<table>
<thead>
<tr>
<th>Topic</th>
<th>Author</th>
</tr>
</thead>
<tbody>
<tr>
<td>Physiopathology of glycine transporters in glycinergic neurotransmission: hyperekplexia and pain</td>
<td>Carmen Aragón Rueda</td>
</tr>
<tr>
<td>Function of microtubular proteins in neurons</td>
<td>Jesús Ávila de Grado</td>
</tr>
<tr>
<td>Physiopathology and Therapy of Neurodegenerative Diseases: Friedreich’s Ataxia</td>
<td>Javier Díaz Nido</td>
</tr>
<tr>
<td>Molecular Bases of Neuronal Plasticity</td>
<td>Fco. Javier Díez Guerra</td>
</tr>
<tr>
<td>Neuronal differentiation and brain aging</td>
<td>Carlos Dotti</td>
</tr>
<tr>
<td>Molecular and cellular mechanisms for synaptic plasticity</td>
<td>José Antonio Esteban García</td>
</tr>
<tr>
<td>Molecular bases of the glutamatergic synapses</td>
<td>Cecilio Giménez Martín</td>
</tr>
<tr>
<td>Lipids in neuronal physiology and pathology</td>
<td>María Dolores Ledesma Muñoz</td>
</tr>
<tr>
<td>Huntington’s disease and other CNS disorders</td>
<td>José Lucas</td>
</tr>
<tr>
<td>Biology of human neural stem cells. Potential for cell and gene therapy in neurodegeneration</td>
<td>Alberto Martínez Serrano</td>
</tr>
<tr>
<td>Calcium signalling in mitochondria and insulin/leptin signalling during ageing</td>
<td>Jorgina Satrústegui Gil-Delgado</td>
</tr>
<tr>
<td>Genetic bases of Alzheimer’s Disease: Genomic study of pathogenic cell models</td>
<td>Fernando Valdivieso / María Jesús Bullido</td>
</tr>
<tr>
<td>Molecular mechanism of neurodegeneration and regeneration</td>
<td>Francisco Wandosell Jurado</td>
</tr>
</tbody>
</table>
Physiopathology of glycine transporters in glycinergic neurotransmission: hyperekplexia and pain

Research Summary

Current work involves the study of functional mechanism, biogenesis, intracellular trafficking, regulation of CNS glycine transporters, GlyTs (GlyT1 and GlyT2), plasma membrane proteins of neurons and astrocytes responsible for the completion of glycinergic transmission. Gene deletion studies have suggested that modification of glycine transporter activity may be beneficial in treating several human disorders, including neuromotor deficiencies (startle disease, myoclonus), and neuropathic pain. Indeed, mutations in the gene encoding GlyT2 can cause hyperekplexia in humans and congenital muscular dystonia type 2 in calves.

Our recent studies have contributed to the identification of a site in the extracellular vestibule of GlyT2 involved in cation selectivity and glycine transport coupling mechanism by bioinformatics tools and directed mutagenesis (in collaboration with Dr. Antonio Morreale (Bioinformatic Unit, CBMSO)). We are also interested in the study of GlyT2 trafficking as a fundamental mechanism in the control of glycinergic activity as it provides a rapid manner to modulate its activity. In this regard we have defined molecular mechanisms underlying constitutive and PKC-regulated trafficking through GlyT2 recycles between the cell surface and the cell interior and the role of membrane rafts and protein kinase C-dependent ubiquitination in the process.

In addition we have reported a coordinated regulation of GlyT1 and GlyT2 by P2Y1R that may result in an increase of the inhibitory pathways over the excitatory pathways leading to anti-nociception.

We have recently identified a new dominant mutation in the GlyT2 gene in eight patients of hyperekplexia. The heterologous expression and characterization of the mutation (Y705C) allowed us to determine changes in trafficking and biochemical properties and maturation of the mutant transporter.
Physiopathology of glycine transporters in glycinergic neurotransmission: hyperekplexia and pain

**Figure 1.** Genetic and structural analysis of the Y705C (GlyT2) mutant in hyperekplexia patients. A, Partial sequences of exon 15 from control and patient, respectively. B, Molecular model of GlyT2 showing the localization of Tyr-705 in transmembrane domain 11 (TM11).

**Figure 2.** Co-localization of GlyT2 wild type (A) and mutation of the C-terminal lysine cluster of GlyT2, 4KR (B) with syntaxin1A and syntenin-1 in transfected hippocampal neurons. Hippocampal neurons were transfected with wild type GlyT2 or with the 4KR mutant at 10 DIV. The cultures were stained for GlyT2 syntaxin1A and syntenin-1 specific antibodies.
Physiopathology of glycine transporters in glycinergic neurotransmission: hyperekplexia and pain

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Physiopathology of glycine transporters in glycinergic neurotransmission: hyperekplexia and pain

Publications


Physiopathology of glycine transporters in glycinergic neurotransmission: hyperekplexia and pain

Other Activities


Physiopathology of glycine transporters in glycinergic neurotransmission: hyperekplexia and pain

Awards

Carmen Aragón. Académico Correspondiente de la Real Academia Nacional de Farmacia (9 de junio de 2011).

Physiopathology of glycine transporters in glycinergic neurotransmission: hyperekplexia and pain

Doctoral Theses

Function of microtubular proteins in neurons

Research Summary

During years a main objective in our group has been the characterization of the function of the cytoskeletal proteins known as microtubule associated proteins (MAPs). One of these MAPs, MAP1B, is a protein that is mainly located at the axon, in neurons. Now, we have found that MAP1B plays an important role in the development of dendritic spines. On the other hand, we are mainly focusing our studies on other MAP, tau protein. Tau protein appears to play a role in Alzheimer disease and other dementias (tauopathies). Tau protein is a suitable substract for GSK3, a protein kinase also known as tau kinase 1 and that is also related to some features of Alzheimer disease. We have several works about tau and GSK3 and we have isolated, some years ago, a transgenic mouse that overexpresses GSK3. Now, using this mouse, we have studied the neurogenesis at the dentate gyrus. Our results have indicated a decrease in that neurogenesis, in the transgenic mouse, that results in degeneration of dentate gyrus. This degeneration is due to cell death but also to the indicated impaired neurogenesis. These features may be the cause of the memory deficits found in the transgenic mouse. Our actual studies will analyze if the memory impairment found in Alzheimer disease patients are also based in a deficient neurogenesis taking place at the dentate gyrus of the patients.
Function of microtubular proteins in neurons

Figure 1. Retrovirus labeled granule neurons in the dentate gyrus of Alzheimer disease mouse model.

Figure 2. Neuroblastoma cells overexpressing tau-GFP (green), stained with anti-EB1 (red). Although both proteins interact with microtubules, tau is a classical MAP that binds along the microtubule lattice, whereas EB1 is a +TIP that associates with microtubule "+" ends.
Function of microtubular proteins in neurons

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Function of microtubular proteins in neurons

Publications (1)


Function of microtubular proteins in neurons

Publications (2)


Function of microtubular proteins in neurons

Other Activities

Miembro del Comité organizador del Simposio internacional en Alzheimer y Parkinson (AD/PD), Barcelona 2011.
Function of microtubular proteins in neurons

Doctoral Theses

**Elena Tortosa Binacua (2011).** Estudio de la función de la proteína asociada a microtúbulos 1B durante el desarrollo neuronal. Universidad Autónoma de Madrid. Directores: María del Mar Pérez, Filip Lim y Laura Sayas.

**Almudena Fuster Matanzo (2011).** Estudio de la neurogénesis adulta en un modelo murino de sobreexpresión condicional de la glucógeno sintasa quinasa-3β. Universidad Autónoma de Madrid. Director: Félix Hernández.
Physiopathology and Therapy of Neurodegenerative Diseases: Friedreich’s Ataxia

Research Summary

Our group studies the dysfunction and death of neurons in the context of neurodegenerative diseases in order to find ways to stimulate neuronal survival and repair, with a view to possible therapeutic applications. Within the wide spectrum of neurodegenerative diseases, we have focused on Friedreich’s ataxia (FA). FA is a hereditary disorder resulting from a deficiency of frataxin, which is a mitochondrial protein encoded for by the nuclear genome. FA is mainly (but not exclusively) a neurodegenerative disease with a very early onset, and it may serve as a very useful model for other neurodegenerative diseases in which mitochondrial dysfunction also plays a very important role.

We have developed distinct neural cell models to study the molecular mechanisms underlying the degenerative process triggered by the frataxin deficiency. In this respect we have used primary cultures of mouse neurons and human neuroblastoma cell lines, as well as cultured olfactory mucosa stem cells derived from biopsies from human donors. These studies may facilitate the identification of novel therapeutic targets and biomarkers not only for FA but also for other neurological diseases characterized by a prominent mitochondrial dysfunction. These cell models are also being used to test potential therapeutic strategies, particularly those focused on identifying molecules (drugs or genes) capable of compensating for the functional defects induced by the loss of frataxin, or that are capable of efficiently increasing the expression of frataxin.

Our group has also considered a gene therapy approach for Friedreich’s ataxia that involves introducing correct copies of the frataxin gene by administering herpes virus vectors carrying the frataxin cDNA or the entire genomic frataxin “locus”. We are now attempting to optimize the route of administration and the delivery and distribution of both viral and non-viral vectors in the spinocerebellar system. Furthermore, we are also investigating the possible application of vectors that may carry other neuroprotective genes.
Physiopathology and Therapy of Neurodegenerative Diseases: Friedreich’s Ataxia

Figure 1. Cultured Purkinje neuron from mouse cerebellum

Figure 2. Mitochondrial localization of overexpressed frataxin in cultured cerebellar granule neurons transduced with a lentiviral vector.
Physiopathology and Therapy of Neurodegenerative Diseases: Friedreich’s Ataxia

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Physiopathology and Therapy of Neurodegenerative Diseases: Friedreich’s Ataxia

Publications


Physiopathology and Therapy of Neurodegenerative Diseases: Friedreich’s Ataxia

Other Activities

Our Research Group also belongs, as Unit 748, to the Genetic Medicine Program of the Centre for Biomedical Research on Rare Diseases (CIBERER), a network structure set up at the initiative of the “Instituto de Salud Carlos III”. More information at the web page: [http://www.ciberer.es/index.php?lang=english](http://www.ciberer.es/index.php?lang=english)

In addition to our research activity, we are strongly committed with the improvement and innovation of learning/teaching in Biomedicine as well as in outreach and communication activities of advances in biomedical research. Javier Díaz-Nido is currently the Coordinator of the Postgraduate Program in Molecular Biosciences at the “Universidad Autónoma de Madrid”. More information on Graduate Studies at the web page: [http://biociencias.bq.uam.es/en/index.php](http://biociencias.bq.uam.es/en/index.php)
Our research is directed towards the understanding of the cellular and molecular mechanisms that operate in neural networks to modulate synaptic efficiency. Our current goal is to disclose how calcium-binding proteins cooperate in the synaptic environment to regulate neuronal plasticity. Synaptic activity triggers intracellular calcium oscillations that initiate a number of signaling pathways, many of them transduced by calmodulin (CaM). Despite its abundance, the availability of CaM against its effectors is regulated by the presence of proteins such as neurogranin (Ng), which sequesters CaM at the post-synaptic site. Ng is an abundant neuronal protein whose ability to concentrate CaM is regulated by calcium levels and phosphorylation by protein kinase C. We propose that Ng act in two ways: one, by preventing activation of CaM effectors in response to low-intensity stimuli and, the other, by favoring and enhancing downstream activation of some CaM-dependent signaling pathways. Currently, we are focused in studying the regulation of Ng translation in dendrites and its intracellular localization. Recently, we have shown that Ng translocates to the nucleus in response to synaptic activity and also that Ng specifically binds to phosphatidic acid (PA), a membrane phospholipid recognized as a signaling molecule. We use cultures of dissociated neurons obtained from murine cerebral cortex and hippocampus, combined with cell biology, biochemistry, molecular biology and advanced microscopy techniques. Ng deficiency is tightly linked to cognitive impairments, which are present in a plethora of neurological diseases, such as hypothyroidism or schizophrenia. We believe that Ng is an excellent target for the design and development of drugs and therapies aimed to improve our cognitive skills. A wider and deeper knowledge on CaM-sequestering proteins in the context of neuronal plasticity and its mechanisms will foster the emergence of new drugs and therapies that enhance the quality of life of aging individuals and patients suffering neurological diseases.
**Molecular Bases of Neuronal Plasticity**

Figure 1. Cultured hippocampal neurons at various stages of development (DIV = days in vitro). Highlighted in blue is the DAPI stain (nuclei) and in green, the expression of Neurogranin (Ng) in some neurons.

Figure 2. Synaptic strength is modulated by Calmodulin (CaM) within dendritic spines. The mobilization of CaM depends on the translocation of Neurogranin (Ng) from the dendritic shaft.
Molecular Bases of Neuronal Plasticity

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Molecular Bases of Neuronal Plasticity

Publications


Neuronal dysfunction during aging

Research Summary

Our laboratory aims at elucidating the pathways and mechanisms involved in neuronal survival and plasticity in the aged brain. As the individual ages, it occurs a gradual but persistent change in the cholesterol and sphingomyelin content in the plasma membrane of hippocampal neurons, an area of the brain strongly implicated in learning and memory. These changes seem to be due to the transcriptional up-regulation of the genes sphingomyelin synthase and 24 cholesterol hydroxylase, in turn the consequence of metabolic stress from excitatory neurotransmission, therefore a physiological response to normal brain activity. Functionally, the cholesterol/sphingomyelin content changes increase the clustering of receptor tyrosine kinase in the plasma membrane of the aging neurons, especially TrkB, helping the survival response of old neurons to exogenous stressors. On the negative side, cholesterol/sphingomyelin content alterations with age result in impaired lateral mobility and internalization of glutamate receptors of the AMPA type, reducing the capacity of old neurons to efficiently support certain forms of electrical response involved in learning. In molecular terms, the lipid imbalance occurring with age leads to the diffusion away from synaptic sites of the cholesterol and PI(4,5)P2 binding molecule MARCKS. Such loss impacts on the amount of PI(4,5)P2 to be hydrolyzed by PLCg and therefore the reduced activation of memory genes. My laboratory has three major goals for the next years: i) to deepen our understanding of the survival and plasticity mechanisms operating in the aging brain, ii) to identify pathways that could improve cognition in the old without interfering with survival strength and iii) to identify the causes of pathological brain aging.
Neuronal dysfunction during aging

Figure 1. Dendritic marker and receptors in hippocampal neuron

Figure 2: Recombinant protein expression in cerebral cortex.
Neuronal dysfunction during aging

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Neuronal dysfunction during aging

Publications


Molecular and cellular mechanisms for synaptic plasticity

Research Summary

We are interested in the molecular bases for learning and memory. Specifically, we investigate how synaptic connections in the brain are modified in response to experience. This process, known as synaptic plasticity, is critical for learning and memory, and is known to be altered in multiple cognitive disorders, such as Alzheimer’s disease, autism and several forms of mental retardation.

Over the years, we have discovered that neurons fine-tune their synapses by moving neurotransmitter receptors in and out of the synaptic membrane. Our group has pioneered the identification of the molecular machinery that executes this movement, including a network of endosomal compartments driven by specific Rab GTPases, molecular motors, and regulators of phosphoinositide signaling. For these experiments, the laboratory employs a combination of molecular biology techniques, together with live fluorescence microscopy and electrophysiology.

In recent years, we have become more interested in how these mechanisms control cognitive function, both under physiological and pathological conditions. Thus, we have described molecular and pharmacological strategies that facilitate neurotransmitter receptor trafficking at synapses, resulting in enhanced learning and memory in rats. Conversely, we have made significant progress in understanding the molecular mechanisms that lead to synaptic dysfunction in Alzheimer’s disease. Indeed, preliminary results from the laboratory provide clues as to how to correct these dysfunctions and restore normal cognitive function in mouse animal models of this disease.

Overall, our laboratory continues to explore how individual molecules and signaling pathways control synaptic function and determine our cognitive abilities in health and disease.
Molecular and cellular mechanisms for synaptic plasticity

Figure 1. FRET (Fluorescence Resonance Energy Transfer) assay for the visualization of PIP3 at dendritic spines. Expression of the FRET reporter Flip-pm (Sato et al., Nat Cell Biol 2003) in hippocampal neurons allows the quantification of PIP3 levels from YFP/CFP fluorescence ratios upon different pharmacological manipulations (Top: false color representation at dendritic spines. Bottom: histogram representation).

Figure 2. Example of a mouse model of Alzheimer’s disease (APP/PS1) pharmacologically treated using mini-osmotic pumps and intracerebroventricular cannulation (left panel, top). A behavioral test for spatial memory (novel object location; left panel, bottom) was carried out on these animals. The results indicate that a specific treatment may rescue cognitive function in these animals (quantification shown in right panel).
Molecular and cellular mechanisms for synaptic plasticity

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Molecular and cellular mechanisms for synaptic plasticity

Publications


Molecular bases of the glutamatergic synapses

Research Summary

For years, a primary focus of our laboratory has been the understanding the basic regulatory mechanisms of glial and neuronal glutamate and glycine transporters in CNS, and how dysregulation of these proteins, or their associated regulatory proteins, could contribute to neurological disorders such as schizophrenia, amyotrophic lateral sclerosis, or ischemic insults to the brain. Neurotransporters for glutamate (GLT1, GLAST, EAAT3) and glycine (GLYT1) control the levels of these neurotransmitters in glutamatergic synapses thereby regulating the activity of NMDA receptors that requires both ligands as obligatory coagonists. While overstimulation of these receptors drives neurodegenerative processes, the hypofunction has been associated to schizophrenia. Thus, the activity of these transporters has a profound impact on the activity of the glutamatergic pathways, and its modulation is considered of great interest in the treatment of dysfunctions that affect to the glutamatergic system. Our work has intended a better understanding the biogenesis mechanisms: exit from the endoplasmic reticulum, transit through the Golgi complex, and asymmetric distribution to different subdomains of the plasma membrane, its anchoring to scaffolding proteins and, finally, the endocytosis either for degradation or recycling. Our studies have contributed to the fine localization of these proteins in the brain as well as to the identification of several associated proteins (PDZ proteins, exocytst) and to the identification of structural motifs involved in the asymmetric distribution of these transporters in polarized cells. Also, we have defined regulatory mechanisms based in posttranscriptional modifications on the transporters such as phosphorylation and ubiquitination / deubiquitination. In addition, we keep interest in the function of glycine as an inhibitory neurotransmitter, and the associated transporter GLYT2, involved in the genetic disease hyperekplexia. These studies, especially those related with trafficking of GLYT2, have been performed in the last few years in collaboration with the group of Dr. C. Aragón.
Molecular bases of the glutamatergic synapses

Figure 1. Constitutive and regulated intracellular trafficking of the glutamate transporter GLT1.
Molecular bases of the glutamatergic synapses

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Molecular bases of the glutamatergic synapses

Publications


Molecular bases of the glutamatergic synapses

Awards

C. Giménez: miembro de la Academia Nacional de Farmacia
Lipids in neuronal physiology and pathology

Research Summary

To accomplish the general goal of our laboratory, which is to understand lipid influence in neuronal physiology and pathology, we have focused during this period in the analysis of cholesterol and sphingomyelin. These lipids are particularly enriched in neurons and changes in their levels have been related to aging and neurodegenerative diseases. Our results demonstrate that one of the causes for the loss of cholesterol observed in the aged hippocampus is synaptic activity. This activity induces oxidative stress that, in turn, activates the cholesterol-degrading enzyme Cyp46. On the other hand, we have found that the increase in sphingomyelin, which takes place in the aged hippocampus, is not only a consequence of the aging process but contributes directly to it through increasing intracellular calcium levels and oxidative stress. We unveil that mice lacking the acid sphingomyelinase, which present high sphingomyelin levels in their brains, show aging signs prematurely. The characterization of the molecular mechanisms underlying the effects of the lipid alterations as well as the establishment of animal models facilitate the development of strategies aimed to prevent deleterious effects. This is our next goal. By pursuing it we hope to contribute with possibilities to delay or reduce the cognitive decline that accompanies old age and/or neurodegenerative diseases.
Lipids in neuronal physiology and pathology

Figure 1. Cultured hippocampal neurons labelled with lysenin, which specifically recognizes sphingomyelin at the cell surface. The images evidence the increase in sphingomyelin levels in aged neurons (28 days in culture) compared to mature neurons (12 days in culture) from wild type mice.
Lipids in neuronal physiology and pathology

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Estefanía Fernández López

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Carolina Melero Jérez
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Lipids in neuronal physiology and pathology

Publications


Lipids in neuronal physiology and pathology

Patents

Huntington’s disease and other CNS disorders

Research Summary

Huntington disease (HD), being inherited monogenic and dominant, is of particular interest to explore the mechanisms of neurodegeneration in general. Our work with the inducible animal model of HD showed that a continuous supply of mutant huntingtin is needed for the progression of disease and that reversal is still possible in advanced stages with neuronal loss (Cell 101:57-66, 2000 and J. Neurosci. 25:9773-9781, 2005). More recently we explored the possible inhibition of ubiquitin proteasome system (UPS) (J. Neurosci. 23:11653-61, 2003, J. Neurosci. 24:9361-71, 2004, Trends Neurosci. 27:66-69, 2004 and J. Neurochem. 98:1585-1596, 2006). By using UPS activity reporter mice we saw that the UPS is not inhibited at steady state in HD mice (PNAS 106:3986-91, 2009). However, in the past years we have seen that, in vivo, mutant huntingtin induces an inhibition of the UPS but this is temporary as it recovers as the neurons build inclusion bodies accumulating mutant huntingtin (J. Neurosci 30:3675-88, 2010). Another issue we explored is the possible alteration of GSK-3 in HD (Cell Death Differ. 17:324-35, 2010) and the possible neuroprotective effect of GSK-3 inhibitors. Regarding the latter, we found that besides the reported antiapoptotic effect, inhibition of GSK-3 also can be proapoptotic in certain paradigms and cell types (including striatal neurons) and that the underlying mechanism involves NFAT and FAS (J. Clin. Invest. 120:2432-45, 2010). We are now combining EH mice with mice with alterations of GSK-3.

Huntington’s disease and other CNS disorders

Figure 1. Expression of the transcription factor ATF5 in neurons of the mouse hippocampus

Figure 2. COS cells transfected with Green Fluorescent protein (GFP)
Huntington's disease and other CNS disorders

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Huntington’s disease and other CNS disorders

Publications


Huntington’s disease and other CNS disorders

Other Activities

Grupo integrante del Centro de Investigación Biomédica en Red sobre Enfermedades Neurodegenerativas (CIBERNED)
http://www.cibernet.es/grupo-lucas-lozano.html

José Lucas nombrado Académico Correspondiente de la Real Academia Nacional de Farmacia, Junio de 2011.
Research Summary

The incidence of neurodegenerative diseases is steadily increasing, particularly in well-developed countries, due to the increase in life expectancy. For some of them, like Parkinson, Huntington diseases, pharmaceutical drugs are useful at early stages of the disease, but none of them really cure the disease, since they do not halt the neuronal atrophy and death process.

In this context, research on the basic biology of human neural stem cells acquires special relevance, with the prospect that healthy stem cell derivatives, after implantation, would either delay disease progression or actually cure the disease.

Our research group is interested in understanding basic self-renewal (niche factors) and developmental events leading to maturation of stem cell derivatives, using: 1) Neural stem cells, obtained from foetal or adult human tissue, and thus instructed as neural cells; 2) Embryonic stem cells derived from the inner cell mass of the blastocist, (hES cells) from which neural stem cells can be derived; and 3) Induced pluripotent stem cells (iPSCs), reprogrammed from somatic adult cells.

Our main research focus is thus on basic cell growth and developmental events involved in the generation of mature cells, particularly of Dopaminergic neurons, to learn how to harness the potential that stem cells may have for therapy of these devastating diseases.

Another aspect in which we are interested on is the modification of the intrinsic properties of the neural stem cells through genetic modification, to turn them into “biological mini-pumps” (for instance for the secretion of neurotrophic factors), or to instruct them or guide their differentiation towards specific, on-demand desired phenotypes after implantation. To this end we are implementing the technology of zinc-finger nucleases, to help to conduct homologous recombination. Last, we are developing nanotools to label and track the cells in vivo, and study their cell biology in culture.
Biology of human neural stem cells. Potential for cell and gene therapy in neurodegeneration

Figure 1. Generation of human dopaminergic neurons from neural stem cells. Top panels are microphotographs of human neurons generated in culture, stained for a general neuronal marker (β-III-tubulin, green) and Tyrosine Hydroxylase (dopaminergic marker, red). The lower panel is a merge of the two photographs, highlighting in yellow the presence of human dopaminergic neurons.
Biology of human neural stem cells. Potential for cell and gene therapy in neurodegeneration

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Biology of human neural stem cells. Potential for cell and gene therapy in neurodegeneration

Publications


Biology of human neural stem cells. Potential for cell and gene therapy in neurodegeneration

Doctoral Theses


Calcium signalling in mitochondria and insulin/leptin signalling during ageing

Research Summary

Ca$^{2+}$ entry in mitochondria through the Ca$^{2+}$ uniporter (CaU) is important in cell Ca$^{2+}$ signaling, but its persistence in mitochondria is associated with mitochondrial dysfunction and cell death. We are interested in the study of systems for Ca$^{2+}$ signaling in mitochondria that do not require Ca$^{2+}$ entry in the organelle, the mitochondrial carriers of aspartate-glutamate carriers (AGC) aralar and citrin, and of ATP-Mg/Pi, or Short CaMCs (SCaMCs). Both AGCs and SCaMCs have calcium binding domains facing the intermembrane space which are activated by Ca$^{2+}$ without the need of calcium entry in mitochondria. Aralar and citrin, the brain and liver AGCs, are components of the malate-aspartate NADH shuttle. We have shown that both AGC isoforms are regulated by Ca$^{2+}$ at concentrations lower than those required to activate CaU and are essential to transmit very small Ca$^{2+}$ signals to brain or beta-cell mitochondria. On the other hand, Ca$^{2+}$ concentrations activating SCaMCs are close to those activating CaU, indicating that these higher Ca$^{2+}$ concentrations may stimulate simultaneously both Ca$^{2+}$ entry along CaU and ATP entry along SCaMCs. We are developing mouse models with deficiencies in each of these mitochondrial transporters in order to study the role of aralar/AGC1 and the different SCaMC isoforms in human pathology.

Ageing associates with overall insulin resistance. We found hyperleptinemia in aged rats and demonstrated that aged animals show central leptin and insulin resistance. Our findings suggest that leptin might be involved in eliciting adipose tissue insulin resistance at early aging acting through the central nervous system. In contrast, at advanced age leptin decrease insulin action in both, fat and muscle, interacting directly with those tissues. We are currently interested in the study of the role of gastrointestinal peptides (ghrelin and CCK) that regulate central leptin and/or insulin action and ingestive behaviour, in the development of insulin resistance during aging and the possibility of reversing age-associated changes by caloric restriction.
Calcium signalling in mitochondria and insulin/leptin signalling during ageing

Figure 1. X-gal staining as reporter for SCaMC-3/slc25a23 expression in mice carrying the lacZ reporter gene. (A) Genomic structure and design of the SCaMC-3/slc25a23-knockout, lacZ-knock-in mouse. (B, C) Coronal sections containing the hippocampus (B) and the cerebellum (C) of the KO mice stained with X-gal. SCaMC-3/slc25a23 expression is found mainly in hippocampus, caudate putamen (CPu), olfactory bulb and cerebellum. The magnified areas show the X-gal staining of neurons of hippocampal areas CA1 and CA2 and the dentate gyrus (DG). In the cerebellum, X-gal staining is found associated to the Purkinje cell layer (pc), but not granular cell (gcl) or molecular layer (ml).
Calcium signalling in mitochondria and insulin/leptin signalling during ageing

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Calcium signalling in mitochondria and insulin/leptin signalling during ageing

Publications (1)


Calcium signalling in mitochondria and insulin/leptin signalling during ageing

Publications (2)


Calcium signalling in mitochondria and insulin/leptin signalling during ageing

Awards

Calcium signalling in mitochondria and insulin/leptin signalling during ageing

Doctoral Theses

Alain Juan De Solís (2011). Acción de la insulina en el músculo esquelético de la rata Wistar: Efecto del envejecimiento, la restricción calórica y la leptina. Universidad Autónoma de Madrid. Director: José M. Carrascosa.
Genetic bases of Alzheimer’s Disease: Genomic study of pathogenic cell models.

Research Summary

In the last years our group has focused on the search of risk factors and/or genes involved in the Alzheimer’s Disease (AD), developing cellular models to obtain candidates that are then validated through genetic association studies in patients. One of the main objectives of the group is to establish the involvement in the pathogenesis of AD of two factors associated with aging, oxidative stress (OS) and infection by HSV-1.

We have found that in the cell models OS regulates the traffic, degradation and proteolytic processing of the Aβ peptide precursor protein (APP), and that this regulation involves the two main cellular proteolytic systems: ubiquitin/proteasome and autophagy/lysosome. On the other hand, HSV-1 is able to reproduce most of the anomalies found in the brains of AD patients, as the hyperphosphorylation of tau protein and alterations of the autophagic process that lead to the intracellular accumulation of Aβ; these effects are increased in the presence of OS.

After the genomic analysis of the cell models, we have focused on the list of genes which expression is modulated by HSV-1 in the presence of OS because this is similar to the infection in aged people for their functional validation and genetic risk association studies. The “cytokine-mediated inflammation” function is highly enriched in this list, which is consistent with the findings of the latest genome wide association studies (GWAs) and suggests that HSV-1 may participate in the pathogenesis of sporadic AD.

Other works of the group during this period include the analysis of cellular models of monogenic AD, and the participation in collaborative genetic association studies, mainly with groups of CIBERNED and as part of the European consortium EADI, which are revealing novel AD risk factors.
Genetic bases of Alzheimer’s Disease: Genomic study of pathogenic cell models.

**Figure 1.** Oxidative stress produces the accumulation of APP protein and its carboxy and amino terminal fragments in SK N MC neuronal cells.
Genetic bases of Alzheimer’s Disease: Genomic study of pathogenic cell models.

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Genetic bases of Alzheimer’s Disease: Genomic study of pathogenic cell models.

Publications


Genetic bases of Alzheimer’s Disease: Genomic study of pathogenic cell models.

Awards

- Fundación Madrid+D. Premio a las mejores patentes; accésit. (2011)
- Sociedad Madrileña de Neurología. Premio Ramón y Cajal de Investigación básica (2011)
Our group, “Molecular mechanisms of Neurodegeneration and Regeneration”, is a line established in the CBM over several years. We are interested in the analysis of the molecular mechanisms fired by processes neurodegenerative, trying to understand the key points of these processes; for a second attempt to design new regenerative alternatives, or to propose new therapeutic targets.

First, our studies of molecular mechanisms of degeneration are focused on the role of PI3K-Akt: GSK3 in neurodegeneration. We have seen that some neuroprotective elements as estradiol modulates elements, such as GSK3 and β-catenin, proteins shared by other signaling pathways like Wnt. And we recently demonstrate that estradiol may trigger Akt-mTORC1 pathway. All these data represent a new signalling pathway, triggered by estradiol that would complement to IGF-1 and Wnt. These findings have led us to extend the analysis of signalling mediated by Estradiol in normal neuronal physiology and in some pathological conditions such as Ischemia model.

Second and complementary we are interested in the molecular mechanisms that regulate the generation and maintenance of the axonal polarity. This morphological polarity appears during development when the neuron differentiates and begins to extend an axon. Subsequently they “mature” and form their initial segment of the axon. Studies of several laboratories, including ours have shown that PI3K-kinase activity allows axonal growth and determining axonal polarity (in collaboration with JJ. Garrido’s group from Cajal Inst.). Our laboratory has helped identify some of the elements that control polarity, such that GSK3, control the polarity. Our work, in this field, will follow to identify upstream and downstream elements that would be essential for this “morphogenetic process”.

In summary, we are focusing on track PI3K-Akt signalling and elements than those elements that control neuron morphogenesis and are modified in pathology.
Molecular mechanism of neurodegeneration and regeneration
Molecular mechanism of neurodegeneration and regeneration

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Molecular mechanism of neurodegeneration and regeneration

Publications


Molecular mechanism of neurodegeneration and regeneration

Other Activities


This group is also part of the Centre for biomedical research in neurodegenerative diseases network (CiberNed):
http://www.ciberned.es/grupofranciscowandosell.aspx

Colaboración con la Industria:
Acuerdo de Colaboración con FAES-FARMA (2011)
Acuerdo de Colaboración con NOSCIRA (2011)
Molecular mechanism of neurodegeneration and regeneration

Doctoral Theses