Assessment of manganese chloride doses in behavioral and MRI experiments

Aguilar Moreno J. Alejandro; Gasca Deysi; Ortíz Juan; Alcauter Solórzano Sarael; Paredes G. Raúl G. Instituto de Neurobiología (INB), UNAM Campus Juriquilla,Querétaro.**Correo electrónico:***josue_aguilar@ucol.mx;***Cel.***3121006507*
Área: Cognición y Comportamiento

*Introduction:*Manganese enhanced magnetic resonance imaging (MEMRI) is a technique that provides structural and functional brain activity byenhancement of magnetic resonance(MR)signal. Manganese (Mn^{+2}) is an analogue of calcium (Ca^{+2}), that can enter the excited cells through Ca^{+2}dependent channels. Since $Mn^{+2}$is a paramagnetic ion, it modifies the local magnetic field, changing the longitudinal relaxation time (T1) of the tissue where accumulates, producing contrast in MR images. There is evidence indicating that at high doses Mn^{+2} could lead to neurotoxicity effects, affecting the motor skills of the subject with Parkinson's disease-like symptoms.
*Objective:*For the adequate use of MEMRI, we first need to find a dose that does not affect the behavior of the subject and that has enough MR contrast to identify the active structures during thebehavioral (in this casecopulatory)sessions.
*Method:*We use 45 female Wistar rats, 250-300grs, without previous sexual experience. They were ovariectomized and supplemented with hormonal treatment and randomly assigned to the following groups: Control (saline), $MnCl_2$ 8mg/kg and $MnCl_2$ 16mg/kg. Females were tested for sexual behavior in conditions where they control the rate of sexual interaction (paced) for 30 minutes.Immediately thereafter they were exposed for 30 minutes to a runningwheel.Finally, they were evaluated in a rotarod. Subjects were testedin the same behavioral sequence once a week for 10 weeks. $MnCl_2$ was administered s.c. on sessions 1, 5 and 10. An additional group was injected with the same $MnCl_2$indicated doses and tested for sexual behavior in the same time frame and scan in weeks 1, 5 and 10.
*Results:*The results show that 16mg/kg of $MnCl_2$does not affect sexual behavior, running wheel or the rotarod test, indicating that this dose can be used for future studies becauseit does not produce behavioral alterations and we obtained a good MR signal.

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Striatal Circuits on Behavioral Flexibility

Alatriste-León Hector, Linares-Garcia Iván, Ramírez-JarquínJosué Orlando, Tecuapetla-Aguilar Fatuel.
Instituto de FisiologíaCelular, UNAM.
Circuito Exterior s/n Ciudad Universitaria, Coyoacán, 04510 México D.F.
http://www.ifc.unam.mx/investigadores/fatuel-tecuapetla
Tel. 55 56225585; Correo: halatriste@email.ifc.unam.mx

The striatum receives the mayor input to the basal ganglia circuits;it has been involved in learning motor skills, habits and goal directedbehaviors. This nucleus is mainly composed of GABAergic projections neurons (95%)and a small proportion of interneurons, of which ~3% are cholinergic. The cholinergicinterneurons and GABAergic projections neurons arenecessary for learning, mediated by reinforces andresponse to relevant stimuli. Recently the cholinergic system had been suggested tocontrol flexibility mechanisms of behavior (the ability that allow animal toadapt to changes in contingencies), crucial for survival.

The aim of this study is to evaluate the activity and the contribution of the striatal cholinergicinterneurons and striatal sub-circuitson theflexibility of behavior.

To evaluate specifically the dynamics of striatal circuits (Cholinergic interneurons and striatal projection neurons: D1 or A2a) on theflexibility of behavior, we standardized a head-fixed switch task to record the striatal activity *in vivo* by monitoring of calcium imaging (using GCaMP6f) and/or electrophysiological recordings.

As preliminary results I will present 1) the head-fixed switch task, showing that mice are able to identify and modify its behavior following the change to a new contingency. 2) The monitoring of GCaMP6f on D1 and A2a neurons and 3) The attempts to record the activity of the cholinergic interneurons in vivo. 4) Optogenetic manipulations of the cholinergic system showing his necessity for the detection of changes in contingencies.

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Title: D-serine administration restores age-related effects on cognitive flexibility and attention in rats.

Calero Vargas Isnarhazni Alejandra, Ramírez-Hernández Noemi, López-Hidalgo Mónica.

Universidad Autónoma de Querétaro. Facultad de Medicina. Departamento de Investigación Biomédica. Clínica del Sistema Nervioso Central.
Dirección postal: Cerro de las campanas S/N. Colonia Las Campanas. Querétaro, Querétaro. CP. 76010
Teléfono: 5571463284
Dirección electrónica: Alejandra1741@hotmail.com.

D-Serine is a D-aminoacid that is considered the main endogenous co-agonist of the NMDA Receptors (NMDAR). D-serine is essential for the induction of many types of synaptic plasticity, and cognitive processes such as learning and memory. While in young rats D-serine levels are highly concentrated saturating their receptors, in old rats D-serine isdecreased. In agreement with this,there is an impairment of cognitive functionsassociated with aging which raises the question about the role ofD-serine in the aged-related deficits on cognitive functions. The aim of this study was to analyze the role of D-serine incognitive flexibility and attention in young (6 month) and middle-age (12 months) rats. First, we characterized the performance of 6 and 12 monthsrats. As we expected, middle-age rats have a decreased in cognitive flexibility an attention scores when compare with 6 month old rats. Interestingly, oral administration of D-serine (300 mg/kg of body weight) improved the cognitive performance of 12-month rats, making it comparable to younger rats. This results suggest that age-related deficits associated with aging is due (at least in part) to a decrease in D-serine levels.

Expression of synaptophysin in monoparental/biparental prairie voles after pair-bonding formation.

Rebeca A. Cortés-Sánchez[1], Francisco Camacho[1], Néstor Díaz[2], Larry J Young[3], Raúl Paredes[1], Wendy Portillo[1]realcrs752416@gmail.com 4421295506.

[1]Instituto de Neurobiología, Universidad Nacional Autónoma de México[2] Instituto Nacional de Perinatología Isidro Espinosa de los Reyes. [3]Department of Psychiatry and Behavioral Sciences, Center for Translational Social Neuroscience, Yerkes National Primate Research Center, Emory University.

The prairie vole (*Microtus ochrogaster*) unlike mice and rats is characterized by lasting pair-bonding formation and biparental care (BP) towards the pups. Previous studies have demonstrated that the absence of the father (monoparental care, MP) can affect the development and adult social behavior of the pups. When MP pups reach adulthood, they have a reduced care towards their own pups, also both males and females take a longer time, to form pair-bonding. The physiological and plastic changes that may explain these behavioral differences are not well understood. On the other hand, synaptophysin (SYP) is marker for synaptic plasticity. Previous studies have demonstrated that a reduction in parental care during the first stages of postnatal development decreases significantly the expression of SYP in rats.

The aim of this study is to evaluate if the absence of paternal care reduces the expression of SYP as well as inhibiting pair-bonding formation. We use 12 males and 12 females with no previous sexual experience to form mating cages. After mating the couples were divided in monoparental families (MP, n=7), in which the males was removed at gestational day 18th, and biparental families (BP, n=5) in which the male remain with the female during all the experiment. At postnatal day 21 the pups were removed from the family cage and left undisturbed until they were 3 months old. For the partner preference test (PPT), females were ovariectomized and received an estradiol treatment throughout the experiment; both males and females were assigned to a sexual partner, not participating in the study, and let them cohabite and mate for 24hrs. The next day we performed the PPT to evaluate if they preferred their assigned partner (P) or a strange (S) prairie vole, 24 hours after the PPT the animals were sacrificed in order to recollect the brain. Accesory olfactory bulb and nucleus accumbens brain sections were processed byinmunofluorescence to visualize SYP.

Our preliminary results demonstrate that BP males show a preference towards their mate (p=0.068) whereas MPshows a preference towards the S (p=0.08). No difference where found either in MP females nor BP females (p=0.22, p=0.08 respectively).Currently we are performing the immunofluorescence and the densitometric analyze; we hypothesize that a low expression in SYP will explain the lack of pair-bonding formation in MP prairie voles.

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Development of a home-made system to quantify physical activity in an experimental rodent model.

Roberto Cuevas-Olguin, Teresa Mares-Barbosa,Marcela Miranda-Morales,Griselda Garcia, Eric Esquivel-Rendon, Jorge Vargas-Mireles,Gabriela,González, Marco Atzori
Universidad Autónoma de San Luis Potosí, Facultad de Ciencias, Programa de Biología, SLP, México
Contacto: marco_atzori@hotmail.com, Avenida Salvador Nava sin número, Zona Universitaria, San Luis Potosi, SLP, 78290, Mexico
Tel.: 444 1739 256, marco_atzori@hotmail.com

An important parameter in animal models of neuropsychiatric disease is physical, motor activity. Several commercial systems are available to carry outsuch a task, using different set-ups and a variety of physical measurement, like path length run in a fixed amount of time, mean speed of the head of the animal, and other parameters. These systems are available to the researcher at considerable cost, with the further drawback of low flexibility, and additional high cost and long waiting for maintenance. We developed a system for measuring the physical activity of mice, based on infrared beam sensor switches and a joy-stick-based interface to measure two parameters:1) the number of revolutions of a wheel inside the cage, 2) the number of crossing in the shorter midline of the cage. The system is made up by a PC connected to a joy-stick based interface which, in turn, is connected to 6 cages in which –correspondingly- up to 6 mice are individually hosted. The use of the joy-stick interface allowed the use of free libraries which are customary part of the endowment of any Window-based PC operating system, being Window 7 the system that we used. Visual Basic 2010 was used interface platform because of the existence of extensive libraries for control of system-pc interface. A graphic interface was created using the same software, to allow individual users to access the program by starting it, terminating its execution, as well as copying data “on fly” and store them on a portable USB drive (or anywhere else).Data are automatically stored at customer selected intervals (we used every 60 minutes), both in the local hard drive, as well as in a remote Dropbox location. Although we only stored data for continuous experiments for up to 2 weeks, the only storage limit of the system is given by the size of its hard drive. A system log file is generated per each individual cage. Each file is structured with one line per motor event. Each line displays: serial number of the event, number of the activated sensor (wheel revolutions or cage-crossings), time of occurrence in milliseconds. Acquisition records so far display and quantify circadian motor activity with excellent agreement with the dark-light phases, with counts in the order of up to hundreds of thousands per day. The system is being used to carry out research on the effects of various types of stress on experimental animal physical activity.

Area: Cognicion y Comportamiento.

Evaluation of the risk behavior and its relation with the academic yield in students of Faculty of Chemical Sciences BUAP

[1] Fortis Valera Monserrat, [2]Alatriste Bueno Victorino
[1,2]Facultad de Ciencias Químicas BUAP
[2] Laboratorio de Neuroendocrinología. Facultad de ciencias Químicas BUAP. Av. San Claudio y 14 sur, sin número. Teléfono 01 (222) 2 29 55 00 e-mail Victorino.alatriste@correo.buap.mx

Área 12, cognition and behavior.

It's described to the risk behavior as the biggest probability of negative consequences for the health having factors like: individual, familiar and community factors. This one is due to the meso limbic circuit that uses dopamine as main neurotransmitter, and includes projections from the tegmental ventral area, grooved body, limbic structures (amygdala) and to the prefrontal crust it is activated provoking a dopamine liberation in the nucleus accumbens. All of these things are generating an intense pleasure sensation, motivating to the subject to the repetition of the above mentioned activities affecting with these its physical and mental integrity. Due to this, this type of conducts are considered of scientific interest.

The target of this job was determine the main conducts of risk carried out by students of the faculty of chemical sciences-BUAP and how they influence on your academic performance. For this we use a test of identification of risk behavior destined to a population of 200 students chosen randomly. Once identified the main risk behavior carried out by the population we divide the populations in groups of men and women by ages for 19 to 24 years and we realize a survey to know the risk behavior with more incidence in the groups, inside of the survey we include the academic average to know if the academic yield turns out to be affected in accordance with the realized conducts.

In the identification test we obtained that the main risk conducts are: not to sleep, not to eat, to consume alcohol and others (not to study/not to enter to classes), of which across the survey we could observe that the biggest incidence of conducts of risk is in the 20 years-old population, followed by the 19 and 21 years-old groups, in the groups from 22 to 24 years one could appreciate the notable decrease of these conducts. With regard to the academic average we obtained that this one decrease in 50%.

Based on the obtained results we can conclude that the risk conducts are a factor that affects to the individual in the academic yield, being predominant in the population from 19 to 21 years and that they are diminishing to the same way as the age increases. This maybe due to the fact that as we are growing up, the prefrontal crust responsible for the regulation and the control of conduct, it matures increasing the estimation of risks, while the aftereffects in the academic yield this maybe due to the oxidative stress which affects to the capacity of memory and learning of the subject.

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Optogenetic manipulation of the direct and indirect pathways of the basal ganglia: dorsomedial striatum *versus* dorsolateral striatum.

Llanos-Moreno Argelia, Cuevas-Vicente Nisa, Ramírez-Jarquín Josué, Tecuapetla Fatuel.

Instituto de Fisiología Celular, UNAM.
Circuito Exterior s/n Ciudad Universitaria, Coyoacán, 04510 México D.F.
http://www.ifc.unam.mx/investigadores/fatuel-tecuapetla
Tel. 55 56225585; Correo: allanos@email.ifc.unam.mx

The basal ganglia is a system of subcortical nuclei involved in the selection of actions and the permance of voluntary movements. Within the basal ganglia, the striatum contains the cell bodies of the two communication pathways of this system; the direct pathway and the indirect pathway. According to the classical model of the operation of these pathways, the first promotes the movement, and the second decreases it. In recent years evidence testing these model presents some inconsistencies with this interpretation, which may be explained if the atributed function of these pathways is not generalized and we identified their specific contributions in sensorimotor *versus* association circuits within the striatum.

In this study, we performed optogenetic manipulations (activation and inhibition using channelrhodopsine or archeorhodosine stimulation, respectively) of the direct and indirect pathways of the basal ganglia in the compartments that harbor the sonsoriomotorcircuits (dorsolateral) *versus* associative compartments (dorsomedial) of the striatum during an action selection task or on locomotion.

As preliminary results we will present the results of the manipulations of the direct and indirect pathways neurons in the dorsomedial versus the dorsolateral striatum; the dorsolateral striatum manipulations contributs on the performance and on the instauration of a habit while the dorsomedial striatum manipulations contributes to the performance and selection of actions. Similarlly the manioulations of the striatal pathways on these compartments during locomotion showed pathway and compartment specific effects.

These results suggest that the functions of these pathways can´t be generalizated, the functions of these pathways depend on wich striatal compartment is being manipulated.

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Sexual hormones are not sufficient to achieve high sexual receptivity in female mice, sexual experience is required.

P. Marco-Manclús, R. G. Paredes, W. Portillo; Laboratorio de Plasticidad y conducta sexual, INB (UNAM)
Marman6288@gmail.com, Boulevard Juriquilla 3001, 76230 Queretaro, QRO 4422381060

Female mice show low levels of sexual receptivity (SR) toward males in their first sexual experience. SR depends on sexual hormones but is not clear if it also depends on sexual experience. The Lordosis posture is indicative of SR and can be used to quantify the receptivity of females: Lordosis Quotient (LQ) = Lordosis displayed/Mounts displayed. The aim of the study was to evaluate if supraphysiological doses of estradiol benzoate (EB) and progesterone (P) are sufficient to increase SR, and evaluate if sexual experience induces changes in the activity of the brain areas that modulate this behavior such as: Accessory olfactory bulb (AOB), Medial Preoptic Area (MPOA), Ventromedial Hypothalamus (VMH), and Anterior Cortical Amygdala (ACo). For this study 60 CF1 ovariectomized female mice were assigned to one of the following groups: a) Experienced females (n=20), which received 6 mating sessions; b) Unexperienced females (n=18), which receive only 1 session; c) Naïve females (n=18), which had no previous sexual experience; and d) Supraphysiological group (n=6), which also received 6 mating sessions. Females from groups a-c, were primed with 1 µg of EB and 100 µg of P, 48h and 4h respectively, before the test, females from group d, receive 10 µg of EB and 1 mg of P. Sexual behavior tests lasted 1h and LQ was registered. After the 6th test each group was divided in 3 other subgroups: i) Mating group, which receive an extra mating session; ii) Olfaction group, which was exposed to bedding from a male's cage; and iii) Control group, which was exposed to clean bedding. Each subgroup was exposed to the correspondent stimulus for 90 min, then animals were euthanized and perfused, and their brains were collected and sliced (20 µm thick). Brain sections were immunostained for c-Fos. Our behavioral results showed that females increase their LQ with repeated sexual experience, the LQ of the first session of the supraphysiological group was low and indistinguishable from the other groups ($p=0.457$). Moreover, the Supraphysiological females showed a lower increase in their LQ over sessions, and apparently males lost interest in these females, because they did not mount them in later sessions. We also found that sexual experience increases the activation of cells in the VMH, and decreases in the MPOA in Inexperienced females (but not in the Experienced ones). Exposure to male odorants increased cell activation in the AOB, but this increase was not seen when females mated. The exposure to male odorant also caused a reduced activation in the ACo suggesting a possible inhibition in this structure. Our results suggest that although sexual hormones are needed to induce sexual receptivity the high levels of this behavior requires some plastic change, as suggested–by the changes observed in c fos expression. This research was supported by grants CONACYT 252756, 167101, 253631; Fronteras 374; UNAM DGAPA- PAPPIT IN203615, IN210215. We thank Francisco Camacho, Martin Garcia, Alejandra Castilla and Deisy Gasca for their technical assistance.

Deficits in cognitive performance are associated with a reduction in serum levels of transaminases in humans

Ramírez-Hernández Noemi, Calero-Vargas Isnarhazni, Reyes-López Julián, Arias García Nallely Amaranta, Riveroll Romero Roberto, López-Hidalgo Mónica

Universidad Autónoma de Querétaro. Facultad de Medicina. Departamento de Investigación Biomédica. Clínica del Sistema Nervioso Central.
Clavel 200, Col. Prados de la Capilla. CP 76176
noemirh_92@hotmail.com
4424666984

The scientific and medical advances has increased the life expectancy of the population. It is expected that by 2050, more than 20% of the world population will be older than 60 years. Associated with aging, there is a decrease in cognitive functions which is associated with a decreased in glutamatergic transmission. Glutamate levels in the cerebrospinal fluidare positive correlated with the levels of serum glutamate as well as with transaminase enzymes (GOT and GPT), enzymes in charge of peripheral glutamate metabolism. Because glutamate levels are determinant for cognitive performance and transaminases could reflect the amount of central glutamate, it would be possible that there is a correlation between transaminase levels and adeterioration of cognitive functions in aged subjects. To answer this question, the levels of GOT and GPT in healthy subjects between 60-80 years old were determined. In parallel, cognitive functions such as working memory, attention, orientation, executive functions, IQ, among others, were evaluated using neuropsychological tests. Preliminary results showed a negative trend of association between attention and executive functions performance with age and level of education. Also, a positive trend of association between Attention and IQ with education level were observed. Interestingly, the cognitive performance(Attention, memory, executive functions, etc) have a positive trend of association with serum levels of transaminases.

Operant conditioning paradigm in head-fixed rats for yuxtasomal recordings in the cerebral cortex

[1]Santana Chávez Gabriela (graduate student), [2] López- Hidalgo Mónica, [1] Olivares-Moreno Rafael, [1]Rojas- Piloni Gerardo.

[1]Departamento de Neurobiología del desarrollo y Neurofisiología, INB UNAM Campus Juriquilla, Querétaro, México.

[2] Clínica del Sistema Nervioso, Facultad de Medicina UAQ. México

Laboratorio de integración sensoriomotora, INB, Campus UNAM Juriquilla Boulevard Juriquilla 3001. Queretaro, Qro. CP 76230. Tel.56234027, mel_schag@hotmail.com

The Head-Fixed technique, in combination with operant conditioning paradigms, involving skilled movements (reach, leap, etc.) and simultaneously in vivo cell recordings, has been successfully used for the analysis of neuronal function and behavior. Considering the multiple uses of this technique, and the natural ability of rodents to execute movements with their forelimbs, here we develop a behavioral task involving planning and execution of a qualified movement under Head-Fixed conditions (lever pressing) with the left forelimb. Wistar rats with acute water deprived were operantly conditioned in daily sessions of 1 hour to perform pressing movements in response to a visual cue. In order to simplify the learning, the task was presented in successive approximations: in the 1st stage, the rats learned to touch a standard lever to acquire the reward (water); in the second stage, the rats learned to associate the cue (light) with the reward reached 80% of efficiency after 20 consecutive sessions. Lower learning efficiency were observed using saccharine as a reward. In the 3rd stage, head-fixed were associated with the reward. Finally, in the 4th stage the rats learned to associate the cue with the reward in head-fixed condition. Our results show that the performance of an operant conditioning task, under Head-Fixed conditions are similar to the free-moving animal tasks and that the presentation in successive stages of conditioning simplifies the adaptation of the animals to the task.

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Can Autophagy Contributeto the Mouse Neural Tube Closure?

Pilar Sarah Acevo-Rodríguez, Diana Escalante-Alcalde and Susana Castro-Obregón

Departamento de Neurodesarrollo y Fisiología, División de Neurociencias, Instituto de Fisiología Celular, Universidad Nacional Autónoma de México (UNAM).Circuito Exterior S/N Ciudad Universitaria, Coyoacán, 04510 Ciudad de México, D.F. Tel (52) (55) 5622 5676. scastro@ifc.unam.mx

Autophagy has been described classically as a lysosomal catabolic process, but it also has other less known functions: it promotes type II programmed cell death, mediates protein traffic to plasma membrane and secretes proteins in an unconventional way. Autophagy has been associated with many processes throughout mammalian development, from implantation to osteogenesis, but their role during early neural development is still unclear.

In vertebrate development the primordium of central nervous system forms in a process known as neurulation. In this process, at the dorsal part of the embryo, the neuroectoderm in proximity to the notochord thickens and flexes at a medial hinge point, forming two concave walls that extend along the embryo'santerior-posterior axis.Later, the dorso-lateral portion of the walls bents causing their tips to meetat the midline and fuseforming a hollow cylinder known as the neural tube. When the neural tube does not form properly neural tube defects occur. Amongthese disorders, anencephaly and spina bifida have the highestincidence in newborns.Autophagy seems to contribute to proper neural tube closure, as the absence of activating autophagy protein (AMBRA1) causes neural tube defects in mouse embryos and lethality. Nevertheless, there are not direct studies to demonstrate whether autophagy, and no other unknown function of AMBRA1, indeed contributes to the fusion of the neural tube, nor what could be the mechanism.

In this work we demonstrate the presence of abundant cells with high autophagic activity along the fusion line of the neural tube of mouse embryos. We will present the results of ex-utero experiments of early embryos, in which we manipulated autophagy to assess alterations in development. The possible mechanism by which autophagy contributes to the neural tube fusion will be discussed.

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CHARACTERIZATION OF THE PROMOTER OF THE*Klf10* GENE IN THE DEVELOPING HYPOTHALAMUS

Garduño-Tamayo Norma Angélica[1], Pedraza-Alva Gustavo[1], Pérez-Martínez Leonor[1]. Laboratorio de Neuroinmunobiología, [1]Departamento de Medicina Molecular y Bioprocesos, Instituto de Biotecnología, Universidad Nacional Autónoma deMéxico (UNAM), Cuernavaca, Morelos, México.
*Corresponding author:E-mail address: leonor@ibt.unam.mx(Perez-Martinez Leonor) Tel.: +52 5255 5 6227658, fax: +52 52777 3172388.

Summary

Neurogenesis is a crucial process for the development of the central nervous system, it is the process by which neurons are generated and form the brain circuits responsible for receiving, integrating, driving and responding to the environmental stimuli to which the organism is exposed. Neurogenesis is a highly regulated program in which transcription factors play a central role. In the hypothalamus, neuronal populations are organized into nuclei and their main function is to maintain the homeostasis of the organism. Although we know the neuronal phenotypes present in the different nuclei that make up the hypothalamus, we know very little about the molecular mechanisms involved in their differentiation.
Furthermore, one of the main interests of our group has been to identify the molecular signals involved in hypothalamic neurogenesis. Recently, we identified the transcription factor KLF10 as an important part of the differentiation program of TRH (thyrotropin-releasing hormone)expressing neurons. However, the signals that control the expression of *klf10* during embryonic hypothalamic neurogenesis are not known. Therefore, in the present study we aim to characterize the promoter of the klf10 gene in an embryonic hypothalamic context.
As our first approach, we found putative binding sites for 154 transcription factors in the *klf10* promoter using different prediction software. Of these transcription factors11 are known regulators of neurogenesis in other brain regions. In particular, we focused on CREB as it is known to be regulated by neurotrophins such as BDNF and NGF, which play an important role in the developing brain. We identified 9 putative binding sites for CREB in the *klf10* promoter. Using the mHypoE-N1 embryonic hypothalamic cell line and luciferase assays, together with targeted mutations in the *klf10*promoter, and ChIP assays we identified that: i) CREB overexpression induces *klf10* promoter activity, ii) CREB regulates the expression of *klf10*by binding directly to the KPG1 (-254 bp) site present in the *klf10* promoter. Accordingly, we found that the neurotrophin BDNF induces the activity of the *klf10*by the interaction of CREB with the *klf10* promoter, whereas NGF negatively regulates the expression of *kfl10*in our hypothalamic cell line. Taken together our data suggest that *klf10* expression is regulated by neurogenic signals in the developing hypothalamus. Ongoing experiments are aimed at elucidating the impact of *klf10* on the differentiation of the different hypothalamic neuronal phenotypes.

KeyWords: Neurogenesis, Hypothalamus, KLF10, CREB, BDNF, NGF

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Impact of senescent astroglial cells upon neuronal functionality

Morales Rosales Sandra Lizbeth[1,2], González Puertos Viridiana Yazmín[2], Massieu Trigo María de Lourdes[3], Moreno Blas Daniel[3], Gerónimo Olvera Cristian[3], Konigsberg Fainstein Mina[2].

[1] Posgrado en Biología experimental, nivel Maestría, Universidad Autónoma Metropolitana, Iztapalapa (UAM-I), Cuidad de México.

[2] Laboratorio de Bioenergética y Envejecimiento Celular, Departamento de Ciencias Biológicas y de la Salud, Universidad Autónoma Metropolitana, Iztapalapa (UAM-I), Cuidad de México San Rafael Atlixco No. 186, Col. Vicentina, Iztapalapa, 09340, México. Tel. 58044732 mkf@xanum.uam.mx

[3] Instituto de Fisiología Celular, Universidad Nacional Autónoma de México (UNAM), Ciudad de México

Aging is a natural irreversible process characterized for a gradual decline in the physiological functions, which compromise the organisms, and it is also the main risk factor for the development of many diseases such as neurodegenerative pathologies. Neurodegeneration is characterized by a chronic subtle inflammation, a decrease of synaptic contacts, mitochondrial dysfunction and neuronal density loss. The aged world population is dramatically increasing and so are neurodegenerative diseases, therefore it is Important to study the cellular processes implicated in aging.

One of the recognized hallmarks of aging is cellular senescence. This is a cellular state characterized by the loss of proliferative capacity, DNA damage, increased β-galactosidase activity and an inflammatory secretory profile known as SASP. In an aged organism senescent cells accumulate due to the decrease of the immune surveillance. The presence of senescent cells in the central nervous system was recently recognized, and the appearance of senescent astrocytes has been related with age and neurodegenerative diseases. However their exact function is still unknown.

The aim of this work was to study if the neuronal function is affected when the astrocytes become senescent and they secrete the SASP. Here we developed two co-culture models to study neuronal functionality. In the first model cortex neurons were isolated from Wistar rats embryos and co-cultured in transwell chambers with cortex astrocytes from neonatal rats, prematurely induced to senesce by oxidative stress. In the second model the isolated neurons were co-cultured with cortex astrocytes obtained from old rats. These models did not allow the cells to have a direct contact and the effects observed were only due to SASP influence. Then neural mitochondrial functionality and the number of synaptic contacts were evaluated.

Our results showed that neurons that were exposed to the SASP from senescent astrocytes decreased their functionality.

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ß-amyloid 1-42 production in vision related structures on aging and Alzheimer's disease; Could the eye be usefull for early detection of Alzheimer's disease?

Montserrat Pérez-Hernández[1,3], Abigail Torres-Romero[1,3],Uriel Martínez- Hernández[1,2], Roberto González Salinas Ph.D[1], Rosario Gulias-CañizoPh.D[1], Rubén Zamora-Alvarado[1], Lenin Ochoa de la Paz[1], Luis F. Hernández-Zimbrón Ph.D.*[1]

[1]Research Department, Asociación Para Evitar la Ceguera en México, I.A.P. Vicente García Torres 46, Barrio San Lucas, Coyoacán, Cd. México, 04030, Tel. (55)5659-6572. [2]Universidad Autónoma Metropolitana, Unidad Iztapalapa, Ciudad de México [3]Universidad Nacional Autónoma de México, Ciudad de México e-mail: monse.perez.hdez@gmail.com, Corresponding autor: lfhernandez@unam.mx

Introduction. Alzheimer disease (AD) is the most common cause of dementia and neurodegeneration in elderly people.The prevalence of EA is linked to the age, being age the main risk factor. AD is characterized by the formation of senile plaque as well as neurofibrillary tangles. Senile plaques are extracellular and intracellular deposits composed mainly of amyloid beta peptide (ßA), which is a peptide derived from the sequential proteolytic cleavage of the amyloid precursor protein (APP)[1].The proteolytic pathway known as non amylodogenic, involves the action of α-secretasa (ADAM) and γ-secretase complexand are involved in theformation of the non toxic form ßA40. On the contrary, in the amyloidogenic pathway are involved the ß-secretase enzyme (BACE) andγ-Secretase, and this is theresponsible pathway of the production of ßA42 peptide[2]; Itforms autoaggregates that constitute the insoluble fibrils found in the senile plaques which cause neuronal loss and synapses in certain areas of the brain[3].

Recently, it have been recognized that accumulations of βA42 also occurs in glaucoma and age-related macular degeneration (AMD) as well in AD patients. However, there are only few reports showing about the participation of this peptide on neurodegenerative processes in the retina and visual cortex of AD patients and on normal aging. Our research is focused to identify changes in the expression of the enzymes of the non-amyloidogenic (alfa-secretase) and amyloidogenic pathways (BACE, γ-Secretase)in areas associated to vision processes such as retina, optic nerve and visual cortex.

Objective. To evaluate changes in the expression of the enzymes involved in the production of ßA42 peptide in the following areas; visual cortex,optic nerve and retina in aging (C57/BL6, 18 Months old) and in AD triple transgenic mice(3xTg-AD).

Methodology. Double immunofluorescence assays on coronary slides(10µm) of visual cortex, retina and optic nerve of young mice (4M) and old mice (25M), as well aswild type mice (4M) and 18M of 3xTg-ADwere performed. Monoclonal antibodies for each one of the enzymes involved were used ; non amyloidogenic (alfa-secretase); ADAM10, ADAM9; Amyloidogenic pathway (β-secretase pathway); BACE, Preseniline2 (The principal enzyme producing ßA42 peptide).Finally,to verifythe expression changes of these enzymes, qRT-PCR assays for each enzyme were performed using mRNA of the same anatomic regions of the animals used.

Results. The results shows a significant expression increase ofthe enzymes involved in the amyloidogenic pathway (BACE, ADAM 9, Preseniline 2)and a reduction of ADAM 10 on aged mice as well as on 3xTg-AD mice.

Conclusions.1) These models are useful to study the production of amyloid beta in retina, optic nerve and visual cortex in aging and in Alzheimer's disease. 2) The increase of the enzymes involved in the amyloidogenic pathway in the retina, optic nerve and visual cortex, suggest that ßA42 accumulation on these regions is a consequence of the overactivation of this pathway in normal aging and Alzheimer´s Disease. 3) These changes could be associated to the visual loss function observed in AD patients.

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Aging-associated changes in synaptic vesicle recycling

Nelly Rodríguez-Corona[1] and Clorinda Arias[1]

[1]Instituto de Investigaciones Biomédicas, Departamento de Medicina Genómica y Toxicología Ambiental. Circuito Mario de la Cueva s/n, Coyoacán, Ciudad Universitaria, Ciudad de México.
E-mail: rodriguezcorona.nelly@gmail.com, phone:0155-562-29215

Brain function largely depends on the ability of neurons to modify their communication efficiency in response to different stimuli. Neurotransmission is based on the exocytic fusion of synaptic vesicles (SVs) followed by endocytic membrane retrieval and the reformation of SVs. These processes are the main sources of activity-driven energy demands. Age-related cognitive decline likely reflects the manifestation of dysregulated synaptic function and ineffective neurotransmission. Moreover, recent evidence suggests connections between alterations in the presynaptic machinery for endo/exocytosis and aged associated disorders such as Parkinson´s and Alzheimer´s disease. In order to analyze changes in synaptic vesicular recycling process and energy metabolism during aging we have obtained synaptosomes from C57BL/6J mice at different ages. Cortical and hippocampal synaptosomes were isolated and the mitochondrial redox capacity was evaluated by MTT reduction. We found a lower mitochondrial activity of the aged synaptosomes (9-10 and 18-20 months) compared to young(2-3 months). The vesicular recycling analysis was assessed by the incorporation of the fluorescent dye FM4-64,measured in real time by confocal microscopy under basal conditions and after depolarization with 50 mM KCl. At all ages, the dye incorporation increased after KCl depolarization, and this incorporation was totally dependent on external Ca2+, indicating a rapid exocytosis event. However, we found changes in the kinetics of the FM4-64 incorporation between synaptosomes at different ages particularly in the velocity of the SVs exocytosis. After depolarization, the young synaptosomes incorporate FM4-64 faster than synaptosomes from old mice. We also observed significant changes inthe protein content of dynamin I/II (DNM I/II), a protein involved in SVs endocytosis. This protein was significantly decreased in hippocampal synaptosomes of C57BL/6J mice at age of 9-10 months and 18-20 months, compared to mice of 2-3 months. At present these results suggest that aging is associated with functional and structural changes of the exocytosis/endocytosis machinery in pre-synapses. Changes in presynaptic calcium influx or cytosolic ATP level, which would affect either synaptic vesicle fusion or docking of new vesicles, might be responsible for the observed changes in the kinetics of synaptic vesicle recycling.

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Prenatal ethanol exposure alters locomotor activity and selectivelypromotes changes in Met-enk expression in adolescent rats

Hernández-Fonseca Karla[1]; Reyes-Guzmán Aurora Cosette[1]; Abate Paula[2]and Méndez Milagros[1].

1. Departamento de Neuroquímica, Subdirección de Investigaciones Clínicas, Instituto Nacional de Psiquiatría Ramón de la Fuente, Calzada México Xochimilco 101, Col. San Lorenzo Huipulco, CP. 14370 Ciudad de México, México. Tel.:(52)5541605459. Email: kfonseca@imp.edu.mx.

2. Laboratorio de Psicología Experimental, Centro de Investigación en Psicología (CIPSi), Facultad de Psicología, Universidad Nacional de Córdoba, CP 5000 Córdoba, Argentina.

Alcohol reinforcement is partially mediated by the ethanol-induced activation of the endogenous opioid system. Several studies indicate that opioid peptides play a main role in ethanol reinforcement in both animals and humansduring different stages of life, and suggest that ethanol intake during adolescence and adulthood may be facilitated by prenatal ethanol exposure (PEE). However, PEE effects on motor activity induced by an ethanol challenge and the participation of opioids in these actions remain to be understood. This work assessed the responsiveness of adolescent rats to prenatal and/or postnatal ethanol exposure in terms of behavioral responses, along with alcohol effects on Met-enk expression in brain areas related to drug reinforcement. Motor parameters (horizontal locomotion, rearings and stereotyped behaviors) in pre- and postnatally ethanol-challenged adolescents were evaluated. During gestational days 17-20, pregnant rats received water or ethanol (2 g/kg). Adolescents at postnatal day 30 (PD30) were tested in a three-trial activity paradigm (habituation, vehicle and drug sessions). Met-enk content was quantitated by radioimmunoassay in several regions: ventral tegmental area [VTA], nucleus accumbens [NAcc], prefrontal cortex [PFC], substantia nigra [SN], caudate-putamen [CP], amygdala, hypothalamus and hippocampus. PEE reduced rearing responses. Ethanol challenge at PD30 reduced horizontal locomotion and showed a trend to reduce rearings and stereotyped behaviors. PEE increasedMet-enk content in the PFC, CP, hypothalamus and hippocampus, but did not alter peptide levels in the amygdala, VTA and NAcc. Ours results suggest that PEE selectively modifies behavioral parameters at PD30 and induces specific changes in Met-enk content in regions of the dopaminergic mesocortical and nigrostriatal systems, as well as the hypothalamus and hippocampus. Prenatal and postnatal ethanol actions on motor activity in adolescents could involve activation of specific neural enkephalinergic pathways. This work was supported by the Consejo Nacional de Ciencia y Tecnología (CONACyT) (82728).

Behavioral effects of sucrose in Wistar rats: Dose-response studies

Milagros Méndez Ubach and Karina Santiago González.

Departamento de Neuroquímica, Subdirección de Investigaciones Clínicas, Instituto Nacional de Psiquiatría Ramón de la Fuente. Calzada México-Xochimilco 101, Col. San Lorenzo Huipulco, 14370 Ciudad de México, México. Phone: (52) 55 41 60 50 91; Email: ubach@imp.edu.mx.

Nowadays, obesity is a global epidemic threatening people´s physical and psychological well-being. Difficulty in controlling overweight and obesity trends has led to the comparison of obesity with drug addiction, suggesting that some foods possess reinforcing properties similar to ethanol and other drugs of abuse. Loss of control over the consumption of foods rich in sugar could explain the prevailing obesity and overweight epidemic. Current research shows that, under certain conditions, sucrose can trigger behavioral and neurochemical changes similarly to those seen with drugs of abuse. However, at the present time, there are no animal models where the effects of acute administration of carbohydrates on motor activity have been studied. Thus, the aim of this work was to investigate the effects of distinct sucrose doses on motor activity in Wistar rats in an open-field. Horizontal movements, rearings and stereotypes after the intraperitoneal injection of sucrose (0.5, 1.0, 2.0, 2.5 and 3.5 g/kg) were recorded. Ethanol (1.0 and 2.5 g/kg) was used as positive control for comparison, since alcohol possess biphasic behavioral effects; low doses of alcohol induce locomotor stimulation, while high doses cause sedation. All sucrose doses increased horizontal and stereotyped movements at different rates. The effect of high doses was characterized by an immediate rise in activity, lasting a short period of time. In contrast, the effect of low doses was delayed, displaying a longer duration than high sucrose doses. The only sucrose dose that induced an increment in rearings was 2.5 g/kg. These findings suggest that the acute response to sucrose involves behavioral changes that could surpass the effects of a drug of abuse such as ethanol, and support the research in the theory of “food addiction”.

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White Matter Alterations in Heavy Cannabis Users

Authors: Gina Patricia Monteverde Muir, Arafat Angulo-Perkins, Laura Nava-Gómez, Sarael Alcauter.
Institutions: Instituto de Neurobiología, Universidad Nacional Autónoma de México, Querétaro, México.

gmonteve@gmail.com
Gerardo Murillo 17, Pueblo Nuevo, Corregidora, Quéretaro, CP 76900
5536488314

Introduction. Cannabis use has increased in recent years, as well as interest in the different mechanisms in which exposure leads to neural changes that affect diverse cognitive processes. Effects are primarily attributed to δ-9-tetrahydrocannabinol (THC), the main psychoactive ingredient, which binds to cannabinoid receptors in the brain, although more than sixty fitocannabinoids have been identified within the plant. Cognitive alterations have been widely reported in cannabis users, however, alterations in the white matter tracts that may support such functions have not been fully explored (Filbey et al., 2014. Jakabek et al., 2016). This study contributes to better understand the effects of heavy Marijuana consumption on the integrity of brain's white matter. We have made use of an MRI Technique called Diffusion Tensor Imaging (DTI), in which white matter integrity can be compared between groups.

Methods. Participants included 33 heavy marijuana users (at least 16 joints per month in the year previous to imaging); and 35 non-consuming controls. Users reported marijuana as their main psychoactive substance of use, and less frequent use of nicotine cigarettes. High resolution (voxel size of 1x1x1 mm^3) T1 weighted images were acquired in addition to 64 diffusion weighted images in a 3T MR scanner. Images were anonymized and processed using FSL´s Diffusion Toolbox and Tract Based Spatial Statistics. Further processing included selecting and analyzing the different tracts based on John Hopkins University Atlas, also included in FSL.

Results. Voxel-wise analysis of the whole white matter tissue using non parametric tests, showed no significant difference between groups. A linear model was also fitted including group, sex and age interaction as explanatory variables. Significance for each explanatory variable fit was defined as $p<0.05$ after correction for multiple comparisons (around 100,000 voxels) using the Threshold-Free Cluster Enhancement. The groups showed no significant differences in age nor sex proportions. However, when analyzing specific tracts individually, a significant difference was identified in the Left Inferior Longitudinal Fasciculus. Our results support previous evidence suggesting that chronic exposure to fitocannabinoids have localized effects instead of a widespread effects across the brain. These focal effects may be related to the cognitive alterations reported in heavy cannabis users.

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Sympathetic regulation of visceral adiposity and metabolism in a rat model of stress induced by sleep restriction

Lucia Engracia Azuara Alvarez, Adrián Ruiz Báez, Ma. Isabel Casillas Santana, Nadia Saderi, Roberto Carlos Salgado Delgado.

Universidad Autónoma de San Luis Potosí, S.L.P. Facultad de Ciencias.Av. Salvador Nava Martínez S/N, Zona Universitaria c.p. 78290San Luis Potosí, S.L.P., México. Tel. 4443 366194
azuarale@gmail.com, roberto.salgado@uaslp.mx

The reduction of the hours dedicated to sleep is one of the most frequent causes of a stress response. Physiologically, stress is mediated by the Hypothalamus-Pituitary-Adrenal axis and the sympathetic nervous system. Prolonged stress, as it occurs because of sleep insufficiency, increases plasma levels of corticosterone and catecholamine tone in the periphery. Metabolic homeostasis is particularly affected by stress and numbers of molecular and functional changes have been described in the adipose tissue (AT). In adipocytes, a mark of metabolic impairment induced by stress is the increased activity of the 11β-Hydroxysteroid dehydrogenase type (11 β HSD1) enzyme, which catalyzes the local synthesis of glucocorticoids. In order to investigate the effect of sleep restriction-induced stress on both metabolic parameters and AT 11 β HSD1 levels/activity, we divided animal in 3 experimental groups: a group which was allowed to sleep for 6 hours (SR); a group that go through to sympathetic denervation of the AT (DX); a group that underwent to both sleep restriction and sympathetic denervation (SRDX), all of which were compared to control intact animals that slept *ad libitum* (CTL). The experimental protocol was carried out for 8 weeks, at the end of which animals were sacrificed. Body weight and food intake were monitored weekly. The glucose tolerance test (GTT) was performed on the eight week, before the sacrifice. Body temperature was registered by mean of a device placed intraperitoneally. During sacrifice, blood and AT samples were collected for the PCR analysis. Results showed that there were no changes in food intake among groups, but SR, DX and SRDX gained less weight; in particular, SRDX showed a significant decrease in AT mass. Denervation, but not sleep restriction, decreased the amplitude of body temperature daily fluctuations. Plasma adiponectin was augmented in SR, whereas leptin concentration was lower in SR and SRDX. These groups also showed glucose intolerance, although recovery was faster in denervated animals. Corticosterone levels were significantly higher in the SR group, whereas SRDX displayed intermediate levers between SR and CTL. In the AT, 11β HSD1mRNA levels were upregulated in both DX and SRDX groups, while the enzymatic activity showed a significant increment only in DX animals.

All these data demonstrate that indeed, sleep restriction induce a stress response and that affects metabolism. In addition we found that the sympathetic innervation has a protective role in the AT local glucocorticoids production.

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Progressive changesin the expression of5-HT$_7$serotoninreceptorin Paraventricular nucleus of hypothalamus are related to differences in spontaneous motor behavior in a rodent model of chronic stress

Burgos Santillán MI$_1$, Rodríguez Córdova VM$_2$, Hernández Montiel HL$_1$, Mendoza Trejo MS$_2$, Hernández Chan NG$_1$

1) Facultad de Medicina, Universidad Autónoma de Querétaro, 2) Instituto de Neurobiología, Universidad Nacional Autónoma de México.
Corresponding autor E-mail: hdez_chan@yahoo.com.mx

Stress is defined as the physiological response to threatening stimuli that living organisms exhibit in order to preserve the individual´s integrity enhancing the chances for survival. Under normal conditions, the exposure to stressful events results in the fast activation of both, sympathetic nervous system and hypothalamus-pituitary-adrenal (HPA) axis,this latter considered as the main neuroendocrine integrator of central nervous system. Several brain regions such as prefrontal cortex, amygdala and hippocampus are involved in processing stressful stimuli, while other brainstem structures such as serotonergic raphe nuclei also participate during stress response.Parvocellular neurons of Paravetricular nucleus of hypothalamus (PVN) start HPA axis acitivation by secreting corticotropin releasing factor (CRF) which induces the hypophyseal release of adrenocorticotropin hormone (ACTH) to bloodstream, action that induces the secretion of cortisol or corticosterone in rodents by the adrenal gland. PVN neurons receive direct serotonergic projections from raphe nuclei and express several serotonin (5HT) receptors such as type 7 (5-HT$_7$).Due to its high biological cost, endocrine stress response is rapidly finished by glucocorticoids in a negative feedback mechanism during acute stress. However, chronic exposure to stress induces important neural and systemic changes in order to adjust physiological and behavioral strategies to cope repetitive or intense threatening stimuli. The aim of this work was to evaluategradual changes in the expression of 5-HT$_7$ receptor in PVN at days 5,10 and 15 of a chronic restraint stress challenge related to changes in spontaneous motor behavior in rats. Adult male Wistar rats (230-250 g, n=32) were exposed to a daily 20 minutesepisode of restriction stressor handling in previously established periods. At the end of the experiment rats exploratory motor behavior was measured using an automatic system. Our results showed some differences in spontaneous motor behavior of 15 days chronic stressed animals compared to 5 days chronic stressed or handling controls. We also found a significant increase of the adrenal glands weight ($P<0.01$) in 15 days chronic stressed animals compared 5 days stressed animals and controls. Additionally, our histology results showeda significant reduction in the number of 5-HT$_7$ immunoreactive neurons in PVN in 15 days chronic stressed animals compared to controls and 5 days stressed animals.

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Área para el congreso: Estrés

Different sources of stress: sex and model type differences.

Torres-Carrillo, Paulina; Vargas-Gómez, Mariana; Miranda-Guzmán, Jennyfer Teresa; Vergel-Munguía, Mariana Denisse; Gómez-Avalos, Hatzumy; Verdin-Ruvalcaba, Luis; Paz-Trejo, Diana Berenice; Ochoa de la Paz, Lenin & Sánchez-Castillo, Hugo.

Institución de Adscripción: Facultad de Psicología, UNAM. Laboratorio de Neuropsicofarmacología y Estimación Temporal.
Correo electrónico:toca.paupau@gmail.com
Dirección postal: Laboratorio de Neurospicofarmacología y Estimación Temporal, Facultad de Psicología, UNAM. Av. Universidad #3004, Ed. B primer piso, Cub. 001. Col. Copilco Universidad. Del. Coyoacán. C.P. 04510.
Teléfonos: 044-55-5156-1326 o 5622-8222 Ext. 41354.

Stress-related mental illnesses are linked to the nature and duration of the stressor. Excessive and/or prolonged stress can lead to neuronal damage in vulnerable brain structures that is reflected with a potential negative impact on learning and memory function as well behavior. Previous studies showed impairment spatial learning and memory in chronic stress exposed male rats whereas in chronic stress exposed females the performance was the same or better. On the other hand, rodents exposed to Predator Scent Stress (PSS) showed impairment on spatial memory retrieval, independent of sex or strain tested on radial arm maze. This suggests that sex and stressor type influences the effects of stress. The aim of this study was to compare male and female rats exposed to two different stressors vs. no-stress condition: CUS, PSS, and control group, respectively. Four months old male and female Wistar rats were used (n=10 per group). Animals of CUS group were exposed to a Chronic Unpredictable Stress Battery (CUSB) during ten consecutive days. The stressors that made up the battery consisted of: 1) placing animals in movement restrictors for 20 min. (3 times per day), 2) swimming in cold water for five minutes (16°C), 3) overnight light exposure (12 hours), 4) placing the rats for 20 hours (overnight) in their home cages with wet bedding, 5) placing the rats for 3 hours in their home cage that was tilted at 45°, and 6) overnight water deprivation (12 hours). The exposure to each stressor was randomized according to the CUSB protocol. Animals of the PSS group were exposed for 10 minutes in an exposure box that contained a bottle with scent tags impregnated with predator urine (Bob Cat). Behavior was assessed with Saccharine Preference Test 24 hours after the final stressor exposure. The results of saccharine preference index showed that males and females had less consumption of saccharine on PSS condition compared with control group. Females had less consumption on PSS condition compared with CUS condition also. Finally, those results supported that stress induces depressive-like behaviors dependent of sex and stressor kind.

Área: Estrés / Cognición y comportamiento.#

Exposure to continuous light elicits depression through an Interleukin-6 transsignaling dependent mechanism in a murine model

Ricardo Velázquez Contreras, Francisco Vega, Jorge Vargas, Gabriela González, Roberto Cuevas Olguín, Eric Esquivel Rendon, Teresa Mares-Barbosa, Marcela Miranda-Morales, Griselda García, Catalina Arenas-Huertero, Nadia Saderi, Roberto Salgado-Delgado, Stefan Rose-John, Marco Atzori, Universidad Autónoma de San Luis Potosí, Facultad de Ciencias, Programa de Biología, SLP, México, Christian Albrecht Universitet, Biochemistry Department, Kiel, Germany
Contacto: marco_atzori@hotmail.com, Avenida Salvador Nava s/n, Zona Universitaria, San Luis Potosi, SLP, 78290, Mexico,Tel.: 444 1739 256, marco_atzori@hotmail.com

Mood disorders contribute to an increasing extent to the burden of disease in modern society. Their molecular mechanisms are still poorly understood. The interleukin network - a biochemical series of links among molecules part of the immune system - has been proposed to be involved in mood disorder etiology. In particular, recent investigation suggests a role for interleukin-6 (IL-6) in depression. In this work we sought a possible link between IL-6 and mood and motor alterations induced by changes in circadian illumination, by exposing experimental animals to continuous light during 4 weeks. IL-6 exerts its action through two extracellularly-mediated mechanisms: the “classic” pathway, and the “trans-signaling”. The former, carried out only by specialized cells, is activated by direct binding of IL-6 to a membrane complex formed by the IL-6 receptor (IL-6R) and its transducer, glycoprotein 130 (gp130). The latter mechanism, present in all nucleated cells, is initiated by binding of IL-6 to IL-6Rs shed by immune cells by a metalloprotease-dependent mechanisms, followed by binding of the IL-6/IL-6R complex to membrane bound gp130. In order to determine the role of IL-6 we compared the behavioral effects of continuous illumination (L:L) vs. normal light cycle (D:L) in wild-type animals (WT) and animals in which trans-signaling was blocked by overexpressing a soluble version of gp130 (gp130Fc) in genetically modified animals (TG). Among other tests, the Porsolt Forced Swimming Test (FST) was used to determine the effect of the altered photoperiod on experimental animals. The efficacy of the model was shown by a significantly longer immobility time (IT) in WT animals (n=5) at 3-week under L:L (IT = 256.9 s ± 17) and 4-week (IT = 273.3 ± 19.3 in L:L). TG animals submitted to L:L (n=6) displayed ITs significantly shorter than WT animals at 3week (IT = 169.4 ± 20) as well as at 4-week (IT = 187.7 ± 18.4) after the change in photoperiod ($p < 0.05$), suggesting that TG animals are less sensitive to the stressor compared to WT. Our results show for the first time that IL-6 is involved in biologic alterations caused by changes in circadian rhythms. This data may open new avenues for more effective, long-sought treatment for depression, anxiety, and bipolar disease. More experiments will be necessary to investigate the relationship between IL-6 and the cellular mechanisms of mood disease, as well as in order to develop new pharmacological treatments for this group of illnesses.

Area: Estres

TEMPORAL PROFILE IN THE EXPRESSION OF GENES INDUCED BY THE FORCED SWIMMING TEST: IMPLICATIONS OF GR, BDNF AND NURR1.

Valery Vilchis Moreno, Elizabeth Ruíz Sánchez, Pedro Montes del Carmen, Rafael Campos Rodríguez y Patricia Rojas Castañeda.

Instituto Nacional de Neurología y Neurocirugía, Manuel Velasco Suárez. Laboratorio de Neurotoxicología.

Insurgentes sur #3877. Col. La Fama 14269, Ciudad de México, México. Teléfono: (55) 56063822 ext. 2036.Correoelectrónico: valbiology@hotmail.com; prcastane@hotmail.com.

Introduction: The Glucocorticoids secreted by a stressful stimulus which induce the expression of genes as transcription factors and neurotrophins, that participate in the adaptive response to stress. Therefore, it is important to analyze the gene expression to associate it with the behavior response in the forced swimming test (FST). **Objective:**Our aim was to determinethe temporal gene expression of GR (Glucocorticoid Receptor), BDNF (Brain Derived Neurotrophic Factor) and Nurr1 during the period between the two sessions of the FST. **Material and Methods:** Adult male Wistar rats of ~ 250g were submitted to FST (acute stress/ "pre-test" 15 min and "test" 5 min). Six groups were evaluated: Group 1 (control, without FST), groups 2 to 5 were submitted to the pre-test only and sacrificed at different recovery times (0 min, 30 min, 3 h and 24 h respectively) and the group 6 was exposed to both sessions of the FST + 30 min recovery. After each experimental condition, blood (plasma) was obtained to quantify corticosterone levels (ELISA). The mRNA and proteins were extracted from the hippocampus, and were analyzed with RT-PCR and Western Blot respectively. **Results:** Corticosterone levels show that groups 2, 3 and 6 weresignificantly higher with respect to the control group. The mRNA levels of GR, BDNF and Nurr1 were not shown statistically differences between the different groups. The analysis to protein nurr1 showed a significant increase in all the experimental groups with respect to the control group. **Discussion and Conclusions:** The increase of Nurr1 protein in all groups suggests its participation in the adaptive response to stress.

Role of silver nanoparticles (AgNPs) and zinc chloride ($ZnCl_2$) on the survival of Glioma C6.

Jose Fernando Garcia Rodrigo, Samuel Salazar Garcia, Gabriel Alejandro Martinez Castañon, Carmen Gonzalez.
Facultad de Ciencias Quimicas, UASLP.
Av. Manuel Nava, #6, San Luis Potosi, SLP, Mexico.
jose.rodrigo.garcia93@gmail.com
(444)8480290

Work area: Glia

Abstract

The objective of this work was to elucidate the effect of <10 nm silver nanoparticles (AgNPs) and zinc chloride ($ZnCl_2$) on C6 rat glioma cells. Glioblastoma multiforme (GBM) is the most aggressive malignant astrocytoma and its prognosis is unfavorable due to an unspecific and aggressive treatment; for this reason, is necessary to investigate new complementary treatment alternatives that involve the use of nanomaterials (NM) as AgNPs. The experimental methods employed were cell viability assay (MTT) and immunocytochemistry (ICC). Increasing concentrations of AgNPs (10 to 100 µg/mL) and/or $ZnCl_2$ (10 to 50 µg/mL) were treated upon Glioma C6 cells during 24 h. We found that AgNPs and $ZnCl_2$ as separate treatments exerted a viability decrease on C6 cells; 45% and 80% respectively in comparison to the control; however AgNPs (50 µg/mL) with $ZnCl_2$ (10 to 50 µg/mL) 24 h pretreatment, as well as concomitant treatment (AgNPs 50 µg/mL + $ZnCl_2$ 10 to 50 µg/mL) showed a major decreasing viability effect than the one showed as individual treatments. This decrement on cell viability was associated with an enhance damage, highlighting that the concomitant treatment showed a significant major negative effect in comparison with the 24 h $ZnCl_2$ pretreatment. We also observed by ICC that damage markers as metallothioneins, a heavy metal detoxificant proteins, were also evaluated and showed that there are no significant changes in their expression under the treatments aforementioned; the lack of increasing expression of this metallo-proteins under $ZnCl_2$ and AgNPs suggest that could be the reason of the C6 cell damage. However furthermore study are underway in order to determine the cellular mechanisms involved in AgNPs and $ZnCl_2$ response.

Mitochondrial Membrane Potential (mΔψ) Response to NMDA and MK-801 inrat cultured cortical astrocytes.

González-Hernández, José Roberto[1*] and Montes de Oca Balderas, Pavel[1]

1 Unidad de Neurobiología Dinámica, Departamento de Neuroquímica, Instituto Nacional de Neurología y Neurocirugía “Manuel Velasco Suárez”, Mexico City, Mexico

* Number: 55-64-14-97-73 E-mail: robert.biology@ciencias.unam.mx

* Presentaddress: Unidad de Neurobiología Dinámica, Departamento de Neuroquímica, Instituto Nacional de Neurología y Neurocirugía, Av. Insurgentes Sur No. 3877, Col. La Fama, Del. Tlalpan, Mexico City, Mexico.

Since tripartite synapse hypothesis was proposed, multiple studies have demonstrated the participation of astrocytes in functions beyond just providing maintenance and support to neurons, positioning them as active players in the synapse regulation, among other functions. Although one of the basis for this hypothesis was the expression of neurotransmitter receptors by the astrocyte, the functional expression of N-methyl-D-aspartate (NMDAR) receptors (NMDAR), a glutamate-selective ionotropic receptor involved in critical functions in neurons, has been subject of debate in astrocytes. Initially, it was found that this receptor was non-functional electrophysiologically in rat cultured astrocytes, but later in acute isolated mouse astrocytes currents were demonstrated. Recently, we reported that rat cultured cortical astrocyte (rCCA) increased iCa2+ and decreased mitochondrial membrane potential (mΔψ)in response to acid-NMDA treatment. Unexpectedly, we found that iCa2+ was due to Ca2+ release from the endoplasmic reticulum (ER), suggesting a metabotropic-like function. In this work, we further characterized mΔψ response to acid-NMDA treatment. Surprisingly, we found that mΔψ was blocked by MK-801 or Ca2+ free solution, in clear contrast with iCa2+ responses, whereas acid treatment did not change mΔψ, and acid-sensing ion channels (ASICs) were not involved in this response. These observations suggested independence of iCa2+ and mΔψ responses to acid-NMDA. Since the apposition of NMDAR to mitochondria has been reported previously in neurons and this could help to explain our findings, we further investigated this question in rCCA. Immunofluorescence experiments show some NMDAR puncta colocalized with mitochondria. In addition we found by electron microscopy (EM) that NMDA treatment induced membrane deformations that contact mitochondria, whereas Western Blot experiments suggested the presence of GluN1 subunit in mitochondria isolated from astrocytes, as has been previously shown for neuronal mitochondria. Together, these results suggest that iCa2+ and mΔψ responses independence in rCCA could be related with mitochondria and NMDAR localization and function.

DEVELOPMENTAL REGULATION OF THE PROLACTIN/VASOINHIBIN AXIS IN HIPPOCAMPAL ASTROCYTES

FernandoMacías[1], Miriam Ulloa[1], Rodrigo ManuelAroña[1], Carmen Clapp[1], GonzaloMartínez de la Escalera[1], Edith Arnold[1,2].
[1]Department of Celular and Molecular Neurobiology;[2]CONACYT-Neurobiology Institute, Universidad Nacional Autónoma de México, Campus UNAM Juriquilla, Querétaro, México.

Prolactin (PRL) and vasoinhibins are two families of hormones associated in a functional axis. Vasoinhibins, named for their inhibitory effect on angiogenesis, vasopermeability and vasodilation, are synthesized through the proteolytic cleavage of PRL, sharing both the N-terminal region. They range in molecular mass between 11 and 18 kDa, depending on the specific sites of action of the proteases involved in their generation, which include cathepsin D, matrix metalloproteinases (MMPs), and bone morphogenic protein-1 (BMP-1). The balance between the concentration of PRL vs vasoinhibins is determined by the presence and activity of this proteolytic enzymes. PRL and vasoinhibin species are detected in the rat hypothalamus, and other regions of the central nervous system where they trigger opposite effects. In this study we explored the activity of the converting enzymes cathepsin D and MMPs, in astrocytes obtained from the hippocampus of mice in 3 different stages of development. Hippocampi were obtained from the brain of 16 days old embryos (E16), neonate,and adult mice. Isolated astrocytes were obtained from E16 and neonate mice. Lysates from each sample were incubated with 50 ng of rat PRL in a pH 5 or 7 buffer during 24hours at 37º C in the presence or absence of pepstatin A, an inhibitor of the action of cathepsin D. PRL and vasoinhibins were determined by Western blot. Our results show that endogenous PRL and vasoinhibins are differentially found in the 3 stages. E16 hippocampus contain 3 bands corresponding to vasoinhibins of 14, 16 and 18 kDa, while neonate hippocampus also contain3 bands but corresponding to vasoinhibins of 14, 16 and 17 kDa, and the adult hippocampus only 2 vasoinhibins of 14 and 17 kDa. Cathepsin D present in the hippocampus and isolated astrocytes cleaved PRL into a vasoinhibin of 16 kDa at all analyzed stages, but the proteolytic efficacy waslowerin astrocytes from E16 than fromneonates. On the other hand, incubation at pH 7 did not produce any vasoinhibin, suggesting that MMPs are not active in hippocampal astrocytes under basal conditions. Altogether these findings show a cathepsin D developmental-related regulation of the PRL/vasoinhibin axis that operates in hippocampal astrocytes of the mice.
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Glutamate-dependent translational control of glutamine synthetase in Bergmann glia cells

Arturo Ortega, Reynaldo Tiburcio-Félix, Miguel Escalante-López, Bruno López-Bayghen, Samule Zinker, Dinorah Hernández-Melchor, Esther López-Bayghen, Luisa C, Hernández-Kelly

Departamento de Toxicología, Centro de Investigación y de Estudios Avanzados del IPN, Apartado Postal 14-740, Ciudad de México. arortega@cinvestav.mx, Av. IPN 2508, San Pedro Zacatenco 07360. CDMX.555747-3800, ext. 5493

Glutamate is the major excitatory transmitter of the vertebrate brain. Exerts its actions through the activation of specific plasma membrane receptors expressed both in neurons and glial cells. Recent evidence has shown that glutamate uptake systems, particularly enriched in glia cells, trigger biochemical cascades in a similar fashion as receptors. A tight regulation of glutamate extracellular levels prevents neuronal over-stimulation and cell death and it is critically involved in glutamate turnover. Glial glutamate transporters are responsible of the majority of the brain glutamate uptake activity. Once internalized, this excitatory amino acid is rapidly metabolized to glutamine *via* the astrocyte enriched enzyme glutamine synthetase. A coupling between glutamate uptake and glutamine synthesis and release has been commonly known as the glutamate/glutamine shuttle. Taking advantage of the established model of cultured Bergmann glia cells, we explored the gene expression regulation of glutamine synthetase. A time and dose dependent regulation of glutamine synthetase protein and activity levels was found. Moreover, glutamate exposure resulted in the transient shift of glutamine synthetase mRNA from the monosomal to the polysomal fraction. These results demonstrate a novel mode of glutamate-dependent glutamine synthetase regulation and strengthen the notion of an exquisite glia neuronal interaction in glutamatergic synapses.

Area 1. Glia

Titanium dioxide nanoparticles are internalized and induced autophagy and nuclear factor kappa B translocation in astrocytes

José Antonio Pérez Arizti, Rebeca López Marure. Instituto Nacional de Cardiología "Ignacio Chavez". Mexico City, Mexico. Juan Badiano Num. 1, Col. Sección XVI, Delegación Tlalpan, Ciudad de México. 55732911 ext. 1278. aarizti@outlook.com.

Area: Glia

Titanium dioxide nanoparticles (TiO_2-Nps) are one of the most common materials used in food and cosmetic industries, among others. Their small size (<100 nm) give them special characteristics such as a major surface area and a better interaction with other molecules. These nanoparticles can enter the body by inhalation, or oral and dermic uptake. TiO_2-Nps can be introduced inside the respiratory tract and crosses the blood-brain barrier to enter into contact with brain cells like neurons and astrocytes; these last one give protection, support and stability to the neurons. Astrocytes dysfunction is an important factor in the development of neurodegenerative pathologies such as Alzheimer or Parkinson diseases. The aim of this study was to evaluate the uptake of TiO2-Nps and their effect on autophagy and nuclear factor kappa B (NF-kB) translocation in primary cultures of rat astrocytes.

Cells were exposed to 20 µg / cm2 TiO_2-Nps of 50 nm for 1, 2, 4, 7 and 10 days and the morphological changes and internalization were evaluated by Transmission Electron Microscopy (TEM). We also evaluated the autophagy by the LC3-II protein fluorescence, a marker of the formation of the autophagosome structure, using confocal microscopy and flow cytometry at 24 and 48 h. Finally, we determined the nuclear translocation of NF-κB 15 and 30 min after exposure by electrophoretic mobility shift assay (EMSA).

We demonstrate that TiO_2-Nps were internalized from 1 h of exposure, induced morphological changes and damage and this was time-dependent. TiO2-Nps internalization was associated with increase of autophagy at 24 h and with NF-κB translocation after 30 min of exposure.

We concluded that TiO_2-Nps internalization promotes the activation of survival cell responses in astrocytes; particularly the translocation of NF-κB, which has been observed in patients with Alzheimer's disease, and the increase in autophagy in astrocytes, which is a cellular event involved in patients with Alzheimer's and Parkinson's disease. Nps finally leading the cells to lost their morphology and to the development of these pathways associated with damage.

Comparative effects on rat primary astrocytes and C6 rat glioma cells cultures after 24 hours exposure to silver nanoparticles (AgNPs)

Samuel Salazar-Garcia, Manuel A. Ramirez-Lee, Hector Rosas-Hernandez, Edgar Rangel-Lopez, Claudia G. Castillo, Abel Santamaria, Gabriel A. Martinez-Castañon, Carmen Gonzalez

Facultad de Ciencias Químicas, UASLP.
Av. Manuel Nava No. 6 Colonia Universitaria, 78210 San Luis Potosí, S.L.P. México. Tel 8 26 24 40 ext. 6421

Email: samuelsg_9@hotmail.com, Cel: 44 42 35 46 39

Work área: Glia

Abstract:

Glioblastoma multiforme (GBM) is one of the most aggressive primary brain tumor and current treatments lead to diverse side-effects; for this reason is imperative to investigate new approaches, including those alternatives provided by nanotechnology, like nanomaterials (NMs) such as silver nanoparticles. The aim of this work was to compare the effects of 24 hours exposure of rat primary astrocytes and C6 rat glioma cells to <10 nm AgNPs (0-100 µg/ml); through evaluate the cell viability and cytotoxicity through colorimetric assays, the proliferation through 5-bromo-2.deoxiuridine (BrdU) incorporation kit, and apoptosis by flow cytometry. Herein, we found that C6 rat glioma cells, but no primary astrocytes, decreased cell viability after AgNPs treatment (50; 75 and 100 µg/ml); however, both cell types diminished their proliferation (50 and 100 µg/ml). The decrease of glioma C6 cells proliferation was related with necrosis, while in primary astrocytes the decreased proliferation was associated with the induction of apoptosis. Ionic form of silver nitrate used as control, exerted a different toxic profile effects than AgNPs; the bulk form did not modify the basal effect in each determination, suggesting that AgNPs *per se*, can confer differential biological effects because of the nano scale. In summary, this work constitutes for the first time a comparative study between C6 rat glioma cells and primary astrocytes employing the same type of AgNPs, emphasizing the contrast between normal and pathological conditions, and provides new perspectives that could contribute in the development of new tools or alternatives based on nanotechnology, contributing in the understanding, impact and use of NMs in targets like glioblastoma.

Simultaneous detection of Bielschowsky silver-staining technique and fluorescent immunohistochemistry in the rat sciatic nerve.

E. Segura-Anaya, R. Flores-Miranda, A. Martínez-Gomez A, M.A.R. Dent
Laboratorio de Neurociencias. Facultad de Medicina. Universidad Autónoma del Estado de México. Apartado postal 428. C.P. 50000 Toluca, Estado de México, México. Tel. 722 2 17 35 52 Ext. 222 E-mail: mdent6@gmail.com

Santiago Ramón y Cajal and Camillo Golgi's main contributions were achieved using the Golgi-silver technique (Golgi's method). Ramón y Cajal improved the technique by using a method he termed "double impregnation" (Cajal's stain). With these techniques dendrites, the cell soma and axons are clearly stained in brown and black and can be followed along their entire length. This allowed neuroanatomists to track connections between neurons and to discover a number of facts about the organization of the nervous system and also the establishment of the 'neuronal doctrine' by Ramón y Cajal. Silver-staining methods have been steadily improved and the Bielschowsky's silver staining technique is very useful for the visualization of nerve fibers. For many years, these silver-staining techniques remained ideal until the localization of specific molecules on histological sections using fluorescent immunohistochemistry techniques, which revolutionized not only research but also routine diagnosis. However, Bielschowsky's method is routinely used to study Alzheimer's disease by staining axons, neurofibrils and senile plaques in the CNS, and is performed together with antibody staining. The way of doing it is staining serial sections using Bielschowsky's technique and immunohistochemistry. Here we have used teased rat sciatic nerves and performed simultaneously the Bielschowsky's method and fluorescent immunohistochemistry. One of the advantages of using this method is the possibility to accurately localize any molecule directly in the silver-staining section of the nerve. The Bielschowsky technique shows not only strong staining in the axons, but a light staining of myelinating Schwann cells, which allows visualization of the entire internodes. We have stained different regions of the nerve, using antibodies against myelin (anti-MBP), non-compact myelin (anti-AQP1), the nodal region (anti-ankyrin-G), and non-myelinated Schwann cells (anti-GFAP). The results show the precise localization of these molecules, and this method works with both antibodies that need to be fixed with paraformaldehyde or with methanol.

ASTROCYTES ACTIVATION INDUCED BY MORIN IMPROVES MEMORY IN HEALTHY MOUSE

Mónica Adriana Torres Ramos[1],Hilda Martínez Coria[2], Norma Serrano García[3], Gabriela Sinaí López Chávez[1], Marisol Orozco Ibarra[3], Alejandra Y. González Muñiz[1], Héctor López Valdés[1].

1Unidad Periférica de Neurociencias, INNN-Facultad de Medicina, UNAM, 2Laboratorio Experimental de Enfermedades Neurodegenerativas INNN-Facultad de Medicina, UNAM,3Laboratorio de Neurobiología Molecular y Celular, INNN.

Introduction: Cognitive improvement has been linked to the consumption of various polyphenols of natural origin. Morin is a polyphenol present in various fruits such as figs and blackberries;it has antioxidant, neuroprotective and anti-inflammatory activities, among others. Morin treatment has been shown to restores cognitive deficits in impaired animal and it could be a potently promising disease-modifying agent for treatment of Alzheimer's disease.

Objective: To characterize the effect of morin on recognition memory in healthy adult mice and their possible molecular mechanism.

Material and Methods:60 healthy adult mice (25-30 g, C57BL / 6) were divided into groups of 10 animals: control (saline solution), vehicle (DMSO) and those treated with morin. Different doses of morin (1, 2.5, 5 and 10 mg / kg / 24 h for 10 days)were administered by I.P. injection. The object recognition memory test was performed after 10 days of treatment.
The brains of the mice were processed for histological characterization and protein expression by immunohistochemistry.

Results: The RI obtained in the different groups was (% ± SEM): Control = 60 ± 1.8, Vehicle = 64 ± 3.0, 1 mg / kg = 79 ± 4.15, 2.5 mg / kg = 72.31 ± 4.58, 5 mg / kg = 69.95 ± 4.05, 10 mg / kg = 64.39 ± 5.61. Significant RI was observed in mice treated with 1 mg / kg of morin relative to control and vehicle ($p < 0.001$ and $p < 0.01$ respectively, ANOVA followed by Turkey's post hoc test). The behavioral effect correlates with increased expression of the IL-4, GFAP and BDNF proteins.

Discussion and Conclusions: Morin, in low concentrations, improves recognition memory in healthy adult mice. We suggest that the molecular mechanism involved is due to the production of BDNF via the activation of astrocytes produced by the increase of IL-4. Activation of astrocytes by IL-4 and IL-13 has been reported to favor the production of BDNF involved in improved memory and increased expression of proteins in the synapse.

monica.atorres@gmail.com
Instituto de Nacional de Neurología y Neurocirugía "Manuel Velasco Suárez"
Insurgentes Sur #3877 Col. La Fama, Tlalpan, C.P. 14269. CDMX
Tel. 56063822 ext. 1096.

PROLACTIN PROTECTS RAT CORTICAL ASTROCYTES AGAINST OXIDATIVE STRESS.

MiriamUlloa[1], FernandoMacías[1], Rodrigo Manuel Aroña[1], Carmen Clapp[1], Gonzalo Martínez de la Escalera[1], Edith Arnold[1,2].

[1]Department of Celular and Molecular Neurobiology;[2] CONACYT-Neurobiology Institute, Universidad Nacional Autónoma de México, Campus UNAM Juriquilla, Querétaro, México.

Astrocytes maintain brain homeostasis by maintaining synaptic integrity, providing protection and metabolic support for neurons, regulating inflammatory response, and promoting cell survival under oxidant conditions. Several types of stress, injuries and brain diseases induce mitochondrial dysfunction and oxidative stress that leads to astrocyte death. Prolactin (PRL) is a stress-related hormone that limits gliosis and degeneration of the neural retina (Arnold et al JN 2014). In this work, we investigated whether PRLprotects cortical astrocytes against oxidative stress and cell death. Primary cultures of cortical astrocytes were isolated from the brain of neonatal Wistar rats and their purity determined by GFAP positivity by immunocytochemistry. The long isoform of the PRL receptor was detected in cortical astrocytes by qRT-PCR. Astrocytes were treated with increasing concentrations of PRL (0.1-100 nM) for 24 hours and then were exposed to oxidative stress induced with 400 μM hydrogen peroxide (H_2O_2) for 3 hours. Incubation of cortical astrocytes with PRL inhibited H_2O_2-induced cytotoxicity, evaluated by the MTT assay, in a dose-dependent manner.Moreover, PRL treatment (10nM) induced an increase in the expression of its receptor, GFAP and antioxidant enzymes such as superoxide dismutase, peroxiredoxin 6 and glutathione peroxidase 1 under basal condition in astrocytes, and thischange in the expression was exacerbated by H_2O_2-induced oxidative stress, evaluated by qRT-PCR. These results indicate that PRL can act directly on astrocytes to protect them against oxidative stress injury.

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Neurodegeneration and metabolic syndrome: role of oxidative stress, insulin resistance and AMP-dependent protein kinase (AMPK)

Karla Carvajal, Luz del Carmen Camacho-Castillo, Bryan Phillips-Farfán, Aidee Baires-López, Gabriela Rosas-Mendoza, Victoria Campos-Peña.
Instituto Nacional de Pediatría, Instituto Nacional de Neurología y Neurocirugía "Manuel Velasco".
Karla Carvajal, Laboratorio de Nutrición Experimental, Instituto Nacional de Pediatría, Insurgentes Sur 3700, Letra C, Col. Insurgentes Cuicuilco, Del. Coyoacán, Cuidad de México 04530. Email: karla_ca@yahoo.com Tel. 55-1084-0900 Ext. 1876

Área 3: Neuropatología

Background. The metabolic syndrome (MetS) is a conglomeration of biochemical and anthropometric alterations, such as glucose intolerance, dyslipidemias, hypertension, obesity and type 2 diabetes. The principal trigger mechanism of the MetS appears to be insulin resistance, which is an inability of target cells to respond to thehormone stimulus. Increasing evidence supports a relationship between MetS and neurodegenerative diseases, such as Alzheimer's disease. The MetS increases the risk of dementia in adults, as well as the metabolism of amyloid β peptide and tau protein, which are responsible for forming the main neuronal lesions characteristic of Alzheimer's disease. An energy imbalance that is part of the MetS and an imbalance in the production of reactive oxygen species are factors that mediate the progression of neurodegenerative diseases. However, the mechanisms and cellular signaling pathways affected have not been resolved.
Objective. In this work we evaluated the effect of oxidative stress (OS), during the early development of MetS, on insulin and AMPK pathways and their relationship with neurodegenerative processes.
Material and methods. We studied the antioxidant capacity and the systems that mediate it in the serum, hippocampus and hypothalamus of rats withMetSinduced by a high sucrosediet. We evaluated the insulin and AMP-dependent protein kinase (AMPK) pathways as well as energy-producing pathways, such as creatine kinase (CK).
Results and Discussion. The hypothalamus and hippocampus of rats with MetShad significant oxidative damage and decreased antioxidant and CK enzymes. The AMPK and insulin pathways were increased, probably as a compensatory effect. The brains of rats with MetS had alterations in the amloid β precursor protein and in the enzyme that cleavesit. Chronic AMPK activation partially reversed OS in the brain of animals with MetS.
Conclusions. OS is an early cellular mechanism that mediates MetS progression and neurodegenerative processes affecting energy signaling pathways such as those mediated by insulin and AMPK.

Involvement of energy metabolism and Sirtuin 1 inhibition in the expression of Alzheimer's disease markers

Manuel Flores-León[1], Martha Pérez-Domínguez[1], Rodrigo González-Barrios[2] and Clorinda Arias[1].

1. Instituto de Investigaciones Biomédicas, Universidad Nacional Autónoma de México. AP: 70-228, 04510. Ciudad de México, México. 2. Instituto Nacional de Cancerología, AP: 14080. Ciudad de México, México.

E-mail: manuel.leoncio75@gmail.com Phone: 015556229215

Metabolic alterations associated with the consumption of high fat diets (HFD) are considered as risk factors for neurodegenerative diseases, including Alzheimer's disease (AD). Some of the deleterious effects of HFD depend on the high content of saturated fat acids such as palmitic acid (PA). It is presumed that PA might be altering the cellular energy metabolism affecting a group of Histone Desacetylaces (Class III – Sirtuins) that are sensors of the energy cell status due to the utilization of NAD^+ as a cofactor. Sirtuin 1 (Sirt1) is responsible for the deacetylation of several cytoplasmic proteins, transcription factors and residues in histone tails, leading to activation or repression of genes. Nevertheless, the mechanism that relates the intake of HFD and the alteration of cellular metabolism to the expression of cellular markers of AD remains to be elucidated. Thus, we have analyzed the effects of neuronal exposure to PA on Sirt1 content and function and the expression of the limiting enzyme of the amyloidogenic APP processing, Bace1. Hippocampal neuronal cultures were exposed to different concentrations of PA or EX527 (Sirt1 inhibitor), followed by protein and RNA extraction for Western Blot and RT-qPCR analyses. The content and cellular localization of Bace-1 was performed by immunofluorescence. Quantification of Bace1 activity was measured with a fluorometric kit. We found that neuronal exposure to PA, at non-neurotoxic doses diminished Sirt1 protein content associated with an increase of NF-κB acetylation. Both, PA and EX527 increased the protein levels and activity of Bace1. Present results suggest that the neuronal metabolism of PA might be implicated in reduced Sirt1 content and function, which in turn may have an impact in the expression of biochemical markers of AD such as the amyloid-β peptide and the activation of NF-κB.

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Connexin 30.2 is expressed in exocrine vascular cells throughout pancreatic postnatal development.

Elia Martha Pérez-Armendariz, Cristina Coronel-Cruz, Beatriz Hernández-Tellez, Ivonne Grisel, Verónica Rodríguez-Mata, E. Andrés Castell-Rodríguez, Departamento de Biología Celular y Tisular, Facultad de Medicina, Universidad Nacional Autónoma de México, emperezarmendariz@aol.com, 55) 5623-3000 ext 45008, Av. Universidad 3000, colonia UNAM, CU, Ciudad de México. 04510, México,

Gap junctions (GJs) are known to regulate the vascular blood tone. Here we investigated whether the GJ protein Cx30.2 is expressed in vascular exocrine pancreatic cells. For this, pancreatic sections were co-incubated with antibodies against Cx30.2 and against CD31, a marker of endothelial cells (ECs), or against smooth muscle α-actin, which is a marker of vascular muscle cells (SMC) and were analyzed by immunohistochemistry (IHC) or immunofluorescence (IF). Cx30.2-IF dots were found in ECs as well as in the SMCs at their junctional membranes. The same cellular distribution of Cx30.2 was also found in the mouse, rat, hamster and adult guinea pig pancreas by IHC. In the mouse, we showed that Cx30.2 is also expressed in these cell types at days 3, 14 and 21 postnatal days. The expression of the Cx30.2 mRNA and protein in the adult pancreas of all these species was confirmed by real-time RT-PCR and Western blot studies. From the above, our results demonstrate the Cx30.2 protein is expressed in the EC and SMC of the exocrine blood vascular pancreas throughout all postnatal development. The subcellular distribution of Cx30.2 in these cell types at the adult stage suggests that this connexin forms junction intercellular channels that intercommunicate electrically these cell types. Acknowledgment: Grant number DGAPA IN225417

Membrane levels of SNAT2 mediated by PKA phosphorylation.

Tayde Gabriela Serrano Cano[1], Adrián Chávez Cano[1], ArturoOrtega[2], Cecilio Gimenez[3], Francisco Zafra[3],Angelina Rodríguez[4*]

[1]Facultad de Química, Universidad Autónoma de Querétaro,[2]Departamento de Toxicología, CINVESTAV, Ciudad de México, [3]Centro de Biología Molecular Severo Ochoa, Madrid, España, [4]Facultad de Ciencias Naturales, Universidad Autónoma de Querétaro

*Facultad de Ciencias Naturales, Universidad Autónoma de Querétaro, Campus Aeropuerto. Carretera Chichimequillas s/n, Ejido Bolaños, Querétaro, Qro. 76140, México. Tel. 4421921200 ext. 65214. Correo electrónico: angelina@uaq.mx

The Sodium-Coupled Neutral Amino Acid Transporter 2 (SNAT2) is a ubiquitous member of the solute carrier 38 family (SLC38) and according to its biochemical/functional mechanism is classified as a system A transporter, carrying out the symporter of a sodium ion and an aminoacid in a 1:1 stoichiometry. This transporter is subject to adaptive regulation by aminoacid deprivation, osmotic stress and hormones, which involve the translocation of this protein to the plasmatic membrane, increasing the amino acid uptake and therefore being important in various cellular processes such as the glutamine/glutamate shuttle in central nervous system, replenishing the glutamine pool in the presynaptic neuron for further conversion to glutamate and used as neurotransmitter. Post-translational modifications, such as phosphorylation, of SNAT2 are linked to its intracellular mobilization and maturation during the adaptive response; therefore, the aim of this study was to evaluate the regulation of membrane levels of SNAT2 mediated by PKA phosphorylation

Palmitic acid produces differential changes in the content of H3K9ac in human neuroblastoma cells

Iker Francisco Soto Santarriaga[1], Manuel Flores-León[1], Rodrigo González-Barrios[2] and Clorinda Arias[1].1. Instituto de Investigaciones Biomédicas, Universidad Nacional Autónoma de México, Coyoacán, Ciudad Universitaria, AP: 04510. Ciudad de México, México. 2. Instituto Nacional de Cancerología, Tlalpan, AP: 14080. Ciudad de México, México.
E-mail: ikerfrancisco@gmail.com Phone: 015556229215

Class III NAD^+ dependent deacetylases (Sirtuins) are considered an important link between metabolism, cell function and gene regulation because they can sense changes in the metabolic state and produce a cellular response either via modifications on the chromatin or by altering the activity of non-histone proteins. In the brain, an organ with a high level of metabolic activity, Sirtuin 1 (SIRT1) is implicated in maintaining neuronal health during development, differentiation and ageing. Previously we have demonstrated that the saturated fatty acid, palmitic acid (PA), decreases both the activity and the protein levels of SIRT1 in cultured hippocampal neurons. This change was accompanied by the appearance of some biochemical markers characteristic of Alzheimer's disease. However, it is still unknown if the reduced activity of SIRT1 mediated by PA affects the acetylation levels of histone targets such as H3K9ac. We used human neuroblastoma cells (MSN) differentiated with retinoic acid and NGF to analyze the effects of PA on histone acetylation. The cells were exposed to different non-toxic concentrations of PA for distinct periods of time. We found that 300 µM of PA induced differential changes in the levels of H3K9ac along time. During the first six hours of exposure to PA, a significant increase in H3K9ac levels was observed. However, 24 hours later this rise was followed by a decrease in the acetylation level of H3K9 below control levels. The aforementioned changes correlate with a reduction of SIRT1 content and an increase in the number of lipid bodies after 24 hours of treatment as shown by western blot assays and oil red staining protocols. Collectively, these results suggest that PA contributes to an altered neural lipid metabolism, which in turn produces a decrease in the protein content of SIRT1. In addition, the reduced levels of H3K9ac after 24 hours of exposure suggest that the activity of other HDACs could be upregulated by PA.

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A dorsomedial thyrotropin-releasing hormone neuronal population senses energy balance alterations: Identifying new TRHergic hypothalamic connections activated by fasting and refeeding

Karla Yamili Vargas Orihuela[a], Lorraine Jaimes-Hoy[a], Fidelia Romero Arteaga[a], Edith Sánchez Jaramillo[b], Patricia Joseph-Bravo[a], Jean-Louis Charli[a]
[a]Instituto de Biotecnología, Universidad Nacional Autónoma de México, Cuernavaca, Morelos; [b]Instituto Nacional de Psiquiatría Ramón de la Fuente Muñíz, Ciudad de México. E-mail: yamili@ibt.unam.mx

Thyrotropin-releasing hormone (TRH) has an important role in several aspects of energy balance maintenance [1]. Central administration of TRH or TRH agonists reduces food intake in normal rodents and hungry rats [2]. These effects may involve hypothalamic targets, since both TRH receptors, TRH-R1 and R2, are expressed in multiple hypothalamic nuclei [3] and the local injection of TRH into medial and lateral hypothalamus (LH) reduces feeding behaviour [4]. In male rats, fasting enhanced TRH-R1 expression in the LH and in female rats fasting decreased TRH expression in the dorsomedial nuclei (DMN); this suggests a fall in the LH-TRHergic tone as a result of fasting [unpublished]. The DMN has been implicated in food intake control and contains a significant number of TRH cells; it receives afferents from the subparaventricular zone, an output region of the suprachiasmatic nucleus, and sends a dense glutamate-TRH projection to lateral hypothalamus area [4]. In vitro, TRH inhibits the activity of melanin-concentrating hormone (MCH) neurons, apparently through an indirect pathway that involves the previous activation of LH-GABAergic neurons, consistent with the presence of TRH terminals on or near LH-GABAergic neurons [5]. Since MCH neurons send orexigenic projections, the TRHergic DMH-LH projection may transmit anorexic signals through the GABAergic neurons.
The purpose of this project is to determine whether the TRHergic DMH-LH projection senses energy balance. In male adult C57BL/6NJ mice the number of DMN cfos positive cells was decreased by 24-h fasting, and this was reversed by 3-h refeeding. The number of TRH neurons was quantified by in situ hybridization. Although fasting did not change the number of TRH positive cells in the DMN, 3-h refeeding produced a 3 fold increase of the number of DMN TRH neurons,. Thus, our preliminary data suggest that a group of TRHergic-DMH neurons senses energy balance and adjust the activity of TRH neurons, presumably to control feeding behaviour. We will test the DMN-LH projection in fasting and refeeding by double staining of retrograde and anterograde markers to confirm that the DMN TRH neurons activated by refeeding are indeed projecting into the LH.

[1] Joseph-Bravo et al 2015. [2] Suzuki et al 1982. [3] Heuer et al 2000. [4] Chou et al 2003. [5] Zhang et al 2012.

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SULPIRIDE PROTECTS AGAINST DIABETIC RETINOPATHY IN RATS BY INCREASING SYSTEMIC PROLACTIN AND THE ACCUMULATION OF OCULAR VASOINHIBINS

Adán-Castro Elva, Ramírez-Hernández Gabriela, Díaz-Lezama Nundehui, Ruíz-Herrera Xarubet, Arnold Edith, Martínez de la Escalera Gonzalo and Clapp Carmen. Instituto de Neurobiología, Universidad Nacional Autónoma de México, Campus UNAM Juriquilla, Querétaro.

Diabetic retinopathy (RD), an ocular complication of diabetes mellitus (DM), is the leading cause of irreversible blindness and visual impairment amongworking-age adults. In RD damage to the retinal microvasculature results in excessive vasopermeability and vascular proliferation (angiogenesis) that compromise vision. Vasoinhibins, a family of 12 to 18 kDa peptidesderived from the proteolytic cleavage of the hormone prolactin (PRL), inhibit angiogenesis and decrease vasopermeability in experimental diabetes. Here, we used the drug sulpiride, an antagonist of dopamine type 2 receptors, that induces hyperprolactinemia, with the hypothesis that high levels of systemic PRL result in the increase of ocular vasoinhibins able to counteract the progression of RD. Daily intraperitoneal (i.p.) injections of sulpiride induced hyperprolactinemia in a dose-dependent manner, with the highest dose (20 mg/Kg) leading to maximal serum PRL levels (117±10.6 ng/mL). We also demonstrated that ocular vasoinhibins increased in sulpiride-treated animals, as indicated by the presence of 16 and 14 kDa PRL-immunoreactive proteins in the vitreous of sulpiride-treated animals that were not found in the absence of the drug. Sulpiride administered for 2 weeks after 4 weeks of having induced diabetes with a single i.p. injection of streptozotocin (STZ) blocked the diabetes-induced increase in retinal vasopermeability evaluated by the Evans blue assay. Sulpiride did not affect retinal vasopermeability in non-diabetic controls or the glucose circulating levels of all animals. Inducing hyperprolactinemia by osmotic minipumps delivering PRL mimicked sulpiride-induced inhibition of retinal vasopermeability in diabetic rats, thereby supporting hyperprolactinemia leading to elevated levels of intraocular vasoinhibins as its mechanism of action. Sulpiride is a prokinetic drug used in diabetic patients andmay be a desirable therapy for DR.
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VASOINHIBINS PROMOTE APOPTOTIC CELL DEATH IN HIPPOCAMPAL NEURONAL PRIMARY CULTURES

Rodrigo Manuel Aroña[1], Edith Arnold[1,2], Fernando Macías[1], Miriam Ulloa[1], Josué Rivera[1], Carmen Clapp[1], Gonzalo Martínez de la Escalera[1].
[1]Department of Celular and Molecular Neurobiology; [2]CONACYT-Neurobiology Institute, Universidad Nacional Autónoma de México, Campus UNAM Juriquilla, Querétaro, México.

Vasoinhibins (Vi) are a family of peptides derived from prolactin that have been shown to act on endothelial cells blocking angiogenesis via inhibition of proliferation and inducing vaso-obliteration through apoptosis. Similarly to other angiogenic regulatory factors,Vi can modulate some functions of the Nervous System. Vi act in the Central Nervous System promoting anxiety and depression behaviors. Additionally, Vi suppress the neurotrophic effects of vascular endothelial growth factor (VEGF) in primary sensory neurons. Both, anxiety and depression, as well as the inhibition of VEGF actions have been associated with hippocampal neurodegeneration. Thus, in the present study we explored whether Vi affect hippocampal neurons.To explore the actions of Vi on neuronal cells, primary hippocampal neurons were isolated from the brain of E16 mice and cultured on plates or coverslips treated with poli-L-lysine. On DIV1 hippocampal cultures were treated with increasing concentrations of Vi (5, 10, 20, 40 nM) for up to 72 hours (DIV1-DIV4) or with a concentration of 20 nM for up to 16 and 24 hours. Incubation of hippocampal cultures with Vi reduced the cell number in a dose-response manner, evaluated by immunocytochemistry for βIII-tubulin, a neuronal marker. Vi induced the activation of caspase 3 at 16 and 24 hours after treatment, evaluated by immunocytochemistry for cleaved caspase 3. Moreover, Vi increased the expression of genes involve in apoptosis, such as CASP3, the transcription factors FOXO1 and FOXO3, and the members of the Bcl-2 family BAD, BAX, BIM and PUMA, evaluated by qRT-PCR. Altogether these findings show that Vi are capable to induce the apoptosis of hippocampal neuronsand suggest that it occurs via the activation of the apoptotic mitochondrial intrinsic pathway, remaining to demonstrate the role of transcription factors FoxO1 and FoxO3 in this mechanism.
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Effect of GH and IGF-1 treatments after hypoxic-ischemic injury in chicken cerebellar cell cultures.

Baltazar-Lara María del Rosario, Ávila-Mendoza José, Martínez-Moreno Carlos Guillermo, Carranza-Salas Martha, Arámburo Carlos, Luna Muñoz Maricela.Department of Cellular and Molecular Biology, Institute of Neurobiology, UNAM Campus Juriquilla, Querétaro, 76230, México.
Phone number: 427 1141518. E-mail: mabal92@comunidad.unam.mx

Perinatal hypoxic–ischemic (HI) brain damage is a major cause of mortality and long-term neurological impairment in children. The rapid growth of the cerebellum in the last half of fetal development makes it more vulnerable to a HI injury. Several studies have shown that growth hormone (GH) and insulin-like growth factor-1 (IGF-1) are upregulated in different brain areas after damage by hypoxia. Moreover, there is increasing evidence suggesting that GH and IGF-1 treatments are able to induce neuroprotection and neural-regeneration in the CNS. In this study, we evaluated the effects of GH and IGF-1 treatment on cell survival after an acute HI injury in primary cell cultures of embryonic chicken cerebellum. In addition, we evaluated the cerebellar expression of GH and IGF-1 mRNA in normal and hypoxia-low glucose (HLG) conditions. To induce neural damage in primary embryonic cerebellar cultures, cells were maintained in hypoxic conditions (0.5-5% O2), and incubated in low glucose media (1 g/L) for 12 h, and subjected to a 24 h of reoxygenation. Cell cultures were treated with 1 nM recombinant chicken GH (rcGH) and/or 40 nM recombinant human IGF-1 (hIGF-1) to examine their neuroprotective effects. We observed that cell incubation of cells in HLG caused a significant decrease in cell viability (51.6 $\pm$ 2.9 %) increase in apoptosis (122.0 $\pm$ 4.5 %,) and necrosis (538.6 $\pm$ 92.1 %), while treatment with GH increased cell viability (76.1 $\pm$ 4.1 %), and decreased apoptosis (105.0 $\pm$ 3.9 %) and necrosis (73.8 $\pm$ 11.1 %), while IGF-1 treatment only increased cell viability (70.1 $\pm$ 4.2 %) without affecting apoptosis (105.0 $\pm$ 3.9 %) and necrosis (73.8 $\pm$ 11.1 %). After incubation in HLG conditions, cerebellar cell cultures increased GH and IGF-1 mRNA expression were increased by (~3 fold). GH gene silencing by small interfering RNA (siRNA) decreased both, GH mRNA expression (1.6-fold) and IGF-1 (0.5-fold) mRNA expression in the HLG group, suggesting that GH regulates IGF-1 expression under HLG conditions. Our results strongly suggest that both, local and exogenous GH act as a neuroprotective factor and they regulate IGF-1 expression under HLG conditions.

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Expression and regulation of membrane progesterone receptorsdelta and epsilon in cells derived from human glioblastomas

José Moisés Capetillo-Velázquez[1], Aylin Del Moral-Morales[1], Valeria Hansberg-Pastor[2], Ignacio Camacho-Arroyo[1].

[1]Unidad de Investigación en Reproducción Humana, Instituto Nacional de Perinatología-Facultad de Química, Universidad Nacional Autónoma de México (UNAM), Ciudad de México, México
[2]Departamento de Biología, Facultad de Química, UNAM, México

Correo electronico: moicapetillo@gmail.com

Progesterone (P4) is a steroid hormone that exerts different effects on the central nervous system, including the growth of glioblastomas, the most frequent and malignant primary brain tumors. The mechanisms involved in P4 effects have been largely studied regarding its intracellular receptor(PR).However, the presence of membrane progesterone receptors (mPRα-ε) in different regions of the brain has opened new research perspectives. In glioblastoma derived cell lines (U87 and U251), we have demonstrated a high expression of mPRα and β, and a very low expression of γ subtype. Both,α and β expression is regulated by P4 at 12 h post-treatment. Nevertheless, the expression and hormone regulation of the remaining mPR subtypes have not been yet described.

In this work, U87 and U251 cells were used to study the expression and hormonal regulation of mPRδ and mPRε using RT-qPCR. We first determined the basal expression levels of both genes. In both cell lines, mPRδ shows a higher expression than mPRε. We then treated the cells with different concentrations of P4 (10 nM, 100 nM, and 1 μM) for 12 and 24 h. Preliminary data show no changes in mPRδ expression after 24 hof treatment, and a tendency to increase mPRε with 1 μM P4 was observed. Our results indicate differences in mPRδand mPRε expression in glioblastoma cell lines that could be regulated by P4.

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Prolactin neuroprotection against glutamate excitotoxicity is mediated in part via NF-kB activation and reducing the $[Ca^{2+}]i$ overload in hippocampal neuronal primary cell cultures

CERBÓN, M[1*]; VÁZQUEZ-MARTÍNEZ, ER[1], RIVERO-SEGURA, NA [1], FLORES, E [2]; GARCÍA DE LA CADENA, S [3]; CABRERA-REYES, EA [1], RINCÓN-HEREDIA, R[3], MARTÍNEZ-PACHECO, H[2],; FERREIRA, DG [4];, LOPES,L [4], MASSIEU, L[3].

[1] Unidad de Investigación en Biología de la Reproducción Instituto Nacional de Perinatología-Facultad de Química, UNAM. [2]Facultad de Medicina, UNAM, [3]Instituto de Fisiología Celular, UNAM.[4] Instituto de Medicina Molecular (IMM-Lisboa).

*E-mail: mcerbon85@yahoo.com.mx; Montes Urales 800, Lomas de Virreyes, 11000, Mexico City, Mexico. Tel. 55209900 ext. 513

Recently, our group has described the neuroprotective effects of prolactin (PRL) hormone against Glu excitotoxicity in both *in vivo* and *in vitro* models. However, the molecular mechanisms of neuroprotection induced by prolactin remain unknown. The aim of the present study was to evaluate whether PRL-treatment prevents the neuronal cell death through the preservation of the mitochondrial activity and the intracellular calcium homeostasis. Furthermore, we assessed the content of proteins (Bcl-2, Bax and Casp-3) involved in the apoptotic pathway, as well as the activation of the transcriptional factor NF-κB, in primary cultures of rat hippocampal neurons exposed to Glu. In addition, we evaluated whether PRL interacts with glutamatergic receptors (AMPA, NMDA and KA) by *in silico* analysis. Our results demonstrated that PRL, significantly reduced both apoptosis and necrosis, induced by Glu excitotoxicity and maintains mitochondrial activity. Importantly, the Glu-induced intracellular calcium overload was attenuated by PRL-treatment. These data correlate with the significant reduction observed in the content of activated Casp-3 and in the pro-apoptotic Bax/Bcl-2 ratio. Accordingly, PRL elicited the activation of NF-κB transcription factor. Interestingly, the *in silico* analysis suggest that PRL binds to glutamatergic receptors. In conclusion, our results demonstrate that PRL exerts neuroprotection against Glu-induced excitotoxicity in primary cultures of rat hippocampal neurons, overexpression of Bcl-2 protein via NF-kB pathway activation and the preservation of [Ca2+]I levels, and possibly through the PRL interaction with glutamatergic receptors.

Sex hormone effects on EZH2 expression in human glioblastoma cells

Aylin Del Moral-Morales[1], Valeria Hansberg-Pastor[2], Ignacio Camacho-Arroyo[1].
[1]Unidad de investigación en Reproducción Humana, Instituto Nacional de Perinatología-Facultad de Química, UNAM. [2]Facultad de Química, Departamento de Biología, UNAM.

aylindmm@hotmail.com Facultad de Química, Ciudad Universitaria, Coyoacán, Ciudad de México, C.P. 04510 Tel 56223732

ABSTRACT

Enhancer of zeste homolog 2 (EZH2) is a methyltransferase subunit of the Polycomb repressive complex 2 (PRC2), which catalyzes the mono- di- or trimethylation of histone 3 at lysine 27 (H3K27). This epigenetic mark has been associated with the transcriptional repression of tissue-specific genes. It has been reported that EZH2 is over expressed in several types of cancer including glioblastomas, the most frequent and aggressive primary brain tumors. EZH2 over expression contributes to glioblastoma progression. Besides, it has been shown that sex hormones such as progesterone (P4)increases proliferation, migration, and invasion of glioblastoma cells. However, their role in the transcriptional regulation of EZH2 in these tumors is unknown. In this study, we first conducted an *in silico* analysis to search for potential binding sites for nuclear hormone receptors in the promoters and sequence of the EZH2 gene, and we found that there are several hormone response elements (estrogen response elements and progesterone/glucocorticoids response elements). To test if P4 or estradiol (E2) could directly regulate EZH2 transcription, we first determined its basal expression in three cell lines derived from human glioblastomas (U251, U87, and D54). We found that all cell lines differentially express EZH2, with U251 showing the highest levels and U87 the lowest. We then treated U251 and U87 cells with P4 10 nM and E210 nM for 12 and 24 hours and evaluated the expression of EZH2 by RT-qPCR. Our data show that P4 decreases EZH2 expression in glioblastoma cells after 12 hours of treatment, suggesting a hormonal regulation of this gene.

Chronic stress inhibits the response of the thyroid axis to cold exposure

Angélica Gutiérrez Mata, Mariana Gutiérrez-Mariscal, Miguel Cisneros, Jean-Louis Charli, Patricia Joseph-Bravo*

Departamento de Genética del Desarrollo y Fisiología Molecular, Instituto de Biotecnología, UNAM.Av. Universidad #2001, Col. Chamilpa C.P. 62210, Cuernavaca, Morelos.gtzmata@ibt.unam.mx,* joseph@ibt.unam.mx(tel 56227632).

The hypothalamus-pituitary-thyroid (HPT) axisis involved in energy homeostasis. It is activated by cold stimulation and inhibited by stress; a previous stress exposure or corticosterone injection blunts the cold-induced activation. Certain types of chronic stress inhibit also the HPT axis, but their effects on the axis response to acute energy demanding stimuli are unknown (1). The aim of this work is to evaluate if chronic exposure to restrain (RES),a psychological stressor, interferes with the response of the HPT axis to cold.

Wistar male rats were introduced into a restrain apparatus every day, during 30 min, for14 days; controls were placed in an isolated cage during the same period. On day 15th at 9.30 AM animals were introduced, in clean individual cages,eitherinto the cold room (5°C), or into a near-by room at room temperature (RT).Parameters of HPT axis function, thyrotrophin-releasing hormone-TRH, thyrotrophin-TSH, and thyroxine-T4, were evaluated as well as those representing the activity of the HPA axis (CRH, corticosterone). Compared to controls, RES decreased body weight gain and TSH serum concentration, whereas that of corticosterone increased. Gene expression was evaluated in the paraventricular nucleus of the hypothalalamus (PVN); cold stimulation diminished that of*Crh,*and increased *Trh* in controls but not in RES animals. At the median eminence level, where hypophysiotropic terminals are concentrated, levels of processed TRH were similar in controls or RES groups remaining at RT but were lower in both groups exposed to cold, supporting cold-induced increased release. In contrast, serum TSH concentration augmented only in the cold-exposed control group. Corticosterone was increased 8.5 fold by cold exposure in controls and by 18 fold in RES. Brown adipose tissue (BAT) is the thermogenic organ that in response to cold-induced adrenergic stimulation activates deiodinase 2 (D2) increasing T3 intracellular levels that stimulate the uncoupling protein UCP-1.Cold increased D2 and UCP1 expression in controls, albeit D2 change did not achieve significance, and RES animals showed no changes. These results corroborate the hyperactivity of the HPA axis to a heterotypic stimulus (cold) in the chronically stressed group (RES) (3) and demonstrate that a chronically stressed animal presents a blunted HPT axis response to cold. Dysfunction of the HPT axis response may contribute to altered energy homeostasis.

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The effects of Progesterone-Induced Blocking Factor (PIBF) in human glioblastoma cell proliferation, migration and invasion

Valeria Hansberg-Pastor[1], Araceli Gutiérrez-Rodríguez[2], and Ignacio Camacho-Arroyo[2]

[1]Facultad de Química, Departamento de Biología, Universidad Nacional Autónoma de México (UNAM).
[2]Unidad de Investigación en Reproducción Humana, Instituto Nacional de Perinatología-Facultad de Química, UNAM.

Facultad de Química, UNAM, Av. Universidad 3000, UNAM, C.U., Coyoacán, 04510, Ciudad de México. Tel: 5622 3732, valeriahp@gmail.com

Glioblastomas are the most frequent and aggressive primary brain tumors, they are highly invasive, and the median patient survival is less than two years. It has been reported that these tumors respond to progesterone (P4, 10 nM) by increasing their proliferation, migration and invasion capabilities. Progesterone-induced blocking factor (PIBF) is a P4-regulated protein expressed in different types of highly proliferative cells including glioblastomas. Besides, PIBF increases the number of glioblastoma cells *in vitro* although there is no evidence regarding its role in tumor cell migration and invasion.

In this work, we first evaluated the P4-dependent regulation of PIBF expression in the human glioblastoma derived cell lines U251 and U87. Then, we determined the effects of PIBF on proliferation, migration, and invasion of these cells. PIBF mRNA expression was up-regulated by P4(10 nM) from 12 to 24 h. At the protein level, glioblastoma cells expressed two PIBF isoforms, 90 and 57 kDa. The content of the shorter isoform was significantly increased after 24 h of P4 treatment, while the progesterone receptor antagonist RU486 (10 μM) blocked this effect. PIBF (100 ng/mL) increased the number of U87 cells on days 4 and 5 of treatment and induced cell proliferation on day 4. Wound-healing assays showed that PIBF promotes the migration of U87 (12–48 h) and U251 (24-48 h) cells. Transwell invasion assays showed that PIBF augmented the number of invasive cells in both cell lines at 24 h. These data suggest that PIBF promotes the proliferation, migration, and invasion of human glioblastoma cells.

The Renin-Angiotensin system is up-regulated in the Arcuatenucleus of rats exposed to a high-fat diet.

Luis Daniel Hernández-Torres[1,2,*], Guadalupe Elena Donjuán …[1,2], Carolina Escobar[3], Roberto Salgado-Delgado[1,2], Nadia Saderi[1,2]

(1) Laboratorio de Neuroanatomía Funcional y Ritmos Biológicos, Facultad de Ciencias, Universidad Autonoma de San Luis Potosí, Av. Dr. Salvador Nava Mtz. s/n (Zona Universitaria), San Luis Potosí, S.L.P., México
(2) Centro de Investigaciones en Ciencias de la Salud y Biomedicina, UASLP
(3) Departamento de Anatomía, Facultad de Medicina, UNAM

*luis.d.hernandez@hotmail.com, +52 444 165 2248

The renin-angiotensin system (RAS) is part of the regulation mechanism of blood pressure and plays a crucial role in the metabolic syndrome-related hypertension. There is evidence that anti-hypertensive treatments regulating RAS, in both metabolic syndrome patients and animal models of this pathology,improve metabolic parameters, such as fasting plasma glucose and leptin.

The Arcuate nucleus of the hypothalamus (ARC) perceives blood-borne metabolic signals and regulates energy homeostasis via the autonomic and neuroendocrine brain output. The ARC also expresses all the components of the RAS. In order to investigate whether the ARC RAS is involved in overweight and hypertension comorbidity, we developed a rat model of metabolic syndrome induced by a high-fat diet (HFD), containing the 30% of fat. Experimental animals were divided in 3 groups (n=5), which underwent to HFD for 4, 8 and 12 weeks, respectively, and then were sacrificed together with the respective control groups. In order to test the effectiveness of the protocol, food intake, bodyweight and water consumption were monitored. In addition, the arterial tail blood pressure was measured for 5 days before the sacrifice, with a non-invasive CODA system to evaluate the hypertension onset. Both body weight and blood pressure became significantly higher at the week 8, with respect to controls. In the ARC, we found that after 12 weeks under the HFD protocol, there is significant increase of both angiotensin-converting enzyme (ACE) and angiotensin-II type 1receptor (AT1-R) immunoreactivity (IR). We also observed that ACE-IR is especially strong laterally to the third ventricle and within the inner layer of the median eminence, and that AT1-R positive cells are surrounded by ACE containing fibers.

Given the pivotal role of the ARC in detecting the peripheral energy state and in transducing it into autonomic adjustments, our results suggest a direct involvement of this nucleus in the overweight-related hypertension subsequent to an unbalanced diet.

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Optimization of tanycyte cultures for expression of the thyrotropin-releasing hormone-degrading ectoenzyme

Abraham López-Barajas[1], Adair Rodríguez-Rodríguez, Beetsi Urrieta-Chávez,Antonieta Cote-Vélez, Patricia Joseph-Bravo, Jean-Louis Charli.
Instituto de Biotecnología, Universidad Nacional Autónoma de México. Av. Universidad #2001, Col. Chamilpa. C.P. 62210. Cuernavaca, Morelos. Tel. 56227620.
E-mail: [1]abarajas@ibt.unam.mx

Tanycytes are a specialized glial cell population which surrounds third ventricle walls, in the medial basal hypothalamus. These cells have been implicated in barrier functions, as they limit the entrance and outflow of signals to the systemic circulation; generation of new hypothalamic neurons in the adult rat, based on their similarity with radial glia; and the regulation of the hypothalamus-pituitary-thyroid (HPT) axis, which sets thyroid hormone levels in serum. In this axis, thyrotropin-releasing hormone (TRH) neurons of the paraventricular nucleus of the hypothalamus project to the median eminence, where TRH is released into portal vessels connected with the anterior pituitary. Pyroglutamyl peptidase II (PPII), the TRH-degrading ectoenzyme, is expressed in the tanycytes of the median eminence, including β2 tanycytes. Signals that decrease HPT axis activity, as systemic administration of thyroid hormones (TH) [1] or fasting [2], increase the expression of PPII in the median eminence, suggesting that tanycyte PPII controls the HPT axis, limiting the amount of TRH that reaches the anterior pituitary. This suggestion has been substantiated experimentally, since PPII inhibition can increase serum thyrotropin concentration [1].
Since median eminence is a blood brain barrier-free area, we intent to identify the local and peripheral signals that regulate PPII expression and activity, as they can possibly affect HPT axis activity. To accomplish this aim, we tested PPII expression and activity in tanycyte primary cultures obtained from the median eminence of 10-day old rats; cultures were initially established in serum supplemented medium and tested 1 or 2 weeks later. Unexpectedly, we could not detect PPII activity, probably because of abnormal overexpression of a truncated isoform of PPII, which exerts a dominant negative activity over the complete isoform [3,4]. We therefore tested a new method, in serum free culture conditions [5].Tanycyte primary cultures were characterized by immunofluorescence, showing the presence of nestin, vimentin and DARPP-32, tanycyte specific markers. Cultures expressed a robust activity of PPII. We also tested organotypic cultures of hypothalamic slices [6], and detected tanycyte markers and PPII activity. Thus, although culture in serum-supplemented conditions favors the expression of the truncated isoform of PPII, serum-free primary and serum-supplemented organotypic cultures preserve PPII activity, and should be useful models to study regulation of PPII activity.

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HYPERPROLACTINEMIA LEADS TO ELEVATED LEVELS OF PROLACTIN IN THE VITREOUS OF PATIENTS WITH DIABETIC RETINOPATHY

Nuñez-Amaro Daniel[1], Ramírez-Neria Paulina[2], Mira-Lorenzo Ximena[2], García-Franco Renata[2], Robles-Osorio Ludivina[1], Clapp Carmen[3]
[1]Facultad de Ciencias Naturales, Universidad Autónoma de Querétaro; [2]Instituto Mexicano de Oftalmología I.A.P., Querétaro; [3]Instituto de Neurobiología, UNAM, Campus Juriquilla, Querétaro, México.

Diabetic retinopathy (DR) is a major microvascular diabetic complication characterized by excessive vasopermeability and the formation of new blood vessels (angiogenesis) that can lead to retinal detachment and blindness. Preclinical studies show that the hormone prolactin (PRL) accesses the retina from the systemic circulation where it is proteolytically cleaved to vasoinhibins, a family of PRL fragments that inhibit ischemia-induced retinal angiogenesis and diabetes-derived retinal vasopermeability. Therefore, medications causing hyperprolactinemia have therapeutic potential in DR. Levosulpiride is a dopamine D2 receptor antagonist effective for inducing hyperprolactinemiaused in diabetic patients due to its prokinetic effects. In this study we investigated whether the oral administration of levosulpiride elevates PRL levels in the vitreous of volunteer patients with DR undergoing medically prescribed vitrectomy. PRL was measured by immunoassay (IMMULITE 2000 XPi). The treatment with levosulpiride (25 mg, 3 times a day) was for 7 days ending on the day of vitrectomy. Levosulpiride elevated circulating (111.7 ± 12.51 vs 10.08 ± 1.46 ng / ml, $p < 0.0001$) and vitreous (3.59 ± 0.45 vs. 1.65 ± 0.26, $P < 0.0008$) levels of PRL compared to placebo treatment. PRL levels in the vitreous correlated directly ($r = 0.56$, $R2 = 0.3140$, $p < 0.05$) with the circulating levels of PRL in the whole study population (16 patients treated with placebo and 15 with levosulpiride). These results show for the first time the presence of PRL in human vitreous and the incorporation of systemic PRL into the eye of patients with DR. In-process studies are evaluating vitreous levels of vasoinhibins and of other vasoactive and proinflammatory mediators known to influence DR. Our work is consistent with the use of levosulpiride to elevate intraocular PRL as potential treatment for DR.
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Sex differences in daily hypothalamic leptin signaling between lean and obese mice *Neotomodon alstoni*

***Moisés Pérez-Mendoza**[1,2], Manuel Miranda-Anaya[1].

[1]Unidad Multidisciplinaria de Docencia e Investigación, Facultad de Ciencias, UNAM-Juriquilla, Qro. CP. 76230. [2]Facultad de Ciencias Naturales, UAQ-Juriquilla.*pememoises@ciencias.unam.mx; Tel. 1926212. Ext. UNAM 34312

This paper compared the effects of spontaneous obesity on the daily profile in the relative amount of the leptin receptor (LepRb), and its output. That is, the precursor Pro-opiomelanocortin (POMC) over a 24-hour period, and compared with differences in locomotion and food intake in periods of artificial light. Differences between lean and obese mice were examined, as were sex differences. Body weight, food intake and locomotor activity were monitored in freely moving lean and obese mice. Hypothalamic tissue was collected at 5h, 10h, 15h, 19h and 24 h. Samples were analyzed by western blotting to determine the relative presence of protein for LepRb, STAT3 phosphorylation (by pSTAT3/STAT3 ratio) and POMC.

Obese mice were 60% less active in locomotion than lean mice during the night. While both locomotor activity and food intake were noticeably greater during the day in obese mice than in lean mice; the hypothalamus in obese mice showed a lower relative abundance of POMC and reduced pSTAT3/STAT3 ratio andleptin receptors. Behavioral and biochemical differences were more evident in obese females than in obese males. These results indicate that obesity in *N. alstoni* affects hypothalamic leptin signaling according to sex.

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Rapid induction of thyrotropin releasing hormone biosynthesis in the paraventricular nucleus of the hypothalamus during obesity development in male rats; role of leptin signaling.

Marlen Asucena Ramírez Bustos, María del Pilar Torres Reyes, Miguel Cisneros Ramírez, DavidAntonio Villaseñor Peña, Patricia Joseph-Bravo, Jean-Louis Charli andRosa María Uribe Villegas. Instituto de Biotecnología-UNAM. Av. Universidad 2001, Col Chamilpa, Cuernavaca, Morelos. CP 62210. marasu@ibt.unam.mx

Hypothalamus-pituitary-thyroid (HPT) axis activity plays an important rolein energy homeostasis. The axisis inhibited by fasting, chronic stress and stimulated by cold stress and obesity. Thyrotropin-releasing hormone (TRH) neurons of the paraventricular nucleus of hypothalamus (PVN) have a pivotal role in control on HPT axis activity. These neurons project to the median eminence, where TRH is released to the portal blood system. TRH stimulates the secretion of thyrotropin from the pituitary, which activates the synthesis and release of thyroid hormones(T_4 and T_3) from the thyroid gland. Orexigenic and anorexigenic signals modulate HPT activityin opposite way, in part through regulation of TRHmRNA levels in the PVN neurons. Neuronal endings containing NPY, AgRP, αMSH, and MCH contact PVN TRH-synthesizing neurons and control their activity. Furthermore, leptin is a peripheral anorexigenic signal that increases PVN TRH mRNA level directly or by activation of αMSH neurons from the arcuate nucleus.
We hypothesized that a positive energy balance increases PVN TRH mRNA levels and that this effect is modulated by leptin.Young adult Wistar male rats were fed with a regular diet (18% Kcal from fat) or a high fat diet (45%Kcal from lard fat) during 3 or 30 days in order to induce obesity. Physical and biochemical parameters were analyzed. In animals maintained with the high fat diet for 30 days bodyweight and weight of white fat depots was increased compared to the rats fed with regular diet. High fat diet in take enhanced leptin and insulin serum concentrations as soon as at day 3, an increase that was maintained up to 30 days; however, serum glucose concentration did not change. Semi-quantitative RT-PCR revealed that feeding with the high fat diet for 3 or 30 days increased TRH mRNA level in the PVN area. This was corroborated by in situ hybridization at day 30. To clarify whether leptin or αMSH can contribute to the increase in PVN TRH mRNA levels in response to positive energy balance, we quantified the number of PVN TRHergic neurons that express phosphorylated Stat-3 or CREB (pStat-3 or pCREB respectively) in rats maintained with the high fat diet for 30 days. We detected significantly more TRHergic neurons positive for pSTAT3 compared with animals fed with the regular diet. In contrast, we did not observe changes in the number of TRHergic neurons that co-localize with pCREB. In conclusion, the data suggest that the early increase in PVN TRHmRNA levels induced by positive energy balance is mediated by a direct action of leptin.

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PROLACTIN PROTECTS NEURORETINAL FUNCTION AND VASCULAR STABILITY IN DIABETIC MICE

Ramírez-Hernández Gabriela, Díaz-Lezama Nundehui, Adán-Castro Elva, Ruiz-Herrera Xarubet, Macotela Yazmín, Arnold Edith, Martínez de la Escalera Gonzalo Clapp Carmen. Instituto de Neurobiología, UNAM Campus Juriquilla, Querétaro.

The diabetic pandemia requires new approaches to understand the pathophysiology and improve the detection, prevention, and treatment of diabetic retinopathy (DR), the cause of blindness in diabetic patients. The pathogenesis of DR includes glucose-mediated neuronal alterations and microvascular damage in the retina. Mechanisms of vascular injury comprise increased microvascular permeability and occlusion leading to ischemia-induced angiogenesis. The hormone prolactin (PRL) is a neurotrophic factor protecting against neuronal cell death and dysfunction in the continuous light-exposure model of retinal degeneration. Also, PRL protects against DR via its proteolytic conversion to vasoinhibins, a family of PRL fragments that inhibit ischemia-induced retinal angiogenesis and diabetes-derived retinal vasopermeability. Here, we used 18-week diabetic mice treated with multiple low-doses of streptozotocin (STZ) to evaluate the electroretinogram (ERG) under hyperprolactinemic conditions. Also, we determined diabetes-induced retinal vasopermeability in 24-week diabetic micethat were null (*Prlr*$^{-/-}$) or not (*Prlr*$^{+/+}$) for the PRL receptor. Hyperprolactinemia (>50 ng/ml) induced by treatment with the dopamine type 2 receptor antagonist, sulpiride, prevented the diabetes-mediated reduction in the B-wave of the ERG but had no effect in non-diabetic controls. Retinal vasopermeability evaluated by the Evans Blue method increased two-fold in diabetic mice and such increase was significantly higher ($p<0.03$) in *Prlr*$^{-/-}$mice compared to *Prlr*$^{+/+}$ mice. Genetic deletion of the PRL receptor may have prevented the incorporation of systemic PRL into the eye, thereby blocking its ocular conversion to vasoinhibins and favoring excessive retinal vasopermeability. Our findings support the protective influence of systemic PRL on retinal tissue and the potential therapeutic effects of drugs inducing hyperprolactinemia in DR.
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Testosterone promotes glioblastoma cell proliferation throughandrogen receptor activation

Dulce Carolina Rodríguez-Lozano[1], Alejandro Cerón Villalva[1], Valeria Hansberg-Pastor[2]; Ignacio Camacho-Arroyo[1]

[1]Unidad de Investigación en Reproducción Humana, Instituto Nacional de Perinatología-Facultad de Química, Universidad Nacional Autónoma de México (UNAM), Ciudad Universitaria, Coyoacán 04510, Ciudad de México, México.
[2]Facultad de Química, Departamento de Biología, UNAM, Ciudad de México, México.

Correo electrónico: dcrl88@gmail.com. Tel. 55 42 29 50 08

Glioblastomas (GBM) are the most aggressive and frequent brain tumors in humans.The incidence of GBM in men is higher thanin women (3:2), but little is knownabout themechanisms underlying theepidemiology of this disease. It has been reported that the expression of theandrogen receptor (AR) is higherin GBM tissue as compared to the periphery normal brain tissue in patients. Wedetermined the effectsof testosterone (T) in the number of cells and proliferationrates of the human glioblastoma derived cell lines U87, U251, and D54.First, we determined the effect of different concentrations of T (1 nM-1 μM) on the growth of the GBM cells. Our results showed that T (100 nM) significantly increased the number of cells of the three GBM lines. This increase was blocked by the treatment withthe AR antagonist, flutamide. Besides, BrdU assaysshowed that the increase in the number of U87, U251, and D54 cells is due to the increase in cell proliferation induced by T at 3 days post-treatment. Finally, by western blot we found that Tdecreasesthe expression of AR at 24 hours, suggesting the ligand-dependent degradation of AR.These data indicate that T induces proliferation in human GBM cells throughthe activation of AR.

Adeno-associated virus as transgenic tools for manipulation of tanycytes and hypophysiotrophic cells

Adair Jonathan Rodríguez Rodríguez[1], Rosa María Uribe, Fidelia Romero, Patricia Joseph-Bravo and Jean Louis Charli
Instituto de Biotecnología, Universidad Nacional Autónoma de México. Av. Universidad #2001, Col. Chamilpa C.P. 62210, Cuernavaca, Morelos. Tel. 56227620. [1]adairr@ibt.unam.mx

Tanycytes are a group of specialized glial cells located in the walls of the third ventricle of the basal medial hypothalamus. These cells are crucial for the regulation of thyroid hormone levels, as they have the highest relative expression of deiodinase II and pyroglutamyl peptidase II, the thyrotropin degrading ectoenzyme, in the brain. Tanycytes can be divided in four subtypes according to their location. α1 and α2 tanycytes connect the dorsomedial and ventromedial regions of the hypothalamus with the third ventricle, respectively. β1tanycytes limit the passage of signals from the median eminence to the arcuate nucleus, while β2 tanycytes act as a barrier between the median eminence and the third ventricle, and contact hypophysiotrophic terminals.
Since options to study tanycyte functions are limited, we evaluated multiple serotypes of recombinant adeno-associated viri (AAV), to express GFP under CMV promoter in different cell types of the basal medial hypothalamus. We injected $1x10^{11}$ viral genomes (vg) of serotypes 1, 2, 4, 5, 6, 8 or 9 in the third ventricle of adult rat brains. One month later, GFP expression was evaluated by fluorescence or immunohistochemistry in slices of rat brains perfused with paraformaldehyde. Profiles of viral transduction were different with each serotype, with AAV5 and 6 being less effective, as transduction was limited to the choroidal plexus cells. Interestingly, serotype 4 transduced mostly hypophysiotrophic neurons, as GFP was found in periventricular neurons and terminal buttons in median eminence, with minimal transduction over α tanycytes. Median eminence cells were more effectively transduced by serotypes 1, 2, 8 and 9. While transduction of serotypes 8 and 9 promoted a broad expression throughout the brain, GFP was detected in non-tanycyte shaped cells in the median eminence. AAV1 was the serotype that led to a more abundant expression of GFP over different types of cells in the median eminence, including β2 tanycytes, while for serotype 2 GFP expression was limited to β2 tanycytes. A lower dose ($2x10^{10}$ vg) of self-complementary versions of AAV1 and AAV2 (scAAV1 and scAAV2) was sufficient to detect a strong GFP signal along the median eminence. These data suggest that scAAV1 is useful for efficient transduction of multiple median eminence cell types, scAAV2 for specific β2 tanycyte transduction, and AAV4 to manipulate hypophysiotropic neurons.
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Social isolation during adolescence induces sex-dependent differences on the activity of hypothalamic-pituitary-thyroid axis, and its response to cold exposure

Diego Rodríguez-Sarmiento, Patricia Joseph-Bravo, Lorraine Jaimes-Hoy*

Departamento de Genetica del Desarrollo y Genetica Molecular, Instituto de Biotecnología, UNAM. diegor@ibt.unam.mx, *ljaimes@ibt.unam.mx (tel 5622-7620). Av. Universidad #2001, Col. Chamilpa C.P. 62210, Cuernavaca, Morelos

Social stressorsin rats such as perinatal maternal separation or social isolation can have long-lasting effects on their neuroendocrine development and response to environmental stressors later in life. Response to socialisolationdepends on the animal's age, sex, duration of the stressor, and to external factors (environmental events). As the hypothalamic-pituitary-thyroid (HPT) axis activity is susceptible to various forms of stress (1), we studied if post-weaning social isolation altered the HPT axis function and its response to an environmental stressor, such as cold, and if changes were dependent on the animal´s sex. The initial response to acute exposure to cold begins with the increased expression of TRH in the paraventricular nucleus (PVN) and hypothalamic release. This, in turn, stimulates the release of TSH from the anterior pituitary gland and thyroid hormones from the thyroid gland.

Male and female Wistar rats were socially isolated at weaning on postnatal day (PND) 23 and through adolescence (2); controls were kept in groups of 2-3/cage.Starting adulthood (PND 60), half of the male and female rats of each group were left at room temperature (RT) orexposed to a cold environment (4°C) for one hour,then immediately sacrificed. mRNA levels of Trh in the PVN and expression of genes associated withbrown adipose tissue (BAT) thermogenesis (β3-AR, PGC1-α, D2 and UCP-1) were semi-quantified by RT-PCR. Serum hormones were quantified by radioimmunoassays or ELISA (3). Compared to controls, isolation increased basal corticosterone levels only in females. Basal HPT axis activity was not affected by social isolation in female rats whereas isolated males had diminished T4 serum concentration.

In response to cold exposure, serum TSH and T4 concentrations were higher in cold-exposed male controls and TSH, lower in isolated than inthose kept at RT. The response of females was more variable probably due to variation in estradiol levels (4), as reported.D2 activity, D2 and PGC1-α mRNA levels increased by cold exposure whether in controls or isolated rats. In conclusion, social isolation induces differences in the HPT axis function depending on the animal´s sex, as the cold-induced activation of the HPT axis was blunted only in male rats.

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Voluntary exercise-induced activation of the thyroid axis is attenuated by chronic stress.

Ma. Felix Salmeron Jimenez, Mariana Gutiérrez-Mariscal,Fidelia Romero, Jean-Louis Charli, Patricia Joseph-Bravo*

Departamento de Genética del Desarrollo y FisiologíaMolecular, Instituto de Biotecnología, UNAM. mafel@ibt.unam.mx,*joseph@ibt.unam.mx (tel 5622-7632).Av. Universidad #2001, Col. Chamilpa C.P. 62210, Cuernavaca, Morelos

Exercise stimulates energy utilization activating lipolysis and glycolysis, events modulated by sympathetic and hormonal influences that include thyroid and glucocorticoid hormones (1,2). Energy homeostasis relies on the efficient response of the hypothalamus-pituitary-thyroid (HPT) and HP-adrenal (HPA)neuroendocrine axes to metabolic and behavioral cues. These axes are controlled by the activity of hypothalamic paraventricular hypophysiotropic neurons expressing thyrotrophin-releasing hormone (TRH) or corticotrophin-releasing hormone (CRH). Acute or chronic stress inhibit basal activity of the HPT axis and we have shown that acute stress or corticosterone injections blunt cold-induced activation of HPT axis and target organs (2). As the response of the HPA axis depends on the previous history of an organism, we have evaluated the effect of chronic exposure to restrain, a psychological stressor,on the metabolic and hormonal responses to voluntary exercise.

Wistar male rats were introduced into a restrain apparatus every day, during 30 min, for19 days (Res); controls (C) were placed in an isolated cage during the same period. At day 15th and for 3 weeks, animals had either access every night to a running wheel (RW) or stayed in individual cages (Sed). Sed controls received the amount of food consumed by the exercised groups.All rats rejoined their cage partner during the day. No differences were detected in body weight gain between groups. Res-RW ran 45% less than C-RW and in consequence, white or brown adipose tissues weights decreased in C-RW but not in Res-RW. Diminished adipose tissue mass was proportional to revolution number in both groupsAs reported, compared to sedentary groups TRH expression increased in the PVN of C-RW (1) but not in Res-RW, so as the expression of uncoupling protein-1 in brown-adipose tissue.

These results support our hypothesis that the hyperactivity of the HPA axis produced by chronic stress inhibits the response of the HPT axis to changes in energy requirements and diminishes physical activity, possibly due to fatigue, a well-known consequence of hypothyroidism (3).Stress-induced dysfunction of the HPT axis response to altered energy homeostasismay contribute to obesity.

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Comparison between the neuroendocrine mechanisms regulating the synthesis and release of reptilian, avian and mammalian pituitary Growth Hormone (GH)

Urban Sosa Valeria Alejandra, Ávila Mendoza José, Carranza Salas Martha Elizabeth, Luna Muñoz Maricela, Martínez-Moreno Carlos Guillermo y Arámburo de la Hoz Carlos

Departamento de Neurobiología Celular y Molecular, Instituto de Neurobiología, UNAM, Campus Juriquilla, Querétaro, CP 76230.Tel (442) 2-38-10-65

urbanv@comunidad.unam.mx

The regulation of GH synthesis and release in the somatotropes is controlled by several hypothalamic neuropeptides, including GHRH, TRH, PACAP, Ghrelin, GnRH and somatostatin. Among vertebrates, these neuropeptides regulate the expression of GH with different potency. However, there are only a few studies devoted to analyze which is the specific role of these neuropeptides upon the pituitary GH regulation during evolution. Therefore, the present study compared the effect of the neuropeptides and its potency in the regulation of GH (in vitro) in three different groups of vertebrates: reptiles (green iguana); birds (chicken) and mammals (rat). The following results were obtained: a) for rat pituitary cultures it was found that 10 nM GHRH stimulated GH release at one and 4 h of incubation, while GH mRNA expression was increased at 2 and 6 h post treatment (9 and 22 times respectively). On the other hand, 10 nM TRH had no significant effect on GH release; however, the expression of GH mRNA increased after 2 and 6 h of incubation (9 and 51 times respectively). In chicken pituitary cultures, the administration of 10 nM GHRH increased GH release up to the 6 h of treatment, while the GH mRNA increased its expression within the first two hours, decreased at fourth, and increased again after 6 h of incubation. TRH (10 nM) stimulated GH secretion at 4 h, while the mRNA expression increased after 4 and 6 h of incubation (4 and 1.5 times respectively). In the case of the iguana pituitary cultures, we found that after 4 h the GHRH (10 nM) stimulated GH release in a parallel manner as the expression of its ARNm (60 times). In contrast, 10 nM TRH had no significant effect on the secretion of GH while the mRNA expression increased 180 times. Regarding PACAP, a tendency to increase both, the release and the mRNA synthesis, was observed, while 10 nM GnRH had not effect upon the regulation of iguana GH. Moreover, Ghrelin administration had no significant effect on GH secretion, whereas it strongly decreased GH mRNA expression (32 times). Finally, somatostatin inhibited both the release of the hormone and the expression of GH mRNA. (240 times) In conclusion, our results indicate that GHRH is conserved during vertebrate evolution as the principal regulator of both the synthesis and release of GH in all the studied species, whereas TRH seems to have a more potent effect in birds than in mammals and reptiles. In iguana, it appears that GHRH and TRH, as well as PACAP, have an important role in regulating GH. As was already known in other species, we confirmed that, in reptiles, somatostatin is the principal negative regulator of GH synthesis and secretion. These data suggest that the control of GH expression and release may vary during the evolution of vertebrates.

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Characterization of energy balance in mice KO for the thyrotropin releasing hormone degrading enzyme; effect of 5 back-crossings with the C57BL/6NJ background

Laura Urzúa-Contla[a1], Miguel Cisneros[b], Patricia Joseph-Bravo[b], Jean-Louis Charli[b] and Antonieta Cote-Velez[b]

[a]Escuela de Biología, Facultad de Ciencias Biológicas de la Universidad

[b]Instituto de Biotecnología, Universidad Nacional Autónoma de México, Cuernavaca, Mor, México. Autónoma del Estado de Morelos.

[1]lcontla@ibt.unam.mx

Hypothalamic paraventricular nucleus (PVN) thyrotropin-releasing hormone (TRH) neurons send axonal projections into the median eminence (ME) of the hypothalamus.In the external layer, TRH enters the hypothalamus-pituitary portal capillaries, and regulates thyrotropin (TSH) secretion from the pituitary. The TRH-degrading enzyme (TRH-DE)is an ectoenzyme expressed in tanycytes of the ME,where it degrades TRH in the extracellular space, switching off the central control of the hypothalamus-pituitary-thyroid (HPT)axis [1].In tanycytes, its activity is regulated by thyroid hormones, and fasting [1,2], suggesting that it controls the activity of the HPT axis in response to energy homeostasis clues.To better understand the role of PPII in energy homeostasis, we made use of a TRH-DE knockout mouse line in a B6129S5 background [3]. We initially compared the phenotype of the adult KO mice with that of heterozygous or wild type C57BL/6NJ mice. In KO mice, the activity of TRH-DE was strongly reduced in the brain, and TRH half-life increased in serum. The body weight of male KO mice was slightly reduced. KO malesate less than WT and heterozygotes. In basal conditions, markers of HPT axis activity were independent of genotype [4]. To test whether these differences were attributable to TRH-DE KO or genetic background, we performed 5 backcrosses of the mutation in the C57BL/6NJ background and compared the phenotype of TRH-DE KO and C57BL/6NJ mice. We confirmed that PPII activity was severely decreased in KO mice. The amount of food ingested was not altered by genotype. Body weight tended to be reduced in female KO mice. There was generally no effect of the genotype on spontaneous locomotor activity, except for an increase locomotion of female KO in the center of the field at the beginning of the experiment; this may be related to the known anxiolytic effect of TRH [5]. Markers of basal HPT axis activity indicated increased expression of thyrotropin (TSH) in the female KO pituitary, but not in circulating TSH. These data suggest that discrete sex-dependent alterations of HPT homeostasis occur in response to genetic interruption of TRH-DE expression.

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PROLACTIN PROMOTESTHE HYPEROXIA-INDUCED INHIBITION OF RETINAL NEOVASCULARIZATION IN NEWBORN MICE

Vázquez-Membrillo Miguel, Díaz-Lezama Nundehui, Adán-Castro Elva, Ramírez-Hernández Gabriela, Ledezma-Colunga Guadalupe, Ruíz-Herrera Xarubet, Martínez de la Escalera Gonzalo, Clapp Carmen.
Instituto de Neurobiología, Universidad Nacional Autónoma de México, Campus UNAM Juriquilla, Querétaro, México.

Retinopathy of prematurity (ROP) is a potentially blinding retinal neovascular disease. Prolactin (PRL) circulating levels are higher in ROP than in control patients and such imbalance mayinfluenceROP progression. PRL accessess the retina from the systemic circulationand is pro-angiogenic but can be cleaved into generate anti-angiogenic fragmentsPRL fragments (vasoinhibins).Like preterm infants, newborn mice have incomplete retinal vascularization at birth and exposure to high oxygen, mimicking supplemental oxygen given to premature infants for respiratory care, inducesa loss of new blood vesselsin the retina that can lead to retinal neovascularization. Here, we have investigated the vascular effects of PRL in the retina of newborn mice exposed to high oxygen. Retinal neovascularization was assessed in mice throughout the first 8 days after birth inflat-mountedretinas immunostained for blood vessels. At postnatal day 1 (P1) the retina is almost devoid of blood vessels, which originate from the optic nerve and migrate radially reaching the periphery at P8. Exposure of P6 mice and nursing mothers to hyperoxia (75% oxygen) inhibited retinal vessel growth as determined by a 40% reduction in vascular density and a significant decrease ($p<0.05$) in the expression of endothelial cell markers (CD31, VEGF). Treatment with PRL (2 µg/gi.p. twice a day from P5 to P8) increased hyperoxia-induced inhibition of retinal vessel growth. The anti-angiogenic effect of PRL may be favored by its higher conversion to vasoinhibins under hyperoxic conditions. Hyperoxia increased the rate of PRL conversion to vasoinhibins by retinal proteases as shown by the incubation of PRL with retinal extracts from newborn rats exposed to hyperoxia. These results suggests that high levels of systemic PRL in ROP favor disease progression by enhancing the retinal accumulation of vasoinhibins promoting the hyperoxia-induced loss of blood vessels that leads to the excessive retinal angiogenesis that characterizes the disease.
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Assessment of fecal lactoferrin in BALB/c mice under immobilization stress

[1]Vega-Bautista Edward Alan, [1]Rodríguez-Paz José Antonio, [2]Rojas-Osornio Sandra Angélica, [2]Cruz-Hernández Teresita Rocío, [2]Campos-Rodríguez Rafael,*[1]Drago-Serrano Maria Elisa (dragome@yahoo.com)

[1]Departamento de Sistemas Biológicos, Universidad Autónoma Metropolitana Unidad Xochimilco (UAM-X) [2]Sección de Estudios de Posgrado e Investigación, Escuela Superior de Medicina, Instituto Politécnico Nacional (IPN).Work place: Laboratorio de Microbiología Molecular, UAM-X, Calzada del Hueso No. 1100 CP 04960, CDMX, Tel +5255 54837000/ext 3652

Introduction. Fecal lactoferrin (Lf) derives from the degranulation of circulating neutrophils infiltrated through the intestinal mucosa and it is regarded as biomarker of inflammation. Fecal-Lf levels increase in infections or in chronic dysfunctions like intestinal bowel disease (IBD). In some stress-associated diseases like intestinal bowel syndrome (IBS) fecal-Lf levels are unchanged **Justification**. Impact of immobilization stress on fecal-Lf fluctuations is unknown **Aim.** To assess the fecal-Lf under immobilization stress. **Methods.** Groups (n=6) of 8-week old female BALB/c mice were either unstressed or stressed by board-immobilization 2 h/4 consecutive days. Before being euthanized with isoflurane and exsanguinated by cardiac-puncture, duplicate samples with two fresh voided fecal pellets from each mouse were collected to prepare fecal extracts. Total protein of fecal-extracts quantified by Bradford assay was normalized at same concentration to assess lactoferrin by an indirect immunoenzimatic assay (EIA). In microplates previously coated with fecal samples and blocked with bovine serum albumin, Lf was detected with rabbit polyclonal anti-lactoferrin and goat anti-rabbit IgG-peroxidase conjugated as first and second antibodies, respectively; for Lf quantitation, standard curve of bovine lactoferrin was done. Lactoferrin content expressed in ng/mL from both mice groups was compared with Mann-Whitney U test and the statistical differences were regarded at $p<0.05$ **Results.** By comparison with the unstressed mice, stressed mice had greater fecal-Lf levels ($p=0.004$). **Discussion**. Elicitation of fecal-Lf levels could result from the release of gut neuropeptides by peripheral endings of afferent (sensory) neurons causing of neurogenic inflammation characterized by arteriolar vasodilatation and extravasation of plasma proteins and neutrophils. **Perspectives.** Study may impact in pharmacological interventions to control stress-associated dysfunctions such as IBS.

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Role of Wnt signaling pathway on hippocampal reorganization after entorhinal cortex lesion

LizbethGarcía-Velázquez,[1] ClorindaArias-Álvarez.[11]Department of Genomic Medicine and Environmental Toxicology, IIB, UNAM.
Circuito exterior, Ciudad Universitaria. CP. 04510
Tel. (+52)55 56229215 Mail: lizgarvel@comunidad.unam.mx

Neurons display structural plasticity through remodeling of spines and axons in a dynamic way. These structural changes are also observed upon synaptic rewiring of a lesioned circuit and contribute to homeostatic maintenance of network activity. Numerous molecules have been proposed to favor these processes, including neurotrophins and cellular adhesion molecules. However, there is a signaling pathway that could play an important role in these processes despite it has been scarcely explored in this sense, the Wnt signaling pathway.
Wnt signaling plays a fundamental role during early and postnatal development regulating cell differentiation, proliferation and plasticity. In recent years, it has been shown that components of canonical or non-canonical Wnt pathways are also expressed in adult brain. There is evidence that emphasizes the involvement of Wnt ligands, particularly Wnt5a and Wnt7a, in the modulation of neuronal circuit assembly at pre- and post-synaptic levels. However, it remains unknown if Wnt pathways are activated in response to a synaptic loss and participate in the synaptic reorganization after damage. In this work, we have evaluated changes in the expression and function of Wnt agonists (Wnt5a, Wnt7a) and antagonists (Dkk1 and Sfrp1) in a model of hippocampal deafferentation. Male Wistar rats of 250-300 g were used throughout the study. Unilateral injection of kainic acid (2 μM) was applied to the right medial entorhinal cortex (EC) (A=27.64, L= 25.4 and V= 25.6). Animals were sacrificed by decapitation 1, 3, 7 and 30 days after lesion and the right hippocampus was dissected. Control animals received 1μL of 10 mM phosphate buffer. Total RNA and protein were obtained using TRizol. Expression analysis of *Wnt5a*, *Wnt7a*, *Sfrp1* and *Dkk1* were carried out by qRT-PCR. Protein levels of Wnt5a, Wnt7a, Sfrp1, Dkk1, CycD1, c-Myc and ABC were analyzed by Western Blot. Structural analysis was assessed using AChE, Fluorojade and Nissl stain protocols.We have found a differential expression of the analyzed genes along time, including an upregulation of Wnt5a during the first 3 days and an increase of Wnt7a at the seventh day after deafferentation. Active β-catenin, a central protein of the canonical pathway, shows a significant increase at the seventh day after lesion, suggesting an activation of this pathway that could be due to the increase in Wnt7a. This activation is reinforced by the increase of CyclinD1, a downstream gene of the canonical Wnt signaling. All these processes are accompanied by changes in the hippocampal structure along time. We can conclude that the hippocampus remodeling after deafferentation is accompanied by changes in the expression of Wnt signaling components, of which Wnt7a increases and seems to induce an activation of the canonical pathway. The silencing of *Wnt7a* gene may support the understanding of its role in hippocampal reorganization after damage.

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The link between PKCα and ERβ activity in a medulloblastoma cell line

Jiménez-Arellano Carolina & González-Arenas Aliesha*.
Departamento de Medicina Genómica y Toxicología Ambiental, Instituto de Investigaciones Biomédicas, Universida Nacional Autónoma de México.
Circuito Mario de la Cueva s/n, Coyoacán, Ciudad Universitaria, Cd. Universitaria, 04510, CDMX.
*alieshag@iibiomedicas.unam.mx; Teléfono: 562-29209

Estrogen receptors (ERs) fulfill physiological functions in the CNS, however, they also participate in the regulation of pathological processes such as cancer. ERs belong to the nuclear receptors superfamily that acts as transcription factors. There are 2 isotypes of ERs: alpha and beta, and different mechanisms are capable of inducing the activation of ERs among which is the receptor phosphorylation mediated by protein kinases such as Akt and Cdk2. *In silico* studies suggest that the protein kinases C (PKCs) could phosphorylate ER beta (REβ), PKCs belong to a family of serine / threonine residue kinases related to signaling pathways that promote tumor proliferation.

Medulloblastomas are the most common tumors of the central nervous system (CNS) in the pediatric population. In these tumors ERβ content and its activity dependent on estradiol have been associated with the regulation of carcinogenic processes. On the other hand, activation of the PKCα isoform is able to increase cell proliferation and migration in these tumors.

The aim of this work was to determine if PKCα is capable of inducing the phosphorylation of ERβ and the effect of such phosphorylation on the receptor activity in the Daoy cell line derived from a human medulloblastoma. We observed that activation of PKCα by 12-O-tetradecanoylphorbol-13-acetate (TPA) increases the levels of phosphorylated ERβ and that PKCα-dependent activation of the receptor increases the content of the receptor for insulin-like growth factor 1 (IGF1R), whose gene is known to be a target for regulation of ERβ. These results suggest the existence of a communication between the signaling pathways of ERβ and PKCα in medulloblastomas.

Palmitic acid-induced neuronal insulin resistance: role of PKCs activation, reactive oxygen species and ceramides production.

Karina Sánchez-Alegría, Patricia Ferrera and Clorinda Arias
Depto. Medicina Genómica y Toxicología Ambiental. Instituto de Investigaciones Biomédicas, Universidad Nacional Autónoma de México. 04510 México, Ciudad de México.
E-mail: carias@unam.mx

The intake of high fat diets (HFDs) contributes to the development of metabolic alterations such as insulin resistance, obesity and type II diabetes mellitus. In the central nervous system (CNS), the HFDs generate structural and functional changes associated to insulin signaling reduction. Insulin regulates several aspects of neuronal function such as neuronal differentiation, survival and plasticity. Palmitic acid (PA) is the most abundant fatty acid in the HFDs and has been found to play an important role in the development of insulin resistance in hypothalamus, liver, pancreas and skeletal muscle. It is believed that PA effects on periphery tissues depends on different metabolic alterations such as reactive oxygen species (ROS) and ceramide production and PKC activation trough the FFAR1 (GPR40) receptor. It has been described that an essential part of the insulin signaling is the generation of a pulse of hydrogen peroxide (H_2O_2) produced in response to the binding of insulin to its receptor. Therefore the presence of excessive ROS may have an inhibitory effect on insulin signaling. It is still unknown if PA also participates in the development of neuronal insulin resistance neither the mechanism involved. Thus in the present study we have evaluated the mechanisms behind the PA-induced neuronal insulin resistance particularly the role of mitochondrial ROS, ceramide metabolism and PKC activation. As neuronal model we used differentiated human neuroblastoma cells. Neurons were exposed to a non-toxic dose of PA (200μM) for 1 h and then we measured the metabolic activity by the MTT reduction method. We found that insulin at 10 μM increased the MTT reduction by approximately 20% compared to control neurons. This insulin-mediated metabolic activation was totally prevented by PA exposure. We also found that PA impeded the phosphorylation of Akt (S473) in response to insulin. The incubation with the mitochondrial antioxidant (MitoTEMPO), PKC inhibitor (BIM) and ceramide synthesis inhibitor (myriocin) prevented the inhibitory effect of PA on insulin metabolic activation in different magnitude. At present these results point to the participation of PA in reducing neuronal insulin signaling as well as the role of ROS production, ceramide, and activation of PKCs in such effect.

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Lysophosphatidic acid signaling induce Protein Kinase C nuclear translocation in glioblastoma cell lines

Silvia Anahi Valdés-Rives, Aliesha González-Arenas

Departamento de Medicina Genómica y Toxicología Ambiental, Instituto de Investigaciones Biomédicas, Universidad Nacional Autónoma de México, Ciudad de México, México.

*Corresponding Author: Aliesha González-Arenas, PhD, Departamento de Medicina Genómica y Toxicología Ambiental, Instituto de Investigaciones Biomédicas, Universidad Nacional Autónoma de México, Ciudad Universitaria, 04510, Ciudad de México, México. E-mail: alieshagonzalez@gmail.com

Glioblastoma multiforme (GBM), a grade IV astrocytoma, is the most aggressive type of brain cancer. It has an incidence of 4.37 per 100,000 individuals and a mean age of 45 years in the Mexican population. GBM aggressiveness is due to increased invasion, migration, proliferation, angiogenesis and a decrease in apoptosis. These processes are regulated by growth factor receptors such as EGFR, PDGFR, nuclear receptors such as progesterone receptor and effector molecules such as PKC. From the latter, PKCα is overexpressed in these tumors compared to normal glia and it has been reported to impact in GBM growth. Despite substantial research of GBM over the last decades, few progresses have been made regarding overall survival in these patients. Therefore, light on new players that could lead to better therapies are vital. Recent studies have shown that lysophosphatidic acid (LPA) signaling through its GPCRs (LPA1-6) can regulated several processes that impact GBM progression. In our group, we aim to study PKCα activation through LPA1 receptor, which is overexpressed in GBM compared to normal brain. The first approach was to evaluate the translocation of PKCα to the nucleus and cell membrane when stimulated with TPA (a well-known PKC activator) and LPA. While TPA induces a translocation of PKCα to nucleus at a short time (5 min) and subsequently to the cell membrane (30 min), PKCα activation through LPA induces its translocation to the nucleus (5 min) and later (30 min) it redistributes to the cytoplasm without accumulating on the cell surface. These results suggest that PKC activation through LPA may have different targets, mainly located in the nucleus.