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# A possible metabolic explanation for drug-induced phospholipidosis

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A large variety of amphiphilic cationic drugs which are in widespread clinical use produce a generalized phospholipidosis when administered for prolonged periods. These drugs, which vary widely in their potency in causing phospholipidosis, include chlorphentermine, fenfluramine, triparanol, *trans*-1,4-bis (2-chlorobenzylaminoethyl)-cyclohexane (A79944), azacosterol, 5,5'-diethylaminoethyoxyhexestrol, 1-chloroamitriptyline, iprindole, 2-*N*-methyl-piperazino-methyl-1,3-diazofluoroanthen 1-oxide (AC 3579), chlorcyclizine, chloroquine, chlorpromazine, thioridazine, imipramine, clomipramine, haloperidol and boxidine (Yamamoto, Adachi & others, 1971a, b; Shikata, Kanetaka & others, 1972; Hruban, Slesers & Ashenbrenner, 1973; Lüllman, Lüllman-Rauch & Wasserman, 1973; Wherrett & Huterer, 1973; De La Iglesia, Feuer & others, 1974; Kasama, Yoshida & others, 1974; Lüllman-Rauch, 1974a, b, 1975; Schmien, Seiler & Wasserman, 1974). Although these drugs have a variety of therapeutic effects they are physicochemically rather similar, in that they all possess both a hydrophobic region and a primary or substituted amine group which can bear a net positive charge. This amphiphilic nature enables the drugs to interact with phospholipids, particularly the anionic phospholipids which are quantitatively minor constituents of membranes (e.g. phosphatidate, phosphatidylinositol, phosphatidylserine, cardiolipin). Their capacity both to cause phospholipidosis and to interact with lipids depends largely on the size and hydrophobicity of the apolar portions of the molecule.

We recently suggested that interactions of these drugs with anionic phospholipids might cause some of the therapeutic actions or side-effects of these drugs (Brindley, Allan & Michell, 1975).

The lipids which accumulate in the lysosomes of a variety of tissues during drug treatment are mainly glycerophospholipids. There are clear indications that compared with normal tissue, these tend to include increased proportions of anionic phospholipids (phosphatidate, phosphatidylinositol, phosphatidylglycerol and lysobisphosphatidate) and decreased proportions of triglyceride and of the major zwitterionic glycerophospholipids (phosphatidylcholine and phosphatidylethanolamine) (Yamamoto & others, 1971a, b; Wherrett & Huterer, 1973; Kasama, Yoshida & others, 1974; Allan & Michell, 1975; Karabelnik & Zbinden, 1975). This pattern of lipid accumulation is in marked contrast to that seen in the classical hereditary lipidoses in which sphingolipids, particularly glycosphingolipids, are the main lipids which accumulate in lysosomes.

One proposed explanation of this effect is that phospholipids are normally degraded in lysosomes by phospholipases, but that when amphiphilic cationic drugs form complexes with the phospholipids this prevents phospholipase attack and the phospholipid-drug complexes therefore accumulate and engorge the lysosomes (Lüllman & others, 1973; Lüllman-Rauch, 1974a). Although this mechanism would explain many of the experimental findings it does not provide a complete explanation. For example, it does not explain

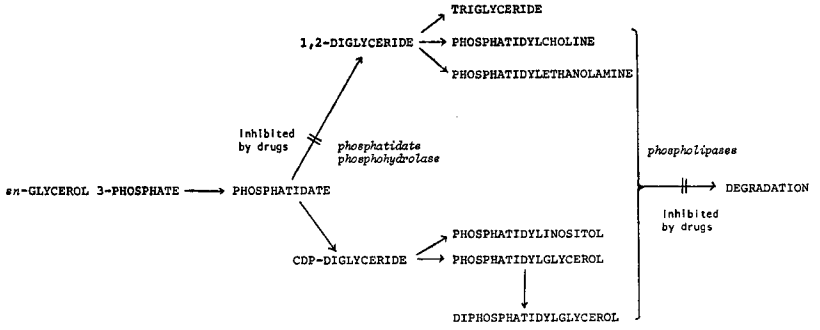


Fig. 1. Effect of amphiphilic cationic drugs on the metabolism of glycerolipids.

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the particular accumulation of anionic lipids and it does not take into account the fact that amphiphilic cations interact much more strongly with anionic lipids than with zwitterionic lipids.

Recent work has shown that a variety of cationic amphiphilic drugs do indeed inhibit at least one important reaction catalysed by a phospholipase. The affected reaction is the conversion of phosphatidate to 1,2-diglyceride by phosphatidate phosphohydrolase (EC 3.1.3.4), an enzyme whose substrate is an anionic phospholipid. The best understood function of this enzyme is in lipid biosynthesis, where it lies at the main branchpoint in the pathways leading to the various glycerolipids (Fig. 1). Thus this enzyme is concerned with the synthesis of glycerolipids rather than with their degradation. Its inhibition has been demonstrated in two ways. First, assays of phosphatidate phosphohydrolase activity in a cell-free system from liver have shown clear inhibition by cationic amphiphilic drugs, but not by other drugs which modify lipid metabolism (Brindley & Bowley, 1975). Second, the cationic amphiphilic drugs cause marked changes in the relative rates of incorporation of metabolic precursors into glycerolipids in intact cells and tissues. These changes involve diversion of glycerolipid synthesis away from triglyceride, phosphatidylcholine and phosphatidylethanolamine, and into phosphatidate, CDP-diglyceride, diphosphatidylglycerol (cardiolipin) and phosphatidylinositol; they are most simply explained as the result of inhibition of phosphatidate phosphohydrolase in intact cells (Fig. 1) (Eichberg & Hauser, 1974; Allan & Michell, 1975; Brindley & Bowley, 1975). The changes in lipid composition which occur during exposure of cells to these drugs either briefly (Allan & Michell, 1975) or for longer periods (Wherrett & Huterer, 1973), particularly the elevated concentration of phosphatidate, phosphatidylinositol and phosphatidylglycerol (Wherrett & Huterer, 1974; Allan & Michell, 1975; Karabelnik & Zbinden, 1975) suggest that in intact cells this inhibition of phosphatidate phosphohydrolase can be sufficient to cause appreciable changes in cellular lipid composition. It therefore seems probable that a major factor in the development of the unusual lipid pattern characteristic of drug-induced phospholipidosis is the inhibition *in vivo* of phosphatidate phosphohydrolase by therapeutic concentrations of drugs. The relationship between this inhibition and the accumulation of lysobisphosphatidate (Yamamoto & others, 1971a, b; Kasama & others, 1974; Wherrett & Huterer, 1973) is not clear since the route of synthesis of this rather unusual anionic phospholipid is not known (Brotherus, Renkonen & others, 1974).

Thus it seems that a biochemical explanation of the pathogenesis of drug-induced phospholipidosis should probably take account of at least two main factors. Inhibition of phosphatidate phosphohydrolase first upsets the balance of glycerolipid biosynthesis in the affected cells, leading to a situation in which the cells accumulate an abnormal pattern of lipids (Fig. 1). Subsequently, the degradation of these phospholipids by lysosomal phospholipases may be impaired, so that large quantities of lipids accumulate in and engorge the lysosomes. The lysosomal phospholipases presumably show this decreased activity because they are not able to handle the unusual substrate with which they are presented. This may be either because the membrane material taken into the lysosomes has large quantities of the administered drug associated with it (Lüllman & others, 1973), or because it contains abnormally high proportions of acidic phospholipids; in either case the physicochemical state (e.g. surface charge) and composition of the lipid phase presented to the enzymes would be markedly abnormal.

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