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# Second Messengers in Neuronal Signaling[a]

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**ABSTRACT: The past decade has witnessed an enormous increase in our knowledge of the variety and complexity of intracellular signaling events that follow receptor binding on the cell surface. This overview emphasizes the phosphoinositidase C–mediated dual messenger pathway in brain and in brain-derived cells, with special reference to possible significance for research on the dementias.**

## INTRODUCTION

The metabolic rate of the human brain is high, accounting for some 20–25% of the body's energy consumption at rest, even though it represents only about 2% of total body mass. This remarkable energy requirement is a reflection of the excitable nature of nervous tissue. That is, it is metabolically costly to maintain the ability to rapidly conduct impluses within neurons and to mediate chemically transmitted signals between them. While neurotransmitter synthesis, storage and release all require ATP, some of the brain's metabolic expense can be attributed to the sequence of intracellular signaling events that occur between neurotransmitter binding to extracellular receptors and the ultimate physiological expression of the message, *e.g.*, initiation or block of secretion, of muscular contraction, of nerve impulse generation or of genomic processing. As these intramembrane and cytosolic chemical messengers are identified, convergence on intracellular $Ca^{2+}$ regulation and protein kinases and phosphatases becomes increasingly apparent. A central role in intracellular signaling is evident for the dual pathway initiated by the phosphodiesteratic cleavage of phosphatidylinositol 4,5-bisphosphate ($PIP_2$) into two moieties, each of which then serves as an intracellular messenger: inositol 1,4,5-trisphosphate (1,4,5-$IP_3$) and 1,2-diacyl-*sn*-glycerol (DAG). This receptor-stimulated, G-protein-mediated cleavage is catalyzed by a phosphoinositide-specific phospholipase C (phosphoinositidase C; PLC). One turn of the "phosphoinositide cycle" whereby $PIP_2$ is regenerated consumes at least

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TABLE 1.[a] Neural Receptors That Activate Phosphoinositidase C-mediated Cleavage of $PIP_2$

**Muscarinic cholinergic ($M_1$ and $M_3$)**
Brain slices, retina, cochlea, primary neuronal cultures, neuroblastoma cells (SK-N-SH and SH-SY-5Y), astrocytoma cells (1321N1), glioma cells ($C_6$), neuroepithelioma cells (SK-N-MC), pheochromocytoma cells (PC-12)

**Adrenergic ($\alpha_{1A}$ and $\alpha_{1B}$)**
Brain slices, primary neuronal and primary glial cultures, SK-N-MC cells, $C_6$ cells

**Histaminergic ($H_1$)**
Primary glial cultures, neuroblastoma cells (NIE-115), $C_6$ cells

**Serotonergic ($5HT_2$ and $5HT_{1C}$)**
Brain (in vivo), brain slices, primary neuronal cultures, pituitary tumor cells (P11), $C_6$ cells

**Glutamatergic (metabotropic)**
Brain slices, retina, synaptoneurosomes, primary glial cultures

**Endothelin**
Brain slices, primary neuronal and primary glial cultures, glial cells ($C_6$ and $A_{172}$), SK-N-MC cells

Above, brain receptor ligands that mediate a robust stimulation of phosphoinositide turnover in neural tissues, generally demonstated by means of preincubation of the listed preparations with $^3$H-inositol, and measurement of accumulated labeled inositol phosphates following further incubation in the presence of the ligand and $Li^+$. Somewhat reduced stimulation is evoked in neural tissues by a number of additional ligands, for which principal evidence of a phosphoinositide-linkage comes from studies in non-neural tissues. They include: purinergic ($P_2$), thromboxane ($A_2$), nerve growth factor, prostaglandin ($E_2$), bradykinin ($B_2$), vasopressin ($V_1$), cholecystokinin, neuropeptide Y, neurotensin, gastrin-releasing peptide, bombesin, substance P, oxytocin, eledoisin, neurokinin, vasointestinal peptide, angiotensin, gonadotropin-releasing hormone, platelet activating factor, and thyrotropin releasing hormone.

[a] From ref. 1, in which specific literature references for each example may be found.

5 high energy phosphate equivalents. Given the large and growing list of ligands shown to bind to receptors that utilize the ligand-stimulated PLC pathway (TABLE 1), we may consider that operation of the pathway and its associated cellular functions likely accounts for a significant fraction of total brain metabolism. This complex dual pathway is of great interest, not only because its elucidation will lead to a better understanding of normal brain function, but also for the possibility that such understanding will then lead to the discovery of correlates of the dementias.

## BIOCHEMICAL ASPECTS OF THE PHOSPHOINOSITIDE CYCLE

The three phosphoinositides, phosphatidylinositol (PI), phosphatidylinositol 4-phosphate (PIP) and $PIP_2$ are a family of quantitatively minor components of eukaryotic membrane lipids, of which PI, the metabolic precursor of PIP and $PIP_2$, is by far the most prevalent. The phosphoinositides are the most acidic of the phospholipids and are synthesized by the cytidine

diphosphodiacylglycerol (CDP-DAG) pathway, as are the phosphatidylglycerol series of mitochondrial lipids. The cyclic sequence of reactions whereby phosphoinositide is broken down and regenerated is catalyzed by six enzymes, two of which are unique to the stimulated turnover, while the remaining four also mediate *de novo* synthesis. The latter are CDP-DAG synthase, PI synthase, PI kinase, and PI 4-kinase. One of the two turnover-specific steps is catalyzed by PLC, initiating the cycle of breakdown and resynthesis. DAG released upon $PIP_2$ cleavage is phosphorylated via DAG kinase to phosphatidate (PA), which in turn serves as substrate for CDP-DAG synthesis, thence to PI, PIP and $PIP_2$, completing the cycle.

Studies in brain and in other tissues indicate the presence of many isoforms of PLC, based upon purification and characterization on the one hand, and upon established amino acid sequence, on the other. The differences in function of various PLC families thus far identified are not yet certain, but it is clear that some isoforms are regulated by G-proteins (and can in turn regulate G-proteins by serving as GTPase-activating proteins), while others are regulated by tyrosine kinases.[2,3]

DAG kinase may regulate the cycle in a number of ways. By phosphorylating DAG, it can serve as an "off" signal that deactivates protein kinase C (PKC). The product, PA, has been reported to stimulate PIP 4-kinase activity.[4] A membrane-bound DAG kinase activity has been described that is highly selective for the 1-stearoyl, 2-arachidonoyl species of DAG that characterizes all six of the phosphoinositide cycle lipids, and may play a crucial role in isolating this unique DAG species from other cellular DAG pools, such as those seen in phosphatidylcholine or phosphatidylethanolamine synthesis and breakdown.[5] We have found that the essential fatty acid arachidonate (20:4ω6*cis*) is deficient in standard cell culture media[6] and is largely replaced by "Mead" acid (20:3ω9*cis*). Cells grown on the deficient media appear normal and show no deficit in phosphoinositide signaling. Since the fatty acid composition of the DAGs has generally been found to have relatively little effect on the DAG's activation of PKC, the effects of arachidonate supplementation on such depleted cells may be useful for investigation of the physiological significance of reported interactions of arachidonate metabolites with the PLC-mediated pathway, as well as with cyclic AMP and other second messenger systems.[7] Similarly, dietary restriction of arachidonate should decrease prostanoid production, but as a result of these cell culture studies, is predicted to have little effect on phosphoinositide-mediated signaling. This observation could be of value in dietary approaches to lowering prostanoid formation *in vivo*.

## OTHER BRAIN INOSITOL LIPIDS

A variety of proteins are bound to all membranes via a PI anchor, in which the 6-position of inositol is glycosylated. The carbohydrate substituent,

containing glucosamine and mannose, is linked to the protein via ethanolamine. While phospholipse C does not act on these lipids, PI anchor-degrading phospholipases have been found in brain.[1]

A family of polyphosphoinositides has been described, primarily in non-neural tissues, in which PI is phosphorylated on the 3 rather than on the 4 position of inositol to form PI3P, PI(3,4)$P_2$ and PI(3,4,5)$P_3$. Roles for these trace lipids in neural function have not yet been indicated, but can be anticipated, since brain has proven to be an excellent source of PI 3-kinase.

## RELEVANCE TO THE DEMENTIAS

A number of investigations, using animal models, postmortem human brain or cultured human cells have been employed to examine the possible involvement of the stimulated PLC pathway in Alzheimer's disease. Since a quantitatively major fraction of the brain's PLC-linked receptors are thought to be muscarinic cholinergic, and further, since defects in acetylcholine metabolism have long been implicated in Alzheimer's disease, there have been numerous investigations on a possible link between the disease and the stimulated PLC pathway. Recent examples include studies in cells tranfected with human brain muscarinic receptors, in which cholinergic stimulation alters amyloid precursor protein metabolism, presumably via PKC activation.[8] From studies in rats and in $GTP_\gamma S$-supplemented postmortem human brain membranes, Crews *et al.*[9] conclude that amyloid β-protein may selectively block cholinergic and serotonergic PLC-mediated signal transduction. Comparing cultured fibroblasts from Alzheimer's disease patients with those of age-matched control patients, Gibson's laboratory has reported evidence for differences in available intracellular $Ca^{2+}$ pools in the two cell populations, based on bradykinin receptor-stimulated, PLC-mediated release of 1,4,5-$IP_3$, together with the effects of ionophores and of other agents.[10]

Whether these studies will lead to a common causal mechanism, or to biological markers of the disease or of its progression, they may well constitute an important step forward in our understanding of the underlying pathologic process, and put us on firmer ground in proposing possible therapeutic interventions.

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