Annex 3
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Review Article

Possible Involvement of TLRs and Hemichannels in Stress-Induced CNS Dysfunction via Mastocytes, and Glia Activation

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In the central nervous system (CNS), mastocytes and glial cells (microglia, astrocytes and oligodendrocytes) function as sensors of neuroinflammatory conditions, responding to stress triggers or becoming sensitized to subsequent proinflammatory challenges. The corticotropin-releasing hormone and glucocorticoids are critical players in stress-induced mastocyte degranulation and potentiation of glial inflammatory responses, respectively. Mastocytes and glial cells express different toll-like receptor (TLR) family members, and their activation via proinflammatory molecules can increase the expression of connexin hemichannels and pannexin channels in glial cells. These membrane pores are oligohexamers of the corresponding protein subunits located in the cell surface. They allow ATP release and Ca^{2+} influx, which are two important elements of inflammation. Consequently, activated microglia and astrocytes release ATP and glutamate, affecting myelinization, neuronal development, and survival. Binding of ligands to TLRs induces a cascade of intracellular events leading to activation of several transcription factors that regulate the expression of many genes involved in inflammation. During pregnancy, the previous responses promoted by viral infections and other proinflammatory conditions are common and might predispose the offspring to develop psychiatric disorders and neurological diseases. Such disorders could eventually be potentiated by stress and might be part of the etiopathogenesis of CNS dysfunctions including autism spectrum disorders and schizophrenia.

1. Introduction

Signaling between nervous and immune systems is in part due to the fact that these two systems share ligands and receptors. The cellular components involved in these interactions within the central nervous system (CNS) are mainly mastocytes, also called mast cells, and glia. In human brain, mastocytes are very scarce and are preferentially located in perivascular territories. By contrast, glial cells comprise about 90% of the total cell content in the CNS and are classified as microglia and macroglia (astrocytes, oligodendrocytes, and ependymal cells) [1]. Representative of the immune system in the CNS are mastocytes and microglia, two cell types derived from hematopoietic cells of the bone marrow that migrate to the brain before closure of the blood brain barrier (BBB) [2, 3].

The CNS challenged by different aggressions frequently elicits immune and inflammatory responses [4, 5]. Mastocytes and microglia are efficient sensors of adverse endogenous or exogenous conditions of the CNS [2, 6]. Moreover, stress conditions induce rapid mastocyte degranulation via the hypothalamic peptide corticotropin-releasing hormone (CRH) [7] and exogenous danger molecules like polyinosinic-polycytidylic acid (poly (I:C)), bacterial lipopolysaccharide (LPS), and peptidoglycan (PGN), which are detected by mastocytes and microglia via toll-like receptors (TLRs) [8, 9]. Also, glucocorticoids (GCs) play a relevant role in stress-induced potentiation of neuroinflammatory responses by sensitizing microglia to proinflammatory stimuli [10]. As part of these responses, glial TLRs, connexin hemichannels (Cx HCs), pannexin (Panx) channels might be key players in acute and chronic neurodegenerative diseases

Journal of Cellular Physiology

FasL-Triggered Death of Jurkat Cells Requires Caspase 8-Induced, ATP-Dependent Cross-Talk Between Fas and the Purinergic Receptor $P2X_7$

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Fas ligation via the ligand FasL activates the caspase-8/caspase-3-dependent extrinsic death pathway. In so-called type II cells, an additional mechanism involving tBid-mediated caspase-9 activation is required to efficiently trigger cell death. Other pathways linking FasL–Fas interaction to activation of the intrinsic cell death pathway remain unknown. However, ATP release and subsequent activation of purinergic $P2X_7$ receptors ($P2X_7Rs$) favors cell death in some cells. Here, we evaluated the possibility that ATP release downstream of caspase-8 via pannexin I hemichannels (Panx1 HCs) and subsequent activation of $P2X_7Rs$ participate in FasL-stimulated cell death. Indeed, upon FasL stimulation, ATP was released from Jurkat cells in a time- and caspase-8-dependent manner. Fas and Panx1 HCs colocalized and inhibition of the latter, but not connexin hemichannels, reduced FasL-induced ATP release. Extracellular apyrase, which hydrolyzes ATP, reduced FasL-induced death. Also, oxidized-ATP or Brilliant Blue G, two $P2X_7R$ blockers, reduced FasL-induced caspase-9 activation and cell death. These results represent the first evidence indicating that the two death receptors, Fas and $P2X_7R$ connect functionally via caspase-8 and Panx1 HC-mediated ATP release to promote caspase-9/caspase-3-dependent cell death in lymphoid cells. Thus, a hitherto unsuspected route was uncovered connecting the extrinsic to the intrinsic pathway to amplify death signals emanating from the Fas receptor in type II cells.

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Apoptosis is an active process that depends on a defined sequence of signaling events and can occur via two different routes, referred to as the extrinsic (death receptor mediated) and the intrinsic (endogenous) pathways. The extrinsic pathway is activated in a manner dependent on ligand binding to death receptors. Fas ligand (FasL) binding to the Fas/CD95 receptor promotes formation of the death-induced signaling complex (DISC) by recruiting Fas-associating death domain (DD) [FADD-containing protein] through its DD domain, which then recruits death-effector domain (DED)-containing initiator caspases, such as caspase-8 and -10. Proximity of pro-caspase molecules is required for autocleavage and activation of initiator caspases, which then go on to proteolytically cleave and activate executioner caspases, including caspase-3, -6, -7 (Scaffidi et al., 1998; Peter and Krammer, 2003).

In some cells, referred to as Type I, this sequence of events is sufficient to trigger apoptosis. Alternatively in Type II cells, caspase-8 activation is weak and signaling downstream of the DISC needs to be amplified via a loop involving cleavage of Bid to generate tBid and activation of the mitochondrial pathway, which generally responds to intracellular stress signals. Changes in mitochondrial membrane potential and mitochondrial membrane permeability induced downstream of tBid formation culminate in release of mitochondrial molecules to the cytosol. Mitochondrial cytochrome c in conjunction with cytosolic apoptosis protease activating factor 1 (Apaf-1) and adenosine triphosphate (ATP) assemble into a multi-protein complex, called the apoptosome, which catalyzes proteolytic auto-activation of the initiator caspase-9 and activation downstream of the executioner caspases (Hengartner, 2000; Samraj et al., 2006).

The purinergic receptor $P2X_7$ ($P2X_7R$), whose ligand is ATP, can trigger either necrosis and/or apoptosis in various cell types, including myeloid cells, dendritic cells, thymocytes, macrophages and lymphocytes (Di Virgilio et al., 1998).

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Seccion 3.2. – Nuevos conocimientos biomédicos y fisiológicos de la práctica deportiva (con énfasis en canales de iones en musculo esquelético)

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El movimiento y la vida están interconectados desde el punto de vista biológico, yendo desde fundamentos de fenomenología y de la observación, hasta descripciones que nos han permitido en años recientes un entendimiento cada vez mayor de sus bases moleculares y fisiológicas. Se describen distintos niveles de movimiento que van desde actividad física a deporte. En esta sección procuraremos definir estos conceptos para luego centrarnos en las modificaciones que se producen a nivel de canales iónicos en el músculo esquelético durante el ejercicio.

3.2.1. Actividad física, ejercicio y deporte, precisiones del lenguaje e importancia a nivel molecular y de la vida y envejecimiento.

Puede ocurrir movimiento en diferentes niveles, que conocemos como actividad física, ejercicio físico o deporte, de acuerdo con sus variaciones de forma, intensidad y frecuencia. Estos tres niveles de movimiento llevan a modificaciones moleculares, celulares y fisiológicas de acuerdo con la forma como son realizados. La comprensión del comportamiento de estas variables ha mejorado mucho con el correr de los años, debido al avance tecnológico. Así entonces, el movimiento o actividad física en general en el campo biológico puede manifestarse a varios niveles entrelazados que conocemos como actividad física, ejercicio o deporte, de acuerdo a sus variantes en formas, intensidad, competitividad y periodicidad de prácticas al respecto. En sí pueden considerarse distintos escalones de conjuntos inclusivos (ver Fig. 1). La **actividad física** puede ser definida como cualquier movimiento corporal producido por la musculatura esquelética, que resulta en un gasto energético por encima de los niveles de reposo. Se ha determinado que la misma es central a la hora de definir los

Octodon degus (Molina 1782): A Model in Comparative Biology and Biomedicine

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One major goal of integrative and comparative biology is to understand and explain the interaction between the performance and behavior of animals in their natural environment. The Caviomorph, *Octodon degu*, is a native rodent species from Chile, and represents a unique model to study physiological and behavioral traits, including cognitive and sensory abilities. Degus live in colonies and have a well-structured social organization, with a mostly diurnal–crepuscular circadian activity pattern. More notable is the fact that in captivity, they reproduce and live between 5 and 7 yr and show hallmarks of neurodegenerative diseases (including Alzheimer's disease), diabetes, and cancer.

BACKGROUND

The Octodontidae family (Rodentia) is endemic to South America and shows a wide range of diversity, from its genes to its communities. Octodontidae fossil records go back to the last Eocene, around 40 million years ago. Currently, they are dispersed in north and central Chile from sea level to ~3500 m altitude, with microhabitats in matorral (bush) with evergreen sclerophyllous shrubs over a seasonal herbaceous layer. This environment forms a characteristic South American scrub ecosystem with diverse flora and fauna. The Octodontidae includes nine species (Spotorno et al. 1995), with a unique morphological gradient ranging from cursorial/generalist to subterranean. The number of chromosomes varies among Octodontidae species from 38 (2N) to 102 and the phylogenetic relationship of Octodontidae has been established by cladistics analysis (Honeycutt et al. 2003). The monophyletic *Octodon* genus has three species: The common *O. degu*, *O. lunatus*, and *O. bridgesi*. All are endemic to Chile and related to the Chinchilloidea and Cavioidea families (Opazo 2005). *Octodon degu* is a small, diurnal, herbivorous rodent that lives in social groups (see Fig. 1).

SOURCES AND HUSBANDRY

The accompanying protocol, **Husbandry and Breeding in the *Octodon degu* (Molina 1782)** (Palacios and Lee 2013), describes successful husbandry and breeding practices based on the experience of the University of Michigan degu colony.

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REVIEW ARTICLE

Properties, Projections, and Tuning of Teleost Olfactory Receptor Neurons

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Abstract In many fishes, the olfactory sense participates in such vital processes as feeding, reproduction, orientation, and predator avoidance. In teleosts, these tasks are fulfilled by a single type of olfactory organ for odorant and pheromone detection, containing ciliated and microvillus receptor neurons, and olfactory crypt cells. Recently, progress was made in understanding crypt cell function with the discovery of a V1R-like odorant receptor expressed in this neuron, an analysis of crypt cell odorant tuning properties, and the dissection of crypt cell connectivity within the telecephalon. Here, we review recent findings on the molecular properties, functions, and associated neural pathways of the three types of teleost olfactory receptor neurons with special emphasis on the crypt cell, and evaluate their roles in the detection of food, social and sexual odorants.

Keywords Olfaction · Smell · Pheromone · Crypt cell · Odorant receptor · Fish

Introduction

About 50 % of all vertebrates on this planet are teleost fishes. As other vertebrates, teleosts have an olfactory system that allows them to detect and discriminate between different kinds of olfactory stimuli, such as amino acids, nucleotides, bile salts, gonadal steroids, and prostaglandins (Sorensen and Caprio, 1998). These distinct odorant classes are related to different behaviors, such as feeding, orientation, and reproduction in fishes. Due to their aqueous habitat, compounds that elicit chemosensory responses in fishes are mostly hydrophilic, and may stimulate either the olfactory or the taste system or both. Traditionally, substances effective at submicromolar concentrations have been considered as odorants, and those with higher functional thresholds as tastants, but this distinction is invalid in fishes, whose taste system is as or more sensitive to certain substances as their olfactory system (Ogawa and Caprio, 2010; Hara, 2011). Notwithstanding this caveat, a long series of behavioral and electrophysiological experiments in different teleost species has provided us with a large body of data on fish olfaction, revealing sometimes astonishing sensitivity and functional sophistication. This review provides an overview of the current knowledge regarding the cellular and molecular properties, odorant tuning, and neural connections of the three types of olfactory receptor neurons (ORNs) in teleosts, with an emphasis on recent findings on the physiology of the olfactory crypt cell (CC).

Teleost ORNs

As opposed to the majority of vertebrates, teleosts lack both a vomeronasal organ and an accessory olfactory bulb. Instead, they have a single pseudostratified olfactory epithelium on each side of the head (Fig. 1a, b), generally forming a multilamellar olfactory rosette, although single plain epithelial sheets also can be found in some species (Hansen and Zielinski, 2005). Three morphologically distinct types of ORN are present in the olfactory epithelium: Ciliated and microvillus bipolar neurons (cORNs and mORNs), and the CCs, which are thought to be absent from any other vertebrate group (Hansen and Finger, 2000; Laberge and Hara, 2001) (Fig. 1c–f). In addition, undifferentiated basal cells in the olfactory epithelium allow the regeneration of sensory neurons and accessory cells, and the growth of the organ with age (Evans et al., 1982) (Fig. 1g).

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Quantitative Analysis of Cell Migration Using Optical Flow

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Abstract

Neural crest cells exhibit dramatic migration behaviors as they populate their distant targets. Using a line of zebrafish expressing green fluorescent protein (*sox10:EGFP*) in neural crest cells we developed an assay to analyze and quantify cell migration as a *population*, and use it here to characterize in detail the subtle defects in cell migration caused by ethanol exposure during early development. The challenge was to quantify changes in the *in vivo* migration of all Sox10:EGFP expressing cells in the visual field of time-lapse movies. To perform this analysis we used an Optical Flow algorithm for motion detection and combined the analysis with a fit to an affine transformation. Through this analysis we detected and quantified significant differences in the cell migrations of Sox10:EGFP positive cranial neural crest populations in ethanol treated versus untreated embryos. Specifically, treatment affected migration by increasing the left-right asymmetry of the migrating cells and by altering the direction of cell movements. Thus, by applying this novel computational analysis, we were able to quantify the movements of populations of cells, allowing us to detect subtle changes in cell behaviors. Because cranial neural crest cells contribute to the formation of the frontal mass these subtle differences may underlie commonly observed facial asymmetries in normal human populations.

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Introduction

The cranial neural crest cells (CNCC) are a population of progenitors that give rise to both craniofacial structures and components of the peripheral nervous system (PNS). These cells migrate from the dorsal neural tube to populate the face. After leaving the neuroepithelium, CNCC split into discrete streams separated by CNCC-free regions [1]. Three main streams are formed according to their relative pre-migratory position along the cranial neural tube. The most anterior stream of CNCC migrates dorsal to the eye and populates the frontal mass (or anterior neurocranium) [2], ganglia of cranial nerves and melanocytes among others. More posterior streams of CNCC populate the branchial arches, where they form the cartilage and bones of the jaw [3]. Much is known about the CNCC migration and differentiation as it relates to the branchial arch derivatives including the jaw elements [4,5]. By contrast, the migration of CNCC that pass dorsal to the eye and contribute to the facial structures has not been extensively described with the exception of the migration of neural crest cells that contribute to specific parts of the neurocranium [2,6,7]. We (this study) and others [8] have found that the migration of the dorsal anterior CNCC appears to be sensitive to environmental factors such as alcohol, smoking, and herbicides. Yet, the cell movements that occur during the migration of this population of CNCC are complex and difficult to describe and characterize quantitatively. For this reason we developed an analytical method to characterize cell migration, and use it here to describe the defects of dorsal anterior CNCC migration caused by alcohol exposure.

There is a large body of evidence demonstrating that ethanol (EtOH) affects CNCC development. The primary defects observed are cell death in both *in vitro* and *in vivo* systems; some studies also report defects in cell migration. *In vivo* EtOH-induced cell death has been reported in regions populated by CNCC in the developing chick embryo [9] and while the number of CNCC is reduced, the migration patterns were unaffected [10]. Additional studies in chick have reported cell death in neural crest cells [11]. *In vitro* analysis of CNCC has shown that EtOH exposure produces permanent changes in cell shape, surface morphology, migration, and cell death [12,13]. Interestingly no analysis of migration defects have been reported *in vivo*, although histological studies have suggested the occurrence of EtOH-induced migration defects, based on the pattern of immunostained neural crest in treated chick embryos [14]. Thus, whereas EtOH induced cell death has consistently been reported in a variety of model systems, the effects on CNCC migration have not been well documented. An *in vivo* real time analysis of the CNCC population contributing to the frontal mass would improve our understanding of CNCC migration in normal and EtOH exposed animals.

Many studies of cell migration use techniques that track single cells and base their analysis on the average migration behavior of single cells. In zebrafish and *Xenopus* embryos the cell-cell interactions of individual CNCC have been analyzed to demonstrate contact inhibition of locomotion in which cells cease migrating after contact with another cell [15]. Because the CNCC

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Measurement of autophagy flux in the nervous system *in vivo*

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Accurate methods to measure autophagic activity *in vivo* in neurons are not available, and most of the studies are based on correlative and static measurements of autophagy markers, leading to conflicting interpretations. Autophagy is an essential homeostatic process involved in the degradation of diverse cellular components including organelles and protein aggregates. Autophagy impairment is emerging as a relevant factor driving neurodegeneration in many diseases. Moreover, strategies to modulate autophagy have been shown to provide protection against neurodegeneration. Here we describe a novel and simple strategy to express an autophagy flux reporter in the nervous system of adult animals by the intraventricular delivery of adeno-associated viruses (AAV) into newborn mice. Using this approach we efficiently expressed a monomeric tandem mCherry-GFP-LC3 construct in neurons of the peripheral and central nervous system, allowing the measurement of autophagy activity in pharmacological and disease settings.

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Subject Category: Neuroscience

Macroautophagy, here referred to as autophagy, is the major catabolic pathway involved in the degradation of damaged or superfluous organelles, abnormal protein aggregates and other cytosolic components.[1] An explosion of literature in the last few years highlights the contribution of autophagy to diverse physiological processes including metabolic control, lipid homeostasis, immunity and myopathies.[2] Autophagy is initiated by the formation of autophagosomes, double membrane vesicles that engulf cytosolic components and fuse with endosomes to form hybrid organelles called amphisomes that later fuse with lysosomes where cargoes are degraded. The mechanisms underlying the regulation of different steps of autophagy are highly complex and dynamic and are reviewed in detail elsewhere.[3,4] In brief, two ubiquitin-like conjugation pathways control autophagy. One of them involves the covalent binding of Atg12 to Atg5, which occurs at the phagophore level, and dissociation after autophagosome consolidation. One of the key steps in the regulation of autophagy is the conjugation of microtubule-associated protein 1 light chain 3 (LC3) to phosphatidylethanolamine to form microtubule-associated protein 1 light chain 3 lipidated form (LC3-II). Lipidated LC3 binds to the expanding phagophore and remains associated with autophagosomes even after fusion with lysosomes. Then, LC3-II can be either delipidated and recycled or degraded by hydrolytic enzymes at the lysosome. Monitoring LC3-II conversion, LC3 distribution or its flux through the autophagy pathway are the gold standards for measuring autophagy activity and are widely used in the field.[4]

The generation of conditional knockout mice for essential autophagy regulatory components in the nervous system revealed a key role of the pathway in the basal maintenance of protein homeostasis in neurons.[5,6] Most prevalent neurodegenerative diseases, including Alzheimer's disease, amyotrophic lateral sclerosis, Parkinson's disease and Huntington's disease are associated with the misfolding and aggregation of specific proteins.[7] Many studies indicate that autophagy operates as an efficient mechanism for the degradation of aggregation-prone proteins linked to neurodegeneration and suggest that pharmacological activation of autophagy offers a promising therapeutic avenue.[8] Accumulating evidence also suggests that autophagy impairment may underlie the etiology of several neurodegenerative diseases.[8,9]

One of the main limitations in the field is the lack of reliable tools to monitor autophagy activity in the nervous system,[4,10] a challenging issue due to barriers for drug penetrance into

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Keywords: Autophagy; adeno-associated vector (AAV); microtubule-associated protein 1 light chain 3 (LC3); nervous system; autophagy flux
Abbreviations: AAV, adeno-associated vectors; LC3, microtubule-associated protein 1 light chain 3; LC3-II, microtubule-associated protein 1 light chain 3 lipidated form; DRP, DNase-resistant particles; ICV, intracerebroventricular; ChAT, choline acetyltransferase; EBSS, Earle's balanced salt solution; IP, intraperitoneal; SCI, spinal cord injury; DRG, dorsal root ganglia; CNS, central nervous system; BBB, blood–brain barrier; DMSO, dimethyl sulfoxide; PBS, phosphate-buffered saline solution; DAPI, 4′,6-diamidino-2-phenylindole
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De novo expression of connexin hemichannels in denervated fast skeletal muscles leads to atrophy

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Denervation of skeletal muscles induces atrophy, preceded by changes in sarcolemma permeability of causes not yet completely understood. Here, we show that denervation-induced Evans blue dye uptake in vivo of fast, but not slow, myofibers was acutely inhibited by connexin (Cx) hemichannel/pannexin1 (Panx1) channel and purinergic ionotropic P2X$_7$ receptor (P2X$_7$R) blockers. Denervated myofibers showed up-regulation of Panx1 and de novo expression of Cx39, Cx43, and Cx45 hemichannels as well as P2X$_7$Rs and transient receptor potential subfamily V, member 2, channels, all of which are permeable to small molecules. The sarcolemma of freshly isolated WT myofibers from denervated muscles also showed high hemichannel-mediated permeability that was slightly reduced by blockade of Panx1 channels or the lack of Panx1 expression, but was completely inhibited by Cx hemichannel or P2X$_7$R blockers, as well as by degradation of extracellular ATP. However, inhibition of transient receptor potential subfamily V, member 2, channels had no significant effect on membrane permeability. Moreover, activation of the transcription factor NFκB and higher mRNA levels of proinflammatory cytokines (TNF-α and IL-1β) were found in denervated WT but not Cx43/Cx45-deficient muscles. The atrophy observed after 7 d of denervation was drastically reduced in Cx43/Cx45-deficient but not Panx1-deficient muscles. Therefore, expression of Cx hemichannels and P2X$_7$R promotes a feed-forward mechanism activated by extracellular ATP, most likely released through hemichannels, that activates the inflammasome. Consequently, Cx hemichannels are potential targets for new therapeutic agents to prevent or reduce musde atrophy induced by denervation of diverse etiologies.

connexons | membrane leakage | purinergic receptors | phosphorylated p65 | inflammation

Denervated skeletal muscles undergo a change in membrane permeability along with a progressive array of metabolic, structural, and functional changes that lead to atrophy (1). For example, at approximately 7 d after denervation, rodent skeletal muscles show a decrease in intracellular K^+ concentration (2) and an increase in intracellular Na^+ concentration (1) and total calcium content (1). In addition, contraction of denervated skeletal muscle depends on extracellular Ca^{2+} as early as 6 d after denervation (3). A possible explanation for this latter result is that denervation induces the expression of the cardiac Ca^{2+} permeable dihydropyridine receptor isoform (4). However, this protein is only expressed from day 25 of denervation. Therefore, the Ca^{2+}-dependency of denervated muscles for a single contraction remains unexplained (4). The increase in dihydropyridine receptors Cav1.1 and ryanodine receptor complex has also been proposed to contribute to the increase in free Ca^{2+} concentration (5), but the denervation-induced reduction in membrane potential (V_m) is not sufficient to activate these channels (6). The changes in intracellular Na^+ and K^+ concentrations and V_m reduction of denervated myofibers might be explained by a deficiency in Na^+/K^+-dependent ATPase pump activity, but ouabain still induces a ~10% reduction in V_m in denervated myofibers (7). Consequently, the transmembrane electrochemical changes induced by denervation are, at present, not fully explained. An alternative mechanism could be the de novo expression of nonselective cation channels, which, to our knowledge, has not been reported. Investigation of this possibility was the main goal of the present work.

To date, treatments with several compounds have only partially reduced the development of atrophy (8). However, substantial reduction of myofiber atrophy has been obtained upon muscle-specific inhibition of NF-κB through expression of IκB-α super-repressor (1) or genetic deletion of either of two muscle-specific E3 ligases, atrogin-1 or muscle ring finger-1 (MurF1) (1). However, the sequence of events that initiates muscle atrophy and the relevance of most changes induced by denervation remain uncertain.

Here, we demonstrated that denervated fast skeletal muscles express de novo the monovalent cation and Ca^{2+} permeable channels connexins (Cxs) 39, 43, and 45, purinergic ionotropic P2X$_7$ receptors (P2X$_7$Rs), as well as transient receptor potential, subfamily V, member 2 (TRPV2), channels, and show an up-regulation of pannexin1 (Panx1). The relevance of functional Cx hemichannels and P2X$_7$Rs in denervation-induced permeabilization of the sarcolemma was also demonstrated. In addition, denervation was found to induce an inflammatory state of myofibers associated with muscular atrophy, and both responses were greatly

PHYSIOLOGY

Significance

In this paper two biological findings are described and explain several muscle changes induced by denervation: (*i*) the sarcolemma of fast myofibers are permeabilized to small molecules such as Evans blue via connexin (Cx) hemichannels and (*ii*) the absence of Cx43/Cx45 hemichannels greatly attenuates the inflammasome activation and muscle atrophy. The first finding explains the activation of proteolysis in denervated muscles. The second demonstrates that muscle inflammation can occur without inflammatory cell infiltration, offering an explanation how denervated muscles can alter other tissues. These findings unveil therapeutic targets to reduce atrophy in diverse clinical conditions. Because Cx hemichannels are permeable to Evans blue, the use of this dye as tracer of cell damage should be reevaluated in different systems.

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The authors declare no conflict of interest.

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Chapter 1
Spike Train Statistics From Empirical Facts To Theory: The Case Of The Retina.

Bruno Cessac and Adrian Palacios

Abstract This chapter focuses on methods from statistical physics and probability theory allowing the analysis of spike trains in neural networks. Taking as an example the retina we present recent works attempting to understand how retina ganglion cells encode the information transmitted to the visual cortex via the optical nerve, by analyzing their spike train statistics. We compare the maximal entropy models used in the literature of retina spike train analysis to rigorous results establishing the exact form of spike train statistics in conductance-based Integrate-and-Fire neural networks.

This chapter is done in the spirit of the course "Neuronal dynamics", given at the Master of Computational Biology, University of Nice, aiming at showing how a specific problem in neuroscience can be addressed on theoretical grounds, and how it can be related to experimental methods and results. As a consequence, this chapter contains both recent biological results and mathematical developments.

1.1 Introduction

Given a stimulus from the external world (visual scene, sound, smell, ...) biological sensors at the periphery of the nervous system are able to transduce the physical manifestations of this stimulus (light emission, air pressure variations, chemical concentrations) into sequences of action potentials (spike trains), which propagate through the nervous system. Then, the brain is able to *analyze* those spike trains and infer crucial information on the nature of the stimulus. Critical - yet unsolved - questions in neuroscience are How is the physical signal encoded by the nervous system ? How does the brain analyze the spike trains ? What are the underlying computational *coding* principles ? At the current stage of scientific knowledge, answering those questions is still a challenge for biology and computational neuroscience.

Among sensory systems the retina provides functionality such as detection of movement, orientation, temporal and spatial prediction, response to flash omissions and contrast, that were up to recently viewed as the exclusive duty of higher brain centers [22]. The retina is an accessible part of the brain [13] and a prominent system to study the neurobiology and the underlying computational capacity of the neural coding. As a matter of fact, there is currently a wide research activity in understanding how the retina encodes visual information. However, basic questions are still open, such as: Are the ganglion cells (which send spikes from the eyes to the brain via the optical nerve), independent signal-encoders or are neural correlations important for coding a visual scene, and how to interpret them ?

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A BK (Slo1) channel journey from molecule to physiology

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Keywords: BK channels, Slo1, auxiliary subunits, voltage sensor, intracellular Ca^{2+}, smooth muscle, diseases

Abbreviations: BK, big conductance voltage and Ca^{2+}-dependent potassium channel; Charybdotoxin, ChTx; Iberotoxin, IbTx; regulator of the conductance of K^+ channels, RCK; voltage sensing domain, VSD; leucine-rich repeat proteins, LRRC; nitric oxide, NO; cyclic guanosin mono-phosphate, cGMP

Calcium and voltage-activated potassium (BK) channels are key actors in cell physiology, both in neuronal and non-neuronal cells and tissues. Through negative feedback between intracellular Ca^{2+} and membrane voltage, BK channels provide a damping mechanism for excitatory signals. Molecular modulation of these channels by alternative splicing, auxiliary subunits and post-translational modifications showed that these channels are subjected to many mechanisms that add diversity to the BK channel α subunit gene. This complexity of interactions modulates BK channel gating, modifying the energetic barrier of voltage sensor domain activation and channel opening. Regions for voltage as well as Ca^{2+} sensitivity have been identified, and the crystal structure generated by the 2 RCK domains contained in the C-terminal of the channel has been described. The linkage of these channels to many intracellular metabolites and pathways, as well as their modulation by extracellular natural agents, has been found to be relevant in many physiological processes. This review includes the hallmarks of BK channel biophysics and its physiological impact on specific cells and tissues, highlighting its relationship with auxiliary subunit expression.

Introduction

BK channels are members of a family of Ca^{2+} and voltage-dependent potassium channels. They are constituted by a tetramer of α subunits that form the conducting pore and are encoded by the *slo1* gene. In several tissues, BK channels have been observed to be modulated by auxiliary subunits, which confer important physiological performance to the channels. BK channels are ubiquitously expressed in cell membranes of mammalian tissues, where they couple signals that result from differences in membrane voltage and intracellular Ca^{2+} concentration, which are both key actors in the physiology of nervous and non-nervous cells. In this review we provide an overview of BK channel function and its relationship with structural cues, the voltage sensor domain and gating properties of the channels, as well as its crosstalk with its auxiliary subunits.

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A Short Story about How BK Channels Were Identified

The first evidence of a K^+ permeability induced by increases in intracellular calcium concentration was obtained in red blood cells.[1] Later, a calcium-dependent K^+ current was reported from experiments where Ca^{2+} was injected in motoneurons, resulting in both an increase in membrane conductance and a decrease in cellular excitability.[2] Moreover, the removal of external Ca^{2+} was found to decrease a voltage-dependent K^+ current in mollusk neurons.[3,4] A few years later, these currents were defined as being carried by a calcium-dependent potassium current,[5] after which their critical role in neuronal firing properties and hyperpolarization was soon acknowledged.[6]

The year 1981 was the *annus mirabilis* of BK channel research, since abundant expression of these channels was found in skeletal muscles and chromaffin cells. Single channel recordings from skeletal muscle and chromaffin cells[7,8] as well as the reconstitution of a calcium-dependent K^+ channel in bilayers[9] revealed the large conductance of BK channels, which ranges within 200 pS. This magnitude gave rise to the names MaxiK or BK, thus representing the large conductance potassium channel.[10] With the advent of the giga-seal patch clamp technique[11] and the possibility of isolating and patching small cells, BK channels were soon found and described in liver, lymphocyte, epithelium, exocrine, and endocrine glands as being linked to excitation-secretion coupling.[8,12-14] Marty et al. discovered that channels of different conductance give rise to Ca^{2+}-dependent potassium currents in rat lacrimal glands and named the largest conductance type channel *BK*.[15] By that time, another important finding regarding this channel's localization was made, namely that it is abundantly

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The Amazing Odontoblast: Activity, Autophagy, and Aging

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ABSTRACT

Odontoblasts are dentin-secreting cells that survive for the whole life of a healthy tooth. Once teeth are completely erupted, odontoblasts transform into a mature stage that allows for their functional conservation for decades, while maintaining the capacity for secondary and reactionary dentin secretion. Odontoblasts are also critically involved in the transmission of sensory stimuli from the dentin-pulp complex and in the cellular defense against pathogens. Their longevity is sustained by an elaborate autophagic-lysosomal system that ensures organelle and protein renewal. However, progressive dysfunction of this system, in part caused by lipofuscin accumulation, reduces the fitness of odontoblasts and eventually impairs their dentin maintenance capacity. Here we review the functional activities assumed by mature odontoblasts throughout life. Understanding the biological basis of age-related changes in human odontoblasts is crucial to improving tooth preservation in the elderly.

KEY WORDS: dentin, human dental pulp, sensory system, lipofuscin.

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INTRODUCTION

Odontoblasts derive from cranial neural crest cells that emerge early during vertebrate evolution (Chai *et al.*, 2000; Hall and Gillis, 2013). Their secretory product, dentin, is one of the first manifestations of extracellular matrix based on biomineralized collagen. This hard compound appeared in the Ordovician period in jawless fish with a dermal skeleton, whose tooth-like denticles contained dentinous tissues similar to the orthodentin that forms the bulk of contemporary vertebrate teeth (Smith and Sansom, 2000; Kawasaki and Weiss, 2008). In mammals, odontoblasts are long-lived post-mitotic cells organized in a continuous cellular palisade at the dentin-pulp interface, where they maintain pre-dentin and dentin apposition for the whole life of a tooth. Together with an impressive network of trigeminal nerve fibers, they form a complex sensory organ, detecting and transmitting changes in temperature, mechanical stimuli, and pain (Byers *et al.*, 2003; Magloire *et al.*, 2010; Farahani *et al.*, 2011).

Increasing life expectancy in humans, exceeding 80 years in several developed countries, means that ever more individuals are reaching old age. From an oral health perspective, the increase in human longevity implies the necessity for prolonged tooth preservation (Tsakos, 2011). Understanding the mechanisms and biological foundation of age-related changes affecting odontoblasts and dentin apposition along life may create novel foundations for dental therapy and the application of adequate protective measures, depending on the age of the tooth.

This article reviews the different activities of odontoblasts during their life cycle, with a focus on their secretory, sensory, and defensive functions. In addition, it aims to define the homeostatic mechanisms that maintain mature odontoblasts for several decades, describing their autophagic-lysosomal system as one of the main pathways for organelle turnover in this cell (Couve, 1986; Couve and Schmachtenberg, 2011; Couve *et al.*, 2012). Finally, the consequences of odontoblast aging for tooth preservation are discussed.

DENTIN-SECRETORY ACTIVITY OF ODONTOBLASTS

Odontoblasts differentiate from embryonic ectomesenchymal cells derived from the cranial neural crest during the initial events of tooth development (Koussoulakou *et al.*, 2009). The development of pre-odontoblasts into terminally differentiated odontoblasts results in highly polarized cells with a columnar shape, about 50 μm in height (Couve, 1986). They are connected by junctional complexes forming a densely packed palisade at the dentin–pulp interface, from where each cell projects an odontoblastic process into the pre-dentin/dentin matrix. In erupted permanent teeth with completely formed crowns, coronal odontoblasts are organized in a pseudostratified palisade,

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Editorial

Beyond the retina neural coding: On models and neural rehabilitation

In the periphery, known sensory systems are at the boundary between physics and biology. The molecular and cellular designs of such systems allow for the generation and propagation of action potentials (spikes) through an intricate neural-coding network to the brain. Among these sensory systems, the retina stands out for its functionalities, such as motion detection, orientation, anticipatory temporal prediction as well as, response to flash omissions and contrast, which were all viewed in the past as being exclusively processed by higher brain areas (Gollisch and Meister, 2008). The retina is an accessible part of the brain (Dowling, 1987) and, thus, has become a unique model system to study phototransduction, shedding light on the first steps into neural coding and its underlying computational capacity. The retina is a tightly packed neural tissue comprised on a rich diversity of neurons and structured into three cellular and two synaptic layers (Masland, 2001a). The anatomical and physiological segregation of different aspects of visual scenes in separate spatial, temporal and chromatic channels start at the retina and relies on local neural networks (Atick, 1992). However, how the precise articulation of this neural network contributes to local solutions and global perception is still largely a mystery. The recent description of a diversity of ganglion cell (GC) neurons (the output of the retina that generate spikes) conveys an important question about the actual early visual capacity of the retina. By using high-density multielectrode arrays (MEAs) over a small piece of retina, a complete population of GC has been characterized in salamanders (Segev et al., 2004) and monkeys Pillow et al. (2008). Nevertheless, only a reduce fraction (5/20) of the existing GC types, including a diversity of non-standard behaviors (Masland, 2001a; Masland, 2001b; Masland and Martin, 2007; Schwartz et al., 2007) has been studied in detail ([e.g.]Gollisch and Meister, 2008).

How does the brain make sense of the physical world? How is a physical signal encoded by the nervous system? To what extent does our natural environment shape our sensory systems? What are the underlying computational coding principles? Is it possible to characterize neural response statistics under a common framework? What are the underlying mechanisms for color vision? How is sensory information is integrated to perform complex computations? Those are some of the question that were reviewed during the II LACONEU summer school (Valparaíso, Chile). Even though the main topic of this summer school was retina oriented, we have included works on neural coding in higher order areas and in visual rehabilitation within this Special Issue of Journal of Physiology – Paris: Neural Coding-Chile 2012.

Sensory systems are endowed with the neural coding capacity to transmit incoming signals from the environment through limited capacity channels all the way to the brain (Attneave, 1954; Barlow, 1961; Shannon, 1948). From the standpoint of biological adaptation, the adequacy of sensory design (Barlow, 1961) can match an optimal solution, where the biological mechanisms are embedded in an intricate chemical and electrical neural network. Are redundancy and noise reduced prior to signal transmission to visual cortex? Or are they both necessary for sensory encoding? In an influential article, Nirenberg et al. (2001) suggest that rat GCs act as independent encoders. However recently the relevance of correlated (or synchrony) responses, and therefore population code, has been fully reconsidered. Population code refers to the computational capacity of a neural assembly in terms of spike probability distribution, considering, for example, noise removal, relevance for critical behavior and predictability (Averbeck et al., 2006; Pouget et al., 2000) to code for visual signals. Evidence supporting an orchestrated (i.e., correlated) GC spiking response comes from pioneer studies by Rodieck (1967) and Mastronarde (1983a); Mastronarde (1983b); Mastronarde (1983c).

One of the first stages of any neural coding analysis is the identification and classification of retinal GCs present in the studied retina. The receptive fields of retinal GCs integrate information from a certain region of the visual scene. This, has been classically treated as a linear mechanism computing a known feature followed by a nonlinear computation normally associated to spike generation. Gollisch (2013), in this issue, reviews the advances in understanding the nonlinear spatial integration performed by some retinal GCs inside their receptive fields. The nonlinear mechanisms here reviewed are not only associated to the spike generation of retinal GCs, but also to the nonlinear mechanism existing between bipolar and GCs. Moreover, these nonlinear interactions have been associated to singular feature extraction performed by some retinal GCs, normally related to relevant natural visual stimuli. Additionally, within the framework of nonlinear computations, one of the features detected by a population of retinal GCs is motion direction. In this issue, the article presented by Escobar et al. (2013) reviews the mathematical, theoretical and biophysical models for motion computation in the retina linking the mathematical constraints needed for motion direction selectivity and the biological mechanisms that can be mapped to accomplish these constraints. This article also reviews the biophysical models existing in the literature, in the effort to explain the underlying mechanisms related to motion direction, as well as to identify the critical actors in this computation.

A critical aspect for neural coding in a population of spiking neurons is to infer the statistical distribution of their response. In fact, the probability distribution associated to sensory spiking neurons has been normally estimated by using the questionable assumption that there is conditional independence between neurons. The article of Cofre and Cessac (2013) reviews the Gibbs distribution, originally used in statistical physics, proposing it as

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Review Paper

Mathematical analysis and modeling of motion direction selectivity in the retina

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ABSTRACT

Motion detection is one of the most important and primitive computations performed by our visual system. Specifically in the retina, ganglion cells producing motion direction-selective responses have been addressed by different disciplines, such as mathematics, neurophysiology and computational modeling, since the beginnings of vision science. Although a number of studies have analyzed theoretical and mathematical considerations for such responses, a clear picture of the underlying cellular mechanisms is only recently emerging. In general, motion direction selectivity is based on a non-linear asymmetric computation inside a receptive field differentiating cell responses between *preferred* and *null* direction stimuli. To what extent can biological findings match these considerations? In this review, we outline theoretical and mathematical studies of motion direction selectivity, aiming to map the properties of the models onto the neural circuitry and synaptic connectivity found in the retina. Additionally, we review several compartmental models that have tried to fill this gap. Finally, we discuss the remaining challenges that computational models will have to tackle in order to fully understand the retinal motion direction-selective circuitry.

Contents

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Diffusion of nitric oxide across cell membranes of the vascular wall requires specific connexin-based channels

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ABSTRACT

NO is generated within cells and frequently must be transferred to responsive neighboring cells, as occurs in the endothelium-dependent relaxation of smooth muscle cells observed in blood vessels. It is thought that NO diffuses freely across cell membranes, but it may also permeate through low resistant membrane pathways. Here, we describe the participation of connexin (Cx)-formed channels in the NO transport across cell membranes and between endothelial and smooth muscle cells. We used a water-soluble NO donor of high molecular weight (S-nitrosylated albumin, BSA-NO) that does not permeate through cell membranes or Cx-based channels and the NO-sensitive dye 4,5-diaminofluorescein diacetate to detect changes of intracellular NO concentration. We found that NO generated in the extracellular space was not detected intracellularly in Cx-deficient HeLa cells, suggesting that cell membrane represents a significant diffusion barrier for NO transfer. However, Cx-based channels provide efficient pathways for NO signaling because NO opened and permeated hemichannels expressed in HeLa cells transfected with Cx43, Cx40, or Cx37. In contrast, NO closed hemichannels of HeLa-Cx32 cells, which otherwise are permeable to NO if are opened by a divalent cation-free extracellular solution. Consistent with this, blockade of Cx-based channels abolished the myoendothelial NO transfer and associated NO-dependent vasodilation induced by acethylcholine. These results indicate that Cx-based channels play a key role in the NO-dependent tonic control of vascular function and may direct the NO signal to specific targets, which provides a novel mechanistic basis for the critical role of Cxs in cell–cell communication in the vessel wall.

This article is part of the Special Issue Section entitled 'Current Pharmacology of Gap Junction Channels and Hemichannels'.

1. Introduction

Nitric oxide (NO) is a critical signaling molecule that plays a central role in the regulation of diverse systems, including immune, nervous and cardiovascular systems (Moncada et al., 1991). In blood vessels, NO is a potent vasodilator involved in the minute-to-minute and long-term control of vascular function (Moncada et al., 1991). Since NO is generated in the endothelium by the endothelial NO synthase (eNOS), it must reach the underlying smooth muscle cells or the lumen of the vessel to exert some of its biological effects (Moncada et al., 1991). The small size and hydrophobicity of NO has led to think that it diffuses freely across cell membranes. However, NO has a very short half-life (~4 s) (Moncada et al., 1991; Thomas et al., 2001), and its concentration in the cell membrane may decrease its bioavailability and efficacy. Although NO may react with other radicals, as well as transition metal centers and oxygen (O_2), in physiological conditions the lifetime of NO mainly depends on the relative abundance of O_2, which has solubility and diffusion properties similar to that of NO (Moller et al., 2005; Subczynski et al., 2009). Consequently, the chemical reaction between O_2 and NO has been estimated to be 2–2.5 times faster when both gases diffuse and concentrate in the plasma membrane compared to in the aqueous phase (Liu et al., 1998; Subczynski et al., 2009). In addition, the diffusion rate and concentration time of NO in the cell membrane are not consistent with the physiological signaling kinetics of NO. In this context, direct measurements of bradykinin-induced NO production using porphyrinic microsensors showed that the NO concentration in the endothelial cell membrane peaks after ~13 s (Malinski et al., 1993).

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Linoleic acid induces opening of connexin26 hemichannels through a PI3K/Akt/Ca^{2+}-dependent pathway

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ABSTRACT

Connexin hemichannel (Cx HC) opening is involved in physiological and pathological processes, allowing the cellular release of autocrine/paracrine signaling molecules. Linoleic acid (LA) is known to modulate the functional state of connexin46 (Cx46) HCs. However, the molecular mechanism involved in this effect, or whether LA affects HCs constituted of other connexins, remains unknown. Here, we report the effects of LA on HCs in HeLa cells that express Cx26, one of the main Cxs in the cochlear sensory epithelium. Cx26 HC activity (dye uptake) was increased in a concentration-dependent manner by bath application of LA and inhibited by HC blockers. Moreover, intracellular BAPTA, a Ca^{2+} chelator, and PI3K/AKT inhibitors were found to reduce the LA-induced Cx26 HC opening, suggesting that the LA effect is mediated by an increase of free intracellular Ca^{2+} concentration and activation of the PI3K/Akt-dependent pathway. The LA-induced increase in free intracellular Ca^{2+} concentration was mainly due to Ca^{2+} influx through Cx26 HCs. In addition, the involvement of −SH groups was ruled out, because dithiothreitol (DTT) did not block the LA-induced dye uptake. LA also increased the membrane current mediated by Cx26 HCs expressed in *Xenopus* oocytes and the dye uptake in HeLa cells expressing Cxs 32, 43 or 45. Since LA is an essential polyunsaturated fatty acid, its effect on HCs might be relevant to cell growth as well as to cellular functions of differentiated cells such as audition.

1. Introduction

A hemichannel (HC) results from the oligomerization of six protein subunits called connexins (Cxs). Serial docking of two HCs, each one located at the cell membrane of two neighboring cells, forms an intercellular gap-junction channel (GJC). This GJC connects the cytoplasm of two adjacent cells, allowing the exchange of ions and small cytoplasmic molecules, including metabolites and second messengers [1]. Until about a decade ago, HCs were thought to remain closed and open only when they are part of GJCs. However, during the past decade growing evidence has shown that HCs open under physiological as well as pathological conditions, providing a mechanism for communication between the cytoplasm and the external microenvironment [2,3]. Through controlled release of signaling molecules, HC opening has been implicated in important physiological events such as cell cycle progression and hence in development and also in pathological processes where they promote and/or accelerate cell death [4].

Connexin26 (Cx26) is expressed in a variety of tissues, including skin and cochlear sensory epithelia, where it fulfills important functions [5,6]. The latter have been unveiled by deafness and skin diseases associated with several mutations in gene GJB2, which encodes Cx26 [7,8].

Polyunsaturated fatty acids (PUFAs), omega-6 and omega-3, and their metabolites influence physiological and pathological processes acting as pro-inflammatory or anti-inflammatory agents, respectively [9]. They are structural components of phospholipids in cell membranes, and can affect their fluidity and flexibility [10]. In addition, recent studies suggest that omega-3 PUFAs influence the expression and phosphorylation status of Cx43 [11].

The functional state of Cx26 HCs and GJCs is modulated by fatty acids. For example, oleamide derivatives of omega-9 unsaturated Oleic acid, inhibit Cx26 GJCs in mouse melanoma BL6 cells [12], while arachidonic acid (AA), an omega-6 PUFA, potentiates the cellular release of ATP promoted by a divalent cation-free solution in HeLa-Cx26 cells [13]. Recently, linoleic acid (LA), an essential unsaturated omega-6 fatty acid, was shown to induce a biphasic effect on the functional state of HCs formed by Cx46 in *Xenopus laevis* oocytes, used as an

Abbreviations: Cxs, connexins; GJCs, gap junction channels; HCs, hemichannels; LA, linoleic acid; Etd, ethidium; DTT, dithiothreitol

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11 Impact of Microglial Activation on Astroglial Connexin Expression and Function in Brain Inflammation

Christian Giaume, Nicolas Froger, Juan Andrés Orellana, Mauricio Retamal, and Juan Carlos Sáez

CONTENTS

11.1 INTRODUCTION

The central nervous system (CNS) is capable of dynamic immune and inflammatory responses mediated by the activation of microglia, the brain resident macrophage population, and astrocyte reactivity. A growing body of evidence shows that the innate immune response exerts a dichotomous role in the brain. Under physiological conditions, microglia exhibit a resting phenotype that is associated with the production of anti-inflammatory and neurotrophic factors. Microglia shift to an activated phenotype in response to a wide range of insults that activate specific signaling pathways, thereby promoting an inflammatory response necessary to further engage the immune system. The sustained inflammation resulting in brain injury and pathology

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Connexin and pannexin hemichannels in brain glial cells: properties, pharmacology, and roles

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Functional interaction between neurons and glia is an exciting field that has expanded tremendously during the past decade. Such partnership has multiple impacts on neuronal activity and survival. Indeed, numerous findings indicate that glial cells interact tightly with neurons in physiological as well as pathological situations. One typical feature of glial cells is their high expression level of gap junction protein subunits, named connexins (Cxs), thus the membrane channels they form may contribute to neuroglial interaction that impacts neuronal activity and survival. While the participation of gap junction channels in neuroglial interactions has been regularly reviewed in the past, the other channel function of Cxs, i.e., hemichannels located at the cell surface, has only recently received attention. Gap junction channels provide the basis for a unique direct cell-to-cell communication, whereas Cx hemichannels allow the exchange of ions and signaling molecules between the cytoplasm and the extracellular medium, thus supporting autocrine and paracrine communication through a process referred to as "gliotransmission," as well as uptake and release of metabolites. More recently, another family of proteins, termed pannexins (Panxs), has been identified. These proteins share similar membrane topology but no sequence homology with Cxs. They form multimeric membrane channels with pharmacology somewhat overlapping with that of Cx hemichannels. Such duality has led to several controversies in the literature concerning the identification of the molecular channel constituents (Cxs versus Panxs) in glia. In the present review, we update and discuss the knowledge of Cx hemichannels and Panx channels in glia, their properties and pharmacology, as well as the understanding of their contribution to neuroglial interactions in brain health and disease.

Keywords: astrocytes, oligodendrocytes, microglia, gap junctions, neuroglial interactions

INTRODUCTION

For a long time, it has been taken as dogma that connexin (Cx) proteins can only function as gap junction channels. Indeed, before the aggregation of Cxs at the junctional plaque and subsequent formation of gap junctions, hexameric rings of Cxs, termed connexons, were initially assumed to remain closed. An obvious reason for this occlusion was that, as gap junction channels are "poorly" selective for ions and permeable to low molecular weight molecules (<1 to 1.2 KDa), if once at the membrane connexons could open, the cell would lose its integrity or at least would have to spend substantial energy to maintain this energetically unfavorable condition. Such statements began to be challenged when evidence for "functional hemichannels," a term proposed to substitute for plasma membrane connexons, were reported in the early 1990s. In these pioneering studies, Cx hemichannels were opened either by large depolarization in *Xenopus* oocytes (Paul et al., 1991) or by lowering the extracellular calcium ion (Ca^{2+}) concentration in horizontal cells (DeVries and Schwartz, 1992). Later on, the occurrence of functional hemichannels (i.e., hemichannels that can be turned into the open state) composed of Cx43 was demonstrated in primary cultures of astrocytes in the absence of external Ca^{2+} (Hofer and Dermietzel, 1998). This observation was followed by the demonstration that metabolic inhibition performed in the presence of normal external Ca^{2+} concentrations (1–2 mM) induced cell permeabilization, due to Cx43 hemichannel opening, before loss of membrane integrity (Contreras et al., 2002). More recently, hemichannel opening in astrocytes was triggered either by treatment with pro-inflammatory cytokines or selective lipopolysaccharide (LPS) stimulation of microglia co-cultured with astrocytes, again in the presence of external Ca^{2+} (Retamal et al., 2007a). The opening of Cx43 hemichannels in astrocytes was also demonstrated to occur in experiments designed to decipher the mechanism of intercellular Ca^{2+} wave propagation in astrocytes to which both gap junction channels and hemichannels contribute (Scemes and Giaume, 2006; Leybaert and Sanderson, 2012). In this case, the release of ATP through Cx43 hemichannels

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Molecular mechanism of voltage sensing in voltage-gated proton channels

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Voltage-gated proton (Hv) channels play an essential role in phagocytic cells by generating a hyperpolarizing proton current that electrically compensates for the depolarizing current generated by the NADPH oxidase during the respiratory burst, thereby ensuring a sustained production of reactive oxygen species by the NADPH oxidase in phagocytes to neutralize engulfed bacteria. Despite the importance of the voltage-dependent Hv current, it is at present unclear which residues in Hv channels are responsible for the voltage activation. Here we show that individual neutralizations of three charged residues in the fourth transmembrane domain, S4, all reduce the voltage dependence of activation. In addition, we show that the middle S4 charged residue moves from a position accessible from the cytosolic solution to a position accessible from the extracellular solution, suggesting that this residue moves across most of the membrane electric field during voltage activation of Hv channels. Our results show for the first time that the charge movement of these three S4 charges accounts for almost all of the measured gating charge in Hv channels.

INTRODUCTION

Voltage-gated proton (Hv) channels are found in phagocytes, neurons, airway epithelia, muscle, and sperm (Decoursey, 2003; Okochi et al., 2009; Ramsey et al., 2009; Iovannisci et al., 2010; Lishko et al., 2010). In phagocytes, Hv channels play an essential role in bacterial clearing. Depolarization-activated H^+ currents through Hv channels in the phagosomal membrane compensate for NADPH oxidase currents, activated during a process called respiratory burst, ensuring sustained production of reactive oxygen species in phagocytes to neutralize engulfed bacteria. The importance of Hv channels is supported by the observation that inhibiting Hv channels prevents further production of reactive oxygen species in the phagosomes. In addition, Hv1 knockout mice are unable to efficiently clear bacterial infections, further showing the essential role Hv channels play in bacterial clearing (Ramsey et al., 2009). Because the voltage dependence of Hv channels determines their physiological role, it is important to determine the molecular structure that defines the voltage sensitivity of Hv channels. At present, it is unknown which charged residues underlie the voltage dependence of Hv channels. Here, using the limiting slope method and accessibility experiments, we have identified the charged residues responsible for voltage activation of Hv channels.

Each subunit in an Hv channel has four transmembrane segments, called S1–S4 (Ramsey et al., 2006; Sasaki et al., 2006). These four transmembrane segments of the Hv channels are homologous to the first four transmembrane segments in voltage-gated potassium (Kv) channels (Ramsey et al., 2006; Sasaki et al., 2006). In Kv channels, the first four transmembrane segments form the voltage-sensing domain, whereas the fifth and the sixth transmembrane segments from all four Kv subunits together form the potassium-conducting pore domain (Jiang et al., 2003; Long et al., 2005). In contrast, Hv channels do not have a fifth or sixth transmembrane segment. In addition, unlike the tetrameric structure of Kv channels, Hv channels are composed of two subunits (Koch et al., 2008; Lee et al., 2008; Tombola et al., 2008). Deletion of the cytosolic domains removes the dimerization of the Hv subunits (Koch et al., 2008; Tombola et al., 2008). Interestingly, the monomeric Hv channel is a functional voltage-gated proton channel. Thus, each subunit comprises a voltage-gated H^+ permeation pathway (Koch et al., 2008; Tombola et al., 2008).

In Kv channels, the fourth transmembrane segment (S4) contains many positively charged residues and has been shown to function as the main voltage sensor in Kv channels (Aggarwal and MacKinnon, 1996; Larsson et al., 1996; Mannuzzu et al., 1996; Seoh et al., 1996; Yang et al., 1996; Tombola et al., 2006). S4 in Hv channels

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Abbreviations used in this paper: FEP, free energy perturbation; MD, molecular dynamics; MTSEA, 2-aminoethyl MTS; MTSET, 2-(trimethylammonium)ethyl MTS; MTSPT, 3-(trimethylammonium)propyl MTS.

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Dynamin-2 Regulates Fusion Pore Expansion and Quantal Release through a Mechanism that Involves Actin Dynamics in Neuroendocrine Chromaffin Cells

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Abstract

Over the past years, dynamin has been implicated in tuning the amount and nature of transmitter released during exocytosis. However, the mechanism involved remains poorly understood. Here, using bovine adrenal chromaffin cells, we investigated whether this mechanism rely on dynamin's ability to remodel actin cytoskeleton. According to this idea, inhibition of dynamin GTPase activity suppressed the calcium-dependent *de novo* cortical actin and altered the cortical actin network. Similarly, expression of a small interfering RNA directed against dynamin-2, an isoform highly expressed in chromaffin cells, changed the cortical actin network pattern. Disruption of dynamin-2 function, as well as the pharmacological inhibition of actin polymerization with cytochalasine-D, slowed down fusion pore expansion and increased the quantal size of individual exocytotic events. The effects of cytochalasine-D and dynamin-2 disruption were not additive indicating that dynamin-2 and F-actin regulate the late steps of exocytosis by a common mechanism. Together our data support a model in which dynamin-2 directs actin polymerization at the exocytosis site where both, in concert, adjust the hormone quantal release to efficiently respond to physiological demands.

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Introduction

Dynamin is a mechano-GTPase encoded by three distinct genes (DNM1, DNM2 and DNM3) that generates membrane deformation and triggers membrane fission [1]. Its best characterized function is the scission of nascent vesicles from the plasma membrane during endocytosis. Of the three dynamin isoforms only dynamin-2 is ubiquitously expressed, while dynamin-1 is exclusively expressed in neuronal tissue, and dynamin-3 is only present in brain, testis, heart and lungs [2], [3]. Studies in knock-out animals show that only dynamin-2 is critical during early embryonic development [4] and that the absence of dynamin-1 or -3 can be compensated by the other isoforms [5]. From these findings arises the idea that the different dynamin isoforms have overlapping roles and their relative contribution to endocytosis in a given tissue is mostly determined by their abundance rather than on structural specialization [5].

Dynamin participates in several cellular processes that are dependent on the actin cytoskeleton dynamics, some of them are actin comet [6], [7] lamellipodia formation [8], T cell activation [9], phagocytosis [10] and different types of endocytosis [11 14]. Furthermore, a functional link between dynamin and actin has been observed during endocytosis, where one regulates the recruitment of the other [14] and both work synergistically to efficiently catalyze membrane scission [12]. The exact mechanism of the crosstalk between dynamin and actin is not completely clear, but some evidences suggest that dynamin binds directly to actin filaments to promote its polymerization by displacing the actin capping protein gelsolin [15]. Additionally, dynamin can control the stability of actin filaments in association with the actin-binding protein cortactin, in a GTP hydrolysis-dependent way [16], [17].

In neuroendocrine cells, both actin and dynamin have been involved in the regulation of the exocytotic process. On one hand, cortical actin network is reorganized during exocytosis [18], wherein it regulates the expansion of the fusion pore [19], an intermediate structure formed during the fusion of the secretory vesicle with the plasma membrane [20]. On the other hand, dynamin appears to regulate both fusion pore expansion [21] and closure [22], [23], and to control the quantal size of release events [24 27]. These actions have been attributed to the neuronal isoform dynamin-1 [21], [23], [25], while dynamin-2 has been proposed to specialize in the regulation of compensatory

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Dynamin-2 function and dysfunction along the secretory pathway

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Dynamin-2 is a ubiquitously expressed mechano-GTPase involved in different stages of the secretory pathway. Its most well-known function relates to the scission of nascent vesicles from the plasma membrane during endocytosis; however, it also participates in the formation of new vesicles from the Golgi network, vesicle trafficking, fusion processes and in the regulation of microtubule, and actin cytoskeleton dynamics. Over the last 8 years, more than 20 mutations in the dynamin-2 gene have been associated to two hereditary neuromuscular disorders: Charcot–Marie–Tooth neuropathy and centronuclear myopathy. Most of these mutations are grouped in the pleckstrin homology domain; however, there are no common mutations associated with both disorders, suggesting that they differently impact on dynamin-2 function in diverse tissues. In this review, we discuss the impact of these disease-related mutations on dynamin-2 function during vesicle trafficking and endocytotic processes.

Keywords: dynamin-2, endocytosis, exocytosis, actin, microtubules, mutations, Charcot–Marie–Tooth neuropathy, centronuclear myopathy

INTRODUCTION

Dynamin was identified for the first time almost 25 years ago as a 100 kDa microtubule-associated protein that induced microtubule bundles and promoted microtubule sliding *in vivo* (1). As described by the same authors, the motor activity of dynamin required ATP and other co-purified polypeptides (1). A year later, the same group cloned and sequenced dynamin, and found that it contained a consensus GTP-binding site (2), and subsequently characterized its GTPase activity (3). At present, three dynamin isoforms encoded by three distinct genes (*DNM1*, *DNM2*, and *DNM3*) have been described in mammals (4). These exhibit approximately 80% homology in their sequences, yet they differ in their tissue expression pattern; dynamin-1 is mainly expressed in neuronal tissue, dynamin-2 is ubiquitously expressed, and dynamin-3 is expressed in brain, testis, and lungs (5). Of these three dynamin isoforms, only dynamin-2 appears to play a pleiotropic role during embryonic development (6). In fact, studies in knock-out animals show that deletion of dynamin-1 or -3 can be compensated by the other dynamin isoforms (7), while the deletion of dynamin-2 causes early embryonic lethality (8). Moreover, as discussed below, mutations in *DMN2* result in severe hereditary neuropathies and myopathies in humans, strongly suggesting that dynamin-2 has more susceptible functions in the nervous and skeletal muscle tissues.

All dynamin isoforms exhibit at least four alternatively spliced variants, resulting in different dynamin proteins (5) that share a primary structure comprising: a large amino-terminal GTPase domain (G-domain) that binds and hydrolyzes GTP; a middle and a GTPase effector domains (GED) that form a "stalk" structurally essential region; a pleckstrin homology domain (PH) that binds inositol phospholipids and a carboxy-terminal proline and arginine rich-domain (PRD) that allows interaction with SH3-domain-containing-proteins (5) (**Figure 1**).

Dynamin function relies on its ability to form high order oligomers, and its self-assembly is necessary to promote its catalytic activity. Purified dynamin has been shown to spontaneously polymerize in the presence of negatively charged tubular templates such as lipid membranes (11), microtubules (3, 12), or actin bundles (13, 14) as well as after incubation in low ionic strength solutions (15). Over the last years several cryo-electron microscopy (16–18) and X-ray crystallographic studies (19, 20) of dynamin and its domains (21–24) have allowed a better understanding of the mechanisms mediating dynamin oligomerization. It appears that the stable dimers formed by the crossed interaction between the "stalk" regions of monomeric dynamins (20, 25) are the basic unit that allows dynamin polymerization (18), thus promoting the GTPase activation required for membrane remodeling and scission in different cellular processes.

In the present review, we discuss the different roles of dynamin during endocytosis, vesicle trafficking, and exocytosis, specially focusing in dynamin-2, and how disease-linked mutations in dynamin-2 gene alter such cellular processes.

DYNAMIN AS A KEY COMPONENT OF ENDOCYTOSIS AND VESICLE RECYCLING

Dynamin is a GTPase that plays a crucial role in the recycling of secretory vesicle in neuroendocrine cells (26).

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Research Article

Boldine Prevents Renal Alterations in Diabetic Rats

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Diabetic nephropathy alters both structure and function of the kidney. These alterations are associated with increased levels of reactive oxygen species, matrix proteins, and proinflammatory molecules. Inflammation decreases gap junctional communication and increases hemichannel activity leading to increased membrane permeability and altering tissue homeostasis. Since current treatments for diabetic nephropathy do not prevent renal damage, we postulated an alternative treatment with boldine, an alkaloid obtained from boldo with antioxidant, anti-inflammatory, and hypoglycemic effects. Streptozotocin-induced diabetic and control rats were treated or not treated with boldine (50 mg/Kg/day) for ten weeks. In addition, mesangial cells were cultured under control conditions or in high glucose concentration plus proinflammatory cytokines, with or without boldine (100 μmol/L). Boldine treatment in diabetic animals prevented the increase in glycemia, blood pressure, renal thiobarbituric acid reactive substances and the urinary protein/creatinine ratio. Boldine also reduced alterations in matrix proteins and markers of renal damage. In mesangial cells, boldine prevented the increase in oxidative stress, the decrease in gap junctional communication, and the increase in cell permeability due to connexin hemichannel activity induced by high glucose and proinflammatory cytokines but did not block gap junction channels. Thus boldine prevented both renal and cellular alterations and could be useful for preventing tissue damage in diabetic subjects.

1. Introduction

Diabetic nephropathy (DN) is considered a microvascular complication of diabetes. It is characterized by a chronic injury to renal tissue, mainly glomerular structures. DN is the major complication associated with type I diabetes mellitus, affecting approximately 30–40% of diabetic patients, and is the leading cause of end-stage renal disease [1].

There are several factors involved in the development of diabetic glomerulopathy but the main initiator of this disease is chronic hyperglycemia, which triggers nonenzymatic protein glycosylation and increased production of reactive oxygen species, increasing oxidative stress and favoring coagulation and fibrotic events [2]. Hyperglycemia also sensitizes the vessel wall of efferent arterioles to the action of vasoconstrictors, which, together with an increase in vascular permeability, lead to hyperfiltration. Also, in the progression of DN persistent proteinuria, glomerular hypertrophy, mesangial expansion, and tubulointerstitial fibrosis occur, which lead to partial or total loss of renal function [3, 4].

In diabetes and in different cell types cultured in high glucose concentration (from now and on named as high glucose), an increase in the inflammatory response is observed. This response is usually initiated by cytokine release and subsequent attraction of macrophages, important actors in the progression of DN [5, 6]. In this and other diseases where inflammation is present, the expression of connexins (Cxs), including Cx43, is altered and increased activity of Cx formed hemichannels is observed. The latter alterations have been linked to a reduction in gap junction activity (essential for intercellular communication) and have been considered a risk factor for certain diseases [7, 8].

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Editorial

We've had important advances in the connexin/pannexin field, yet there is still much to do

In recent years, we have seen major advances in the field of connexin (Cx) and pannexin (Panx) proteins. New methods have been developed or adapted to work in this area of research and a number of Cx mutations linked to human disease have been found. Moreover, work on Cx hemichannels (Cx HCs, prior to docking and the formation of gap junction (GJ) channels) has exploded, having implications for a variety of biological processes. Studies of Panx channels have also captivated the interest of investigators and expanded the area of research on cell–cell communication via membrane channels. In addition, several rumors indicate that Panxs also form GJ channels and studies may soon be reported clarifying this gossip. This special issue is composed of original and review articles that correspond to a small sample of progress in specific subareas of the field and undoubtedly underscore the overall advances made thus far.

It is worth emphasizing, however, especially for the students, postdoctoral fellows and scientists from other areas who have turned their attention to this field, that there is yet much to do to answer the fundamental questions of the day. Some of the most attractive questions are: What other diseases are linked to Cx/Panx defects? What are the mutations in Cxs and Panxs telling us about the functional roles of these proteins in living cells? What are we learning as the structural models for Cx/Panx channels are refined further? What may the development of pharmacological approaches to Cx/Panx-based channels reveal about: a) the physiological roles of these channels and b) design therapies for different human diseases? And the list goes on. For relevant progress on the last question, see in this issue: Dahl et al., 2013; Verselis and Srinivas, 2013; Iyyathurai et al., 2013; Liu et al., 2013; Riquelme et al., 2013a).

We would like to highlight another set of topics in the field. In this case, the questions might not be obvious, but we believe they are just as important. We suggest that in all fields of biological research, investigators are dealing with an array of complex questions, ongoing studies and dreams of identifying responsible regulatory mechanisms and functions. Obviously, certain aspects are better understood than others. Embroiled in their research, as they seek to build models, there is a tendency for investigators to simplify some aspects that would benefit from a more detailed analysis. Thus, the current understanding of particular issues is not based on thorough experimental demonstrations, but rather on assumptions that fit well with one's favorite idea. Over time, these simplifications have a way of sending down roots and pervading the thinking in the field – even reaching into academic classrooms, textbooks and presentations at scientific meetings. Along with many other areas in biology, the field of cell communication, GJs and HCs has been impacted in this way.

An excellent reminder comes from the history of work on HCs over the last ~15 years. This history illustrates how we can be misled when we rely on only theoretical analyses or an incomplete set of experimental data relevant to a fundamental question. For many years, the idea had been that any significant opening of HCs would lead to a major cell catastrophe. And, in fact, the opening of HCs for extended periods of time can be deleterious and in injured cells can accelerate the process of cell death (see in this issue: Orellana et al., 2013; Kozoriz et al., 2013). However, and this was completely unexpected, we have learned that HCs do open to the external milieu with high open probability in healthy cells (Franco et al., 2001; Pearson et al., 2005; Schalper et al., 2008; Huckstepp et al., 2010a; Orellana et al., 2011; Riquelme et al., 2013b), even *in vivo* (Huckstepp et al., 2010b; Li et al., 2011; Riquelme et al., 2013b), where they play essential roles in a variety of cellular processes. Of critical importance, the health of these cells does not appear to be compromised as previously hypothesized. Thus, future theoretical and experimental analysis will be required to explain how cells handle the predicted detrimental conditions caused by HC openings. An even more unexpected finding is that Cx43 HCs have been found to confer resistance to glioblastome multiform upon treatment with temozolomide used in chemotherapy (Gielen et al., 2013).

We take this opportunity to identify a few ideas in the GJ/HC/Cx/Panx field that are widely held, but not actually supported by a rigorous set of experimental data. Certainly, different investigators describe these ideas in different ways in the literature and at scientific meetings. However, our overall treatment of them and our growing understanding of these topics would benefit from being alert to both the limitations of the existing data and the assumptions incorporated into our models. In the spirit of constructive scientific exchange, we offer the following examples of topics that deserve cautious treatment and further scientific scrutiny.

Common Belief 1: A popular Idea is that a wide variety of small molecules of biological importance pass between cells via GJs and through the cell membrane via HCs.

Problem 1: We obviously do not know at this time what different molecules pass and which ones are excluded by each channel type.

Problem 2: We still do not know the intrinsic permeability properties of GJ channels and high resolution structural features need to be improved; therefore, we cannot predict what passes through and what is excluded by these channels.

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Cerebral ischemic injury is enhanced in a model of oculodentodigital dysplasia

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ABSTRACT

Oculodentodigital dysplasia (ODDD) is a rare autosomal dominant disease that results in visible developmental anomalies of the limbs, face, eyes and teeth. Recently analysis of human connexin43 (Cx43) DNA sequences has revealed a number of different missense, duplication and frame shift mutations resulting in this phenotype. A mouse model of this disorder has been created with a missense point mutation of the glycine amino acid at position 60 to serine (G60S). Heterozygote +/G60S mice exhibit a similar ODDD phenotype as observed in humans. In addition to the malformations listed above, ODDD patients often have neurological findings. In the brain, Cx43 is highly expressed in astrocytes and has been shown to play a role in neuroprotection. We were interested in determining the effect of the +/G60S mutation following stroke. Four days after middle cerebral artery occlusion the volume of infarct was larger in mice with the +/G60S mutation. In astrocyte-neuron co-cultures, exposure to glutamate also resulted in greater cellular death in the +/G60S mutants. Protein levels of Cx43 in the mutant mouse were found to be reduced when compared to the normal tissue. Cx43 protein was observed as a continual line of small punctate aggregates in the plasma membrane with increased intracellular localization, which is distinct from the larger plaques seen in the normal mouse astrocytes. Functionally, primary +/G60S astrocytes exhibited reduced gap junctional coupling and increased hemichannel activity, which may underlie the mechanism of increased damage during stroke.

This article is part of a Special Issue entitled 'Connexin based channels'.

1. Introduction

Oculodentodigital dysplasia (ODDD) was first described by Meyer-Schwickerath et al. (1957) and further defined by Gorlin et al. (1963). Common external features in this syndrome include ocular, nasal, dental and digital abnormalities (Paznekas et al., 2009). It is now known that this syndrome is caused by mutation of the gene encoding the gap junction protein connexin43 (Cx43). Currently, 62 different Cx43 mutations have been identified in ODDD patients, and in the majority of cases the mutation is dominant (Paznekas et al., 2009).

Cx43 is expressed in numerous tissues and plays an important role in cellular communication. Cx43 proteins oligomerize into hexamers to form connexons, which are inserted into the plasma membrane of single cells to form hemichannels, or coupled to the connexons in neighboring cells to form gap junction channels that provide cytoplasmic continuity between cells (Sáez et al., 2003). The effect of ODDD Cx43 mutations has been studied by several groups and in general these mutants show reduced gap junction formation and increased hemichannel activity (Dobrowolski et al., 2007; McLachlan et al., 2005). Also, mutated Cx43 often exerts a

Abbreviations: BSA, bovine serum albumin; Cx43, connexin43; DAPI, 4′,6-diamidino-2-phenylindole; DMEM, Dulbecco's modified Eagle's medium; EBSS, Earle's balanced salt solution; Etd, ethidium; PI, propidium iodide; La^{3+}, lanthanum ion; G60S, missense point mutation of the glycine amino acid at position 60 conversion to serine; GFAP, glial fibrillary acidic protein; HBSS, Hank's buffered salt solution; IBA-1, ionized calcium-binding adaptor molecule 1; LDH, lactate dehydrogenase; MCAO, middle cerebral artery occlusion; ODDD, oculodentodigital dysplasia; RIPA, radioimmune precipitation lysis buffer; TBS-T, Tris-buffered saline with tween.

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C

Ca^{2+} Activation of K^+ Channels: RCK Domains

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Introduction

Some K^+ channels can be activated by a rise in cytosolic Ca^{2+} and, in two cases (the K^+ channels from the archeon *Methanobacterium thermoautotrophicum* (MthK) and the large conductance voltage- and Ca^{2+}-activated K^+ (BK) channel), the Ca^{2+}-binding sites are contained within the *regulatory domains for K^+ conductance* (RCK). The MthK channel is a tetramer formed by subunits containing two transmembrane domains and a C-terminal domain containing a single RCK domain (Jiang et al. 2002). The functional MthK channel, however, contains eight RCK domains, and the green domains in Fig. 1a come from the cytoplasmic milieu.

The structure of the RCK domain of a six transmembrane domain K^+ channel from *E. coli* (solved at 2.4 Å resolution) has a Rossmann-fold topology, which is a very common structural motif of enzymes and ligand-binding proteins (see ▸ Structural Motifs). Rossmann-fold secondary structures are organized into two linked β-α-β-α-β units (see Fig. 1b) and were first identified in a number of NAD^+-dependent dehydrogenases. This is the type of structure present in the MthK and in the *Drosophila*, mouse, and human Slo1 (commonly known as BK; see ▸ Potassium Channels (Kv, Kir, KCa, and K(2P) Channels) channels (Fig. 1b). The cloning of the BK channel from *Drosophila* showed that it is a member of the S4 superfamily encompassing voltage-dependent K^+ (Kv), Na^+, and Ca^{2+} channels. The gene coding for BK was called *Slowpoke* or *Slo* (later renamed *Slo1* after the cloning and expression of *Slo2* and *Slo3* (Salkoff et al. 2006)). In the case of Kv channels, the channel-forming protein possesses six transmembrane domains (S1–S6) containing the pore-forming domain S5-P-S6, and an S4 voltage-sensing element (S1-S4). As Kv channels, the BK channel is a tetramer; however, unlike Kv channels, the BK channel protein consists of seven transmembrane (S0–S6) domains with an exoplasmic N-terminus (Fig. 1c). The intracellular C-terminal domain, comprising two thirds of the protein, contains four hydrophobic segments (S7–S10) and the Ca^{2+}- and Mg^{2+}-binding sites. The C-terminus of BK channels consists of two tandem RCK domains (only RCK1 is shown in Fig. 1b). The RCK domain in the BK channel was initially unveiled by MacKinnon's group (Jiang et al. 2001) by multiple sequence alignment of the BK channel with prokaryotic K^+ channels and other proteins known to possess the RCK domain motif. Based on their primary sequence, the C-terminal domains of K^+ channels *Slo2* and *Slo3* are believed to be structured as two RCK domains in tandem (Salkoff et al. 2006). Unlike *Slo1*, however, *Slo2* is activated by internal Na^+ and Cl^-, and *Slo3* is activated by protons. The actual structural nature of the C-termini of these two channels is unknown at present and will not be discussed further here.

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Keeping you healthy: BK channel activation by omega-3 fatty acids

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In the exuberant world of K^+ channels, the Ca^{2+}- and voltage-activated K^+ (BK, MaxiK, Slo1) channel stands alone. It is coded by a single gene (*slo1* or *KCNMA1*), and the pore-forming α subunit has seven transmembrane segments instead of six as found in voltage-dependent K^+ channels (Atkinson et al., 1991; Adelman et al., 1992; Butler et al., 1993; Wallner et al., 1996). Being activated by depolarizing voltages and cytoplasmic Ca^{2+}, the BK channel is the perfect molecular machine to retard or to simply stop excitatory signals. The negative feedback (hyperpolarization) created by the opening of these K^+ channels is caused by the perfect tuning between Ca^{2+} and voltage sensors. The communication between these two types of sensors is allosterically established, that is, voltage or internal Ca^{2+} alone is able to open the BK channel, but channel opening is increasingly facilitated as more Ca^{2+} and voltage sensors are activated (Horrigan and Aldrich, 2002; Horrigan, 2012) (Fig. 1 A). Another important difference between BK channels and Kv channels, where opening is tightly coupled to voltage-sensor activation (Soler-Llavina et al., 2003) (Fig. 1 B), is that, albeit with a very low probability, BK channels can open in a voltage sensor– and Ca^{2+}-independent manner (reaction C↔O defined by the equilibrium constant *L* in Fig. 1 A).

Despite being coded by a single gene, BK channel diversity is large. Alternative splicing, posttranslational modifications, and/or the presence of modulatory β or γ subunits create this diversity (Orio et al., 2002; Salkoff et al., 2006; Yan and Aldrich, 2010, 2012). In particular, modifications induced in BK gating kinetics by the β1, β2, and β4 subunits proved to be of crucial importance in many physiological processes, ranging from shaping neuronal excitability and neurosecretion to smooth muscle tone, and in others not so physiological, such as alcohol tolerance (Brenner et al., 2000; Hu et al., 2001; Gollasch et al., 2002; Grimm and Sansom, 2007; Martin et al., 2008). The expression of β subunits is highly tissue specific; β1 is the only β subunit expressed in smooth muscle, and β4 is mainly expressed in the nervous system (Orio et al., 2002; Wu and Marx, 2010). In vascular smooth muscle cells (SMCs), the presence of β1 plays a vital role in vasoregulation, and its lack leads to hypertension (Brenner et al., 2000; Fernández-Fernández et al., 2004; Nelson and Bonev, 2004). β1 and β2 have been observed to dramatically slow down activation and deactivation kinetics as well as increase the apparent Ca^{2+} sensitivity of the BK channel. Although β4 also decelerates BK activation and deactivation kinetics, even more so than β1, Ca^{2+} sensitivity of channels formed by α/β4 subunits is complex. Channels are less sensitive to Ca^{2+} at low internal Ca^{2+} concentrations (<10 μM) than channels formed by the α subunit alone. However, Ca^{2+} is more effective in activating α/β4 channels at higher Ca^{2+} concentrations (Ha et al., 2004; Wang et al., 2006).

In addition to their effects on channel gating, β subunits grant BK channels sensitivity to several physiologically important compounds, thus making these subunits targets for possible pharmacological interventions. For example, β1-containing BK channels but not channels formed by the α subunit alone appear to be the target of 17β-estradiol and other compounds such as estrogen analogues, anti-estrogens, and the bile salt component lithocholic acid (Valverde et al., 1999; Dick et al., 2001; Bukiya et al., 2009; Maher et al., 2013). The activation of BK channels by 17β-estradiol has been proposed as the possible mechanism that mediates the acute relaxation of vascular smooth muscle induced by the hormone (White et al., 1995; Ruehlmann et al., 1998). On the other hand, stress steroids activate channels formed by the α/β4 complex but not by α/β2 (King et al., 2006). Polyunsaturated fatty acids such as arachidonic acid (AA) are also able to directly activate BK channels, but in this case, AA enhances BK current in the presence of either β2 or β3 (Sun et al., 2007). Findings by Sun et al. (2007) also show that AA is able to remove inactivation, suggesting that this fatty acid is interacting with the β2-inactivating peptide. Tissue specificity of β subunits and their particular capacity to endow BK channels with different pharmacological profiles have greatly increased the importance of BK channels in maintaining the adequate cellular electrical homeostasis in different tissues.

Docosahexaenoic acid (DHA), an omega-3 fatty acid known to be associated with beneficial cardiovascular effects, was reported to be a potent activator of BK currents in rat coronary artery SMCs and to promote dilation of isolated small coronary arteries (Lai et al., 2009;

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Signal Transduction–Dependent Channels 4

Ramon Latorre, Carlos González, and Patricio Rojas

Brief History

From the moment the first member of this ion channel family, Slo1, was discovered, the scientific world was confronted with a molecular Pandora's box: once opened, its electrical language left scientists bewitched. They were fascinated with this "monster" of a single-channel conductance (250 pS in symmetrical 100 mM K^+) close to the ceiling imposed by simple diffusion combined with an exquisite K^+ selectivity. Slo1 channels are essentially impermeable to Na^+ and conduct K^+ 10- and 200-fold more effectively than Rb^+ and Cs^+, respectively, though it was previously thought that large conductance channels were not supposed to be so selective. At the same time, the channel was activated by voltage and cytoplasmic Ca^{2+}. This latter property led Meech in 1978 to hypothesize that this conductance system was perfect link between cell metabolism and electrical activity, and he was right on the mark. Because of its large conductance, this voltage- and calcium-activated K^+ channel was christened "maxi-K" or "BK" (for big K^+). This high single-channel

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a0010 # Voltage-Dependent K^+ Channels

Au1,2 **R Latorre, F J Morera, and C Zaelzer**

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Glossary

dt0010 **Delayed rectifier** A K^+ channel that changes the membrane conductance with a delay after a depolarizing voltage step.

dt0015 **Gating** The opening or closing of a channel in response to some stimulus.

dt0020 **Inward rectifier** A K^+ channel that opens when the membrane is hyperpolarized.

dt0025 **Long QT syndrome** An inherited cardiac arrhythmia.

dt0030 **Missense mutation** A mutation in which an incorrect amino acid is incorporated into the protein.

dt0035 **Voltage sensor** A channel structure able to detect changes in membrane potential. In voltage-dependent ion channels, the voltage-sensing elements are located in the S4 segment.

s0010 ## Structure and Diversity

p0010 Voltage-dependent K^+ channels are members of the voltage-dependent cation channel family, which includes the voltage-dependent Na^+, Ca^{2+}, and K^+ channels. The first voltage-dependent K^+ channel was cloned in 1987 from a fruit fly, *Drosophila*, based on a mutation that causes the *Shaker* phenotype. After that, they have been identified in an immense number of organisms including prokaryotes and eukaryotes.

p0015 The 78 members of the K^+ channel superfamily can be divided into four structural types based on their mode of activation and the number of their transmembrane segments: (1) voltage-gated six-transmembrane K^+ channels (K_v), (2) voltage- and Ca^{2+}-activated seven-transmembrane K^+ channels (K_{Ca}), (3) Inward rectifying two-transmembrane K^+ channels (K_{ir}), and (4) two-pore four-transmembrane K^+ channels (K_{2P}). Only the K_v and K_{Ca} families have channel-forming proteins containing intrinsic voltage sensors (see below).

p0020 All the voltage-dependent K^+ channels are tetrameric assemblies. In the K_v channels, each subunit consists of six hydrophobic segments, S1–S6. The primary sequence of these hydrophobic segments shows similarities between all of the K_v channels, including the voltage-sensor domain (VSD, S1–S4) and the ion-conduction pore domain (S5–S6) (**Figure 1(a)**). Au3 Au4

p0025 All K^+ channels have a consensus amino acid sequence inside the pore region, TVGYGD, dubbed the 'signature sequence'. The residues, TVGYG, line the selectivity filter (in some K^+ channels, the tyrosine (Y) residue is replaced by phenylalanine (F), e.g., in the ether-a-go-go (eag) channel discussed later on). In the selectivity filter, carbonyl oxygen atoms are directed toward the pore to coordinate dehydrated K^+ ions. Hydrophobic chains of valine and tyrosine directed toward the hydrophobic core surrounding the filter stabilize the main chain.

p0030 Gating in the K_v channels is conferred through the attachment of gating domains to the pore. The basic function of these gating domains is to perform mechanical work on the ion-conduction pore to change its conformation between closed and opened states. In voltage-dependent channels, a VSD (S1–S4) is present on each subunit. Thus, a voltage sensor converts energy stored in the membrane electric field into mechanical work. There is strong evidence that the positive charges contained in S4 are the voltage-sensing elements. Thus, ion-channel gating is essentially an electromechanical coupling between a gating unit and a pore unit.

p0035 The crystal structure of a mammalian voltage-dependent K^+ channel ($K_v1.2$) had initially been resolved at 2.9 Å and further improved to 2.4 Å using a chimeric $K_v1.2$–$K_v2.1$ channel. In the latter case, the channel was crystallized in complex with lipids. These structures showed that the helices of the ion-conduction pore (S5–S6) related to the helices of the VSD (S1–S4) in a special way. The VSD of one subunit is located near the pore domain of an adjacent subunit (**Figure 1(b)**). The connection between the pore and the VSD is made by the S4–S5 linker helix, which runs parallel to the intracellular membrane surface.

p0040 Voltage-dependent K^+ channels are ubiquitously distributed in different cells and tissues, therefore, K^+ channel diversity is of great importance in determining the variety of electrical responses produced by cells when subjected to stimuli. The possible mechanisms that originate the immense voltage-dependent K^+ channel diversity are: (1) multiple genes; (2) alternative splicing; (3) formation of heteromultimeric channels; and (4) coexpression with regulatory β-subunits.

p0045 Voltage-dependent K^+ channel diversity seems to have accompanied animal cells through evolution. Some K^+ channels are the most conserved proteins in eukaryotes. Potassium channels became fundamental to animal cell physiology very early in evolution, and through voltage-dependent K^+ channel control of membrane potential they couple the cell inner dynamics to the outer environment. A differential expression of voltage-dependent K^+ channel messenger RNAs (mRNAs) is found to accompany the events of animal development and also occurs in adult animal tissues.

p0050 The diversity of voltage-dependent K^+ channel genes is shown in the dendrogram shown in **Figure 2**. The dendrogram shows the major classes of voltage-dependent K^+ channels. The ramification of voltage-dependent K^+ channels was constructed by carrying out sequence alignments. To compare the amino acid sequences of the different voltage-dependent K^+ channels, the sequences were aligned using at least 300 amino acids from six hydrophobic segment (S1–S6) channels and

REM SLEEP PHASE PREFERENCE IN THE OCTODON DEGUS

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REM Sleep Phase Preference in the Crepuscular *Octodon degus* Assessed by Selective REM Sleep Deprivation

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Study Objectives: To determine rapid eye movement (REM) sleep phase preference in a crepuscular mammal (*Octodon degus*) by challenging the specific REM sleep homeostatic response during the diurnal and nocturnal anticrepuscular rest phases.

Design: We have investigated REM sleep rebound, recovery, and documented REM sleep propensity measures during and after diurnal and nocturnal selective REM sleep deprivations.

Subjects: Nine male wild-captured *O. degus* prepared for polysomnographic recordings

Interventions: Animals were recorded during four consecutive baseline and two separate diurnal or nocturnal deprivation days, under a 12:12 light-dark schedule. Three-h selective REM sleep deprivations were performed, starting at midday (zeitgeber time 6) or midnight (zeitgeber time 18).

Measurements and Results: Diurnal and nocturnal REM sleep deprivations provoked equivalent amounts of REM sleep debt, but a consistent REM sleep rebound was found only after nocturnal deprivation. The nocturnal rebound was characterized by a complete recovery of REM sleep associated with an augment in REM/total sleep time ratio and enhancement in REM sleep episode consolidation.

Conclusions: Our results support the notion that the circadian system actively promotes REM sleep. We propose that the sleep-wake cycle of *O. degus* is modulated by a chorus of circadian oscillators with a bimodal crepuscular modulation of arousal and a unimodal promotion of nocturnal REM sleep.

Keywords: crepuscular chronotype, *Octodon degus*, REM sleep, REM sleep homeostasis, REM sleep rebound

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INTRODUCTION

Crepuscular mammals exhibit a characteristic bimodal profile of activity, with activity bouts peaking just before dawn (morning peak) and dusk (evening peak). The crepuscular chronotype of rest-activity rhythm is widely distributed among mammals and has been reported in both free-ranging and captive animals.[1–3] Physiological and behavioral measures such as body temperature,[4] wheel-running,[5,6] cortisol secretion,[7] and electroencephalographic (EEG) data[8] have been also found to display bimodal temporal profiles among crepuscular species.

A crepuscular activity pattern has been described in *Octodon degus* (Rodentia: Hystricognatha) living in natural conditions and confinement.[1,9] Polysomnographic recordings have shown a bimodal crepuscular profile of wakefulness, with phase-opposed modes at zeitgeber time (ZT)11 and ZT23 under a 12:12 light-dark cycle.[10] Wake bouts delimit two sleep-predominant rest plateaus occurring outside the crepuscular periods (anticrepuscular rest phases, as defined by McElhinny et al.[4]). No significant diurnal-nocturnal difference has been found for the amount of rapid eye movement (REM) or non-REM (NREM) sleep,[10,11] casting doubts on the idea of a diurnal or nocturnal phase sleep state preference. The crepuscular bimodal sleep-wake pattern departs from the typical unimodal profile observed among nocturnal and diurnal mammals. In particular, REM sleep of diurnal and nocturnal mammals is under strong unimodal circadian modulation, with the acrophase coinciding with core body temperature nadir.[12,13] The opponent process model proposes that circadian modulation of the sleep-wake cycle is explained by an active promotion of arousal by the circadian system that opposes the homeostatic sleep pressure accumulated during wakefulness.[14] If this is true, diurnal and nocturnal anticrepuscular sleep-predominant rest intervals observed in *O. degus* occur permissively, mirroring the bimodal crepuscular promotion of arousal.

However, some evidence suggests that REM sleep may be actively promoted by the circadian system, as homeostatic REM sleep recovery after sleep deprivation is facilitated during the corresponding rest phase and impaired during the active phase.[15,16] To explore phase-dependent REM sleep modulation in the crepuscular *O. degus*, in this report we take advantage of a property of REM sleep: REM sleep quickly compensates for state debts after selective REM sleep deprivation during the rest phase, as has been observed in the rat.[15,17,18] If REM sleep during the anticrepuscular rest phases occurs permissively, without circadian REM sleep promotion, as predicted by the opponent process model, REM sleep deprivation during those intervals would be compensated for by an equivalent homeostatic response. Alternatively, asymmetric diurnal versus nocturnal REM sleep homeostatic responses must be interpreted as the manifestation of phase-specific REM sleep promotion, supporting the notion that sleep states are actively promoted by the circadian system.

METHODS

Animal handling and experimentation was performed according to local institutional animal care guidelines and autho-

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Invited review

Gap junction channels and hemichannels in the CNS: Regulation by signaling molecules

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ABSTRACT

Coordinated interaction among cells is critical to develop the extremely complex and dynamic tasks performed by the central nervous system (CNS). Cell synchronization is in part mediated by connexins and pannexins; two different protein families that form gap junction channels and hemichannels. Whereas gap junction channels connect the cytoplasm of contacting cells and coordinate electric and metabolic activities, hemichannels communicate intra- and extra-cellular compartments and serve as diffusional pathways for ions and small molecules. Cells in the CNS depend on paracrine/autocrine communication via several extracellular signaling molecules, such as, cytokines, growth factors, transmitters and free radical species to sense changes in microenvironment as well as to adapt to them. These signaling molecules modulate crucial processes of the CNS, including, cellular migration and differentiation, synaptic transmission and plasticity, glial activation, cell viability and microvascular blood flow. Gap junction channels and hemichannels are affected by different signaling transduction pathways triggered by these paracrine/autocrine signaling molecules. Most of the modulatory effects induced by these signaling molecules are specific to the cell type and the connexin and pannexin subtype expressed in different brain areas. In this review, we summarized and discussed most of the relevant and recently published information on the effects of signaling molecules on connexin or pannexin based channels and their possible relevance in CNS physiology and pathology.

This article is part of the Special Issue Section entitled 'Current Pharmacology of Gap Junction Channels and Hemichannels'.

1. Introduction

Coordinated interaction among cells is critical to perform the extremely complex and dynamic tasks performed by the brain. Cell ability to sense local and neighboring microenvironments has evolved in different ways in more complex organisms. In vertebrates, cell interaction and synchronization is in part mediated by intercellular communication via connexin- and pannexin-based channels. Connexins and pannexins comprise two different gap junction protein families, which in mammals are composed of about 20 and 3 members, respectively (Abascal and Zardoya, 2012). Eumetazoans, with the only exception of echinoderms, express pannexins (called innexins in non-chordates), whereas the connexin family is exclusive to chordates (Abascal and Zardoya, 2012; Phelan and Starich, 2001; Shestopalov and Panchin, 2008). Despite the fact that connexins and pannexins do not share a relevantly homologous primary structure, they have similar secondary and tertiary structures with four α-helical transmembrane domains, connected by one cytoplasmic and two extracellular loops, where both N- and C-termini are intracellular (Fig. 1). Pannexins and connexins oligomerize into hexamers to constitute single hemichannels, except for pannexin2 (Panx2), which seems to form octamers (Ambrosi et al., 2010).

After assembly, connexin hemichannels are transported to the non-junctional plasma membrane and diffuse laterally to dock with connexin hemichannels from a neighboring cell to form gap junction channels (Sáez et al., 2003a) (Fig. 1). Gap junctions are aggregates of these intercellular channels and mediate an important form of direct intercellular communication in the animal kingdom. Gap junction channels favor the intercellular exchange of metabolites (e.g., ADP, glucose, glutamate and glutathione), second messengers (e.g., cAMP and IP_3) and ions, allowing the intercellular spread of electrotonic potentials in excitable and non-excitable tissues (Evans et al., 2006; Sáez et al., 2003a; Sohl et al., 2005). For a long time, the main function attributed to connexin

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JLB

Article

Pannexin1 hemichannels are critical for HIV infection of human primary CD4$^+$ T lymphocytes

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ABSTRACT

HIV is a major public health issue, and infection of CD4$^+$ T lymphocytes is one of its key features. Whereas several cellular proteins have been identified that facilitate viral infection and replication, the role of hemichannels in these processes has not been fully characterized. We now show that the HIV isolates, R5 and X4, induced a transient-early (5–30 min) and a later, persistent (48–120 h) opening of Panx1 hemichannels, which was dependent on the binding of HIV to CD4 and CCR5/CXCR4 receptors. Blocking Panx1 hemichannels by reducing their opening or protein expression inhibited HIV replication in CD4$^+$ T lymphocytes. Thus, our findings demonstrate that Panx1 hemichannels play an essential role in HIV infection. *J. Leukoc. Biol.* 94: 399–407; 2013.

Introduction

HIV infects mostly immune cells by binding the viral env protein gp120 to the host cellular proteins, CD4 and CCR5 and/or CXCR4, resulting in fusion of the viral env with the cellular membrane. To date, in addition to CD4, CCR5, and/or CXCR4, no other plasma membrane proteins have been identified to participate directly in the process of viral entry [1]. However, a few studies indicated that upon binding of the virus to its cellular receptors, intracellular-free Ca^{2+} levels rise [2–4], and opening of nonselective cation channels, as well as Ca^{2+}-activated K^+ channels, occurs [5], suggesting that signaling and activation of other proteins may be required for infection and replication. Recently, studies in cell lines, PBMCs, and human macrophages indicated that ATP release through pannexin-1 (Panx1) hemichannels is required for HIV replication [6, 7], supporting the hypothesis that additional host proteins are required for infection/replication.

Hemichannels are plasma membrane channels that can be opened at the unapposed cell surface, forming aqueous conduits permeable to ions and small molecules (e.g., ATP, glutamate, NAD^+, and PGE_2). They allow diffusional exchange between the intra- and extracellular compartments, constituting a route for autocrine/paracrine cellular communication [8]. Hemichannels are constituted by the oligomerization of six protein subunits, termed Cxs or Panxs, both highly conserved protein families encoded by 21 or three genes in humans, respectively [9, 10]. Panx1 hemichannels, in concert with purinergic receptors, have been described to be important in different immune functions, including cellular activation [11–13], apoptosis [14], stress signals [15], secretion of inflammatory cytokines [16], and HIV replication [7]. However, how HIV infection changes the opening of these channels in primary human CD4$^+$ T lymphocytes—one of the main targets of HIV—remains to be elucidated. Here, we show that depending on the HIV isolate, the interaction of CD4 and CCR5 and/or CXCR4 is crucial for the biphasic Panx1 hemichannel opening induced by HIV in human primary CD4$^+$ T lymphocytes. We also found that chemokines that bind CCR5 and CXCR4 increase Panx1 hemichannel activity, but only early, and more transiently, as compared with the biphasic opening of Panx1 hemichannels in response to HIV infection. Down-regulation or pharmacological blockade of Panx1 hemichannels inhibited HIV replication in CD4$^+$ T lymphocytes,

Abbreviations: AU=arbitrary unit(s), C_T=comparative threshold, Cx=connexin, env=envelope, Etd=ethidium, F1=fluorescence intensity in each cell, FB=background fluorescence-intensity measurement, La^{3+}=lanthanum ion, NIAID=National Institute of Allergy and Infectious Diseases, NIMH=National Institute of Mental Health, Panx1=pannexin1, Prob=probenecid, qRT-PCR=quantitative RT-PCR, scr=scrambled, SDF-1α=stroma cell-derived factor-1α, siRNA=small interfering RNA

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Calcium-activated chloride channels do not contribute to the odorant transduction current in the marine teleost *Isacia conceptionis*

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This study compared the contribution of the Ca^{2+}-activated Cl^- conductance to the electro-olfactogram (EOG) evoked by different odorant classes between the marine Cabinza grunt *Isacia conceptionis* and rainbow trout *Oncorhynchus mykiss*. The Ca^{2+}-activated Cl^- channel blocker niflumic acid significantly diminished odorant responses in *O. mykiss*, but had no effect on the EOG in *I. conceptionis*, supporting the notion that Ca^{2+}-activated Cl^- channels may not operate as odorant transduction current amplifiers in this marine teleost.

Key words: fish; olfaction; olfactory receptor neuron; rainbow trout.

Twenty years ago, two independent publications in the journal *Nature* reported the contribution of a Ca^{2+}-activated Cl^- conductance to the odorant transduction cascade in vertebrate olfactory receptor neurons (Kurahashi & Yau, 1993; Lowe & Gold, 1993). This conductance, which had first been described in olfactory cilia from the northern grass frog *Rana pipiens* (Kleene & Gesteland, 1991), has now been established to contribute a significant portion to the odorant transduction current in all terrestrial and freshwater mammals and amphibians tested (Schild & Restrepo, 1998; Kleene, 2008). Recently, the molecular identity of the olfactory Ca^{2+}-activated Cl^- channel has been tentatively identified as anoctamin2 (Ano2) in mammals, also known as TMEM16B (Stephan *et al.*, 2009; Pifferi *et al.*, 2012). In fishes, the largest group of vertebrates, evidence remains limited to one patch-clamp study in dissociated olfactory receptor neurons from the rainbow trout *Oncorhynchus mykiss* (Walbaum 1792), that demonstrated the presence of a Ca^{2+}-activated Cl^- conductance in these cells and its participation in odorant transduction (Sato & Suzuki, 2000). To date, however, no study has addressed the possible role of Ca^{2+}-activated Cl^- channels in the olfactory transduction cascade of marine or saltwater-adapted euryhaline teleosts. Owing to the elevated chloride concentration in the marine environment, *c.* 500 mM, the chloride reversal potential is shifted to negative values compared to

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Protocol

Husbandry and Breeding in the *Octodon degu* (Molina 1782)

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The *Octodon degu* is a native rodent species from South America, which lives in colonies with a well-structured social organization grouping of 5–10 young and 2–5 adult animals sharing a burrow system. They show a temperature-dependent diurnal-crepuscular activity pattern. In nature they rarely survive 2 yr, mostly because of predation. However, in captivity, females reproduce for 4–4.5 yr, and both sexes live for 5–7 yr. Males remain fertile until death. Some care is required to maintain healthy degus, particularly breeding females. Here we describe husbandry and breeding guidelines from the experience of the University of Michigan degu colony. With the husbandry practices described here, 90% of pups born in our colony reach maturity (6 mo of age), and no diarrheal diseases are apparent in our adult population.

MATERIALS

It is essential that you consult the appropriate Material Safety Data Sheets and your institution's Environmental Health and Safety Office for proper handling of equipment and hazardous materials used in this protocol.

Reagents

Alfalfa

Animals

There is no commercial breeder selling degus. Laboratories and zoos that maintain colonies are usually able to provide some pairs to begin a breeding group. Degus can also be found in some pet stores in the United States as well as in Europe.

Reagent for acidification of water

Rodent chow (e.g., LabDiet 5001, PMI International)

Wheat-based PMI diet (ProLab RMH 2000 5P06)

Equipment

Bedding (preferably an inert material such as corn cob or paper chips)

Bathing dust (e.g., LM Chinchilla Dust Bath, purchased from pet stores) can also be used (see Step 1).

Breeding cages (opaque polypropylene; 20 in × 20 in × 8 in)

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SVCT2 vitamin C transporter expression in progenitor cells of the postnatal neurogenic niche

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Known as a critical antioxidant, recent studies suggest that vitamin C plays an important role in stem cell generation, proliferation and differentiation. Vitamin C also enhances neural differentiation during cerebral development, a function that has not been studied in brain precursor cells. We observed that the rat neurogenic niche is structurally organized at day 15 of postnatal development, and proliferation and neural differentiation increase at day 21. In the human brain, a similar subventricular niche was observed at 1-month of postnatal development. Using immunohistochemistry, sodium-vitamin C cotransporter 2 (SVCT2) expression was detected in the subventricular zone (SVZ) and rostral migratory stream (RMS). Low co-distribution of SVCT2 and βIII-tubulin in neuroblasts or type-A cells was detected, and minimal co-localization of SVCT2 and GFAP in type-B or precursor cells was observed. Similar results were obtained in the human neurogenic niche. However, BrdU-positive cells also expressed SVCT2, suggesting a role of vitamin C in neural progenitor proliferation. Primary neurospheres prepared from rat brain and the P19 teratocarcinoma cell line, which forms neurospheres *in vitro*, were used to analyze the effect of vitamin C in neural stem cells. Both cell types expressed functional SVCT2 *in vitro*, and ascorbic acid (AA) induced their neural differentiation, increased βIII-tubulin and SVCT2 expression, and amplified vitamin C uptake.

Keywords: SVCT2, vitamin C, brain, niche, stem cells, progenitor, ependymal cells

INTRODUCTION

Active neurogenesis occurs within the anterior wall of the lateral ventricle in the adult mammalian brain (Lois and Alvarez-Buylla, 1993; Doetsch et al., 1999). Neurogenic precursors have also been found in the human brain, specifically located in the periventricular region before 18 months of age (Johansson et al., 1999; Nunes et al., 2003; Sanai et al., 2011; Bergmann et al., 2012). The formation of new neurons, which are βIII-tubulin-positive, occurs in restricted, organized compartments termed neurogenic niches (Doetsch et al., 1997, 1999; Alvarez-Buylla and Garcia-Verdugo, 2002; Conover and Notti, 2008; Mirzadeh et al., 2008; Nualart et al., 2012). The neuroblasts formed in this region migrate tangentially in chains throughout the rostral migratory stream (RMS), where the presence of neurogenic progenitors and astrocytes has also been described (Doetsch and Alvarez-Buylla, 1996). The neuroblasts present in the RMS reach the olfactory bulb, where they differentiate into interneurons (Lois et al., 1996; Alvarez-Buylla and Garcia-Verdugo, 2002; Lledo et al., 2008). Ultrastructural, immunohistochemistry, and proliferation analyses of the cytoarchitecture of the neurogenic niche (Doetsch et al., 1997) have revealed the presence of four cell types. B-type cells or astrocytes (GFAP- and nestin-positive) are preferentially located in the subventricular zone (SVZ) and are precursor cells. C-type cells are intermediate transient neuronal cells (nIPC) that proliferate rapidly (Eisch and Mandyam, 2007; Ihrie and Alvarez-Buylla, 2008) and differentiate into neuroblasts or type-A cells (Doetsch et al., 1999; Tramontin et al., 2003; Chojnacki et al., 2009; Kriegstein and Alvarez-Buylla, 2009; Nualart et al., 2012). E-type cells, which are cube-shaped and multiciliated, are ependymocytes. B-type cells are found in the ependymal layer, projecting cilium to the ventricular lumen, similar to what has been described in the radial glia (Tramontin et al., 2003; Spassky et al., 2005); they also have a close relationship with blood vessels (Mirzadeh et al., 2008). Precursor cells reactive to GFAP have been identified in the SVZ of the human brain (Roy et al., 2000; Gibbons and Dragunow, 2010), and some are in direct contact with the cerebrospinal fluid (CSF) (Sanai et al., 2004, 2011; Quinones-Hinojosa et al., 2006).

Vitamin C, which is present in high concentrations in the CSF (Spector and Lorenzo, 1974; Kratzing et al., 1985), may be important in postnatal neural differentiation. An important role for vitamin C in embryonic cerebral development and in the differentiation of dopaminergic and serotoninergic neurons has been described (Lee et al., 2000; Yan et al., 2001). Embryonic precursors supplemented with vitamin C show an increase in neural and glial markers (Lee et al., 2003). Recently, Esteban et al. (2010) found that vitamin C favored the generation of induced pluripotent stem cells (iPS) (Esteban et al., 2010). Furthermore, cells grown *in vitro* in the presence of vitamin C expressed two histone demethylases, Jhdm1a and Jhdm1b (Wang et al., 2011), which are required for iPS cell production. Together, these results suggest that vitamin C is able to positively regulate stem cell generation and proliferation.

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Subunit Interactions during Cooperative Opening of Voltage-Gated Proton Channels

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SUMMARY

Voltage-gated proton (Hv1) channels are dimers, where each subunit has a separate permeation pathway. However, opening of the two pathways is highly cooperative. It is unclear how Hv1 channels open their permeation pathways, because Hv1 channels lack a classic pore domain. Using voltage-clamp fluorometry, we here detect two conformational changes reported by a fluorophore attached to the voltage sensor S4 in Hv1 channels. The first is voltage dependent and precedes channel opening, with properties consistent with reporting on independent S4 charge movements in the two subunits. The second is less voltage dependent and closely correlates with channel opening. Mutations that reduce dimerization or alter the intersubunit interface affect both the second conformational change and channel opening. These observations suggest that, following an initial S4 charge movement in the two subunits, there is a second, cooperative conformational change, involving interactions between subunits, that opens both pathways in Hv1 channels.

INTRODUCTION

Voltage-gated proton (Hv1) channels were first discovered in snail neurons (Thomas and Meech, 1982). Subsequently, Hv1 have been found in a variety of cell types and have been implicated in a wide variety of biological functions, such as the respiratory burst in phagocytic cells and the capacitation of the sperm (DeCoursey, 2010; El Chemaly et al., 2010; Iovannisci et al., 2010; Lishko et al., 2010; Morgan et al., 2009). In neurons, Hv1 channels are thought to play a role in the control of intracellular pH by extruding protons in response to excess intracellular acidification (Decoursey, 2003) and have been recently implicated in enhancement of brain damage in ischemic stroke (Wu et al., 2012). Hv1 channels belong to the superfamily of voltage-gated cation channels (Ramsey et al., 2006; Sasaki et al., 2006). However, in contrast to other members of this superfamily, such as Kv and Nav channels, Hv1 channel subunits have just four transmembrane segments (S1–S4; Figure 1A), which correspond to the S1-S4 voltage sensor domain in Kv channels (Ramsey et al., 2006; Sasaki et al., 2006). Hv1 channels lack the characteristic pore domain that is made up of transmembrane segments S5 and S6 in other voltage-gated cation channels (Jiang et al., 2003; Long et al., 2005). In other voltage-gated cation channels, the intracellular end of S6 constitutes the activation gate that opens and closes the pore (Liu et al., 1997). The lack of S6 in Hv1 channels makes it unclear what constitutes the gate in Hv1 channels. We here characterize conformational changes that are coupled to opening and closing (i.e., gating) of Hv1 channels, in order to elucidate how Hv1 channels are gated.

Hv1 channel subunits oligomerize as dimers (Koch et al., 2008; Lee et al., 2008; Tombola et al., 2008), not tetramers as other members of the superfamily of voltage-gated cation channels (Jiang et al., 2003; Long et al., 2005). This dimerization is most likely through the formation of a coiled-coil structure of the two C-termini from the two subunits in the dimer (Fujiwara et al., 2012; Koch et al., 2008; Lee et al., 2008; Tombola et al., 2008). Each subunit of the dimer has its own permeation pathway (Koch et al., 2008; Tombola et al., 2008), but there is a strong cooperativity between the two subunits during channel activation (Fujiwara et al., 2012; Gonzalez et al., 2010; Tombola et al., 2010). Using voltage-clamp fluorometry and cysteine accessibility to thiol reagents on Hv1 channels, we previously showed that the fourth transmembrane segment S4 with its three positively charged residues moves outward in response to depolarizations, as if S4 functions as the voltage sensor (Gonzalez et al., 2010). Cysteines introduced in the external end of S4 are exposed to the extracellular solution at depolarized membrane potentials, but not at hyperpolarized potentials. Conversely, cysteines introduced in the internal end of S4 are exposed to the intracellular solution at hyperpolarized membrane potentials, but not at depolarized potentials (Gonzalez et al., 2010). In addition, in response to depolarizations, a fluorophore attached close to S4 displays a fluorescence decrease that precedes the proton current (Gonzalez et al., 2010), suggesting that the fluorescence decrease reports on S4 charge movement preceding channel opening. The time course of the fluorescence decrease raised to a power of two overlaps well the time course of the proton currents for moderate depolarizations (Gonzalez et al., 2010). Using a Hodgkin-Huxley type analysis, we previously interpreted the time courses of the fluorescence and currents to denote that S4s in both subunits need to activate before either of the two proton permeation pathways in the two subunits is activated. This suggests that there is a high degree of cooperativity between the two subunits in the Hv1 dimer during channel opening. Using linked Hv1 subunits with different

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Penicillium purpurogenum produces two GH family 43 enzymes with β-xylosidase activity, one monofunctional and the other bifunctional: Biochemical and structural analyses explain the difference

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ABSTRACT

β-Xylosidases participate in xylan biodegradation, liberating xylose from the non-reducing end of xylooligosaccharides. The fungus *Penicillium purpurogenum* secretes two enzymes with β-D-xylosidase activity belonging to family 43 of the glycosyl hydrolases. One of these enzymes, arabinofuranosidase 3 (ABF3), is a bifunctional α-L-arabinofuranosidase/xylobiohydrolase active on p-nitrophenyl-α-L-arabinofuranoside (pNPAra) and p-nitrophenyl-β-D-xylopyranoside (pNPXyl) with a K_M of 0.65 and 12 mM, respectively. The other, β-D-xylosidase 1 (XYL1), is only active on pNPXyl with a K_M of 0.55 mM. The *xyl1* gene was expressed in *Pichia pastoris*, purified and characterized. The properties of both enzymes were compared in order to explain their difference in substrate specificity. Structural models for each protein were built using homology modeling tools. Molecular docking simulations were used to analyze the interactions defining the affinity of the proteins to both ligands. The structural analysis shows that active complexes (ABF3–pNPXyl, ABF3–pNPAra and XYL1–pNPXyl) possess specific interactions between substrates and catalytic residues, which are absent in the inactive complex (XYL1–pNPAra), while other interactions with non-catalytic residues are found in all complexes. pNPAra is a competitive inhibitor for XYL1 (K_i = 2.5 mM), confirming that pNPAra does bind to the active site but not to the catalytic residues.

Introduction

Lignocellulose is a major component of plants and it represents the main source of renewable organic matter. There is considerable interest in the exploitation of lignocellulosic materials as a source of feed, fuels and chemical feedstocks. Both the abundance and renewable nature of lignocellulosic materials have provided input for intensive research over recent years. It can be converted to value-added products through saccharification by lignocellulolytic enzymes [1]. Lignocellulose is composed of lignin, pectin, cellulose and hemicelluloses. Xylan is the main hemicellulose of annual plants and hardwoods, and it is composed of a linear chain of xylopyranose residues joined by β (1 → 4) glycosidic linkages. The main chain is substituted by a variety of compounds, such as arabinofuranose, methyl glucuronate and acetate, and the arabinoses may be linked to hydroxycinnamic acids [2].

The biodegradation of xylan is a complex process. The main chain is hydrolyzed by the action of endoxylanases (EC 3.2.1.8), which liberate xylooligosaccharides of different length and are eventually hydrolyzed to xylose by β-xylosidases (EC 3.2.1.37). These enzymes are produced by fungi and bacteria and are mainly extracellular [3]. β-Xylosidases are active against artificial substrates such as p-nitrophenyl glycosides; most of them are very specific for p-nitrophenyl-β-D-xylopyranoside (pNPXyl)[1] [4–6]. Others are able to cleave p-nitrophenyl-α-L-arabinofuranoside (pNPAra) [7–10], p-nitrophenyl-β-D-galactopyranoside [9] or p-nitrophenyl-α-D-glucopyranoside [5,11,12]. β-Xylosidases are grouped in families, according to their amino acid sequence similarities, in the Carbohydrate Active Enzymes database (CAZy; http://www.cazy.org); they are classified in eight families of glycoside hydrolases (GH): 3, 30, 39, 43, 52, 54, 116 and 120 [13–16]. Filamentous fungal β-xylosidases have been described only for families 3, 43 and 54 [5,12,17]. Members of GH families 3 and 54 operate with retention of the anomeric configuration, while family GH43 contains inverting glycoside hydrolases. These last enzymes possess a proton donor acting as general acid and a nucleophile as general base; in

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[1] *Abbreviations used:* ABF3, arabinofuranosidase 3; XYL1, β-xylosidase; pNPXyl, p-nitrophenyl-β-D-xylopyranoside; pNPAra, p-nitrophenyl-α-L-arabinofuranoside; GH, glycoside hydrolases.

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The ATP required for potentiation of skeletal muscle contraction is released via pannexin hemichannels☆

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Contractil force

ABSTRACT

During repetitive stimulation of skeletal muscle, extracellular ATP levels raise, activating purinergic receptors, increasing Ca^{2+} influx, and enhancing contractile force, a response called potentiation. We found that ATP appears to be released through pannexin1 hemichannels (Panx1 HCs). Immunocytochemical analyses and function were consistent with pannexin1 localization to T-tubules intercalated with dihydropyridine and ryanodine receptors in slow (*soleus*) and fast (*extensor digitorum longus, EDL*) muscles. Isolated myofibers took up ethidium (Etd^+) and released small molecules (as ATP) during electrical stimulation. Consistent with two glucose uptake pathways, induced uptake of 2-NBDG, a fluorescent glucose derivative, was decreased by inhibition of HCs or glucose transporter (GLUT4), and blocked by dual blockade. Adult skeletal muscles apparently do not express connexins, making it unlikely that connexin hemichannels contribute to the uptake and release of small molecules. ATP release, Etd^+ uptake, and potentiation induced by repetitive electrical stimulation were blocked by HC blockers and did not occur in muscles of pannexin1 knockout mice. MRS2179, a $P2Y_1R$ blocker, prevented potentiation in *EDL*, but not *soleus* muscles, suggesting that in fast muscles ATP activates $P2Y_1$ but not P2X receptors. Phosphorylation on Ser and Thr residues of pannexin1 was increased during potentiation, possibly mediating HC opening. Opening of Panx1 HCs during repetitive activation allows efflux of ATP, influx of glucose and possibly Ca^{2+} too, which are required for potentiation of contraction.

This article is part of the Special Issue Section entitled 'Current Pharmacology of Gap Junction Channels and Hemichannels'.

1. Introduction

A single twitch of a vertebrate skeletal muscle can occur in the absence of extracellular Ca^{2+}, and the required rise in intracellular free Ca^{2+}, $[Ca^{2+}]_i$, is released from intracellular stores (Araya et al., 2003). Repetitive twitches lead to potentiation of the contraction through accumulation of free Ca^{2+} in the cytoplasm (Sandona et al., 2005; Zhi et al., 2005). ATP signaling is involved in both, fast and slow muscle potentiation. In slow skeletal muscles, potentiation depends on activation of purinergic $P2X_4$ receptors present in the T-tubule membrane and entry of extracellular Ca^{2+} (Sandona et al., 2005). However, $P2X_4$ receptors are not found in fast skeletal muscle (Sandona et al., 2005), and potentiation in these muscles occurs without of extracellular Ca^{2+} (Louboutin

Abbreviations: Panx1 HCs, pannexin1 hemichannels; Etd^+, ethidium.

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Disruption in Connexin-Based Communication Is Associated with Intracellular Ca^{2+} Signal Alterations in Astrocytes from Niemann-Pick Type C Mice

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Abstract

Reduced astrocytic gap junctional communication and enhanced hemichannel activity were recently shown to increase astroglial and neuronal vulnerability to neuroinflammation. Moreover, increasing evidence suggests that neuroinflammation plays a pivotal role in the development of Niemann-Pick type C (NPC) disease, an autosomal lethal neurodegenerative disorder that is mainly caused by mutations in the *NPC1* gene. Therefore, we investigated whether the lack of NPC1 expression in murine astrocytes affects the functional state of gap junction channels and hemichannels. Cultured cortical astrocytes of NPC1 knock-out mice ($Npc1^{-/-}$) showed reduced intercellular communication via gap junctions and increased hemichannel activity. Similarly, astrocytes of newborn $Npc1^{-/-}$ hippocampal slices presented high hemichannel activity, which was completely abrogated by connexin 43 hemichannel blockers and was resistant to inhibitors of pannexin 1 hemichannels. $Npc1^{-/-}$ astrocytes also showed more intracellular Ca^{2+} signal oscillations mediated by functional connexin 43 hemichannels and $P2Y_1$ receptors. Therefore, $Npc1^{-/-}$ astrocytes present features of connexin based channels compatible with those of reactive astrocytes and hemichannels might be a novel therapeutic target to reduce neuroinflammation in NPC disease.

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Introduction

Niemann-Pick type C (NPC) disease is an autosomal recessive neurodegenerative disorder that is caused by mutations in the *NPC1* or *NPC2* genes [1]. Most cases of NPC disease are caused by mutations in the *NPC 1* gene that yields a dysfunctional protein [1,2]. NPC1 and NPC2 proteins are required for the trafficking of cholesterol; hence, a loss of function in these proteins results in the intracellular accumulation of free cholesterol and other lipids in late endosomes/lysosomes [3]. Progressive neurodegeneration, hepatosplenomegaly, and dysfunction of other organs are observed in patients affected with NPC disease [2]. These symptoms are also observed in a murine model of NPC disease [2,4].

$Npc1^{-/-}$ mice show hippocampal and cortical neuronal dysfunction [5 7], apoptosis of Purkinje neurons of the cerebellum and neuronal death in different brain regions [8 10]. Astrocytes express NPC1; and in the $Npc1^{-/-}$ mouse brain, $Npc1^{-/-}$ astrocytes exhibit morphological changes and become activated [11,12]. The global neuronal deletion of NPC1, but not astrocyte-specific NPC1 deficiency, leads to the complete development of NPC neuropathology [13], which suggests that neuronal NPC1 deficiency is sufficient to mediate neurodegeneration. However, rescuing NPC1 expression in astrocytes delays neuronal loss and prolongs the life span in $Npc1^{-/-}$ mice [14], suggesting that astrocytes may play an important role in the neuroinflammatory state of NPC disease. Neuroinflammation is present in $Npc1^{-/-}$ mouse brain at an early post-natal age and is characterized by an enhanced number of microglia, increased levels of interleukin-1β and the presence of activated astrocytes [15]. Because astrocytes form extensive communicating networks [16], it is conceivable that NPC-induced neurodegeneration could depend on intercellular signaling and coordination among astrocytes. Such intercellular communication between astrocytes is partially attained by sharing cytoplasmic content through gap junction channels (GJCs); these intercellular channels allow direct but selective cytoplasmic communication between contacting cells, thereby promoting the exchange of metabolites and second messengers [17]. Each GJC is formed by the serial docking of two hemichannels (HCs), each contributed by one of two adjacent cells. HCs are composed of six protein subunits termed connexins (Cxs) [18]. Under defined conditions HCs mediate the uptake or release of ions and small molecules such as Ca^{2+} and ATP, respectively [19]. *In vivo*,

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Research Article

ATP Is Required and Advances Cytokine-Induced Gap Junction Formation in Microglia In Vitro

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Microglia are the immune cells in the central nervous system. After injury microglia release bioactive molecules, including cytokines and ATP, which modify the functional state of hemichannels (HCs) and gap junction channels (GJCs), affecting the intercellular communication via extracellular and intracellular compartments, respectively. Here, we studied the role of extracellular ATP and several cytokines as modulators of the functional state of microglial HCs and GJCs using dye uptake and dye coupling techniques, respectively. In microglia and the microglia cell line EOC20, ATP advanced the TNF-α/IFN-γ-induced dye coupling, probably through the induction of IL-1β release. Moreover, TNF-α/IFN-γ, but not TNF-α plus ATP, increased dye uptake in EOC20 cells. Blockade of Cx43 and Panx1 HCs prevented dye coupling induced by TNF-α/IFN-γ, but not TNF-α plus ATP. In addition, IL-6 prevented the induction of dye coupling and HC activity induced by TNF-α/IFN-γ in EOC20 cells. Our data support the notion that extracellular ATP affects the cellular communication between microglia through autocrine and paracrine mechanisms, which might affect the timing of immune response under neuroinflammatory conditions.

1. Introduction

Microglia are the major immune effectors in the central nervous system (CNS). Under resting conditions, surveillance microglia have a ramified morphology and monitor their local microenvironment [1, 2]. However, microglia can rapidly become activated in response to diverse stimuli and danger signals, such as ATP or bacterial lipopolysaccharide (LPS) [1–3]. Consistently, microglia are activated in neuroinflammatory conditions and are a common hallmark in many neurodegenerative diseases [1, 2, 4].

Microglial cell activation includes morphological changes, proliferation, recruitment to the site of injury, and expression of specific proteins including MHC II molecules and cell adhesion molecules [1, 2]. Activated microglia also release cytokines, including TNF-α, IL-1β, IL-6, IFN-γ, and other soluble molecules, such as glutamate and ATP [5–9]. Many of these pro-inflammatory molecules act in an autocrine manner and show synergism, increasing the activation of microglia [10–12].

Many studies have focused on ATP release mechanisms and the subsequent receptors activation at the CNS, because they promote the release of other pro-inflammatory molecules, such as TNF-α and IL-1β [13]. These cytokines mediate cell communication and Ca^{2+} signaling among microglia, as well as among microglia and astrocytes [14–16]. Microglia sense extracellular ATP through P2Y and P2X receptors [1]. Under control conditions, microglia express

4 Gap Junctions in Antigen-Presenting Cells

Pablo J. Sáez, Kenji F. Shoji, and Juan Carlos Sáez

CONTENTS

4.1 INTRODUCTION

Dendritic cells (DCs) constitute a heterogeneous cell population that emerges in the bone marrow (BM) from a macrophage and DC precursor, generating the following: (1) a common DC precursor and (2) a monocyte precursor (Liu and Nussenzweig 2010). In addition, DCs can also emerge from monocytes under inflammatory conditions (Shortman and Naik 2007). Two major categories of DCs have been established as follows: (1) conventional (cDCs) and (2) inflammatory DCs. DC differentiation is reached by the former during resting steady-state conditions, while the latter do so during inflammatory conditions (Merad and Manz 2009). In addition, cDCs can be classified into several subtypes that share the ability to pick up, process, and present antigens to T cells. DC subtypes can be recognized through the expression of different cell surface markers, pattern cytokine secretions, migration pathways, locations, and functions (Shortman and Naik 2007). Although plasmacytoid DCs (pDCs) are

In Silico Analysis of Putative Paralytic Shellfish Poisoning Toxins Export Proteins in Cyanobacteria

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Abstract

Paralytic shellfish poisoning toxins (PSTs) are a family of more than 30 natural alkaloids synthesized by dinoflagellates and cyanobacteria whose toxicity in animals is mediated by voltage-gated Na^+ channel blocking. The export of PST analogues may be through SxtF and SxtM, two putative MATE (multidrug and toxic compound extrusion) family transporters encoded in PSTs biosynthetic gene cluster (*sxt*). *sxtM* is present in every *sxt* cluster analyzed; however, *sxtF* is only present in the *Cylindrospermopsis-Raphidiopsis* clade. These transporters are energetically coupled with an electrochemical gradient of proton (H^+) or sodium (Na^+) ions across membranes. Because the functional role of PSTs remains unknown and methods for genetic manipulation in PST-producing organisms have not yet been developed, protein structure analyses will allow us to understand their function. By analyzing the *sxt* cluster of eight PST-producing cyanobacteria, we found no correlation between the presence of *sxtF* or *sxtM* and a specific PSTs profile. Phylogenetic analyses of SxtF/M showed a high conservation of SxtF in the *Cylindrospermopsis-Raphidiopsis* clade, suggesting conserved substrate affinity. Two domains involved in Na^+ and drug recognition from NorM proteins (MATE family) of *Vibrio parahaemolyticus* and *V. cholerae* are present in SxtF/M. The Na^+ recognition domain was conserved in both SxtF/M, indicating that Na^+ can maintain the role as a cation anti-transporter. Consensus motifs for toxin binding differed between SxtF and SxtM implying differential substrate binding. Through protein modeling and docking analysis, we found that there is no marked affinity between the recognition domain and a specific PST analogue. This agrees with our previous results of PST export in *R. brookii* D9, where we observed that the response to Na^+ incubation was similar to different analogues. These results reassert the hypothesis regarding the involvement of Na^+ in toxin export, as well as the motifs L^{398}XGLQD^{403} (SxtM) and L^{390}VGLRD^{395} (SxtF) in toxin recognition.

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Introduction

Cyanobacteria are a biochemically and morphologically diverse clade of photosynthetic bacteria, with major environmental and economic roles. They are main primary producers in marine and freshwater ecosystems, key biocatalysts in the N_2 cycle [1] and some are capable of producing toxins. Toxin production can present waterborne health hazards for humans and animals. This has become of particular interest lately, due to the proliferation and dominance of harmful blooms of cyanobacteria; a result of the increase of eutrophication and climate change among others [2]. In addition, toxic strains of *Anabaena*, *Anabaenopsis*, *Microcystis* and *Nodularia* are salt tolerant, and increasing halotolerant HABs have been observed more frequently [2].

Major cyanobacterial toxins include microcystins, cylindrospermopsins, nodularin, anatoxins and saxitoxins (STX) [3]. STX and its analogues (more than 30) have been detected in filter-feeding bivalve mollusks. In humans, these toxins cause paralytic shellfish poisoning (PSP). PSP-toxins (PSTs) produce symptoms that vary from a slight tingling sensation or numbness around the lips to a fatal respiratory paralysis. The long-established molecular target of PSTs is the voltage-gated sodium channel in nerve and muscle cells, to which PSTs bind with analogue-dependent affinity, blocking Na^+ channels in nanomolar concentrations [4]. STX has also been shown to target voltage-gated potassium [5] and calcium [6] ion channels.

STX is a trialkyltetrahydropurine molecule (Figure 1), with two pKa's of 8.22 and 11.28 in aqueous solution, which belong to the 7,8,9 and 1,2,3 guanidinium groups, respectively. At physiological pH, the 1,2,3-guanidine carry a positive charge, whereas the 7,8,9-guanidine group is partially deprotonated. STX and its analogs can be structurally classified into several classes such as non-sulfated (STX, neoSTX), mono-sulfated (gonyautoxins-GTX1 6), di-sulfated (C1 4), and decarbamoylated, each with varying levels of toxicity. Of the decarbamoyl variants of these analogs, there are decarbamoyl-saxitoxins (dcSTX, dcneoSTX) and decarbamoyl-gonyautoxins (dcGTXs 1 4).

Emerging Role of Calcium-Activated Potassium Channel in the Regulation of Cell Viability Following Potassium Ions Challenge in HEK293 Cells and Pharmacological Modulation

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Abstract

Emerging evidences suggest that Ca^{2+}activated-K^+-(BK) channel is involved in the regulation of cell viability. The changes of the cell viability observed under hyperkalemia (15 mEq/L) or hypokalemia (0.55 mEq/L) conditions were investigated in HEK293 cells expressing the hslo subunit (hslo-HEK293) in the presence or absence of BK channel modulators. The BK channel openers(10^{-11}-10^{-3}M) were: acetazolamide(ACTZ), Dichlorphenamide(DCP), methazolamide(MTZ), bendroflumethiazide(BFT), ethoxzolamide(ETX), hydrochlorthiazide(HCT), quercetin(QUERC), resveratrol(RESV) and NS1619; and the BK channel blockers($2x10^{-7}$M-$5x10^{-3}$M) were: tetraethylammonium(TEA), iberiotoxin(IbTx) and charybdotoxin(ChTX). Experiments on cell viability and channel currents were performed using cell counting kit-8 and patch-clamp techniques, respectively. Hslo whole-cell current was potentiated by BK channel openers with different potency and efficacy in hslo-HEK293. The efficacy ranking of the openers at -60 mV(Vm) was BFT> ACTZ >DCP ≥RESV≥ ETX> NS1619> MTZ≥ QUERC; HCT was not effective. Cell viability after 24 h of incubation under hyperkalemia was enhanced by 82±6% and 33±7% in hslo-HEK293 cells and HEK293 cells, respectively. IbTx, ChTX and TEA enhanced cell viability in hslo-HEK293. BK openers prevented the enhancement of the cell viability induced by hyperkalemia or IbTx in hslo-HEK293 showing an efficacy which was comparable with that observed as BK openers. BK channel modulators failed to affect cell currents and viability under hyperkalemia conditions in the absence of hslo subunit. In contrast, under hypokalemia cell viability was reduced by -22±4% and -23±6% in hslo-HEK293 and HEK293 cells, respectively; the BK channel modulators failed to affect this parameter in these cells. In conclusion, BK channel regulates cell viability under hyperkalemia but not hypokalemia conditions. BFT and ACTZ were the most potent drugs either in activating the BK current and in preventing the cell proliferation induced by hyperkalemia. These findings may have relevance in disorders associated with abnormal K^+ ion homeostasis including periodic paralysis and myotonia.

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Introduction

Potassium ions regulate inflammation, oxidative stress, vascular biology and blood pressure, the excitability of the cells, exerting beneficial effects on different tissues [1–3]. Abnormalities in the serum potassium ion levels are associated with acquired and congenital diseases affecting several apparatus including skeletal muscle [4].

Severe hyperkalemia characterizes the hyperkalemic renal tubular Acidosis (type IV), mineralocorticoid deficiency (hypoaldosteronism states) as well as tumor lysis syndrome, rhabdomyolysis, marked leucocytosis and thrombocytosis, trauma and burns [5]. Disease progression and increased hearth mortality are observed in chronic kidney disease under hypokalemia or hyperkalemia conditions and these effects are gender and race dependent [6]. Severe nephropathy with renal

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Review Article

Role of Gap Junctions and Hemichannels in Parasitic Infections

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In vertebrates, connexins (Cxs) and pannexins (Panxs) are proteins that form gap junction channels and/or hemichannels located at cell-cell interfaces and cell surface, respectively. Similar channel types are formed by innexins in invertebrate cells. These channels serve as pathways for cellular communication that coordinate diverse physiologic processes. However, it is known that many acquired and inherited diseases deregulate Cx and/or Panx channels, condition that frequently worsens the pathological state of vertebrates. Recent evidences suggest that Cx and/or Panx hemichannels play a relevant role in bacterial and viral infections. Nonetheless, little is known about the role of Cx- and Panx-based channels in parasitic infections of vertebrates. In this review, available data on changes in Cx and gap junction channel changes induced by parasitic infections are summarized. Additionally, we describe recent findings that suggest possible roles of hemichannels in parasitic infections. Finally, the possibility of new therapeutic designs based on hemichannel blokers is presented.

1. Introduction

Members of gap junction (GJ) familiy proteins form intercellular communication channels, which connect the cytoplasm of neighboring cells and hemichannels, which connect the intra- and extracellular milieu [1]. Both intercellular channels and hemichannels participate in physiologic and pathologic processes including electrical conduction [2], inflammation [3], immune system activation [4], tissue repair/remodeling [5], and response to bacterial [6, 7] and viral infections [8]. However, little is known about the role of GJ channels in parasite infection and studies on the possible role of hemichannels are not available. Herein, we summarize the available data on the role of GJ channels in parasitic diseases and we also present new data suggesting that hemichannels might serve as key paracrine communication pathway during parasitic infections.

2. Gap Junction Channels and Hemichannels

Connexins (Cxs) and pannexins (Panxs) are members of two different GJ protein families in vertebrates [1]. Both protein subtypes can form channels that serve as pathways of cellular communication [1, 9]. Cxs and Panxs show similar membrane topology but only modest sequence homology [1]. In rodents and humans Cxs are encoded by 20 and 21 genes, respectively [10], whereas Panxs include only three members [11]. Moreover, innexins (Inxs) are members of a GJ family expressed only in invertebrates (Figure 1) [12]. They show similar membrane topology with Cxs and Panxs and can also form intercellular channels and hemichannels [13]. Inxs were originally identified in *Drosophila melanogaster* and *Caenorhabditis elegans*; however, Inx genes have been cloned recently from several other invertebrates (reviewed in [13]).

Does Nocturnality Drive Binocular Vision? Octodontine Rodents as a Case Study

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Abstract

Binocular vision is a visual property that allows fine discrimination of in-depth distance (stereopsis), as well as enhanced light and contrast sensitivity. In mammals enhanced binocular vision is structurally associated with a large degree of frontal binocular overlap, the presence of a corresponding retinal specialization containing a fovea or an area centralis, and well-developed ipsilateral retinal projections to the lateral thalamus (GLd). We compared these visual traits in two visually active species of the genus Octodon that exhibit contrasting visual habits: the diurnal *Octodon degus*, and the nocturnal *Octodon lunatus*. The *O. lunatus* visual field has a prominent 100° frontal binocular overlap, much larger than the 50° of overlap found in *O. degus*. Cells in the retinal ganglion cell layer were 40% fewer in *O. lunatus* (180,000) than in *O. degus* (300,000). *O. lunatus* has a poorly developed visual streak, but a well developed area centralis, located centrally near the optic disk (peak density of 4,352 cells/mm^2). *O. degus* has a highly developed visual streak, and an area centralis located more temporally (peak density of 6,384 cells/mm^2). The volumes of the contralateral GLd and superior colliculus (SC) are 15% larger in *O. degus* compared to *O. lunatus*. However, the ipsilateral projections to GLd and SC are 500% larger in *O. lunatus* than in *O. degus*. Other retinorecipient structures related to ocular movements and circadian activity showed no statistical differences between species. Our findings strongly suggest that nocturnal visual behavior leads to an enhancement of the structures associated with binocular vision, at least in the case of these rodents. Expansion of the binocular visual field in nocturnal species may have a beneficial effect in light and contrast sensitivity, but not necessarily in stereopsis. We discuss whether these conclusions can be extended to other mammalian and non-mammalian amniotes.

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Introduction

Among vertebrates, the size, shape and position of the eyes and their orbits exhibit a high degree of variability, ranging from the small, unilaterally placed eyes of the flatfishes (*Achirus lineatus*), to the large, highly convergent frontal eyes of humans and other apes [1,2]. Peculiar (from a human perspective) placement of the eyes is not exceptional among fishes, perhaps reflecting the high diversity of morphological features present in these taxa. For instance, the eccentric lateral placement of the eyes of the hammerhead sharks (Sphyrnidae) endows them with a 360° span of the visual field in the horizontal and vertical dimensions, while retaining at the same time a significant amount of binocular overlap [3].

Among amniotes it is a well-established fact that convergent, more frontally oriented eyes, grant higher degrees of binocular overlap, while lateralized placement of eyes result in narrow binocular visual fields [1,4,5]. The degree of ocular convergence, in turn, is associated with differences in other functional aspects of the visual system, such as the presence and position of retinal specializations and the relative emphasis of the different retinal projections. In mammals, the position of the area centralis (the retinal specialization that subserves the binocular area of the visual field) varies from temporal in species with lateralized eyes to pericentral in species with frontal eyes [6–8]. Furthermore, in birds and mammals, the relative size of the visual thalamofugal projection is larger in species with frontally-oriented eyes [4,9,10]. In mammals the retinal ipsilateral projections to the thalamic nucleus geniculatus lateralis pars dorsalis (GLd), the visual pathway that mediates functional binocular vision, is positively correlated with the degree of binocular overlap [11–14].

Various observations show that among amniotes feeding behavior influences eye orientation. Aerial and terrestrial predators (e.g. Felidae, Strigiformes and some Caprimulgiformes) have a larger degree of binocular overlap than their prey (ranging from large herbivores to small ground feeding birds) [4,15–17]. In addition, comparative studies indicate that irrespective of their feeding habits, nocturnal animals also exhibit a high degree of binocularity. Different taxa of nocturnal ammniotes such as the grey-headed flying fox (Megachiroptera), the Galago (Galagidae), and even the nocturnal parrot Kakapo (Strigopidae), can be cited as representative examples of this tendency [18–20].

THE JOURNAL OF PHYSICAL CHEMISTRY B

Article

pubs.acs.org/JPCB

Computationally Efficient Methodology for Atomic-Level Characterization of Dendrimer–Drug Complexes: A Comparison of Amine- and Acetyl-Terminated PAMAM

Ariela Vergara-Jaque,[†] Jeffrey Comer,[‡,§] Luis Monsalve,[†] Fernando D. González-Nilo,[§,∥,‡] and Claudia Sandoval*[,§]

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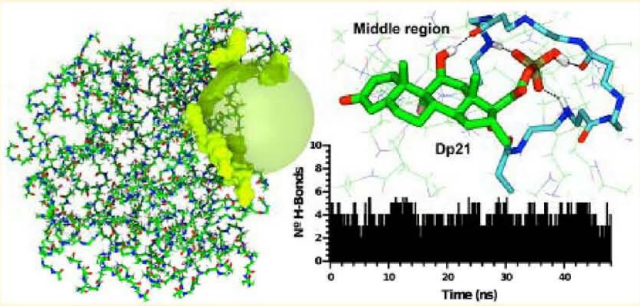
Supporting Information

ABSTRACT: PAMAM dendrimers have been widely studied as a novel means for controlled drug delivery; however, computational study of dendrimer–drug complexation is made difficult by the conformational flexibility of dendrimers and the nonspecific nature of the dendrimer–drug interactions. Conventional protocols for studying drug binding have been designed primarily for protein substrates, and, therefore, there is a need to establish new protocols to deal with the unique aspects of dendrimers. In this work, we generate cavities in generation-5 polyamidoamine (PAMAM) dendrimers at selected distances from the center of mass of the dendrimer for the insertion of the model drug: dexamethasone 21-phosphate or Dp21. The complexes are then allowed to equilibrate with distance between centers of mass of the drug and dendrimers confined to selected ranges; the free energy of complexation is estimated by the MM-GBSA (MM, molecular mechanics; GB, generalized Born; SA, surface area) method. For both amine- and modified acetyl-terminated PAMAM at both low and neutral pH, the most favorable free energy of complexation is associated with Dp21 at distance of 15–20 Å from the center of mass of the dendrimer and that smaller or larger distances yield considerably weaker affinity. In agreement with experimental results, we find acetyl-terminated PAMAM at neutral pH to form the least stable complex with Dp21. The greatest affinity is seen in the case of acetyl-terminated PAMAM at low pH, which appears to be due a complex balance of different contributions, which cannot be attributed to electrostatics, van der Waals interactions, hydrogen bonds, or charge–charge interactions alone.



INTRODUCTION

A recent trend in the pharmaceutical industry has been the focus on improving the properties of drugs that are currently used in various treatments, rather than focus on the creation of new drugs. Side effects in most drugs are considerable, in some cases more severe than the malady the drug is chosen to treat. To provide a solution to this problem, diverse nanocarriers have been proposed, such as micelles,[1] polymersomes,[2−4] carbon nanotubes,[5,6] nanospheres,[7,8] and many others. These nanocarriers can reduce the side effects while improving targeting and controlled release all without changing the chemical structure of the drug.[9]

Dendritic nanoparticles are actually one of the most studied for use as nanocarriers,[10,11] because these can enhance the transport and release of drugs due to their particular multifunctional structure. Dendrimers are synthetic hyper-branched structures with a globular architecture. They may have hydrophobic or hydrophilic internal cavities, and their terminal groups can be modified with different molecular moieties in order to decrease cytotoxicity, improve bioavailability, and optimize transport of particular drugs.[12−14] Dendrimers may encapsulate a drug through intermolecular interactions or, alternatively, form covalent bonds with a drug, and in this way enhance the uptake, cellular trafficking,[15] and controlled release of the drugs. Polyamidoamine (PAMAM)[10,11] is one of the most studied classes of dendrimers,[16,17] and has widely been investigated for delivery of drugs targeting a diverse set of diseases.[18] Studies have

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Paclitaxel-PHBV nanoparticles and their toxicity to endometrial and primary ovarian cancer cells

Cristian Vilos [a,c], Francisco A. Morales [a], Paula A. Solar [a], Natalia S. Herrera [a], Fernando D. Gonzalez-Nilo [b], Daniel A. Aguayo [b], Hegaly L. Mendoza [b], Jeffrey Comer [b,h], Maria L. Bravo [d], Pamela A. Gonzalez [d], Sumie Kato [e], Mauricio A. Cuello [e], Catalina Alonso [f], Erasmo J. Bravo [f], Eva I. Bustamante [g], Gareth I. Owen [d], Luis A. Velasquez [a,c,*]

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ABSTRACT

This report is an integrated study to include the molecular simulation, physicochemical characterization and biological analysis of a paclitaxel-loaded PHBV nanoparticle that demonstrates uptake, release and cytotoxicity in cancer cell lines. Taking this nanoparticle one step closer to its use in a clinical setting, we demonstrate that it causes significant cell death in primary cultures of stage IIIc serous ovarian cancer cells isolated from six patients. Molecular simulations revealed a high affinity of paclitaxel for the water–polymer interface, thus the drug is delivered only when the polymer near it is degraded. The Fourier transform infrared spectroscopy suggests the formation of a short-lived crystalline phase, also observed in the CG simulations, and transmission electron microscopy revealed branched structures on the surface of particles, which disappeared after 4 days. Biological analyses indicated that these particles have a 48-h window of toxicity protection, allowing for the endocytosis of the particle by the cells; this finding was corroborated by confocal microscopy and flow cytometry. The low cost to synthesize PHBV using microorganisms and the potential chemical modifications of the polymer make it attractive for inexpensive, large-scale pharmaceutical production.

1. Introduction

The worldwide cancer burden continues to grow and is becoming a major economic expenditure for all developed countries. Although the 5-year survival for ovarian cancer patients has improved to nearly 30%, this disease remains the fifth deadliest cancer among American women and is the leading cause of death from gynecologic malignancy. In 2010, 21,880 new cases and 13,850 new deaths were recorded in the United States [1,2].

The major obstacles in the field of oncology include the tremendous effort that is required for early detection, the development of many new drugs and drug delivery systems and the existence of effective chemotherapeutic agents [3,4]. One additional challenge is the development of nanoparticles that meet the clinical demands of therapeutic agents, such as biocompatibility, biodegradability, a clinically relevant circulating half-life, a low rate of intravascular aggregation, and a long-term storage capacity [5]. During the last decade, drug delivery systems based on polymeric nanoparticles have been central to many significant advances in nanomedicine [6]. Accordingly, these particles have generated interest related to their potential use in preclinical and clinical cancer drug development [5].

Polymeric nanoparticles have numerous uses in drug delivery, including the specific and targeted delivery of therapeutic agents,

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Annex 4
Organization of Scientific Events

CINV

II MEETING MILLENNIUM INSTITUTE CINV

"The science of magic, when attention meets illusion"

Organized by the PhD students of the Millennium Institute
"Centro Interdisciplinario de Neurociencia de Valparaíso"

August 1, 2013
Valparaíso, Chile

Sistemas Complejos

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ICM

CENTRO INTERDISCIPLINARIO DE
Neurociencia de
Valparaíso

September 23rd - 30th
CINV - Universidad de Valparaíso

More information:
www.cinv.cl

For funding and scholarships contact:
Alejandra Pinto (alejandra.pinto@cinv.cl)

Deadline: August 1st, 2013



Introduction,
types of ion channels, convergence; essential techniques for studying ion channels and Transporters

Function
Electrophysiology - Voltage clamp techniques.

Structure
Biochemistry - Membrane Proteins Biochemistry
Molecular Biology of ion channels

Fluorescence - Voltage clamp fluorometry, LRET, patch clamp fluorometry, real time fluorescence spectroscopy.

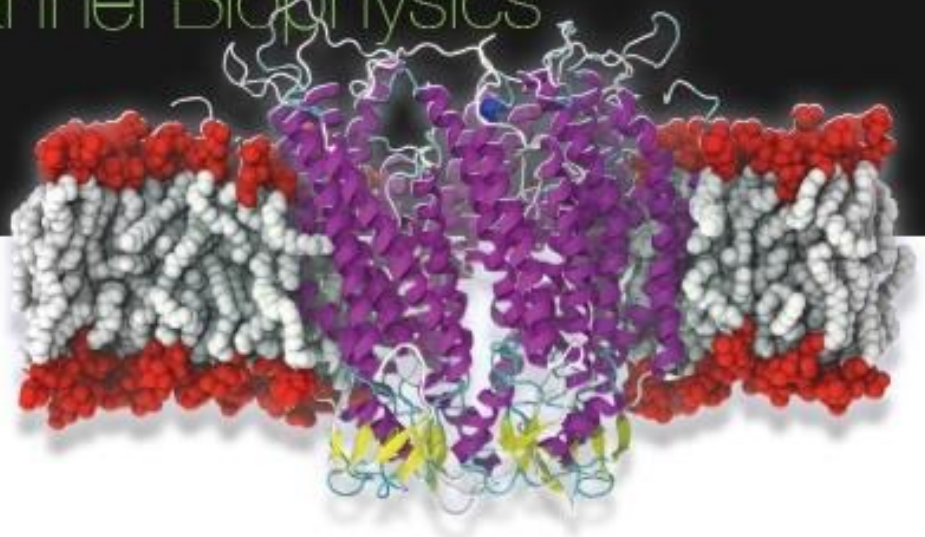
Electronic paramagnetic resonance
Crystalography

Channels in native systems
Smooth Muscle
Nanotechnology - Remote control of ion channels

Channels and diseases
Potassium channels
Hemichannels, connexins and pannexins

Course / Workshop - Ion Channels: Structure, function and disease

Latin american school on Ion Channel Biophysics



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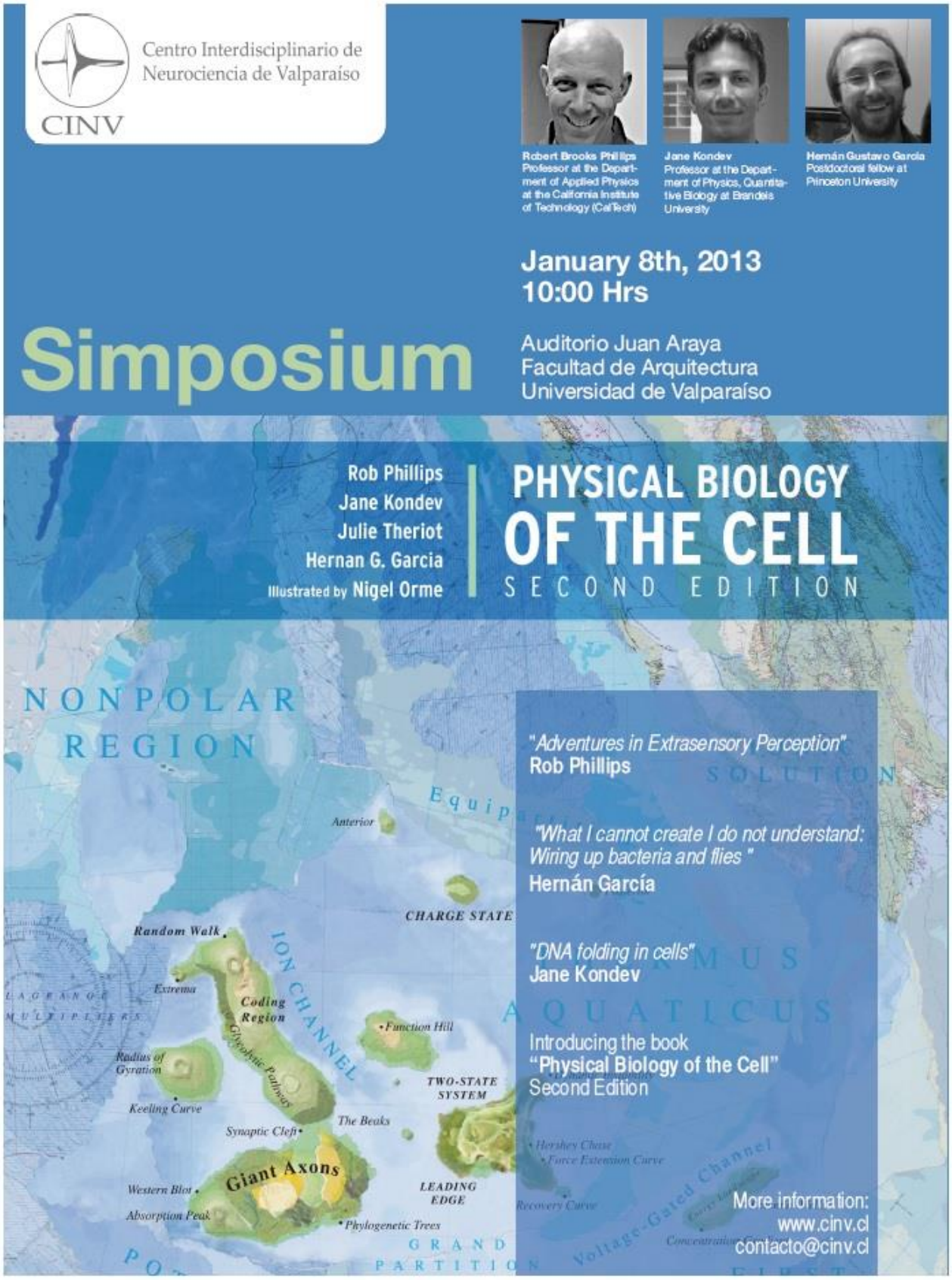
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CINV
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Professor at the Department of Applied Physics at the California Institute of Technology (CalTech)
Jane Kondev
Professor at the Department of Physics, Quantitative Biology at Brandeis University
Hernán Gustavo Garcia
Postdoctoral fellow at Princeton University
January 8th, 2013
10:00 Hrs
Simposium
Auditorio Juan Araya
Facultad de Arquitectura
Universidad de Valparaíso
Rob Phillips
Jane Kondev
Julie Theriot
Hernan G. Garcia
Illustrated by Nigel Orme
PHYSICAL BIOLOGY
OF THE CELL
SECOND EDITION
NONPOLAR
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"Adventures in Extrasensory Perception"
Rob Phillips
"What I cannot create I do not understand: Wiring up bacteria and flies"
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"DNA folding in cells"
Jane Kondev
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Western Blot
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Phylogenetic Trees
LEADING EDGE
CHARGE STATE
ION CHANNEL
Anterior
Hershey Chase
Force Extension Curve
Recovery Curve
Voltage-Gated Channel
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VIII Congreso Iberoamericano de Biofísica

IX Reunión Anual Sociedad Chilena de Neurociencia

1º - 4 de Octubre, 2013
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CHILE

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New insights on calcium signaling - *A. Escobar, E. Ríos*
Diversity of chemical senses - *J. Bacigalupo, J. Alcayaga*
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Synaptic plasticity - *P. Muñoz, C. Hidalgo*
Protein structure - *E. Perozo*
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Lightning the structure and function of ion channels - *T. Giráldez, C. González*

Más información en: www.**socneurociencia**.cl | Inscripciones hasta el **31 de agosto 2013**

Respaldado por

CENTRO INTERDISCIPLINARIO DE Neurociencia de Valparaíso

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Annex 7.3
Articles and Interviews

Title: "In Valparaiso was launched TV seriesabout neuroscience and life mysteries"
Media: www.envalparaiso.cl Informative web page
Date: June 21st, 2013

envalparaiso gastronomía hospedaje artesanía salas y museos

Lanzan serie científica acerca la neurociencia y los misterios de la vida

Lanzamiento de serie científica acerca la neurociencia y los misterios de la vida

viernes 21 de junio de 2013

Abordar de forma interactiva temas de interés universal como la belleza, la ilusión, las emociones, entre otros, desde las perspectivas de la neurociencia y de la innovación es el objetivo del Centro Interdisciplinario de Neurociencia (CINV) de la Universidad de Valparaíso en conjunto con Novasur, Televisión Educativo del Consejo Nacional de Televisión (CNTV) con el lanzamiento de la serie "Neuromantes. Exploradores de la vida" que se realizará el próximo jueves 27 de junio.

El proyecto televisivo, el cual fue financiado gracias al Primer Concurso de Divulgación y Valoración de la Ciencia y la Tecnología del Programa EXPLORA CONICYT, se complementa con la visión de personalidades relevantes de otros ámbitos de la cultura y de las artes de la región (ver información anexa en dossier).

Valparaíso es otro de los protagonistas, ya que en cada capítulo se hace referencia a la ciudad patrimonial, la cual sirve como locación o telón de fondo para cada capítulo.

Además, la serie, a través de Novasur, tiene como fin proporcionar material audiovisual y complementario en apoyo al currículum escolar en materias como neurociencias, innovación, arte, literatura e historia, además de transmitir y proyectar a nivel nacional e internacional el conocimiento científico generado por el CINV.

La actividad contará con la presencia de autoridades de las diferentes instituciones involucradas, así como también del gobierno regional y en especial, la del alcalde de la Ilustre Municipalidad de Valparaíso, Jorge Castro, quien firmará un convenio de emisión de la serie en las diferentes oficinas municipales con atención de público y que cuentan con televisor.

El evento se llevará a cabo en la Biblioteca Santiago Severín de Valparaíso a las 19 horas y es abierta a todo público.

Title: "Scientific TV Series set in Valparaíso tries to resolve life mysteries"

Media: www.elmartutino.cl Online Newspaper
Date: June 24th, 2013

Serie científica hecha en Valparaíso intenta resolver los misterios de la vida

El proyecto televisivo es realizado por el Centro Interdisciplinario de Neurociencia (CINV) de la Universidad de Valparaíso en conjunto con Novasur, Televisión Educativa del Consejo Nacional de Televisión (CNTV)

Por El Martutino
523 Lecturas

24 de Junio, 2013 00:06
Comentar

Abordar de forma interactiva temas de interés universal como la belleza, la ilusión, las emociones, entre otros, desde las perspectivas de la neurociencia y de la innovación es el objetivo del Centro Interdisciplinario de Neurociencia (CINV) de la Universidad de Valparaíso en conjunto con Novasur, Televisión Educativa del Consejo Nacional de Televisión (CNTV) con el lanzamiento de la serie "Neuromantes. Exploradores de la vida" que se realizará el próximo jueves 27 de junio.

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Title: "CINV and Novasur launched Neuromantes"
Media: www.elmartutino.cl Online Newspaper
Date: June 24th, 2013

CINV y Novasur lanzan Neuromantes

Como programa de divulgación científica, *Neuromantes* tiene como objetivo dar a conocer a la región como un polo de la generación de conocimiento de nivel mundial.

Por Novasur Chile
369 Lecturas

26 de Junio, 2013 09:06
Comentar

El año 2012 Novasur la televisión educativa del Consejo Nacional de Televisión, CNTV, convocó en cada región del país a actores significativos de la comunidad para co-producir audiovisuales educativos, que fueran representativos de los temas e intereses propios de las región y de las comunidades locales.

En la quinta región, gracias a una alianza con el Centro Interdisciplinario de Neurociencias de Valparaíso, CINV de la Universidad de Valparaíso, surgió *Neuromantes. Exploradores de la Vida*, serie de divulgación científica que trata temas universales como la *Belleza*, *Memoria*, *Percepción* y otros desde la mirada de la neurociencia, la innovación y la cultura. El nombre del programa viene de las palabras *neuro*:sistema nervioso o mente y *mantes*: magia.

Este próximo 27 de junio se realizará el lanzamiento de la serie, que consta de seis capítulos y que fue financiada por Explora-CONICYT -a través del Primer Concurso de Productos de Apropiación y Divulgación de la Ciencia y la Tecnología- y por el CNTV. La producción estuvo a cargo de Cábala Producciones, reconocidos realizadores con amplia experiencia, creadores de programas como *Cuaderno de Ciencias* y *Cambio Global*.

Title: "Launch: Neuromantes, Exploradores de la vida "
Media: www.sientevalpo.cl Informative web page
Date: June 26th, 2013

SIENTE VALPO

TAGS MUSICA TEATRO

LANZAMIENTO: NEUROMANTES, EXPLORADORES DE LA VIDA

26 junio, 2013 11:17 pm Publicado por Vivi Gutiérrez B. | Deje sus pensamientos

Participa este **jueves 27 de junio** en el lanzamiento de la serie "**Neuromantes. Exploradores de la vida**", que se realizará en la **biblioteca Santiago Severín de Valparaíso** a las **19 horas.**

Esta nueva serie científica toca de forma interactiva temas de interés universal como la belleza, la ilusión, las emociones, entre otros. El proyecto está bajo la perspectiva del **Centro Interdisciplinario de Neurociencia** de la **U. de Valparaíso** junto con **Novasur**, Televisión Educativo del Consejo Nacional de Televisión.

Entrada liberada

¡Están todos invitados a explorar nuestra ciudad patrimonial de una manera distinta y nueva!

En estudio:

Title: "Launch of scientific series that tries resolving life mysteries"
Media: www.ucv.cl
Date: June 27th, 2013

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Lanzamiento de serie científica intenta resolver los misterios de la vida

27 de junio de 2013

Abordar de forma interactiva temas de interés universal como la belleza, la ilusión, las emociones, entre otros, desde las perspectivas de la neurociencia y de la innovación es el objetivo del Centro Interdisciplinario de Neurociencia (CINV) de la Universidad de Valparaíso en conjunto con Novasur, Televisión Educativa del Consejo Nacional de Televisión (CNTV) con el lanzamiento de la serie "Neuromantes. Exploradores de la vida" que se realizará el próximo jueves 27 de junio.

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Además, la serie, a través de Novasur, tiene como fin proporcionar material audiovisual y complementario en apoyo al currículum escolar en materias como neurociencias, innovación, arte, literatura e historia, además de transmitir y proyectar a nivel nacional e internacional el conocimiento científico generado por el CINV.

La actividad contará con la presencia de autoridades de las diferentes instituciones involucradas, así como también del gobierno regional y en especial, la del alcalde de la Ilustre Municipalidad de Valparaíso, Jorge Castro, quien firmará un convenio de emisión de la serie en las diferentes oficinas municipales con atención de público y que cuentan con televisor.

El evento se llevará a cabo en la Biblioteca Santiago Severín de Valparaíso a las 19 horas y es abierta a todo público.

Title: "Universidad de Valparaíso and Novasur will broadcast scientific serie "Neuromantes: Exploradores de la vida "
Media: www.elepicentro.cl, online newspaper
Date: June 28th, 2013

Universidad de Valparaíso y Novasur emitirá serie científica "Neuromantes: exploradores de la vida"

"Neuromantes: exploradores de la vida" es el nombre de la nueva serie científica que a partir de julio se emitirá a través de las redes asociadas a Novasur, canal educativo del Consejo Nacional de Televisión. La serie la protagonizan científicos del Centro Interdisciplinario de Neurociencia de la Universidad de Valparaíso (CINV), junto a personajes del mundo del arte y la cultura.

"El objetivo es mostrar la ciencia como parte de nuestra cultura", explicó el doctor Ramón Latorre, director del CINV, en el lanzamiento de la serie, realizado en la tarde del jueves en la Biblioteca Severín.

"Esto es un empujón que nos da, por un lado, el Instituto Milenium, nuestro Centro Interdisciplinario de Neurociencia de Valparaíso (CINV), y por otro la Universidad de Valparaíso, para llevar la ciencia a la calle, la ciencia al ciudadano, la ciencia a los niños. Nuestro deber como miembros de esta sociedad, porque recibimos un financiamiento del Estado, es comunicar a la gente lo que hacemos, y tratamos de transmitirlo de una manera cariñosa, de una manera que llegue naturalmente y que no confunda la ciencia con la tecnología, sino que la ciencia se perciba más bien como hermana del arte, de las humanidades y de la cosa social como un todo, porque la ciencia es parte de la cultura", afirmó Latorre.

Title: "Squid is used to study the epilepsy and muscular paralysis"
Media: www.soyvalparaiso.cl Online Newspaper
Date: October 07th, 2013

Usan jibias para estudiar el impulso eléctrico de las neuronas

07.10.2013

Centro de investigación de Montemar desarrolla investigación sobre este calmar desde hace 40 años.

Seguir a @soyvalparaiso

La jibia no sólo es un molusco que busca imponerse en la cocina de numerosos países. Es además, el objeto de estudio de científicos nacionales que desde Montemar en el camino costero Viña-Concón, investigan las propiedades eléctricas de las neuronas y la importancia de este impulso para múltiples funciones del organismo, entre ellas, el movimiento muscular.

Uno de los científicos que se encuentra estudiando al calamar es el chileno Miguel Holmgren, quien está radicado en Estados Unidos. Según comentó, junto con estar "lleno de electricidad", la jibia posee los axones más grandes de la naturaleza -de 1 mm aproximadamente-, los que pueden ser percibidos por el ojo humano.

"Una vez generada la señal eléctrica, las neuronas tienen la propiedad de poder conducirla a través de estas prolongaciones llamadas axones las que, a modo de graficar, serían los cables del circuito eléctrico", comenta Holmgren, quien además es presidente de la Sociedad de Biofísicos Latinoamericanos (Sobla).

Title: "Open green building in house of Playa Ancha"
Media: La Estrella de Valparaíso, Newspaper
Date: October 08th, 2013

Actualidad

LA ESTRELLA | MARTES 8 DE OCTUBRE DE 2013 5

DURANTE LA JORNADA DE AYER, EL LOCAL FUNCIONÓ SÓLO LA RESPECTIVA MAMPARA.

Auto loco provocó caos en un McDonald's y dejó heridos a dos brasileños

El hecho ocurrió en Placilla y fue provocado por un choque en cadena.

Cinthia Matus O.

EL MOMENTO EN QUE EL VEHÍCULO TRASPASÓ EL VENTANAL.

MUCHO FRÍO

Durante la mañana de ayer, afuera del local de comida rápida aún quedaban restos de vidrios como evidencia del insólito accidente. De la mampara ya no quedaba absolutamente nada, por lo que algunos trabajadores comentaban que sentían frío, ya que a esa hora las temperaturas eran bajas. También señalaron que quedaron asustados, porque les parecía "de película" lo que sucedió.

Emergencia agrícola en 21 comunas

AGRICULTOR AFECTADO

Las inusuales heladas que cayeron en las últimas semanas y que han complicado la producción de frutas, hortalizas y hasta flores, motivaron que el ministerio de Agricultura decretara emergencia agrícola para 21 comunas de la V Región. Éstas son: Calle Larga, Los Andes, Rinconada, San Esteban, Limache, Olmué, Cabildo, La Ligua, Petorca, Zapallar, Hijuelas, La Cruz, Nogales, Quillota, Cartagena, San Antonio, Catemu, Putaendo, San Felipe, Santa María y Casablanca.

La mujer que cultiva el "alimento sagrado"

EL AMARANTO CRECE EN TARQUA.

Cristina Pizarro no es una agricultora convencional. Después de haber vivido en Santiago, Brasil y Estados Unidos, sintió un día la necesidad de volver a su tierra, Cabildo, e iniciar un viaje espiritual, donde se encontró con lo que hoy es su fuente de trabajo: el amaranto, alimento sagrado de los incas, que hoy compite con la quinoa en cuanto a poderes medicinales y, de paso, es el emblema de los ecologistas, pues la planta devora hectáreas de cultivos transgénicos en Estados Unidos.

Una noche, instalada en el valle de Paihuén, en la V Región interior, Cristina tuvo un sueño revelador: un hombre le habló del "alimento del futuro". Se trataba de la kiwicha inca, alimento que posee varias propiedades medicinales que actúan sobre el cuerpo y, dicen, también el espíritu. Su primera siembra de amaranto dio como resultado 250 kilos de semillas. Hoy, Cristina tiene un invernadero y su emprendimiento, levantado con apoyo de Indap, crece.

"Siento que el amaranto va a ser de mucha utilidad a la humanidad al ser una alternativa eficaz de alimentación que nutre y sana el alma", asegura la cabildana.

Inauguran edificio verde en casona de Playa Ancha

Mañana será inaugurado oficialmente el edificio verde de "Ciencia Al Tiro", un nuevo espacio tecnológico y sustentable, dirigido a la comunidad escolar y de estudiantes, ubicado en calle Pedro León Gallo, en Playa Ancha. La edificación, una casa patrimonial del sector, permitirá la realización de charlas y talleres que promuevan el intercambio de ideas a nivel local, nacional e internacional.

Recolector de basura traficaba droga en Placeres

Carabineros del retén Cabo 1° Juan Silva Toro, de cerro Los Placeres, arrestó a un recolector de basura municipal por microtráfico. La policía sorprendió a R.A.D.Z., de 52 años, alias "Conejo", en la población Las Palmas. Andaba trabajando en el aseo, pero a la vez vendía droga a adictos. Portaba 22 envoltorios de marihuana prensada y $71.600 de las ganancias. En su hogar de Rodelillo se hallaron otros 280 gramos de la yerba a granel. Pasó al juzgado de garantía.

EL EFECTIVO POLICIAL ES ASESTADO AL PERIODO SU VEHÍCULO.

Motorista de Carabineros se accidentó en patrullaje

Con lesiones de consideración resultó ayer un efectivo policial en un accidente de tránsito.

El siniestro se registró pasadas las 17.30 horas en calle Noruega N°350, en dirección al cerro, donde se vieron involucrados un motorista de Carabineros, un taxi colectivo y una furgoneta. El jefe (s) de la Tercera Comisaría Norte, capitán Pablo Pinochet, expresó que el cabo 2° de esa repartición se encontraba en el segundo turno de patrullaje preventivo cuando ocurrió el incidente. Al parecer, el taxi colectivo se detuvo en un paradero a dejar pasajeros, generando que el vehículo particular que lo antecedía frenara en forma intempestiva. Este fue impactado por el motorista que cayó al suelo y quedó con contusiones en la cervical. Fue trasladado al hospital Van Buren a constatar lesiones. Los choferes fueron al mismo centro de salud para los exámenes de alcoholemia. Personal de la Sitat efectuó peritajes.

Inauguran edificio verde en casona de Playa Ancha

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Title: "Provide house for children learn funny science "
Media: El Mercurio de Valparaíso, Newspaper
Date: October 10th, 2013

RECICLAJE.— Con materiales nobles rescatados de demoliciones, una vieja casona del barrio de Playa Ancha fue recuperada y dotada de tecnologías eficientes y sustentables para fomentar la ciencia entre los niños.

Edificio Verde, en Valparaíso:

Equipan casa para que niños aprendan ciencia entretenida

Programa "Ciencia al tiro" fomenta el interés científico bajo el alero del Centro Interdisciplinario de Neurociencia.

HERNÁN CISTERNAS ARELLANO

Cientos de alumnos de 7° y 8° año básico que estudian en escuelas municipales de sectores vulnerables de Valparaíso protagonizan una experiencia científica única en Chile y que, desde ayer, cuenta con un espacio propio: el Edificio Verde.

Se trata de una tradicional casona del barrio de Playa Ancha que fue reciclada y equipada con tecnologías eficientes y sustentables.

Allí los monitores, que son candidatos a doctorados y magísteres del Centro Interdisciplinario de Neurociencia de Valparaíso (CINV) —que dirige el biofísico Ramón Latorre—, realizarán talleres que buscan interesar a los niños en materias científicas.

Estos estudiantes ya participan en forma periódica de las iniciativas del proyecto "Ciencia al tiro" que creó la neurobióloga norteamericana y profesora del CINV Kathleen Witlock.

La experiencia partió el 2008 en la escuela de la población Montedónico, uno de los sectores de mayor vulnerabilidad del puerto.

En estos talleres, los alumnos aprendieron que a través de un horno solar podían calentar agua, cocinar galletas o preparar huevos duros sin necesidad de otro tipo de energía. También generaron biogás a partir de los desechos orgánicos.

Incluso avanzaron en conocimientos sobre genética, el desarrollo del sistema nervioso y su funcionamiento, y la acuaponía (sistema sustentable de producción de plantas y peces) a través de su trabajo con moscas del vino y peces cebra.

"Aquí los niños pueden utilizar los equipos, microscopios y lupas, lo que es 'bacán'. En otras partes les prohíben tocar todo. Descubren que la ciencia es superinteresante y divertida", subraya Kathleen Witlock.

Ramón Latorre, Premio Nacional de Ciencias 2002, expresó al respecto que "un país sin ciencia es un país cojo. Chile tiene poca ciencia. Lo que está haciendo Kate es un proyecto que llega a los niños. Es ahí donde uno tiene que empezar a enseñar la ciencia, a despertar la curiosidad".

Title: "Green Building of Playa Ancha turns schoolchild into small Scientists"
Media: El Mercurio de Valparaíso, Newspaper
Date: November 06th, 2013

Fotografía: Media Training Consultores
pemoreno@mercuriovalpo.cl

Con la presencia del alcalde de Valparaíso, Jorge Castro; el director regional del Consejo Nacional de la Cultura y las Artes, Rafael Torres; el rector de la Universidad de Valparaíso, Aldo Valle, y el director del Centro Interdisciplinario de Neurociencia de Valparaíso, Ramón Latorre, se realizó la inauguración del primer edificio verde en Chile, dedicado a la educación de la ciencia. El espacio ubicado en el Cerro Playa Ancha, pertenece a la iniciativa Ciencia Al Tiro, que dirige la Dra. Kathleen Whitlock, quien además es integrante del CINV.

Ramón Latorre, director Centro Interdisciplinario Neurociencia de Valparaíso; Aldo Valle, rector Universidad de Valparaíso; Kathleen Withlock, directora Ciencia Al Tiro, y Jorge Castro, alcalde de Valparaíso.

Kathleen Withlock, directora Ciencia Al Tiro, junto a alumnos de la escuela básica Pacífico de Playa Ancha, Valparaíso.

Moisés Acevedo, Centro Interdisciplinario Neurociencia de Valparaíso; Pablo Muñoz, Universidad de Valparaíso; Alejandra Pinto, Universidad de Valparaíso, y David Carrillo, director de Extensión y Comunicaciones Universidad de Valparaíso.

Carlos Briceño, consejero regional; Víctor Fuentes, director regional de Corfo, y Agustín Martínez, Centro Interdisciplinario Neurociencia de Valparaíso.

Juan Carlos García, Centro Interdisciplinario Neurociencia de Valparaíso; Rodrigo Sepúlveda, seremi de Energía; Patricia Arancibia, directora edificio verde Ciencia Al Tiro, y John Ewer, Centro Interdisciplinario Neurociencia de Valparaíso.

Title: "TV Series set in Valparaíso talks about beauty from the science"
Media: www.biobiochile.cl
Date: December 08th, 2013

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MUJER

Domingo 8 diciembre 2013 | 20:47 · *Actualizado: 21:48*

Serie televisiva ambientada en Valparaíso aborda el concepto de belleza desde la ciencia

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Title: "TV series Neuromantes"
Media: www.24horas.cl
Date: December 11th, 2013

Valparaíso: Neuromantes, exploradores de la vida

Es la serie científica que produce el Centro de Neurociencia de la Universidad de Valparaíso y que busca acercar la ciencia a las personas. ¿Quiere saber de qué se trata? Entonces ponga atención a la siguiente nota.

POR NATALIA GUTIÉRREZ

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11
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Title: " Iberoamerican Congress of Biophysics in Valparaíso"
Media: www.soyvalparaiso.cl
Date: September 30th, 2013

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Comienza en Valparaíso el Congreso Iberoamericano de Biofísicos

Destacado científicos del área se darán cita hasta el jueves en la ciudad Puerto. Mañana el doctor Miguel Holmgren dictará conferencia sobre la electricidad como motor de los seres vivos.

30.09.2013
#BIOFÍSICOS

Seguir a @soyvalparaiso

En el Parque Cultural de Valparaíso (ex Cárcel) se desarrollará entre mañana y el viernes el VIII Congreso Iberoamericano de Biofísicos, que en esta ocasión estará dirigido a unos 300 escolares de la Región. Para mañana destaca la conferencia sobre la importancia de la electricidad en seres vivos, que dictará el científico chileno que trabaja en Estados Unidos, Miguel Holmgren, quien además es Presidente de la Sociedad de Biofísicos Latinoamericanos.

La jornada inaugural también será presidida por Emanuel Mora, científico cubano experto en audición y bioacústica de humanos, y diversos animales tales como: murciélagos, mariposas, ranas y delfines.

El VIII Congreso Iberoamericano de Biofísicos se realizará de forma conjunta con la IX Reunión Anual de la Sociedad Chilena de Neurociencias. Se trata de la primera vez que esta cita se desarrollará en Valparaíso. El evento contará, además, con la participación de 80 científicos de Chile y el extranjero.

Entre los principales expositores destacan: Paul Brehm y Gail Mandel, deVollum Institute, Portland, Estados Unidos; Enrico Nasi, de la Universidad Nacional de Colombia; Marcelo Morales, de la Universidad Federal de Espíritu Santo, Brasil; y Ramón Latorre, Director del Centro Interdisciplinario de Neurociencia de la Universidad de Valparaíso, CINV, y Premio Nacional de Ciencias.

Alan Neely, subdirector del Centro Interdisciplinario de Neurociencia de Valparaíso (CINV), sostuvo que las expectativas respecto de la realización del encuentro científico se elevadas y se orientan a "generar intercambio de ideas, fortalecer redes, y sobre todo, lograr una actualización de conocimientos".

soyvalparaíso.cl
Me gusta

Title: "Valparaiso role in the develop of science"
Media: El Mercurio de Valparaíso, Newspaper
Date: October 02nd, 2013

8 | Actualidad

EL MERCURIO DE VALPARAÍSO | Miércoles 2 de octubre de 2013

Merval tiene el primer tren antimicrobiano del mundo

REGIÓN. *El proyecto piloto consta de la cobrerización de los cinco tipos de pasamanos en dos coches. Así se eliminan virus y bacterias.*

Osvaldo Álvarez
osvaldo@mercurioval.cl

¿Se imagina la comodidad de manos que cada minuto tocan los pasamanos y manillas de los carros de los trenes de Metro Valparaíso, con el consiguiente intercambio de gérmenes? Ahora el 99,9% de los virus y bacterias presentes en dichas superficies serán eliminados gracias a un innovador proyecto que considera el uso del cobre.

Se trata del primer tren antimicrobiano del mundo, que ya está operando en dos carros, con capacidad total de 800 pasajeros, lo que expresa una intervención de 56 manillas y pasamanos de cinco tipos diferentes de diseño, fabricados en Chile. El proyecto piloto demandó más de 250 metros lineales de cobre, ofreciendo a los usuarios superficies de contacto sanificadas.

Estas iniciativas fueron inauguradas por otro viaje entre las estaciones Puerto y Hospital por el ministro de Transportes y Telecomunicaciones, Pedro Pablo Errázuriz; su par de Minería, Hernán de Solminihac; el presidente ejecutivo de Codelco, Thomas Keller; el presidente del Directorio Metro Valparaíso, José Luis Domínguez, y el vicepresidente de Alstom Cono Sur, Julio Friedmann.

Las autoridades y ejecutivos destacaron la propiedad antimicrobiana del cobre, que elimina virus y bacterias casi en un cien por ciento, lo que tendrá un impacto positivo en el resguardo de la salud de los usuarios del Metro Valparaíso.

99,9% de eficacia. Estudios científicos han demostrado que el cobre crea una zona natural de protección.

18 millones de pasajeros transportó Metro Valparaíso durante el año 2012 en sus 27 trenes.

CERTIFICADO

El cobre es el único metal certificado como antimicrobiano y seguro para el medio ambiente por la Environmental Protection Agency (EPA) de los EEUU. Son propiedades que han sido demostradas, comprobadas y reconocidas ampliamente.

La iniciativa del cobre antimicrobiano en los espacios públicos es de carácter mundial y en Chile, a través de Codelco, ingenieros y médicos exponente con experiencias desarrolladas en el Hospital de Urgencia de Asistencia Pública en Punta Central, Roberto del Río, Hospital del Cobre en Calama, en la estación Santiago Buenas del Metro Santiago (pasamanos) y ahora en el tren de Metro Valparaíso.

Así lo destacó el ministro de Minería, Hernán de Solminihac, quien explicó que el cobre tiene múltiples posibilidades de uso, haciendo ver que incluso la corbata que utilizaba estaba hecha con dicho producto. "Gracias a las propiedades antimicrobianas del cobre, las familias chilenas tienen la oportunidad de experimentar directamente los beneficios que se desprenden del cobre. Estamos convencidos que aplicar cobre en las superficies de mayor contacto entre las personas significará un gran cambio en los estándares del transporte público".

A su vez, el ministro de Transportes y Telecomunicaciones, Pedro Pablo Errázuriz, resaltó que es "una iniciativa importante, que se suma a los avances que está realizando Metro Valparaíso para mejorar la experiencia de viaje, como el mejoramiento de las principales estaciones, las nuevas estaciones multimodales y la llegada de nuevos trenes".

José Luis Domínguez, presidente de Merval, afirmó que la idea es "apertar con una alternativa que impacta positivamente en la calidad de vida del gran público de la Quinta Región. Por ello, decidimos implementar este tren antimicrobiano, que permitirá hacer un aporte concreto en la salud de las miles de personas que diariamente ocupan el Metro Valparaíso". CR

"Hoy damos el primer paso al poner en marcha el primer tren de cobre del mundo y esperamos que otras redes se interesen"

Julio Friedmann
Vicepresidente de Alstom Cono Sur

LAS AUTORIDADES EXPLICARON QUE SE ESTUDIARÁ IMPLEMENTAR EL PRODUCTO EN NUEVOS TRENES.

Características del proyecto con cobre

- El proyecto consta de la cobrerización de los cinco tipos de pasamanos (56 unidades en total) en un tren completo de dos coches, con una capacidad por unidad de 400 pasajeros.
- La elaboración de estas manillas y tubos -construidos con una aleación de cobre, níquel y manganeso- demandó la utilización de más de 150 metros lineales de cobre. La fabricación de los pasamanos se realizó íntegramente en Chile.

LA GERENTA DEL RAMO, GLORIA BASCUÑÉCIO, EXPLICÓ EL SISTEMA.

Región capacita a 54 examinadores para examen de conducir

NOVEDAD. *En noviembre parte nueva metodología para el test "práctico".*

En meses de sesenta días entrará en vigencia en todo el país un nuevo examen práctico para quienes postulen a licencia de conductor no profesional (clase B) por primera vez, por lo cual el Ministerio de Transportes y Telecomunicaciones ya capacitó a 54 examinadores de las 31 municipalidades de la Región de Valparaíso completó para entregar dicho documento.

Así lo dio a conocer la secretaria regional ministerial del ramo, Gloria Bascuñécio, quien ayer entregó en la Intendencia las primeras credenciales a los examinadores que se capacitaron en las comunas del Gran Valparaíso.

La secretaria dijo que antes existía "el examen de la vuelta a la manzana, que duraba 5 minutos y se decían si reprobaba o no". La diferencia con el nuevo examen práctico es que ahora "presenta un mayor nivel de exigencia que permite evaluar las redes destrezas de los postulantes al volante. A través de estas modificaciones a la obtención de licencia buscamos prevenir accidentes y proteger la vida de las personas", recalcó.

El nuevo examen durará a lo menos 25 minutos en los cuales se deberán recorrer por lo menos 5 kilómetros. El desarrollo del examen se dividirá en dos etapas de conducción una libre y una guiada. La prueba será complementada con un material audiovisual de estudio que estará disponible para los postulantes a licencias de conducir clase B a través de la página web www.conaset.cl. Consiste en videos que muestran maniobras básicas que debe conocer el postulante. CR

Destacan rol porteño en desarrollo de la ciencia

VALPARAÍSO. *Científicos de varios países participan en Congreso de Biofísica.*

Alumnos de séptimo a primero medio asistieron a la conferencia inaugural del VIII Congreso de Biofísica, que organizaron en conjunto el Centro Interdisciplinario de Neurociencia de la Universidad de Valparaíso, la Asociación Chilena de Neurociencia y la Sociedad de Biofísica de América Latina.

El congreso se inició con la charla "Electricidad, luz y vida", que dio cuenta sobre la importancia de la electricidad en los seres vivos.

La conferencia estuvo a cargo del científico chileno, que trabaja en Estados Unidos, Miguel Holmgren, quien además es presidente de la Sociedad de Biofísicos Latinoamericanos. El doctor Holmgren señaló que "Valparaíso en la ciencia ha sido muy importante en los años 60 y 70. Aquí se hacían cosas que no se podían hacer en ningún lugar del mundo, y recordar esas tradiciones a los niños de ahora, quizás estimule a algunos a seguir en la rama de la ciencia en el futuro".

El rector de la Universidad de Valparaíso, Aldo Valle, destacó el rol de la ciencia y cómo la universidad a través de sus científicos tiene como misión difundir los avances, acercando el conocimiento a la comunidad. "Es un orgullo contar con los académicos que se dedican a la neurociencia en la Universidad de Valparaíso", expuso. CR

Title: "With a magic session ended the last Tertulias Porteñas of this 2013"
Media: www.uv.cl
Date: December 20th, 2013

Ciclo de charlas del CINV de la Universidad de Valparaíso

Con mágica jornada terminó ciclo 2013 de Tertulias Porteñas

Última charla convocó a cerca de 200 personas en el Parque Cultural de Valparaíso.

20 Me gusta Twittear 2

El ilusionismo complementó las visiones de la ciencia y el arte en la última jornada del ciclo 2013 del ya tradicional encuentro Tertulias Porteñas, que organiza el Centro Interdisciplinario de Neurociencia de la Universidad de Valparaíso y que en esta ocasión abordó, desde las distintas miradas, el tema de la percepción sensorial.

IMÁGENES

En una inédita jornada Tertulias Porteñas reunió al mago Juan Esteban Varela, quien ha destacado a nivel mundial por su innovador acto de magia para ciegos; al científico Pedro Maldonado, quien se ha concentrado en estudiar la percepción visual a través de mecanismos de la percepción sensorial, y al reconocido escultor Francisco Gazitúa, autor de numerosas obras que ornamentan modernas edificaciones, parques y plazas de Chile y el extranjero.

Reinventándose

La última jornada de Tertulias Porteñas del ciclo 2013, moderada por el editor Ernesto Pfeiffer, quien en esta versión reemplazó a Cristián Warnken, reveló el espíritu de sus organizadores por reinventarse e innovar en la puesta en escena, esta vez incorporando apoyo audiovisual, para contextualizar el trabajo desarrollado por el neurobiólogo Pedro Maldonado y las esculturas de Francisco Gazitúa, cuyo sello es la utilización de materiales autóctonos, como témpanos de hielo de la Antártica y rocas de la precordillera, que se sumaron a la imagen en vivo, en pantalla gigante, de los sorprendentes juegos de magia de Juan Estaban Varela.

Este último dijo estar maravillado con la experiencia: "¿Sabes lo que más me gusta? Es la fusión de puntos de vistas. Cuando uno piensa en el aprendizaje, en la palabra universidad, es justamente esa universalidad, una manera de enriquecer el conocimiento muy profunda y maravillosa. El hecho de que la magia, que yo hago con locura desde los seis años, esté presente en esta integración de puntos de vista para mí es un honor y un privilegio. Y además que la gente ha sido muy cariñosa", afirmó.

Con respecto a cómo influiría esta experiencia en el arte, afirmó que "es una fuente constante de inspiración". Además, fundamentó: "A veces es simplemente un disparo inspirador, otras veces el conocimiento duro. Está demostrado científicamente que tenemos mala capacidad para enfocar el movimiento; es una conclusión importante para un mago, qué bueno que esté demostrado científicamente, ya lo sospechábamos, pero de alguna manera son cosas que son más a nivel inspiracional y otras de nivel más técnico. Pero sobre todo la mayor riqueza viene a nivel humano, a nivel de las conexiones que estás haciendo, las conversaciones que vas teniendo, eso te va enriqueciendo. Si te enriquece como persona te va a enriquecer como profesional. Sin ninguna duda".

Title: "Squid is used to study the epilepsy and muscular paralysis"
Media: www.24horas.cl
Date: December 20th, 2013

24 HORAS > TENDENCIAS > Ciencia

Imagen

Con jibias chilenas estudian la epilepsia y parálisis muscular

El doctor Miguel Holmgren, chileno radicado en EE.UU, explicó que estos calamares poseen los axones más grandes entre todos los animales.

Mediante el estudio de **jibias** que habitan en la **región de Valparaíso**, **científicos chilenos** indagan en las propiedades eléctricas de las neuronas y la importancia de este impulso para múltiples funciones del organismo, entre ellas, el **movimiento muscular**.

"La electricidad es generadora de vida", señala Miguel Holmgren, científico chileno radicado en Estados Unidos y quien participó en el Octavo Congreso de Biofísicos Latinoamericanos realizado en la ciudad porteña. Esta cualidad es tan vital en el organismo de los seres vivos, que cuando existen fallas en la transmisión eléctrica, es posible que ocurran algunos tipos de epilepsia, parálisis musculares y migraña. Por esta razón, el científico explica que conocer los mecanismos de la bioelectricidad y especies como la jibia, puede contribuir al mejor entendimiento de patologías como éstas, y su manejo terapéutico".

La exploración de este calamar gigante constituye un hito en la biofísica de Chile y el mundo. Esto, debido a que el animal, junto con estar "lleno de electricidad", posee los axones más grandes de la naturaleza -de 1 mm aproximadamente-, los que pueden ser percibidos por el ojo humano.

·Dicha característica, el interés de diversos investigadores cómo se transmite el impulso nervioso . "Una vez generada la señal eléctrica, las neuronas tienen la propiedad de poder conducirla a través de prolongaciones llamadas axones las que, modo de , serían los cables del circuito eléctrico", comenta el Dr. Holmgren, quien además es Presidente de la Sociedad de Biofísicos Latinoamericanos –

Media: www.aricahoy.cl
Date: October 16th, 2013

ESTUDIO CIENTÍFICO A JIBIAS

Escrito Por Administrador En Octubre 16th, 2013 05:51 PM | Destacado, Nacional, Región

Investigan propiedades eléctricas de las neuronas y la importancia de este impulso para múltiples funciones del organismo, entre ellas, el movimiento muscular.

Mediante el estudio de jibias, calamar que habita en nuestras costas, científicos chilenos, investigan las propiedades eléctricas de las neuronas y la importancia de este impulso para múltiples funciones del organismo, entre ellas, el movimiento muscular.
Este trabajo está siendo desarrollado en la zona de Montemar, comuna de Concón (Valparaíso), por especialistas del Centro Interdisciplinario de la Neurociencia, CINV, con apoyo del científico chileno radicado en Estados Unidos, Dr. Miguel Holmgren.
"La electricidad es generadora de vida. Esta cualidad es tan vital en el organismo de los seres vivos, que cuando existen fallas en la transmisión eléctrica, es posible que ocurran algunos tipos de epilepsia, parálisis musculares y migraña" señaló el especialista.
Por esta razón, el científico explica que conocer los mecanismos de la bioelectricidad y especies como la jibia, puede contribuir al mejor entendimiento de patologías como éstas, y su manejo terapéutico.
La exploración de este calamar gigante constituye un hito en la biofísica de Chile y el mundo. Esto, debido a que el animal, junto con estar "lleno de electricidad", posee los axones más grandes de la naturaleza -de 1 mm aproximadamente-, los que pueden ser percibidos por el ojo humano.

Title: "Squid will allows study the epilepsy and muscular paralysis"
Media: www.lanación.cl
Date: October 19th, 2013

Nación.cl BETA

Portada Noticias | Opinión | Economía | Triunfo | Tecnología | Cultura & Entretención | Vida

País | Mundo | Reportajes | Regiones | Flujo de Noticias | Elecciones 2013

Inicio » Tecnología » Ciencia

19/10/2013 | ENVIAR | IMPRIMIR

JIBIAS "ELÉCTRICAS" PERMITIRÁN ESTUDIAR EPILEPSIA Y PARÁLISIS MUSCULAR

La exploración de este calamar gigante constituye un hito en la biofísica de Chile y el mundo. Esto, debido a que el animal, junto con estar "lleno de electricidad", posee los axones más grandes de la naturaleza -de 1 mm aproximadamente-, los que pueden ser percibidos por el ojo humano. La investigación es llevada a cabo por el Centro Interdisciplinario de Neurociencia de Valparaíso.

Sábado 19 de octubre de 2013 | por Nacion.cl + Sigue a Nación.cl en Facebook y Twitter

Mediante el estudio de **jibias que habitan en la Región de Valparaíso, científicos chilenos indagan las propiedades eléctricas de las neuronas** y la importancia de este impulso para múltiples funciones del organismo, entre ellas, el movimiento muscular.

"La electricidad es generadora de vida", señala Miguel Holmgren, científico chileno radicado en Estados Unidos - y quien participó en el Octavo Congreso de Biofísicos Latinoamericanos, realizado en la ciudad porteña-.

Esta **cualidad es tan vital en el organismo de los seres vivos**, que cuando existen fallas en la transmisión eléctrica, es posible que ocurran algunos tipos de epilepsia, parálisis musculares y migraña. Por esta razón, el científico explica que conocer los mecanismos de la bioelectricidad y especies como la jibia, puede contribuir al mejor entendimiento de patologías como éstas, y su manejo terapéutico.

La exploración de este calamar gigante **constituye un hito en la biofísica de Chile y el mundo.** Esto, debido a que el animal, junto con estar "lleno de electricidad", **posee los axones más grandes de la naturaleza -de 1 mm aproximadamente-**, los que pueden ser percibidos por el ojo humano.

Dicha característica captó el interés de diversos investigadores tanto en Europa con en EEUU y fueron estos axones los que permitieron dilucidar **cómo se transmite el impulso nervioso**, estudios que le dieron el Premio Nobel a los Ingleses, Alan Hodgkin y Andrew Huxley en Medicina y Fisiología el año 1962.

"Una vez generada la señal eléctrica, las neuronas tienen la propiedad de poder conducirla a través de sus prolongaciones llamadas axones, las que de un modo de gráfico serían los cables del circuito eléctrico", comenta el doctor Holmgren, quien además es Presidente de la Sociedad de Biofísicos Latinoamericanos (SOBLA).

Respecto a cómo sucede este proceso, los expertos determinaron que se debe a la **permeabilidad de la membrana celular,** la cual contiene unas proteínas que permiten el flujo constante de cargas eléctricas, desde el interior y hacia el exterior de la célula.

ELECTRICIDAD, MOTOR DE VIDA

Respecto al poder de la electricidad, el biofísico explica que **cada célula del cuerpo es una especie de batería eléctrica de aproximadamente 0.1 voltios** y cuyo polo negativo se ubica al interior de la célula.

Gracias a esto, se generan una serie de eventos, desde la ocurrencia de una idea, hasta el movimiento. "Además, la mayoría de las células usan la electricidad para alimentarse y descartar toxinas, entre otras funciones. Asimismo, las plantas también utilizan electricidad para poder sobrevivir".

Title: "Green Building turns schoolchild into small Scientists "
Media: www.lanación.cl
Date: October 20th, 2013

Nación.cl

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20/10/2013 | ENVIAR | IMPRIMIR

EDIFICIO VERDE CONVIERTE A ESCOLARES EN PEQUEÑOS CIENTÍFICOS

Alumnos de escuelas públicas de Valparaíso podrán experimentar en laboratorios y aprender sobre neurociencia, genética y tecnologías sustentables, con estudiantes de Doctorado en Neurociencia de la Universidad de Valparaíso.

Domingo 20 de octubre de 2013 | por Nación.cl + Sigue a Nación.cl en Facebook y Twitter

Una **casa especialmente equipada con tecnología sustentable y laboratorios**, es el nuevo proyecto que tendrá por protagonistas a **niños de escuelas vulnerables en Valparaíso**.

Se trata del **Edificio Verde de** Ciencia Al Tiro, iniciativa dirigida por la **doctora Kathleen Whitlock**, del **Centro Interdisciplinario de Neurociencia de Valparaíso**, que busca enseñar y acercar la ciencia de forma cercana y entretenida.

Este lugar, una antigua **casona del Cerro Playa Ancha** que fue refaccionada, **abrió sus puertas para que alumnos, principalmente de séptimo y octavo básico**, puedan experimentar y conocer aspectos de la neurociencia, la genética, la energía inteligente y la acuaponia: un sistema sustentable de producción de plantas acuáticas y peces.

CIENCIA PARA TODOS

Según explica Kathleen Whitlock, neurobióloga norteamericana, el proyecto responde a un "fuerte **compromiso social que busca apoyar a niños de escasos recursos**", incentivándoles a aprender ciencia de manera didáctica, y generando oportunidades para desarrollar sus capacidades.

"Nos interesa transmitir la idea de que **la ciencia está en todas partes y es entretenida**. Además, creemos que ésta puede ser útil en sus vidas", comenta.

Al interior de este edificio también, se permitirá la realización de charlas y talleres que promuevan el intercambio de ideas a nivel local, nacional e internacional. Esto, a fin de **continuar y profundizar la tarea ya iniciada a partir del 2008 por Ciencia Al Tiro**, cuando se dio curso a las actividades con la Escuela Básica Pacífico y la Escuela Árabe República de Siria.

El programa **Ciencia al Tiro es reconocido por la Universidad de Valparaíso** como parte de la formación de los estudiantes de doctorado, incluyendo créditos de la malla curricular.

Title: "Chilean Squid is used to study the epilepsy and muscular paralysis"
Media: El Día de la Serena, Newspaper
Date: October 18th, 2013

elDía
VIERNES
18 DE OCTUBRE 2013
Fundado el
1 de Abril de 1944
AÑO LXX Nº 25.349 IV Región La Serena 40 páginas $300. www.diarioeldia.cl

INVESTIGACIÓN DEL CENTRO INTERDISCIPLINARIO DE LA NEUROCIENCIA

Electricidad de jibia chilena permitirá estudiar epilepsia y parálisis muscular

FOTO: CARLOS VILLAVICENCIO

Científicos buscan conocer los mecanismos de la bioelectricidad. Especies como la jibia son claves.

Exploración de este calamar gigante, que también está en la Región de Coquimbo, constituye un hito en biofísica de Chile y el mundo

La Serena

Mediante el estudio de jibias, calamar que habita en nuestras costas, científicos chilenos, se encuentran indagando en las propiedades eléctricas de las neuronas y la importancia de este impulso para múltiples funciones del organismo, entre ellas, el movimiento muscular.

Este trabajo está siendo desarrollado en la zona de Montemar, comuna de Concón (Valparaíso), por especialistas del Centro Interdisciplinario de la Neurociencia, con apoyo del científico chileno radicado en Estados Unidos, Dr. Miguel Holmgren.

"La electricidad es generadora de vida. Esta cualidad es tan vital en el organismo de los seres vivos, que cuando existen fallas en la transmisión eléctrica es posible que ocurran algunos tipos de epilepsia, parálisis musculares y migraña" señaló el especialista.

Por esta razón, el científico explica que conocer los mecanismos de la bioelectricidad y especies como la jibia puede contribuir al mejor entendimiento de patologías como éstas, y su manejo terapéutico.

La exploración de este calamar gigante constituye un hito en la biofísica de Chile y el mundo. Esto, debido a que el animal, junto con estar "lleno de electricidad", posee los axones más grandes de la naturaleza -de 1 mm aproximadamente-, los que pueden ser percibidos por el ojo humano.

"Generada la señal eléctrica, las neuronas tienen la propiedad de poder conducirla a través de sus prolongaciones llamadas axones las que, de un modo de gráfico, serían los cables del circuito eléctrico", comenta el Dr. Holmgren, quien, además, es presidente de la Sociedad de Biofísicos Latinoamericanos.

Respecto a cómo sucede este proceso, los expertos determinaron que se debe a la permeabilidad de la membrana celular, la cual contiene unas proteínas que permiten el flujo constante de cargas eléctricas, desde el interior y hacia el exterior de la célula.

Dicha característica, captó el interés de diversos investigadores tanto en Europa como en los EE.UU. y fueron estos axones los que permitieron dilucidar cómo se transmite el impulso nervioso, estudios que le dieron el Premio Nobel a dos ingleses, Alan Hodgkin y Andrew Huxley en Medicina y Fisiología el año 1962. #104

Title: "Squid whet the appetite of Scientifics: It is used to study the epilepsy "
Media: La Cuarta, Newspaper
Date: October 25th, 2013

NICOLAS MERIKOVICH

Son buenas pa'l buche

Además de ser muy ricas con mayo, las jibias sirven para entender y tratar enfermedades nerviosas como la epilepsia.

Chilenos cacharon que, al estudiarla, se entienden enfermedades nerviosas

La jibia les abrió el apetito a los científicos: sirve para la epilepsia

Por ***Nicole Salvatierra***

Aunque cuando la cocinan deja la casa *pasá* a mariscos, la jibia es una delicia marina que no sólo sirve al paladar. El tremendo calamar es tan pulento que incluso llama la atención de científicos.

Justo ahora hay unos capos chilenos estudiando los nervios de la jibia para entender y tratar enfermedades cuáticas que afectan el sistema nervioso, como la epilepsia. Todo un hito en la biofísica chilensis y del mundo.

"La electricidad es generadora de vida. Esta cualidad es tan vital en el organismo de los seres vivos que cuando existen fallas en la transmisión eléctrica, es posible que ocurran algunos tipos de epilepsia, parálisis musculares y migraña", soltó el doc Miguel Holmgren.

El especialista, radicado en Estados Unidos pero más chileno que los porotos, apoya la investigación que realizan los científicos del Centro Interdisciplinario de la Neurociencia, CINV, en la zona de Montemar, ubicado en las costas de Concón.

Ocurre que el cefalópodo está dotado con los nervios más grandes del reino animal, perceptibles al ojo humano. Por eso, la jibia sirve pa' cachar las propiedades eléctricas de las neuronas y la importancia de este impulso para múltiples funciones del organismo, entre ellas, el movimiento muscular.

Para hacerla más clarita, el biofísico explica que cada célula del cuerpo es una especie de batería eléctrica de aproximadamente 0.1 voltios, cuyo polo negativo se ubica al interior de la célula. Gracias a esto, el ser humano puede pensar y actuar. O sea, se aviva con una idea y la lleva al movimiento.

"Además, la mayoría de las células usan la electricidad para alimentarse y descartar toxinas, entre otras funciones. Asimismo, las plantas también utilizan electricidad para poder sobrevivir", versea Holmgren.

El hombre de ciencia, en todo caso, también disfruta de la jibia con mayo. ¡Obvio!

Title: "Chilean scientist discover that organism's Protein Causes Genetics Deafness"
Media: www.24horas.cl
Date: November 15th, 2013

24 HORAS.CL
VIERNES 15 DE NOVIEMBRE

NACIONAL POLITICA ECONOMIA INTERNACIONAL DEPORTES TENDENCIAS REGIONES VIDEOS

Imagen

Chileno descubre que proteína produciría sordera

El doctor Agustín Martínez, del Centro Interdisciplinario de Neurociencia de la Universidad de Valparaíso, espera que sus hallazgos contribuyan a la creación de terapias.

POR 24HORAS.CL

Twittear 26 Recomendar 17 Compartir 1

Title: With the Severin Building try to recover patrimonial sector of Valparaiso.
Media: www.biobiochile.cl
Date: December 12th, 2013

NACIONAL

Lunes 2 diciembre 2013 | 13:04 · *Actualizado: 13:50*

Buscan recuperar sector patrimonial del puerto con obras en Edificio Severín de Valparaíso

3 Recomendar | 7 Twittear | 0 +1 | 28 Visitas


Raúl Zamora | Agencia UNO

Recuperar el sector patrimonial del barrio puerto, donde fue asaltado el experto argentino de la Unesco, es el principal objetivo de los trabajos que se realizan en el edificio Severín, que queda atrás de la iglesia La Matriz, lugar en donde funcionaría el Centro Interdisciplinario de Neurociencia de la Universidad de Valparaíso.

Este proyecto ya está terminado y fue presentado a la comisión de ICOMOS de la Unesco, compuesta por el experto argentino y brasilero, a quienes un día antes del robo se les informó la peligrosidad del sector y su necesidad de recuperarlo. Así lo dio a conocer a La Radio Ramón Latorre, director y gerente del Centro Interdisciplinario de Neurociencia de la Universidad de Valparaíso.

00:00 | 00:00

La latente peligrosidad en el sector, que es donde se funda Valparaíso, gatilló que los científicos presentaran esta nueva iniciativa que ya cuenta con una inversión de mil quinientos millones de pesos, por lo que se espera solicitar fondos al Gobierno Regional, puesto que la infraestructura tiene un costo total de 5 millones de pesos aproximadamente.

El edificio Severín fue elegido porque este lugar- según explicó Latorre, fue sede del primer Congreso Bicameral de Chile en 1828, inscribiéndose la Constitución de 1925, por lo cual presentaría las ventajas para generar la recuperación.

En tanto, Ramón Latorre, enfatizó que la construcción del Centro no solo es un beneficio patrimonial y cultural sino que también social porque las dependencias pueden ser utilizadas por la comunidad, entregando incluso wi-fi a un perímetro del barrio puerto.

00:00 | 00:00

De obtener los recursos para la ejecución, se espera que la construcción comience a mediados del 2014 para estar terminada a más tardar el 2015, donde sería un espacio para la comunidad, la ciencia y la cultura.