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ÍNDICE DE TRABAJOS PRESENTADOS

Biología Aplicada

- *Cambios de la cobertura arbórea en localidades con implementación de producción agroindustrial (provincia de Córdoba, Argentina): degradación ambiental y riesgos para la salud* 7
- *El rol de los bosques nativos en la regulación de metales atmosféricos asociados a diferentes prácticas agrícolas* 8

Bioquímica y Biofísica Molecular

- *Synthesis and characterization of nanoparticles and nanofibers from whey protein concentrate* 10
- *Biogénesis de "lipid droplets" y ampollas hidrofílicas en bicapas: mojabilidad de monocapas por lentes líquidas* 11
- *Comparative study of insect and mammals neuronal membranes: microviscosity, interfacial behavior and morphology of the transferred films.* 12
- *Effect of natural terpenes on Bovine erythrocyte acetylcholinesterase (BEA) activity from bovine erythrocyte ghost membranes (BEM). Possible unspecific mechanism that tunes the BEA catalytic activity.* 13
- *The gabaergic insecticide fipronil interacts with membrane lipids: a langmuir film study* 14
- *Using virtual screening for the discovery of new gabaergic insecticides: assessment of RDL homology models and docking scoring functions* 15
- *Producción de péptidos con actividad microbiana a partir de suero lácteo* 16

Biología Celular y Molecular

- *Mecanismos que regulan la fecundación en mamíferos: modulación de la quimiotaxis espermática en bovinos mediada por acetato de ulipristal y zinc* 18
- *Equine spermatozoa at optimum physiological state are selected by chemotaxis toward progesterone* 19
- *REACTIVE OXYGEN SPECIES MAY BE INVOLVED IN THE SIGNALING OF EQUINE SPERM CHEMOTAXIS* 20
- *Detección de la unión de caltrin de rata a espermatozoides de epididimo mediante diferentes técnicas* 22
- *Activity budget, behavioural activities of pairs and adrenocorticotrophin-induced adrenocortical response in captive dolichotus patagonum* 23
- *Expression of aggressiveness modulate mesencephalic c-fos activation during a social interaction test in japanese quail (coturnix coturnix) reared in enriched or plain environments* 24

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Effect of natural terpenes on Bovine erythrocyte acetylcholinesterase (BEA) activity from bovine erythrocyte ghost membranes (BEM). Possible unspecific mechanism that tunes the BEA catalytic activity.

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BEA is a GPI-anchored enzyme that hydrolyzes acetylcholine. The ‘anionic’ subsite in the active site determines the specificity with respect to the choline moiety through electrostatic interactions. Since a) changes on the molecular environment of GPI-anchored enzymes affect their kinetic parameters and b) monoterpenes (MT) affects biomembrane order and electrostatics according to their dipole moment modulus and orientation, here we tested the effects of MTs (1-8 cineol, CIN and camphor, CAM) on the hydrolysis of acetylthiocholine (ATC, Ellman's method) catalyzed by BEA present in BEM. The affinity of the BEA-ATC complex in the absence of MTs ($K_M=0.1$) was significantly affected by CIN which resulted a stronger inhibitor ($K_M=0.81$) than CAM ($K_M=0.11$) (both at 0.3mM). Moreover, CIN exhibited an $IC_{50}=0.3mM$ whereas the IC_{50} of CAM was $\gg 0.6mM$. Measurements of the fluorescence anisotropy (A) of DPH and TMA-DPH in BEM, demonstrated that both MTs affected the organization of the inner regions of the bilayer (both MTs reduced about a 10% the A_{DPH}) but not the polar head group region ($A_{TMA-DPH}$ was almost unaffected). The effect of MTs on the lateral pressure (π) and surface potential (DV) vs Area compression isotherms in Langmuir films were also studied. In the presence of CIN, the transition found in the control π -A isotherm become less cooperative and the $\pi_{collapse}$ decreased. At low π , the slopes of both isotherms (π -A and DV-A) changed; e.g. we found a $DDV \sim 20mV$ with respect to the control without CIN. At high π , CIN and control isotherms converged suggesting the CIN molecules expulsion from the film upon compression. CAM did not produce significant effects on DV, but expanded slightly the whole π -A isotherm up to the collapse point. Concluding, the inhibitory activity of CIN on BEA may be related with its effect on the membrane order and electrostatics which may be interfering unspecifically with the BEA-ATC electrostatic interaction at the active site.

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