conditions compared to basal, as neuronal firing was reduced by more than 50% at 10 uM while no effects were noticed under the basal conditions. The observed effect could be related to the direct inhibition of the Nav channels, as benzydamine is a known blocker; but a direct or indirectly action via proalgesic channels or receptors like ASIC or TRP, sensitized by the inflammatory environment, cannot be excluded. In conclusion, benzydamine has evident synergistic and complementary effects in the treatment of local inflammatory symptoms as well as in the painful processes, not only by reducing the inflammation cascade but also by reducing the inflammatory-mediated neuronal signalling, closing the local circle of inflammation and pain.

2443-Pos

Fine-tuning the immunological signature of mast cells using TRPC photopharmacology

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Canonical transient receptor potential (TRPC) channels are elements of the immune cell Ca^{2+} handling machinery with specific functions limited to particular cell phenotypes. Mast cells play a complex role within the tumour microenvironment. NFAT transcriptional activation in mast cells is considered beneficial for immune defence, while secretion of certain mast cell mediators by degranulation is likely promoting tumour progression. Here we set out to establish a pharmaco-optogenetic protocol for specific control over mast cell function. RBL-2H3 mast cells were found to lack appreciable Ca^{2+}/NFAT signalling in response to benzimidazole TRPC activators. Genetic modification of the cells to overexpress the TRPC6 isoform generated benzimidazole sensitivity and provided the basis for photopharmacological control of NFAT activity. Expression of TRPC6 enabled light-mediated generation of well-defined Ca^{2+} signalling patterns. Importantly, cells overexpressing TRPC6 retained essentially low basal levels of NFAT activity and displayed rapid and efficient NFAT nuclear translocation upon OptoBI-1 photoactivation. In striking contrast to classical pharmacological activation, with a benzimidazole TRPC channel activator (GSK1702934A), Opto-BI-induced activation using a sequence of short (15s) light pulses did not induce degranulation of the mast cells. Our results demonstrate that a TRPC6-OptoBI-1-based strategy of sculpturing mast cell Ca^{2+} signals enables specific control over transcriptional activation, while preventing potentially undesired innate immune responses.

2444-Pos

Mechanical and pharmacological activation of PIEZO1 channels characterized by high throughput electrophysiology

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PIEZO channels are mechanically-activated cation channels that play important roles in biological functions including touch, proprioception, shear stress and stretch sensation as well as blood pressure regulation. Mutations in Homo sapiens PIEZO1 channels are associated with anemia, malarial resistance, lymphatic dysplasia and varicose vein disease, suggesting important red blood cells and vascular roles in humans.

The pharmacology of the PIEZO1 channels is in its infancy. Here we sought high throughout methodology for investigating small-molecule modulation in combination with mechanical stimulation.

A bottleneck in PIEZO drug development has been the lack of mechanical stimulation in automated patch clamp. Here we show how the optimization of pipetting parameters and the modification of the NPC-384 chip of the SyncroPatch 384 lead to the possibility to mechanically stimulate PIEZO1 channels using high throughput electrophysiology. Data of mouse and human PIEZO1 channels expressed in HEK T-REX 293 cells activated by either mechanical or chemical stimuli will be shown as well as the combination of both methods.

Under voltage-clamp we were able to show reliable quantification of PIEZO1 activation by fluid flow, Yoda1 (a small-molecule PIEZO1 agonist) and a Yoda1 analogue.

To our knowledge, this is the first time that mechanical stimulation of PIEZO channels in a high throughput planar patch clamp system has been shown. The possibility of comparing and combining mechanical and chemical stimulation in a high throughput electrophysiological assay facilitates the biophysical and pharmacological studies of PIEZO channels.

This work was supported by research grants from the Wellcome Trust, British Heart Foundation and has received funding from the European Union's Horizon 2020 research and innovation program.

2445-Pos

Ion pathways in the human pannexin 1 channel

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Pannexin 1 is a large pore anion channel located on the plasma membrane. It transports not only small halides but also large molecules such as ATP. Pannexin 1 has an important role in apoptosis signaling and several mutations in the gene cause infertility in human females. Pannexin 1 can activate with a variety of stimuli. Activation by membrane depolarization typically evokes very small chloride currents in the absence of any ATP release. ATP can be released when pannexin 1 is activated by caspase cleavage of its C-terminal tail. Several pannexin 1 cryo-EM structures by several groups including ours reveal a heptameric assembly with a wide main pathway in parallel with the axis of symmetry. For full-length Pannexin 1, the main pathway is plugged from the intracellular side by the C-terminal tail. A 4.4 Å radius opening is seen at the extracellular side formed by residues W74 and R75, which are important ion selectivity and pore block by carbenoxolone. We observed narrow side tunnels located underneath the transmembrane region and between adjacent subunits. These tunnels join the main pathway and involve residues between the amino-terminal helix and the first transmembrane domain. Residues at this junction were critical for ion permeation and gating. The discovery of multiple ion pathways endowed in the Pannexin 1 complex has unveiled an unforeseen and exquisite biophysical feature uncommonly seen in most other ion channels.

2446-Pos

Ion channels and myogenesis in Duchenne muscular dystrophy: a first characterization in differentiating wild-type and dystrophic myocytes

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In skeletal muscle, several ion channels interact with the dystrophin complex, which is disrupted in Duchenne muscular dystrophy (DMD). However, little is known about the early involvement of ion channels in DMD, with particular focus to myogenesis. Here, we performed a pilot study to characterize the electrophysiological asset of myoblasts and myocytes by using two immortalized mouse satellite-derived cell lines: the wild-type 2B4 and the dystrophic SF1. For each cell line, Inward and outward currents were recorded at the proliferative state (myoblasts) and at different time points of differentiation (2, 4, 6 and 11 days) by whole-cell patch clamp. 2B4 cells showed an increment of fast inward currents as the differentiation program progresses. In SF1 myocytes, inward currents increased up to the 6th day of differentiation, similar to 2B4 cells. However, day-11 SF1 myocytes showed 50% lower inward currents compared to day-11 2B4 myocytes (3.3 nA vs 6.8 nA for Ipeak at −40/20 mV). High concentration of tetrodotoxin blocked these inward currents in both day-6 2B4/SF1 and day-11 2B4. Outward currents were clearly detectable in day-11 2B4 myocytes but were very small at previous time points. Conversely, SF1 outward currents reached the highest value at day-6 (being 3-fold higher than in 2B4 cells) but decreased by day-11. $BaCl_2$ effectively blocked outward currents in day-6 SF1 cells; however, its effects were poorly appreciable at day-11 in both 2B4 and SF1. This preliminary data demonstrate that inward and outward currents could play an essential role during myoblasts differentiation and that their impairment could correlate with altered myogenesis in DMD. Further pharmacological and molecular biology experiments are ongoing, to identify the main ion channels involved at different time points. (Supported by BTS grant 2018-PDR-00351 to OC)

2447-Pos

Negative allosteric modulation of GluN3-containing NMDA receptors with a novel mechanism of action

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N-methyl-D-aspartate receptors (NMDARs) are ionotropic glutamate receptors involved in many physiological and neuropathological processes in the central nervous system. NMDARs are tertrameric assemblies of two GluN1 subunits and two GluN2 or two GluN3 subunits, with each receptor composition endowing distinct biophysical and pharmacological properties. GluN1 and GluN3A-B subunits bind glycine, while GluN2A-D subunits bind glutamate. The well understood GluN1/2 NMDARs require binding of both glutamate and glycine for activation, whereas the less studied GluN1/3 receptors only need glycine binding for activation. The physiological roles of GluN1/3 NMDARs remain elusive, in part due to their cryptic functional properties. Glycine binding to the GluN1 subunit results in strong desensitization, whereas glycine binding