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BIOPHYSICAL CHARACTERISTICS OF TESTICULAR SPHINGOMYELINS WITH VERY LONG CHAIN POLYUNSATURATED FATTY

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Very long-chain (C24 to C36) polyunsaturated fatty acids (VLCPUFA) are important constituents of sphingomyelin (SM) of mammalian spermatogenic cells and spermatozoa. The most abundant VLCPUFA in rat testicular SM are 28:4n-6 and 30:5n-6, followed by 32:5n-6. In order to study some of the biophysical properties of these molecular species, liposomes were prepared from three rat testis SM subfractions differing in the fatty acids bound to sphingosine: I) C16:0-C18:0, II) C22:0-24:0, and III) C28-C32 VLCPUFA. Their thermal behavior was measured by determining the generalized polarization (GP) of the fluorescent probe Laurdan as a function of temperature. Despite the difference of about 6 carbon atoms in their acyl chains, subfractions I and II showed a similar transition temperature (Tt), around 45°C. In contrast, SM fraction III (on average 7 carbon longer than fraction II) showed a significantly lower Tt, about 25°C. Thus, the degree of unsaturation in the amide-bound fatty acid of SM overrides the effect of chain length, causing a low GP value in the liposomes at physiological temperatures. Opposite to the typical ability of most naturally occurring species of SM to form highly-ordered, closely-packed lipid domains, VLCPUFA-rich SMs may form liquid-disordered lipid domains in liposomes and perhaps also in bilayers of the specific germ cells and gametes in which they occur.

LI-P10.

LIPIDS WITH VERY LONG CHAIN FATTY ACIDS (VLCPUFA) IN GERM CELLS AND RESIDUAL BODIES <u>Oresti GM¹</u>, Reyes JG², Luquez JM¹, Pediconi MF¹, Aveldaño MI¹.

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In seminiferous tubules, Sertoli cells and spermatogenic cells at different stages of differentiation coexist. In this work pachytene spermatocytes (PS) and round spermatids (RS) were isolated to study how ceramides (Cer) and sphingomyelin (SM) with VLCPUFA are formed in the testis. Although the concentration of phospholipids including SM was similar in both cell types, there were many differences in their polyenoic fatty acids (FA). In glycerophopholipids, the 20:4n-6/22:5n-6 ratio was significantly higher in PS than in RS. In SM (and Cer), 28:4n-6, 30:5n-6, 32:5n-6 were the main FA in PS, whereas 28:4n-6 predominated in RS. By contrast, in the latter, an important proportion of SM (and Cer) FA were recovered as a-hydroxylated versions of VLCPUFA (2-OH 28:4 to 32:5). The residual bodies (RB) contain materials -including lipids- that are discarded from late spermatids (elongated forms) as they differentiate to spermatozoa, to be phagocytized and "recycled" in Sertoli cells. The RB were very rich in glycerophopholipids and triglycerides with 22:5n-6. The SM of RB contained virtually no VLCPUFA but was extremely rich in 2-OH VLCPUFA. The results point to fatty acid desaturase, elongase and hydroxylase activities that are highly specific for long-chain and very long-chain polyenoic fatty acids encoded by genes that are expressed sequentially as celldifferentiation proceeds.

LI-P11.

RAPID AND PERMANENT LIPID CHANGES AFTER DEATH OF TESTICULAR CELLS INDUCED BY EXPOSURE TO CADMIUM

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Because it selectively damages testicular capillary endothelial cells, exposure to cadmium results in hemorrhagic ischemia and hemato-testicular barrier disruption. Tight junctions between Sertoli cells are disassembled, followed by sloughing of dead Sertoli and germ cells to seminiferous tubule lumen. Here we studied the early and late effects of CdCl₂ on rat testicular lipids. The early effects were similar to those of ischemia induced by testicular artery ligation. Two days after a single CdCl₂ dose, visible hemorrhage and inflammatory edema was accompanied by a massive hydrolysis of germ cell 22:5-rich glycerophospholipids (GPL) (to free fatty acids and diacylglycerols), and of sphingomyelins (SM) containing normal and hydroxylated polyenoic fatty acids (to the corresponding ceramides, Cer). Thirty and 45 days later, the testicular weight was reduced to less than one third and most of the original 22:5-GPL and their products had been removed from the testis. Cholesterol esters and triglycerides mirrored the GPL changes, suggesting they were formed as GPL fatty acids were eliminated, probably by phagocytes. By contrast, the Cer initially produced remained high and unchanged in the tissue. Whether this is due to physical seclusion of Cer in a space inaccessible to phagocytes or to a Cd-related inhibition of ceramidase remains to be established.

LI-P12.

DIMETHOATE-INDUCED LIPID PEROXIDATION IN LEYDIG CELL IS REVERTED BY *IN VITRO* TOCOPHEROLADDITION

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Dimethoate (D) is involved in deleterious effects on testicular physiology. We studied the impact of a sub-chronic administration of D to Wistar rats (15 mg/Kg body weight ip, 5 weeks) on the neutral (N) and polar (P) lipid composition, antioxidant status and peroxidability of microsomal (Mi) and mitochondrial (Mt) membranes isolated from Leydig cells. N and P fatty acyl chains from Mi membranes were enriched in PUFAs compared with Mt fraction. D treatment did not modify their composition; however, it produced a significant decrease in the tocopherol (Toc) content. The loss of Toc correlated with both an increased TBARS production, and a high sensitivity to peroxidation (PX). The time-curse of the in vitro t-BHP-induced PX was measured as TBARS generation under standard incubation conditions. At zero time, membranes isolated from D-treated rats showed an increase of TBARS. The lag of induction almost disappeared in cells isolated form intoxicated animals and the rate of propagation and the maximal production of TBARS were significantly increased. These alterations were almost reverted by in vitro Toc addition. The protective effect was dose-dependent and followed a sigmoidal behavior. Toc may be effective in the protection of Mi and Mt membranes of Dintoxicated cells and in the preservation of PUFAs intimately involved in androgenic function and spermatogenesis.