Comp. Biochem. Physiol. Vol. 90B, No. 2, pp. 307–309, 1988
Printed in Great Britain

0305-0491/88 $3.00 + 0.00

LIPID COMPOSITION OF MINIATURE PIG PLATELETS

F. MATA,* P. GARCIA-BARRENO and A. M. MUNICIO
Medicina Y Cirugia Experimental, Hospital General Gregorio Marañon; Universidad Complutense, 28007 Madrid, Spain

(*Received* 4 *August* 1987)

Abstract—1. Analyses of platelet lipid composition were carried out on material pooled from male and female miniature pigs.

2. The cholesterol/phospholipid molar ratio was 0.6.

3. Phosphatidylcholine represents the major class of phospholipids (47%) and phosphatidylinositol the minor (2%).

4. The main fatty acids of phosphatidylcholine, phosphatidylethanolamine, phosphatidylserine, phosphatidylinositol and sphingomyelin were: palmitic, stearic, oleic, linoleic and arachidonic acids.

5. The ratios of saturated to unsaturated fatty acids were: sphingomyeline, 1.7; phosphatidylcholine, 1.2; phosphatidylserine, 0.9; phosphatidylethanolamine and phosphatidylinositol, 0.6.

6. Our results suggests that human and miniature pig platelet lipids bear several characteristics in common. This fact would allow miniature pig to be used as a new experimental model.

INTRODUCTION

Platelet lipids are thought to be critical components of platelet activity in normal platelet aggregation and in thrombosis. Phospholipids represent a major constituent of the platelet plasma membrane (Barber and Jamieson, 1970; Taylor and Crawford, 1976; Broekman *et al.*, 1976). Beside their structural function, they have been shown to participate in various platelet responses. The mechanism of regulation of platelet lipid composition is not known. It has been reported that alterations in the fat content of the diet can produce changes in the fatty acid content of platelet phospholipids (Andreoli and Miras, 1971). However, in contrast, *in vitro* studies suggest that platelets incorporate exogenous fatty acids primarily into phosphatidylcholine and phosphatidylinositol, and to a lesser extent into phosphatidylethanolamine and phosphatidylserine (Cohen *et al.*, 1970; Spector *et al.*, 1970; Bills *et al.*, 1976). Furthermore the pathways of phospholipid synthesis in platelets from *de novo* precursors (Lewis and Mejerus, 1969; Cohen *et al.*, 1971; Lloyd *et al.*, 1972) also suggest that platelet lipid metabolism is concerned largely with phosphatidylcholine, phosphatidylinositol and phosphatidic acid. Therefore, the platelet may be limited in its ability to regulate its lipid content. The purpose of the present work is to assess the lipid composition of miniature pig platelets.

MATERIALS AND METHODS

Preparation of platelets

Blood was obtained from the anaesthetized miniature pig after a 1 day fast. Blood was withdrawn from the femoral artery into plastic tubes containing 1/6 volume of ACD anticoagulant (64 mM citric acid, 85 mM trisodium citrate, 111 mM dextrose). The plastic tubes were used to avoid activation of platelets. The citrated blood was then centrifuged at 150 *g* for 20 min at room temperature. The supernatant (platelet enriched plasma, PRP) was removed and centrifuged at 850 *g* for 15 min at 4°C. Platelet pellets derived from 30 ml of PRP were resuspended in Tyrode buffer (134 nm NaCl, 12 mM $NaHCO_3$, 2.9 mM KCl, 0.36 mM NaH_2PO_4, 1 mM $MgCl_2$, 5 mM HEPES, 1 mM EGTA, 8.5 mM dextrose, pH 7.4). The suspension thus obtained was found to be free of any cell contaminants by microscopic examination. The platelet pellets were used for lipid analysis.

Lipid extraction

Platelet pellets derived from 30 ml of PRP were used for the extraction of lipids following the method of Folch *et al.* (1957). Nineteen millilitres of chloroform–methanol (2:1, v/v) were used for each extraction. The lipid extracts were concentrated under vacuum and dried under nitrogen. The total lipid extracts were weighed and resuspended in a known volume of chloroform–methanol (2:1, v/v). Equal volume aliquots of the resuspension were used for determination of cholesterol, phosphorus and for thin layer chromatography (TLC).

Analysis of total lipid extract

Total cholesterol was determined by the modified method of Zlatkis *et al.* (1953), using known amounts of lipids isolated from platelets.

Total lipid extracts were analysed for organic phosphate content by the method of Rouser *et al.* (1966).

Fractionation of lipids

Lipid extracts were resolved into their constituent phosphatides by TLC. TLC layers (DC-Plasticfolien Kiesel gel 60, Merck) were impregnated with 0.2 M ammonic sulphate and activated for 30 min at 110°C. The individual phospholipids were resolved by developing the sample in a bidimensional thin layer chromatography. The first system was chloroform–methanol–acetic acid–water (50:25:8:1, v/v/v/v) and the second chloroform–methanol–ammonium

*Present address: Departamento de Bioquimica y Biologia Molecular I, Facultad de Ciencias Quimicas, Universidad Complutense de Madrid, 28040 Madrid, Spain.