may be degraded to cIP_2, thence to cIP_1, and finally to IP_1. The argument is given support by the recent isolation from placenta of a phosphodiesterase that acts on cIP_1 but that is not active against cyclic derivatives of IP_2 or IP_3 (Ross and Majerus, 1986). The brain also contains D-3-IP_1 (the same compound as L-1-IP_1), derived from the reductive cy-

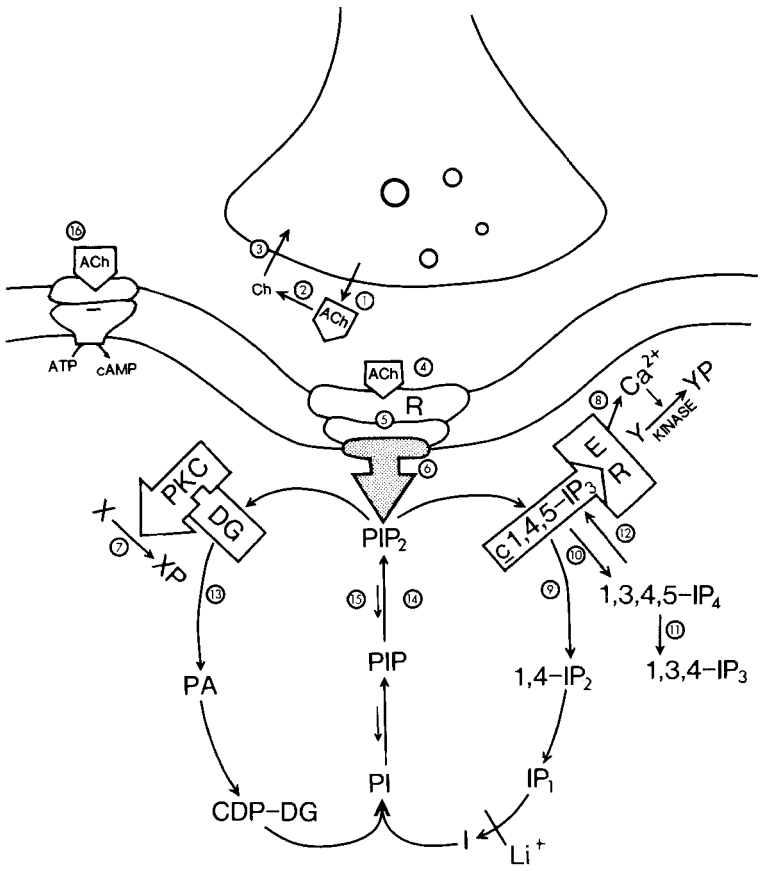

FIG. 3. Diagram of ACh release, muscarinic receptor activation, and second messenger actions in the CNS. ACh released presynaptically (1) is either hydrolyzed by acetylcholinesterase (2) followed by uptake of choline (Ch) (3) or, alternatively, interacts with the muscarinic receptor on the postsynaptic cell surface (4). The activated receptor-ligand complex, possibly acting through a G-protein (5), leads to phosphodiesteratic cleavage (6) of PIP_2. A product, DG, activates PK-C (7), which then catalyzes protein (X) phosphorylation in the presence of Ca^{2+}, leading to physiological cell responses by as yet unestablished mechanisms. The other product of PIP_2 cleavage is 1,4,5-IP_3, and, very likely, its 1,2 cyclic derivative (see text). 1,4,5-IP_3 binds to the endoplasmic reticulum (ER) resulting in increased cytosolic Ca^{2+} (8), thus activating Ca^{2+}-dependent protein kinases that phosphorylate other classes of protein substrates (Y). The action of 1,4,5-IP_3 can be terminated by a membrane-bound 5′-phosphohydrolase (9), yielding 1,4-IP_2, which has no Ca^{2+}-mobilizing action; 1,4,5-IP_3 may alternatively be phosphorylated to IP_4 via a 3′-kinase (10) present in brain cytosol. The fate of IP_4 is not known, but it is tempting to speculate that there exist alternatives: The 5′-phosphohydrolase is expected to degrade IP_4 to the known inactive isomer 1,3,4-IP_3 (11), whereas a hypothetical 3′-phosphohydrolase (12) would reconvert the IP_4 to 1,4,5-IP_3. DG is inactivated by its conversion, via DG kinase (13), to PA, thus initiating recycling to PIP_2 via CDP-DG, PI, and PIP. PIP kinase (14) and PIP_2 phosphatase (15) together constitute an enchange system that rapidly equilibrates the 5′-phosphate of PIP_2 with the γ phosphate of ATP. Also shown is the muscarinic receptor that is negatively coupled to adenylate cyclase (16). cAMP, cyclic AMP; R, receptor.

clization of glucose 6-phosphate (Eisenberg, 1967). The brain, like testis, may be a net producer of inositol. The same phosphatase cleaves both D-1- and D-3-IP_1 and is inhibited by Li^+ (Hallcher and Sherman, 1980). This observation was used to advantage by Berridge et al. (1982) in devising an assay for stimulated inositol lipid turnover with [^{3}H]inositol. The presence of Li^+ permits the accumulation of labeled IP_1, which otherwise would be degraded by the phosphatase. Of the various inositol phosphates, 1,4,5-IP_3 has been of most interest because of its demonstrated ability to mobilize Ca^{2+} from intracellular stores in the endoplasmic reticulum (Streb et al., 1983; Burgess et al., 1984; Thomas et al., 1984; Prentki et al., 1984), probably through its interaction with specific receptor sites (Baukul et al., 1985; Spat et al., 1986*a,b*). The effect of IP_3 on endoplasmic reticulum may be mediated by a guanine nucleotide regulatory mechanism (Dawson, 1985; Gill et al., 1986). Because the highly charged molecule cannot readily enter cells, demonstration of its effects has required permeabilization of cells by various means or, in the case of large cells such as oocytes (Oron et al., 1985), direct injection of IP_3. Irvine et al. (1984) found the presence of a form of IP_3 that is inactive in Ca^{2+} mobilization and, in a series of ingenious experiments, established its structure as the 1,3,4 isomer. It now appears that the source of the 1,3,4 isomer is 1,4,5-IP_3: 1,4,5-IP_3 is phosphorylated by a specific kinase present in brain extracts to form D-*myo*-inositol 1,3,4,5-tetrakisphosphate (1,3,4,5-IP_4) (Batty et al., 1985; Irvine et al., 1986). It can then be selectively dephosphorylated by the specific 5′-phosphohydrolase in brain (Erneaux et al., 1986) to yield the 1,3,4 isomer. What, then, is the significance of these reactions? At present, it appears that IP_4 does not mobilize Ca^{2+} from intracellular stores, although it has been postulated that it may actually gate Ca^{2+} across the plasma membrane (Hansen et al., 1986). One may speculate that, in addition to the known 5′-phosphohydrolase, thought to be the "off" enzyme of the Ca^{2+} mobilization signal of 1,4,5-IP_3 (Connolly et al., 1985), there may also be a 3′-phosphohydrolase. If this proves to be the case, then IP_4 might be preferentially converted back to the 1,4,5 isomer via the action of the putative 3′-phosphohydrolase, rather than to the inactive isomer 1,3,4-IP_3, even though this does not appear to be the case in the parotid gland (Hawkins et al., 1986). These possibilities are shown in Fig. 5, which also entertains the alternative that these various steps are occurring with the 1,2 cyclic derivatives

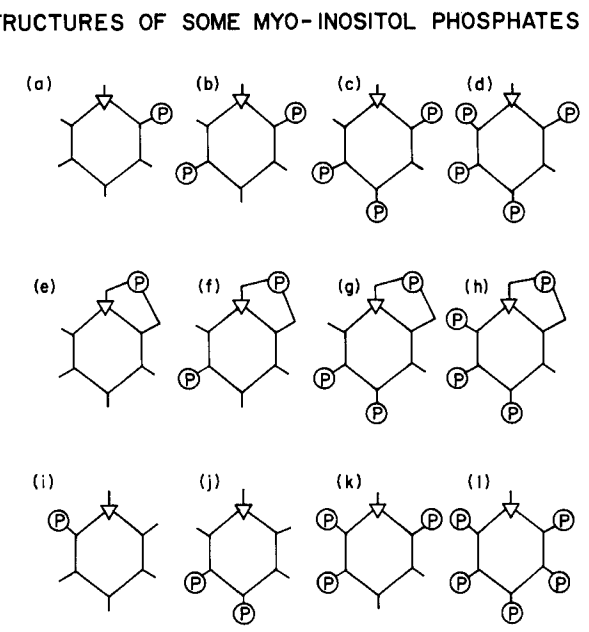

FIG. 4. Higher phosphates of D-*myo*-inositol. Structures (a), (b), and (c) depict IP_1, IP_2, and IP_3, respectively. These are the cleavage products of PI, PIP, and PIP_2. Structure (d) is IP_4, produced by phosphorylation at the D-3 position via IP_3 kinase. Structures (e), (f), (g), and (h) represent 1,2 cyclic derivatives of compounds (a), (b), (c), and (d), respectively. (c1,2)-IP_1 is the best characterized, and there is increasing evidence for the occurrence in tissues of (c1,2)4-IP_2 and (c1,2)4,5-IP_3. The latter compound was first proposed many years ago (Agranoff and Seguin, 1974). The existence of (c1,2)3,4,5-IP_4 is presently speculated. Structure (i) is D-*myo*-inositol 3-phosphate, also known as L-1-IP_1. This ester is formed from cyclization of glucose 6-phosphate. Structure (j) is D-4,5-IP_2. Structure (k) is 1,3,4-IP_3, an inactive isomer of IP_3 (see text). Structure (l) is D-*myo*-inositol 1,3,4,5,6-pentakisphosphate. This isomer is present in avian erythrocytes in relatively high amounts, but may possibly be present in other tissues.

of IP_3 and IP_4 (Connolly et al., 1986). It is of interest, in this regard, that when (c1,2)4,5-IP_3 is injected into the photoreceptor cells of the horseshoe crab, *Limulus,* a membrane conductance change is seen similar to that induced by light, with a potency five times that seen with the noncyclic derivative, 1,4,5-IP_3 (Wilson et al., 1985*a*).

One may wonder why it is IP_3, rather than IP_2 or IP_1, that is recognized by the cell as the signal for Ca^{2+} mobilization. In this regard, it has been hypothesized (Holmsen et al., 1985) that the relative amounts of membrane PI, PIP, and PIP_2 reflect the energy charge of the cell, such that PIP_2 content is reduced at low energy levels. Thus, we may speculate further that a cell is protected from increasing its intracellular Ca^{2+} content when its ATP content is low: When membrane PIP_2 content is reduced, IP_3 production is also reduced. This safety device would then protect the cell from self-destruction, because increasing intracellular Ca^{2+} levels under conditions when this cation cannot be quickly removed (i.e., low ATP levels) would lead to activation of Ca^{2+}-mediated proteases, of phospholipase A_2, etc., which, if sustained, would result in irreparable cellular damage.

INTRACELLULAR MESSENGER GENERATION AND BIOCHEMICAL SEQUELAE

Calcium signaling

It is perhaps a reflection of the daunting complexity of the CNS that direct evidence linking PIP_2 breakdown and the generation of Ca^{2+} signals has, thus far, been restricted to simple neurotumor cells, such as the pheochromocytoma PC-12, neuroblastoma N1E-115, neuroblastoma-glioma hybrid NG-108-15, and astrocytoma 1321N1 cells. In each of these cell types, addition of agonists known to promote the breakdown of inositol lipids also elicits a demonstrable increase in intracellular [Ca^{2+}], even though the intensity and duration of the Ca^{2+} signal differs considerably, depending on cell type and ligand in question (Masters et al., 1984; Yano et al., 1984; Orellana et al., 1985; Vicentini et al., 1985; Snider et al., 1986). The possible linkage of PIP_2 breakdown and Ca^{2+} signals has also been examined in bovine chromaffin cells. Activation of muscarinic receptors in these cells results in an increased turnover of inositol lipids (Fisher et al., 1981*b*; Mohd. Adnan and Hawthorne, 1981), but only a modest increase in intracellular [Ca^{2+}], from 100 to 150 n*M* (Kao and Schneider, 1985), which is apparently insufficient to elicit an increase in catecholamine release (Fisher et al., 1981*b*; Cheek and Burgoyne, 1985). Because IP_3 addition to permeabilized chromaffin cells results in the mobilization of intracellular Ca^{2+}, the failure of muscarinic acetylcho-

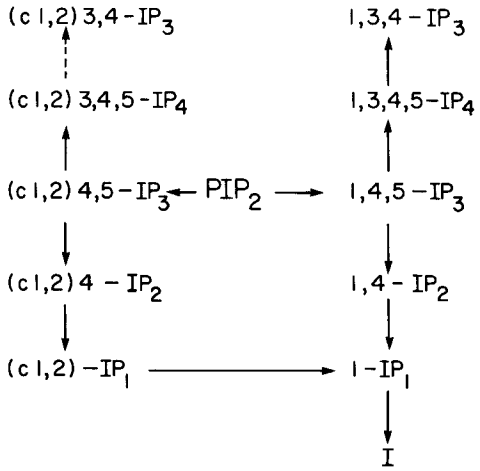

FIG. 5. Metabolic interconversion of inositol phosphates. IP_3 produced by phosphodiesteratic cleavage of PIP_2 can be converted to IP_4 via IP_3 kinase. By action of a 5'-phosphohydrolase, IP_4 can be converted to the inactive isomer 1,3,4-IP_3. It is also possible that the sole initial product of PIP_2 cleavage is cIP_3 and that the kinase and phosphatases act on the 1,2 cyclic derivatives, as indicated.

line (ACh) receptor (mAChR) activation to initiate catecholamine release clearly does not result from the absence of IP_3 "receptors" on intracellular membranes (Stoehr et al., 1986). The situation is further complicated by the fact that distinct species differences exist for the biochemical and physiological consequences of mAChR activation in chromaffin cells. Thus, in the guinea pig, mAChR activation results in catecholamine release (Role and Perlman, 1983), as well as an enhanced turnover of inositol lipids (Figueiredo et al., 1986). One implication from this finding is that in those species, e.g., rat or guinea pig, in which muscarinic receptor activation results in catecholamine release, more IP_3 is produced or less is degraded than in the bovine cell. However, because addition of phorbol esters such as 4β-phorbol 12-myristate 13-acetate (PMA) to guinea pig cells can mimic the effect of muscarinic stimulation on catecholamine release both temporally and quantitatively (Figueiredo et al., 1986), the further possibility must be considered that activation of PK-C is of primary relevance for initiation of exocytotic events.

Production of DG: role in protein phosphorylation

It is now well established that DG released from the breakdown of PIP_2 may serve to activate PK-C, and that as a result, its affinity for Ca^{2+} is lowered, such that in the presence of phosphatidylserine, concentrations of Ca^{2+} as low as 0.1 μM fully activate the enzyme (Nishizuka, 1984; Hirasawa and Nishizuka, 1985). What is much less clear is the identity of specific substrate proteins for PK-C. Gispen and colleagues have proposed that a membrane protein designated B_{50} may be one such substrate (Van Dongen et al., 1985) and that the phosphorylated form inhibits PIP kinase. Addition of corticotropin (1–24)tetracosapeptide (ACTH) inhibits B_{50} phosphorylation, with the net effect that addition of the neuropeptide leads to an increase in PIP_2 synthesis. This result suggests that DG, acting through PK-C, is a regulator of PIP_2 steady-state concentrations in neural membranes. Although in this case DG ultimately reduces PIP_2 concentrations, it may also inhibit receptor-mediated breakdown of PIP_2. Labarca et al. (1984) demonstrated that addition of PMA at nanomolar concentrations results in an inhibition of carbachol-induced release of inositol phosphates from hippocampal slices, whereas the basal turnover of the lipid pool is unaffected. A similar result was obtained by Kanba et al. (1986) with N1E-115 cells. In cultured astrocytoma cells, phorbol esters block both the carbamoylcholine-stimulated breakdown of inositol lipids and the resulting Ca^{2+} signal (Orellana et al., 1985). The mechanism of this inhibition remains unknown for most cells, but in the DDT_1 MF-2 smooth muscle cell line, PMA addition results in phosphorylation of the α_1-adrenergic receptor itself (Leeb-Lundberg et al., 1985). That phorbol esters are capable of modulating neuronal physiology is supported by the recent demonstration that addition of PMA can alter ionic conductance in hippocampal pyramidal cells (Baraban et al., 1985) and in *Aplysia* bag cells (De Riemer et al., 1985). However, application of phorbol esters to hippocampal pyramidal cells can mimic some, but not all, of the electrophysiological responses associated with activation by a receptor agonist (Malenka et al., 1986).

Evidence that *both* DG and IP_3 may play a pivotal role in neuronal signaling was recently obtained by Higashida and Brown (1986) using the NG108-15 neuroblastoma-glioma hybrid cell line. In these cells, addition of bradykinin results in both a hyperpolarization and a depolarization of the membrane. The hyperpolarization response is mimicked by intracellular injection of either Ca^{2+} or IP_3, whereas depolarization can be elicited by addition of phorbol esters or 1-oleoyl-2-acetylglycerol. These results suggest that in NG108-15 cells, the initial breakdown of PIP_2 results in message formation that first inhibits and then accelerates neuronal discharge, and that in this way, neuronal signaling is modified.

PHARMACOLOGICAL CHARACTERISTICS

The availability of novel selective ligands together with the development of more specific assay methods for phosphoinositide turnover has resulted in significant advances in our understanding of receptor coupling to inositol lipid turnover. The number of receptors implicated in inositol lipid turnover in the CNS continues to grow, and in several instances, there is evidence for the involvement of a specific receptor subtype. Furthermore, the relationship between the binding of ligands to the receptor and the ensuing activation of phospholipase C has begun to unfold. At least 17 pharmacologically distinct receptor types have been claimed to be coupled to inositol lipid turnover (Table 1), but the amount of evidence supporting the involvement of each receptor in question varies considerably. They have been subdivided into two groups on the basis of the strength of available supportive evidence. In addition, a third group of receptors is identified for which there is good evidence that they are *not* linked to activation of phosphoinositide phosphodiesterase.

A. Receptors established to be coupled to phosphoinositide turnover

Muscarinic cholinergic. The initial observations that activation of mAChRs in brain slices resulted in an increase in phosphoinositide turnover has since been amply confirmed in a variety of neural preparations, including nerve ending preparations, brain minces, and primary cultures of neurons and glia, as well as transformed cells of neural origin, such as neuroblastoma and astrocytoma (for review, see Fisher and Agranoff, 1985). Within the CNS, there exists a strong correlation between the density of mAChRs, as determined by radioligand binding techniques, and

TABLE 1. *Pharmacological profile of receptor-activated phosphoinositide turnover in CNS and neural-related tissues*

Receptor	Subtype(s)	Tissue	Reference
A. Receptors demonstrated to be coupled to phosphoinositide turnover			
Muscarinic cholinergic	M_1 and M_2	Brain slices	Berridge et al. (1982), Brown et al. (1984), Fisher et al. (1984), Gonzales and Crews (1984), Fisher and Bartus (1985), Gil and Wolfe (1985), Jacobson et al. (1985), Lazareno et al. (1985), Eva and Costa (1986), Heacock et al. (1987)
		Primary neuronal cultures	Gonzales and Crews (1985)
		Primary glial cultures	Pearce et al. (1985)
		Synaptoneurosomes	Gusovsky et al. (1986)
		Astrocytoma 1321N1	Masters et al. (1984), Evans et al. (1985)
		Neuroblastoma N1E-115	Cohen et al. (1983), Kanba et al. (1986)
		Neuroblastoma-glioma NG108-15	Siman and Klein (1981)
		Superior cervical ganglion	Bone et al. (1984), Horwitz et al. (1984)
		Pheochromocytoma	Vicentini et al. (1985)
		Adrenal medulla	Fisher et al. (1981*b*), Mohd. Adnan and Hawthorne (1981), Ohsako and Deguchi (1983), Eberhard and Holz (1987)
		Pituitary	Young et al. (1979), Hauser and Parks (1983)
		Pituitary tumor cells	Akiyama et al. (1986)
Adrenergic	α_1	Brain slices	Berridge et al. (1982), Schoepp et al. (1984), Johnson and Minneman (1985), Kendall et al. (1985), Minneman and Johnson (1984), Fowler et al. (1986), Kemp and Downes (1986)
		Synaptoneurosomes	Gusovsky et al. (1986)
Histaminergic	H_1	Brain slices	Daum et al. (1983, 1984), Carswell and Young (1986), Claro et al. (1986), Donaldson and Hill (1986)
		Neuroblastoma N1E-115	Cohen et al. (1983), Snider et al. (1984)
		Astrocytoma 1321N1	Nakahata et al. (1986)
Serotonergic	5-HT_2 and 5-HT_{1c}	Brain slices (cerebral cortex)	Conn and Sanders-Bush (1984, 1985, 1986), Kendall and Nahorski (1985*b*)
		Choroid plexus	Conn et al. (1986)
Glutamatergic	?	Brain slices	Nicoletti et al. (1985, 1986*a,b*)
		Primary cultures	
		Cerebellum	Nicoletti et al. (1986*c*)
		Striatum	Sladeczek et al. (1985)
B. Receptors purported to be coupled to phosphoinositide turnover[a]			
Substance K	—	Brain slices	Hunter et al. (1985)
Neurotensin	—	Brain slices	Goedert et al. (1984)
		Neuroblastoma N1E-115	Snider et al. (1986)
CCK	—	Brain slices	Downes (1983)
Substance P	P	Brain slices	Watson and Downes (1983), Mantyh et al. (1984)
Vasopressin	V_1	Brain slices (hippocampus)	Downes (1983), Stephens and Logan (1986)
		Superior cervical ganglion	Bone et al. (1984)
Bradykinin	—	Neuroblastoma-glioma	Yano et al. (1984), Osugi et al. (1986)
TRH	—	GH_3 pituitary cells	Martin (1983), Drummond and Raeburn (1984), Gershengorn and Paul (1986)
		Anterior pituitary	Simmonds and Strange (1985)
NGF	—	Superior cervical ganglion	Lakshamanan (1978, 1979)
		Pheochromocytoma	Traynor et al. (1982)
VIP	—	Superior cervical ganglion	Audigier et al. (1986)
Angiotensin	—	Anterior pituitary	Enjalbert et al. (1986)

CCK, cholecystokinin; NGF, nerve growth factor; VIP, vasoactive intestinal peptide.

[a] Receptors listed in the B category are those that when activated elicit a minimal increase in phosphoinositide turnover in tissue preparations from the CNS, even though much larger stimulations of inositol lipid turnover are, on occasion, observed in neurotumor cells or in other neural-related tissues.

the magnitude of stimulated inositol lipid turnover (Downes, 1982, 1983). Increases in inositol lipid turnover in response to cholinergic agonists is primarily, if not entirely, due to the activation of mAChRs. In those instances where a nicotinic cholinergic involvement has been implicated, e.g., superior cervical ganglion (Briggs et al., 1985) or the bovine chromaffin cell (Eberhard and Holz, 1987), this effect may be due to indirect effects of the nicotinic agonist—such as the release of a secondary neurotransmitter or activation

of phospholipase C resulting from the elevation of cytosolic Ca^{2+} concentration. Further support for the sole direct involvement of mAChRs is derived from experiments using antagonists. Addition of muscarinic antagonists such as scopolamine or atropine inhibits the phosphoinositide response, whereas nicotinic antagonists such as *d*-tubocurarine are largely without effect. Muscarinic agonists differ considerably in their ability to enhance phosphoinositide turnover in brain. In the cerebral cortex, a group of agonists, including ACh, carbamoylcholine, and oxotremorine-M, is more effective than several others, including pilocarpine, bethanechol, and oxotremorine (Fisher et al., 1983, 1984; Gonzales and Crews, 1984; Jacobson et al., 1985). The former group of agonists have been termed "group A" agonists, and the latter, "group B." Not only are group B agonists less effective when added alone, but when present in incubations containing group A agonists, they block the effect of the more efficacious group A agonists and are, therefore, considered to be partial agonists. It is of interest to note that this pattern of agonist efficacy is very different from that for another biochemical consequence of mAChR activation, inhibition of adenylate cyclase (Olianas et al., 1983; Brown and Brown, 1984), but is very similar to that described for mAChR stimulation of guanylate cyclase (McKinney et al., 1985). The ability of muscarinic agonists to enhance inositol lipid turnover is highly correlated with the binding characteristics of the agonist. In cerebral cortex, group A agonists readily discriminate at least two affinity forms (high and low) of the mAChR, whereas group B agonists bind predominantly to a single affinity form (Fisher, 1986). This observation indicates that muscarinic agonists differ in their ability to produce an optimal conformational change in the mAChR and that this is related to the subsequent activation of the phosphodiesterase. There are also indications that there are regional, developmental, and tissue differences in the way mAChRs are coupled to inositol lipid turnover. In the guinea pig neostriatum (Fisher and Bartus, 1985), in neonatal rat cerebral cortex (Heacock et al., 1987), and in the human neuroblastoma SK-N-SH cell line (Fisher and Snider, 1986), group A agonists are more potent stimulators of phosphoinositide hydrolysis than in the adult cortex, whereas, conversely, several group B agonists are more effective.

In both the CNS and nonneural tissues, the existence of M_1 and M_2 mAChR subtypes has been proposed on the basis of the presumed selectivity of the antagonist pirenzepine (Watson et al., 1985). A suggestion that the phosphoinositide response in the CNS is a function of activation of the M_1 subtype alone appears unwarranted (Vickroy et al., 1984); both M_1 (pirenzepine-sensitive) and M_2 (pirenzepine-insensitive) characteristics are observed for stimulated lipid turnover. Thus, whereas pirenzepine is a potent inhibitor of the phosphoinositide response in the cerebral cortex, hippocampus, and AtT-20/D16-6 pituitary cells (K_i, ~ 10–20 n*M*) (Gonzales and Crews, 1984; Fisher and Bartus, 1985; Gil and Wolfe, 1985; Smith and Yamamura, 1985; Akiyama et al., 1986), it is considerably less potent in the neostriatum, astrocytoma cell, and brainstem (K_i, 100–300 n*M*) (Brown et al., 1985; Fisher and Bartus, 1985; Lazareno et al., 1985). Both putative M_1 and M_2 mAChR subtypes may be coupled to phosphoinositide turnover in neural tissues.

Adrenergic. There is good evidence that the α_1-adrenergic receptor subtype is specifically coupled to stimulated inositol lipid turnover. Phosphoinositide hydrolysis elicited by norepinephrine can be blocked by the α_1-adrenergic blockers prazosin or phentolamine, but not by yohimbine, an α_2-adrenergic blocker, or by propranolol, a β-adrenergic antagonist (Brown et al., 1984; Minneman and Johnson, 1984; Shoepp et al., 1984; Kendall et al., 1985). As is observed for the muscarinic response, adrenergic agonists differ in their ability to enhance inositol lipid turnover. Methoxamine and phenylephrine are partial agonists, whereas other known α_1-selective agonists, such as oxymetazoline, and ephedrine have no measurable effect (Minneman and Johnson, 1984). The α_1-adrenergic response is detectable in most, if not all, brain regions, but the magnitude of the effect does not correlate well with the density of α_1-adrenergic receptors, a result suggesting that receptor density alone is not the sole determinant of response magnitude (Johnson and Minneman, 1985). An explanation may lie in the recent demonstration of α_1-receptor subtypes that are differentially sensitive to the effect of the alkylating agent chlorethylclonidine. One of the α_1 subtypes appears to be coupled to phosphoinositide turnover, whereas the other is linked to activation of adenylate cyclase (Minneman and Johnson, 1986).

Histaminergic. In the CNS and neuroblastoma and astrocytoma cells, the phosphoinositide response appears to be mediated through an H_1 receptor, because the stimulatory effect of histamine can be blocked by inclusion of pyrilamine, an H_1 antagonist, whereas the H_2 antagonist cimetidine is ineffective (Subramanian et al., 1980). Furthermore, the magnitude of histamine-stimulated inositide turnover mirrors that of the density of H_1 receptors (Daum et al., 1983, 1984). Regional differences in coupling characteristics (EC_{50} values and partial agonist efficacies) of H_1 receptors linked to phosphoinositide turnover have recently been observed (Carswell and Young, 1986).

Serotonergic. In the cerebral cortex, 5-HT_2 receptors appear to be coupled to inositide turnover, because addition of the potent 5-HT_2 antagonist ketanserin blocks the response (Conn and Sanders-Bush, 1984, 1985, 1986; Kendall and Nahorski, 1985*b*). The situation is less clear in all other brain regions examined, because there appears to be little correlation between the density of [^{3}H]ketanserin binding sites and

magnitude of the phosphoinositide response. Furthermore, ketanserin does not readily antagonize the response in these brain regions. Recently, a new serotonin subtype, 5-HT_{1c}, has been implicated in stimulation of phosphoinositide turnover elicited by addition of serotonin to the choroid plexus (Conn et al., 1986). This receptor subtype differs from the 5-HT_2 receptor in its greater affinity (10-fold) for serotonin and its relative insensitivity to ketanserin and spiperone. It would appear, then, that there are at least two 5-hydroxytryptamine (5-HT) subtypes coupled to phosphoinositide turnover.

Glutamatergic. Nicoletti et al. (1985, 1986*a*) have demonstrated that addition of glutamate or ibotenate, its rigid structural analog, elicits a large increase in the accumulation of inositol phosphates. This effect is most evident in neonatal tissue and declines with maturity. In hippocampus, quisqualate is a partial agonist, whereas *N*-methyl-D-aspartate (NMDA), kainate, quinolinate, and *N*-acetylaspartylglutamate are inactive. The pharmacological characteristics of ibotenate-stimulated phosphoinositide turnover are not consistent with three glutamate receptor subtypes characterized primarily electrophysiologically ("quisqualate," "NMDA," or "kainate"), but rather share some similarities with the putative DL-2-amino-4-phosphonobutyric acid (APB) receptor, as determined from recent electrophysiological and radioligand binding studies. APB antagonizes ibotenate stimulation of inositol lipid turnover. However, although DL-2-amino-5-phosphonovalerate (APV) does not antagonize ibotenate-mediated inositol lipid turnover, it displaces [^{3}H]APB, an observation indicating additional complexity. Using cultured striatal neurons, Sladeczek et al. (1985) have also observed that addition of glutamate enhanced the release of inositol phosphate. However, the glutamate agonists' profile is different from that observed in hippocampus: Whereas glutamate and quisqualate are full agonists in the striatum, NMDA and kainate are partial agonists. APV inhibited the reaction to NMDA, but not to glutamate, kainate, or quisqualate. These results suggest that a quisqualate or NMDA receptor subtype is involved, although it is probable that the activation of several glutamate receptor subtypes can enhance inositol lipid turnover. As a result of these findings, it is now generally accepted that some glutamate receptor subtypes, long thought to couple directly to plasma membrane ionophores, may instead operate through the generation of a second messenger signal.

B. Receptors purported to be coupled to phosphoinositide turnover

Several neuropeptide receptors may be coupled to inositol lipid turnover in brain, but a more definite statement must await independent confirmation of what are, at best, marginal effects on labeled inositol phosphate release. In this category are included substance K, neuromedin (Hunter et al., 1985), neurotensin (Goedert et al., 1984), and cholecystokinin receptors (Downes, 1983). The evidence suggesting a linkage between substance P receptors and inositol lipid turnover is somewhat more convincing. Mantyh et al. (1984) have demonstrated a correlation between the receptor density of substance P receptors, as determined autoradiographically, and the magnitude of inositol phosphate release. In the hypothalamus, the substance P-P receptor subtype has been implicated in inositol lipid turnover on the basis of agonist studies (Watson and Downes, 1983). Addition of vasopressin to hippocampal slices elicits only a small increase in inositol phosphate release (150% of control) (Stephens and Logan, 1986), whereas its effect is much greater in the superior cervical ganglion (Bone et al., 1984), even though vasopressin may not be a natural ligand for this receptor (Hanley et al., 1984). In transformed cells of neural origin, such as neuroblastoma N1E-115, neuroblastoma-glioma hybrid, or GH_3 pituitary cells, there is strong evidence favoring linkage of neurotensin, bradykinin, and thyrotropin-releasing hormone (TRH) receptors to stimulated inositol lipid turnover (Martin, 1983; Yano et al., 1984; Snider et al., 1986).

C. Receptors not directly coupled to stimulated phosphoinositide turnover

The list of receptors that have been studied in which no evidence could be found for linked phosphoinositide turnover include several that are either positively or negatively coupled to adenylate cyclase, e.g., H_2-histaminergic, 5-HT_1-serotonergic, β-adrenergic, and α_2-adrenergic, in addition to the nicotinic cholinergic and GABAergic receptors.

D. Receptor occupancy requirements for phosphoinositide hydrolysis

A close relationship exists in the CNS between occupancy of the receptor by the agonist and the degree of stimulation of inositol lipid turnover. Two lines of evidence support this statement. First, the abilities of either muscarinic or adrenergic ligands to stimulate the release of labeled inositol phosphates correlates well with their abilities to displace radiolabeled antagonists from specific membrane binding sites (Fisher et al., 1983, 1984; Minneman and Johnson, 1984). Second, the loss of receptor number either in vivo following neurotoxin treatment (Fisher et al., 1981*a*) or in vitro following irreversible inactivation of a proportion of the muscarinic, α_1-adrenergic, 5-HT_2-serotonergic, or H_1-histaminergic receptors results in a comparable loss of receptor-stimulated phosphoinositide turnover (Johnson and Minneman, 1985; Kendall and Nahorski, 1985*b*; Kendall et al., 1985; Claro et al., 1986; Fisher and Snider, 1986). Some indication that this normal tight coupling between receptor occupancy and response might be capable of adaptive change was obtained by Kendall et al. (1985). Following central noradrenergic denervation, the EC_{50} for

norepinephrine in the hippocampus was significantly reduced, and the partial agonist phenylephrine became more efficacious. Moreover, α_1-adrenergic receptor alkylation with phenoxybenzamine resulted in a greater loss of receptor number than of the inositide response. These results can be interpreted as indicative of the development of a small receptor reserve for the phosphoinositide response following denervation. The observation that 10-fold lower concentrations of norepinephrine (but not of carbamoylcholine) are required to elicit a half-maximal increase in IP_3 accumulation in denervated iris smooth muscle is also consistent with the development of a "receptor reserve" for the α_1-adrenergic receptor (Akhtar and Abdel-Latif, 1986).

E. Signal transduction

In nonneural tissues, there presently exists a significant amount of evidence to support the involvement of a guanine nucleotide binding (G) protein in the transduction process (Joseph, 1985; Litosch et al., 1985), even though the identity of the G-proteins remains to be determined. There is little direct evidence to invoke such a mechanism for neural receptors at present. However, the inositide phosphodiesterase in brain has been shown to be stimulated by GTP or its nonhydrolyzable analogs (Gonzales and Crews, 1985). Outside the CNS, addition of GTPγS to permeabilized chromaffin cells results in a release of IP_3, IP_2, and IP_1 (Eberhard and Holz, 1987), whereas GTP potentiates the effect of TRH in cell-free preparations from GH_3 pituitary cells (Martin et al., 1986). In human astrocytoma cells, a strong correlation exists between the ability of muscarinic agonists to enhance phosphoinositide turnover and their propensity to induce the appearance of a high-affinity GTP-sensitive form of the mAChR (Evans et al., 1985). These results, taken collectively, suggest a role for G-proteins in signal transduction at inositide-linked receptors in the CNS. A recent theory proposed by Rodbell (1985) envisages that, on receptor activation, the α subunit of the G-protein is released from the membrane and that subsequent modifications occur to yield new forms of the protein with differing functions. If correct, the implication of this theory is that these "programmable messengers" are, in fact, the true second messengers of hormone action and that IP_3 and DG comprise tertiary signals.

F. Desensitization

A characteristic of stimulated inositol lipid turnover is its persistence, so long as the agonist continues to occupy the receptor. In brain slices, for example, the continued release of inositol phosphates is observed for up to 120 min following addition of carbamoylcholine (Fisher and Bartus, 1985). Masters et al. (1984) have demonstrated that in human astrocytoma cells, preexposure of the cells to carbamoylcholine results in a loss of stimulated Ca^{2+} efflux but not of the production of inositol phosphates, a result suggesting that desensitization occurs distal to PIP_2 hydrolysis. However, some caution is necessary before this interpretation is universally applied, because in most studies, accumulation of IP_1 rather than IP_3 or its isomers is measured.

Modulation of phosphoinositide turnover by inhibitory receptors

In addition to those receptors that can enhance the breakdown and resynthesis of inositol lipids, there may be a separate class of receptors that regulate the extent of stimulated inositol lipid turnover. For example, in the platelet, agents that elevate the tissue cyclic AMP concentration, such as prostaglandin E and forskolin, inhibit thrombin-mediated PIP_2 breakdown, the generation of Ca^{2+} signals, and subsequent physiological responses (Nishizuka, 1984). Comparable observations in the CNS are limited and often equivocal. Thus, although forskolin failed to reduce either carbamoylcholine- or norepinephrine-mediated stimulation of inositol lipid turnover in cerebral cortex slices (Hollingsworth and Daly, 1985), an inhibitory effect was observed by Nicchitta and Williamson (1986), using nerve ending preparations. Similarly, although it has been claimed that administration of excitatory amino acids (kainic acid, NMDA, or DL-homocysteate) exerts an inhibitory effect on phosphoinositide turnover elicited by carbamoylcholine, histamine, or K^+ depolarization, and not by norepinephrine (Baudry et al., 1986), an inhibition of norepinephrine-linked, and not of carbamoylcholine-linked, stimulation was observed by Nicoletti et al. (1986*b*).

In the retina, light stimulation elicits an increased phosphoinositide turnover that is mediated in part by release of ACh (Anderson and Hollyfield, 1981; Anderson et al., 1983; Schmidt, 1983*a,b*) and can be blocked by administration of glycine (Anderson and Hollyfield, 1984). The inhibitory effect of glycine is, in turn, reversed by preincubation with strychnine. In the anterior pituitary, activation of D_2-dopaminergic receptors results in both an inhibition of adenylate cyclase and a 40% reduction in the stimulated turnover of inositol lipids elicited by TRH or its analog DN 1417 (Simmonds and Strange, 1985). For the same tissue, Enjalbert et al. (1986) have observed that D_2-receptor activation in lactotrophs results in a 50% inhibition of angiotensin II-stimulated inositol phosphate release. To date, the D_2 receptor represents the best-documented example of a receptor-mediated inhibition of inositol phosphate release.

Cellular and synaptic localization of receptors coupled to phosphoinositide turnover

Until recently, a role for glia in stimulated phospholipid turnover in the CNS has largely been ignored, despite earlier indications that inositide turnover in both neuronal and glial cell-enriched fractions could be enhanced by addition of neurotransmitters (Woelk

et al., 1974; Abdel-Latif et al., 1974) and the fact that the number of glial cells may exceed that of neurons by severalfold in the adult mammalian CNS. The possible involvement of glia in receptor-mediated phosphoinositide turnover has been examined in primary cultures of cortical astrocytes judged to be at least 85% pure by immunocytochemical criteria (Pearce et al., 1985). In these cultures, addition of muscarinic cholinergic and α_1-adrenergic ligands stimulated the release of inositol phosphates, and in terms of agonist affinities and Ca^{2+} sensitivity, the characteristics of the glial response were very similar to those observed previously for brain slices. However, using primary cultures of both neurons and glia, Gonzales et al. (1985) concluded that the phosphoinositide response is primarily neuronal. Nevertheless, the demonstration of functionally coupled muscarinic receptors on both neuroblastoma (Cohen et al., 1983) and astrocytoma (Masters et al., 1984) cells indicates that glial elements may also play a significant role in stimulated inositol lipid turnover in the CNS.

Receptors are found at both presynaptic and postsynaptic sites, but most of the limited information presently available points to a postsynaptic localization for receptor-enhanced inositol lipid turnover. Early studies on the superior cervical ganglion indicated that much of the increased [^{3}H]inositol incorporation into inositol lipids that occurs after ACh addition was localized autoradiographically to the cell body (Hokin, 1965). Removal of presynaptic elements failed to reduce appreciably stimulation of lipid labeling elicited by addition of either ACh or nerve growth factor, a result indicating a postsynaptic site for both receptors (Hokin, 1966; Lakshamanan, 1978, 1979). More recent studies on the hippocampus support this conclusion. Lesion of the adrenergic input by means of microinjection of 6-hydroxydopamine, systemic treatment with the noradrenergic neurotoxin DSP4, or surgical lesion of the medial forebrain bundle did not reduce the magnitude of α_1-adrenergic stimulated inositol phosphate release (Janowsky et al., 1984; Kendall et al., 1985; Fowler et al., 1986). Surgical lesion of the guinea pig fornix-fimbria also failed to reduce the muscarinic stimulation of inositol lipid turnover, even though activity of the presynaptic marker enzyme choline acetyltransferase was reduced by 80% (Fisher et al., 1980). In contrast, lesion of postsynaptic structures with the neurotoxin ibotenate resulted in a substantial loss of both muscarinic receptor number and stimulated PA and PI turnover (Fisher et al., 1981*a*). Kemp and Downes (1986) also concluded that stimulated phosphoinositide turnover elicited by α_1-adrenergic agonists in the lateral geniculate nucleus is postsynaptic in location, because lesion of interneurons and cell bodies of afferent neurons with kainic acid reduced the magnitude of phosphoinositide hydrolysis.

Despite the prevailing evidence, the possibility is not excluded that there are also presynaptic receptors coupled to phosphoinositide turnover.

POSSIBLE RELEVANCE OF PHOSPHOINOSITIDE TURNOVER TO NEURAL FUNCTION

Neuronal plasticity

Rapidly axonally transported proteins associated with neuronal growth and regeneration have been identified (Skene and Willard, 1981; Heacock and Agranoff, 1982; Benowitz and Lewis, 1983; Katz et al., 1985). More recently, it has been demonstrated that one of these proteins, designated growth-associated protein 43 (GAP43), is characteristic of growth cones (Meiri et al., 1986). It is identical with the B_{50} protein found in plasma membrane (Gispen et al., 1985) and shown to be phosphorylated by PK-C (Meiri et al., 1986). Because the phosphorylated form of B_{50} inhibits conversion of PIP to PIP_2, this may constitute a regulatory mechanism (Van Dongen et al., 1985) of relevance to brain function. There are other instances of interaction between IP_3 turnover and PK-C. For example, the 5′-phosphohydrolase in platelets has been postulated to be phosphorylated by PK-C, thus activating it (Connolly and Majerus, 1986; Molina y Vedia and Lapetina, 1986). GAP43 phosphorylation has also been implicated in a behavioral memory model, long-term potentiation in the rat hippocampus (Nelson and Routtenberg, 1985).

A major question in understanding the biochemical basis of memory formation is how information expressed in transduction processes associated with synaptic transmission can eventually lead to changes in macromolecular synthesis that can mediate long-term behavioral changes (Agranoff, 1981). A possible mechanism is provided by the phosphoinositide second messengers, which in nonneural systems have been shown to lead to permanent commitment to growth, via oncogenes. By analogy, in neurons, a positive feedback loop may be induced via an increased intracellular Ca^{2+} content, which converts a behaviorally induced transient synaptic response to a permanent one (Berridge, 1986; Goelet et al., 1986).

Nerve-impulse conduction: role of inositide turnover in diabetic neuropathy

The known localization of PIP and PIP_2 in plasma membranes and the propensity of these lipids to bind divalent cations have led to the speculation that PIP-PIP_2 interconversions might regulate the amount of Ca^{2+} bound to specific membrane sites and, thereby, influence the operation of Na^+ and K^+ channels (Hawthorne and Kai, 1969). However, it is unclear how responsive the polyphosphoinositides are to changes in nerve conduction, and conflicting results have been obtained from a variety of neural preparations subjected to electrical stimulation (Goswami and Gould, 1985; for review, see Fisher and Agranoff,

1985). Furthermore, Mg^{2+}, a cation present in the cytosol in much greater concentrations than Ca^{2+}, also binds to the polyphosphoinositides. Indirect evidence for a role of the polyphosphoinositides in nerve conduction has come from recent studies on sciatic nerves of rats made diabetic by streptozotocin, a treatment that leads to a diabetic neuropathy. Altered PI turnover has been previously implicated in diabetes (Palmano et al., 1977; Whiting et al., 1977), and recently Eichberg and colleagues (Bell et al., 1982; Berti-Mattera et al., 1985) have demonstrated a substantial increase in $^{32}P_i$ incorporation into PIP_2 in diabetic animals. The changes could be reversed by insulin administration. A reduction in Na^+,K^+-ATPase activity has been observed for diabetic sciatic nerve (Greene and Lattimer, 1983, 1985); furthermore, the ouabain-sensitive component of respiration, which is reduced in this tissue, can be normalized by in vitro administration of PMA (Greene and Lattimer, 1986). Collectively, these results suggest a link among changes in phosphoinositide turnover, axonal conduction, and transmembrane ion gradients in the nerve.

PI as an anchor for membrane proteins

Low and colleagues have provided convincing evidence that certain membrane proteins and enzymes are anchored within the membrane through a covalent linkage to a ring hydroxyl of PI (Low and Finean, 1978; Low and Zilversmit, 1980; Low et al., 1986). The same group has recently demonstrated that the attachment of Thy-1, a cell-surface glycoprotein, to neural membranes also involves a PI linkage (Low and Kincade, 1985). A phospholipid tentatively identified as either PIP or PIP_2 is also implicated in a covalent linkage to myelin basic protein (Yang et al., 1986). This observation assumes importance in light of the known high polyphosphoinositide content of myelin and the suggestion that this is the site of a metabolically inactive pool of these lipids (Eichberg and Hauser, 1973).

Cuatrecasas and colleagues have demonstrated that, in liver, PI may also be linked to a carbohydrate moiety. Following activation of phospholipase C by insulin, it is proposed that two second messengers are formed: in addition to DG, there is an inositol phosphate glycan that regulates cyclic AMP phosphodiesterase (Saltiel and Cuatrecasas, 1986; Saltiel et al., 1986). These findings attest further to the diversity of functions that inositol lipids are already known to play in cellular physiology.

IS THERE A LINK BETWEEN PHOSPHOINOSITIDE HYDROLYSIS AND PSYCHIATRIC DISORDERS?

Role of lithium

Despite the long-term use of Li^+ in the treatment of manic depressive disorders, only recently has any hint of the underlying mechanism of its action become apparent. Largely as a result of the studies of Sherman and colleagues (Allison and Blisner, 1976; Allison et al., 1976; Hallcher and Sherman, 1980), it is now established that administration of therapeutically relevant concentrations of Li^+ results in the accumulation of inositol monophosphate in brain and that this is the result of the selective inhibition of inositol 1-phosphatase (Hallcher and Sherman, 1980). The net effect of Li^+ administration is a decrease in *myo*-inositol availability, which theoretically could result in a reduction of PI synthesis, especially in view of the relative impermeability of the blood–brain barrier to inositol (Spector and Lorenzo, 1975). It has been proposed that Li^+ may preferentially regulate those Ca^{2+}-mobilizing receptors that are "hyperactive" and thus act as a calcistat (Berridge et al., 1982). Of interest is the recent observation that chronic administration of Li^+ results in an increased activity of brain inositol 1-phosphatase, a mechanism presumably designed to compensate for the inhibitory effect of Li^+ (Renshaw et al., 1986). It should be pointed out that the key regulated step in the cycle is likely to be the resynthesis of PIP_2 rather than PI. In this context, it is noteworthy that Downes and Stone (1986) have observed that, in the parotid gland, administration of Li^+ resulted in an accumulation of CDP-DG (due to inositol depletion) but had no effect on receptor-mediated PIP_2 breakdown. Thus, it appears that the agonist-sensitive pool of phosphoinositides is largely spared the effects of Li^+. A similar conclusion was reached by Drummond and Raeburn (1984) in studies with GH_3 pituitary cells. As in parotid gland, GH_3 cells preserve their cellular levels of PIP_2, even when faced with a large reduction in the cellular content of PI. Despite these observations, it remains possible that a reduction in intracellular inositol content could result in an adverse effect on the steady-state level of PIP_2. Simultaneous determination of the chemical mass of inositol, of inositol lipids, and of Li^+ content in a defined cell type would do much to elucidate the interrelationship between Li^+ and the phosphoinositides. A key development for future studies will be better separation and quantitative estimation of the inositol phosphates. Almost all of the present reports are based on ^{32}P- or [^{3}H]inositol-labeled products, which are poorly characterized and without chemical mass measurements.

Receptor regulation

In addition to providing a better insight into the possible physiological function of a large group of CNS receptors, measurement of phosphoinositide turnover also provides a convenient biochemical measure of receptor activity. This is of particular importance when one considers that many psychotherapeutic agents, including antidepressants and neuroleptics, are known from radioligand binding assays to interact with more than one inositide-linked receptor. Inhibition by these agents of in vitro ligand binding to the mAChR and 5-HT_2, α_1-adrenergic, and H_1 recep-

tors has been documented (Peroutka and Snyder, 1980; Richelson and Nelson, 1984*a,b*). Although the magnitude of the regulatory effects of these agents on receptor density following chronic administration may be modest, available assay techniques have sufficient precision to detect them. Moreover, there may be instances in which the changes in phosphoinositide turnover are larger than those in receptor density, for example, as is seen in down-regulation of 5-HT_2 receptor number and inositide stimulation following iprindole treatment (Kendall and Nahorski, 1985*b*). Such studies may well shed light on the long-term regulation of inositide second messenger generation and on adaptive changes in chemical signaling within the CNS.

CONCLUDING REMARKS

The advantage of reviewing an active research area is that such a review will be timely; the disadvantage is that it will soon be outdated. It is our hope this review will first have served a useful function. If one compares the inositide-linked second messenger systems with an earlier neurochemical era—the elucidation of cyclic AMP mechanisms—we might safely predict that much will be learned about the complexities of signal transduction in the brain. Our new knowledge in this area should also lead to the development of useful pharmacological screens for the development of new drugs.

It is noteworthy that our understanding of the phosphoinositides began with the studies of a pioneer neurochemist, Jordi Folch-Pi, who figures centrally in recent historical reviews of the International Society for Neurochemistry (McIlwain, 1985) and the American Society for Neurochemistry (Tower, 1987).

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REFERENCES

Abdel-Latif A. A. (1986) Calcium mobilizing receptors, polyphosphoinositides, and the generation of second messengers. *Pharmacol. Rev.* **38,** 227–272.

Abdel-Latif A. A., Yau S.-J., and Smith J. P. (1974) Effect of neurotransmitters on phospholipid metabolism in rat cerebral-cortex slices—cellular and subcellular distribution. *J. Neurochem.* **22,** 383–393.

Agranoff B. W. (1978) Cyclitol confusion. *Trends Biochem. Sci.* **3,** N283–N285.

Agranoff B. W. (1981) Learning and memory: biochemical approaches, in *Basic Neurochemistry* (Siegel G. J., Albers R. W., Agranoff B. W., and Katzman R., eds), pp. 801–820. Little, Brown and Co., Boston.

Agranoff B. W. (1987) Receptor-mediated phosphoinositide metabolism, in *Advances in Experimental Biology and Medicine: Molecular Mechanisms of Neuronal Responsiveness* (Ehrlich Y. H., Berry W., and Lenox R. H., eds), in press. Plenum Press, New York.

Agranoff B. W. and Seguin E. B. (1974) Preparation of inositol trisphosphate from brain: GLC of trimethylsilyl derivative. *Prep. Biochem.* **4,** 359–366.

Agranoff B. W., Bradley R. M., and Brady R. O. (1958) The enzymatic synthesis of inositol phosphatide. *J. Biol. Chem.* **233,** 1077–1083.

Agranoff B. W., Murthy P., and Seguin E. B. (1983) Thrombin-induced phosphodiesteratic cleavage of phosphatidylinositol bisphosphate in human platelets. *J. Biol. Chem.* **258,** 2076–2078.

Agranoff B. W., Eisenberg F. Jr., Hauser G., Hawthorne J. N., and Michell R. H. (1985) Comment on abbreviations, in *Cyclitols and Inositides* (Bleasdale J., Hauser G., and Eichberg J., eds), pp. xxi–xxii. Humana Press, Clifton, New Jersey.

Akhtar R. A. and Abdel-Latif A. A. (1980) Requirement for calcium ions in acetylcholine-stimulated phosphodiesteratic cleavage of phosphatidyl-myo-inositol 4,5-bisphosphate in rabbit iris smooth muscle. *Biochem. J.* **192,** 783–791.

Akhtar R. A. and Abdel-Latif A. A. (1986) Surgical sympathetic denervation increases α_1-adrenoceptor-mediated accumulation of *myo*-inositol trisphosphate and muscle contraction in rabbit iris dilator smooth muscle. *J. Neurochem.* **46,** 96–104.

Akiyama K., Vickroy T. W., Watson M., Roeske W. R., Reisine T. D., Smith T. L., and Yamamura H. I. (1986) Muscarinic cholinergic ligand binding to intact mouse pituitary tumor cells (AtT-20/D16-16) coupling with two biochemical effectors: adenylate cyclase and phosphatidylinositol turnover. *J. Pharmacol. Exp. Ther.* **236,** 653–661.

Allison J. H. and Blisner M. E. (1976) Inhibition of the effect of lithium on brain inositol by atropine and scopolamine. *Biochem. Biophys. Res. Commun.* **68,** 1332–1338.

Allison J. H., Blisner M. E., Holland W. H., Hipps P. P., and Sherman W. R. (1976) Increased brain myo-inositol 1-phosphate in lithium-treated rats. *Biochem. Biophys. Res. Commun.* **71,** 664–670.

Anderson R. E. and Hollyfield J. G. (1981) Light stimulates the incorporation of inositol into phosphatidylinositol in the retina. *Biochim. Biophys. Acta* **665,** 619–622.

Anderson R. E. and Hollyfield J. G. (1984) Inositol incorporation into phosphoinositides in retinal horizontal cells of *Xenopus laevis:* enhancement by acetylcholine, inhibition by glycine. *J. Cell Biol.* **99,** 686–691.

Anderson R. E., Maude M. B., Kelleher P. A., Rayborn M. E., and Hollyfield J. G. (1983) Phosphoinositide metabolism in the retina: localization to horizontal cells and regulation by light and divalent cations. *J. Neurochem.* **41,** 764–771.

Audigier S., Barberis C., and Jard S. (1986) Vasoactive intestinal polypeptide increases inositol phospholipid breakdown in the rat superior cervical ganglion. *Brain Res.* **376,** 363–367.

Baraban J. M., Snyder S. H., and Alger B. E. (1985) Protein kinase C regulates ionic conductance in hippocampal pyramidal neurons: electrophysiological effects of phorbol esters. *Proc. Natl. Acad. Sci. USA* **82,** 2538–2542.

Batty I. R., Nahorski S. R., and Irvine R. F. (1985) Rapid formation of inositol 1,3,4,5-tetrakisphosphate following muscarinic receptor stimulation of rat cerebral cortical slices. *Biochem. J.* **232,** 211–215.

Baudry M., Evans J., and Lynch G. (1986) Excitatory amino acids inhibit stimulation of phosphatidylinositol metabolism by aminergic agonists in hippocampus. *Nature* **319,** 329–331.

Baukal A. J., Guillemette G., Rubin R., Spat A., and Catt K. J. (1985) Binding sites for inositol trisphosphate in the bovine adrenal cortex. *Biochem. Biophys. Res. Commun.* **133,** 532–538.

Bell M. E., Peterson R. G., and Eichberg J. (1982) Metabolism of phospholipids in peripheral nerve from rats with chronic streptozotocin-induced diabetes: increased turnover of phosphatidylinositol-4,5-bisphosphate. *J. Neurochem.* **39,** 192–200.

Benjamins J. A. and Agranoff B. W. (1969) Distribution and properties of CDP-diglyceride: inositol transferase from brain. *J. Neurochem.* **16,** 513–527.

Benowitz L. I. and Lewis E. R. (1983) Increased transport of 44,000-

to 49,000-dalton acidic proteins during regeneration of the goldfish optic nerve: a two-dimensional gel analysis. *J. Neurosci.* **3,** 2153–2163.

Berridge M. J. (1984) Inositol trisphosphate and diacylglycerol as second messengers. *Biochem. J.* **220,** 345–360.

Berridge M. (1986) Second messenger dualism in neuromodulation and memory. *Nature* **323,** 294–295.

Berridge M. J. and Irvine R. F. (1984) Inositol trisphosphate, a novel second messenger in cellular signal transduction. *Nature* **312,** 315–321.

Berridge M. J., Downes C. P., and Hanley M. R. (1982) Lithium amplified agonist-dependent phosphatidylinositol responses in brain and salivary glands. *Biochem. J.* **206,** 587–595.

Berridge M. J., Dawson R. M. C., Downes C. P., Heslop J. P., and Irvine R. F. (1983) Changes in the levels of inositol phosphates after agonist-dependent hydrolysis of membrane phosphoinositides. *Biochem. J.* **212,** 473–482.

Berti-Mattera L., Peterson R., Bell M., and Eichberg J. (1985) Effect of hyperglycemia and its prevention by insulin treatment on the incorporation of ^{32}P into polyphosphoinositides and other phospholipids in peripheral nerve of the streptozotocin diabetic rat. *J. Neurochem.* **45,** 1692–1698.

Bone E. A. and Michell R. H. (1985) Accumulation of inositol phosphates in sympathetic ganglia. Effects of depolarization and of amine peptide neurotransmitters. *Biochem. J.* **227,** 263–269.

Bone E. A., Fretten P., Palmer S., Kirk C. J., and Michell R. H. (1984) Rapid accumulation of inositol phosphates in isolated rat superior cervical sympathetic ganglia exposed to V_1-vasopressin and muscarinic cholinergic stimuli. *Biochem. J.* **221,** 803–811.

Briggs C. A., Horwitz J., McAfee D. A., Tsymbalov S., and Perlman R. L. (1985) Effects of neuronal activity on inositol phospholipid metabolism in the rat autonomic nervous system. *J. Neurochem.* **44,** 731–739.

Brown E., Kendall D. A., and Nahorski S. R. (1984) Inositol phospholipid hydrolysis in rat cerebral cortical slices: 1. Receptor characterisation. *J. Neurochem.* **42,** 1379–1387.

Brown J. H. and Brown S. L. (1984) Agonists differentiate muscarinic receptors that inhibit cyclic AMP formation from those that stimulate phosphoinositide metabolism. *J. Biol. Chem.* **259,** 3777–3781.

Brown J. H., Goldstein D., and Masters S. B. (1985) The putative M1 muscarinic receptor does not regulate phosphoinositide hydrolysis: studies with pirenzepine and McN-A-343 in chick heart and astrocytoma cells. *Mol. Pharmacol.* **27,** 525–531.

Burgess G. M., Godfrey P. P., McKinney J. S., Berridge M. J., Irvine R. F., and Putney J. W. Jr. (1984) The second messenger linking receptor activation to internal Ca release in liver. *Nature* **309,** 63–66.

Carswell H. and Young J. M. (1986) Regional variation in the characteristics of histamine H_1-agonist-mediated breakdown of inositol phospholipids in guinea-pig brain. *Br. J. Pharmacol.* **89,** 809–817.

Carter J. R. and Kennedy E. P. (1966) Enzymatic synthesis of cytidine diphosphate diglyceride. *J. Lipid Res.* **7,** 678–683.

Cheek T. R. and Burgoyne R. D. (1985) Effect of activation of muscarinic receptors on intracellular free calcium and secretion in bovine adrenal chromaffin cells. *Biochim. Biophys. Acta* **846,** 167–173.

Claro E., Arbones L., Garcia A., and Picatoste F. (1986) Phosphoinositide hydrolysis mediated by histamine H_1-receptors in rat brain cortex. *Eur. J. Pharmacol.* **123,** 187–196.

Cohen N. M., Schmidt D. M., McGlennen R. C., and Klein W. L. (1983) Receptor-mediated increases in phosphatidylinositol turnover in neuron-like cell lines. *J. Neurochem.* **40,** 547–554.

Colodzin M. and Kennedy E. P. (1965) Biosynthesis of diphosphoinositide in brain. *J. Biol. Chem.* **240,** 3771–3780.

Conn P. J. and Sanders-Bush E. (1984) Selective 5HT-2 antagonists inhibit serotonin stimulated phosphatidylinositol metabolism in cerebral cortex. *Neuropharmacology* **23,** 993–996.

Conn P. J. and Sanders-Bush E. (1985) Serotonin-stimulated phosphoinositide turnover: mediation by the S_2 binding site in rat cerebral cortex but not in subcortical regions. *J. Pharmacol. Exp. Ther.* **234,** 195–203.

Conn P. J. and Sanders-Bush E. (1986) Biochemical characterization of serotonin stimulated phosphoinositide turnover. *Life Sci.* **38,** 663–669.

Conn P. J., Sanders-Bush E., Hoffman B. J., and Hartig P. R. (1986) A unique serotonin receptor in choroid plexus is linked to phosphatidylinositol turnover. *Proc. Natl. Acad. Sci. USA* **83,** 4086–4088.

Connolly T. M. and Majerus P. W. (1986) Protein kinase C (PKC) phosphorylates human platelet inositol trisphosphate 5'-phosphomonoesterase (IP_3 5'-p'tase) increasing phosphatase activity. *Fed. Proc.* **45,** 1872.

Connolly T. M., Bross T. E., and Majerus P. W. (1985) Isolation of a phosphomonoesterase from human platelets that specifically hydrolyzes the 5-phosphate of inositol 1,4,5-trisphosphate. *J. Biol. Chem.* **260,** 7868–7874.

Connolly T. M., Wilson D. B., Bross T. E., and Majerus P. W. (1986) Isolation and characterization of the inositol cyclic phosphate products of phosphoinositide cleavage by phospholipase C. *J. Biol. Chem.* **261,** 122–126.

Daniel L. W., Waite M., and Wykle R. L. (1986) A novel mechanism of diglyceride formation. *J. Biol. Chem.* **261,** 9128–9132.

Daum P. R., Downes C. P., and Young J. M. (1983) Histamine-induced inositol phospholipid breakdown mirrors H_1-receptor density in brain. *Eur. J. Pharmacol.* **87,** 497–498.

Daum P. R., Downes C. P., and Young J. M. (1984) Histamine stimulation of inositol 1-phosphate accumulation in lithium-treated slices from regions of guinea pig brain. *J. Neurochem.* **43,** 25–32.

Dawson A. P. (1985) GTP enhances inositol trisphosphate-stimulated Ca^{2+} release from rat liver microsomes. *FEBS Lett.* **185,** 147–150.

Dawson R. M. C. (1959) Studies on the enzymic hydrolysis of monophosphoinositide by phospholipase preparations from *P. notatum* and ox pancreas. *Biochim. Biophys. Acta* **33,** 68–77.

Dawson R. M. C., Freinkel N., Jungalwala F. B., and Clarke N. (1971) The enzymic formation of *myo*inositol 1:2 cyclic phosphate from phosphatidylinositol. *Biochem. J.* **122,** 605–607.

De Riemer S. A., Strong J. A., Albert K. A., Greengard P., and Kaczmarek L. K. (1986) Enhancement of calcium current in *Aplysia* neurones by phorbol ester and protein kinase C. *Nature* **313,** 313–316.

Donaldson J. and Hill S. J. (1986) Histamine-induced hydrolysis of polyphosphoinositides in guinea-pig ileum and brain. *Eur. J. Pharmacol.* **124,** 255–265.

Downes C. P. (1982) Receptor-stimulated inositol phospholipid metabolism in the central nervous system. *Cell Calcium* **3,** 413–428.

Downes C. P. (1983) Inositol phospholipids and neurotransmitter-receptor signalling mechanisms. *Trends Neurosci* **6,** 313–316.

Downes C. P. (1986) Inositol phosphates: concord or confusion? *Trends Neurosci.* **9,** 394–396.

Downes C. P. and Stone M. A. (1986) Lithium-induced reduction in intracellular inositol supply in cholinergically stimulated parotid gland. *Biochem. J.* **234,** 199–204.

Drummond A. H. and Raeburn C. A. (1984) The interaction of lithium with thyrotropin-releasing hormone-stimulated lipid metabolism in GH_3 pituitary tumour cells. *Biochem. J.* **224,** 129–136.

Eberhard D. and Holz R. W. (1987) Nicotinic and muscarinic receptor activation and micromolar Ca^{2+} stimulate production of inositol phosphates in bovine adrenal chromaffin cells. *J. Neurochem.* (in press).

Eichberg J. and Hauser G. (1973) The subcellular distribution of polyphosphoinositides in myelinated and unmyelinated rat brain. *Biochim. Biophys. Acta* **326,** 210–223.

Eisenberg F. Jr. (1967) D-Myoinositol 1-phosphate as product of cyclization of glucose 6-phosphate and substrate for a specific phosphatase in rat testis. *J. Biol. Chem.* **242,** 1375–1382.

Enjalbert A., Sladeczek F., Guillon G., Bertrand P., Shu C., Epelbaum J., Garcia-Sainz A., Jard S., Lombard C., Kordon C., and Bockaert J. (1986) Angiotensin II and dopamine modulate both cAMP and inositol phosphate productions in anterior pituitary cells. *J. Biol. Chem.* **261,** 4071–4075.

Erneaux C., Delvaux A., Moreau C., and Dumont J. E. (1986) Characterization of D-myo-inositol 1,4,5-trisphosphate phosphatase in rat brain. *Biochem. Biophys. Res. Commun.* **134,** 351–358.

Eva C. and Costa E. (1986) Potassium ion facilitation of phosphoinositide turnover activation by muscarinic receptor agonists in rat brain. *J. Neurochem.* **46,** 1429–1435.

Evans T., Hepler J. R., Masters S. B., Brown J. H., and Harden T. K. (1985) Guanine nucleotide regulation of agonist binding to muscarinic cholinergic receptors. *Biochem. J.* **232,** 751–757.

Figueiredo J. C., Fisher S. K., and Horowitz M. I. (1986) Modulation of muscarinic and nicotinic cholinergic receptor mediated catecholamine secretion in guinea pig chromaffin cells by phorbol esters. *Fed. Proc.* **45,** 505.

Fisher S. K. (1986) Inositol lipids and signal transduction at CNS muscarinic receptors. *Trends Pharmacol. Sci. Suppl.: Subtypes of Muscarinic Receptors* **II,** 61–65.

Fisher S. K. and Agranoff B. W. (1980) Calcium and the muscarinic synaptosomal phospholipid labeling effect. *J. Neurochem.* **34,** 1231–1240.

Fisher S. K. and Agranoff B. W. (1981) Enhancement of the muscarinic synaptosomal phospholipid labeling effect by the ionophore A23187. *J. Neurochem.* **37,** 968–977.

Fisher S. K. and Agranoff B. W. (1985) The biochemical basis and functional significance of enhanced phosphatidate and phosphoinositide turnover, in *Phospholipids in Nervous Tissues* (Eichberg J., ed), pp. 241–295. John Wiley, New York.

Fisher S. K. and Agranoff B. W. (1986) Phosphoinositide turnover in the CNS and in neural-related tissues, in *Receptor Biochemistry and Methodology: Receptors and Phosphoinositides* (Putney J. W. Jr., ed), pp. 219–243. Alan R. Liss, New York.

Fisher S. K. and Bartus R. T. (1985) Regional differences in the coupling of muscarinic receptors to inositol phospholipid hydrolysis in guinea pig brain. *J. Neurochem.* **45,** 1085–1095.

Fisher S. K. and Snider R. M. (1986) Receptor occupancy requirements for muscarinic receptor-stimulated phosphoinositide turnover in brain and in neuroblastoma. (Abstr) *Soc. Neurosci. Abstr.* **12,** 491.

Fisher S. K., Boast C. A., and Agranoff B. W. (1980) The muscarinic stimulation of phospholipid labeling is independent of its cholinergic input. *Brain Res.* **189,** 284–288.

Fisher S. K., Frey K. A., and Agranoff B. W. (1981*a*) Loss of muscarinic receptors and of stimulated phospholipid labeling in ibotenate-treated hippocampus. *J. Neurosci.* **1,** 1407–1413.

Fisher S. K., Holz R. W., and Agranoff B. W. (1981*b*) Muscarinic receptors in chromaffin cell cultures mediate enhanced phospholipid labeling but not catecholamine secretion. *J. Neurochem.* **37,** 491–497.

Fisher S. K., Klinger P. D., and Agranoff B. W. (1983) Muscarinic agonist binding and phospholipid turnover in brain. *J. Biol. Chem.* **258,** 7358–7363.

Fisher S. K., Figueiredo J. C., and Bartus R. T. (1984) Differential stimulation of inositol phospholipid turnover in brain by analogs of oxotremorine. *J. Neurochem.* **43,** 1171–1179.

Fowler C. J., Magnusson O., Mohammed A. K., Danysz W., and Archer T. (1986) The effect of selective noradrenergic lesions upon the stimulation by noradrenaline of inositol phospholipid breakdown in rat hippocampal miniprisms. *Eur. J. Pharmacol.* **123,** 401–407.

Gershengorn M. and Paul M. E. (1986) Evidence for tight coupling of receptor occupancy by thyrotropin-releasing hormone to phospholipase C-mediated phosphoinositide hydrolysis in rat pituitary cells: use of chlordiazepoxide as a competitive antagonist. *Endocrinology* **119,** 833–839.

Gil D. W. and Wolfe B. B. (1985) Pirenzepine distinguishes between muscarinic receptor-mediated phosphoinositide breakdown and inhibition of adenylate cyclase. *J. Pharmacol. Exp. Ther.* **232,** 608–616.

Gill D. L., Ueda T., Chueh S.-H., and Noel M. W. (1986) Ca^{2+} release from endoplasmic reticulum is mediated by a guanine nucleotide regulatory mechanism. *Nature* **320,** 461–464.

Gispen W. H., Leunissen J. L. M., Oestreicher A. B., Verkleij A. J., and Zwiers H. (1985) Presynaptic localization of B-50 phosphoprotein: the (ACTH)-sensitive protein kinase substrate involved in rat brain polyphosphoinositide metabolism. *Brain Res.* **328,** 381–385.

Goedert M., Pinnock R. D., Downes C. P., Mantyh P. W., and Emson P. C. (1984) Neurotensin stimulates inositol phospholipid hydrolysis in rat brain slices. *Brain Res.* **323,** 193–197.

Goelet P., Castellucci V. F., Schacher S., and Kandel E. R. (1986) The long and the short of long-term memory—a molecular framework. *Nature* **322,** 419–422.

Gonzales R. A. and Crews F. T. (1984) Characterization of the cholinergic stimulation of phosphoinositide hydrolysis of rat brain slices. *J. Neurosci.* **4,** 3120–3127.

Gonzales R. A. and Crews F. T. (1985) Guanine nucleotides stimulate production of inositol trisphosphate in rat cortical membranes. *Biochem. J.* **232,** 799–804.

Gonzales R. A., Feldstein J. B., Crews F. T., and Raizada M. K. (1985) Receptor mediated inositide hydrolysis is a neuronal response: comparison of primary neuronal and glial cultures. *Brain Res* **345,** 350–355.

Goswami S. K. and Gould R. M. (1985) Effect of electrical stimulation on phosphoinositide metabolism in rat sciatic nerve *in vivo*. *J. Neurochem.* **44,** 941–946.

Greene D. A. and Lattimer S. A. (1983) Impaired rat sciatic nerve sodium-potassium adenosine triphosphate in acute streptozotocin diabetes and its correction by myo-inositol supplementation. *J. Clin. Invest.* **72,** 1058–1063.

Greene D. A. and Lattimer S. A. (1985) Altered nerve myo-inositol metabolism in experimental diabetes and its relationship to nerve function, in *Inositol and Phosphoinositides: Metabolism and Biological Regulation* (Bleasdale J. E., Eichberg J., and Hauser G., eds), pp. 563–582. Humana Press, Clifton, New Jersey.

Greene D. A. and Lattimer S. A. (1986) Protein kinase C agonists acutely normalize decreased ouabain-inhibitable respiration in diabetic rabbit nerve: implications for (Na,K)-ATPase regulation and diabetic complications. *Diabetes* **35,** 242–245.

Griffin H. D. and Hawthorne J. N. (1978) Calcium-activated hydrolysis of phosphatidyl-*myo*-inositol 4-phosphate and phosphatidyl-*myo*-inositol 4,5-bisphosphate in guinea pig synaptosomes. *Biochem. J.* **176,** 541–552.

Griffin H. D., Hawthorne J. N., and Sykes M. (1979) A calcium requirement for the phosphatidylinositol response following activation of presynaptic muscarinic receptors. *Biochem. Pharmacol.* **28,** 1143–1147.

Gusovsky F., Hollingsworth E. B., and Daly J. W. (1986) Regulation of phosphatidylinositol turnover in brain synaptoneurosomes: stimulatory effects of agents that enhance influx of sodium ions. *Proc. Natl. Acad. Sci. USA* **83,** 3003–3007.

Hajra A. K., Fisher S. K., and Agranoff B. W. (1987) Isolation, separation and analysis of phosphoinositides from biological sources, in *Neuromethods (Neurochemistry), Vol. 8: Lipids and Related Compounds* (Boulton A. A., Baker G. B., and Horrocks L. A., eds), in press. Humana Press, Clifton, New Jersey.

Hallcher L. M. and Sherman W. R. (1980) The effects of lithium ion and other agents on the activity of *myo*-inositol-1-phosphatase from bovine brain. *J. Biol. Chem.* **255,** 10896–10901.

Hanley M. R., Benton H. P., Lightman S. L., Todd K., Bone E. A., Fretten P., Palmer S., Kirk C. J., and Michell R. H. (1984) A vasopressin-like peptide in the mammalian sympathetic nervous system. *Nature* **309,** 258–261.

Hansen C. A., Mah S., and Williamson J. R. (1986) Formation and metabolism of inositol 1,3,4,5-tetrakisphosphate in liver. *J. Biol. Chem.* **261,** 8100–8103.

Hauser G. and Parks J. M. (1983) Evidence from phospholipid metabolism changes for muscarinic cholinergic receptors on rat anterior pituitary cells. *J. Neurosci. Res.* **10,** 295–302.

Hawkins P. T., Stephens L., and Downes C. P. (1986) Rapid formation of inositol 1,3,4,5-tetrakisphosphate and inositol 1,3,4-trisphosphate in rat parotid glands may both result indirectly from receptor-stimulated release of inositol 1,4,5-trisphosphate from phosphatidylinositol 4,5-bisphosphate. *Biochem. J.* **238,** 507–516.

Hawthorne J. N. (1986) Does receptor-linked phosphoinositide metabolism provide messengers mobilizing calcium in nervous tissue? *Int. Rev. Neurobiol.* **28,** 241–273.

Hawthorne J. N. and Kai M. (1969) Metabolism of phosphoinositides, in *Handbook of Neurochemistry, Vol. 3* (Lajtha A., ed), pp. 491–508. Plenum Press, New York.

Hawthorne J. N. and Pickard M. R. (1979) Phospholipids in synaptic function. *J. Neurochem.* **32,** 5–14.

Heacock A. M. and Agranoff B. W. (1982) Protein synthesis and transport in the regenerating goldfish visual system. *Neurochem. Res.* **7,** 771–788.

Heacock A. M., Fisher S. K., and Agranoff B. W. (1987) Enhanced coupling of neonatal muscarinic receptors in rat brain to phosphoinositide turnover. *J. Neurochem.* (in press).

Higashida H. and Brown D. A. (1986) Two polyphosphatidylinositol metabolites control two K^+ currents in a neuronal cell. *Nature* **323,** 333–335.

Hirasawa K. and Nishizuka Y. (1985) Phosphatidylinositol turnover in receptor mechanism and signal transduction. *Annu. Rev. Pharmacol. Toxicol.* **25,** 147–170.

Hokin L. E. (1965) Autoradiographic localization of the acetylcholine-stimulated synthesis of phosphatidylinositol in the superior cervical ganglion. *Proc. Natl. Acad. Sci. USA* **53,** 1369–1376.

Hokin L. E. (1966) Effects of acetylcholine on the incorporation of ^{32}P into various phospholipids in slices of normal and denervated superior cervical ganglia of the cat. *J. Neurochem.* **13,** 179–184.

Hokin L. E. (1985) Receptors and phosphoinositide-generated second messengers. *Annu. Rev. Biochem.* **54,** 205–235.

Hokin L. E. and Hokin M. R. (1955) Effects of acetylcholine on the turnover of phosphoryl units in individual phospholipids of pancreas slices and brain cortex slices. *Biochim. Biophys. Acta* **18,** 102–110.

Hokin M. R. and Hokin L. E. (1959) The synthesis of phosphatidic acid from diglyceride and adenosine triphosphate in extracts of brain microsomes. *J. Biol. Chem.* **234,** 1381–1386.

Hokin M. R. and Hokin L. E. (1964) Interconversions of phosphatidylinositol and phosphatidic acid involved in the response to acetylcholine in the salt gland, in *Metabolism and Physiological Significance of Lipids* (Dawson R. M. C. and Rhodes D. N., eds), pp. 423–434. John Wiley and Sons, New York.

Hollingsworth E. B. and Daly J. W. (1985) Accumulation of inositol phosphates and cyclic AMP in guinea-pig cerebral cortical preparations. Effects of norepinephrine, histamine, carbamylcholine and 2-chloroadenosine. *Biochim. Biophys. Acta* **847,** 207–216.

Holmsen H., Nilsen A. O., and Rongved S. (1985) Energy requirements for stimulus-response coupling. *Adv. Exp. Med. Biol.* **192,** 215–233.

Horwitz J., Tsymbalov S., and Perlman R. L. (1984) Muscarine stimulates the hydrolysis of inositol-containing phospholipids in the superior cervical ganglion. *J. Pharmacol. Exp. Ther.* **233,** 235–241.

Hunter J. C., Goedert M., and Pinnock R. D. (1985) Mammalian tachykinin-induced hydrolysis of inositol phospholipids in rat brain slices. *Biochem. Biophys. Res. Commun.* **127,** 616–622.

Irvine R. F., Letcher A. J., Lander D. J., and Downes C. P. (1984) Inositol trisphosphates in carbachol-stimulated rat parotid glands. *Biochem. J.* **223,** 237–243.

Irvine R. F., Letcher A. J., Heslop J. P., and Berridge M. J. (1986) The inositol tris/tetrakis phosphate pathway—demonstration of inositol (1,4,5)trisphosphate-3-kinase activity in animal tissues. *Nature* **320,** 631–634.

Jacobson M. D., Wusteman M., and Downes C. P. (1985) Muscarinic receptors and hydrolysis of inositol phospholipids in rat cerebral cortex and parotid gland. *J. Neurochem.* **44,** 465–472.

Janowsky A., Labarca R., and Paul S. M. (1984) Noradrenergic denervation increases α_1-adrenoreceptor-mediated inositol-phosphate accumulation in the hippocampus. *Eur. J. Pharmacol.* **102,** 193–194.

Johnson R. D. and Minneman K. P. (1985) α_1-Adrenergic receptors and stimulation of [^{3}H]inositol metabolism in rat brain: regional distribution and parallel inactivation. *Brain Res.* **341,** 7–15.

Jolles J., Schrama L. H., and Gispen W. H. (1981) Calcium-dependent turnover of brain polyphosphoinositides in vitro after prelabelling in vivo. *Biochim. Biophys. Acta* **666,** 90–98.

Joseph S. K. (1985) Receptor-stimulated phosphoinositide metabolism: a role for GTP-binding proteins? *Trends Biochem. Sci.* **10,** 297–298.

Kai M., White G. L., and Hawthorne J. N. (1966) The phosphatidylinositol kinase of rat brain. *Biochem. J.* **101,** 328–337.

Kai M., Salway J. G., and Hawthorne J. N. (1968) The diphosphoinositide kinase of rat brain. *Biochem. J.* **106,** 791–801.

Kanba S., Kanba K. S., and Richelson E. (1986) The protein kinase C activator, 12-O-tetradecanoylphorbol-13-acetate (TPA), inhibits muscarinic (M_1) receptor-mediated inositol phosphate release and cyclic GMP formation in murine neuroblastoma cells (clone N1E-115). *Eur. J. Pharmacol.* **125,** 155–156.

Kao L. S. and Schneider A. S. (1985) Muscarinic receptors on bovine chromaffin cells mediate a rise in cytosolic calcium that is independent of extracellular calcium. *J. Biol. Chem.* **260,** 2019–2022.

Katz F., Ellis L., and Pfenninger K. H. (1985) Nerve growth cones isolated from fetal rat brain. III. Calcium-dependent protein phosphorylation. *J. Neurosci.* **5,** 1402–1414.

Kemp J. A. and Downes C. P. (1986) Noradrenaline-stimulated inositol phospholipid breakdown in rat dorsal lateral geniculate nucleus neurones. *Brain Res.* **371,** 314–318.

Kemp P., Hubscher G., and Hawthorne J. N. (1959) A liver phospholipase hydrolysing phosphoinositides. *Biochim. Biophys. Acta* **31,** 585–586.

Kendall D. A. and Nahorski S. R. (1984) Inositol phospholipid hydrolysis in rat cerebral cortical slices: II. Calcium requirement. *J. Neurochem.* **42,** 1388–1394.

Kendall D. A. and Nahorski S. R. (1985*a*) Dihydropyridine calcium channel activators and antagonists influence depolarization-evoked inositol phospholipid hydrolysis in brain. *Eur. J. Pharmacol.* **115,** 31–36.

Kendall D. A. and Nahorski S. R. (1985*b*) 5-Hydroxytryptamine-stimulated inositol phospholipid hydrolysis in rat cerebral cortex slices: pharmacological characterization and effects of antidepressants. *J. Pharmacol. Exp. Ther.* **233,** 473–479.

Kendall D. A., Brown E., and Nahorski S. R. (1985) α_1-Adrenoceptor-mediated inositol phospholipid hydrolysis in rat cerebral cortex: relationship between receptor occupancy and response and effects of denervation. *Eur. J. Pharmacol.* **114,** 41–52.

Labarca R., Janowsky A., Patel J., and Paul S. M. (1984) Phorbol esters inhibit agonist-induced [^{3}H]inositol-1-phosphate accumulation in rat hippocampal slices. *Biochem. Biophys. Res. Commun.* **123,** 703–709.

Lakshmanan J. (1978) Nerve growth factor induced phosphatidylinositol turnover effect of 6-hydroxydopamine treatment. *FEBS Lett.* **92,** 159–162.

Lakshmanan J. (1979) Post-synaptic PI-effect of nerve growth factor in rat superior cervical ganglia. *J. Neurochem.* **32,** 1599–1601.

Lazareno S., Kendall D. A., and Nahorski S. R. (1985) Pirenzepine

indicates heterogeneity of muscarinic receptors linked to cerebral inositol phospholipid metabolism. *Neuropharmacology* **24,** 593–595.

Leeb-Lundberg L. M. F., Cotecchia S., Lomasney J. W., De Bernadis J. F., Lefkowitz R. J., and Caron M. G. (1985) Phorbol esters promote α_1-adrenergic receptor phosphorylation and receptor uncoupling from inositol phospholipid metabolism. *Proc. Natl. Acad. Sci. USA* **82,** 5651–5655.

Litosch I., Wallis C., and Fain J. N. (1985) 5-Hydroxytryptamine stimulates inositol phosphate production in a cell free system from blowfly salivary glands: evidence for a role of GTP in coupling receptor activation to phosphoinositide breakdown. *J. Biol. Chem.* **260,** 5464–5471.

Low M. G. and Finean J. B. (1978) Specific release of plasma membrane enzymes by a phosphatidylinositol-specific phospholipase C. *Biochim. Biophys. Acta* **508,** 565–570.

Low M. G. and Kincade P. W. (1985) Phosphatidylinositol is the membrane-anchoring domain of the Thy-1 glycoprotein. *Nature* **318,** 62–64.

Low M. G. and Zilversmit D. B. (1980) Role of phosphatidylinositol in attachment of alkaline phosphatase to membranes. *Biochemistry* **19,** 3913–3918.

Low M. G., Ferguson M. A. J., Futerman A. H., and Silman I. (1986) Covalently attached phosphoinositol as a hydrophobic anchor for membrane proteins. *TIBS* **11,** 212–214.

Malenka R. C., Madison D. V., Andrade R., and Nicoll R. A. (1986) Phorbol esters mimic some cholinergic actions in hippocampal pyramidal neurons. *J. Neurosci.* **6,** 475–480.

Mantyh P. W., Pinnock R. D., Downes C. P., Goedert M., and Hunt S. P. (1984) Correlation between inositol phospholipid hydrolysis and substance P receptors in rat CNS. *Nature* **309,** 795–797.

Martin T. F. J. (1983) Thyrotropin releasing hormone rapidly activates the phosphodiester hydrolysis of polyphosphoinositides in GH_3 pituitary cells. *J. Biol. Chem.* **258,** 14816–14822.

Martin T. F. J., Lucas D. O., Bajjalieh S. M., and Kowalchyk J. A. (1986) Thyrotropin-releasing hormone activates a Ca^{2+}-dependent polyphosphoinositide phosphodiesterase in permeable GH_3 cells. GTPγS potentiation by a cholera and pertussis toxin-insensitive mechanism. *J. Biol. Chem.* **261,** 2918–2927.

Masters S. B., Harden T. K., and Brown J. H. (1984) Relationships between phosphoinositide and calcium responses to muscarinic agonists in 1321N1 astrocytoma cells. *Mol. Pharmacol.* **26,** 149–155.

McIlwain H. (1985) In the beginning: to celebrate 20 years of the International Society for Neurochemistry (ISN). *J. Neurochem.* **45,** 1–10.

McKinney M., Stenstrom S., and Richelson E. (1985) Muscarinic responses and binding in a murine neuroblastoma clone (N1E-115). Mediation of separate responses by high affinity and low affinity agonist-receptor conformations. *Mol. Pharmacol.* **27,** 223–235.

Meiri K. F., Pfenninger K. H., and Willard M. B. (1986) Growth-associated protein, GAP-43, a polypeptide that is induced when neurons extend axons, is a component of growth cones and corresponds to pp46, a major polypeptide of a subcellular fraction enriched in growth cones. *Proc. Natl. Acad. Sci. USA* **83,** 3537–3541.

Minneman K. P. and Johnson R. D. (1984) Characterization of *alpha*-1 adrenergic receptors linked to [^{3}H]inositol metabolism in rat cerebral cortex. *J. Pharmacol. Exp. Ther.* **230,** 317–323.

Minneman K. P. and Johnson R. D. (1986) α_1-Adrenergic receptors linked to inositol phosphate and cyclic AMP accumulation in rat brain (Abstr), in *Abstracts of the Sixth European Society of Neurochemistry Meeting,* p. 103. European Society of Neurochemistry, Prague.

Mohd. Adnan N. A. and Hawthorne J. N. (1981) Phosphatidylinositol labeling in response to activation of muscarinic receptors in bovine adrenal medulla. *J. Neurochem.* **36,** 1858–1860.

Molina y Vedia L. M. and Lapetina E. G. (1986) Phorbol 12,13-dibutyrate and 1-oleyl-2-acetyldiacyl-glycerol stimulate inositol trisphosphate dephosphorylation in human platelets. *J. Biol. Chem.* **261,** 10493–10495.

Mori T., Taki Y., Yu B., Takabashi J., Nishizuka Y., and Fujikura T. (1982) Specificity of fatty acyl moieties of diacylglycerol for the activation of calcium-activated, phospholipid dependent protein kinase. *J. Biochem. (Tokyo)* **91,** 427–431.

Nahorski S. R., Kendall D. A., and Batty I. (1986) Receptors and phosphoinositide metabolism in the central nervous system. *Biochem. Pharmacol.* **35,** 2447–2453.

Nakahata N., Martin M. W., Hughes A. R., Hepler J. R., and Harden T. K. (1986) H_1-histamine receptors on human astrocytoma cells. *Mol. Pharmacol.* **29,** 188–195.

Nelson R. B. and Routtenberg A. (1985) Characterization of protein F1 (47 kDa, 4.5 pI): a kinase C substrate directly related to neural plasticity. *Exp. Neurol.* **89,** 213–224.

Nicchitta C. V. and Williamson J. R. (1986) Cyclic nucleotide regulation of inositol lipid metabolism in rat cerebral cortex. *Fed. Proc.* **45,** 1827.

Nicoletti F., Meek J. L., Chuang D. M., Iodarola M., Roth B. L., and Costa E. (1985) Ibotenic acid stimulates inositol phospholipid turnover in rat hippocampal slices: an effect mediated by "APB-sensitive" receptors. *Fed. Proc.* **44,** Abstr 480.

Nicoletti F., Meek J. L., Iadarola M. J., Chuang D. M., Roth B. L., and Costa E. (1986*a*) Coupling of inositol phospholipid metabolism with excitatory amino acid recognition sites in rat hippocampus. *J. Neurochem.* **46,** 40–46.

Nicoletti F., Iadarola M. J., Wroblewski J. T., and Costa E. (1986*b*) Excitatory amino acid recognition sites coupled with inositol phospholipid metabolism: developmental changes and interaction with α_1-adrenoceptors. *Proc. Natl. Acad. Sci. USA* **83,** 1931–1935.

Nicoletti F., Wroblewski J. T., Novelli A., Alho H., Guidotti A., and Costa E. (1986*c*) The activation of inositol phospholipid metabolism as a signal-transducing system for excitatory amino acids in primary cultures of cerebellar granule cells. *J. Neurosci.* **6,** 1905–1911.

Nishizuka Y. (1984) Turnover of inositol phospholipids and signal transduction. *Science* **225,** 1365–1370.

Nishizuka Y. (1986) Studies and perspectives of protein kinase C. *Science* **233,** 305–312.

Ohsako S. and Deguchi T. (1983) Phosphatidic acid mimicks the muscarinic actions of acetylcholine in cultured bovine chromaffin cells. *FEBS Lett.* **152,** 62–66.

Olianas M. C., Onali P., Neff N. H., and Costa E. (1983) Adenylate cyclase activity of synaptic membranes from rat striatum. Inhibition by muscarinic receptor agonists. *Mol. Pharmacol.* **23,** 393–398.

Orellana S. A., Solski P. A., and Brown J. H. (1985) Phorbol ester inhibits phosphoinositide hydrolysis and calcium mobilization in cultured astrocytoma cells. *J. Biol. Chem.* **260,** 5236–5239.

Oron Y., Dascal N., Nadler E., and Lupu M. (1985) Inositol 1,4,5-trisphosphate mimics muscarinic responses in *Xenopus* oocytes. *Nature* **313,** 141–143.

Osugi T., Uchida S., Imaizumi T., and Yoshida H. (1986) Bradykinin-induced intracellular Ca^{2+} elevation in neuroblastoma × glioma hybrid NG108-15 cells: relationship to the action of inositol phospholipids metabolites. *Brain Res.* **379,** 84–89.

Palmano K. P., Whiting P. H., and Hawthorne J. N. (1977) Free and lipid *myo*-inositol in tissues from rats with acute and less severe streptozotocin-induced diabetes. *Biochem. J.* **167,** 229–235.

Pearce B., Cambray-Deakin M., Morrow C., Grimble J., and Murphy S. (1985) Activation of muscarinic and of α_1-adrenergic receptors on astrocytes results in the accumulation of inositol phosphates. *J. Neurochem.* **45,** 1534–1540.

Peroutka S. J. and Snyder S. H. (1980) Long-term antidepressant treatment lowers spiroperidol labelled serotonin receptor binding. *Science* **210,** 88–90.

Petzold G. L. and Agranoff B. W. (1965) Studies on the formation of CDP-diglyceride. *Fed. Proc.* **24,** 476.

Petzold G. L. and Agranoff B. W. (1967) The biosynthesis of cyti-

dine diphosphate diglyceride by embryonic chick brain. *J. Biol. Chem.* **242,** 1187–1191.

Pizer F. L. and Ballou C. E. (1959) Studies on myo-inositol phosphates of natural origin. *J. Am. Chem. Soc.* **81,** 915–921.

Prentki M., Biden T. J., Janjic D., Irvine R. F., Berridge M. J., and Wollheim C. B. (1984) Rapid mobilization of Ca^{2+} from rat insulinoma microsomes by inositol-1,4,5-trisphosphate. *Nature* **309,** 562–564.

Renshaw P. F., Joseph N. E., and Leigh J. S. Jr. (1986) Chronic dietary lithium induces increased levels of myo-inositol-1-phosphatase activity in rat cerebral cortex homogenates. *Brain Res.* **380,** 401–404.

Richelson E. and Nelson A. (1984*a*) Antagonism by antidepressants of neurotransmitter receptors of normal human brain in vitro. *J. Pharmacol. Exp. Ther.* **230,** 94–102.

Richelson E. and Nelson A. (1984*b*) Antagonism by neuroleptics of neurotransmitter receptors of normal human brain in vitro. *Eur. J. Pharmacol.* **103,** 197–204.

Rodbell M. (1985) Programmable messengers: a new theory of hormone action. *Trends Biochem. Sci.* **11,** 461–464.

Role L. W. and Perlman R. L. (1983) Both nicotinic and muscarinic receptors mediate catecholamine secretion by isolated guinea-pig chromaffin cells. *Neuroscience* **10,** 979–985.

Ross T. S. and Majerus P. W. (1986) Isolation of D-*myo*-inositol 1:2-cyclic phosphate 2-inositolphosphohydrolase from human placenta. *J. Biol. Chem.* **261,** 11119–11123.

Saltiel A. R. and Cuatrecasas P. (1986) Insulin stimulates the generation from hepatic plasma membranes of modulators derived from an inositol glycolipid. *Proc. Natl. Acad. Sci. USA* **83,** 5793–5797.

Saltiel A. R., Fox J. A., Sherline P., and Cuatrecasas P. (1986) Insulin-stimulated hydrolysis of a novel glycolipid generates modulators of cAMP phosphodiesterase. *Science* **233,** 967–972.

Schmidt S. Y. (1983*a*) Light- and cytidine-dependent phosphatidylinositol synthesis in photoreceptor cells of the rat. *J. Cell Biol.* **97,** 832–837.

Schmidt S. Y. (1983*b*) Phosphatidylinositol synthesis and phosphorylation are enhanced by light in rat retinas. *J. Biol. Chem.* **258,** 6863–6868.

Schoepp D. D., Knepper S. M., and Rutledge C. O. (1984) Norepinephrine stimulation of phosphoinositide hydrolysis in rat cerebral cortex is associated with the $alpha_1$-adrenoceptor. *J. Neurochem.* **43,** 1758–1761.

Sherman W. R., Stewart M. A., Jurien M. M., and Goodwin S. L. (1968) The measurement of myo-inositol, myoinosose-2 and scyllo-inositol in mammalian tissues. *Biochim. Biophys. Acta* **158,** 197–205.

Siman R. G. and Klein W. L. (1981) Specificity of muscarinic acetylcholine receptor regulation by receptor activity. *J. Neurochem.* **37,** 1099–1108.

Simmonds S. H. and Strange P. G. (1985) Inhibition of inositol phospholipid breakdown by D_2 dopamine receptors in dissociated bovine anterior pituitary cells. *Neurosci. Lett.* **60,** 267–272.

Skene J. H. P. and Willard M. (1981) Changes in axonally transported proteins during axon regeneration in toad retinal ganglion cells. *J. Cell. Biol.* **89,** 86–95.

Sladeczek F., Pin J.-P., Recasens M., Bockaert J., and Weiss S. (1985) Glutamate stimulates inositol phosphate formation in striatal neurons. *Nature* **317,** 717–719.

Smith T. L. and Yamamura H. I. (1985) Carbachol stimulation of phosphatidic acid synthesis: competitive inhibition by pirenzepine in synaptosomes from rat cerebral cortex. *Biochem. Biophys. Res. Commun.* **130,** 282–285.

Snider R. M., Kyes S. A., Seguin E. B., and Agranoff B. W. (1984) Inositol lipid labeling produced by muscarinic, histamine H_1 and thrombin in stimulation in neuroblastoma cells. (Abstr) *Soc. Neurosci. Abstr.* **10,** 276.

Snider R. M., Forray C., Pfenning M., and Richelson E. (1986) Neurotensin stimulates inositol phospholipid metabolism and calcium mobilization in murine neuroblastoma clone N1E-115. *J. Neurochem.* **47,** 1214–1218.

Snider R. M., Fisher S. K., and Agranoff B. W. (1987) Inositide-linked second messengers in the central nervous system, in *Psychopharmacology: The Third Generation of Progress* (Meltzer A. A., Bunney B. B., Coyle C. C., Davis D. D., Kopin E. E., Schuster F. F., Shader G. G., and Simpson H. H., eds), in press. Raven Press, New York.

Spat A., Bradford P. G., McKinney J. S., Rubin R. P., and Putney J. W. Jr. (1986*a*) A saturable receptor for ^{32}P-inositol-1,4,5-trisphosphate in hepatocytes and neutrophils. *Nature* **319,** 514–516.

Spat A., Fabiato A., and Rubin R. P. (1986*b*) Binding of inositol trisphosphate by a liver microsomal fraction. *Biochem. J.* **233,** 929–932.

Spector R. and Lorenzo A. V. (1975) *myo*-Inositol transport in the central nervous system. *Am. J. Physiol.* **228,** 1510–1518.

Stephens L. R. and Logan S. D. (1986) Arginine-vasopressin stimulates inositol phospholipid metabolism in rat hippocampus. *J. Neurochem.* **46,** 649–651.

Stoehr S. J., Smolen J. E., Holz R. W., and Agranoff B. W. (1986) Inositol trisphosphate mobilizes intracellular calcium in permeabilized adrenal chromaffin cells. *J. Neurochem.* **46,** 637–640.

Streb H., Irvine R. F., Berridge M. J., and Schulz I. (1983) Release of Ca^{2+} from a nonmitochondrial intracellular store in pancreatic acinar cells by inositol-1,4,5-trisphosphate. *Nature* **306,** 67–69.

Subramanian N., Whitmore W. L., Seidler F. J., and Slotkin T. A. (1980) Histamine stimulates brain phospholipid turnover through a direct H-1 receptor-mediated mechanism. *Life Sci.* **27,** 1315–1319.

Thomas A. P., Alexander J., and Williamson J. R. (1984) Relationship between inositol polyphosphate production and the increase of cytosolic free Ca^{2+} induced by vasopressin in isolated hepatocytes. *J. Biol. Chem.* **259,** 5574–5584.

Tower D. B. (1987) The American Society for Neurochemistry (ASN): antecedents, founding, and early years. *J. Neurochem.* **48,** 313–326.

Traynor A. E., Schubert D., and Allen W. R. (1982) Alterations of lipid metabolism in response to nerve growth factors. *J. Neurochem.* **39,** 1677–1683.

Van Dongen C., Zwiers H., Oestreicher A. B., and Gispen W. H. (1985) ACTH, phosphoprotein B-50, and polyphosphoinositide metabolism in rat brain membranes, in *Phospholipids in the Nervous System, Vol. 2: Physiological Roles* (Horrocks L. A., Kanfer J. N., and Porcellati G., eds), pp. 49–59. Raven Press, New York.

Van Rooijen L. A. A., Seguin E. B., and Agranoff B. W. (1983) Phosphodiesteratic breakdown of endogenous polyphosphoinositides in nerve ending membranes. *Biochem. Biophys. Res. Commun.* **112,** 919–926.

Van Rooijen L. A. A., Hajra A. K., and Agranoff B. W. (1985) Tetraenoic species are conserved in muscarinically enhanced inositide turnover. *J. Neurochem.* **44,** 540–543.

Vicentini L. M., Ambrosini A., Di Virgilio F., Pozzan T., and Meldolesi J. (1985) Muscarinic receptor-induced phosphoinositide hydrolysis at resting cytosolic Ca^{2+} concentration in PC12 cells. *J. Cell Biol.* **100,** 1330–1333.

Vickroy T., Watson M., Yamamura H. I., and Roeske W. R. (1984) Agonist binding to multiple muscarinic receptors. *Fed. Proc.* **43,** 2785–2790.

Watson M., Vickroy T. W., Roeske W. R., and Yamamura H. I. (1985) Functional and biochemical basis for multiple muscarinic acetylcholine receptors. *Prog. Neuropsychopharmacol. Biol. Psychiatry* **9,** 569–574.

Watson S. P. and Downes C. P. (1983) Substance P induced hydrolysis of inositol phospholipids in guinea-pig ileum and rat hypothalamus. *Eur. J. Pharmacol.* **93,** 245–253.

Whiting P. H., Palmano K. P., and Hawthorne J. N. (1977) Enzymes of *myo*-inositol and inositol lipid metabolism in rats

with streptozotocin-induced diabetes. *Biochem. J.* **179,** 549–553.

Williamson J. R. (1986) Role of inositol lipid breakdown in the generation of intracellular signals. *Hypertension* **8** (Suppl II), 140–156.

Wilson D. B., Bross T. E., Hofmann S. L., and Majerus P. W. (1984) Hydrolysis of polyphosphoinositides by purified sheep seminal vesicle phospholipase C enzymes. *J. Biol. Chem.* **259,** 11718–11724.

Wilson D. B., Connolly T. M., Bross T. E., Majerus P. W., Sherman W. R., Tyler A., Rubin L. J., and Brown J. E. (1985*a*) Isolation and characterization of the inositol cyclic phosphate products of polyphosphoinositide cleavage by phospholipase C. Physiological effects in permeabilized platelets and *Limulus* photoreceptor cells. *J. Biol. Chem.* **260,** 13496–13501.

Wilson D. B., Neufeld E. J., and Majerus P. W. (1985*b*) Phosphoinositide interconversion in thrombin-stimulated human platelets. *J. Biol. Chem.* **260,** 1046–1051.

Woelk H., Kanig K., and Peiler-Ichikawa K. (1974) Incorporation of ^{32}P into the phospholipids of neuronal and glial cell enriched fractions isolated from rabbit cerebral cortex. *J. Neurochem.* **23,** 1057–1063.

Yang J. C., Chang P. C., Fujitaki J. M., Chiu K. C., and Smith R. A. (1986) Covalent linkage of phospholipid to myelin basic protein: identification of phosphatidylinositol bisphosphate as the attached phospholipid. *Biochemistry* **25,** 2677–2681.

Yano K., Higashida H., Inoue R., and Nozawa Y. (1984) Bradykinin-induced rapid breakdown of phosphatidylinositol 4,5-bisphosphate in neuroblastoma × glioma hybrid NG 108-15 cells. *J. Biol. Chem.* **259,** 10201–10207.

Young P. W., Bicknell R. J., and Schofield J. G. (1979) Acetylcholine stimulates growth hormone secretion, phosphatidylinositol labelling, $^{45}Ca^{2+}$ efflux and cyclic GMP accumulation in bovine anterior pituitary cells. *J. Endocrinol.* **80,** 203–213.