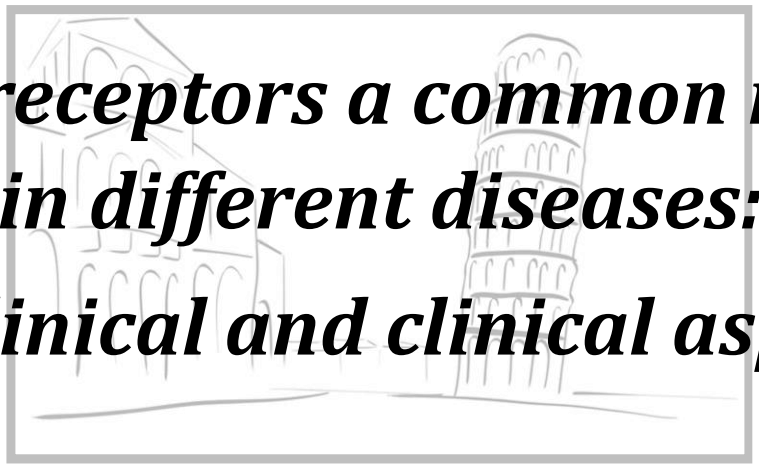
2nd PRESTO COST Action CA21130 Meeting

ABSTRACT BOOK



P2X receptors a common route in different diseases: preclinical and clinical aspects

Pisa, 5th-7th September 2023

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P2X receptors a common route in different diseases: preclinical and clinical aspects

(Sex- dependent) expression levels of P2X4 and P2X7 in PBMC of multiple sclerosis patients

Airi Rump[1], Kristel Ratas[1,2], Tuuli Katarina Lepasepp[1], Jaanus Suurväli[3], Olli- Pekka Smolander[1], Katrin Gross- Paju[4,5], Toomas Toomsoo[6,7] Jean Kanellopoulos[8], Sirje Rüütel Boudinot[1]

[1] Department of Chemistry and Biotechnology, Tallinn University of Technology, Tallinn, Estonia

[2] Synlab, Tallinn, Estonia

[3] Department of Biological Sciences, University of Manitoba, Winnipeg, Manitoba, Canada

[4] Department of Health Technologies, School of Information Technologies, Tallinn University of Technology, Tallinn, Estonia

[5] West- Tallinn Central Hospital, Tallinn, Estonia

[6] School of Natural Sciences and Health, Tallinn University, Tallinn, Estonia

[7] Confido Medical Center, Tallinn, Estonia

[8] Institute for Integrative Biology of the Cell (I2BC), CEA, CNRS, Université Paris- Saclay, France

Abstract

Multiple sclerosis (MS) is an inflammatory autoimmune disorder of the central nervous system and the leading cause of progressive neurological disability in young adults. It decreases the patient's lifespan by about 10 years and affects women more than men. No medication entirely restricts or reverses neurological degradation. However, early diagnosis and treatment increase the possibility of a better outcome. To identify new MS biomarkers, we tested the expression of P2X4 and P2X7 using qPCR in peripheral blood mononuclear cells (PBMC) of MS patients treated with interferon β (IFNβ), with glatiramer acetate (GA) or untreated. We showed that P2X7 is significantly induced in MS patients. In contrast, the expression of P2X4 was not significantly modified by MS but induced by IFNβ treatment in PBMC. P2X7 is essentially induced in female patients and the P2X4 expression induction by IFNβ occurs in male patients only. Our data point to the differential, sex-dependent value of MS markers and treatment effects. The strong upregulation of P2X4 and P2X7 induced in

the spinal cord of WT mice by EAE was abrogated in rgs16KO mice suggesting that rgs16 is required for P2X4 and P2X7 induction by neurological diseases.

2nd PRESTO COST Action CA21130 Meeting
P2X receptors a common route in different diseases: preclinical and clinical aspects

Adenosine and seizures: going beyond A1 receptors

Ana M Sebastião[1]

[1] Institute of Pharmacology and Neurosciences, and Institute of Molecular Medicine, Faculty of Medicine, University of Lisbon, Portugal

Abstract

Adenosine is an endogenous anticonvulsant, being produced from catabolism of ATP or released/taken up through equilibrative transporters, the inward gradient being maintained by adenosine kinase (ADK). When ADK levels are pathologically increased, the resulting extracellular adenosine deficiency translates into increased susceptibility to seizures. Surprisingly ADK deficiency also leads to seizures. We demonstrated that ADK activity, besides influencing the basal adenosinergic tone over inhibitory A1Rs, also modulates the adenosine A2AR-dependent facilitatory effects of BDNF on hippocampal synaptic transmission and plasticity, and that increased seizure susceptibility caused by ADK deficiency could be related to decreased A1R expression and enhanced excitatory A2AR and BDNF-TrkB signalling. Being intrigued by the anticonvulsant action of an agonist of inhibitory adenosine receptors, MRS5474, which lacked cardiac side effects, we assessed its action at the hippocampus. MRS5474 inhibited excitatory transmission under hyperexcitable conditions but not in control ones, precluding an A1R mediated action. MRS5474 inhibited GABA uptake in hippocampal slices, an effect likely mediated by adenosine A3R since it was prevented by an A3R antagonist and mimicked by an A3R agonist. Importantly, the expression of A3Rs is increased in epileptic tissue from the human hippocampus, and MRS5474 enhanced GABAergic currents in human tissue taken from the human epileptic hippocampal tissue but not from control tissue. Summarizing, A1Rs play a major role in control of excitability under physiologic conditions, but A3Rs may play an additional anticonvulsant action under epileptic conditions. A2ARs increase seizure susceptibility under chronically enhanced adenosinergic tonus.

ATP-induced calcium signalling and regulation of mesenchymal stem cells

Lin-Hua JIANG

[1]Department of Physiology and Pathophysiology, Xinxiang Medical University, China; and School of Biomedical Sciences, University of Leeds, the United Kingdom

Abstract

Mesenchymal stem cells (MSC) exist in stem cell niche of various adult tissue that are crucial in adult tissue homeostasis and become an attractive source of stem cells for regenerative medicine. Accumulating evidence shows ATP release from MSC as an autocrine or paracrine signaling molecule by inducing intracellular calcium signal and, importantly, ATP-induced purinergic calcium signalling regulates multiple MSC functions. My presentation will discuss our ongoing efforts to understand the molecular mechanisms mediating ATP-induced purinergic calcium signalling in MSC, derived from human dental pulp tissues, and their role in regulating MSC migration and differentiation.

Brain-wide study of P2X7R expression in the progressive form of experimental autoimmune encephalomyelitis in the rat

Andjela Stekic[1], Milorad Dragic[1], Nadezda Nedeljkovic[1]

[1]Laboratory for Neurobiology, Department for General Physiology and Biophysics, Faculty of Biology, University of Belgrade, 11000 Belgrade, Serbia

Abstract

Accumulation of adenosine triphosphate (ATP) at sites of tissue injury and inflammation can lead to activation of plasma membrane receptors named P2 receptors, among which $P2X_7$ receptor ($P2X_7R$) is known as the most involved receptor in inflammatory processes. Experimental autoimmune encephalomyelitis (EAE) is an animal model of multiple sclerosis (MS), a common immune-mediated, neuroinflammatory and neurodegenerative disease of the central nervous system. Most EAE studies focus on the spinal cord, but it is also demonstrated that pathological events such as inflammation, demyelination, axonal loss and neurodegeneration are noticeable in the brain. Due to its relevance for inflammation, our study aimed to give insight into $P2X_7R$ abundance in different rat brain regions such as olfactory bulb, prefrontal cortex, caudoputamen, hippocampus, cerebellum and brainstem in the peak of EAE. To screen the expression of $P2X_7R$ in listed brain structures, immunoblot analysis was performed. The results showed that $P2X_7R$ abundance in the peak of EAE was decreased in olfactory bulb, prefrontal cortex, cerebellum and brainstem, but increased in caudoputamen. On the other side, hippocampus analyses did not demonstrate a statistically significant change in $P2X_7R$ abundance. The changes in $P2X_7R$ expression indicate that these brain regions presumably undergo pathological alterations, thus, the meaning and importance of these changes are yet to be elucidated.

Cell type-specific targeting of the P2X7 receptor for better seizure control in epilepsy

Tobias Engel[1,2]

[1] Department of Physiology & Medical Physics, RCSI University of Medicine & Health Sciences, Dublin D02 YN77, Ireland

[2] FutureNeuro, SFI Research Centre for Chronic and Rare Neurological Diseases, RCSI University of Medicine and Health Sciences, Dublin D02 YN77, Ireland

Abstract

The purinergic P2X7 receptor (P2X7R) has been postulated as treatment target for numerous diseases of the central nervous system (CNS) including epilepsy, where it has been shown to contribute to neuroinflammation and the generation of hyperexcitable neuronal networks. However, inconsistent data reported over the past years showing both anti-convulsant and pro-convulsant functions of this receptor in rodent models has hampered its progression into a clinical scenario for epilepsy treatment. To understand the basis for these opposing actions, we generated mice lacking exon 2 of the P2rx7 gene in either microglia (P2rx7:Cx3cr1-Cre) or neurons (P2rx7:Thy-1-Cre). Mice with deleted P2rx7 in microglia displayed less severe acute seizures and developed a milder form of epilepsy and a molecular profile characteristic of an anti-inflammatory phenotype in microglia. In contrast, mice lacking P2rx7 in neurons showed a more severe seizure phenotype. Analysis of single cell expression data revealed that human P2RX7 expression is elevated in the hippocampus of patients with temporal lobe epilepsy in excitatory and inhibitory neurons. Functional studies determined that GABAergic interneurons display increased responses to P2X7R activation in experimental epilepsy. Our results provide an explanation for the dual and opposing actions of P2X7R in epilepsy and suggest P2X7R overexpression as a novel therapeutic strategy for epilepsy.

Cellular and animal models to study the role of P2X receptors in retinal degeneration

Natalia Martínez-Gil[1]; Pedro Lax[1] and Victoria Maneu[2]

[1] Physiology, Genetics and Microbiology Department, University of Alicante, Spain.

[2] Optics, Pharmacology and Anatomy Department, University of Alicante, Spain.

Abstract

The purinergic P2X receptors activation by ATP triggers different downstream pathways that finally promote inflammation and cell death mechanisms. In the central nervous system, under pathological conditions, the ATP release by neurons and glial cells induces the P2X receptors overexpression, which participates in the neuroinflammation that is always present in neurodegenerative process. Therefore, the study of P2X receptors in retinal diseases is increasing in recent years. Since now, the implication of P2X7 and P2X4 receptors has been shown in different retinal pathologies such as glaucoma, age-related macular degeneration (AMD) or diabetic retinopathy. More recently, our group demonstrated the possible relationship between the upregulation of both receptors and the progression of the retinitis pigmentosa, an inherited neurodegenerative disease. One of the main objectives of our group is to understand the role of P2X receptors in the neurodegenerative processes that occur in retinal diseases, with the ultimate goal of using them as a therapeutic target for these pathologies. To reach our aims, we study the expression pattern of P2X receptors in different experimental models of retinal degeneration: from cell lines and animal models to human tissue. Altogether, our results suggest that the upregulation of P2X receptors promotes the retinal degeneration by glial activation and photoreceptor cell death. Thus, P2X receptors could be considered as potential targets in the pharmacological treatment of retinal diseases. However, more studies are needed to elucidate the mechanism that are involved in their activation and inhibition.

Development of P2X receptor-based biosensor as tools for drug screening and monitoring extracellular ATP release

François Rassendren[1], Matthias Ollivier[1], Sandra Pierredon[1], Yan Percherancier[2] and Vincent Compan[1].

[1] Institute of Functional Genomics, CNRS UMR5203, INSERM U1991, University of Montpellier. Montpellier, France

[2] Laboratory of Integration from Materials to System, CNRS UMR 5218, University of Bordeaux. Talence, France

Abstract

Development of high throughput drug screening assays for ligand-gated channels is often challenging. This is particularly true in the case of the ATP-gated channels P2X receptors for which the use of intracellular calcium assays is limited by the presence, in most cell lines, of endogenous P2Y metabotropic purinergic receptors coupled to intracellular calcium release. We have developed a series of P2X-based sensors that can track with high fidelity and specificity gating of any P2X receptors. These sensors are based on the fusion at the C-termini of P2X subunits of either a fluorescent calcium reporter (GCaMP6), or bioluminescent BRET modules that can specifically detect calcium or potassium flux through P2X permeation pathways. The P2X-GCaMPs sensors display high fidelity, with minimal activation of the GCaMP6 by other source of intracellular calcium increase than calcium flux through P2X pore. Calcium-sensitive BRET sensors show share similar features. Owing to their sensitivity and high signal-to noise-ratio, these sensors are ideal for drug screening. They can also be used to monitor ions flux through P2X permeation pathways. Finally, by introducing specific gain or loss of function mutations, these sensors can detect nano- to milli-molar concentrations of extracellular ATP and can be used to follow the spatiotemporal dynamic of extracellular ATP in vitro or in vivo.

Effect of high fat diet on brain areas involved in neurocognitive dysfunction: a multifaceted role of the P2X7 receptor (P2X7R)

C. Rossi[1], MR Distaso[1], F. Raggi[1], C. Kusmic[2], F. Faita[2], A. Solini[1]

[1] Department of Surgical, Medical, Molecular and Critical Area Pathology University of Pisa, Italy

[2] CNR, Pisa, Italy

Abstract

Dietary fats consumption, involved in the pathogenesis of insulin resistance and impaired glucose metabolism, is linked with decline of cognitive functions, dementia and development of Parkinson and Alzheimer. Mature IL-1β, requiring the activation of the P2X7R-inflammasome complex, is a key mediator of neuroinflammation. To test whether P2X7R activation might interfere with systemic and cerebral metabolic homeostasis, we treated WT and P2X7R KO mice with high fat diet (HFD) for 16 weeks, evaluating the effects on Substantia Nigra and Hippocampus, target areas of damage in Parkinson and Alzheimer. HFD-treated WT and P2X7R KO mice showed a different brain mRNA profile of Insulin and Igf-1, with these genes and relative receptors more expressed in KO mice Hippocampus (Insulin KO 2.58±0.8 vs WT 0.87±0.7, Igf1R KO 2.63±0.7 vs 1.63±0.5). Unlike P2X7R KO mice, WT treated with HFD displayed a diameter reduction of dopaminergic neurons in Substantia Nigra (WN 24.64±3.3 vs WH 18.75±1.8 μm), accompanied by an increased IBA1 expression in this area (WN 0.68±0.4 vs WH 2.33±1.1 % threshold area); they also showed poor performances during Y-Maze and Morris Water Maze, tasks involving Hippocampus activity. Conversely, Parkin, whose reduction might promote neuronal cell death, was increased in the Hippocampus of P2X7R KO animals (WH 0.69±0.3 vs KH 1.30±0.6). In summary, we report for the first time that HFD induces a damage in dopaminergic neurons of Substantia Nigra and a Hippocampus, related to a worse cognitive performance and both attenuated in absence of P2X7R. The involved mechanisms might differ in the two brain areas, with a predominant role of inflammation in Substantia Nigra and a metabolic derangement in Hippocampus.

Extracellular vesicles released upon P2X7-receptor activation from microglia: pathogenic players and diagnostic tools in neuroinflammatory diseases

Martina Gabrielli, Mariateresa Golia, Federica Scaroni, Claudia Verderio

Abstract

Extensive evidence indicates that activation of P2X7 receptor, an ATP-gated ion channel highly expressed in microglia is strictly associated with the release of extracellular vesicles into the microenvironment. Extracellular vesicles are a heterogeneous group of membranous structures which directly bud from the plasma membrane (microvesicles) or originate in the endocytic compartment (exosomes) carrying a selection of donor cell components (proteins, lipids, genetic materials, and metabolites). Through P2X7-dependent extracellular vesicle release, microglia regulate non classical protein secretion and transfer bioactive molecules to other cells, including misfolded proteins, participating in inflammatory and neurodegenerative diseases. I will summarize our recent studies showing that quantification of microglial EVs in body fluids and analysis of their miRNA cargo may help stratification of patients affected by multiple sclerosis. In addition, I will show that extracellular vesicles carrying the misfolded protein Aβ contribute to progression of cognitive deficit in the mouse brain by propagating Aβ-related synaptic dysfunction among connected brain regions.

Hypoxia Aggravates Inhibition of Alveolar Epithelial Na-Transport by Lipopolysaccharide-Stimulation of Alveolar Macrophages

Emel Baloglu[1,2], Kalpana Velineni [2], Ezgi Ermis-Kaya [2] and Heimo Mairbäurl [2,3,4]

[1] Department of Medical Pharmacology, School of Medicine, Acibadem Mehmet Ali Aydinlar University, 34752 Istanbul, Turkey

[2] Translational Lung Research Center Heidelberg (TLRC-H), Part of the German Center for Lung Research (DZL), 69120 Heidelberg, Germany

[3] Medical Clinic VII, Sports Medicine, University Hospital Heidelberg, 69120 Heidelberg, Germany

[4] Translational Pneumology, University Hospital Heidelberg, 69120 Heidelberg, Germany

Abstract

Inflammation and hypoxia impair alveolar barrier tightness, inhibit Na- and fluid reabsorption, and cause edema. We tested whether stimulated alveolar macrophages affect alveolar Na-transport and whether hypoxia aggravates the effects of inflammation and tested for involved signalling pathways. Primary rat alveolar type II cells (rA2) were co-cultured with rat alveolar macrophages (NR8383) or treated with NR8383-conditioned media after stimulation with lipopolysaccharide (LPS; 1 µg/mL) and exposed to normoxia and hypoxia (1.5% O2). LPS caused a fast, transient increase in TNF-α and IL-6 mRNA in macrophages and a sustained increase in inducible nitric oxide synthase (NOS2) mRNA in macrophages and in rA2 cells resulting in elevated nitrite levels and secretion of TNF- α and IL-6 into culture media. In normoxia, 24 h of LPS treated NR8383 decreased the transepithelial electrical resistance (TEER) of co-cultures, of amiloride-sensitive short circuit current (ISCΔamil); whereas Na/K-ATPase activity was not affected. Inhibition was also seen with conditioned media from LPS-stimulated NR8383 on rA2 but was less pronounced after dialysis to remove small molecules and nitrite. The effect of LPS-stimulated macrophages on TEER and Na-transport was fully prevented by the iNOS-inhibitor L-NMMA applied to co-cultures and to rA2 mono-cultures. Hypoxia in combination with LPS-stimulated NR8383 totally abolished TEER and ISCΔamil. These results indicate that the LPS-stimulation of alveolar macrophages impairs alveolar epithelial Na-transport by NO-

dependent mechanisms, where part of the NO is produced by rA2 induced by signals from LPS stimulated alveolar macrophages.

Inhibition of the P2RX7 by noncanonical nicotinic receptors of mononuclear phagocytes

Katrin Richter[1], Anna Zakrzewicz[1], Marion Meixner[1], NilayAsci[1], Sanaria H. Yakoob[2], Juliane Liese[1], Andreas Hecker[1], Klaus-Dieter Schlüter[3], Axel Gödecke[4], Ivan Manzini[5], Günther Schmalzing[2], Veronika Grau[1]

[1] Laboratory of Experimental Surgery, Department of General and Thoracic Surgery, Justus-Liebig-University Giessen, German Centre for Lung Research, Giessen, Germany;

[2] Institute of Clinical Pharmacology, RWTH Aachen University, Aachen, Germany;

[3] Institute of Physiology, Justus-Liebig-University Giessen, Germany;

[4] Institute of Cardiovascular Physiology, University Hospital Düsseldorf, Düsseldorf, Germany;

[5] Department of Animal Physiology and Molecular Biomedicine, Justus-Liebig-University Giessen, Germany.

Abstract

Stimulation of the P2X7 receptor (P2RX7) by high concentrations of extracellular ATP causes a cation flux in activated mononuclear phagocytes, followed by NLRP3 inflammasome assembly, activation of caspase-1, maturation and release of the pro-inflammatory cytokine interleukin-1β (IL-1β). Controlling the P2RX7 function is of outstanding clinical interest, because IL-1β released in response to ATP importantly contributes to trauma-induced, life-threatening systemic inflammation. We identified a cholinergic mechanism that inhibits the ionotropic function of the P2RX7 via non-canonical nicotinic acetylcholine receptors (nAChRs) containing subunits α7, α9 and/or α10. Here, we report on the metabotropic signalling pathway linking nAChR activation to P2RX7 inhibition. Lipopolysaccharide-primed human monocytic U937 and THP-1 cells, THP-1 cell-derived M1-like macrophages, primary human and murine mononuclear phagocytes were stimulated with ATP to induce IL-1β release. Agonists of nAChRs inhibited the ATP-mediated IL-1β release. This effect was efficiently blunted by diverse NO synthase inhibitors, siRNA silencing of endothelial NO synthase expression and its genetic knock-out. In HEK cells overexpressing the human P2XR7, the ATP-induced ionotropic P2RX7 activity was measured in patch-clamp experiments. The ATP-induced ion currents were strongly reduced by the NO/peroxynitrite donor SIN-1. In contrast, in HEK cells expressing the

human P2RX7 containing a point mutation at cysteine C377, SIN-1 did not impair the ATP-induced ion flux, suggesting that nitrosylation at this cysteine inhibits the ionotropic P2RX7 function. As C377 is a part of the palmitoylated C-terminal cysteine-rich domain that prevents P2RX7 desensitization, we suggest that a competition between nitrosylation and palmitoylation at C377 regulates the ionotropic function of the P2RX7.

Lichen metabolites as potential modulators of P2X receptors during depression-like states in laboratory animals

Terezia Kiskova, Patrik Simko, Nicol Urbanska, Simona Mattova

Department of Animal Physiology, Institute of Biology and Ecology, Faculty of Sciences, Pavol Jozef Safarik University in Kosice, Slovakia

Abstract

Depressive disorder is a prevalent mental illness that affects millions of individuals worldwide. It is characterized by a range of severe side effects, including suicidal thoughts, physical pain, and detrimental impacts on both behaviour and overall health. Additionally, it carries significant economic importance. Purinergic receptors, specifically P2X receptors, have been associated with various symptoms observed in patients with depressive and bipolar disorders. Preclinical models have demonstrated that pharmacological blockade of P2RX7 receptors may induce antidepressant-like effects. Some secondary lichen metabolites, such as vulpinic or usnic acid have been previously shown as classical uncouplers of oxidative phosphorylation, showing dose-dependent pattern in inducing the uncoupling by acting on the inner mitochondrial membrane through their lipophilic properties and protonophoric activities. Thus, our project aims to analyse the levels of different P2X receptors in the brain following the chronic administration of selected lichen metabolites. We will utilize an unpredictable chronic mild stress-induced depression model in Sprague-Dawley rats, as the animals represent an essential part of preclinical research and create the conditions for uncovering the mechanisms of action of drugs and drug discovery.

Macrocyclic cage molecules as delivery vehicles for drug substances-molecular modeling approach

Valya Nikolova[1], Stefan Dobrev[2], Nikoleta Kircheva[2], Victoria Yordanova[1], Todor Dudev[1] and Silvia Angelova[2]

[1] Faculty of Chemistry and Pharmacy, Sofia University "St. Kliment Ohridski", 1164, Sofia, Bulgaria

[2] Institute of Optical Materials and Technologies "Acad. J. Malinowski", Bulgarian Academy of Sciences, 1113, Sofia, Bulgaria

Abstract

Cucurbiturils (CBs) are useful macrocyclic excipients in eye drop formulations: they can increase the water solubility of the drug, enhance drug absorption into the eye, improve aqueous stability and reduce local irritation. Effective and safe drug delivery is, however, a challenge and the information on the host (CBs)/guest (tropicamide and atropine) interactions can help improving the existing treatments and develop novel therapies not limited only to eye diseases/conditions. Since this carrier system can easily modify the properties of the drug and ensure its delivery at the targeted ocular tissue, further insight into the intimate mechanism of the host-guest recognition is crucial. The present DFT/SMD study focuses on the role of numerous factors governing this process, namely the specific position of the guest molecule in the cavity of the cucurbituril, the ionization form (non/protonated) of the antimuscarinic drug, the dielectric constant of the medium, and the size of the cavitant pore. The obtained results are in line with experimental observations and shed light on the mechanism, at atomic resolution, of recognition between the CBs and the two parasympatholytic drugs.

New insights into ATP metabolism and signaling in microglial cells

Gennady G. Yegutkin

MediCity Research Laboratory and InFLAMES Flagship, University of Turku, Turku, Finland

Abstract

Microglia are the resident phagocytes of the central nervous system that are involved in the pathogenesis of Alzheimer's disease (AD) and other neurodegenerative disorders. During the course of ageing and chronic inflammation, resting microglial cells lose their homeostatic signature and become inflammatory, resulting in both protective and disease-associated microglia (DAM). However, the critical extra- and intracellular molecules that orchestrate neuroprotective functions of DAM remain poorly understood. Extracellular ATP and its metabolite adenosine are important signalling molecules involved in a wide range of (patho)physiological activities in virtually all organs and tissues, including the brain. By using transgenic APP/PS1-21 and APP23 mice as appropriate rodent models of AD, as well as human brain with AD, this study was undertaken to identify a link between cerebral amyloidosis and extracellular ATP metabolism and signalling. We showed that mouse microglial cells express several key components of purinergic machinery, including P2Y12R, P2X4R, and NTPDase1/CD39 and in addition, create a spatially arranged network with $CD73^{+}$ amyloid plaques and plaque-associated $NTPDase^{2+}$ astrocytes. These findings suggest that DAM cope with inflammation by scavenging plaque-associated ATP in the mouse brain with cerebral amyloidosis but not in the human Alzheimer's brain.

NLRP3 inflammasome inhibition prevents Aβ- and pTau-driven neuroinflammation and neuronal death by pyroptosis in acute hippocampal slices

Tiago Ferreira[1,2], Ana M. Sebastião[1,2] and Cláudia A. Valente[1,2]

[1]Instituto de Farmacologia e Neurociências, Faculdade de Medicina, Universidade de Lisboa, Lisboa, Portugal

[2]Instituto de Medicina Molecular João Lobo Antunes, Faculdade de Medicina, Universidade de Lisboa, Lisboa, Portugal

Abstract

Alzheimer's disease (AD) is the most prevalent neurodegenerative disease. It is characterized by synaptic loss, neuronal death, and progressive cognitive impairment, attributed to the extracellular accumulation of amyloid-β (Aβ) plaques and neurofibrillary tangles of hyperphosphorylated tau (pTau). Neuroinflammation has been associated to AD pathophysiology, with the NLRP3 inflammasome (NLRP3), a cytoplasmatic multiprotein complex, as one key player. NLRP3 can be activated by perturbations of intracellular ion homeostasis, such as K^+ efflux through P2X7 receptor. NLRP3 activation leads to Caspase-1 processing, which generates pro-inflammatory cytokines and Gasdermin D (GSDMD)-mediated cell death by pyroptosis. In AD, pyroptosis of microglia cells is known to occur, but pyroptotic neuronal death was scarcely explored. Herein, neuronal death by pyroptosis and the impact of NLRP3 inhibition were explored in an *ex vivo* model of AD. Acute hippocampal slices were incubated for 4h with Aβ oligomers ($Aβ_{olig}$, 200 nM), Okadaic Acid (OKA, 25 nM), and both stimuli with MCC950 (1 μM), a selective NLRP3 inhibitor. OKA significantly increased Tau phosphorylation, compared to $Aβ_{olig}$-exposed slices. The presence of $Aβ_{olig}$/pTau induced gliosis, Caspase-1 production, GSDMD cleavage and IL-1β release. Furthermore, GSDMD localization with the neuronal marker NeuN suggested neuronal death by pyroptosis. All these events were decreased by MCC950. This work shows that $Aβ_{olig}$/pTau presence exacerbates the $Aβ_{olig}$ harmful effects, most likely through NLRP3 overactivation. It provides evidence of neuronal death by pyroptosis, hinting that neurons may be instigators of neuroinflammation. Finally, it demonstrates that NLRP3 inhibition

strongly prevents the $A\beta_{olig}$/pTau-induced pathological events, suggesting this pathway as a potential therapeutic target for AD.

Origin, distribution, and function of three frequent mutants of human P2X7

Waldemar Schäfer, Tobias Stähler, Carolina Pinto Espinoza, Welbeck Danquah, Jan Hendrik Knop, Björn Rissiek, Friedrich Haag, Friedrich Koch-Nolte

Institute of Immunology, University-Medical Center Hamburg-Eppendorf, Hamburg, Germany

Abstract

The P2X7 ion channel is expressed on the plasma membrane of immune cells and plays important roles in inflammation and apoptosis. In contrast to other members of the P2X family, non-synonymous polymorphisms in P2X7 are common. Three such coding mutations occur at overall frequencies of more than 25%. The affected residues lie in the extracellular "head"-domain of P2X7 (155 Y/H), its "lower body" (270 R/H), and its "tail" in the second transmembrane domain (348 T/A). Comparison of the P2X7 orthologues of human and other great apes indicates that the ancestral allele is Y-R-T (at 155-270-348). The originally published reference sequence of human P2X7, in contrast is H-H-A, i.e. this so-called "wildtype" human P2X7 differs from the ancestral allele at all three positions. Data of the 1,000 Genome Project from ~ 500 persons from each of the five major continental regions shows that the ancestral variants occur in all analyzed human populations, albeit at strikingly different frequencies (e.g., 25%-59% for Y155, 59%-77% for R270, and 13%-47% for T348). These results suggest that most individuals co-express two copies of P2X7 that differ in one or more amino acids at positions 155, 270, and 348. Interestingly, the ancestral Y-R-T variant displays higher ATP sensitivity than "wildtype" P2X7 and all of the single or double amino acids variants. Other, less frequent, allelic variants of P2X7 display a near complete loss of ATP-sensitivity. Thus, it is important to consider variants of P2X7 carried by participants in clinical trials targeting P2X7.

P2RX gene in PGC and ConLiGen studies of bipolar disorder and unipolar major depression

Maria Grigoroiu-Serbanescu

Alexandru Obregia Clinical Psychiatric Hospital, Bucharest, Romania

Abstract

The purinergic receptor P2X7 (P2RX7 gene), located on chromosome 12q24, encodes a purinergic brain-expressed receptor and is involved in Ca^{2+}-dependent signalling pathways. P2X7 receptors mediate neuroinflammatory response, glutamate release, neuroplasticity, and are expressed in brain and other tissues. Non-synonymous SNPs of this gene were related to major mood disorders [bipolar disorder (BP); unipolar major depression (MDD)] in candidate gene studies. Nevertheless, this association was not replicated in several candidate-gene studies. Presentation of the results of recent genome-wide association studies (GWAS) of major affective disorders conducted on large-scale international samples by two consortia: Psychiatric Genomics Consortium and Consortium on Lithium Genetics. Analysis of several GWAS of BP, MDD, and lithium-treatment response conducted over the last 5 years on samples from four continents. Romanian samples were included in all BP-GWAS. None of the GWAS identified SNPs of P2RX7-gene significantly associated with BP, MDD or lithium-treatment response. But in the BP-GWAS and MDD-GWAS the P2RX7-gene appeared in significant pathways underlying the liability to these disorders. Although the large-scale GWAS advanced the knowledge of genetic basis of major affective disorders, they have limitations created by including not strictly defined phenotypes and population genetic heterogeneity.

P2X receptors: Early Stages of Clinical Development

Prof. Jasmina Trojachanec, MD, PhD

Department of Preclinical and Clinical Pharmacology and Toxicology, Medical Faculty, Skopje, R.N. Macedonia

Abstract

P2X receptors are a family of seven (P2X1R-P2X7R) cation permeable ligand-gated ion channels (LGICs) that open in response to binding by the extracellular ligand, adenosine 5'-triphosphate (ATP). This ion channel family was seen by many to offer a potential to deliver much needed therapeutic options for many poorly met diseases, syndromes, and symptoms. The development of new drugs is one of the most challenging steps which includes extensive preclinical and clinical trials. The transition from basic scientific discoveries into therapeutic applications is the advancement of a drug candidate from preclinical studies to initial human testing. First-in-human (FIH) trials serve as the link to advance new promising drug candidates and are conducted primarily to determine the safe dose range for further clinical development. Cross-functional collaboration is essential to ensure efficient and successful FIH trials. The purpose of FIH trials is to evaluate an investigational medicinal product (IMP) in humans for the first time, to study the human pharmacology, tolerability and safety of the IMP and to compare how effects seen in non-clinical studies translate into humans. In this presentation, an emphasis is placed on FIH trial design considerations, including starting dose selection, study size and population, dose escalation scheme, and implementation of adaptive designs according to the recent revision of the European Medicines Agency (EMA) guideline on FIH trials to promote safety and mitigate risk.

P2X4 and P2X7 receptors in prostate cancer bone metastasis

Ning Wang

Department of Oncology & Metabolism, University of Sheffield, UK

Abstract

Prostate cancer (PCa) is the main leading cause of cancer death in men worldwide and preferentially metastasize into the skeleton. P2X4 receptor (P2X4R) and P2X7 receptor (P2X7R) were found to be significantly up-regulated at transcription level in a subpopulation of PCa cells (metastasis-initiating cells) which are responsible for bone metastasis. Therefore, we hypothesized that P2X4R/7R plays pro-tumour roles during PCa bone metastasis. To test this hypothesis, we genetically modified P2X4R and P2X7R in PCa cells using the CRISPR/Cas9 system. Results showed that P2X4R knockout (KO) cells had significantly reduced cell proliferation and invasiveness but increased apoptosis, compared to wild-type controls in vitro. BALB/c immunocompromised mice received an intracardiac injection of P2X4R KO cells showed slower tumour progression and the absence of bone metastases. In contrast to P2X4R, P2X7R is bi-functional, possessing both anti- and pro-tumorigenic properties linked to its long intracellular C-terminus. Disrupting the P2X7R C-terminus by generating frameshifts (ΔC) in exon 13 reduced pore permeabilization and apoptosis in PCa cells but enhanced the presence of metastasis-initiating cell subpopulations. Mice injected with P2X7R ΔC cells exhibited an increased incidence of PCa bone metastasis and reduced survival. These findings are consistent with our cross-sectional study using clinical samples, suggesting that the C-terminal truncated P2X7RB isoforms, but not the wild-type full-length P2X7RA, were positively associated with PCa progression and bone metastases. In summary, our data shows that both P2X4R and P2X7R play a vital role in PCa cell biology, indicating their potential as clinical targets and prognostic/predictive biomarkers for PCa bone metastasis.

P2X4 and P2X7 receptors in the expression and release of interleukin-1β by mononuclear phagocytes

Luca J. Ettischer[1], Marie S. Dippel[1], Anna I. Herbolsheimer[1], Andreas Hecker[1], Juliane Liese[1], Christa E. Müller[2], Fritz Markwardt[3], Günther Schmalzing[4], Veronika Grau[1], Katrin Richter[1]

[1] Laboratory of Experimental Surgery, Department of General and Thoracic Surgery, Justus-Liebig-University Giessen, German Centre for Lung Research (DZL), Cardio-Pulmonary Institute (CPI), Giessen, Germany

[2] Pharmaceutical Institute, Pharmaceutical & Medicinal Chemistry, University of Bonn, Bonn, Germany

[3] Julius-Bernstein-Institute for Physiology, Martin-Luther-University, Halle-Wittenberg, Germany

[4] Institute of Clinical Pharmacology, RWTH Aachen University, Aachen, Germany

Abstract

In mononuclear phagocytes activation of the ATP receptor P2RX7 regulates the lipopolysaccharide-induced biosynthesis of pro-inflammatory cytokines. Under pathological conditions such as major surgery or trauma, the extensive release of ATP by injured cells induces P2RX7 activation in innate immune cells, culminating in cytokine release including interleukin (IL)-1β. There is some evidence that the P2RX4 contributes to this process. We set out to clarify the role of the P2RX7 and P2RX4 in the lipopolysaccharide-mediated priming of mononuclear phagocytes and the ATP-dependent secretion of IL-1β. 5-BDBD, an P2RX4 inhibitor, enhanced the lipopolysaccharide-induced mRNA expression of IL1B by human monocytic THP-1 cells and THP-1-derived macrophages. Accordingly, the secretion of IL-1β in response to an ATP-independent stimulus (the pore-forming toxin nigericin) was increased. When the P2RX4 inhibitor PSB-15417 or the P2RX7-specific inhibitor A438079 were used, a minimal increase in IL-1β secretion was detected. These data suggest that the P2RX4 down-modulates the lipopolysaccharide-induced biosynthesis of pro-IL-1β. More experiments are, however, warranted to confirm this conclusion and to explain the functional differences between 5-BDBD and PSB-15417. When lipopolysaccharide-primed mononuclear phagocytes were stimulated with ATP, A438079 fully inhibited ATP-dependent IL-1β secretion by monocytic THP-1 cells, THP-1-derived macrophages, and primary human and murine mononuclear phagocytes. Moreover, 5-BDBD and PSB-15417 dose-dependently blunted the ATP-dependent secretion of IL-1β, suggesting that the

P2RX4 contributes to the signalling of ATP in this context. Our findings might be of eminent clinical relevance, because lipopolysaccharide-driven IL-1β expression is essential for host defence against pathogens, while the ATP-induced IL-1β secretion contributes to trauma-associated hyperinflammation.

P2X7 receptor–induced NLRP3 inflammasome as a biomarker of prognosis in sepsis: Viva IVD approach

Veronica Canovas[1], Pablo Gonzalez-Polanco[1], Maria C. Baños-Gregori[1], Diego Angosto-Bazarra[1,2], Laura Hurtado-Navarro[1,2], Juan J Martínez-García[1,2,3], Pablo Pelegrin[1,2,3]

[1] VIVA IN VITRO DIAGNOSTICS S.L., Parque Científico de Murcia, 30100 Murcia, Spain

[2] Instituto Murciano de Investigación Biosanitaria IMIB, Hospital Clínico Universitario Virgen de la Arrixaca, 30120 Murcia, Spain.

[3] Departamento de Bioquímica y Biología Molecular B e Inmunología, Facultad de Medicina, Universidad de Murcia, 30120 Murcia, Spain

Abstract

Sepsis is a life-threatening organ dysfunction caused by a dysregulated immune host response to infection. In 2017, the World Health Organization reported 49 million cases and 11 million sepsis-related deaths, accounting for approximately 20% of all annual deaths globally. The mortality rate increases with delayed diagnosis or with inappropriate antibiotic therapy, which implies, besides the human losses, a high economic cost for the healthcare systems. The NLRP3 inflammasome is a key component of the innate immune system that mediates caspase-1 activation and the secretion of proinflammatory cytokines in response to microbial infection and cellular damage. The activation of the purinergic P2X7 receptor is a potent trigger of NRLP3 inflammasome, that culminates with the formation of a multiprotein complex composed of the innate immune receptor protein NLRP3, adapter protein ASC and inflammatory protease caspase-1. The formation of this assembled multi-protein complex is a distinguishing and critical feature of inflammasome activation. VIVA IN VITRO DIAGNOSTICS (VIVAIVD) exploits a crucial discovery in septic patients: during the first 24 hours of the inflammatory response in sepsis, some septic patients show early impairment of the P2X7–induced NLRP3 inflammasome activation, which is linked to over 80% of sepsis-related deaths. Building on this critical finding, VIVAIVD is developing an in vitro diagnostic (IVD) device for the fast detection of the P2X7–induced NLRP3 inflammasome activation in whole blood samples based on the detection of the assembled NLRP3 complex in monocytes.

P2X7R: a key determinant of microparticles and mitochondria trafficking in mouse microglia

Valentina Vultaggio-Poma[1], Paola Chiozzi[1], Simonetta Falzoni[1], Giada Salvi[1], Mario Tarantini[1], Elena Adinolfi[1], Paola Boldrini[2], Anna Lisa Giuliani[1], Dariusz C. Gorecki[3], Francesco Di Virgilio[1]

[1] Department of Medical Sciences and [2]Center for Electron Microscopy, University of Ferrara, Italy

[3] School of Pharmacy and Biomedical Sciences, University of Portsmouth, UK

Abstract

Microparticles (MPs) are ubiquitously secreted by all cells and play a fundamental role in numerous biological processes such as cell-to-cell communication, cell differentiation, inflammation, and cell energy transfer. Stimulation by extracellular ATP (eATP) of the P2X7 receptor (P2X7R) is one of the most powerful stimuli for MP release. We investigated MP release in the P2X7R-expressing mouse microglial cell line (N13- P2X7RHigh) and in the N13R microglial cell line selected for low P2X7R expression (N13- P2X7RLow). Released MPs were investigated using confocal microscopy, electron microscopy and western blot analysis. N13-P2X7RHigh cells release a massive amount of MPs in response to stimulation with eATP, that was largely reduced in the N13-P2X7RLow. Released MPs are a heterogenous population of small vesicles, mitochondria-containing vesicles, and naked mitochondria, that are transferred to recipient cells in a P2X7R-dependent fashion. MPs released from N13-P2X7RHigh (MP-P2X7RHigh) had a much higher content of mitochondria than in those released from N13-P2X7RLow (MPP2X7RLow). MP-P2X7RHigh transferred the P2X7R to N13-P2X7RLow and restored P2X7R-dependent responses, among which eATP-dependent reversible plasma membrane depolarization and ability to generate multinucleated giant cells (MGCs), a hallmark of chronic inflammation. These data show that the P2X7R is a master regulator of intercellular MP trafficking and MP-mediated transfer between microglial cells might be an efficient mechanism for the modulation of neuroinflammation.

P2X7R activation by extracellular ATP rapidly regulates mitochondrial Pyruvate Dehydrogenase complex

Fatima Martin-Sanchez[1], Rodrigo Diaz-Pino[1], Oliver Wagstaff[1], Jo Watson[2], JeanMarc Schwartz[2], Chiara Francavilla[3], Gloria Lopez-Castejon[1].

[1]Lydia Becker Institute of Immunology and Inflammation, Division of Immunology, Infection and Respiratory Medicine, School of Biological Sciences, FBMH, University of Manchester, UK.

[2]Division of Evolutions, Infection and genomics, School of Biological Sciences, FBMH University of Manchester, UK.

[3]Division of Molecular and Cellular Functions, School of Biological Sciences, FBMH University of Manchester, UK.

Abstract

Activation of P2X7R by extracellular ATP in macrophages mediates important inflammatory responses such as, but not limited to, inflammasome activation and cell death. A relation between P2X7R activity and mitochondrial functions, including links to metabolic homeostasis have been reported. However, these remain poorly understood. Here we set to investigate the regulation of the mitochondrial Pyruvate Dehydrogenase (PDH) complex, a key player in the TCA cycle by converting Pyruvate to Acetyl-CoA, by the P2X7R. We focused on the regulation of PDHa a key enzyme controlling the function of this complex. We found that in wild type, but not P2X7R deficient macrophages, treatment with high ATP concentrations led to PDHa dephosphorylation at sites conductive to its activation. This occurred in macrophages in a resting as well as a pro-inflammatory state induced by treatment with LPS. We characterised the dynamics of this regulation and found that exposure to high ATP concentrations for 5 min was sufficient to trigger dephosphorylation of PDHa and that de-phosphorylation was lost upon 60 min of continued ATP activation. Intensity of P2X7R activation by ATP was also important as lower ATP concentrations were less efficient at phospho-regulation of PDHa despite activation of the receptor. All of this suggests that activation of P2X7R by high concentrations of ATP lead to an alteration on mitochondrial

metabolic pathways, which could be linked to mitochondrial disruption associated to inflammation. Our work sheds light into new mechanisms of activation of PDH complex in macrophages under stress responses during inflammation.

Pharmacological characterization of the P2X7 receptor radioligand [3H]JNJ-64413739: Species differences and variation in a human population

Jens D. Mikkelsen[1,2] and Sanjay S. Arapika[1]

[1] Neurobiology Research Unit, University Hospital Rigshospitalet, Copenhagen, Denmark

[2] Institute of Neuroscience, University of Copenhagen, Denmark

Abstract

P2X7R is expressed in the brain and increased under neuroinflammation. Because P2X7R is increased under inflammation and expressed in microglia the radioligand [18F]JNJ-64413739 has been developed to determine neuroinflammation in the living human brain. In this study, we tritiated JNJ-64413739 and characterized its binding properties in the human brain in comparison to mouse brain using autoradiography. In the human temporal cortex, the saturation profile indicated one binding site that is saturable in concentrations up to 100 nM. The Bmax in the white matter was ~40% higher than Bmax in the grey matter. The binding showed a high affinity and the KD for the two compartments was similar and calculated to be around 7 nM. Three ligands were able to reach full displacement of [3H]JNJ-64413739 with calculated best fit IC50: 20 nM for JNJ-64413739, 70 nM for JNJ-47965567, and 90 nM for Lu AF27139, respectively. In contrast, saturation of [3H]JNJ-64413739 was not be achieved in the mouse brain, indicating that the binding pocket in the mouse P2X7 receptor is different from human. In human cortical tissue sections resected from a total of 48 patients with treatment resistant epilepsy a large variability in saturable binding among the samples was found. The Bmax values were not correlated to either age, sex, or the duration of the disease. These data demonstrate that [3H]JNJ-64413739 is a suitable radioligand for evaluating the distribution and expression of the P2X7R in the human brain, but that the level of binding is highly variable in a population.

Possible Presence of P2X7R on Mammalian Cardiomyocytes

Sinan Değirmenci[1], Gül Şimşek[1], Mehmet Uğur[1].

[1] Ankara Üniv. Faculty of Medicine, Dpt. Biophysics, Ankara Türkey.

Abstract

It has been shown by using immuno-histochemistry that P2X7 receptor (P2X7R) staining is present on cell membranes of both mice atrial cardiomyocytes and HL-1 cells. HL-1 is a mouse atrial cardiomyocyte-derived immortal cell line that preserves many characteristics of cardiomyocytes. However, whether this published immuno-staining corresponds to an actual functional P2X7R had not been investigated. By using whole cell patch clamp and live cell fluorescent microscopy techniques, we have shown that HL-1 cells actually have a functional ATP-activated receptor which shows pharmacological and electrophysiological properties quite similar to P2X7R. Both agonist and antagonist specificities of this receptor exactly match that of the P2X7R. These findings show the presence of functional P2X7R on the HL-1 cell line. However, we were unable to show the presence of functional P2X7 receptors on the dissociated atrial cardiomyocytes. This negative result may indicate nonspecific staining by the anti-P2X7R Abs. Alternatively, it may be due to a very restricted expression of this receptor in the atrial tissue (for example only within the SA node). This point requires further investigation.

Relevance of CD73 in melanoma as crucial checkpoint in the conversion of extracellular ATP into adenosine

Roberta Turiello[1], Caterina Giraulo[2], Lavinia Orlando[2], Raffaella Belvedere[2], Silvana Morello[2]

[1] Institute of Experimental Oncology, University Hospital Bonn (UKB), University of Bonn, Germany

[2] Department of Pharmacy, University of Salerno, Italy

Abstract

Within tumour microenvironment extracellular adenosine is mainly generated by hydrolysis of ATP via two ectoenzymes CD39 and CD73. CD73 represents a crucial checkpoint in the conversion of proinflammatory ATP into immunosuppressive adenosine, which inhibits the activity of T cells mainly through A2A adenosine receptor, in a cAMP-dependent manner, limiting in turn the efficacy of T cell-targeted therapies. The CD73 has been found overexpressed in different types of cancer. Ongoing studies conducted in our laboratory are focused on investigating the clinicopathological relevance of CD73 in melanoma. Results show that patients with melanoma show high levels of extracellular CD73 compared with healthy subjects and high serum CD73 activity resulted associated with no-response to immunotherapy and with reduced overall survival and progression-free survival. In serum of melanoma patients CD73 is also expressed on exosomes, and its expression and activity resulted increased in patients who do not respond to therapy. Moreover, within melanoma lesions CD73 is highly expressed in dedifferentiated cells. Further investigations performed in vitro in human melanoma cells, demonstrate that cells exposed to nutrient deprivation, which is a common feature of the tumour microenvironment, expressed high levels of CD73, accompanied by acquisition of an EMT-like signature. At the same time, we observed that in this condition CD73 is highly released in a soluble form, contributing to produce elevated levels of adenosine. These results provide new insights into the expression profile of CD73 in melanoma for a better understanding of its prognostic value and therapeutic potential in cancer.

Rescuing Tri-Heteromeric NMDA Receptor Function: The potential of Pregnenolone-Sulfate in Loss-of-Function GRIN2B Mutations

Shai Kellner, Shai Berlin

Department of Neuroscience, Ruth and Bruce Rappaport Faculty of Medicine, Technion- Israel Institute of Technology, Israel.

Abstract

N-methyl-D-aspartate receptors (NMDARs) are tetrameric receptors that typically consist of GluN1 and GluN2(A-D) subunits, giving rise to di- and tri-heteromeric forms. During prenatal development, the GluN2B subunit predominates in di-heteromeric receptors composed of two GluN1 and two GluN2B subunits, whereas during postnatal stages, GluN2A emerges leading to the appearance of tri-heteromers containing GluN1, GluN2A, and GluN2B subunits. Mutations in different NMDAR subunits are closely associated with severe pediatric neurodevelopmental disorders and encephalopathies known as GRINopathies, to which disease-modifying treatments are lacking. Despite extensive research on purely mutant di-heteromeric receptors, the impact of GRIN variants on mixed di and tri-heteromers remains poorly understood. Here, we investigated the effects of two de novo GRIN2B variants in pure, mixed di- and tri-heteromeric receptors by employing a molecular method to control channel stoichiometry at the membrane. We find that incorporation of a single variant in mixed di-heteromers or tri-heteromers results in a dominant negative effect over glutamate potency. In contrast, the variants do not impair the ability of the receptors to respond to positive allosteric modulators, particularly pregnenolone-sulfate (PS). PS had a strong and positive effect over current amplitude and synaptic activity in cultured neurons expressing the variants. Together, our study represents the first investigation of severe loss-of-function GRIN2B mutations in the context of mixed di- and tri-heteromeric receptors and provides the initial demonstration of the beneficial effects of a GRIN2B-relevant potentiator on tri-heteromers. Together, our study contributes to the ongoing efforts to understand the pathophysiology of GRINopathies and offers insights into potential treatment strategies.

Role of P2X7 receptor as intriguing pharmacological target in retinal neurodegenerations

Chiara Bianca Maria Platania[1,2], Francesca Lazzara[1], Federica Conti[1], Giovanni Luca Romano[1,2], Filippo Drago[1,2], Claudio Bucolo[1,2]

[1] Department of Biomedical and Biotechnological Sciences, School of Medicine, University of Catania.

[2] Center for Research in Ocular Pharmacology-CERFO, University of Catania, Catania, Italy.

Abstract

The P2X7 receptor is a trimeric ATP-gated cation channel and its activation results in several downstream events. Particularly, P2X7 receptor activates inflammatory pathways, then participating with a pivotal role in etiopathogenesis of neurodegenerative diseases. Several studies evidenced that P2X7 receptor activation is implicated in pathophysiology of retinal neurodegenerations, such as glaucoma and age-related macular degeneration. Furthermore, other preclinical studies provided evidence on detrimental role of P2X7 receptor in diabetic retinopathy. These studies have shown that P2X7 receptor would be involved not only in triggering retinal inflammation, but also in the modulation of pathological angiogenesis and blood retinal barrier breakdown. These mentioned retinal diseases are devastating conditions with huge impact on patient quality of life. Moreover, retinal degenerations are associated to economic burden, linked to direct and indirect costs for irreversible vision loss and disease's management. The role of the P2X7 receptor in retinal age-related conditions such as glaucoma, age-related macular degeneration, and diabetic retinopathy will be discussed. Furthermore, results on pharmacological modulation of the P2X7 receptor activity in in-vitro and in-vivo models of retinal diseases will be presented, providing evidence that P2X7 receptor antagonists might have a relevant clinical impact to treat retinal diseases.

Synthesis and biologically evaluation of aminopyridine derivatives targeting P2X receptors

Murat Güney[1,2], Deniz Ekinci,[3] Murat Şentürk[4]

[1]Ağrı İbrahim Çeçen University, Faculty of Science and Art, Department of Chemistry, 04100, AĞRI, TÜRKİYE

[2] Ağrı İbrahim Çeçen University, Faculty of Pharmacy, 04100, AĞRI, TÜRKİYE

[3] Ondokuz Mayıs University, Department of Agricultural Biotechnology, Faculty of Agriculture, 55139, SAMSUN, TÜRKİYE

[4] Ağrı İbrahim Çeçen University, Faculty of Pharmacy, Department of Biochemistry, 04100, AĞRI, TÜRKİYE

Abstract

Divided into seven subunits, P2X receptors are ligand-gated, non-selective cation channel receptors involved in a variety of physiological functions, including the cardiovascular, neuronal, and immune system.[1] The synthesis of molecules that can be antagonists or agonists for P2X receptors is also of great interest by researchers.[2] For example, Dichloroarylpurinyletanones were synthesized as antagonists for P2X7, which occurs in the pathogenesis of many neurodegenerative diseases, and their pharmacological properties were investigated.[3] Considering all of these, we aimed to synthesize the aminopyridine derivatives given in figure 1 below and examine their effects on P2X receptors.

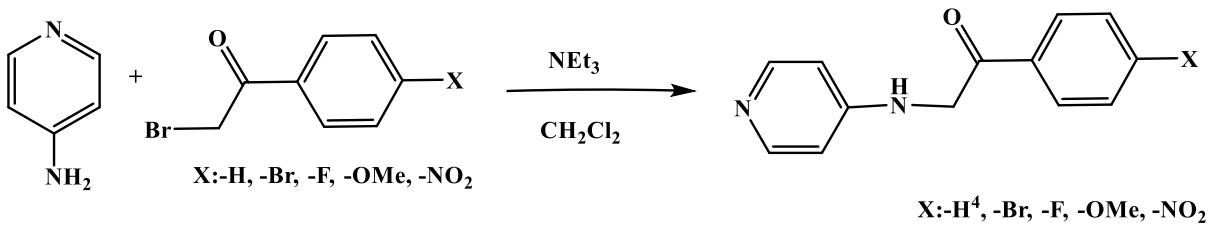

Fig. 1. Aminopyridine derivatives targeting P2X receptors.

Targeting Adenosine Signaling and Generation: What has and has not worked?

Kris Sachsenmeier

Precision and Translational Medicine, Oncology Therapeutic Unit, Takeda Pharmaceuticals

Abstract

Over the past two decades, a series of both preclinical and clinical studies have examined the role of adenosine generation and signalling in cancer. This work has formed a compelling scientific narrative, yet with mixed clinical results in cancer. Highlights have included clear demonstration of adenosine inhibition on various aspects of anti-tumour immunology – including both innate and adaptive immunity in both lymphoid and myeloid cell lineages. Targeting the adenosine axis clearly enhances both T cell mediated activity and antigen presentation. And yet the range of anti-tumour activities seen in preclinical settings has not led to a matching range in patients. Data presented here reviews these studies and concludes with a brief discussion of a clinical setting in which targeting adenosine generation is still being pursued and the importance of accounting for tumour-intrinsic factors in adenosine biology.

The P2X7receptor: the physiopathological function of the macropore unveiled (maybe)

Francesco Di Virgilio

Department of Medical Sciences, University of Ferrara (Italy)

Abstract

The P2X7 receptor (P2X7R) is now an (almost) generally accepted player in inflammation, yet one of its most peculiar features, the ability to form the so called “macropore”, remains little understood. Ever since the early ages we proposed that, due to macropore activity, the P2X7R (or P2Z, as it was once referred to) is: a) a cytotoxic receptor; b) a fusogen; c) a stimulant of the NLRP3 inflammasome, thanks to its impressive K^+ permeability; c) a pathway for ATP release. Now, more data reinforce the key role of the P2X7R macropore in inflammation. Yan and coworkers showed that the P2X7R pore is needed to allow cellular uptake of the STING stimulant cGAMP, and therefore to elicit a proper anti-tumoral (or antiviral) immune response. Altogether, these findings may help better understand the physiopathological function of the enigmatic P2X7R macropore.

The PML-NLRP3-P2X7R axis modulates the anti-cancer response

Sonia Missiroli[1], Mariasole Perrone[1], Roberta Gafà[2], Francesco Nicoli[3], Massimo Bonora[1], Giampaolo Morciano[1], Caterina Boncompagni[1], Saverio Marchi[4], Magdalena Lebiedzinska-Arciszewska[5], Bianca Vezzani[1], Giovanni Lanza[2], Franz Kricek[6], Alessandro Borghi[7], Francesco Fiorica[8], Keisuke Ito[9], Mariusz R. Wieckowski[5], Francesco Di Virgilio[1], Luigi Abelli[10], Paolo Pinton[1] and Carlotta Giorgi[1]

[1]*Department of Medical Sciences, Section of Experimental Medicine and Laboratory for Technologies of Advanced Therapies (LTTA), University of Ferrara, Ferrara, Italy.*

[2]*Department of Translational Medicine, University of Ferrara, Ferrara, Italy; Anatomic Pathology Unit, University Hospital of Ferrara, Ferrara, Italy.*

[3]*Department of Chemical, Pharmaceutical and Agricultural Sciences, University of Ferrara, Ferrara, Italy.*

[4]*Department of Clinical and Molecular Sciences, Marche Polytechnic University, Ancona, Italy.*

[5]*Laboratory of Mitochondrial Biology and Metabolism, Nencki Institute of Experimental Biology, Warsaw, Poland.*

[6]*NBS-C Bioscience & Consulting GmbH, Vienna, Austria.*

[7]*Department of Medical Sciences, Section of Dermatology and Infectious Diseases, University Hospital of Ferrara, Ferrara, Italy*

[8]*Department of Radiation Oncology and Nuclear Medicine, AULSS 9 Scaligera, Verona, Italy.*

[9]*Ruth L. and David S. Gottesman Institute for Stem Cell and Regenerative Medicine Research, Departments of Cell Biology and Medicine, Albert Einstein College of Medicine, Bronx, New York.*

[10]*Department of Life Sciences and Biotechnology, Section of Biology and Evolution, University of Ferrara, Italy.*

Abstract

Uncontrolled inflammatory response arising from the tumor microenvironment (TME) significantly contributes to cancer progression, prompting an investigation and careful evaluation of counter-regulatory mechanisms. In this study, we revealed a previously

unknown localization of purinergic P2X7 receptor at the endoplasmic reticulum (ER)/ mitochondria-associated membranes (MAMs) where directly interacts with both NLRP3 and Promyelocytic Leukemia Protein (PML), forming a triumvirate complex. PML downregulation promotes an exacerbated immune response to stress conditions due to the increased redistribution and interaction of NLRP3 and P2X7R at MAMs. This in turn promotes tumor growth, exerting a direct effect on cancer cells. Tumor growth was strongly reduced by inhibiting host NLRP3, acting on P2X7R, a major inflammasome activator, or directly impeding NLRP3 assembly.

The role of P2X7 receptor in mouse models of depression

Cecilia Csölle, PhD[1] Lilla Otrokocsi, PhD[1] Bernadett Iring-Varga[1] Pál Tod, PhD[1] Prof. Beáta Sperlágh, DSc[1]

[1] Laboratory of Molecular Pharmacology, Institute of Experimental Medicine, Budapest, Hungary

Abstract

The purinergic system is implicated in various physiological and pathological processes of the central nervous system. Growing body of evidence suggests that ATP-sensing ionotropic P2X or metabotropic P2Y receptors play a major role in neurodegenerative diseases or depression. Our aim was to study the effect of P2X7 receptor (P2X7R) loss in depression. Adult (2-3 month old) C57BL/6J wild-type (WT) and P2X7R knockout (KO) mice were used. To investigate the depression-like behaviour the following test were performed: tail suspension test, forced swim test, sucrose preference test, and learned helplessness test (LHT). The effect of the genetic deletion of P2X7R on the neurotransmitter release was measured in living tissue perfusion technique. Monoamine content of various brain areas were measured by HPLC. Immunohistochemistry and electron microscopy were used to visualize the impact of P2X7R loss on the cellular level in depression. A whole genome microarray analysis was performed for the study of genetic alterations in depression. The relevant findings were further examined using RT-qPCR and Western blot or ELISA methods. P2X7R loss attenuated the depression-like behaviour in KO mice compared to WT animals. P2X7R partly mediated the antidepressant effect of zinc. P2X7R deletion restored the loss of the spine synapse density after LHT. The glutamatergic signalling and monoamine levels was influenced by the loss of P2X7R in various brain areas. The microarray and protein analysis revealed several genetic alterations important in synaptic function and neuroplasticity. In conclusion, P2X7 receptors may play a role in depression through alteration of neuronal plasticity and neuronal signalling.

The shed P2X7R (SP2X7R) is an index of adverse clinical outcome in COVID-19 patients

Anna Lisa Giuliani[1], Valentina Vultaggio-Poma[1], Juana Sanz Molina[2], Sara Ghisellini[3], Simonetta Falzoni[1], Angelina Passaro[4], Francesco Di Virgilio[1]

[1] Department of Medical Sciences, University of Ferrara, Ferrara, Italy

[2] Department of Chemical, Pharmaceutical and Agricultural Sciences, University of Ferrara, Ferrara, Italy

[3] Laboratory Division of the S. Anna Hospital, University of Ferrara, Ferrara, Italy

[4] Department of Translational Medicine, University of Ferrara, Ferrara, Italy

Abstract

Despite the great number of studies, the pathophysiology of the Corona Virus Disease 2019 (COVID-19), caused by SARS-CoV-2 infection, is nowadays incompletely known. A robust inflammatory response caused by viral replication is a main cause of the acute lung and multiorgan injury observed in critical patients. The P2X7R, as a main activator of the NLRP3 inflammasome, of the ensuing release of inflammatory cytokines and of cell death by pyroptosis, has been implicated in COVID-19-dependent hyperinflammation and in the associated multiorgan damage. Shed forms of P2X7R (sP2X7R) and NLRP3 (sNLRP3) have been previously detected in plasma and other body fluids, especially during infection and inflammation. The aim of this study was to investigate whether the sera levels of sP2X7R and sNLRP3 might be related to, and possibly predict, the COVID-19 progression and outcomes. Blood samples from 96 patients with confirmed SARS-CoV-2 infection with various degrees of disease severity were tested at diagnosis at hospital admission. Standard haematological parameters and IL-6, IL-10, IL-1β, sP2X7R and sNLRP3 levels, were measured, compared to reference values, and correlated to disease progression and clinical outcome. Most COVID-19 patients included in this study presented lymphopenia, eosinopenia, neutrophilia, increased inflammatory and coagulation indexes and augmented IL-6 and IL-10 levels, as expected.

sP2X7R and sNLRP3 blood levels were also increased. Interestingly, sP2X7R significantly positively correlated with lymphopenia, IL-10, and procalcitonin, a useful marker of infection. Increased sP2X7R levels at diagnosis were more frequent in patients who showed fever and respiratory symptoms, were more often transferred to Pneumology division, required mechanical ventilation, and had a higher likelihood to die during hospitalization. sP2X7R might therefore be a useful marker of COVID-19 disease progression.

The synthesis and P2X receptor pharmacology of endogenous steroids bearing an amide-based structural motif

Hana Zemkova[1] and Eva Kudova[2]

[1] Institute of Physiology, Czech Academy of Sciences, Prague, Czech Republic

[2] Institute of Organic Chemistry and Biochemistry, Czech Academy of Sciences, Prague, Czech Republic

Abstract

Neurosteroids are steroids synthesized de novo in the brain from cholesterol. The term "neuroactive steroid" includes also newly synthesized analogues of steroids that modify neuronal activities by rapid modulation of membrane receptors and ion channels including P2X. Many neurosteroids are currently widely studied as potential neuroprotective therapeutics affecting the process of recovery from brain/CNS injury or disease, and drugs that could be used for treatment of neurodegenerative disorders. We designed, synthesized, and tested in vitro 15 neurosteroids with amide structural motif that could improve allosteric modulatory effect of these drugs. We examined the effect of steroidal amides in HEK293 cells expressing P2X2, P2X4 and P2X7, as well as in pituitary cells and hypothalamic neurons endogenously expressing these receptors. We found that androstanes bearing C-3 amino acid substituent that is connected with steroidal skeleton via metabolically stable amide moiety effectively potentiate P2X receptors expressed in HEK cells while androstanes bearing C-3 amino acid substituents connected with steroidal skeleton via ester bond, that should improve the solubility of these compounds as compared with amides, are ineffective. Potentiating effect of steroidal androstanes could be further improved by free NH2 group in C-3 dicarboxylic acid substituent. Steroids potentiated equally recombinant P2X2, P2X4 and P2X7, as well as endogenously expressed P2X receptors. These results showed that steroids with an amide-based structural motif can be used as general activators of P2X, and that positive allosteric modulatory effect of these drugs on P2X have to be considered when these steroids are tested for their potential clinical use.

Towards a Positron Emission Tomography (PET) tracer for purinergic P2X7 receptor for molecular imaging of neuroinflammation

Enza Lacivita[1], Imane Ghafir El Idrissi[1], Francesca Intranuovo[2], Diego Dal Ben[2], Andrea Spinaci[2], Michela Buccioni[2], Rosaria Volpini[2], Marcello Leopoldo[1]

[1] Dipartimento di Farmacia-Scienze del Farmaco, Università degli Studi di Bari Aldo Moro, Via Orabona 4, 70125 Bari (Italy)

[2] Scuola di Scienze del Farmaco, Università di Camerino, Via Madonna delle carceri, 62032 Camerino (Italy)

Abstract

Neuroinflammation is an adaptive response of the Central Nervous System to diverse potentially harmful stimuli, closely associated with neurodegeneration and typically characterized by activation of microglia and astrocytes. Even though neuroinflammation is a key pathological hallmark of numerous neurological diseases, its exact role in vivo is not fully understood. Positron Emission Tomography (PET) has emerged as a non-invasive and translational imaging technique to visualize and quantify in vivo biochemical processes in real time. As such, PET could help study neuroinflammation and its role in neurological disorders. The purinergic P2X7 receptor (P2X7R) is among the targets actively investigated as PET biomarkers of neuroinflammation because of its crucial role in microglia activation. Several P2X7R antagonists have been radiolabelled and studied as potential PET radioligands in the last decade. Most of these radioligands have poor metabolic stability, low brain uptake in preclinical models, or limitations in clinical studies. To identify an effective P2X7R PET tracer, we selected from the literature three different chemotypes showing the promise for high P2X7R potency and selectivity, tunable physicochemical characteristics to fit the profile of a PET radioligand, and ease of radionuclide incorporation. Here we describe our design strategy to obtain potential P2X7R PET tracers and the activity data of the newly synthesized compounds.

Translating the roles of P2X4 and P2X7 in demyelination and remyelination into multiple sclerosis therapies

Carlos Matute, Fernando Pérez-Cerdá and Alberto Pérez-Samartín

Achucarro Basque Center for Neuroscience, University of the Basque Country and CIBERNED, 48940-Leioa, Spain

Abstract

We and others found that P2X4 and P2X7 receptors are expressed in microglia and oligodendrocytes, two cellular targets key to multiple sclerosis (MS) pathology and therapeutics. Our laboratory observed that P2X7 receptors mediate excitotoxity both in neurons and in oligodendrocytes in experimental models of stroke and MS. Notably, blockade of P2X7 receptors ameliorates the symptoms and neuropathology in both disease paradigms. These findings add to previous results by us showing that glutamate receptors also contribute to oligodendrocyte excitotoxicity in progressive MS. With the ultimate aim of developing a complete preclinical study, we are exploring the possibility that the combine effect of both glutamate and P2X receptors can generate further protection in two MS models, the cuprizone and the experimental autoimmune encephalitis (EAE). Ideally, novel more selective/specific and potent P2X7 antagonist should be tested for which we seek partnership with our PRESTO partners. On the other hand, we also found that P2X4 receptors control the fate and survival of activated microglia and their phenotype in response to injury. Thus, P2X4 receptor levels and function are upregulated in activated microglia in inflammatory foci in EAE as well as in MS lesions. In turn, enhanced P2X4 receptor signalling in activated microglia by the positive allosteric modulator ivermectin promotes a regenerative phenotype of microglia that contributes to amelioration of EAE symptoms. Again, novel more selective/specific and potent P2X4 allosteric modulators should be tested for which we seek partnership with our PRESTO partners. Together, these findings indicate that targeting simultaneously P2X4 and P2X7 receptors with combined therapies may greatly improve progressive MS both primary and

secondary, a disease condition for which there is current treatment. Supported by ARSEP, Spanish Ministry of Education, Basque Government and CIBERNED.

Use of mouse models to study P2X7 localization and function- Caveats and pitfalls in P2X7 research

Annette Nicke

Walther Straub Institute of Pharmacology and Toxicology, LMU Munich, Germany

Abstract

The P2X7 receptor subtype is present in epithelia, glia and immune cells, and is involved in cytokine release and T-cell differentiation. P2X7 up-regulation is observed under pathophysiological conditions and P2X7 blockade or deletion reduces inflammation and tissue damage in a variety of disease models. In contrast to other P2X receptor subtypes, the P2X7 has a low ATP sensitivity and a large intracellular domain that triggers multiple short and long-term effects like cytokine release, gene transcription, cell proliferation and cell death. These effects appear to be cell type-dependent. Since P2X7 is activated upon tissue damage, when high concentrations of ATP are present, it is considered as a promising drug target. However, the sites and duration of P2X7 activation and its activity under (patho)-physiological conditions have been hampered by the use of nonspecific ligands as well as a lack of sensitive and selective antibodies. In contrast to its generally accepted roles in T cell differentiation and interleukin secretion by phagocytic cells, its functions in epithelia, oligodendrocytes and astrocytes are less well investigated and its presence and functions in neurons and other cell types remain debated. To address this issue, a variety of mouse models have been generated. In this presentation, the generation and characterization of the available mouse models will be described, and possible caveats will be discussed.

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