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Muscarinic Receptor Activation of Phosphatidylcholine Hydrolysis

RELATIONSHIP TO PHOSPHOINOSITIDE HYDROLYSIS AND DIACYLGLYCEROL METABOLISM*

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We examined the relationship between phosphatidylcholine (PC) hydrolysis, phosphoinositide hydrolysis, and diacylglycerol (DAG) formation in response to muscarinic acetylcholine receptor (mAChR) stimulation in 1321N1 astrocytoma cells. Carbachol increases the release of [^{3}H]choline and [^{3}H]phosphorylcholine ([^{3}H]Pchol) from cells containing [^{3}H]choline-labeled PC. The production of Pchol is rapid and transient, while choline production continues for at least 30 min. mAChR-stimulated release of Pchol is reduced in cells that have been depleted of intracellular Ca^{2+} stores by ionomycin pretreatment, whereas choline release is unaffected by this pretreatment. Phorbol 12-myristate 13-acetate (PMA) increases the release of choline, but not Pchol, from 1321N1 cells, and down-regulation of protein kinase C blocks the ability of carbachol to stimulate choline production. Taken together, these results suggest that Ca^{2+} mobilization is involved in mAChR-mediated hydrolysis of PC by a phospholipase C, whereas protein kinase C activation is required for mAChR-stimulated hydrolysis of PC by a phospholipase D.

Both carbachol and PMA rapidly increase the formation of [^{3}H]phosphatidic acid ([^{3}H]PA) in cells containing [^{3}H]myristate-labeled PC. [^{3}H]Diacylglycerol ([^{3}H]DAG) levels increase more slowly, suggesting that the predominant pathway for PC hydrolysis is via phospholipase D. When cells are labeled with [^{3}H]myristate and [^{14}C]arachidonate such that there is a much greater $^3H/^{14}C$ ratio in PC compared with the phosphoinositides, the $^3H/^{14}C$ ratio in DAG and PA increases with PMA treatment but decreases in response to carbachol. By analyzing the increase in ^{3}H *versus* ^{14}C in DAG, we estimate that the DAG that is formed in response to PMA arises largely from PC. Muscarinic receptor activation also causes formation of DAG from PC, but approximately 20% of carbachol-stimulated DAG appears to arise from hydrolysis of the phosphoinositides.

The ability of a variety of drugs and hormones to stimulate breakdown of the inositol phospholipids is well documented (1), but recently much attention has been given to the hydrolysis of phosphatidylcholine (2, 3). Agonist-stimulated hydrolysis of phosphatidylcholine (PC) could lead to the production of diacylglycerol (DAG), thought to be an important signal for activation of protein kinase C (4), or perhaps to the generation of other potential second messengers such as phosphatidic acid (5, 6).

Phosphatidylcholine constitutes the largest fraction of total plasma membrane phospholipid and was previously thought to be metabolically stable relative to the phosphoinositides (7, 8). However, recent studies of the regulation of PC metabolism have revealed that phorbol esters and certain hormones are able to influence the synthesis and breakdown of choline phospholipids. Besterman *et al.* (9), Irving and Exton (10), and others (11–13) have reported that stimulation of hormone receptors or protein kinase C leads to hydrolysis of PC by a phospholipase C, producing phosphorylcholine (Pchol) and DAG. In contrast, other work has shown that PC can be hydrolyzed by a phospholipase D to form choline and phosphatidic acid (PA) (14–18). The latter work includes studies by Löffelholz and coworkers (19–21) that demonstrate that muscarinic receptor activation leads to the release of choline from perfused heart and brain preparations. The second messenger DAG could also be produced following phospholipase D activation, because it has been demonstrated that DAG can arise from PA by the action of a PA phosphohydrolase (14, 18, 22).

Although hydrolysis of PC in response to stimulation of hormone receptors is known to occur, the mechanism underlying hormone-mediated PC breakdown is not clear. Some studies (9) have suggested that second messengers, such as DAG or Ca^{2+}, are involved, whereas other results (10, 14) indicate that a guanine nucleotide regulatory protein (G-protein) mediates the response. We used 1321N1 astrocytoma cells to determine whether the rapid stimulation of phosphoinositide breakdown (and generation of second messengers) might be involved in the subsequent hydrolysis of PC. Muscarinic receptor activation of 1321N1 cells leads to production of inositol 1,4,5-trisphosphate and mobilization of intracellular Ca^{2+} (23–25) as well as an increase in DAG and redistribution of protein kinase C (26); any of these second messengers could be responsible for stimulating PC hydrolysis.

It was also of interest to determine how DAG is increased in response to muscarinic receptor activation in a system where a single stimulus results in hydrolysis of both PC and the phosphoinositides. By using fatty acids to differentially label phospholipids (11, 27), we obtained an estimate of the contribution of these two phospholipids to muscarinic receptor-stimulated formation of DAG in 1321N1 cells.

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[1] The abbreviations used are: PC, phosphatidylcholine; DAG, 1,2-*sn*-diacylglycerol; Pchol, phosphorylcholine; PA, phosphatidic acid; G-protein, guanine nucleotide regulatory protein; Hepes, 4-(2-hydroxyethyl)-1-piperazineethanesulfonic acid; TLC, thin-layer chromatography; PI, phosphatidylinositol; PMA, phorbol 12-myristate 13-acetate.