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Libretto degli Abstracts

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A NEW F1/FO-ATP SYNTHASE INHIBITOR FOR THE TREATMENT OF MYOCARDIAL REPERFUSION INJURY

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Recent cardiology research studies have reported that the opening of a large pore in the mitochondrial membrane, namely the mitochondrial permeability transition pore (mPTP), is one of the major contributory factors of the myocardial reperfusion injury (RI), phenomenon, following myocardial infarction, that results from the blood flow restoration to the ischemic area, responsible for mitochondrial and cardiomyocyte death. We provided evidences that c subunit of the F1/FO-ATP synthase owns a pivotal role in mPTP formation and activity and thus we sought to test a new mPTP opening inhibitor directed against the c subunit for the treatment of RI. We first synthesized and explored the potential activity of a small-molecule analogue of oligomycin A, IB13, compound that has been identified as mPTP inhibitor. After proving its ability to accumulate selectively into mitochondria and to inhibit the mPTP activity by binding the c subunit, we tested its cardioprotective effect in an *ex-vivo* model of RI, by using the Langendorff system. In isolated hearts, perfusion at constant volume of 10μM IB13 resulted in a decrease in coronary perfusion pressure (CPP, -17.5±3.4%) and in end-diastolic pressure (EDP, -72±9.86%) with an increased left ventricle developed pressure (LVDP, +36.4±3.9%) that mark a reduction of the diastolic stiffness, vasoconstriction and the deterioration of myocardial performance. In RI group, 64% of cardiomyocytes were TUNEL positive, a percentage that was significantly reduced in the presence of IB13. These findings confirmed the ability of IB13 to inhibit mPTP opening *ex-vivo* and to limit the detrimental effect of myocardial RI.

Photocatalytic activity of polymer nanoparticles modulates intracellular calcium dynamics and reactive oxygen species in HEK-293 cells

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Optical modulation of living cells activity by light-absorbing exogenous materials is gaining increasing interest, due to the possibility both to achieve high spatial and temporal resolution with a minimally invasive and reversible technique and to avoid the need of viral transfection with light-sensitive proteins. In this context, conjugated polymers represent ideal candidates for photo-transduction, due to their excellent optoelectronic and biocompatibility properties. In this work, we demonstrate that organic polymer nanoparticles, based on poly(3-hexylthiophene) conjugated polymer, establish a functional interaction with an *in vitro* cell model (Human Embryonic Kidney cells, HEK-293). They display photocatalytic activity in aqueous environment and, once internalized within the cell cytosol, efficiently generate reactive oxygen species (ROS) upon visible light excitation, without affecting cell viability. Interestingly, light-activated ROS generation deterministically triggers modulation of intracellular calcium ion flux, successfully controlled at the single cell level.
In perspective, the capability of polymer NPs to produce ROS and to modulate Ca^{2+} dynamics by illumination on-demand, at non-toxic levels, may open the path to the study of biological processes with a gene-less approach and unprecedented spatio-temporal resolution, as well as to the development of new biotechnology tools for cell optical modulation.

The human amniotic fluid stem cell secretome triggers pro-angiogenic intracellular Ca^{2+} oscillations in human endothelial colony forming cells

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II trimester human amniotic fluid stem cells (hAFS) have shown to possess remarkable cardioprotective paracrine potential in different preclinical models of myocardial infarction (MI) and drug-induced cardiotoxicity. Preliminary unpublished results from our team also showed that the hAFS secretome, namely the hAFS-conditioned medium (hAFS-CM), can also strongly sustain in vivo angiogenesis in a murine model of acute MI. Herein, we characterized in details the role of hAFS-CM in triggering pro-angiogenic intracellular Ca^{2+} oscillations in human endothelial colony forming cells (ECFCs), which are mobilized in circulation upon a hypoxic stimulus to replace damaged endothelium and restore local blood perfusion. We found that the secretome obtained by hAFS undergoing hypoxic preconditioning induced *in vitro* tubulogenesis and intracellular Ca^{2+} oscillations in ECFCs. BAPTA, a membrane-permeant buffer of intracellular Ca^{2+} levels, prevented hAFS-CM-induced tube formation, thereby hinting at a crucial role for Ca^{2+}. The oscillatory Ca^{2+} response to hAFS-CM was triggered by extracellular Ca^{2+} entry and supported by rhythmical Ca^{2+} release from endogenous stores in the majority of cells. Pharmacological manipulation revealed that hAFS-CM induced intracellular Ca^{2+} oscillations were initiated by Vanilloid Transient Receptor Potential 4 (TRPV4). TRPV4-mediated Ca^{2+} entry, in turn, promoted the concerted interplay between inositol-1,4,5-trisphosphate ($InsP_3$)- and nicotinic acid adenine dinucleotide phosphate (NAADP)-Ca^{2+} release and store-operated Ca^{2+} entry (SOCE). These data demonstrated for the first time that hAFS secretome is able to promote angiogenesis via a specific Ca^{2+}-dependent manner.

Type 2 Diabetes alters Ca^{2+} handling in rat aortic vascular smooth muscle cells

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Diabetes mellitus (DM) is a group of metabolic diseases which are mainly characterized by hyperglycemia. Worldwide, type 2 DM (T2DM) constitutes one of the main causes of death, mainly due to associated cardiovascular complications. Vascular dysfunction, which is typical among individuals with diabetes, increases the risk of stroke, heart attack and diabetic end-organ damage. It is known that chronic hyperglycemia can lead to the development of hypertension, atherosclerosis and coronary disease through the damage of the vascular wall. In particular, T2DM affects vascular smooth muscle cells (VSMC), which regulate the vascular tone by contracting/relaxing as well contribute to vascular repair and angiogenesis by proliferating and migrating. All of these processes are dependent on intracellular Ca^{2+} changes. Therefore, the maintenance of Ca^{2+} homeostasis is crucial for correct functioning of VSMC. There is still not enough information on how exactly T2DM affects Ca^{2+} handling in VSMC. In this study, we found that intracellular Ca^{2+} handling is severely altered in cultured VSMC of Zucker Diabetic Fatty rats, a widespread model of T2DM. Inositol-1,4,5-trisphosphate-dependent Ca^{2+} release from the endoplasmic reticulum and Store-Operated Calcium Entry (SOCE) were significantly enhanced. Likewise, the activity of the three major Ca^{2+} extrusion systems, SERCA, PMCA and NCX, was increased, possibly to compensate the increased Ca^{2+} activity. Nevertheless, the duration of the ATP-induced Ca^{2+} transient was remarkably elongated. These results provide the first demonstration that the intracellular Ca^{2+} handling machinery is affected by T2DM, which could contribute to explain the chronic increase in blood pressure observed in the patients.

Histamine activates a Ca^{2+} signal in normal fetal lung fibroblasts

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Pulmonary fibrosis is a progressive interstitial lung disease characterized by accelerated remodeling of the lung architecture. Several studies have documented mast cells accumulation in the lungs of patients with fibrosis. Mast cells are a major source of histamine. In patients with pulmonary fibrosis, the increase in mast cells number is correlated with increased histamine concentration in the bronchoalveolar lavage fluid. In *in vitro* studies, histamine was able to stimulate lung fibroblast collagen synthesis, migration and proliferation. However, the transduction mechanisms leading histamine to these effects in lung fibroblasts are still unclear. An increase in intracellular free Ca^{2+} concentration ($[Ca^{2+}]_i$) is an important signal for many cellular processes. It has been shown that Ca^{2+} signaling mediates lung fibroblasts proliferation, migration, apoptosis and collagen production. Accordingly, this study aimed to examine the mechanisms underlying histamine-induced increase in $[Ca^{2+}]_i$ in normal fetal lung fibroblasts WI-38. WI-38 fibroblast cells were loaded with 3 μM Fura-2/AM and the $[Ca^{2+}]_i$ was monitored by microfluorimeric techniques. Histamine caused a concentration-dependent increase in $[Ca^{2+}]$. The application of maximal histamine concentration, i.e. 300 μM, elicited different patterns of Ca^{2+} signals in WI-38 fibroblasts. Pharmacological manipulation revealed that histamine activates a Ca^{2+} signal through Ca^{2+} release from intracellular stores mediated by phospholipase C and inositol 1,4,5-trisphosphate receptors and Ca^{2+} influx via a store-operated pathway. These results suggest that intracellular Ca^{2+} signals could mediate histamine-induced collagen production, proliferation and migration in human fibroblasts.

Altered firing and Cav1.2 channel inactivation of hippocampal neurons and adrenal chromaffin cells in the Timothy syndrome type 2 (TS2-neo) autistic mouse model: rescuing effects by DHP antagonists

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Timothy syndrome (TS) is a rare genetic disease caused by a single point mutation in the pore forming α1-subunit of L-type Cav1.2 calcium channels (CACNA1C) that, in TS2-type form occurs either at gly406 (G406R) or gly402 (G402S) within exon 8 (Splawski et al. PNAS, 2005) generating long-QT and autism spectrum disorder.
Here, we show that L-type currents of MCCs of TS2-neo mice exhibit slower inactivation and leftward shifted V-dependent activation and inactivation, causing an increased "window" Ca2+ current at resting potentials. Conditions that are responsible for the altered spontaneous and evoked firing pattern in TS2-neo vs. WT cells.
In good agreement with this, the L-type currents of TS2-neo mice hippocampal neurons (HNs) appear markedly less inactivating and leftward shifted as compared to WT when recorded in young neurons (3-7 days in vitro, DIV). Current-clamp data indicate that only 50% of TS2-neo young neurons exhibit evocked regular firing, as compared to 100% WT. Moreover, young TS2-neo HNs(7 DIV) recorded by means of MEA exhibit lower mean frequency as compared to WT accompanied by a significant higher number of "bursts" and "bursts duration". Overall, our findings suggest that, as in MCCs, also mutated HNs are severely impaired.
In addition, overnight incubation (15-18 h) with low doses of nifedipine (300 nM) completely restore the normal firing activity both in MCCs and in HNs, suggesting novel possible therapeutic approaches for autism using DHP antagonists.
Our study provides clear evidence that the TS2-neo mouse is a valid animal model for studying the altered molecular components regulating neuronal excitability and neurotransmitter release in genetic diseases associated with autism spectrum disorder.

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New insights on the role of Store-operated calcium-entry in rare genetic diseases

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Abstract:

Calcium is a ubiquitous intracellular second messenger with a central role in a wide range of processes, which abnormality are related to a number of human diseases. In recent years STIM and Orai, the key proteins that mediate Store-Operated Calcium Entry (SOCE, the ability of cells to sense a decrease in endoplasmic reticulum luminal calcium and induce Ca^{2+}-entry across the plasma membrane), have been associated to a number of disorders and suggested to be druggable targets.
Although SOCE is present in all type cells, this intracellular mechanism has an essential role in muscle cells and platelets. Gain-of-function STIM1/Orai1 mutations cause severe health issues, including muscle defects, and disturbances, platelet dysfunction and bleeding disorders.
Three separate disorders, tubular aggregate myopathy (TAM), Stormorken and York platelet syndrome, can be re-conducted to gain-of–function point mutations of the STIM1 or Orai1 genes and currently they represent an unmet medical need. Affected patients exhibit a wide and likely continuous spectrum of symptoms that affect multiple organs and are different in intensity, progression and in age of onset, although skeletal muscle and platelets are mainly affected.
In this work, we have identified hit compounds, synthetized by *click-chemistry* approach, with druggable pharmacodynamics/pharmacokinetic profiles able to restore SOCE to physiological levels in cultured cells, in cells that harbour gain-of-function mutations and on a biopsy of a patient affected by TAM. Last, an only-world mouse model for TAM and Stormorken syndrome (KI-STIM1^{I115F}) has been created and it presents a phenotype compatible to the human disease and pathology.

Guanylate Cyclase Activating Protein 1 mutants associated with retinal dystrophy: a biochemical investigation

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Guanylate Cyclase Activating Protein 1 (GCAP1) is a Ca^{2+}-sensor protein involved in the regulation of the target enzyme Guanylate Cyclase (GC), an important modulator of the phototransduction cascade, which initiates the visual process. To date, fifteen missense mutations have been found to be associated with cone and cone-rod dystrophy (COD and CORD), degenerative retinal diseases characterized by progressive loss of central vision and defective color perception. In order to provide new insights into functional effects of GCAP1 mutations, a combination of biochemical and biophysical techniques was used. After the expression and purification of the recombinant human WT GCAP1 and three mutants (D100G, E155G and I143N) structural and functional analysis were performed by using circular dichroism (CD), polyacrylamide gel electrophoresis (PAGE, both in presence and absence of SDS), dynamic light scattering (DLS), fluorescence and absorption spectroscopy and, finally, analytical size exclusion chromatography. Our data clearly show that both WT and COD/CORD-related human GCAP1 forms a constitutive dimer in reducing conditions and at each tested concentration (2-90 μM). From a structural point of view, CD spectra did not display significant differences in thermal stability, secondary or tertiary structures for WT and mutated GCAP1 variants; the only exception is D100G-GCAP1, for which a reduced Ca^{2+}-induced response was observed. The most apparent difference between WT and dystrophy-related GCAP1 mutants is the affinity for Ca^{2+}: except for I143N, which shows a WT-like affinity, D100G and E155G are characterized by lower apparent affinity. These results will constitute an important starting point for the future design of molecules capable to restore physiological conditions.

Calcium and the regulation of the visual sensory system: a multiscale investigation from molecules to networks

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Ca^{2+} plays an important role as intracellular messenger in a variety of cellular processes. Dynamic variations in its concentration are rapidly detected by calcium sensor proteins, which switch to specific conformations suitable for target recognition and activation/inhibition. Subtle variations in intracellular Ca^{2+} constitute the molecular basis for the fine regulation of the signaling processes occurring in vertebrate phototransduction and provide the ground for feedback mechanisms regulating light adaptation. The Biochemistry and Molecular Biophysics research group at the University of Verona (https://sites.google.com/view/bmbunivr/home) has a long-standing interest in the structure/function relationships of calcium sensors involved in phototransduction, both under physiological and altered conditions, an in the way the delicate balance between the two second messengers, Ca^{2+} and cGMP, leads to normal or altered homeostasis in photoreceptors. The poster will show the current projects and the main approaches used in our research, which range from classical biochemistry to computational biophysics and systems biology, with the final goal to reach a system-level description of the biological system under investigation by comprehensive and deep knowledge of its main components.

The interaction of SiO_2 nanoparticles with the neuronal plasmamembrane: modulation of ionic currents and calcium influx

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ABSTRACT
INTRODUCTION: SiO_2 nanoparticles (SiO_2 NPs) are one of the most promising tools in the field of nanomedicine. We have previously shown that non-toxic doses of SiO_2 NPs (50 nm) induce membrane potential depolarization, modulating the electrical activity of neuroendocrine cells. Since this process is dependent on Ca^{2+} influx, we investigated which calcium-permeable channels are activated by SiO_2 NPs.
MATERIALS& METHODS: combining Ca^{2+} imaging and patch clamp techniques with a pharmacological approach, we obtained a detailed biophysical characterization of the multiple pathways activated by SiO_2 NPs.
RESULTS: both Ca^{2+} imaging data on cell populations and electrophysiological recordings at single channel and whole cell levels suggest that TRPV4, Cx and Panx channels are the major components of inward currents elicited by SiO_2 NPs. Furthermore, pre-incubation with the antioxidant N-acetyl-L-cysteine (NAC) strongly reduce $[Ca^{2+}]_i$ increase.
CONCLUSIONS: our findings suggest that SiO_2 NPs directly activate a complex set of calcium-permeable channels, possibly by free radicals production. The mechanisms of interaction between the SiO_2 NPs and their targets is a prerequisite to the rational design of safe and efficient nanotools for laboratory and clinical applications.

Lysosomal Ca^{2+} signaling in metastatic colorectal carcinoma

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Lysosomes play an emerging role in tumorigenesis by driving many crucial cancer hallmarks, such as migration, metastasis and angiogenesis. Recent work showed that lysosomes control metastasis by releasing Ca^{2+} through the nicotinic acid adenine dinucleotide phosphate (NAADP)-gated two pore channels 1 and 2 (TPC1-2). It is, however, unclear whether NAADP-induced Ca^{2+} release may be amplified by the endoplasmic reticulum (ER), the largest endogenous Ca^{2+} reservoir, through the Ca^{2+}-induced Ca^{2+} release (CICR) process. Herein, we aimed at assessing for the first time the role of lysosomal Ca^{2+} signaling in primary cultures of human metastatic colorectal carcinoma (mCRC). We found that the lysosomotropic agent, Gly-Phe β-naphthylamide (GPN), caused a robust increase in intracellular Ca^{2+} concentration ($[Ca^{2+}]_i$). Likewise, nigericin, which dissipates the ΔpH that drives Ca^{2+} refilling of acidic organelles, induced a sizeable elevation in $[Ca^{2+}]_i$. The Ca^{2+} response to GPN was inhibited by 2-APB, which blocks inositol-1,4,5-trisphosphate receptors ($InsP_3Rs$) under $0Ca^{2+}$ conditions. Liposomal delivery of NAADP induced an increase in $[Ca^{2+}]_i$ that was reduced by GPN and NED-19, a selective TPC antagonist. Accordingly, genetic silencing of TPC1, the only TPC isoform expressed by mCRC cells, suppressed the Ca^{2+} response to NAADP. Similar to GPN, 2-APB also hindered NAADP-induced Ca^{2+} signals in mCRC cells. Finally, NED-19 and genetic silencing of TPC1 reduced foetal calf serum-induced Ca^{2+} signals and proliferation in mCRC cells. Collectively, these data demonstrate that NAADP-gated TPC1 controls mCRC cell proliferation and could be regarded as a novel target for alternative therapies to treat this disease.

TRP EXPRESSION SIGNATURE IN TUMOR-DERIVED ENDOTHELIAL CELLS: FUNCTIONAL ROLES IN PROSTATE CANCER ANGIOGENESIS

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Introduction: TRP channels play a key role in cancer progression, modulating cell proliferation and survival, cancer invasion of surrounding tissues and angiogenesis. TRP expression could therefore characterize the prostate cancer (PCa) cell phenotype. Another well-established concept is that TRPs deeply modulate endothelial cell (EC) biology and tumor angiogenesis. However, a specific TRP expression signature of PCa angiogenesis is still lacking. Our aim was therefore to define a TRP expression signature during PCa angiogenesis providing novel therapeutic targets.
Methods: By means of a qPCR screening and Western blotting, as well as immunohistochemistry, we fully profiled the expression of all TRPs in normal ECs and tumor endothelial cells (TECs) derived from PCa, as well as from breast and renal tumors. Moreover, we characterized the role of the 'prostate specific' TRPs in the modulation of EC biological processes such as cell proliferation, motility and ability to form tubules *in vitro*, as well as *in vivo* angiogenesis.
Results: We identified five 'prostate specific' trp genes whose expression is deregulated in PCa-derived ECs compared to their healthy counterpart. We the specifically characterized the role of each TRP channel in both *in vitro* and *in vivo* angiogenesis, EC proliferation and migration as well as their role in PCa cell attraction by TECs.
Conclusions: Taken together, our results propose novel molecular players to selectively target PCa progression and angiogenesis. Indeed, our expression profiling and functional data could explain the transition of prostate endothelial cells to their aggressive tumor phenotype.

Modulation of intracellular Ca^{2+} concentration in brain microvascular endothelial cells actively induced by brain targeted liposomes

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AIMS
The aim of our study is to evaluate the interaction at the neurovascular unit of liposomes (mApoE-PA-LIP) functionalized with ApoE-derived peptide (mApoE) and phosphatidic acid (PA). In light of our previous results (Re et al.,2010), we assess mApoE-PA-LIP activities on human cerebral microvascular cells (hCMEC/D3) as an in vitro human BBB model.
METHODS
The intracellular Ca^{2+} concentration was measured by digital imaging microscopy in hCMEC/D3 maintained in a low-profile chamber in presence of PSS solution (NaCl 150 mM; KCl 6 mM; MgCl2 1mM; CaCl2 1.5mM; HEPES 10mM; Glucose 10mM). We pre-incubated hCMEC/D3 cells with 4µm acetoxy-methyl-ester Fura-2 AM for 30 minutes at 37°C. Afterwards, we perfused the cells with mApoE-LIP or mApoE-PA-LIP (in PSS) to evaluate the ATP (50µM) evoked calcium waves.
RESULTS
The interaction of mApoE-PA-LIP with the hCMEC/D3 actively induced a modulation in the duration of the ATP induced calcium waves. We found an increase (mean ± se, 136 ± 3.75 sec, n=52, p-value <0.05) of the duration of the ATP evoked calcium waves in presence of mApoE-PA-LIP in comparison to mApoE-LIP perfusion (mean ± se, 125 ± 1.95 sec, n=52). mApoE-LIP and "PSS alone" do not prolong ATP evoked calcium waves.
CONCLUSIONS
Our data confirm that the specific liposome functionalization with phosphatidic acid may be linked to the enhanced calcium waves evoked in hCMEC/D3 by ATP. This finding suggests an intriguing issue involving PA intracellular pathways and its possible implications in the modulation of calcium waves duration in hCMEC/D3 cells.

Bioactive molecules extracted from olive pomace protect cells from calcium mediated damages

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Alterations in intracellular Ca^{2+} homeostasis can promote cytotoxic effects inducing aberrant activation of the Ca^{2+}-dependent enzyme calpain and NO synthases. In the present study, we are reporting that molecules extracted from olive pomace prevent cell death in Ca^{2+}-overloaded human neuroblastoma SKNBE and mouse brain endothelioma bEnd5 cells. These molecules counteract the Ca^{2+}-induced cell damages, by reducing the Ca^{2+}-dependent proteolysis and the abnormal activation of NO synthase (1). Experiments carried out on cells exposed to toxic concentration of calcium ionophore A23187 suggest that the molecules extracted from olive pomace maintain the intracellular calcium level close to physiological concentrations. Further studies are in progress in order to identify the molecule(s) responsible for this protective effect and to shine a light on calcium pathway target(s) affected by these bioactive molecule(s).

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Control of neuronal protein expression and functions by astroglial calcineurin

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Astrocytes play fundamental homeostatic functions in the brain. Calcineurin (CaN) is a Ca^{2+}-activated phosphatase which, in astrocytes, is known to trigger reactive gliosis and neuroinflammation. However, physiological role of CaN in astrocytes is currently unknown. Here, we report the generation of a mouse with CaN KO specific for GFAP-expressing astrocytes (Astro-CaN-KO). At 2 month of age Astro-CaN-KO mice exhibit a deficit of episodic memory as tested by Novel Object Recognition test. Within 5 mo all mice tested exhibit interictal spikes and by 10 mo 50% of males (22 of 42) exhibit tonic-clonic seizures and premature death. Of note, 100 % of cerebellar astrocytes are GFAP-positive. Therefore, we assessed the impact of astrocyte CaN loss in granule cells excitability and passive properties. Patch clamp recordings revealed that loss of astroglial CaN converted high-frequency tonic firing into adaptive firing due to progressive reduction in K+ currents-mediated after hyperpolarization, with no change in passive properties. Proteomic analysis of differentially expressed proteins using Sequential Windowed Acquisition of All Theoretical Fragment Ion Mass Spectrometry (SWATH-MS) revealed that most of the proteins with changed expression were neuronal or neuron-enriched proteins. Gene ontology analysis revealed that several differentially expressed proteins in Astro-CaN-KO mice belonged to KEGG pathways for neurodegenerative diseases, suggesting a role for CaN in neurodegeneration. In conclusion, **astroglial CaN controls neuronal phenotype and functions and, therefore, is fundamental for normal physiological functions of the central nervous system**.

Neuronal activity-induced activation of calcineurin in astrocytes

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Astrocytes communicate with neurons and respond to neuronal activity by increases of intracellular Ca^{2+}, release of gliotransmitters and by morphological changes, directed to adaptation of astrocytes to the increased synaptic activity. Although Ca^{2+} elevations, mediated by astroglial mGluR5, have been implicated in astrocyte-neuronal crosstalk and in structural plasticity of astrocytes, molecular mechanisms of these changes are currently unknown. We hypothesized that a Ca^{2+}/calmodulin-dependent phosphatase calcineurin (CaN), which is responsible for remodeling of astroglial Ca^{2+} signaling toolkit in neurodegenerative diseases such as Alzheimer's disease, may be a mediator of astroglial plasticity. We tested this hypothesis by applying a chemical LTP protocol to mixed astrocyte-neuronal hippocampal murine cultures and monitoring CaN activation as well as cytoplasmic calcium signals in astrocytes. Using lentiviral chimeric vector mCherry-H2Bc-NFATΔ-EYFP as a CaN sensor, we found that cLTP, but not cLTD, induced robust activation of astroglial CaN in astrocytes located in proximity to neurons, but not in distal astrocytes or pure astroglial cultures. Fura-2 Ca^{2+} imaging revealed sustained elevation of Ca^{2+} in astrocytes 5-30 min after application of cLTP paradigm. Both, Ca^{2+} signals and CaN activation were blocked by application of i) inhibitor of NMDA receptors MK801, ii) inhibitor of group I metabotropic glutamate receptors MTEP, iii) inhibitors of store-operated calcium entry (SOCE) 2APB, Pyr3 or Pyr6. mRNA for iNOS and TNFα, both strongly involved in regulation of synaptic function, were robustly induced in astrocytes after cLTP. We conclude that CaN is activated in astrocytes in response to neuronal activity and LTP through Ca^{2+} entry via store- and/or receptor-operated mechanism, and speculate that tis may mediate adaptational processes in astrocytes during neuronal activity and plasticity.

Optical control of Endothelial Progenitor Cells fate mediated by light sensitive conjugated polymers

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Direct control of cells homing to damaged myocardium after an ischemic insult is an overarching goal in the cardiac repair field because this will allow to prime the re-activation of the injured myocardium and vasculature. Here we propose a novel strategy to gain optical control of Endothelial Progenitor Cell (EPC) fate, avoiding the drawbacks associated with current approaches, mainly based on cell therapy and electro-mechanical stimulation.
We exploited the use of Endothelial Colony Forming Cells (ECFCs), which represent the only known EPCs subset truly belonging to the endothelial lineage showing robust *in vitro* proliferation and overwhelming vessel formation *in vivo*.
Our strategy is based on the combination of light sensitive conjugated polymers (CPs), used as photo-actuators, with the advantages offered by optical stimulation. At variance with cell therapy and electro-mechanical stimulation, light modulation offers unprecedented spatial and temporal resolution, permitting lower invasiveness and higher selectivity and allows to provide either excitation/inhibition of the cell activity. Furthermore CPs are highly stable in a biological environment, very well tolerated *in vivo* and easily processed by solution-based techniques. We demonstrated that polymer-mediated optical excitation is able to induce a robust enhancement of lumen formation *in vitro*. We identified the pathways leading to this effective enhancement in ECFCs network formation, as due to light induced activation of TRPV1 channel. Altogether our results represent the first report of use of semiconducting polymer-based optical modulation to restore cardiac function *in vitro*, by modulating cellular activities of one of the main characters involved in cardiac repair.

Early impairments of NMDA receptors function induced by Abeta42 oligomers

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We have recently defined the mechanisms of calcium dyshomeostasis induced by Abeta42 showing that, while Abeta42 stimulates Ca2+ release from ryanodine receptors (RyRs), it inhibits Ca2+ entry through voltage gated Ca2+ channels (VGCCs) and NMDA receptors (NMDARs). It is known that NMDARs are important for controlling neuronal plasticity, learning and memory processes and that these brain functions are altered during aging as well as in AD. These previous observations suggested to perform a more detailed study of the effects of Abeta42 on NMDARs activated currents. Patch clamp experiments revealed that the average inward current carried by NMDARs previously activated by the selective agonist NMDA (50 μM) was significantly decreased by Abeta42 oligomers. We next estimated the unitary current and the average number of NMDARs expressed in hippocampal neurons by performing variance analysis and observed that Abeta42 decreased the total number of NMDARs without altering the single NMDAR activated current. Further experiments revealed that Ca2+ entry through NMDARs was accompanied by Ca2+ release from the stores. When we focused on the role of RyRs we observed that the overall amount of [Ca2+]i increase, measured after NMDA administration, was half-dependent by RyRs and that Abeta42 did not change this proportion. We concluded that Abeta42 impairs NMDARs function and that this may occur during the early stages of AD onset. The development of selective modulators of these receptors may be useful for developing effective therapies that could enhance the quality of life of AD patients.

Intra vs. intermolecular communication in proteins revealed by Molecular Dynamics simulations: a GCAP1 story

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Guanylate Cyclase Activating Protein 1 (GCAP1) is a neuronal Ca^{2+}-sensor protein that regulates the phototransduction cascade in vertebrates upon subtle conformational changes, by switching between activator and inhibitor of the target guanylate cyclase (GC) in a Ca^{2+}-dependent manner. GCAP1 is also target of several mutations causing cone/rod dystrophies, degenerative retinal diseases ultimately leading to blindness. Here we carried out exhaustive molecular dynamics simulations of GCAP1 and determined the intramolecular communication pathways involved in the specific GC activator/inhibitor switch. The switch was found to depend on the Mg^{2+}/Ca^{2+} loading states of the three EF hands and on the way the information is transferred from each EF hand to specific residues at the GCAP1/GC interface. Post-translational myristoylation is fundamental to mediate long range allosteric interactions including the EF2-EF4 coupling and the communication between EF4 and the GC binding interface. The investigation of the functional role of key residues in the protein network topology revealed that some hubs are the target of retinal dystrophy mutations, suggesting that the lack of complete inhibition of GC observed in many cases is likely due to the perturbation of intra/intermolecular communication routes[1]. Moreover, since this protein was recently found to be a dimer in solution and the dimerization interface partially overlaps the GC interface, we investigated the effects of the quaternary structure on the communication between EF hands and the target-regulating interface.

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Defective Ca^{2+} homeostasis in different cell models of amyotrophic lateral sclerosis

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Amyotrophic lateral sclerosis (ALS) is a fatal neurodegenerative disease characterised by the selective damage and death of motor neurons (MNs). In recent years, important steps forward in the elucidation of ALS pathomechanisms have been done, including: (i), the identification of several ALS-associated genes, which also prompted the development of animal and cell disease models; (ii), the understanding that cell types other than MNs (e.g., astrocytes and skeletal myocytes) play a primary role in the pathology; (iii), the identification of Ca^{2+} dysmetabolism as a common theme in different ALS forms.
In light of this notion, we have recently undertaken the following experimental approaches aimed at identifying possible alterations of Ca^{2+} metabolism in different ALS paradigms.
1) We have generated and functionally validated adeno-associated viral vectors for the expression of fluorescent cameleon Ca^{2+} probes targeted to different cell domains under the transcriptional control of a MN-specific promoter. Preliminary analyses using such tools showed altered Ca^{2+} responses following AMPA stimulation in primary MNs from a genetic ALS mouse model (i.e., expressing the ALS-associated human SOD1(G93A) mutant).
2) We have performed aequorin-based analyses of Ca^{2+} fluxes in primary spinal astrocytes and skeletal myocytes, finding altered Ca^{2+} responses in SOD1(G93A) cells that suggest defective mitochondrial Ca^{2+} uptake/buffering capacity and/or endo/sarcoplasmic reticulum Ca^{2+} handling.
3) Finally, aequorin-based Ca^{2+} measurements also highlighted defective Ca^{2+} release from the ER, and uptake into mitochondria, in HeLa cells in which TDP43 (another ALS-related protein) was downregulated by RNA-silencing.
The above defects of Ca^{2+} homeostasis are often associated to altered expression of specific Ca^{2+}-transporting systems. We will describe and discuss such a complex Ca^{2+}- related phenomenology in the context of the different ALS cell models.

Honey mediated wound healing: H_2O_2 entry through AQP3 determines extracellular Ca^{2+} influx.

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Honey has long been used in 'folk medicine', but only in last decades, the therapeutic virtues of honey have been rediscovered and are gaining acceptance. Literature reports show successful use of honey on infections not responding to standard antiseptic and antibiotic therapy, because of its intrinsic H_2O_2 production.

In our study, we demonstrated the involvement of H_2O_2 as a main mediator of honey regenerative effects on keratinocytes.

We observed that this extracellularly released H_2O_2 could pass across the plasma membrane through a specific aquaporin (i.e. AQP3). Once in the cytoplasm H_2O_2, in turn, induces the entry of extracellular Ca^{2+} through TRPM2 and Orai1 channels by a ROS-dependent mechanism. Honey-induced extracellular Ca^{2+} entry results in wound healing, which is consistent with the role played by Ca^{2+} signaling in tissue regeneration.

This is the first report showing that honey exposure affects $[Ca^{2+}]_i$ regulation, due to H_2O_2 production and redox regulation of ion channels, opening up a new horizon for the further use of the honey as a therapeutic tool.

Acetylcholine induces Ca^{2+} signals and nitric oxide release in human brain microvascular endothelial cells

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Basal forebrain neurons control cerebral blood flow (CBF) by releasing acetylcholine (Ach), which binds to endothelial muscarinic receptors to induce nitric (NO) release and vasodilation in intraparenchymal arterioles. Nevertheless, the mechanism whereby Ach stimulates human brain microvascular endothelial cells to produce NO is still unknown. Herein, we sought to assess whether Ach stimulates NO production in a Ca^{2+}-dependent manner also in hCMEC/D3 cells, a widespread model of human brain microvascular endothelial cells. Ach induced an increase in intracellular Ca^{2+} concentration ($[Ca^{2+}]_i$) that was prevented by the genetic blockade of M5 muscarinic receptors (M5-mAchRs), the only mAchR isoform coupled to phospholipase Cβ (PLCβ) present in hCMEC/D3 cells. A comprehensive real time-polymerase chain reaction analysis revealed the expression of the transcripts encoding for type 3 inositol-1,4,5-trisphosphate receptors ($InsP_3R3$), two-pore channels 1 and 2 (TPC1-2), Stim2, Orai1-3. Pharmacological manipulation showed that the Ca^{2+} response to Ach was mediated by $InsP_3R3$, TPC1-2, and store-operated Ca^{2+} entry (SOCE). Ach-induced NO release, in turn, was inhibited in cells deficient of M5-mAchRs. Likewise, Ach failed to increase NO levels in the presence of L-NAME, a selective NOS inhibitor, or BAPTA, a membrane-permeant intracellular Ca^{2+} buffer. Moreover, the pharmacological blockade of the Ca^{2+} response to Ach also inhibited the accompanying NO production. These data demonstrate for the first time that synaptically-released Ach may trigger NO release in human brain microvascular endothelial cells by stimulating a Ca^{2+} signal via M5-mAchRs.

Phospholipases cause transient increases of H^+ and Ca^{2+} concentrations.

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Abstract

Phospholipases produce acids and then release protons. Furthermore, protons compete with Ca2+ for the same binding site in the cell, thus causing the release of Ca2+. It is possible to assume that there is a direct biochemical interconnection between the activity of phospholipases and the cellular concentrations of both H^+ and Ca^{2+}.

Spatiotemporal heterogeneity of mitochondrial Ca^{2+} signals activated by cancer photodynamic therapy *in vivo*

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Photodynamic therapy (PDT) is a valid treatment for superficial neoplasms. The molecular mechanism by which PDT induces apoptosis or necrosis depends on several factors, such as cellular context and photosensitizer agent. A better understanding of intracellular pathways activated by PDT is critical to improve treatment for different types of tumors.
Here, we performed live cell imaging experiments using new generations of genetically-encoded fluorescent Ca2+ probes targeted to endoplasmic reticulum or mitochondria (G-CEPIA1er, R-CEPIA1er, CEPIA2mt, LAR-GECO1.2) to visualize the transfer of Ca2+ between these subcellular compartments during PDT on mouse melanoma cell line B16-F10. In addition, using specific fluorescent biosensors, we show that apoptotic cell death induced by PDT involves activation of caspase-9 and caspase-3 within seconds of photosensitizer activation. Finally, we investigated Ca2+ response to PDT *in vivo* by intravital microscopy using B16-F10 cells expressing CEPIA biosensors to generate bulk tumors. We visualized the effects of photodynamic treatment *in vivo* with high spatial and temporal resolution in the dorsal skinfold chamber, surgically implanted on the tumor-bearing mouse back. We show that PDT-induced stress responses in B16-F10 cells involve Ca2+ release from endoplasmic reticulum and recurrent variations of Ca2+ concentration in mitochondria microdomains both *in vitro* and in tumor microenvironment *in vivo*.
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A portable, low cost aequorinometer based on Silicon Photomultipliers

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Sensing of intracellular calcium concentration variations based on aequorin chemiluminescence offers outstanding advantages with respect to more widespread fluorescence-based assays. The response of aequorin is proportional to the Ca^{2+} intracellular concentration. Furthermore, aequorin experiments are not plagued by cell photodamage and are safe with respect to systematics related to detection of excitation stray light. Finally, absolute Ca^{2+} concentration can be assessed as long as normalisation of the luminescence spike, sensitive to the variability in aequorin expression and cell number, is provided. This is achieved by delayed disruption of cells with surfactant to consume the residual amount of aequorin through reaction with extracellular Ca^{2+}. The main difficulty of aequorin assays is the low level of emitted light, with signals consisting in single-photon pulses. This necessarily requires the use of single photon sensitive detectors, typically Photo-Multiplier Tubes (PMT), integrated in custom engineered instruments at high cost, limited flexibility and portability. A valuable alternative to PMT is offered by Silicon Photo-Multipliers (SiPM). SiPM essentially consist in an array of p-n junctions operated beyond the breakdown voltage, in quenched Geiger-Mueller regime. They are single photon sensitive, photon number resolving, endowed with high temporal resolution, with gains in excess of 10^6 at biasing voltages not exceeding 60V, cost-effective and highly customizable. Here we present a proof-of-concept SiPM-based set-up for aequorin-based calcium sensing, relying on customized front-end electronics. The response in counting and gated current integration mode is qualified. A critical analysis intended to define the pre-requisites for the development of a dedicated platform is performed.

Calcium handling in porcine coronary endothelial cells by monomeric adiponectin

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AIMS: Perivascular adipose tissue can be involved in the process of cardiovascular pathology through the release of adipokines, namely adiponectins. Monomeric adiponectin has been shown to increase coronary blood flow in anesthetized pigs through increased nitric oxide (NO) release and the involvement of adiponectin receptor 1 (AdipoR1). Recently, in microvessels and capillaries, monomeric adiponectin has been found to improve endothelial function through the activation of endothelial NO synthase (eNOS) related signalling. As eNOS, is Ca^{2+}-dependent, and monomeric adiponectin increases Ca^{2+} in myocytes, we examined the effects of monomeric adiponectin on Ca-heandling in aortic endothelial cells (PAEs) in normal/high glucose conditions.
METHODS: PAEs were treated with monomeric adiponectin alone or in the presence of intracellular kinases blockers, AdipoR1 and Ca^{2+}-ATPase pump inhibitors. The role of Na^+/Ca^{2+} exchanger was examined in experiments performed in zero Na^+medium. Intracellular $[Ca^{2+}]_c$ was measured through specific probes.
RESULTS: In PAE cultured in normal glucose conditions, monomeric adiponectin elevated $[Ca^{2+}]_c$. Similar effects were observed in high glucose conditions, although the response was lower and not transient. The Ca^{2+} mobilized by monomeric adiponectin originated from an intracellular pool thapsigargin and ATP-sensitive and from the extracellular space. Moreover, the effects of monomeric adiponectin were prevented by kinase blockers and AdipoR1 inhibitor. Finally, in normal glucose condition, a role for Na^+/Ca^{2+} exchanger and Ca^{2+}-ATPase pump in restoring $[Ca^{2+}]_c$ was found.
CONCLUSION: Our results add new information about the mechanism at the basis of the endothelial NO release by monomeric adiponectin, which would be achieved by modulation of Ca^{2+} transients.

Endothelial cells in tissue regeneration: the mastermind role of intracellular calcium in a PL-driven wound healing process.

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Tissue regeneration requires precise coordination among endothelial, epithelial and mesenchymal morphogenesis. The healing process relies on a complex integration of some cellular signalling, requiring multiple growth factors and cytokines stimuli.

Platelets contain a series of factors able to influence cellular activities at wounded sites, including growth factors, cytokines and chemokines.

An interesting platelet derivative is platelet lysate (PL) that has shown potential clinical application. PL is obtained from repeated freezing thawing of platelet enriched blood samples.

Our previous data showed that PL accelerates wound closure in endothelial cell monolayers. A more in depth analysis showed that the effect of PL occurs through the stimulation of cell proliferation and migration that are strictly dependent on intracellular Ca^{2+} regulation.

The aim of this study if to further characterize the wound healing processes induces by PL exposure as well as the involvement of Ca^{2+} toolkit in PL-boosted regeneration. Unveiling the molecular nature of the pathway gating Ca^{2+} influx into PL-induced endothelium is an imperative challenge to utilize Ca^{2+} signals for therapeutic purposes.

Oxaliplatin induces pH acidification in dorsal root ganglia neurons

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Abstract
Oxaliplatin-induced peripheral neurotoxicity (OIPN) is characterized by an acute cold-induced syndrome characterized by cramps, paresthesias/dysesthesias in the distal limbs and perioral region that develops rapidly and lasts up to one week affecting nearly all the patients as well as by long-lasting symptoms. It has been previously shown that pharmacological or genetic ablation of TRPA1 responses reduces oxaliplatin-induced peripheral neurotoxicity in mouse models. In the present report, we show that treatment with concentrations of oxaliplatin (OHP) similar to those found in plasma of treated patients leads to an acidification of the cytosol of mouse dorsal root ganglia neurons in culture and this in turn is responsible for sensitization of TRPA1 channels, thereby providing a mechanistic explanation to acute toxicity of OHP. Reversal of the acidification indeed leads to a significantly reduced activity of TRPA1 channels. Last, acidification occurs also in vivo after a single injection of therapeutically-relevant doses of OHP.

Astrocytes as cell targets for therapeutic intervention in ALS

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Collective evidence indicates that motor neuron degeneration in Amyotrophic Lateral Sclerosis (ALS) is non-cell-autonomous and requires the interaction with the neighboring astrocytes. Astrocytes can hurt motor neurons by secreting neurotoxic factors, but they can play deleterious roles also by losing functions that are supportive for neurons.
Recently, we reported that stimulation of inositol 1,4,5 triphosphate (IP_3)-generating group I metabotropic glutamate receptors in ALS astrocytes triggers abundant and persistent elevations of intracellular Ca^{2+} concentrations in the absence of spontaneous oscillations. This correlates with mitochondrial disarrangement and cell death in subsets of astrocytes. The interaction of IP_3 receptors with the anti-apoptotic protein Bcl-X_L was previously described to prevent cell death by generating pro-survival Ca^{2+} oscillations. In ALS astrocytes, we found that the sole BH4 domain of Bcl-X_L, fused to the protein transduction domain of the HIV-1 TAT protein (TAT-BH4), is sufficient to restore sustained Ca^{2+} oscillations and cell death resistance. Furthermore, chronic treatment of ALS mice with the TAT-BH4 peptide exerts a positive impact on the disease manifestations.

Time-Frequency analysis of calcium oscillations in nerve cells

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Cytosolic calcium signals control a large set of cellular functions. In particular, in nerve cells they can regulate many processes such as synaptic communication, integration of information at the cell body, transcriptional events, neurite growth and formation of neural circuitry. These signals often have complex time courses and information they convey can be coded both in amplitude and frequency. For this reason, their quantitative analysis is not easily accomplished and, in particular, it may be difficult to highlight subtle differences in their temporal patterns. In general, spectral analysis is mandatory, but it provides a mean of analysis solely in the domain of frequencies, typically unsuitable for non-stationary signals. To overcome this limitation we developed new tools based on wavelet analysis in order to extract information on the structure of $[Ca^{2+}]_i$ oscillations. In particular we derived a set of indices by which different $[Ca^{2+}]_i$ oscillatory patterns and their change in time can be detected and quantitatively evaluated. This approach has been validated through some experimental recordings from chick ciliary ganglion (CG) glial cells showing changes in $[Ca^{2+}]_i$ oscillatory behavior when stimulated with nicotinic acid adenine dinucleotide phosphate (NAADP), a calcium-releasing agonist. Then we applied this method to the oscillatory responses induced in CG neurons by basic fibroblast growth factor (FGF-2), in particular focusing on growth cone activity. Moreover, we quantified the effects of SKF-96365 (a blocker of TRPC channels), nifedipine and ω-conotoxin (blockers of voltage-operated Ca^{2+} channels) on FGF2-induced calcium response, trying to correlate them to biological responses, such as neurite growth. Finally we extended this approach including spatial variable in the analysis, in order to better compare $[Ca^{2+}]_i$ oscillations in distinct cellular subdomains (such as growth cone, neurites and soma) and to related the signals to the local geometry of the cell (e.g. surface-to-volume ratio). By this way, it is possible to provide a full characterization in terms of frequency, temporal activity and spatial localization of the different oscillatory components making up the global calcium signal.

$P2XR_7$-DEPENDENT PURINERGIC REGULATION OF TUMOR VASCULARIZATION

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Background: Tumor microenvironment is characterized by low O_2 and pH levels, together with ATP accumulation. Tumor-derived Endothelial Cells (TEC) play a pivotal role during cancer progression sustaining angiogenesis and are strongly influenced by tumor microenvironment. In this study we focus our attention on $P2X_7$ purinergic receptor ($P2RX_7$), previously shown to be involved in remodelling of tumor-derived human EC from breast carcinoma (BTEC).
Aims: We investigate whether and how $P2RX_7$ may act as a sensor or tumor hypoxia and acidosis thus regulating endothelial function.
Methods: Calcium imaging, electrophysiology, cell migration assays, tubulogenesis.
Results: The selective $P2RX_7$ agonist BzATP reduced migration of different TEC types (from breast, kidney and other human carcinomas). Low extracellular pH levels (pH=6.40) partially rescued the anti-migratory effect and affected the calcium signals triggered by the agonist. Interestingly, the same anti-migratory effect was obtained in HMEC co-coltured for 72h with breast cancer derived tumor cells (MCF7). Even more interestingly, the functional activity of BzATP on TEC was altered by severe hypoxia (1% O_2) and low pH.
Conclusion: $P2RX_7$ could play a key role in vascular remodelling occurring during tumor progression modulating the calcium-dependent endothelial events finely tuned by hypoxia and acidosis, two hallmarks of cancer.

Supra-linear Ca^{2+} dependence of the neurotransmitter release at mammalian vestibular ribbon synapses

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Abstract:
Inner ear sensory synapses faithfully transduce information over a wide range of stimulus intensities for prolonged periods of time. The efficiency of such demanding and stringent exocytotic activity depends on the presence of specialised presynaptic ribbons in the sensory hair cells. Ribbons are electron dense structures able to tether a large number of releasable vesicles at the synaptic active zone and able to maintain high rates of vesicle release. Calcium entry through $Ca_V1.3$ (L-type) Ca^{2+} channels in response to cell depolarization causes local increase in Ca^{2+} at the ribbon synapses, which is detected by the exocytotic Ca^{2+} sensors. We studied the Ca^{2+} dependence of exocytosis and the release kinetics of different vesicle pool populations in mature mouse VHCs using patch-clamp capacitance measurements under physiological recording conditions. Exocytosis in VHCs showed a high order dependence on Ca^{2+} entry, which contrasts with the linear Ca^{2+} dependence observed in adult mammalian auditory inner hair cells (IHCs). Our findings show that the coupling between Ca^{2+} influx and neurotransmitter release at VHC ribbon synapses is described by a non-linear relation that is likely to be more appropriate for the faithful encoding of low frequency vestibular information, consistent with that observed in very low frequency mammalian IHCs.

Bitter taste receptor hTAS2R46 activation regulates mitochondrial Ca^{2+}-buffering in airway smooth muscle cells

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Bitter taste receptors (TAS2R) are discovered to be expressed in extraoral tissues such as the respiratory system, in which is involved in relaxation. We have explored the mechanism by which absinthin, the highly specific agonist of hTAS2R46, could counteract the response induced by histamine in airway smooth muscle cell (ASM). We show that absinthin is able to reduce cytosolic histamine-induced Ca^{2+}-rises. To investigate this mechanism, we infected ASM with aequorin-based Ca^{2+} probes targeted to the cytosol, sub-plasma membrane domains and the mitochondrial matrix showing that such reduction is a consequence of an increased Ca^{2+}-influx into mitochondria. Cytosolic Ca^{2+}-decreases and simultaneous mitochondrial Ca^{2+}-increases are sensitive to a mitochondrial uncoupler, an inhibitor of the mitochondrial uniporter calcium, and to the cytoskeletal disrupter latrunculin; it is inhibited by a hTAS2R46 antagonist and is no longer evident in hTAS2R46-silenced cells, demonstrating that it is hTAS2R46-dependent. All these data demonstrated that mitochondrial Ca^{2+}-uptake can be modulated via a G-protein receptor, thereby adding to the complexity of Ca^{2+}-signalling.

Gene expression, proteome and calcium signalling alterations in immortalized hippocampal astrocytes from an Alzheimer's disease mouse model.

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Alzheimer's disease (AD) is a neurodegenerative disorders, characterized by progressive cognitive decline, neuronal and synaptic loss. Growing evidence suggest that astroglial cells may have a role in the pathogenesis of AD. But it has not been already cleared if astroglial cells activation and function could be positive or negative during diseases progression. To better study astrocyte function, we decided to set up an in vitro model of astroglial AD cells.
We have established immortalized astroglial cell lines from the hippocampus of 3xTg-AD and wild type control mice (3Tg-iAstro and WT-iAstro, respectively).
Both 3Tg-iAstro and WT-iAstro maintain an astroglial phenotype and markers (glutamine synthetase, Aldh1l1 and AQP4) and display proliferative potential until at least passage 25. Furthermore, these cell lines maintain the potassium inward rectifying (Kir) current and present transcriptional and proteomic profiles compatible with primary astrocytes. Importantly, differences between the 3Tg-iAstro and WT-iAstro cell lines in terms of calcium signaling and in terms of transcriptional changes can be re-conducted to the changes previously reported in primary astroglial cells.
To illustrate the versatility of this model we performed shotgun mass spectrometry proteomic analysis and found that proteins related to RNA binding and ribosome are differentially expressed in 3Tg-iAstro vs WT-iAstro. In summary, we present here immortalized hippocampal astrocytes from WT and 3xTg-AD mice that might be a useful model to speed up research on the role of astrocytes in AD.

FROM PATCH-CLAMP TO MICRO-GRAPHITIC ARRAYS TO STUDY THE ACTIVITY OF DOPAMINERGIC NEURONS

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Electrophysiological and secretory properties of cultured dopaminergic neurons from TH-GFP positive mice have been investigated by merging different approaches: (i) *voltage-* and *current-clamp* measurements; (ii) micro-graphitic arrays (μG-SCD-MEAs), aimed to monitor intracellular Ca^{2+} transients, action potential firing and oxidizable neurotransmitter release. These prototypes are able to simultaneously acquire data from 16 or 60 rectangularly-shaped graphitic microelectrodes (20 × 3.5 μm^2), separated by 200 μm gaps. In particular, here we show that μG-SCD-MEAs can combine amperometric and potentiometric recording.

(i) Ca^{2+}-dependence of secretion from **single dopaminergic neurons** was quantified by depolarization-evoked membrane capacitance increases, using pulses of different length (*voltage-clamp)*. We found that the dopamine precursor L-dopa increased the overall secretion without altering Ca^{2+} entry, suggesting a potentiated Ca^{2+}-dependent efficiency of exocytosis. Concerning the electrical activity, (*current-clamp* recordings), we found that dopaminergic neurons were spontaneously firing at low frequencies (*1-2 Hz*), while L-Dopa administration inhibited this activity within few minutes; this inhibitory effect was reversed by the D_2-antagonist sulpiride, suggesting a D_2-autoreceptor mediated pathway.

(ii) We used two prototypes of micro-graphitic arrays (respectively with 16 and 60 channels) to investigate the functional properties of the **dopaminergic network** from cultured dopaminergic neurons and from SN slices. In the potentiometric configuration, the 60ch μG-SCD-MEAs were suitable to detect the spontaneous electrical activity. While in the amperometric configuration the 16ch μG-SCD-MEAs allowed the measurement of quantal dopamine release events, under spontaneous and KCl-evoked exocytosis.

These data confirm the versatility of μG-SCD-MEAs and their suitability as multi-task sensing devices for neuroscience.

Preferential binding of Mg^{2+} over Ca^{2+} to CIB2 triggers an allosteric switch impaired in Usher syndrome type 1J

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Calcium- and integrin- binding protein 2 (CIB2) is a ubiquitous Ca^{2+} and Mg^{2+} sensor protein whose function is not completely clear. Recently, a CIB2 variant, namely p.E64D (p. Glu64Asp), was found to be associated to Usher syndrome J1 (USH1J), a disease leading to hearing loss and blindness. The present study reports a detailed biochemical *in vitro* characterization of recombinant wild type (WT) CIB2 and p.E64D variant using size exclusion chromatography and spectroscopic techniques, i.e., dynamic light scattering, circular dichroism, nuclear magnetic resonance and fluorescence. It was found that WT CIB2 does not possibly work as a Ca^{2+} sensor under physiological conditions, because its affinity for Ca^{2+} (K_d^{app} = 0.5 mM) is too low for detecting normal intracellular levels. Instead, WT CIB2 has a fairly high affinity for Mg^{2+} (K_d^{app} = 290 µM), and it is probably Mg^{2+} -bound under physiological conditions. CIB2 forms a non-covalent dimer under conditions that mimic the physiological ones. The Mg^{2+} binding to the WT protein creates a long range allosteric communication between the residue E64, located at the N-terminal domain, and the metal cation binding site EF3, located at the C-terminal domain. The conservative p.E64D mutation breaks up such inter-domain communication resulting in the impaired ability of CIB2 to switch to its Mg^{2+}-bound form. The ability to bind the target integrin α7b peptide was substantially conserved for p.E64D, therefore the present study suggests that the molecular defect associated to USH1J resides in its inability to sense Mg^{2+} and adopt the required conformation.

Synthesis and characterization of novel TRPM8 agonists to target prostate cancer progression

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Prostate cancer (PCa) is the most common non-cutaneous human malignancy and the second most lethal tumour among men, with the highest incidence in industrialized countries. Due to its multifaceted role in the control of cell proliferation and motility, Ca^{2+} signalling is implicated in tumour progression as well as in the regulation of angiogenesis. The discovery of Transient Receptor Potential (TRP) superfamily of channels provided putative candidates for non-voltage-gated Ca^{2+} entry mechanisms. In particular Transient Receptor Potential Melastatin 8 (TRPM8) has recently been proposed to play a protective role in prostate cancer by impairing cell motility of both cancer cells and endothelial cells. TRPM8 is activated by mild cold temperatures and cooling compounds such as menthol, and synthetic cooling mimetic agents commercially available like icilin and WS-12 which has been described as one of the most selective agonist to date in the scientific literature. The aim of my work is to synthesize and characterize new agonist for TRPM8 in order to better improve their properties for a potential use as drug to target prostate cancer progression. We therefore designed a new set of TRPM8 agonists based on structures of Menthol and WS-12 in order to obtain new selective and active molecules and to better understand the key chemical features of the activators that are important for TRPM8 activation and selectivity. Our results show that the newly designed compounds present higher solubility as compared with WS-12. Moreover, we identified at least two compounds with similar activity and selectivity on TRPM8 channels as compared to WS-12, opening up new perspectives on their use in the clinical and academic field.