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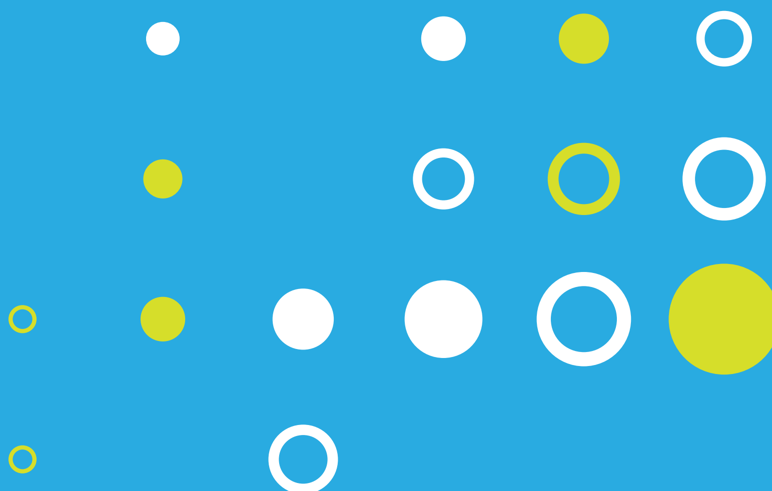

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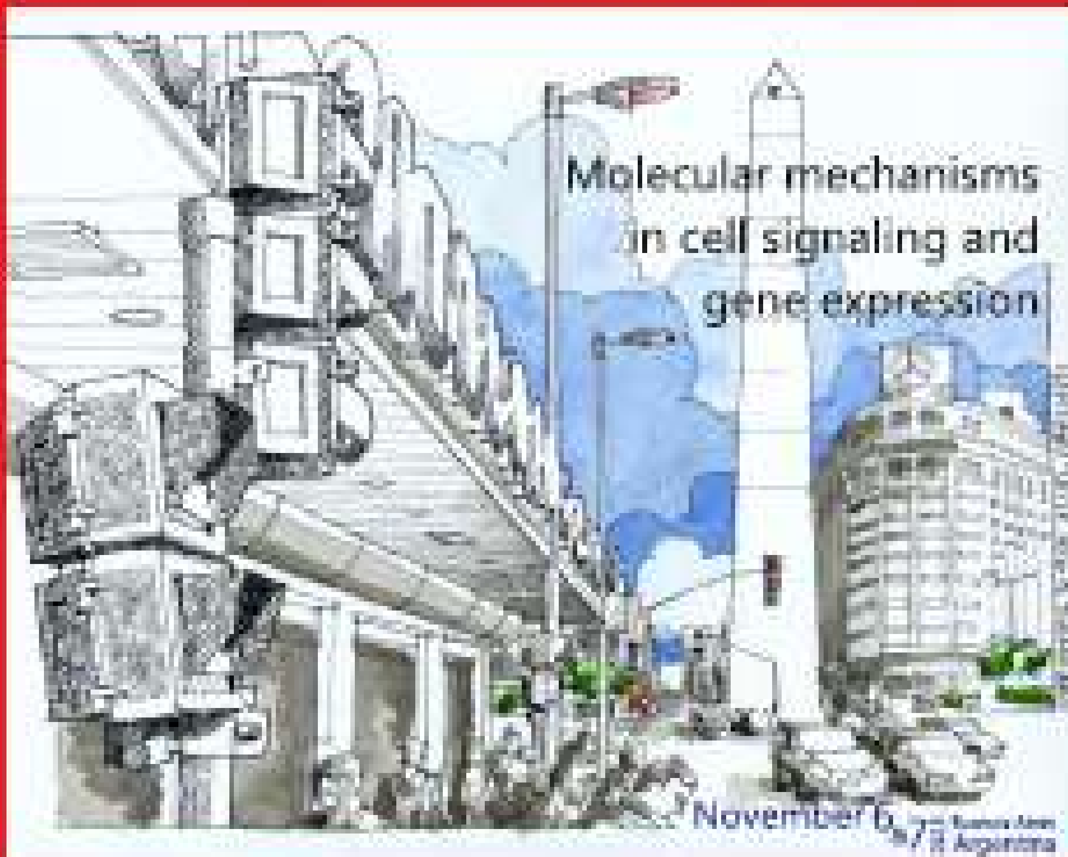

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«Molecular mechanisms in cell signaling and gene expression»

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LI-P09.

MACROPHAGE DIFFERENTIATION INCREASES GLYCEROLIPID SYNTHESIS IN ACCORDANCE WITH GPAT UP-REGULATION

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Type-2 diabetes and obesity are characterized by an excessive accumulation of triacylglycerols (TAG) partially caused by a deregulation of glycerol-3-phosphate acyltransferases (GPATs) that catalyze the first step in *de novo* glycerolipid synthesis, and carnitine palmitoyl transferase 1 (CPT1) that regulates fatty acid oxidization. In order to study the roles of these enzymes in monocyte-macrophage(MP)-foam-cell transition (a model of TAG accumulation during atherogenesis) we tested their expression by qRT-PCR in the murine MP RAW264 and the human THP-1 monocyte cell lines differentiated into foam cells by oxidized LDL (oxLDL) and into MP by PMA, respectively. Mitochondrial GPAT1 and 2 expressions did not change in either model. We then analyzed the ER isoforms GPAT3 and 4. Interestingly, only GPAT3 expression significantly increased in monocyte to MP transition. These results were consistent with GPAT activity assays, since N-ethylmaleimide (NEM) sensitive activity (GPAT2, 3 and 4) but not NEM resistant activity (GPAT1) increased after oxLDL treatment. We could also prove that CPT1a was up-regulated during RAW264 cell differentiation to foam cells while a significant decrease was observed in the human MP derived from THP1 monocytes, suggesting that β-oxidation is not that active in MP, consistently with the anaerobic metabolism hallmark of M1 pro-inflammatory MP.

LI-P10.

SYNAPTIC PKC ISA DOWNSTREAM EFFECTOR OF PLD DURING IRON-INDUCED OXIDATIVE STRESS. EFFECT OF AGING

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We have previously demonstrated that iron-induced oxidative stress activates phospholipase D (PLD) signaling in cerebral cortex synaptic endings (Syn). The purpose of this work was to study the PLD downstream signaling events during iron-induced oxidative stress in Syn obtained from adult (4 months old) and aged (28 months old) rats. Diacylglycerol (DAG) production was increased in Syn from adult rats exposed to iron. This rise in DAG formation was dependent on PLD1 and PLD2 activities. Western blot assays showed that iron overload activates synaptic PKCα/βII and PKD1 and reduces glutamate uptake, both in adult and aged rats. In adult rats, PLD1 and PLD2 modulated PKCα/βII and PKD1 activation. In contrast, in senile rats, DAG formation catalyzed by PLDs did not participate in PKD1 and PKCα/βII activation, but it was dependent on PKC activities. Moreover, PLD1 and PKC inhibition (with 0.15 μM EVJ and 10 μM BIM, respectively) restored glutamate uptake to control levels only in Syn from aged rats. On the contrary, PLD2 inhibition (with 0.5 μM APV) reduced even more the uptake of glutamate in adult and aged Syn. Our results show a differential regulation of PKCα/βII and PKD1 by PLDs during iron-induced oxidative stress as a consequence of aging.

LI-P11.

DIFFERENT PPARs AGONISTS MODIFY NUCLEAR LIPID METABOLISM IN RAT CEREBELLUM

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Previous research from our lab demonstrated an active phosphatidic acid (PA) metabolism in isolated nuclei from rat cerebellum. We detected and characterized several enzymatic activities related to its metabolism, such as lipid phosphate phosphatase (LPP), diacylglicerol lipase (DAGL), monoacylglycerol lipase (MAGL), phospholipase A (PLA) and lysophosphate phosphatase (LPAPase). Interestingly, we also showed that they are differently regulated by trans-retinoic acid through an unknown non-genomic mechanism. Thus, in this work we study the modulation of these enzymatic activities by different fatty acids (FA) and their derivatives which could interact with nuclear receptors, such as peroxisome proliferator-activated receptors (PPARs). To this end, adult rat cerebellums were homogenized and highly purified nuclei were isolated by sucrose-density ultracentrifugation. Using the respective radiolobeled substrates co-incubated with these agonists, we observed that arachidonic acid and docosahexaenoic acid stimulate DAGL activity by 80%. PGE2 also produced a stimulatory effect on this activity but to a major extent (128%) while slightly diminished LPP activity (19%). Taken together, these results demonstrate a lipid metabolism regulated by FA and related molecules in rat cerebellum nuclei that could be involved in gene expression and differentiation, apoptosis or inflammation processes.

LI-P12.

CHANGES IN CERAMIDE METABOLISM CONTRIBUTE TO MDCK CELL DIFFERENTIATION

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Ceramide (Cer) and Glucosylceramide (GlcCer) are involved in many important cellular processes. We have previously demonstrated that GlcCer derived from recycled Cer is essential in MDCK cell differentiation induced by hypertonicity. Cer species are synthesized by six Cer synthases (CerS1-6), each one with different acyl CoA specificity. The aim of this study was to evaluate the expression of CerS1-6, and the different species of Cer and GlcCer during MDCK cell differentiation. Confluent MDCK cells were cultured under isotonicity or subjected to hypertonic media. RT-PCR analyses showed that MDCK cells express CerS2, CerS4 and CerS6 in both experimental conditions. We analyzed the fatty acid composition of Cer and GlcCer species by MALDI-TOF MS. We identified four mayor species of Cer (C16:0, C24:1, C24:0 and C22:0) and six mayor species of GlcCer (C16:0, C24:1, C24:0, C22:0, C20:0 and C18:0). These results are in accordance with the profile of CerS expression detected. Although the composition of sphingolipids was qualitatively the same under both conditions, we found that C16:0 GlcCer have the highest relative intensity in control cells, whereas hypertonicity-cultured cells showed an increase in the relative intensity in C24:0 GlcCer. These changes in Cer metabolism of MDCK cells could provide new clues to understanding the mechanism of renal epithelial cell differentiation.