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COMMENTARY

RECEPTORS AND PHOSPHOINOSITIDE METABOLISM IN THE CENTRAL NERVOUS SYSTEM

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Our understanding of the characteristics and regulation of chemical transmission in the central nervous system has had to undergo changes not only because of the ever-increasing complexity of the chemical signals themselves but also because of our better appreciation of the mechanisms for recognition and translation of such signals into appropriate intracellular responses. Those receptors that use cyclic AMP as a second messenger are probably the best understood, and there is now substantial information about the role played by GTP binding proteins in transmembrane signalling from cell-surface receptor to adenylate cyclase located on the cytoplasmic face of the membrane. Specific proteins appear to be associated with stimulatory and inhibitory effects on this enzyme and both of these receptor-mediated responses have been observed in brain [1].

On the other hand, until recently, very little was known about the mechanisms of signal transduction and the second messengers used by another large group of receptors—those that appear to hydrolyse membrane inositol phospholipids. Although it has been known for many years that stimulation of inositol phospholipid turnover accompanied activation of several receptors, it is only in the last two or three years that there has been rapid and dramatic progress in our understanding of these particular receptor mechanisms (see Ref. 2 for the background to this new information and its application to CNS function). It is the intention of this review to indicate briefly some of the developments that have occurred in our understanding of inositol phospholipid metabolism over the last couple of years. Primarily, however, we emphasise how this better appreciation of inositol phospholipids has provided new information on the nature and regulation of several neurotransmitter receptors and voltage-sensitive calcium channels in cerebral tissue.

New directions in phosphoinositide metabolism: inositol trisphosphate isomers and inositol tetrakisphosphate

Originally it was thought that extracellular agonists acted at appropriate receptors to stimulate the hydrolysis of phosphatidylinositol (Ptd Ins), but there is now evidence that the primary phospholipase C attack is directed against phosphatidylinositol 4,5-bisphosphate (Ptd Ins 4,5-P_2) generating inositol 1,4,5-trisphosphate (Ins 1,4,5-P_3) and diacylglycerol (DG), two potential second messengers. Ins 1,4,5-P_3 is released into the cytoplasm and has been shown to be able to mobilise bound intracellular calcium in a number of tissues (though not yet in neuronal cells) (see reviews [3, 4]). Ins 1,4,5-P_3 is degraded by sequential dephosphorylation to inositol which can be reincorporated into Ptd Ins (see Fig. 1). However, this cyclic scheme of inositol metabolism has been complicated by the description of a second trisphosphate isomer, inositol 1,3,4-trisphosphate (Ins 1,3,4-P_3), which was first shown to accumulate together with Ins 1,4,5-P_3 in rat parotid glands following muscarinic receptor stimulation [5]. The source and function of Ins 1,3,4-P_3 are unknown. Figure 1 indicates two potential metabolic routes for the production of this molecule: (a) phospholipase C-catalysed hydrolysis of the corresponding phospholipid, phosphatidylinositol 3,4-bisphosphate (Ptd Ins 3,4-P_2), or (b) isomerisation of Ins 1,4,5-P_3. To date there is no evidence to support either pathway.

Very recent experiments in our laboratory have revealed that muscarinic receptor stimulation of rat cerebral cortical or parotid slices is rapidly followed by the accumulation of a compound more polar than Ins P_3 [6]. This has now been identified in brain, at least, as a novel metabolite, inositol 1,3,4,5-tetrakisphosphate (Ins 1,3,4,5-P_4), and this now raises a number of potential alternatives to the currently accepted scheme of inositol metabolism. The rate at which Ins 1,3,4,5-P_4 accumulates may be consistent with a second messenger function for this molecule, and whether such a role would be related to control of intracellular Ca^{2+} will be of considerable future interest. The structure and rapid accumulation of Ins 1,3,4,5-P_4 in cerebral cortex suggest that it could be the precursor of both Ins 1,4,5-P_3 and Ins 1,3,4-P_3. An inositol trisphosphate phosphatase from human erythrocytes specific for the 5-phosphate of Ins 1,4,5-P_3 will also degrade Ins 1,3,4,5-P_4, yielding Ins 1,3,4-P_3 [6]. A similar enzymic activity in brain has not yet been demonstrated nor has the capacity of this or other tissues for specific removal of the 3-phosphate of Ins 1,3,4,5-P_4 to generate Ins 1,4,5-P_3 been reported. Indeed, the stimulated accumulation of Ins

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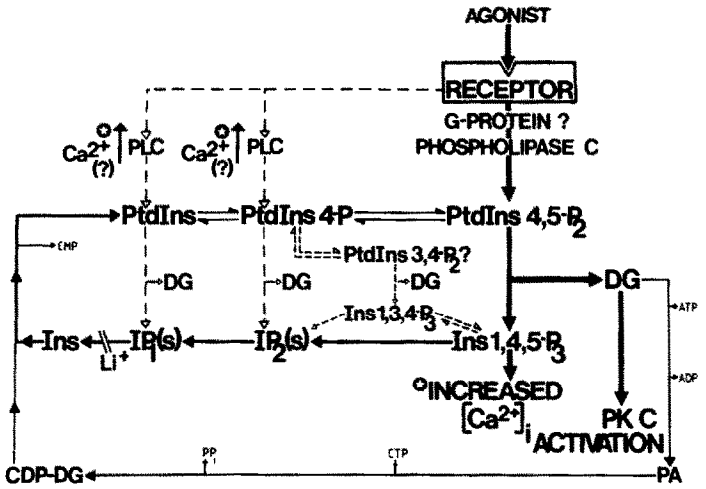

Fig. 1. Model (see Ref. 4) for receptor-mediated hydrolysis of phosphoinositides. The primary event is agonist–receptor activation (via GTP protein(s)?) of phospholipase C (PLC) and hydrolysis of phosphatidylinositol 4,5-bisphosphate (Ptd Ins 4,5-P_2) to yield inositol 1,4,5-trisphosphate (Ins 1,4,5-P_3) and diacylglycerol (DG). The possibility that a subsequent (calcium-dependent) PLC hydrolysis of phosphatidylinositol (Ptd Ins) and phosphatidylinositol-4-phosphate (Ptd Ins 4-P) yielding inositol-1-monophosphate (IP_1) and inositol 1,4-bisphosphate (IP_2) and more DG is also shown. The complicating issue of Ins 1,3,4-P_3 is also shown with potential but unproven sources (dotted lines).

1,3,4,5-P_4 in tissues other than cerebral cortex and, probably, parotid remains to be established. However, heterogeneous distribution of phosphatase activities specific for the 3 and 5 phosphates of Ins 1,3,4,5-P_4 would provide a simple explanation of apparent differences in the proportions of Ins 1,4,5-P_3:Ins 1,3,4-P_3 accumulating in separate tissues.

At present, the source of Ins 1,3,4,5-P_4 remains unclear. One obvious possibility is phospholipase C catalysed hydrolysis of an appropriate phospholipid, phosphatidylinositol 3,4,5-trisphosphate (Ptd Ins 3,4,5-P_3), as indicated in Fig. 2. The recent study demonstrating stimulated Ins 1,3,4,5-P_4 accumulation in rat cerebral cortex [6] was unable to identify a Ptd Ins 3,4,5-P_3 in lipid extracts from small samples (20–30 mg protein) of [^{3}H]-inositol-labelled tissue, but this difficulty may be related to the extremely low concentrations of such a lipid and/or possible inadequacies in extraction procedures. However, preliminary evidence for an inositol lipid containing inositol and phosphate in a ratio of 4:1 was reported by Santiago-Calvo *et al.* [7] following bulk extraction of bovine brain. Should a Ptd Ins 3,4,5-P_3 exist, then it may provide either a complementary or an alternative substrate to Ptd Ins 4,5-P_2 for the primary receptor-mediated hydrolysis of the phosphoinositides (see Fig. 2). However, in the absence of an identified lipid precursor for Ins 1,3,4,5-P_4 other potential sources, such as phosphorylation of inositol trisphosphates or dephosphorylation of higher inositol phosphates, should be examined. One anticipates active work in this area, particularly regarding potential biological effects of Ins 1,3,4,5-P_4.

As indicated in Fig. 1 and 2, it also is premature to dismiss phospholipase C catalysed Ptd Ins and Ptd Ins 4-P hydrolysis which may occur as a secondary event subsequent to an increase in Ca^{2+}. As recently pointed out by Majerus *et al.* [8], since the cellular mass of Ptd Ins is so much greater than that of the polyphosphoinositides, a secondary hydrolysis of this lipid could be the more important source of diacylglycerol. The latter has also been suggested to play a second messenger role by activating a specific protein kinase C, increasing the proportion of this enzyme bound to the plasma membrane [9]. Although protein kinase C is found in high concentrations in neuronal tissue and can catalyse the phosphorylation of several protein substrates, the precise significance of these changes in the central nervous system remains to be established. However, phorbol diesters, which also stimulate protein kinase C, are proving useful tools in this direction. These agents have been shown recently to mimick, in part, the action of muscarinic agonists on hippocampal neurones by blocking a calcium-dependent potassium conductance and the late hyperpolarisation elicited by synaptic stimulation [10]. In another study, phorbol esters have been shown to increase the amplitude of the calcium-dependent action potentials in *Aplysia* bag cells [11]. However, it should be considered that, unlike diacylglycerol, which is produced and confined to the plasma membrane upon cell-surface receptor activation, phorbol esters can permeate to intracellular membranes to bind and activate protein kinase C.

It should be emphasised that much of our understanding of the genereration and action of potential second messengers generated from phosphoinositides has come mainly from the study of peripheral tissues or isolated cells derived from them. In the central nervous system, progress has been slower probably because interpretation of results has been complicated by the heterogeneity of the cell type and the complexity of the potential cell–cell interactions. Nevertheless, our better understanding of cerebral phosphoinositide metabolism must provide new

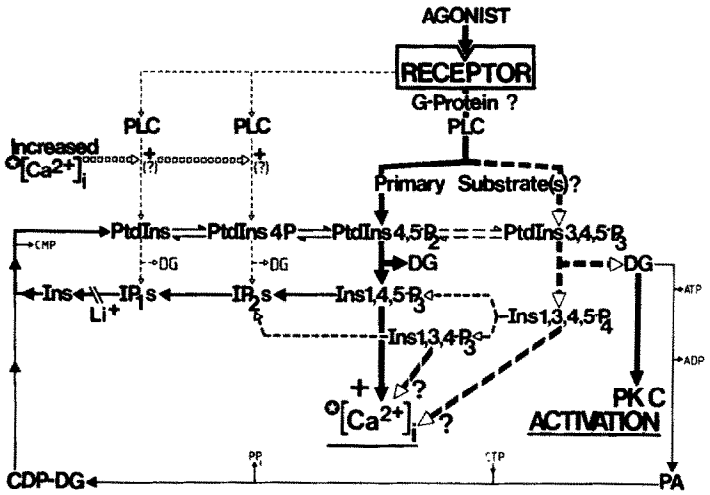

Fig. 2. Alternative metabolic scheme of receptor-mediated phosphoinositide hydrolysis. Broken lines indicate some potential alternatives to the accepted cycle of inositol metabolism suggested by the stimulated accumulation of Ins 1,3,4,5-P_4 and Ins 1,3,4-P_3. The hypothetical Ptd Ins 3,4,5-P_3 is shown as a possible complementary or alternative primary substrate for phospholipase (C) yielding Ins 1,3,4,5-P_4 and DG. Ins 1,3,4,5-P_4 is indicated as the metabolic precursor of Ins 1,3,4-P_3 and as an additional or alternative source of Ins 1,4,5-P_3. *Note that the potential generation of Ins 1,3,4,5-P_4 via kinase or phosphatase steps is not indicated.*

information regarding neurotransmission and possibly other forms of cell to cell chemical signalling in the central nervous system.

Cerebral neurotransmitter receptors and inositol phospholipid hydrolysis

A large number of neurotransmitter receptors appear to be linked to inositol phospholipid metabolism in brain (Table 1), and it is probable that, by extrapolation of our understanding in peripheral tissues, Ins 1,4,5-P_3, diacylglycerol and perhaps Ins 1,3,4-P_3 and Ins 1,3,4,5-P_4 play second messenger roles in cerebral cells. Although one can anticipate difficulties in establishing precise cellular roles for these messengers in the CNS, our understanding of a variety of central receptors has been greatly enhanced by studying these systems.

For some years now, it has been known that several neurotransmitters stimulate the rate of incorporation of radiolabel into brain inositol lipids [23]. These changes, however, were generally small, very rarely characterised and, because they only indirectly reflect stimulated hydrolysis of the lipids, were difficult to interpret. An important development in this area was made by Berridge and colleagues [24] when they exploited the capacity of lithium to block the breakdown of *myo* inositol-1-phosphate and, hence, to greatly amplify the detection of agonist-stimulated phospholipase C-specific inositol phospholipid hydrolysis in tissue slices. In this assay, lipids are labelled with [^{3}H]-inositol, and the soluble inositol phosphates are detected in the presence of lithium. As in other tissues, recent evidence suggests that Ptd Ins 4,5-P_2 (or possibly Ptd Ins 3,4,5-P_3, see Fig. 2) is probably a primary target for phospholipase C attack following agonist–receptor interaction in brain [12, 25], and we have recently detected increased Ins 1,4,5-P_3 and Ins 1,3,4,5-P_4 in rat cerebral cortical slices as early as 5 sec after adding carbachol, a muscarinic cholinergic receptor agonist [6]. However, one should be careful not to dismiss direct or indirect effects on Ptd Ins 4-P and Ptd Ins (Figs. 1 and 2). Care should be taken with the use of lithium in assays, as prolonged exposure of cortical slices to this monovalent ion and receptor agonists can suppress Ins P_3/Ins P_4 accumulation while concomitantly potentiating Ins P_2 and Ins P_1 [25].

A variety of small amine transmitters as well as various neuropeptides can stimulate phosphoinositide hydrolysis in brain. Acetylcholine markedly stimulates inositol phosphate accumulation in several brain regions, and pharmacological analysis of the response indicates mediation by muscarinic receptors [12, 13]. The potencies of agonists in producing this response appear to be closely related to the low affinity component at muscarinic receptor binding sites and suggest that in brain there may be a simple linear relationship between agonist occupation of receptor and inositol phospholipid hydrolysis, i.e. there are no "spare receptors". This suggests that receptors may be very tightly "coupled" to phospholipase C in brain but whether this also applies to other tissues [12] or when cerebral receptors are undergoing adaptive changes [15, 17] (see later) appears less likely. These relationships are of crucial importance in our understanding of the nature and "valency" of receptor–phospholipase C coupling in tissues.

The recent interest in the role and significance of muscarinic receptor subtypes has been extended to central nervous tissue with the view to perhaps developing selective agonists and antagonists for brain disorders such as senile dementia. Muscarinic receptors appear to be linked negatively to adenylate

Table 1. Receptor-coupled inositol phospholipid hydrolysis in neuronal tissue

Transmitter	Receptor	Agonist	Antagonist	Location	Refs.
Acetylcholine	Muscarinic (M_1 and M_2)*	Carbachol	Atropine Pirenzepine*	Most cerebral regions but particularly cortex hippocampus, striatum	[12–14]
Noradrenaline Adrenaline	$Alpha_1$	Methoxamine	Prazosin	Most cerebral regions but particularly cortex, hippocampus, brainstem	[15, 16]
5-Hydroxytryptamine	5-HT_2†	Quipazine	Ketanserin	Cortex and hippocampus	[17]
Histamine	Histamine H_1	2-Methyl-histamine	Mepyramine	Most cerebral regions but particularly cortex and cerebellum	[13, 18]
Substance P	SP-P and SP-E?			Hypothalamus, striatum, substantia nigra	[19, 20]
Vasopressin	Vasopressin V_1	{1-(β-Mercapto-β, β-cyclopentamethylene proprionic acid) 8-argine} vasopressin		Hippocampus, superior cervical ganglion	[2, 21]
Neurotensin				Most cerebral regions but particularly cortex and hypothalamus	[22]
CCK-Octapeptide				Cortex	2

* M_1 antagonist pirenzepine displays differential affinities against carbachol in different cerebral regions.
† Some properties of receptor differ from binding sites labelled by [^{3}H]ketanserin in membranes.

cyclase in some tissues as well as almost invariably being linked to inositol phospholipid metabolism in most tissues [26]. The possibility that the so-called M_1 and M_2 receptors (differentiated by the more potent antagonism of the former by drugs like pirenzepine) might be differentially coupled to these two effector systems seems unlikely as *both* so-called M_1 and M_2 sites appear to be linked to phosphoinositide hydrolysis in brain tissue [14, 27].

The relationship between agonist occupancy of receptors and inositol phospholipid hydrolysis has been directly examined in the case of the $alpha_1$ adrenoceptor in cerebral cortex [15, 16]. As with the muscarinic receptor, there is a linear relationship between agonist occupation of the $alpha_1$ adrenoceptor and inositol phospholipid hydrolysis. Inactivation of the receptor sites with the alkylating antagonist phenoxybenzamine results in a close agreement between the extent of receptors lost and the fall in maximal phosphoinositide response to alpha agonists [15]. This suggests a very close coupling of receptors to inositol phospholipid metabolism and again emphasises that, as a primary response to receptor activation, it is ideally placed as an effector mechanism.

Although 5-hydroxytryptamine (5-HT) has been known to be a transmitter in several areas of the central nervous system, much confusion and speculation have surrounded the classification of 5-HT receptors. Perhaps the most widely discussed classification is that of Peroutka and Snyder [28] in which it is proposed, based on ligand binding to receptor sites in cerebral membranes, that two groups (5-HT_1 and 5-HT_2 receptors) exist. There is still much controversy regarding this classification because there has been little evidence for a biochemical effector system associated with either of these receptor subtypes. Recent studies suggest that 5-HT_2 receptors may be linked to phospholipase C and that agonist occupation of these receptors leads to hydrolysis of inositol phospholipids in brain slices [17]. Although there appears to be good qualitative agreement between the affinities of antagonists at 5-HT_2 binding sites in membranes and in suppressing 5-HT-stimulated inositol phosphate accumulation in cortical slices, significant differences were also observed [17]. This perhaps emphasises the problems of comparing intact cells with a potential difference across their membranes and isolated membranes in which receptor and effector proteins sense zero potential. On the other hand, the complexity of the brain slice may accommodate partially indirect effects of 5-HT (perhaps in the release of other mediators) on phospholipid metabolism.

Histamine is another biogenic amine neurotransmitter candidate in the mammalian central nervous system and it is now well established that its cellular actions are mediated by two receptors, H_1 and H_2, that can be clearly differentiated by pharmacological antagonists. It now seems certain that, whereas H_2 receptors are linked positively to adenylate cyclase, histamine can stimulate the hydrolysis of cerebral inositol phospholipids by interacting with H_1 sites [15, 18].

A large number of small peptides are now known

to occur in central neurones, and it seems certain that at least some of these neuropeptides play a role in chemical signalling in the brain. At present we know little of neuropeptide receptors and their mechanism, but it does seem probable that several peptides can stimulate hydrolysis of inositol phospholipids in brain. These include neurotensin, cholecystokinin, vasopressin and substance P, but it is only with the latter that any detailed study has been made. There appears to be a close relationship between the density of specific [^{3}H]-substance P binding sites and the rate of substance P-induced hydrolysis of inositol phospholipids in several cerebral regions of the rat [19]. In addition, the good agreement in rank order of potency of various tachykinins on inositol lipid hydrolysis with that seen functionally in guinea pig ileum, in which it is believed that contraction is mediated by both SP-P and SP-E receptors, suggests that both putative receptor subtypes are linked to phospholipase C [20].

Although original studies on rat cortical slices failed to observe a significant effect of the amino acid glutamate on phosphoinositide hydrolysis [13], very recent studies on striatal neurones in primary culture [29] reveal a glutamate-evoked increase in inositol phosphates mediated by quisqualate and *N*-methylaspartate receptors. It remains to be established whether there is a direct link of these receptors to phosphoinositide hydrolysis or whether the stimulation is secondary to depolarisation.

Calcium requirements

An important argument in support of the hypothesis that the agonist-induced primary phospholipase C-mediated hydrolysis of inositol phospholipids precedes rather than results from calcium mobilisation is that this response should not be dependent upon an increase in intracellular calcium. There are complex effects of these ions on inositol phospholipid metabolism, and only recently by using a direct assay could this question be addressed in brain [30]. It appears that there is a requirement for a low (μM) concentration of calcium for all receptor-mediated responses, but we were also able to establish that there were different requirements for this ion when various receptors were stimulated. Thus, for example, histamine H_1 responses were very much more dependent on extracellular calcium than muscarinic responses. This could indicate fundamental differences in the receptor responses or perhaps that histamine produces inositol phospholipid hydrolysis indirectly by releasing another agonist by a calcium-dependent mechanism. In this context, there is evidence that the calcium dependency of Ptd Ins P_2 phospholipase C differs dramatically between preparations. For example, soluble rat brain enzyme is only stimulated when calcium approaches millimolar concentrations [31], whereas membrane-bound enzyme from sea urchin eggs begins to be stimulated when calcium is micromolar [32]. Since there is now increasing evidence for a role played by guanine-nucleotide proteins in phosphoinositide metabolism (see Ref. 4), perhaps the association of such proteins with the enzyme alters the ability of calcium to activate the hydrolysis of polyphosphoinositides.

Whatever the mechanism, it is of interest that the calcium ionophore A23187 can induce a small accumulation of inositol phosphates in brain slices [30]. Although this could, in part, be related to an indirect transmitter-releasing effect, it was at least additive to the effects of exogenous receptor agonists, suggesting that the latter do not primarily stimulate phosphoinositide hydrolysis by initiating an increase in intracellular calcium.

Depolarising stimuli and voltage-sensitive calcium channels

Depolarising stimuli have been shown to stimulate inositol phospholipid metabolism in the rat superior cervical ganglia [33]. Since this response can be suppressed, at least in part, by muscarinic antagonists, it is likely to result from the release of acetylcholine which can initiate hydrolysis by a receptor-mediated mechanism. Furthermore, in cerebral cortical slices, potassium or veratrine-induced depolarisation is accompanied by an increased inositol phosphate accumulation, a response that can be enhanced by a cholinesterase inhibitor, and this effect suppressed by atropine [34]. However, it is not certain whether all of the effects of depolarising stimuli can be attributed to transmitter release. Certainly, a substantial response still persists in the presence of a mixture of receptor antagonist drugs. However, at the present time we cannot exclude the possibility that the effects of depolarising stimuli on inositol phospholipid breakdown are indirectly mediated by the presynaptic release of an unidentified transmitter. Recent studies in the superior cervical ganglion [21] suggest release of an unknown proteinase-sensitive (neuropeptide?) substance by high potassium, and subsequent receptor-stimulated inositol phospholipid metabolism. On the other hand, possible direct effects on phosphoinositide metabolism of calcium entering through voltage-sensitive channels may need to be considered. Whatever the site and mechanism involved, recent studies [35] have shown that the increased breakdown of cerebral inositol phospholipids induced by depolarising concentrations of potassium is stereospecifically suppressed by 1,4-dihydropyridine calcium antagonists and enhanced by related channel activators such as BAY-K-8644. These data provide evidence for functional voltage-sensitive calcium channels in brain and add significance to the high density of specific [^{3}H]-dihydropyridine binding sites in this tissue. Until recently, there has been virtually no evidence that these binding sites represent functionally relevant calcium channels [36]. The possibility could now be entertained that depolarisation-evoked inositol phospholipid metabolism is part of an amplification system to raise intracellular calcium in cerebral cells. These effects on phosphoinositide breakdown in cortical slices and those on transmitter release [37] should focus attention on the action of calcium antagonists and activators in the central nervous system.

Cellular localisation

Studies of inositol phospholipid metabolism in the CNS are complicated by the cellular heterogeneity of the tissue brain slice preparation. There is no

reason to suppose that agonist-stimulated phospholipid hydrolysis should be localised in a single cell type in cerebral tissue given the widespread nature of the response in peripheral cell types, and there is now growing evidence that the response may occur in both neuronal and non-neuronal cells. Receptor-mediated responses have been demonstrated in human astrocytoma and neuroblastoma [38, 39] cell lines. Recently, responses to a number of neurotransmitters have been observed in primary astrocyte cultures prepared from embryonic rat brain [40], although the physiological significance of these responses in non-neuronal elements remains unknown.

Support for neuronally located phospholipid hydrolysis has come from studies employing synaptosomes. On the basis of these synaptosomal experiments, it was suggested that muscarinic receptor-mediated inositol lipid metabolism may occur at a mainly presynaptic location possibly associated with neurotransmitter release [23]. However, synaptosomal fractions are often contaminated with post-synaptic membranes or contain vesicles representing resealed dendrites or possibly glial cells. Studies in the periphery suggest an almost exclusive post-synaptic location for the inositol phospholipid response. The latter view is also more consistent with the results from lesion experiments in the CNS. Muscarinic responses in hippocampal synaptosomes are unaltered after destruction of the cholinergic input from the septum [41] but lost after lesions of neuronal cell bodies [42]. Alpha$_1$-adrenoceptor mediated phospholipid hydrolysis in the cerebral cortex is also predominantly post-synaptic as this response becomes supersensitive after destruction of noradrenergic terminals by intraventricular injection of 6-hydroxydopamine [15].

Arachidonic acid metabolism and cyclic GMP

The inositol phospholipids are enriched in arachidonate and it is accepted that these lipids may serve as a source of eicosanoids following appropriate stimulation [43]. Since certain arachidonate metabolites are potent stimulators of inositol phospholipid hydrolysis in some cell types, the possibility could therefore be entertained that arachidonate metabolites could be formed in cerebral tissue and mediate a feed forward or amplification mechanism to enhance the signal carried by a calcium-mobilising neurotransmitter. The formation of cyclooxygenase or lipoxygenase products of arachidonate would also provide a means whereby information could be transmitted from a stimulated cell to nearby cells having the appropriate eicosanoid receptors.

These schemes, at present, are speculative for cerebral tissue and, indeed, there is some evidence that is inconsistent with their existence. Thus, it appears that, in guinea pig cerebral cortical synaptosomes stimulated by the cholinergic agonist carbachol, diacylglycerol is conserved by re-synthesis via phosphatidate to inositol phospholipid, i.e. there is, at best, limited release of arachidonate under these conditions [44].

Cyclic GMP frequently accumulates as a result of stimulating calcium-mobilising receptors, but its function and the precise way in which the signal for its formation is generated are largely unknown. Recently, however, Snider and colleagues [45] suggested that, in murine neuroblastoma clone NIE-115 cells, cyclic GMP formation stimulated by cholinergic or histaminergic agonists is mediated by a lipoxygenase metabolite or arachidonate, apparently released by phospholipase A_2 attack on an unidentified phospholipid. However, since cyclic GMP formation is not a universal consequence of the stimulation of cerebral calcium-mobilising receptors [1], this does not seem to be a general mechanism in the central nervous system.

Adaptive responses

The close coupling of inositol phospholipid metabolism to several neurotransmitter receptors not only provides a primary biochemical response for activation of these receptors but should be crucial in any evaluation of adaptive changes occurring, for example, during chronic drug treatment, ageing or development. At the present time, there is little information available concerning changes which occur during development and ageing but there is some evidence that receptor-mediated inositol phospholipid response can exhibit plasticity. Noradrenergic denervation has been known to only produce a small elevation of alpha$_1$ adrenoceptors in cerebral membranes. However, recent studies have revealed markedly increased alpha$_1$-mediated inositol phosphate accumulation in cortex or hippocampus after electrolytic or 6-hydroxydopamine lesions. In the hippocampus, an elevation of the maximal response to noradrenaline was observed [46], whereas in cortex an agonist specific shift of the dose–response curve was observed [15]. In the latter study, there is evidence that the linear relationship between agonist occupation and phospholipid response may be altered by denervation. The mechanism of such a change in "receptor–effector" coupling is not understood, though the possible involvement of GTP-binding proteins in inositol lipid responses [4] may imply some similarities with changes in receptor–adenylate cyclase coupling.

Another example of adaptive response in cerebral inositol phospholipid metabolism follows the chronic treatment with antidepressant drugs. It has been known for some time that chronic but not acute antidepressant treatment leads to a reduction in 5-HT$_2$ receptor binding sites in brain [47]. It has been shown recently that this is accompanied by a reduction in the maximal responses of 5-HT-stimulated inositol phosphate accumulation [17]. However, the magnitude of this reduced response is greater than the "down-regulation" of receptors assayed in the same experiment. This suggests that either the radioligand labels an additional population of sites not linked to inositol phosphilipid breakdown or there is decreased receptor–effector coupling. Whatever the mechanism, this provides the first biochemical evidence that 5-HT responsiveness is selectively impaired after chronic antidepressant treatment.

The ability of lithium to inhibit inositol-1-phosphatase is well established and has greatly assisted receptor characterisation by amplifying agonist responses in brain. It has also been argued that

prolonged exposure to this monovalent ion may limit the availability of inositol rather selectively in brain since blood-borne inositol does not gain easy access to cerebral tissues [24]. This could alter the sensitivity of those cerebral receptor systems that use inositol lipids. Such a hypothesis is now clearly testable.

Concluding remarks

The last few years have seen a dramatic development in our understanding of a large group of receptors that appear to be closely linked to inositol phospholipid metabolism. This has not only provided new information about the characteristics and regulation of these receptors but has probably revealed two important second messengers, Ins 1,4,5-P_3 and diacylglycerol. Furthermore, very recent studies have revealed that the production of another potential second messenger, Ins 1,3,4,5-P_4, may be a very early consequence of receptor stimulation in brain. The full impact of this has yet to be realised in nervous tissue, and one can anticipate significant efforts in the next few years to establish receptor-linked calcium mobilisation and protein kinase C phosphorylation.

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Note added in press. Very recent studies (R. F. Irvine, A. J. Letcher, J. P. Heslop and M. J. Berridge, Nature **320**, 631 (1986) have established that a specific soluble 3-kinase from brain can phosphorylate Ins 1,4,5, P_3 to Ins 1,3,4,5-P_4. In the absence of evidence for a Ptd 3,4,5-P_3 lipid (see above), a kinase step is the more likely source of Ins 1,3,4,5-P_4.

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