1525

ONTOGENATIONAL CHANGES IN THE COMPOSITION OF HUMAN SURFACTANT PHOSPHOLIPIDS. S.A. Shelley*, J.E. Paciga*, and J.U. Balis. Dept. Pathology, Univ. South Florida and VAH, Tampa, FL 33612

Lung surfactant was isolated from a variety of human sources including adult, newborn and stillborn lungs, tracheal aspirates of neonates, and amniotic fluids. With the exception of infants with hyaline membrane disease (HMD), surfactant phosphatidylcholine (PC) contained more than 63% palmitic acid regardless of gestational or postnatal age. PC from mature fetal surfactant contained twice as much arachidonic acid and 40% less palmitoleic acid than adult surfactant. These differences were more pronounced in surfactant of amniotic fluids from preterm and diabetic pregnancies. The PC fatty acid pattern of surfactant in control newborns (24-40 weeks gestation) was the same as mature fetal surfactant. However, surfactant from infants with HMD contained much less palmitic and palmitoleic acids and more arachidonic acid. Phosphatidylglycerol (PG), present in adult surfactant as 9% of the total phospholipid, was found in smaller amounts, 2-5%, in surfactant from term amniotic fluids, and was frequently absent in preterm and HMD samples. The fatty acid composition of PG differed greatly from that of PC, containing less than 35% palmitic acid and more than 40% oleic acid. The findings suggest that although surfactant matures early with respect to PC palmitic acid content, there are qualitative changes in the composition of surfactant during both prenatal and postnatal lung development. (Supported in part by NIH HD-13278, HL-23320; MRS-VA).

1526

ANTI-TUMOR ACTION OF B CAROTENE (B Car). E. Seifter, G. Rettura, F. Stratford, S. M. Levenson. Albert Einstein College of Medicine, Bronx, New York 10461

Because of our finding of the prophylactic (chemopreventive) action of B Car against C3HBA tumor induction in mice, we examined the influence of supplemental B Car started after tumors developed. Two groups (20/group) of CBA male mice were inoculated with $2x10^5$ tumor cells. Thirteen days later (tumor size, 6.1 mm in diameter), one group was continued on lab chow which contains 2x the NRC RDA of vitamin A and B Car. The other group received the same chow supplemented with 90 mg B Car/kg diet. B Car had two notable effects: (1) tumor growth was slowed, and (2) survival time was increased (45 days vs 27.5, $p < 0.001$). When mice were subjected to 3000 R local X-radiation of the tumor (6.1 mm) on day 13, partial tumor regression occurred (2.7 mm) and tumor growth ceased for 3 weeks. Tumor bearing mice that received local radiation and were then started on B Car showed (1) complete tumor regression and (2) no evidence of regrowth. B Car supplementation also improved the tumor growth inhibiting action of cyclophosphamide. It is not known if these actions of B Car require that it be converted to vit. A. (We have shown that supplemental vit. A produces similar anti-tumor responses). Although the mouse converts B Car to vit. A, the presence of significant B Car levels in their body fluids may contribute to its observed anti-tumor actions. Supported by NIH Grant 5K06 GM14208 (SML) and Dept. of Army DADA 17-70C.

1527

THE ROLE OF THE PHOSPHATIDYLINOSITOL CYCLE IN THE REGULATION OF PRODUCTION OF ARACHIDONIC ACID. Eduardo G. Lapetina, M.M. Billah* and P. Cuatrecasas. Dept. of Molecular Biology, The Wellcome Research Laboratories, Research Triangle Park, NC 27709

An increase in the metabolism of phosphatidylinositol occurs in a wide variety of tissues by the action of specific ligands. In platelets, the interaction of thrombin with its receptor initiates the degradation of phosphatidylinositol by the action of a specific phospholipase C. Under normal conditions of stimulation, the resultant 1,2-diacylglycerol is rapidly and completely phosphorylated to phosphatidic acid. The phosphatidate produced is degraded by a specific phospholipase A_2, resulting in the production of arachidonic acid and lysophosphatidic acid. The lysophosphatidate produced may serve as a substrate for the transfer of arachidonate directly from other phospholipids to form new phosphatidate which in turn can release more arachidonate. Overall, such a sequence would be equivalent to phospholipase A_2 activation of other phospholipids. When the release of arachidonic acid is completely inhibited by cyclic-AMP or quinacrine, phosphatidic acid is redirected entirely to phosphatidylinositol and there is no production of arachidonate. Under these conditions, the availability of calcium might be profoundly restricted. The correlation in platelets of a phosphatidylinositol response and the deacylation of the resultant phosphatidate by a specific phospholipase A_2 might suggest that these phenomena may be applicable to activations in other cells.

1528

PHOSPHOLIPID ACTIVATION OF PHOSPHOLIPASE A_2 FROM COBRA VENOM. Andreas Plückthun* and Edward A. Dennis. Department of Chemistry (M-001), University of California at San Diego, La Jolla, CA 92093.

The enzymatic hydrolysis of phosphatidylethanolamine (PE) in mixed micelles, which is a very poor substrate alone, can be greatly increased by addition of dibutyryl phosphatidylcholine (DiC_4PC), which is an even poorer substrate. ^{31}P-NMR studies have demonstrated that in these mixtures only PE is hydrolyzed and that DiC_4PC is not incorporated into the Triton X-100/PE mixed micelles that form the substrate. The activation by DiC_4PC shows saturation behavior with a maximal velocity similar to the optimal substrate phosphatidylcholine (PC). The activator only increases V_{max} but does not change K_m. If increasing amounts of Triton X-100 are added, the rate of hydrolysis decreases if the substrate is PC, PE or PE activated by sphingomyelin, which is in the micelle, but cannot be hydrolyzed. With sufficient amounts of monomeric DiC_4PC as an activator, however, no decrease in activity with higher detergent concentrations is observed. If monomeric DiC_4PC is the substrate and micellar sphingomyelin the activator, a detergent dependence is not observed. These findings are explained in terms of surface dilution kinetics and two functional sites on the enzyme: an activator site and a catalytic site. Those functional sites may be on different subunits of an enzyme dimer or constitute separate functional domains on a monomeric enzyme. (Supported by NIH grant GM-20,501)

1529

A SULFHYDRYL-DEPENDENT ACTIVATION OF A PHOSPHOLIPASE A_2 IN HOMOGENATES OF MOUSE PERITONEAL MACROPHAGES. P.D.Wightman,* M.E.Dahlgren* and R.J.Bonney*(Spon.:S.L.Steelman)Dept.of Immun.,Merck Institute for Therap.Research,Rahway,NJ 07065.

We have recently characterized three phospholipase activities in homogenates of resident mouse peritoneal macrophages (Mø); a phosphatidylinositol-specific phospholipase C (PLC) active at neutral pH and two phospholipase A_2 activities optimal at pH 4.5 ($PLA_{4.5}$) and pH 8.5 ($PLA_{8.5}$), respectively. The $PLA_{8.5}$ activity in fresh homogenates of resident mouse peritoneal Mø can be dramatically increased from 5 nmol/h/mg protein to over 100 nmol/h/mg protein by incubating fresh homogenates at 37°C. This augmentation of specific activity occurs maximally at pH 7.5 and requires, optimally, 4 mM Ca^{2+}. This activation of $PLA_{8.5}$ is inhibited by Zn^{2+}, EGTA and 1,10-phenanthroline. The sulfhydryl-specific reagents N-ethylmaleimide and p-hydroxymercuribenzoate each inhibit the $PLA_{8.5}$ activation by greater than 95% at a concentration of 1 mM but have no effect on the fully activated $PLA_{8.5}$ itself. The serine proteinase inhibitors phenylmethanesulfonylfluoride and soybean trypsin inhibitor, the carboxypeptidase inhibitor pepstatin, and the metalloendopeptidase inhibitor phosphoramidon have no effect on the activation of $PLA_{8.5}$. Furthermore, the activation can be inhibited by eluting fresh homogenate from Thiopropyl Sepharose, a covalent chromatography support specific for thiol-containing proteins. The activation of $PLA_{8.5}$ in cell homogenates has, therefore, a Ca^{2+} and a sulfhydryl requirement. Mø homogenates incubated under conditions optimal for $PLA_{8.5}$ activation exhibit no change in the level of $PLA_{4.5}$ activity and a marked decrease in PLC activity. The relevance of $PLA_{8.5}$ activation in Mø homogenates to the response of the intact cell to inflammatory stimuli and subsequent release of oxygenated products of arachidonic acid remains to be determined.

1530

CELLULAR AND ENZYMIC STUDIES OF SPHINGOMYELIN BIOSYNTHESIS. Dennis R. Voelker* and Eugene P. Kennedy. Harvard Medical School, Boston, MA 02115

Pulse-chase experiments with [methyl-^{3}H]-choline in BHK cells reveal that the specific activity of sphingomyelin continues to increase during the period of chase while the specific activities of phosphocholine and CDP-choline decline. Addition of [methyl-^{3}H]-methionine to cells that have been grown in 1 mM dimethylethanolamine effectively labels phosphatidylcholine and sphingomyelin, but not phosphocholine and CDP-choline. These *in vivo* results demonstrate that the source of the phosphocholine moiety of sphingomyelin is phosphatidylcholine, a reaction first elucidated *in vitro* by Ullman and Radin [J. Biol. Chem. (1974) 249, 1506]. The enzymic synthesis of sphingomyelin has been studied in membrane fractions from rat liver. When [^{3}H]-phosphatidylcholine liposomes are used as the substrate, sphingomyelin synthesis is almost entirely dependent upon added phospholipid exchange protein. Synthesis can also be measured using [^{3}H]-ceramide and detergents in the absence of phospholipid exchange proteins. The highest enzymic activity is found in the plasma membrane fraction. (Supported in part by USPHS NIH Grants GM 19,822-21 and GM 22,057-06, and The American Cancer Society PF-1708.)