2017

medicina

BUENOS AIRES VOL. 77 Supl. I - 2017

MEDICINA

Volumen 77, Supl. I, págs. 1-620

medicina

BUENOS AIRES, VOL. 77 Supl. I - 2017

La Tapa (Ver p. IV)
Imagen ígnea, 1996.
María Esther Gené

MEDICINA (Buenos Aires) – Revista bimestral – ISSN 1669-9106 (En línea)

REVISTA BIMESTRAL
Registro de la Propiedad Intelectual N° 5324261
Personería Jurídica N° C-7497
Publicación de la Fundación Revista Medicina (Buenos Aires)
Propietario de la publicación: Fundación Revista Medicina
Queda hecho el depósito que establece la Ley 11723

Publicada con el apoyo del Ministerio de Ciencia, Tecnología e Innovación Productiva.
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Incluida en el Núcleo Básico de Revistas Científicas Argentinas del CONICET.

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Tel. 5287-3827 Int. 73919 y 4523-6619
e-mail: revmedbuenosaires@gmail.com – http//: www.medicinabuenosaires.com

Vol. 77, N° 5, Noviembre 2017

Edición realizada por
GRAFICA TADDEO – Charrúa 3480 – Buenos Aires – Tel: 4918.6300 l 4918.1675 l 4918.0482
e-mail: ctp@graficataddeo.com.ar – www.graficataddeo.com.ar

medicina
BUENOS AIRES, VOL 77 Supl. I - 2017

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XXIX REUNIÓN ANUAL DE LA SOCIEDAD ARGENTINA DE PROTOZOOLOGÍA (SAP)

13-17 de noviembre de 2017
Palais Rouge– Buenos Aires

on respiratory response and breathing disturbances commonly observed in neonate rats and humans, such as Sudden Infant Death Syndrome.

(1772) **IMPAIRMENT OF NITRIC OXIDE METABOLISM AT CENTRAL SYNAPSES BY LEVOCABASTINE, AN ANTAGONIST FOR NEUROTENSINERGIC NTS2 RECEPTOR**

Alicia Gutnisky (1), Analía G Karadayian (2), Silvia Lores Arnaiz (2), Georgina Rodríguez De Lores Arnaiz (1)
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Neurotensin is able to modulate ionic gradient equilibria through neuronal membranes because it inhibits the activity of the sodium pump. Some properties of Na^+, K^+-ATPase are modified by the administration of L-NAME (Nω-nitro-L-arginine methyl ester), an inhibitor of nitric oxide synthase (NOS), and by levocabastine, an antagonist for neurotensinergic NTS2 receptor. In the search of a relationship between the activity of neurotensin NTS2 receptor and nitric oxide (NO) synthesis, levocabastine was administered to rats and the activity and expression of NOS were evaluated. Wistar rats injected (i.p.) with levocabastine (50 μg/kg) or saline solution (controls) were decapitated 30 min, 18 or 36 hours later. Cerebral cortices were processed by differential and sucrose gradient centrifugation to obtain synaptosomal membrane fractions. Nitric oxide production by NOS was measured following the oxidation of oxyhemoglobin to methemoglobin at 577-591 nm in a double-beam dual-wavelength spectrophotometer. Neuronal and inducible NOS expression were evaluated by Western blot assays. In synaptosomal membrane fractions NOS activity decreased 46% and 74% at 30 min and 18 hours after levocabastine administration ($p<0.05$). These changes were reversible in time. *In vitro* incubation of control synaptosomal membranes with 1 μM levocabastine also decreased the activity of NOS. At 30 min and 18 hours after levocabastine administration the expression of neuronal NOS protein decreased by 18% and 56% ($p<0.05$). These changes did not reverse in time. Concomitantly, an enhancement of inducible NOS expression was recorded after levocabastine treatment. Altogether, the results allow us to suggest that the NTS2 antagonist levocabastine markedly modified NOS activity and expression at CNS synapses, showing a possible interrelationship between the activities of neurotensinergic and nitrergic systems.

Keywords: cerebral cortex, nitric oxide synthase, synaptosomal membranes, neurotensin receptor, levocabastine

(951) **KETAMINE EFFECT ON CALCIUM CONCENTRATIONS AND CELL METABOLISM IN PC12 CELLS**

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Ketamine an inhibitor of NMDAR; has coupled ion channels highly permeable to calcium ions. Changes in intracellular [Ca^{2+}], are essential for neurophysiological processes, being indicators of physiological functions in neurons. This work evaluates the changes in [Ca^{2+}] induced by depolarization after 24 h exposure of PC12 cells to 100, 500 and 1000 μM ketamine. Ketamine effects on mitochondrial membrane potential ($\Delta\Psi m$) and nitric oxide (NO) levels were also analyzed. Methods using, Fluo-4 AM, epifluorescent microscopy, multiple-label counter and flow cytometry were used. Micrographies quantification showed that ketamine 100 μM and 500 μM decreased the number of positive fluorescent cells. Meanwhile, 1000 μM ketamine showed highest fluorescence intensity, with highest values of positive fluorescent cells, being significantly different from control cells ($p<0.05$). The results obtained by multilabel counter GloMax®-Multi Detection System, and by flow cytometry showed that low ketamine concentrations decreased the calcium fluorescent signal significantly ($p<0.05$) as compared with control, and similar differences were recorded with both methods, being in absolute intracellular [Ca^{2+}], for control, 100 and 500 μM ketamine: 612±60 nM, 239±15 nM and 258±8 nM versus 567±33 nM, 99±10 and 118±7 nM respectively for each method. With 1000 μM ketamine the absolute intracellular [Ca^{2+}] was significantly ($p<0.05$) different as compared with control, being 1035±234 nM and 2175±443 nM for the two methods respectively, and was accompanied by cellular morphology typical of apoptotic death as predicted by SSC and FSC. Metabolic alterations, with a significant decrease ($p<0.05$) in $\Delta\Psi m$ and NO levels were observed after all ketamine doses as compared with control. We conclude that ketamine has consequences on intracellular calcium homeostasis, probably mediated by NMDARs blockage, inducing drastic changes in neurons.Keywords: ketamine, calcium concentration, mitochondria and nitric oxide.

(219) **EARLY ANALYSIS OF CEREBELLAR MICROVASCULAR AND ASTROCYTIC DAMAGE BY SHIGA TOXIN 2**

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Shiga Toxin 2 (Stx2) is secreted by enterohaemorragic *Escherichia coli* (EHEC) and produces damage in several organs of the human body, including the cerebellum. The toxin travels through the blood stream and reaches several organs. The objective of this work is to observe whether Stx2 reaches the parenchyma of the cerebellum, and once in, if it can produce cell damage. For this work, NIH male mice were used. They were divided and injected intravenously in two groups (n=4): 1ηg/mice of Stx2 or saline solution (control group). Two days after the toxin was injected, they were sacrificed and their brains were fixed with PFA 4%. Then, their cerebellums were extracted and cut in slices 20μm thick. They were then subjected to histofluorescence technique with lectins to determine the microvasculature profile, anti-GFAP (glial fibrillary acidic protein) to identify reactive astrocytes and anti-Stx2 to identify immunopositive Stx2 cells. The group treated with Stx2 showed a decrease in the area occupied by the microvasculature (18.27 ±0.57 AU control and 5.77 ±0.2 AU Stx2, in IOD) along with an increase in the expression levels of GFAP (20.27 ±0.54 AU control and 36.35 ±0.95 AU Stx2, in IOD) $p<0,01$. The presence of Stx2 was detected in the Stx2 treated group and not in the control one, in the cerebellar cortex. This work proved that Stx2 damages the microvasculature of the cerebellum, and it produces astrocytary reaction (hiperthrophy and hyperplasia), which suggests a disruption on the Blood Brain Barrier of the cerebellum, and damages its parenchyma.

Key Words: Purkinje, Gfap, Damage, Cns, Immunofluorescence

(669) **NEURODEGENERATIVE EFFECTS OF THE CYANOTOXIN Β-N METHYLAMINO-L-ALANINE (BMAA) ON RETINAL NEURONS**

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The non-proteic aminoacid BMAA is a cyanotoxin released by many cyanobacteria occurring in most dams and water resources around the world. Human chronic intake of this toxin has been linked with the development of neurodegenerative diseases, like Amyotrophic Lateral Sclerosis (ALS); Parkinson and Alzheimer Disease. Multiple studies have shown the effects of BMAA on live animals; however, its effects at the cellular or molecular levelsía are still mostly unknown. We here investigated the effects of BMAA on retinal amacrine and photoreceptor neurons and Muller glial cells (MGC) in vitro. We incubated pure neuronal cultures and mixed neuro-glial cultures obtained from newborn rat retinas, with 400 nM BMAA for

2 days. We then evaluated cell death and apoptosis by Propidium Iodide (PI) TUNEL assays, and DAPI staining. Mitochondrial activity was assessed by using Mitotracker and cytoskeleton integrity and axonal outgrowth were analyzed by immunocytochemical methods. In `pure neuronal cultures, BMAA increased the percentage of apoptotic amacrine and photoreceptor neurons, from 21.6% to 64.5% and from 29.3% to 65.7%, respectively, in controls and BMAA-treated cultures. Noteworthy, functional mitochondria decreased from 55% to 35% in photoreceptors but only slightly in amacrine neurons. In addition, BMAA treatment disrupted the organized assembly of tubulin in axons. BMAA addition to mixed neuro-glial cultures induced lamellipodia retraction and loss of mitochondrial membrane potential in MGC, but did not increase their cell death. Moreover, MGC partially prevented neuronal death.

These results suggest that BMAA induces subcellular changes in both neurons and glial cells, and markedly affects the viability of retinal neurons, confirming its threat to human health as a potential inducer of neurodegenerative damages.

Keywords: BMAA, cyanotoxins, apoptosis; retina neurons; glial cells

(206) **PHOTODAMAGE INDUCES MITOCHONDRIAL QUALITY CONTROL DYSREGULATION IN RETINAL PIGMENT EPITHELIUM.**
IMPLICATIONS FOR AGE-RELATED MACULAR DEGENERATION

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Age-related macular degeneration (AMD) is a late-onset neurodegenerative retinal disease. Accumulated lipofuscin fluorophore A2E in retinal pigment epithelium (RPE) confers susceptibility to blue light-mediated damage, a risk factor for AMD. Cells have developed well-coordinated Mitochondrial Quality Control (MQC) mechanisms in order to limit mitochondrial injury induced by oxidative stress, ensure mitochondrial integrity and preserve cellular homeostasis. RPE dysfunction is a critical event in AMD. Thus, our study was designed to evaluate MQC status, including mitochondrial dynamics (fusion/fission) and mitophagy, under photooxidative damage conditions in RPE.

Light-emitting diodes irradiation (λ=445nm; 1.7mW/cm^2, 30min) significantly reduced the viability of both non-pigmented and 10 or 25µM A2E-containing human RPE cells (MTT assay: 52%, 46%, 31%, respectively) as well as increased mitochondrial anion superoxide production (MitoSOX). A2E (25μM), along with blue light, induced apoptosis measured by Annexin V/PI-flow cytometry (6-fold, $p<0.001$) and by procaspase-3 levels (38%, $p<0.01$). Photodamage produced fusion/fission imbalance towards mitochondrial network fragmentation in non-pigmented cells (72%) and 25µM A2E-fed cells (92%) ($p<0.01$) which correlates with deregulation of mitochondria-shaping proteins levels. We detected a dramatic rise in the expression of fission protein DRP1 and a decrease in the mitochondrial fusion and anti-apoptotic OPA1 protein levels. Mitochondrial $\Delta\phi m$ loss activates OMA1, a MQC protease that cleaves OPA1 to inhibit fusion and facilitate apoptosis. Particularly, A2E and blue light stimuli lead to OMA1 activation, revealed by it self-cleavage (5-fold, $p<0.01$). Finally, TOM20 and LC3 ICC analysis suggested an altered mitophagy. Our work reveals for the first time, that photodamage cause RPE dysfunction through MQC dysregulation which may contribute to AMD pathology. Moreover, the possibility to develop MQC-based therapies is outlined.

Keywords: AGE-RELATED macular degeneration, A2E, blue light, mitochondrial quality control, retinal pigment epithelial cells

CARDIOVASCULAR AND RESPIRATORY SYSTEMS 2

(1205) **HEART MITOCHONDRIAL FUNCTION IN RATS WITH THYROID DISORDER AND HYPOVOLEMIA**

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Cardiovascular system regulation would be crucial in pathological situations such as hypovolemia. The actions of thyroid hormones would be relevant during this condition considering its actions on cellular respiration and mitochondrial function. The aim of the present work was to examine the effect of thyroid state on rat heart mitochondria function during hypovolemia. Sprague-Dawley rats aged 2 months old treated with T3 (hyper, 20 µg/100 g body weight) or 0.02% methimazole (hypo, w/v) during 28 days. Hypovolemic state was induced by a loss of 20% of blood volume during 2 minutes. Hearts were removed for mitochondria isolation and determination of O2 uptake, enzyme activity of complex I and protein levels (mt-NOS, akt T and akt P). The malate-glutamate-supported state 3 respiration decreased and increased in hypo and Hyper rats. Hemorrhage did not change this parameter. Malate-glutamate-supported state 4 did not change. No differences in succinate-supported state 4 and 3 respiration were observed in all groups. Hypothyroidism increased nNOS protein levels. This protein levels did not change in Hyper animals. Withdrawal decreased and increased nNOS protein levels in hypo and Hyper rats, respectively. Hyper increased complex I activity and hemorrhage did not change this activity. No differences were observed between Eut and hypo rats. Hemorrhage only increased complex I activity in hypo group. Thyroid disorders increased aktT protein levels. Hemorrhage did not change this parameter. Hyper increased akt P protein levels meanwhile hemorrhage induced a decreased of this protein levels. In hypothyroidism the lowest NO production would be responsible for increasing cellular respiration and guaranteeing the supply of oxygen to the tissues. The opposite would happen in the hyperthyroid where high levels of NO try to decrease the oxygen consumption as in the euthyroid. Alterations of complex I activity as well as AKT pathway could mediate these effects.

(615) **INFLUENCE OF NITRIC OXIDE AND ADRENERGIC STIMULATION ON THE CARDIOPROTECTION OF HYPOTHYROIDISM IN RAT HEARTS EXPOSED TO ISCHEMIA-REPERFUSION**

María Inés Ragone, Lucía Clavelino, Matías Bayley, Alicia Elvira Consolini
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Hypothyroidism (HypoT) reduced the stunning in severe ischemia-reperfusion (I/R), but increased the sensitivity of mitochondria to the Ca^{2+} overload-induced dysfuntion (SAFE 2016). The aims of this work were to evaluate whether HypoT cardioprotection was due to nitric oxide (NO) release, and if it remains under more physiological adrenergic conditions. HypoT was induced by drinking methimazole (0.02%) for 15 days. Isolated hearts were perfused inside a calorimeter at 37°C to measure left ventricular pressure (LVP, in mmHg) and total heat rate (Ht, in mW.g^{-1}) during the exposition to severe I/R (30 minI/ 45min R). The role of NO was evaluated by perfusing HypoT hearts with 30 μM L-NAME (to inhibit NO-synthase) before I/R. L-NAME improved the postischemic contractile recovery (PICR) to 84.8 ± 6.7% of initial pressure (Pi) ($p<0.05$ vs 53.9 ± 4.7% in non treated C-HypoT, n=8-5) and total muscle economy (Eco=P/Ht) to 4.0 ± 0.7 mmHg.g.mW^{-1} (vs 2.8 ± 0.3 mmHg.g.mW^{-1}) without changing the diastolic tone. Nevertheless, the treatment with 10 μM nitroprussate (NO-donor) did not change the behavior of HypoT hearts on stunning (n=5). Moreover, perfusing 50 nM adrenaline before I/R reduced PICR to 20.0 ± 7.9 % of Pi (vs 53.9 ± 4.7 % in HypoT-C, $p<0.05$, n=6-5) and Eco to 1.2 ±0.6 mmHg.g.mW^{-1} (vs 2.85 ± 0.3 in C-HypoT) with diastolic contracture (ΔLVEDP = 7.8 ± 0.5 mmHg, $p<0.05$ vs 0). Results suggest that: a) The NO release is not a cause of the cardioprotection of HypoT; b) There would be