Letters

Probes of the distribution of phospholipids in endoplasmic reticulum membranes

In a recent *TIBS* article[1] Dr Van Meer criticizes the use of phospholipase C as a probe for the distribution of phospholipids across the endoplasmic reticulum (ER) membrane. However, there is a considerable amount of experimental evidence using this probe which is difficult to dismiss completely as artefactual. Phospholipase C hydrolyses approximately 50% of the phospholipid of ER vesicles. The vesicles remain closed, as indicated by retention of mannose-6-phosphatase latency, retention of soluble protein contents, and appearance in the electron microscope: the bilayer structure is retained in both sectioned material and freeze–fracture preparations[2]. It is true that more than two decades ago it was demonstrated that phospholipase C treatment of microsomes produces relatively large diglyceride droplets either associated or separated from the membranes. However, the microsomes were derived from muscle and not liver and different conditions were used[3]. Phospholipase C treatment of liver microsomes causes several morphological changes. Approximately 10% of the vesicles exhibit amorphous droplets associated with the bilayer. However, the most striking changes are association of several vesicles so that they share a common outer leaflet and formation of small vesicles apparently by pinching from the microsomes. These changes are consistent with stabilization of the membrane bilayer in which the area of the outer leaflet is reduced compared with that of the inner leaflet. Thus the endoplasmic reticulum membrane appears to act as a bilayer couple.

The most serious artefact that can occur is if the probe used causes rearrangement of the membrane bilayer. This can only be satisfactorily excluded by preparing inside-out vesicles and demonstrating that the distribution of their phospholipids is the reverse of that of outside-out vesicles determined under the same conditions. Such experiments have been performed with red blood cell membranes using phospholipase C as a probe and in myoblasts or fibroblasts using trinitrobenzene sulphonate (TNBS)[4,5]. In these membranes neither phospholipase C nor TNBS causes a rearrangement of the phospholipid and the bilayer is stabilized in some way after hydrolysis of the outer leaflet head groups. Unfortunately it has not been possible to prepare inside-out vesicles of ER. However, there is evidence suggesting that phospholipids are not translocated by phospholipase C treatment and therefore that these membranes may also be stabilized. Phospholipase C hydrolyses approximately 30% of the phosphatidylethanolamine of closed ER membranes and TNBS reacts with a similar proportion of this phospholipid. If, however, the microsomal vesicles are pretreated with phospholipase C the amount of phosphatidylethanolamine available for reaction with TNBS is reduced in proportion to the extent of hydrolysis of the phospholipids[6].

Recently, Olaisson *et al.*[7] have determined the distribution of phospholipids in gastric mucosal membranes using phospholipase C (from *Clostridium welchii* or *Bacillus cereus*) or TNBS. The membrane vesicles remained closed as indicated by electron microscopy or turbidimetry studies and the phospholipids remained in a lamellar arrangement as indicated by ^{31}P-NMR. Thus a variety of different experimental approaches supported their conclusion that the membrane is not rearranged by the probes used.

Newly synthesized phosphatidylethanolamine is initially incorporated into a pool of phospholipid available for hydrolysis by phospholipase C or for reaction with TNBS and rapidly equilibrates with the remaining pool of phosphatidylcholine[8,9]. These results suggest that phosphatidylethanolamine is initially synthesized at the outer leaflet of the membrane bilayer and rapidly equilibrates with the inner leaflet. This is also consistent with the site of the enzymes involved in the biosynthesis of phospholipids[10]. These results would be difficult to interpret if phospholipase C or TNBS caused extensive rearrangement of phospholipids which would obliterate any differences in the labelling patterns of phospholipid pools.

References

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Reply from Van Meer

The effects of phospholipase C on the lipid organization in membranes are complex and undefined at the molecular level. Diglycerides segregate into membrane associated droplets, massive shrinkage of the membrane vesicles occurs and fusion intermediates are induced during the hydrolysis[1–3]. These observations rule out a simple hydrolysis of the outer leaflet phospholipids which would result in vesicles with an intact internal leaflet of phospholipids and an external leaflet exclusively consisting of diglycerides. Any findings concerning selective hydrolysis by phospholipases C should therefore be interpreted with caution.

References

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