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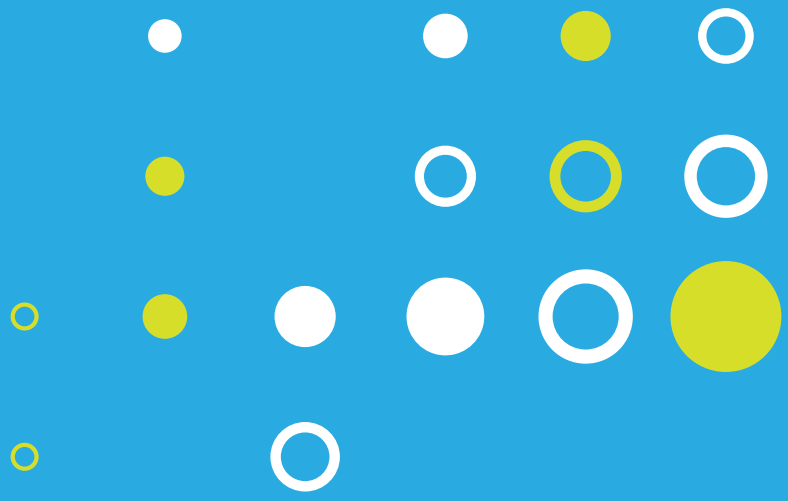

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LI-P17.
RETINOIC ACID MODIFIES LIPID METABOLISM IN ISOLATED ADULT AND AGED RAT CEREBELLUM NUCLEI
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Nuclear lipid metabolism gives rise to several lipid second messengers that seem to be involved in the regulation of nuclear structure and gene expression. The purpose of the present research was therefore to study the metabolic pathways involved in the metabolism of phosphatidic acid (PA) and its regulation in isolated nuclei from the central nervous system. This study was also conducted in isolated nuclei from aged animals allowing us to analyze the effects of neurodegeneration processes on PA metabolism as a result of ageing. Adult (4 mo) and aged (28 mo) rat cerebellums were homogenized and highly purified nuclei were isolated by sucrose-density ultracentrifugation. Using radiolabelled substrates we demonstrated lipid phosphate phosphatases (LPPs), diacylglycerol lipase (DAGL), monoacylglycerol lipase (MAGL), lysophosphatidate phosphohydrolase (LPAPase) and phospholipase (LPAase) as well as PA-phospholipase type A (PLA) activities. We further studied their regulation by the nuclear agonist retinoic acid (RA), which was observed to decrease DAGL and MAGL activities. Significant aged-related changes in the above-mentioned enzymatic activities as well as in its regulation by RA were observed. Taken together, our results demonstrate a RA-regulated PA metabolism in rat cerebellum nuclei which could be involved in neurodegeneration processes.

LI-P18.
AGED-RELATED CHANGES IN 2-AG METABOLISM ENZYMES EXPRESSION AND ACTIVITY IN RAT CEREBRAL CORTEX
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Endocannabinoid 2-arachidonoylglycerol (2-AG) is synthesized by the enzymes diacylglycerol lipase (DAGL) and lysophosphatidate phosphohydrolase (LPAase). Its hydrolysis is carried out by monoacylglycerol lipase (MAGL) although other enzymes, such as fatty acid amide hydrolase (FAAH) and serine hydrolase ABHD may also be involved. The aim of this study was to analyze 2-AG synthesis and hydrolysis during physiological aging. Cerebral cortex membrane and soluble fractions; and synaptosomes from adult (3 mo) and aged (28 mo) rats were isolated by differential centrifugation and the synaptosomes were purified in ficoll gradients. LPAase, DAGL, and MAGL activities were assayed using radiolabeled substrates, and their products were quantified from aqueous or lipid phase, previously separated by TLC. The expression of DAGL, MAGL and FAAH was analyzed by Western Blot. Both DAGL α and ß were expressed in membranes while the second was only expressed in synaptosomes. The expression and activitiy of DAGL changed during aging. 2-AG hydrolysis showed no changes in the membrane fraction during aging in coincidence with the absence of changes in MAGL expression. 2-AG hydrolysis was observed to be higher in synaptosomes whereas MAGL expression decreased during aging. Our results show that the expression and activity of the enzymes involved in 2-AG metabolism are differently modulated by aging.

LI-P19.
METAL-INDUCED OXIDATIVE STRESS ACTIVATES DIFFERENT LIPID SIGNALING PATHWAYS IN DOPAMINERGIC NEURONS
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The characterization of the mechanisms mediating the effects of metal-induced oxidative stress on neuronal dysfunction and death is central in the understanding of the pathology of several neurodegenerative disorders such as Parkinson's disease. In this work, we characterized the cellular responses that operate in dopaminergic neurons (N27 cells) exposed to an overload of transition metals such as iron (Fe, 1 mM), copper (Cu, 10 and 50 µM) or their combination for 24 hs. Under these experimental conditions, reactive oxygen species measured by fluorescence microscopy, and lipid peroxidation levels increased as a function of metal concentration. Maximum levels of lipid peroxides were observed in the presence of Fe + Cu. Cell viability, determined by MTT reduction, strongly decreased in the presence of Cu and with the combination of both metals. Under these experimental conditions, an increase in the levels of Akt phosphorylation in Ser-473 was observed. Bcl-2 expression showed the same profile that Akt phosphorylation. In addition, the expression and the activation of the secretory and cytosolic isoforms of phospholipase A2 (PLA2) were differentially affected by metal overload. Our results demonstrate that phospholipid deacylation processes catalyzed by PLA2s and PI3K activation are involved in the response of dopaminergic neurons to metal-induced oxidative stress.

LI-P20.
SPHINGOMYELIN SYNTHASE1 ACTIVITY IS IMPLICATED IN MDCK CELLS EPITHELIAL-MESENCHYMAL TRANSITION
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We have demonstrated that sphingomyelin (SM) biosynthesis is essential for hypertonicity-induced MDCK cell differentiation. Under inhibition of SM synthesis, MDCK cells instead of differentiate switch to mesenchymal phenotype, thus performing an epithelial to mesenchymal transition (EMT). We aim to study the sphingolipid metabolic pathway as well as the sphingomyelin synthase isoform 1 (SMS1) involvement in such process. MDCK cells were subjected to hypertonicity and concomitantly treated or not (control) with 15 µM D609 (SMS inhibitor) or siRNA-SMS1. Sphingolipid metabolism was determined by using radioactive precursors in the presence or absence of cicloserine (CS) or Fumonisin B1 (FB1). By using D609 as well as siRNA SMS1 the characteristic polarized phenotype of the cells was lost and it was not retrieved by a concomitant treatment with CS or FB1; suggesting no intermediates accumulation participation. Acquirement of mesenchymal phenotype was accompanied by alterations in amount and localization of the epithelial markers (E-Cadh, Cad16 and ZO-1) and mesenchymal marker Vimentin. These results demonstrate implication of SM synthesis in the EMT. Is important to note that EMT has been implicated in the development of cancer and renal fibrosis, consequently SMS1 activity emerges as a possible target molecule for the study of such important human pathologies.