CONICET UBA

FACULTAD DE MEDICINA

IFIBIO HOUSSAY

Workshop IFIBIO

Libro de abstracts

Facultad de Medicina

20 de diciembre 2019

Programa

10.00 - 10.45 hs: **Apertura. La mujer en la Ciencia**

Verónica De La Fuente

10.45 - 11.30 hs: **Resultados Encuesta: Situación y condiciones laborales en el IFIBIO**

Actividad coordinada x los Becarios

11.30 - 12.30 hs: **¿Qué actividades llevamos a cabo los CPAs en el IFIBIO?**

Actividad coordinada x los CPAs

12.30 - 13.30 hs: **Almuerzo y Brindis**

13.30 - 15.00 hs: **Sesión de Posters**

15.00 – 16.00 hs: **Nuevos proyectos interdisciplinarios surgidos de la colaboración entre grupos del IFIBIO**

- ✓ **Uso de Biomarcadores para la Detección temprana del desarrollo del Síndrome Urémico Hemolítico y el Diseño de Estrategias de Prevención: Avances y dificultades (PUE2017).**

  C. Ibarra, M. Marta Amaral, C. Silberstein, E Zotta, R. Dorr, J. Goldstein

- ✓ **La glía y el aprendizaje: Rol de la AQP4.**

  Valeria Della Maggiore, Lorena Rela, Claudia Capurro

16.00 – 17.00 hs: **Principales logros del IFIBIO**

Gustavo Murer

Políticas Públicas de Medicamentos y medicamentos de alto costo,

Patricio de Urraza

Abstracts

(1) Renal effects of Shiga toxin type 2 in pregnant and non-pregnant female rats.

Lilian K Fischer Sigel, *Flavia Sacerdoti, Alicia Aráoz, Daiana S Sánchez, Cristina Ibarra, Elsa Zotta, Claudia Silberstein*

Shiga toxin-producing Escherichia coli cause acute renal failure and Hemolytic Uremic Syndrome. Previous studies showed that Shiga toxin type 2 (Stx2) intraperitoneally (ip) injected in rats during the early gestation period produces kidney damage. After a renal injury, tubular epithelial cells have the capacity of proliferate and repair. The aim of the work was to study the effects of Stx2 on damage and tubular proliferation in kidneys of pregnant rats during the early gestation period and compare with non-pregnant rats. Pregnant Sprague-Dawley rats, at the eighth day of gestation, were inoculated ip with a sublethal dose of Stx2 (PS) (0.5 ng/g body weight) or diluent (PC). Non-pregnant rats were injected with the same dose of Stx2 (NPS) or diluent (NPC). Rats were placed in metabolic cages, urine samples were collected, and rats were euthanized 1 to 4 days post-injection (dpi). The kidneys were removed for histopathological observations and Ki67 expression, as proliferation marker, was evaluated by immunofluorescence. Tubular necrosis was observed in renal cortex of PS and NPS rats from 2 dpi, which increased significantly at 4 dpi, with respect to PC and NPC rats ($p<0.05$). Medullar tubules of both NPS and PS did not show significant necrosis. However, a significant increase in Ki67 expression was observed in tubular cells of renal medulla of NPS (8.0±2.0 %) and PS (6.4±0.4 %) with respect to NPC (0.7±0.6 %) and PC (0.7±0.1 %) ($n= 4$, $p<0.05$), indicating an increase in medullar and not cortical tubular proliferation. NPS showed a significant increase in urinary flow compared to NPC (36.2±2.6 ml/d vs 17.0±3.5 ml/d) that was not observed in pregnant rats. Our results showed a similar increase in tubular proliferation in renal medulla of NPS and PS rats. We propose that Stx2 may produce a milder damage in medulla than in cortex, allowing proliferation and repair of the tubular epithelium.

(2) Eliglustat, an inhibitor of Gb3 receptor synthesis, protects human microvascular endothelial cells from Shiga toxin type 2 cytotoxicity.

Fernando Gómez, *Daniel Vélez Gutiérrez, Alejandro Balestracci, Cristina Ibarra, María Marta Amaral*

Hemolytic Uremic Syndrome associated to Shiga toxin (Stx)-producing *E. coli* infection is the most common cause of acute renal failure (ARF) in children in Argentina. Stx2 binds the globotriaosylceramide (Gb3) receptor and causes direct damages on human renal microvascular endothelial cells (HGEC). In this work, we assayed the action of a Gb3 synthesis inhibitor, Eliglustat (EG), to prevent the Stx2 cytotoxicity on human renal cells. Cell viability was analyzed by neutral red uptake and data are expressed as mean ± SEM. Cell morphology analysis was evaluated by light microscopy after staining with H&E. Cell counts were performed on five fields and cell area values were obtained using Image J software. Necrosis and apoptosis were detected by flow cytometry after Annexin V-FITC/PI double staining assay. Non-cytotoxic concentrations of EG were established on HGEC treated with EG (0.05µM-50µM) for 120 h. While EG (50µM) caused a significant decreased of cell viability (8.3 ± 0.9% *vs* Ctrl: 100 ± 2.7%, $n = 3$, $p <0.05$), EG (0.05-25µM) did not exhibit any cytotoxic effect. Next, HGEC were pre-treated with non-cytotoxic EG concentrations at different times (2, 4, 6, 24 and 48 h) and then incubated with Stx2 (0.5 ng/ml) for 72 h and in the presence of EG. At all the times, EG (0.5-10 µM) prevented the decrease in HGEC viability caused by Stx2 ($n=5$, $p <0.05$) and pre-incubation with EG (5 µM) for only 2 h was enough to protect the HGEC viability in about 73%. The maximum protection (100%) was obtained after 24 h and 48 h of pre-treatment with 5 µM EG (EG 24 h and 48 h: 100.0 ± 2.6% *vs* Stx2: 49.0 ± 7.9%, $n = 5$, $p<0.05$). Furthermore, EG (5 µM, 24 h) prevented the cell detachment in 80% and the swelling in 81%. Finally, a significant prevention (86%) of necrosis induced by Stx2 was obtained with EG (1µM, 24 h). We propose EG as a therapy to avoid the renal damage and the consequent ARF.

Abstracts

(3) Presence of microvesicles carried Shiga toxin type 2 in patients with post-diarrheal Hemolytic Uremic Syndrome.

***Fernando Gómez*[1]**, *Melina Porporato*[2], *Cristina Ibarra*[1], *Flavia Sacerdoti*[1], *María Marta Amaral*[1]

[1]Laboratorio de Fisiopatogenia, IFIBIO-Houssay, Departamento de Fisiología, Facultad de Medicina, Universidad de Buenos Aires, Buenos Aires, Argentina. [2]Hospital Prof. Dr. Alejandro Posadas.

Shiga toxin (Stx) producing Escherichia coli (STEC) is responsible for bloody diarrhea, hemorrhagic colitis and hemolytic uremic syndrome (HUS). HUS mainly affects children under 5 years old and it is characterized by kidney and brain damage caused by Stx. So far, there is no a clinical marker for establish an early diagnosis of this pathology. Microvesicles (MVs) are small (<1 µm), pro-inflammatory vesicles shed by host cells during activation and apoptosis. We previously were able to detect the presence of circulating MVs bound to Stx2 (MVs-Stx2) in a rat model of sublethal HUS (Sacerdoti et al. Medicina 77 (supl I): 558 (a 1404), 2017). The objective of this work was to analyze whether the finding of MVs-Stx2, in HUS patient's blood samples, could be useful as an early clinical biomarker to diagnose this disease. Two children who developed bloody diarrhea were admitted to the hospital (P1 and P2). Two days after admission, blood samples were obtained and sequentially ultracentrifuged in order to obtain a MVs-enriched suspension samples. Five age-matched healthy controls (Ctrl) were recruited. Then, MVs were labeled with Annexin V-FITC and MVs-Stx2 were detected by a mouse monoclonal anti-Stx2 antibody and a secondary antibody labeled with Alexa Fluor 647. Finally, MVs were analyzed by flow cytometry. Data are expressed as the percentage of positive MVs-Stx2. From the controls, a cut-off point for MVs-Stx2 was established (1.02-1.90 %, n = 5). A significant higher percentage of MVs-Stx2 in both patients was detected (P1: 3.63%, P2: 5.20%, $p<0.05$). These results indicate that MVs-Stx2 could be a clinical biomaker for the diagnosis of HUS in the early stage. The detection of MVs-Stx2 in combination with STEC and free fecal Stx2 in stool culture considerably can improve diagnosis.

(4) Effect of alkaline gradient on clear renal cell carcinoma mortality: role of isoform 1 of Na^+/H^+ exchanger function.

Marina Mazzocchi*, *Gisela Di Giusto, Alejandro Pizzoni, Natalia Beltramone, Paula Ford, Claudia Capurro, Valeria Rivarola

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The association between cell death and intracellular pH elicits the possibility that extracellular pH (pHe) may modify cell survival. Moreover, as tumor extracellular acidity is a hallmark of cancers, is probable that pHe affects differently cancer or normal cells. Our previous studies showed that cells derived from renal cell carcinoma (RCC) were more sensible to 24h alkalosis than normal cells. The aim of this study was to investigate further the best alkaline condition to kill cancer cells without affecting normal ones. We use three renal cell models: HK2, derived from normal human proximal epithelial cells, 786-O and Caki-1, both derived from human RCC. We exposed cells to media with different alkaline pHe during 48 to 96h. Then, we estimated cell survival by acrydin orange–ethidium bromide experiments. Our results show that normal HK2 cells could survive up to 72h in very mild alkaline conditions (4.8mM NaOH, + 0.05 pH units) without being affected (94±16 % of living cells respect to control, n=6). At the same conditions, both RCC derived cell lines had significant fewer % of living cells respect to control (64±5 % for 786-O and 72±7 % for Caki-1 cells, both with n=6). With 72h exposition to 9.6 mM NaOH (+ 0.1 pH units) the percentage of living RCC cells respect to control became minimal (23±8 % for 786-O and 16±5 % for Caki-1 cells, both with n=6). However, at this condition HK2 cells were also affected (28±5 % of living cells respect to control, n=6). NHE1 isoform of Na^+/H^+ exchanger favors apoptotic process in some experimental models. Then, we inhibited the transporter during the 72h exposition to 9.6 mM NaOH. NHE1 inhibition partially reverted the effects of alkalosis only in normal HK2 cells (% of living cells respect to control: +NHE1 28±5 vs –NHE1 54±6, $p<0.01$, n=12). In summary, the combination of alkali plus NHE1 treatment might improve tumor control with less normal tissue damage.

Abstracts

(5) Dexamethasone blocks motor impairment and brain morphological changes induced by the mixture of taurine and alcohol in a mice model of alcohol hangover.

Alipio Pinto, Silvia Carbone, Jorge Goldstein, Rodolfo A Cutrera

Instituto de Fisiología y Biofísica "Bernardo Houssay" (IFIBIO) UBA-CONICET. Facultad de Medicina, Universidad de Buenos Aires. Argentina.

It has been proposed that inflammatory mechanisms could be involved in alcohol hangover (AH). In previous work we demonstrated that at the beginning of AH mice treated with alcohol (OH) and taurine (TAU), the main component of energy drinks, showed behavioral and morphological changes in the brain. The aim of this work was to study if the pretreatment with dexamethasone (DEXA) could block the motor disabilities and the neural stress produced by TAU in an experimental model of AH. Mice (n= 8-12) were ip pretreated with DEXA (7.5 mg/kg) 24 h before the ip injection of OH (3.8 mg/kg) and/or TAU (190 mg/kg). Controls were injected with saline. The Tight rope (TR) and Hanging wire (HW) tests were used to study neuromuscular coordination and muscle tension, respectively. Another group of mice (n=8) were perfused and the brains were subjected to immunofluorescence with an anti-GFAP antibody (glial fibrillary acidic protein) and an anti-MBP antibody (myelin basic protein) to identify reactive astrocytes and myelin sheath respectively. All the studies were performed 6 h after treatment with OH and/or TAU (beginning of AH). It was observed that DEXA blocked significantly (ANOVA-Tukey $p<0.001$): A) In HW test, the decrease in latency to fall (sec) in OH and OH+TAU groups; B) In TR test, the loss of neuromuscular coordination (sec) in animals with OH and a tendency to improve the performance in DEXA+OH+TAU. It was also observed a significant increase ($p<0.001$) in astrocytic reaction and a significant decrease in MBP in OH, TAU and OH+TAU treated mice compared to the control ones. On the other hand, DEXA significatively achieved to block the astrocytic reaction in OH+TAU treated mice and the reduction of MBP in TAU and OH+TAU treated mice. These results suggest that an inflammatory process may be mediated by the decrease in muscle tension and morphological changes in the brain caused by combined treatment with OH and TAU evidenced at the beginning of AH in mice.

(6) Eliglustat prevents against the cytotoxic effects of Shiga toxin type 2 in human renal tubular epithelial cells.

***Daiana S Sánchez**, Lilian K Fischer Sigel, Alejandro Balestracci, Cristina Ibarra, María Marta Amaral, Claudia Silberstein*

Instituto de Fisiología y Biofísica "Bernardo Houssay" (IFIBIO) UBA-CONICET. Facultad de Medicina, Universidad de Buenos Aires. Argentina

In Argentina, post-diarrhea Hemolytic Uremic Syndrome due to Shiga toxin-producing Escherichia Coli is a common cause of acute renal failure in children. Shiga toxin type 2 (Stx2) binds to the globotriaosylceramide (Gb3) receptor on the surface of renal cells. We have previously shown that the compound C-9 (Genzyme), an inhibitor of glucosylceramide synthase (GLI-1), decreased Gb3 expression and prevents the cytotoxic actions of Stx2 in renal cells. The aim of the present work was to study whether Eliglustat (EG, MedKoo Biosci, USA), a new potent inhibitor of GLI-1, prevents the cytotoxic effects of Stx2 on primary cultures of human renal tubular epithelial cells (HRTEC) and HK-2, a human renal proximal tubule cell line. HRTEC were developed from nephrectomies performed at the Hospital de Niños Pedro de Elizalde. Cells were pre-incubated with or without EG (1-1000 nM, 6-24h), followed by co-incubation with EG and Stx2 (10 ng/ml, 24-72h). Cell viability and proliferation were measured by neutral red and bromodeoxyuridine uptake, respectively. Apoptosis was evaluated by acridine orange/ethidium bromide staining. Treatment with Stx2 significantly decreased cell viability and proliferation and increased cell apoptosis in HRTEC and HK-2 compared to control cells ($p<0.01$). The cytotoxic dose of Stx2 to kill 50% of cells at 72h was 1 ng/ml for HRTEC and 10 ng/ml for HK-2. Pre-incubation of HRTEC and HK-2 with EG, from a dose 10 nM for 24h and 100 nM for 6h, significantly prevented the effects of Stx2 on cell viability, cell proliferation and apoptosis ($p<0.05$). However, C-9 prevented the Stx2 damage at doses 100 times higher than EG. In conclusion, EG protects the human renal tubular epithelium against the activity of Stx2, being more effective than C-9. Decrease Gb3 expression by compounds like Eliglustat could be a novel substrate inhibition therapy to neutralize Stx2 action in renal cells.

Abstracts

(7) Human Mesenchymal Stem Cell-Derived Conditioned Media (MC-MSCs) protects human microvascular endothelial cells from Shiga toxin type 2 cytotoxicity.

Daniel Vélez Gutiérrez, *Esteban Fiore, Fernando Gómez, Mariana García, Cristina Ibarra, María Marta Amaral*

Hemolytic uremic syndrome (HUS) is the clinical triad of thrombocytopenia, anemia, and acute renal failure (ARF). HUS is classically associated with Shiga toxin–producing Escherichia coli infection and affects mainly children under 5 years old. Argentina exhibits the highest incidence rate in the world. HUS lacks a specific treatment and many patients develop chronic kidney disease. Recently, mesenchymal stem/stromal cells (MSCs) have been proposed to treat the ARF. MSCs release several antiapoptotic and proliferative mediators that could mitigate the cytotoxic effects caused by Stx2 on renal cells. The objectives of this work were to isolate human MSCs and to analyze if whether the human mesenchymal Stem Cell-Derived Conditioned Media (MC-MSCs) would be able to protect human glomerular endothelial cells (HGEC) from the detrimental effects of Stx2. MSCs were isolated by culturing explants of Warthon's Jelly from human umbilical cord. Then, cells were subcultured and MC-MSCs was collected after 24 h of incubation. HGEC were treated with Stx2 (0.5 ng/ml) and in the presence or not of MC-MSCs. After 72 h, cell viability was analyzed by neutral red uptake. Cell morphology analysis was evaluated by light microscopy after staining with H&E. Cell counts were performed on four fields and cell area values were obtained using Image J software. Results are expressed as percentage respect to controls (100%). MC-MSCs significantly protected in about 30% the HGEC viability ($p<0.05$). Also, prevented cell morphologic disturbances, since Stx2 in presence of MC-MSCs caused less swelling and retraction. HGEC cell area was preserved in about 62% ($p<0.05$) and cell detachment was reduced in 46% ($p<0.05$). In conclusion, MC-MSCs were able to avoid the injury caused by Stx2 on HGEC. Therefore, MSCs could be considered as a therapeutic strategy to prevent the renal damage caused by Stx2.

(8) Aquaporin-4 facilitates cell proliferation in retinal Müller cells: Implications in Neuromyelitis Optica.

Azul Cocca, *Tomás Molina Ponce, Gisela Di Giusto, Juan Fernández, Paula Ford, Miriam Echeverría, Claudia Capurro, Vanina Netti*

Müller cells are involved in controlling extracellular homeostasis in the retina, regulating cell swelling by a regulatory volume decrease (RVD) mechanism that depends on the efflux of solutes and water through Aquaporin-4 (AQP4). Müller cells are also important for retinal integrity, as they respond to injury byre-entering the cell cycle for tissue repair. Since AQP4 was reported to modulate cell volume during cell cycle progression and facilitate proliferation in astrocytes, the aim of this study was to evaluate, using the novel inhibitor TGN-020, if AQP4 was involved in human Müller cells' proliferation in physiological conditions. Considering that AQP4 is the target of autoantibody IgG-NMO present in the sera of patients with Neuromyelitis Optica (NMO), we also evaluated if cell proliferation was altered in the presence of IgG-NMO. MIO-M1 human Müller cells were exposed to 100 nM TNG-020 or vehicle or to 1/50 dilution of IgG-NMO positive or control sera. Cell volume (videomicroscopy) and cell proliferation (cell count, cell cycle analysis by flow cytometry and BrdU incorporation by immunofluorescence) were measured. AQP4 inhibition with TGN-020 reduced osmotic water permeability (Pf, µm/s) from 20.3±1.2 to 12.2±0.4 ($n=5$, $p<0.001$) and %RVD 15min from 54±4 to 17±3 ($n=5$, $p<0.001$). MIO-M1 cell proliferation was decreased by TGN-020 (doubling time in hours, control vs. TGN-020: 31±1 vs. 40±3, $n=4$, $p<0.05$) without affecting cell viability. TGN-020 also increased the % of cells in G1/G0 phase, decreased the S phase of cell cycle and reduced BrdU incorporation by 20%. IgG-NMO positive sera decreased AQP4 plasma membrane expression in MIO-M1 cells, reducing Pf from 22.4±1.5 to 15.9±0.6 µm/s ($n=6$, $p<0.001$) and %RVD 15min from 66±5 to 48±4 ($n=6$, $p<0.005$), as well as cell proliferation (doubling time in hours, control vs. IgG-NMO: 59±5 vs. 86±4, $n=3$, $p<0.05$) in comparison to control sera. We propose that inhibition or removal of AQP4 from the plasma membrane reduces AQP4-mediated water permeability altering cell proliferation. This is of particular importance in NMO, as the decreased ability of Müller cells to proliferate may affect retinal tissue repair.

Abstracts

(9) Release of ATP by TRPV4 activation is dependent upon the expression of AQP2 in renal cells.

Alejandro Pizzoni, *Zaher Bazzi, Gisela Di Giusto, Valeria Rivarola, Claudia Capurro, Pablo J Schwarzbaum, Paula Ford*

The involvement of purinergic signalling in kidney physiology and pathophysiology is rapidly gaining recognition. Purinergic signalling influences water and electrolyte transport in all segments of the renal tubule. In several tissues, there is increasing evidence that ATP release is dependent upon activation of the transient receptor potential cation channel (TRPV4). Because we have recently found that TRPV4 physical and functional interacts with the water channel AQP2 in cortical collecting ducts cells (CCD)[1], the aim of this work was to examine the possibility that TRPV4/AQP2 interaction influences ATP release in these cells. We used two rat CCD cell lines expressing AQP2 (AQP2-RCCD1) or not (WT-RCCD1). Extracellular ATP (ATPe) measurements were carried out with cells laid on coverslips that were mounted in the assay chamber of a custom-built luminometer. Cells were stimulated with the specific TRPV4 activator GSK1016790A (GSK, 10 nM) and ATPe was measured using firefly luciferase. We found that GSK stimulate ATP release only in AQP2- expressing cells (MaxAQP2 = 222.9 ± 32 nM (n=10)). ATP release stimulated by GSK in AQP2-RCCD1 cells was inhibited by the TRPV4 specific antagonist HC-067047 (1 µM) and by extracellular calcium removal. In order to identify if ATP release occurs via a conductive or an exocytic route, before stimulating cells with GSK, we incubated the cells with carbenoxolone (100 µM, to block pannexin 1 and connexin hemi-channels) or brefeldin A (5 µg/ml, an intracellular vesicular transport inhibitor). We found a similar reduction of ATP release with both inhibitors. Interestingly, when we tested both inhibitors together, an additive reduction was observed, suggesting that both mechanisms function independently. In addition, blocking purinoceptors with PPADS (20 µM) strongly reduced ATP release. In conclusion, these findings suggest that in CCD cells AQP2 is critical for the release of ATP induced by TRPV4 activation. Moreover, ATPe, in turn, acts in an autocrine and/or paracrine manner to stimulate PPADS-sensitive purinergic receptors leading to ATPe-induced ATP release.

[1]*Pizzoni et al, J Cell Biochem 2018 119(5): 4120-4133*

(10) Activation of D1R reduces the Kv1.3 current and contributes to the hypercholinergic state of parkinsonism.

Cecilia Tubert. *RM. Paz, MG Murer*

Balanced actions of dopamine (DA) and acetylcholine (ACh) shape striatal function. In Parkinson's disease (PD) this balance is lost, leading to a hypercholinergic state. Striatal cholinergic interneurons (ChIs) are the main source of striatal ACh. Previously we found that ChIs are hyperexcitable in a mouse model of PD as a result of a reduction in a current mediated by Kv1.3 channels, which is not explained by reduced channel expression. Our aim is to identify the mechanisms that underlie this hyperexcitability. With ex-vivo electrophysiological recordings, we found that SKF81297 (SKF), a DA D1-type receptor (D1R) agonist, increases the excitability of ChIs, and this effect is occluded by Margatoxin (MgTx), a blocker of Kv1.3 channels. The reduction in the current after application of SKF is also occluded by MgTx, suggesting a shared signaling pathway. Preliminary results suggest that this common pathway is adenylatecyclase (AC) dependent, since activating it with Fosrkolin increases the excitability and reduces the current in ChIs in the same way as SKF and MgTx do, and either of the effects are occluded by them. Our results suggest that the activation of D1R promotes the activation of the AC and, probably through PKA, induces the reduction of the Kv1.3 current and the subsequent hyperexcitability of ChIs. Further experiments in a mouse model of PD will be necessary to evaluate if an alteration of this pathway produces the ChIs hyperexcitability observed in this condition.

Abstracts

(11) A transcellular Gb3 dependent pathway is mainly responsible for Shiga toxin-2 cytotoxicity and translocation across human intestinal epithelial cells infected with E. coli O157:H7.

Nicolás Garimano, *María Marta Amaral, Cristina Ibarra*

Shiga toxin-2 (Stx2) is produced and released by E. coli O157:H7 (O157:H7) into the intestinal lumen after colonization, and is able to translocate to the circulatory system and reach target cells causing hemolytic uremic syndrome. Our aim was to elucidate which pathways were involved in Stx2 endocytosis and translocation across intestinal cells infected with STEC. HCT-8 cells grown on 96-well plates were preincubated with specific endocytosis inhibitors such as Eliglustat (EG), Dynasore (DY), MβCD or Amiloride (AM). Then, cells were washed and incubated for 4 h with 100 ng/ml Stx2 alone or in the presence of O157:H7 mutant lacking stx2 gene (O157:H7Δstx2). Stx2 uptake was detected by flow cytometry and its cytotoxicity by neutral red uptake assay. Translocation of Stx2 was evaluated by incubation of HCT-8, grown as monolayers on Millicell inserts, with O157:H7Δstx2. Then Stx2 cytotoxicity was quantified in the media lower chamber by Vero cells, To analyze inhibitors effect on bacteria attachment, bacterial adherence assays were performed on HCT-8 monolayers cultured on 24-wells plates. EG caused the maximum decrease of Stx2 cytotoxic activity, followed by MβCD. AM and DY significantly neutralized the Stx2 cytotoxicity but only in presence of O157:H7Δstx2. Furthermore, Stx2 uptake was reduced when cells were pre-incubated with EG or MβCD compared to DY or AM So, Stx2 uptake may depend on Gb3 receptor, and to a lesser extent, on cholesterol, which is consistent with a necessary interaction between Stx2 and the Gb3 receptor express on HCT-8 cells, to cause cytotoxicity. Moreover, both dynamin-dependent endocytosis and Gb3-independent macropinocytosis became relevant only when bacteria were present, suggesting that these mechanisms are sensible to bacterial infection. Taken together, our study suggests that the mechanisms responsible for enhanced cytotoxicity and transcytosis during infection may have the same endocytic origin.

(12) Detection of Shiga toxin type 2 binds to microvesicles in the plasma sample of a patient with Shigellosis.

Melina Porporato[1], *Fernando Gomez*[2], *Elsa Isern*[3], *Mariana Pellegrini*[3], *Flavia Sacerdoti*[2], *María Marta Amaral*[2], *Cristina ibarra*[2], *Elsa Zotta*[1]

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Shiga toxin type 2 (Stx2) are mainly produced by Shiga toxin–producing Escherichia coli (STEC) that it has been reported to be highly pathogenic and to be associated with hemorrhagic colitis hemolytic and uremic syndrome (HUS). At this moment, an early method of diagnosis of HUS is not exist. Our laboratory developed a method of detection of Stx2 bind to microvesicles. The aim was to detect plasma Stx2 bounded to microvesicles (MVs-Stx2) in a patient with Shigellosis and systemic complications. A 10-year-old girl was admitted to the hospital because of mucous diarrhea with bloody stretch marks over two days. Her laboratory admission showed normal hematocrit, hemoglobin and platelet count. Renal function was conserved without hematuria and proteinuria. Coproculture sample was positive for Shigella flexneri. Two days after admission, hematocrit (30.7%), hemoglobin (9.8 g/dl) and platelet count (187,000/mm^3) were decreased. Take into account this results it was suspected a development of HUS. A peripheral blood smear was performed and did not show schistocytosis. We obtained blood samples in order to detect the presence of plasma Stx2. Samples were sequentially ultracentrifuged to obtain microvesicles (MVs)-enriched suspension. Then, MVs carrying Stx2 were analyzed by flow cytometry. Data are expressed as the percentage of positives MVs-Stx2. From the controls, a cut-off range for MVs-Stx2 was established (1.02-1.90 %, n = 5). A significant higher percentage of MVs-Stx2 (13.6 %) was detected. The patient was not present renal function alterations during this time. These results indicate that the systemic alterations observed in this patient could be explain by the effects of circulating Stx2. Based on this, it is essential to incorporate Stx2 detection to patients with bacterial infection such as Shigellosis complementing the clinical and laboratory study.

Abstracts

(13) Prevalence of Escherichia coli and analysis of virulence factors in endocervical cultures from pregnant women.

M. Luján Scalise[1], Patricia Leonino[2], Adriana Pereyra[2], Roberto Casale[2], José A. Ferreiros[2], Flavia Sacerdoti[1], Cristina Ibarra[1]

1Laboratorio de Fisiopatogenia, IFIBIO-Houssay (UBA-CONICET), Facultad de Medicina, Universidad de Buenos Aires, Argentina 2Departamento de Obstetricia, Hospital Nacional "Profesor Alejandro Posadas", Buenos Aires, Argentina.

Presence of E.coli in the endocervical microbiome has been associated to pregnancy complications. We have previously reported that Shiga toxin (Stx) producing E. coli (STEC) infections during pregnancy may cause maternal or fetal damage mediated by Stx2 in rats in early stage of gestation. Moreover, Stx2 inhibits migration, invasion and cell viability in extravillous trophoblast human cells of first trimester. Therefore, we propose to study the presence of STEC in female genital tract in the pregnant women since might be risk factor during gestation. Our objective was to identify different virulence factors of STEC cultures of endocervix of pregnant women. Endocervical swabs from 103 asymptomatic pregnant women with gestational age of 14 to 30 weeks from the National Hospital Posadas were enrolled. Samples were enriched in Tryptic Soy Broth and sub-cultivated on sorbitol-MacConkey (SMAC) agar in order to detect no sorbitol fermenting colonies, characteristic of STEC. Genomic DNA was purified from colonies and the presence of the uidA gene, exclusive for E. coli was analyzed by polymerase chain reaction (PCR). Positive colonies for uidA were checked for rfbO157, lpfAO113, hcp, eae, stx1, stx2 genes. STEC strains positive for stx2 genes were also cultured in the presence of mitomycin C (2µg/ml) to evaluate expression of Stx2 by viability assays on Vero cells. The PCR results showed that 16/103 samples were positive for SMAC agar and 15/103 were positive for the uidA gene. Furthermore, 6/15 E. coli expressed lpfAO113 and hcp, and 9/15. All of them were negative for rfbO157, eae. stx1 genes. One STEC strain positive for stx2 gene showed cytotoxic effects even in absence of mitomycin C. These results suggest that STEC strains could colonize the endocervix of pregnant women.

(14) Increased levels of nitrated AQPs in villous trophoblast impairs its function.

Yollyseth Medina[1], Mauricio Di Paola[1,2], Carolina Anud[2], Mauricio Castro Parodi [2], Alicia E Damiano[1,2]

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Preeclampsia (PE) is a multisystem syndrome unique to human pregnancy highly studied, however its etiopathogenesis remains unclear. Placentas of women with PE present an intermittent perfusion is established which leads to an ischemia/reperfusion injury in the syncytiotrophoblast. This fluctuations in O2 tensions may increase the generation of reactive oxygen species (ROS) and reactive nitrogen species (RNS) by promoting the formation of peroxynitrite (ONOO-) that covalently modifies proteins by nitration of tyrosine residues. Previous evidence indicates that nitration affects placental signal transduction enzymes and transporters. Recently, we reported that placental explants exposed to hypoxia/reoxygenation showed an overexpression of AQP9 with a lack of functionality similar to that observed in preeclamptic placentas. This channel, is permeable not only to water but also to no charged solutes such as lactate, which acts as an energy substrate and as a source of NADH, a scavenger of ROS. Our aim was to evaluate the effect of nitrative stress mediated by ONOO on AQP9 expression and compare it with samples of preeclamptic and normal patients. This study was approved by the ethics committee of the Hospital Nacional Dr. Prof. A. Posadas. Explants from normal term placentas were maintained in culture under conditions of nitrativo stress induced by 100µM de ONOO and incubated at 37°C for 18h. In this model we evaluated the damage cells indexes by lipid peroxidation and expressions of antioxidant enzymes, AQP9 molecular expression, and the levels of 3NTp-AQP9 and water uptake were evaluated in these explants. The cell viability was determined by MTT assay and the cytotoxicity by LDH release in culture medium. The survival of trophoblast cells was evaluated in explants cultured in in media with different substrates as energy sources, in the presence of an AQP inhibitor (0.3mM HgCl2) and a specific inhibitor of AQP9 (0.5mM Phloretin).

In addition, the amount of 3NTp-AQP9 in trophoblast samples of preeclamptic and normal patients, was determined using an immunoprecipitation assay (Pierce Crosslink IP Kit). In the presence of 100 µM of ONOO-, AQP9 expression significantly increased. In this condition, TBARS increased 3-fold, while SOD-1 showed a 70.35 ± 16.51% decrease (n=5, p <0.02). In this condition of nitrative stress we detected a significant decrease in uptake of water in the explants. However, the lack of sensitive to $HgCl_2$ on the incorporation of water, suggest that AQPs are not functional in these explants. We observed a significant increase of 3-nitrotyrosine AQP9 in preeclamptic placentas. The effect of 3 NytAQP9 formation induced apoptosis in trophoblast cells. The impaired lactate transfer by APQ9 reduced not only energy production but also the amount of cellular ROS scavenger NADH, resulting in the increased death rate of trophoblast cells. Conclusions: In preeclamptic placentas, nitrative stress may induce the nitration of placental AQPs resulting in non-functional proteins. In particular, the lack of functionality of AQP9, impair the transfer of lactate, and induce cell death, possibly due to promoting the accumulation of reactive oxygen species. This could be altered the turnover of villous trophoblast and may play a key role in the pathogenesis of preeclampsia.

(15) Osmotic stress and human amnion aquaporins.

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Amniotic fluid (AF) is essential for normal fetal growth. Aquaporins (AQPs) may have a key role in the AF volume homeostasis. Therefore, altered expression of these proteins might be associated with pathologies as oligo and polyhydramnios. However, the etiology of these syndromes remains unknown. Recently, we demonstrated that AQPs facilitate water transport across human amnion, being the AQP1 the most important contributor. Objective: To study the effect of osmotic stress on the expression and function of human amnion aquaporins (AQPs) and its consequences on cell survival. This study was approved by the ethics committee of the Hospital Nacional Prof. Dr. A. Posadas. Human amnion explants and WISH cell line were cultured in hypo (150 mOsm) and hyperosmolar (400 mOsm) conditions. .Materials and Methods: Net transepithelial water movements across the human amnion were measured using a modified Ussing chamber and osmotic permeability (pOsm) was calculated. AQP1, AQP3, AQP8 and AQP9 expressions were assessed by Western Blot and semiquantitative RTPCR. In some experiments, Tetraethylammonium (TEA) and CuSO4 were used as selective inhibitors of AQP1 and AQP3, respectively. Cell viability and integrity were studied by the MTT and LDH assays. Nfkb expression by Western Blot was also assayed. Results: In human amnion, hyperosmolar condition showed a significantly decreased in AQP1 and AQP9 expressions (n=7; p<0.01), while AQP8 significantly increased (n=7; p<0.05), and AQP3 did not change. However, in hypoosmolar condition, AQP1, AQP8 and AQP9 expressions significantly increased (n=6; p<0.01) while AQP3 significantly decreased (n=6; p<0.001). Similar results were observed in WISH cell line. Accordingly, pOsm decreased 44% (n=5; p<0.001) in hyperosmolar condition, while pOsm increased 48% (n=5; p<0.001) in hypoosmolar condition. In both stress conditions the blocking of each AQP negatively affected cell viability (n=5; p<0.01), however cell integrity was not altered. Conclusions: Our findings showed for the first time, that the expression and function of human amnion AQPs are regulated by changes in osmolarity, and may have an important role on the amniotic cells survival. Our work provides new evidence that changes in human amnion AQP expressions may represent an adaptive response to amniotic fluid volume abnormalities such as oligohydramnios and polyhydramnios.

(16) Role of caveolae in the formation of the placental microvasculature and its interaction with AQP1 and VEGFR2.

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A proper development of the placental vasculature is necessary to ensure a successful pregnancy. In the human placenta, there are two types of endothelial cells: the human placental microvascular endothelial cells (hPMECs) which are in the chorionic villi, and the macrovascular endothelial cells which are in the umbilical cord.

Caveolae are membrane domains that compartmentalize intracellular signaling pathways to orchestrate different cellular events, such as cell migration and invasion. Caveolae are a type of lipid rafts, coated with caveolin-1 (Cav-1) protein which can bind to different membrane receptor proteins and signaling molecules and concentrate these molecules in the caveolae. VEGFR2 is known to regulate endothelial survival, proliferation, migration and formation of the vascular tube. On the other hand, Aquaporin-1 (AQP1) is a transmembrane water channel which moves water in response to osmotic gradients. Recently, it was suggested that VEGFR2, AQPs and Cav-1 are also indispensable for efficient cell migration. Our hypothesis is that AQP1 and VEGFR2 located in the caveolae are essential for the proper formation of the human placental microvasculature. Objective: To evaluate the role of caveolae in the formation of the placental microvasculature and its interaction with AQP1 and VEGFR2. Materials and Methods: Primary culture of placental microvascular endothelial cells (hPMEC) was cultured in complete M199. This study was approved by the ethics committee of the Hospital Nacional Prof. Dr. A. Posadas. In Silico analysis of Cav-1 binding site in AQP1 and VEGFR2 was performed using BioEdit and PyMol programs. Gene expression of Cav-1 (sense 5'-TCTCTACACCGTTCCCATCC-3'; antisense 5'-CACAGACGGTGTGGACGTAG-3'), AQP1 (sense 5'-GAGTATGACCTGGATGCCGA-3'; antisense 5'-GGCCAGCTTGTCAGAGTGT-3') and VEGFR2 (sense 5'-CTTCGAACGATCAGCATAAGAAACT-3'; antisense 5'-TGGTCATCAGCCCACTGGAT-3') in microvascular and macrovascular endothelial cells was evaluated by RT-PCR. Protein expression and localization were studied by Western Blot and immunofluorescence. Co-localization of Cav-1 with AQP1 and VEGFR2 was assessed by immunoprecipitation assay. Cells were treated with 5 mM methyl-β-cyclodextrin (MβCD) to disrupt caveolae and with Tetraethylammonium chloride (TEA) to block AQP1. Cell viability was evaluated by 3- (4,5-Dimethylthiazol-2-yl)-2,5-Diphenyltetrazolium Bromide (MTT) assay. Macrovasculature cell line EA.hy926 (ATCC® CRL-2922 ™) derived from the fusion of human umbilical vein macrovascular endothelial cells (HUVEC) with the human cell line A549 were used as controls. Results: hPMEC cells express Cav-1, AQP1 and VEGFR2. All these proteins were localized in the cell membrane of hPMEC and AQP1 and VEGFR2 co-localize with Cav-1. MβCD significantly reduced cell migration in EA.hy926 cells (n=4; $p<0.05$) and in hPMEC cells (n=4; $p<0.0001$), while no changes were observed when cells were treated with TEA. Conclusions: AQP1 and VEGFR2 are present in caveolae of placental microvascular endothelial cells. Although cell migration is not related to AQP1, an intact caveolar structure is required for the appropriate migration of placental endothelial cells. Any perturbations might result in aberrant angiogenesis leading to serious pregnancy disorders such as preeclampsia or fetal growth restriction.

(17) Aquaporin-2 and Na^+/H^+ exchanger isoform 1 modulate the efficiency of renal cell migration.

Di Giusto Gisela, *Pizzoni Alejandro, Rivarola Valeria, Beltramone Natalia, White Alan, Ford Paula, Capurro Claudia*

Aquaporin-2 (AQP2), in addition to its canonical role as a water channel, promotes renal cell migration by the modulation of integrin β1 trafficking and the turnover of focal adhesions. This novel role described for AQP2 opens the possibility to further investigate if AQP2 also works in concert with other components of the cell migration machinery. Na^+/H^+ exchanger isoform 1 (NHE1) is a well-known protein involved in the regulation of cell migration, which is proposed to act in the leading-edge membrane to direct migration. NHE1 activity is highly modulated by Ca2+ and we recently showed a physical interaction between AQP2 and the Ca^{+2} channel TRPV4. Then, the aim of our work was to investigate the possible crosstalk between AQP2, its mechanosensitive partner TRPV4, and NHE1 to regulate cell migration. We used two renal cell models: one not expressing AQPs and another one expressing AQP2. We performed wound healing and cell tracking assays to evaluate cell migration; immunofluorescence assays to evaluate lamellipodia volume, focal adhesions, and assembly of F-actin; and fluorescence videomicroscopy to measure lamellipodia pHi and NHE activity. Our results confirm that AQP2 promotes renal cell migration and during wound closure, AQP2-expressing cells follow a less tortuous route compared with AQP2-null cells. Lamellipodia of AQP2-expressing cells exhibit significantly smaller volumes and size of focal adhesions and more alkaline pHi due to increased NHE1 activity than AQP2-null cells.

The blockage of AQP2 or TRPV4 significantly reduced lamellipodia NHE1 activity. Also, the blockage of NHE1 significantly reduced the rate of cell migration, the number of lamellipodia and the assembly of F-actin only in AQP2-expressing cells. Altogether these results let us propose that during lamellipodia protrusion the presence of AQP2 activates it's partner TRPV4, leading to Ca^{2+} entry and to the consequent activation of NHE1. It is likely that the interplay between AQP2, TRPV4, and NHE1 defines the pH dependent-actin polymerization, providing mechanical stability to delineate lamellipodia structure and consequently the speed and directionality of cells, promoting the migration.

(18) Text mining applied to PubMed searches on Hemolytic Uremic Syndrome.

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Human Hemolytic Uremic Syndrome (HUS) is characterized by the simultaneous development of nonimmune hemolytic anemia, thrombocytopenia, and acute renal failure. Different causes lead to the syndrome, but the more frequent is the infection caused by Shiga toxin-producing Escherichia coli (STEC), present in food and water supplies. STEC causes human gastrointestinal infections and the developing of HUS in 15% of the cases. According to the World Health Organization, Argentina has the highest global incidence rate of HUS in children under five. HUS can cause death and is the leading cause of acute renal failure in pediatric patients. From the first publication in 1955 a great number of papers have contributed to the understanding of HUS. At the same time, with the exponential increase in the number of articles published each year on biomedical topics, it is raised as a necessity in science to build automated systems to extract information from them. Our hypothesis holds that text-mining on scientific databases offers a powerful tool to analyze behaviors, track tendencies and make predictions. To test, we have carried out an in-depth data-mining analysis on the results of a search on HUS of all publications indexed in MEDLINE up to 2018. Our main goal was to analyze the underlying text at the level of the descriptors used in searches and to discover information structures and nonexplicit (often hidden) patterns. Different informatic tools were applied: Knime Analytics Platform; Voyant tools and AntConc. We show the text mining results on a set from 7989 original articles with more than 3.2 x 106 words. We analyzed 5949 abstracts, 40851 authors and 6191 affiliations to obtaining new valuable information in the study of HUS. Also, we worked on bag of words to analyze temporal frequencies and to do forecasting, and we applied unsupervised computing techniques in topic extraction. As a conclusion, we believe that text mining is an important tool to enriching understanding and promoting disease prevention.

(19) Medial prefrontal cortex encoding of contextual information in a mice model of schizophrenia.

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The medial prefrontal cortex (mPFC) is involved in various forms of cognition that depend on contextual information to guide goal directed behaviors. To do so, the mPFC provides a global representation of the spatial context, incorporating emotional elements to form a complex contextual representation. This representation can be dynamically rearranged depending on cognitive loads and behavioral demands. Altered mPFC function has been associated to the pathophysiology of schizophrenia (SZ) and may be subjacent to the profound cognitive impairments displayed by these patients. Little is known about how mPFC encodes contextual representations and how these representations are altered in SZ. Here, we recorded mPFC neurons activity in a validated SZ-mouse model (NMDA receptors ablated in corticolimbic PV+ interneurons, KO) and control mice while performing exploratory tasks under different degrees of cognitive and emotional load. We observed differences between control and KO regarding their contextual representation (including firing rate and number of engaged units) while analyzing activity of putative pyramidal mPFC neurons. The differences between control and KO depend on the task's cognitive load and are exacerbated when salient social stimuli are incorporated into the environment.
These findings can help us understand how the mPFC integrates relevant contextual information and regulates exploratory behaviors in normal and pathological conditions.

Abstracts

(20) Implementation of an axotomy paradigm in Drosophila wings to study the role of FKBP2 in neurodegeneration

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FKBP51 and FKBP52 are immunophilins that bind immunosuppressive drugs such as FK506. FKBP51/52 are abundant in the nervous system, are not related to immunosuppression and their function at the neuronal level is unclear. FK506 protects and regenerates the nervous system upon several types of injuries. Recently, we found that FK506 promotes in vitro neurodifferentiation and regeneration of murine neurons in a FKBP52-dependent manner. However, mechanisms involved in this effect have not been elucidated and in vivo studies are necessary. Here, we implemented a model of axotomy in Drosophila wing to investigate the role of FKBP52 in neuronal degeneration. In this model, glial cells or neurons expressing fluorescent proteins can be easily visualized over time and changes after nerve injury can be recorded. Using this model, we observed that 2 dpa (days post axotomy) there is an increase in pigment spots in the veins, a sign of inflammatory processes. 7 dpa there is an increase in intensity and discontinuous fluorescence patterns in glia cells. Finally, 2 and 7 dpa, the L1 nerve thickness is reduced and there is a fluorescence discontinuity and reduction of glutamatergic axons. Toxicity studies showed that treatments with FK506 for 3 days at concentrations ranging from 0.01 µM to 1 mM do not alter the survival of adult flies. This model will allow us to examine the effect of FK506 in vivo and the underlying mechanisms of FKBP52 in nerve injury.

(21) Lower density of perisomatic GABAergic boutons containing α1 subunit and Excitation/Inhibition imbalance in a mouse model of schizophrenia

Nicolas M Fulginiti, Carlos A Pretell Annan, Juan E Belforte y Diego E Pafundo

Schizophrenia in characterized by cognitive symptoms that are present before the onset of psychosis. Cognitive processes correlate with synchronous activity, which at the neuronal level is represented by membrane potential oscillations, critical for neuron firing and produced by excitatory and inhibitory inputs. Importantly the excitation (E) is balanced by inhibition (I), i.e. when E increases, I proportionally increases and is maintained in each cycle in a wide range of synaptic conductance. Parvalbumin interneuron (PVI) activity seems crucial for the E/I balance, and also, PV dysfunction may lead to cognitive deficits. Thus, PVI function deficits may produce a new E/I steady state or an altered dynamic range of E/I balance, and thus alter the circuit function.

We used a model of PVI dysfunction by selectively ablating the NMDAR in corticolimbic PVIs to test if the E/I balance in the adult mPFC is altered by a PV dysfunction early. The results show that KO mice show altered E/I balance at the functional connectivity level that can be compensated only under low network activity. Here we propose to find a structural correlate to the E/I changes in the KO mice by estimating the GABA synapses in the mPFC. We found that mPFC neurons of KO mice have less α1 subunit perisomatic GABA synapses, whereas there is no change in those containing the α2 subunit or PV. Finally, we found differences in the frequency of I inputs vs the number of perisomatic α1 GABAergic synapses correlation.

Abstracts

(22) Modulation of ultrasonic vocalizations by chemical stimuli to assess olfactory function in mouse pups

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The rate of ultrasonic vocalizations emitted by mouse pups can be modulated by a variety of conditions, including age, maternal separation and exposure to olfactory stimuli, and the degree of modulation varies depending on mouse strain. Here we addressed whether neonatal ultrasonic vocalizations were susceptible to maternal potentiation and to inhibition by the presentation of aversive olfactory stimuli in a transgenic mouse line that fluorescently reports glial cells (S100B-GFP mice), previously uncharacterized for these responses. We found that the rate of ultrasonic vocalizations during the first 5 minutes after nest separation peaked at the end of the first postnatal week. Nest separation of 1-week-old mouse pups during 5 minutes followed by re-exposure to the nest (mother plus littermates) for another 5 minutes produced potentiation of the rate of ultrasonic vocalizations, emitted in the 75-85 kHz frequency band, by 90 ± 31 %, from a basal value of 12 ± 8 vocalizations per minute. In addition, the aversive odorant citral diluted at 10 % produced an inhibition of 76 ± 10 % of the rate of vocalizations after maternal potentiation, while citral diluted at 1 % had no inhibitory effect. These data are consistent with published work characterizing other mouse strains and sets the starting point to explore the sensitivity of ultrasonic vocalization inhibition by aversive olfactory stimuli to evaluate differences in olfactory thresholds.

(23) Behavioral alterations in an NMDA receptor knockout mouse model of schizophrenia mainly emerge after adolescence.

Carlos A. Pretell Annan, *Diego Pafundo, Juan E Belforte*

Schizophrenia (SZ) is a chronic mental disorder usually emerging during adolescence and early adulthood that encompasses disruptions in various symptomatic domains, sometimes leading to profound disability. Although its etiology is not yet thoroughly understood, cortical parvalbumin expressing interneurons (PVs) have been pathophysiologically implicated. Also, it is known that PVs normally complete their maturation around SZ onset. We have shown that early postnatal ablation of NMDA receptors in cortical PVs results in an adult SZ-like behavioral phenotype, although its developmental trajectory had not been fully described. Therefore, we aimed to characterize the time course of behavioral alterations, with a special emphasis in presymptomatic stages. To address this, we evaluated mice behavior throughout development, from PND 25 to 20 weeks of age, by means of an open field (OF), a spontaneous alternation Y-maze (YM), nesting and marble burying (MB). Here we confirmed previous findings for OF, YM and nesting, with hyperlocomotion and memory deficit emerging at adulthood. We also found this altered behavior in MB. Remarkably, we found a transient deficit in YM occurring before adolescence. Also, differences were seen between males and females for the first time. In conclusion, while the majority of SZ-related phenotypes induced by early postnatal NMDA receptor ablation emerge in adulthood, some traits may be present at early stages as preclinical entities.

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Abstracts

(24) Behavioral characterization of nociceptive sensitization in an animal model of neuropathic chronic pain.

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Chronic pain is a debilitating neurological condition of high clinical relevance and the treatments currently available show limited efficacy. The transition to chronic pain remodels neuronal circuits in the brain regions that mediate pain perception. Although initially those changes were believed to reflect peripheral and spinal cord sensitization, brain imaging reports in patients and preclinical studies in animal models suggest that brain neuronal plasticity may actively contribute to the development and persistence of chronic pain. Pain is a complex subjective experience which includes sensory, affective and emotional components mediated by distributed brain networks. Maladaptive changes in all those systems may participate in the chronification of pain. However, the behavioral manifestation of pain was traditionally evaluated by means of reflexive responses, which prevents a proper understanding of the broad mechanisms involved in pain perception. The objective of this work was to set a behavioral paradigm which will enable to differentiate reflex from affective responses toward a diverse variety of stimuli (e.g., noxious vs. neutral) in an animal model of chronic neuropathic pain. For that, we assessed in detail mice behavioral repertoires after stimulation of an injured hind paw. Our preliminary results show that injured animals exhibit affective manifestations after noxious stimuli that do not occur in sham animals. We conclude that this experimental design will allow us to investigate the mechanisms that mediate the different aspects of chronic pain. Keywords: neuropathic pain, nociceptive sensitization, reflex responses, affective responses.

(25) Unsupervised cluster analysis of task solving strategies using an animal model of striatal cholinergic interneurons ablation.

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Chronic pain is a debilitating neurological condition of high clinical relevance and the treatments currently available show limited efficacy. The transition to chronic pain remodels neuronal circuits in the brain regions that mediate pain perception. Although initially those changes were believed to reflect peripheral and spinal cord sensitization, brain imaging reports in patients and preclinical studies in animal models suggest that brain neuronal plasticity may actively contribute to the development and persistence of chronic pain. Pain is a complex subjective experience which includes sensory, affective and emotional components mediated by distributed brain networks. Maladaptive changes in all those systems may participate in the chronification of pain. However, the behavioral manifestation of pain was traditionally evaluated by means of reflexive responses, which prevents a proper understanding of the broad mechanisms involved in pain perception. The objective of this work was to set a behavioral paradigm which will enable to differentiate reflex from affective responses toward a diverse variety of stimuli (e.g., noxious vs. neutral) in an animal model of chronic neuropathic pain. For that, we assessed in detail mice behavioral repertoires after stimulation of an injured hind paw. Our preliminary results show that injured animals exhibit affective manifestations after noxious stimuli that do not occur in sham animals. We conclude that this experimental design will allow us to investigate the mechanisms that mediate the different aspects of chronic pain.

Abstracts

(26) Cortical spiking activity entrainment with beta oscillations is enhanced after nigrostriatal degeneration and when L-DOPA effects have worn off.

Daniela Piña Novo, *Mariano Belluscio, Gustavo Murer*

Abnormal involuntary movements known as L-DOPA-induced dyskinesia (LID) are a common complication in Parkinson's disease (PD) after prolonged treatment with L-DOPA, which is the gold standard medication. Little is known about the oscillatory activity associated with LID, especially in the motor cortex (MC). However recent studies show that exaggerated beta activity (15–35 Hz) who emerge in the basal ganglia after nigrostriatal degeneration, correlate with motor impairment in PD and can be suppressed by LID. Our previous characterization in MC disclosed a similar pattern, with an increased number, duration and power of beta events. Interestingly this pattern was reverted during the acute effect of L-DOPA, but reappeared when L-DOPA effects have worn off. Here we sought to identify cortical neuronal populations related to this rhythm. We performed recordings of single unit activity by means of high density electrodes in primary MC of parkinsonian mice before and after L-DOPA regime that induced LID. We found an increased mean firing rate in both conditions. Also, phase preference of spiking activity to beta oscillations was higher in lesioned than in sham animals. This pattern was present both in putative pyramidal neurons and interneurons. These results reveal a better entrainment of neuronal activity with beta oscillations in the parkinsonian condition, which is not reversed by chronic L-DOPA administration, and could explain the increased beta power previously observed.

(27) Consolidación nocturna de memorias adaptación visuomotora: Efectos diferenciales sobre la retención de la memoria y la persistencia.

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La adaptación motora es un tipo de aprendizaje que permite mantener un control motor preciso frente a perturbaciones del ambiente y/o internas. Al igual que otros tipos de aprendizaje, la adaptación motora puede conducir a interferencia o facilitación dependiendo del nivel de congruencia del material aprendido secuencialmente. En trabajos previos hemos demostrado que la adaptación a rotaciones visuales opuestas, A y B, deteriora la capacidad de aprendizaje de B y la memoria a largo plazo medida 24 horas después. Aquí, examinamos el impacto del sueño en la retención y persistencia de los recuerdos de adaptación visuomotora, en presencia o ausencia de una perturbación conflictiva. Para ello realizamos dos experimentos en los que manipulamos la proximidad entre el entrenamiento y el sueño (PAS). Se plantea la hipótesis de que si el sueño participa en la consolidación de la adaptación visuomotora, entonces debería haber un beneficio de la promixidad del sueño sobre la retención de la memoria y/o la persistencia. Para examinar esta posibilidad realizamos dos experimentos: en el Experimento 1, los sujetos realizaron una tarea de adaptación visuomotora (perturbación de 30 grados CW); en el Experimento 2, un grupo diferente de participantes realizaron la misma tarea pero bajo un protocolo de interferencia anterógrada en el que se adaptaron a dos rotaciones visuales opuestas (30 grados CCW seguido de 30 grados CW) separadas por un intervalo de 5 minutos. En cada experimento, un grupo de sujetos realizaba la tarea por la mañana con una proximidad aprendizaje-sueño de unas 14 hs (PAS-14hs) y el otro grupo por la noche, justo antes de irse a dormir (PAS-10min). Todos los sujetos participaron en un estudio polisomnográfico de noche entera en el laboratorio del sueño, y regresaron al laboratorio 24 horas y 2 semanas después del entrenamiento para evaluar la retención de la memoria y la persistencia, respectivamente.

Los resultados del Experimento 1 revelaron que el grupo PAS-10min mostró un mayor nivel de retención y persistencia de la memoria que el grupo PAS-14hs (RM-ANOVA: PAS: F(1,37)=4.2227, p=0.046*, Test: F(1,37)=17.3587, p=0.0001*, PAS*Test: F(1,37)=0.0828, p=0.775). Se observa ademas un efecto facilitador de la memoria memoria entre el primer y segundo ciclo que ocurre sólo en el grupo PAS-10min (RM-ANOVA: PAS*Número.Ciclo: F(1,37)=4.7094, p=0.036*). En el Experimento 2, en coincidencia con reportes anteriores, los resultados indicaron que el nivel general de retención de la memoria cayó un 70% respecto a la retención observada en el experimento 1. Al analizar el nivel de retención y persistencia de los Grupos PAS-14hs y PAS-10min que participaron del Experimento 2, se obtuvo una interacción estadísticamente significativa entre el grupo (PAS-14hs y PAS-10min), el test (Día 2 y Día 15) y el número de ciclo (RM-ANOVA: PAS*Test*Número.Ciclo: F(1,32)=4.9421, p=0.033*). Dicha interacción se explica por una caída del nivel de retención entre el primer y segundo ciclo en el test del Día 2 para el Grupo PAS-14hs, a diferencia del Grupo PAS-10min que presentó una caída en el nivel de persistencia en el test del Día 15. Sin embargo, no se observó un efecto robusto que refleje un beneficio de la proximidad aprendizaje-sueño en una situación de interferencia entre memorias opuestas, consistente con la protección de la memoria. Los resultados sugieren que la proximidad entre el aprendizaje y el sueño favorece el nivel de retención de la memoria 24 horas después (retención a largo plazo) y su persistencia evaluada 15 días después de aprender. Estos resultados son consistentes con un efecto del sueño sobre la consolidación de la memoria de tipo AM. Sin embargo, no hay evidencia suficiente para sugerir un efecto de protección o atenuación del impacto de la interferencia anterógrada entre memorias asociadas a perturbaciones opuestas.

(28) Stx2 from EHEC produces Hemolytic Uremic Syndrome and neurologic alterations including cerebellar involvement in patients.

Vanina Velardo, Clara Valentina Berdasco, Fernando Correa, Adriana Cangelosi, Patricia Geoghegan, Jorge Goldstein

The aim of this study was to determine the mechanisms by which Stx2 causes cell damage in the cerebellum. Mice were injected intravenously with 1ng of Stx2 or 100 µl of saline. Fixed cerebellums were subjected to staining with lectins (microvasculature profile) and immunofluorescence with anti-GFAP (astrocytosis marker) and anti-MBP (myelin protein marker). ELISA kits measured TNFα and IL-10. Stx2 reached the Purkinje and granular layers. Stx2 significantly: decreased the area occupied by the microvasculature (12.58 ± 0.73 Control vs 7.71 ± 0.72 Stx2, day 2, in µm2); increased the expression of GFAP (11.3 ± 0.3 Control vs 17.97 ± 0.87 Stx2, day 2, and 12.11 ± 0.67 Control vs 14.8 ± 0.6 Stx2 day 4, in IOD),and decreased the expression of MBP (67.6 ± 2.4 Control vs 35.4 ± 0.9 Stx2, day 2, and 62.6 ± 2.5 Control vs 46.2 ± 1.4 Stx2 day 4, in IOD); p<0.001. Stx2 increased the expression of TNFα at day 2 (4.8 ± 1.7 Control vs 12.4 ± 2.1 Stx2, in pg/mg protein), while IL-10 expression was increased at day 4 (25.05 ± 3.9 Control vs 67.2 ± 10.8 Stx2, in pg/mg protein); p<0.001. Finally, Stx2 damaged the cells that integrate the vascular unit, with inflammatory involvement. Further studies are being conducted to elucidate the observed cell events.

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(29) Neural mechanism involved in contextual memory: role of CA3 and CA1 remapping.

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The Hippocampus (HP) is involved in encoding, consolidation and retrieval of episodic memories. Some hippocampal neurons, place cells (PC) are tuned to spatial location and generally change their tunning when an animal change context (remapping). It has been suggested that the hippocampal ability of storing and distinguishing between different situations and contexts can be related with place cell's remapping.
Several studies have shown how PC can either remap or not as a consequence of changes in the environment. It is also known that there are differences between CA1 and CA3 (two hippocampal regions) in spatial codification. Still, there is no study showing the link between the memory that the animal is expressing and the activity of its neurons. In other words, It's still unknown whether when an animal recognizes a certain context as new, there is remapping in the HP or not.
The aim of this project is to understand how the differential remapping observed in CA1 and CA3 correlates with the behavioral response. To answer this question we use A behavioral task that allowed us to discriminate if an animal recognizes a context as new, or as one they already knows. We carried out electrophysiological recordings in CA3 and CA1 region of the HP while they were performing the tasks in order to correlate the remapping and the evocation of different contexts.

(30) Stx2 from enterohemorrhagic E. coli induces NF-kappaB activation in reactive astrocytes

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Shiga toxin 2 (Stx2) from enterohemorrhagic E. coli causes hemolytic uremic syndrome (HUS) and acute encephalopathy, which may lead to fatal outcomes in patients. When neurological symptoms are evidenced mortality rate may rise up to 40%. The mechanism by which the encephalopathy emerges in patients with HUS is still unknown. Reactive astrogliosis is a widespread glial response to brain injury and NF-kappa B activation was related to the proinflammatory-neurodegenerative astroglial polarization. The aim of this study was to determine whether Stx2, LPS or a combination of both produce astrocyte reactivity in vitro, and whether this reactivity involves the activation of NF-kappaB pathway. Primary cortical astrocytes were obtained from P3-P5 C57 mice. Confluent astroglial cultures were incubated either with control (saline solution), LPS (50 ng/ml), Stx2 (50 or 200 ng/ml), or a combination of both toxins. GFAP expression and astroglial cell morphology was evaluated by immunocytochemistry. Reactive astrogliosis was observed following the treatment with 200 ng of Stx2 plus 50 ng of LPS in comparison to the control (0. 058 ± 0.006 control vs 0.088 ± 0.006 Stx2+LPS, measured as the number of filamentous astrocytes per total number of astrocytes). Nuclear translocation of p65 NF-kappaB subunit was measured as an index of NF-kappaB activation. The 3h treatment with 50 ng/ml LPS, 200 ng/ml Stx2, and 200 ng/ml Stx2 plus LPS showed a significantive NF-kappa B activation in primary astrocytes when compared with controls (63.19±4.51, 23.77±1.97, 55.30±4.2, 1.33±0.51 respectively; expressed as a ratio of nuclear p65 vs. total number of astrocytes). We conclude that Stx2 causes reactive gliosis in vitro and NF-kappaB activation which it may be involved in the proinflammatory astroglial polarization known to produce neurodegenerative effects.

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(31) Abnormal neuron nuclear morphology after transgene suppression in a conditional TDP-43 mouse model of neurodegenerative disease.

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Dysregulation of TDP-43 is a key feature of frontotemporal dementia (FTD) as well as amyotrophic lateral sclerosis (ALS). Previously, using a transgenic mice conditionally overexpressing human wild-type TDP-43 protein (hTDP-43-WT) we analyzed the region-specific neuronal loss. We found a decrease in CA1 and dentate gyrus (DG) NeuN+ cells width accompanied by decreased neuronal number, indicating mild neurodegeneration after 1 month transgene (TG) expression (1mo). In this study, we evaluated in this model the nuclear morphology of neurons from hippocampal regions (DG and CA1), and somatosensory cortex layers after a TG suppression protocol. We found an increased percentage of abnormal nuclei in the suppressed group compared to controls and 1mo mice in all analyzed regions. This suggest that the cellular mechanisms coping with hTDP-43 overexpression and recovery after suppression might differ. In this context, we are currently assessing how TG suppression modulates neurodegeneration by evaluation of neuronal survival. Additionally, we studied the impact of corticospinal tract degeneration (observed in hTDP-43-NLS mice expressing a cytoplasmically-localized form in the forebrain) on lower motor neuron (LMN) health in the spinal cord (SC). Using Nissl staining, we analyzed LMN area in the anterior horn from different SC segments and found no differences in TG TDP-43-NLS mice compared to controls. In summary, these results contribute to our understanding of FTD/ALS pathology.

(32) Tracking the time course of structural plasticity in motor learning using DWI: skill learning vs adaptation

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Structural remodelling induced by motor learning is a rapid, dynamic process associated with synaptogenesis and enlarged astrocytic volume [1, 2, 3]. In the last years, Diffusion Weighted Imaging (DWI) has gained popularity as a non-invasive tool to quantify neuroplasticity in the cortex. Sagi and collaborators have demonstrated that learning-specific reductions in mean diffusivity (MD) reflect astrocytic hypertrophy, and therefore can be used as a reliable marker of plasticity in humans [4]. Here, we used MD maps to track the time course of structural plasticity in two motor tasks, tapping on different neural substrates: motor sequence learning and visuomotor adaptation. In order to contrast LTP-like plasticity with structural changes induced by memory consolidation, subjects were scanned before, 30 minutes and 24 hours post-training.

We trained 21 healthy subjects (11 female, age 23,6±3,1) in two well-characterized motor tasks: motor sequence learning (MSL) and visuomotor adaptation (VMA). DWI images were obtained before, 30 min and 24 hours after training to asymptotic performance (~15 min. for MSL and ~25 min. for VMA). We ran two voxel-wise statistical analyses aimed at distinguishing structural changes that were common from those that were different between the motor learning tasks. Furthermore, functional magnetic resonance images (fMRI) were acquired during MSL to assess how the brain regions that participate in the acquisition of this motor task are related to those areas showing microstructural plasticity. A marked reduction in MD over the lateral posterior parietal cortex 30 min. post-training, that persisted at 24 hours, distinguished MSL from VMA. Changes in the precuneus area associated with MSL proved to be long lasting, as they were still present after 7 days.

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These results were specific to motor learning, since they were not present in a control condition. This finding was in contrast with short-lasting changes occurring for both motor learning tasks. Specifically, MSL and VMA showed a reduction of MD in the right lateral cerebellum 30 min. post-learning that returned to baseline at 24 hours. Surprisingly, the left hippocampus was reliably modified in both motor tasks during a short time window. Short-term MD changes associated with VMA in the hippocampus and the cerebellum were correlated with memory retention at 24 hours (R=-0.6, p=0.0082 for the hippocampus; R=-0.54, p=0.02 for the cerebellum), which indicates that the observed neuroplasticity is related to memory consolidation. In turn, brain regions showing MD changes in MSL overlapped with areas active during task acquisition, which suggests that the same network that is activated during learning is modified during memory consolidation and is expressed as changes in brain microstructure. In particular, microstructural changes were associated with a higher functional activation during the periods of rest interspersed between blocks of sequence execution. A higher activation in the hippocampus during rest was correlated with a greater decrease in MD 30 minutes post-learning (R=-0.42; p=0.065). This is related to the existence of micro-offline gains during MSL [7], evidenced by the correlation between activity in the hippocampus and the precuneus in early MSL and the level of "micro-offline" gains (R=0.51, p=0.035 in the hippocampus; R=0.6, p=0.012 in the precuneus). Although there is increasing literature pointing to a function of the hippocampus in the acquisition and/or consolidation of MSL when learned explicitly [8, 9], there is no evidence for a role of the hippocampus in VMA. The transfer of rapidly encoded information from the hippocampus to long-term storage sites in the neocortex has been shown to play a key role in consolidation of declarative and spatial memories [10]. However, recent work has highlighted the involvement of the hippocampus in the stabilization of non-hippocampal memories [11, 12]. Our results shed light on the time course of structural plasticity elicited by motor learning, and adds to the current debate challenging the traditional role of the hippocampus in explicit memory encoding.

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(33) TDP-43 overexpression affects global brain translation,

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TDP-43 is a RNA-binding protein that, amongst other functions, participates in mRNA metabolism, and it is a major component of inclusions observed in neurodegenerative diseases like frontotemporal dementia (FTD) and amyotrophic lateral sclerosis (ALS). Previous results from our lab showed a decrease in global mRNA translation as compared to wild-type animals, revealed by polysome profiling of brain cortex from hTDP-43 expressing mice. To further understand the role of TDP-43 in mRNA and protein metabolism, we used a combined approach with animal and cellular models. Application of SUNSET method (which assesses ongoing translation) in brain slices from control and hTDP-43-ΔNLS expressing mice revealed a decrease in puromycin incorporation in brain cortex cells of ΔNLS mice when compared to control animals. Complementary immunoblot analysis corroborates that puromycin is actively incorporated during translation of new proteins. The Unfolded Protein Response (UPR) is a major cellular process that also regulates translation. To assess in vitro how TDP-43 modulates the UPR, HEK293 cells were transfected with TDP-43 variants and treated with vehicle or ER stress inducers. We are currently analyzing ATF4 and ATF6 pathways; preliminary data corroborate that MG132 induces ATF6 cleavage and ATF4 protein levels. These results suggest that dysregulation of TDP-43 might alter global translation and that cytotoxic effects in FTD/ALS might be due to alterations in proteostasis by TDP-43.

Índice de Primeros Autores