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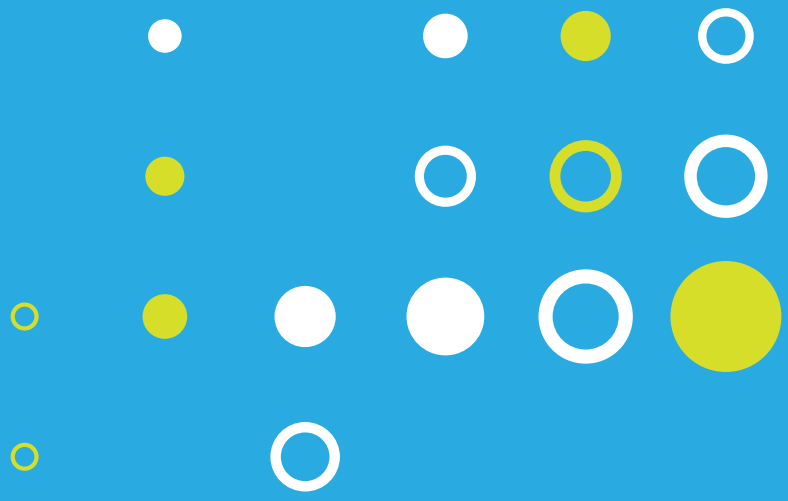

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LI-P09

IDENTIFICATION, CLONING AND EXPRESSION OF A NOVEL PHOSPHOLIPASE A FROM *Leishmania braziliensis*

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We have reported in *Trypanosoma cruzi* infective stages the presence of Phospholipase A1 (PLA1), a membrane and secreted activity that modified Vero cells lipid profile and activated PKC, suggesting its involvement in the signaling events of parasite invasion (Belaunzarán *et al.*, 2007). To extend these studies to *Leishmania braziliensis*, we here describe the cloning and expression of PLA from this parasite. We identify in the genome database TriTrypDb (http://tritrypdb.org/tritrypdb/) 2 putative genes with lipase motif GXSXG: LbrM33_V2.1730 and LbrM31_V2.2750. As the first codifies for a putative precursor, LbrM31_V2.2750 was chosen. Primers were designed and PCR was done using genomic DNA of the reference clone MHOM/BR/75M2904. A unique band (1129 bp) was obtained and cloned into pGEMT vector, sequence was corroborated and then cloned into pET30a and pGEX-6p1 plasmids. Expression was assayed at 21-37ºC and 0.05-1mM IPTG. The best yields were obtained with pET30a in BL21C43(DE3)pLysS cells at 1mM IPTG, 37°C, 3 hs. Protein identity was confirmed by Western blot with anti-*T. cruzi* and anti-*T. brucei* PLA1 antibodies. Enzyme activity was assay with 14C Phosphatidylcholine confirming that the expressed gene has PLA activity. Further studies will allow us to determine the role of PLA in L. braziliensis biology as well as its potential pathogenic effects.

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LI-P10

HYPERTONICITY INDUCES OPPOSITE REGULATION OF PPARγ AND CYCLOOXYGENASE 2 EXPRESSION IN RENAL CELLS

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Peroxisome proliferator-activated receptors (PPARs) regulates the transcription of lipid metabolism related-genes including cyclooxygenase (COX2) which is considered an osmoprotective molecule in renal cells. Although PPARγ was demonstrated to be present in renal cells, the relationship between both proteins has not been established. The present work explores whether PPARγ regulates COX2 expression in renal epithelial cells in isotonic and hypertonic conditions. MDCK cell cultures were grown in isotonic (298 mOsm/Kg H2O) and NaCl-hypertonic (500 mOsm/Kg H2O) media in the absence or presence of PPARγ agonists, rosiglitazone (Rosi) and 15-deoxy-Δ12,14-PGJ2 (PGJ2), or antagonist, GW9662 (GW). After 24 h, treated cells were collected and submitted to westernblot analysis for PPARγ and COX2 In parallel experiments, immunofluorescence microscopy was performed. In isotonicity, Rosi and PGJ2 decreased COX2 expression, but they did not affect PPARγ protein. Therefore, the activation of PPARγ represses COX2 expression. In hypertonicity, the NaCl-induced increase of COX2 level was not prevented by GW, suggesting that PPARγ is not involved. But, when PPARγ was evaluated, the active form of PPARγ 2 was withdrawn. Taken together these results suggest that the up-regulation of the osmoprotective gene COX2 in cells submitted to high-NaCl medium only occurs after

LI-P11

THERMAL BEHAVIOR OF SPHINGOMYELINS WITH VERY LONG CHAIN POLYUNSATURATED FATTY ACIDS (VLCPUFA)

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Rat germ cells and spermatozoa contain species of SM and Cer with 28:4n-6 and 30:5n-6, followed by 32:5n-6, as main nonhydroxy (n) and 2-hydroxy (2-OH) VLCPUFA. The aim of this study is to gain some insight into the properties of these unique sphingolipids in membranes. Total SM from rat testis was partially separated by TLC into two main fractions: SM1, containing n-VLCPUFA, and SM2, containing similar proportions of 16:0 and 18:0 as well as 2-OH VLCPUFA. Each was then separated into molecular species by HPLC. Liposomes formed with these fractions or species were prepared and the generalized polarization of the fluorescent probe Laurdan as a function of temperature was followed. The effects of adding various % of these SM upon the transition temperature (Tt) of dipalmitoyl and dimiristoyl phosphatidylcholine (DPPC, DMPC) were also determined. The Tt of SM1 was significantly lower than that of SM2. In turn, the Tt of SM2 was lower than that of 16:0-SM, 18:0-SM, or a 50:50 mixture of 16:0-SM/18:0-SM, indicating that the presence of SM with 2-OH VLCPUFA in SM2 accounts for this decrease. The Tt of DPPC and DMPC decreased and increased with SM2, respectively, and decreased when SM1 or species with n-VLCPUFA were added. The potential increase in Tt that could have resulted from their unusually long carbon chains is thus overriden by the effect the double bonds have in decreasing it

LI-P12

LIPIDS WITH LONG CHAIN POLYENOIC FATTY ACIDS (PUFA) OF THE n-9 SERIES IN RAT EPIDIDYMAL CELLS

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In rats, epididymal sperm maturation is associated with the acquisition of glycerophospholipids (GPL) with uncommon C20-C24 carbon PUFA of the n-9 series, particularly 22:4n-9-rich plasmenylcholine. Little is known about how the epididymal epithelium participates in this accretion. In this study, three epididymal regions (caput, corpus, cauda) were isolated for lipid and fatty acid analysis. Unexpectedly, the main n-9 PUFA-rich epididymal GPL including plasmalogens were those of ethanolamine rather than those of choline. Epididymal triacylglycerols (TAG) were highly enriched in 18:1n-9, a precursor of 22:4n-9, whereas the ether-linked triglycerides (ADG), as the GPL, were rich in C20-C24 n-9 PUFA (mainly 22:4n-9). The epididymal n-9 PUFA-rich ADG were made up by important proportions of 1-O-alkyl and 1-alk-1´enyl diacylglycerols. This contrasts with the ADG of testis, where the former subclass predominates over the latter and both are rich in 22:5n-6. Although 22:4n-9-rich GPL and ADG were present in the three epididymal segments, they were significantly more concentrated in the corpus, the metabolically most active region of the epididymis. Our results suggest an active involvement of epididymal cells in the lipid remodeling undergone by spermatozoa during their maturation, most of which takes place before the mature gametes are stored in the cauda region.