Ethanolamine as well as choline is released to the external medium on phorbol ester and foetal calf serum stimulation of glial cells in primary culture.

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Stimulation of protein kinase C (PKC) in a variety of cells causes enhanced catabolism of phosphatidylethanolamine [1,2]. Diacylglycerol (DAG) released from phosphatidylcholine turnover functions as a second messenger in plasma membranes activating PKC [3]. In the CNS at least, choline released extracellularly as a result of enhanced phosphatidylcholine (PC) turnover may provide free choline for acetylcholine synthesis [4]. Basal turnover of phosphatidylcholine in primary glial cells may occur through lysophosphatidylcholine suggesting a phospholipase D-mediated mechanism [5]. However, phorbol ester treatment of glial cultures activates a phospholipase D [6]. Phosphatidylethanolamine (PE) in several cell lines also shows increased catabolism on stimulation with phorbol esters [7]. Here we show that phorbol ester and foetal calf serum stimulate the turnover of PE and PC in glial cells in primary culture. As a result of this enhanced catabolism ethanolamine, as well as choline, is released to the external medium.

Gliai cell cultures were prepared from cerebrum of 1-2 day rat pups [8] and cells grown in 6-well plates (Nunc). Preliminary experiments (data not shown) revealed that incorporation of 3H-choline or 3H-ethanolamine into lipids reached equilibrium after about 12 hours. For routine labelling of lipids, cultures at seven days in DMEM containing 0.1% FCS and 2% glutamine were incubated with 5Ci per well of either 3H-choline or 3H-ethanolamine (Amersham plc) for 24 hours at 37°C. After incubation cultures were rinsed with DMEM and fresh DMEM (2%) added. After agonist stimulation ethanolamine and choline metabolites in the extracellular and intracellular phases were recovered by solvent extraction and characterised using a modified cation exchange column method [9].

Labelling of glial cell cultures with either 3H-choline or 3H-ethanolamine led to greater than 90% of the total recoverable label being incorporated into PC or PE respectively. When 3H-choline labelled cells were treated with TPA (25nM) or 10% FCS for 30 min we found no significant increase over controls for glycerophosphocholine (GPC) or phosphatidylcholine (PCho) or choline (Chol) in the intracellular phase. Choline was released to the extracellular medium, however, (Fig.1) at levels some 2-3 fold above control unstimulated cells. There was no significant increase of GPC or PCho in the extracellular medium (Fig.1). Stimulation of choline release to the extracellular medium was partially inhibited (3%) by prior treatment of cells with staurosporin (5µM), a PKC inhibitor. TPA and 10% FCS also caused a 2-3 fold increase over controls in the release of ethanolamine to the extracellular medium (Fig.2) from 3H-ethanolamine labelled glial cells. TLC analysis showed that virtually no label was present in choline in the extracellular medium in such experiments.

Our results for phorbol ester and FCS stimulated choline turnover in glial cells with a choline release to the external medium, are in agreement with many reports [1,2]. The stimulated release of choline and ethanolamine with no detection of other derivatises, however, suggests that a phospholipase D may produce the observed effects. Phospholipase D activity has been identified in astrocytes [10] and in a variety of cell lines [11]. Here we have noted phosphatidylethanolamine formation from our glial cultures are stimulated with phorbol esters in the presence of ethanol (Rumsby & McNulty, unpublished), indicating phospholipase D activity.

The present findings confirm that stimulation of glial cells in primary culture results in increased catabolism of choline-containing phospholipids probably through a PKC-mediated activation of phospholipase D. We have found that at the same time TPA and FCS increase the catabolism of ethanolamine phosphoglycerides. Thus both choline and ethanolamine headgroups are released to the external medium and perhaps both PC and PE species contribute DAG to maintain a sustained PKC response during signal transduction processes. Activation of a phospholipase D would initially lead to the formation of phosphatidate from PC and PE, the DAG being formed by the subsequent action of phosphatidate phosphohydrolase. Choline released to the extracellular medium from agonist stimulated PC turnover may, in the CNS contribute to acetylcholine formation [4] or may be transported back into the cells for PC biosynthesis [5]. No obvious role exists for the ethanolamine headgroup also released to the extracellular medium on increased PE catabolism.

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**Fig.1.** Release of phosphatidylethanolamine metabolites to the extracellular medium on TPA stimulation of C6 cells [□] and primary glial cultures[■]. Details in text.

**Fig.2.** Release of ethanolamine to the extracellular medium after TPA and FCS stimulation of oligodendrocytes [OL], Type I astrocytes [TA] and primary glial cell cultures [PG]. See text for details.