(10-90 mM) were inhibitory. Further, this activating mechanism was not intrinsic to the PC2 channel but instead seems to be mediated by PC2-associated proteins. Channel function of the *in vitro* translated PC2 protein with Ca^{2+} concentrations of 10-15 mM was non-responsive to lowering cytoplasmic Ca^{2+} with either EGTA or BAPTA. Our data are consistent with a regulatory role of both cytoplasmic and external Ca^{2+} in PC2 channel function, which does not involve putative Ca^{2+}-binding sites on the channel protein, but instead external sites to the channel protein.

1363-Pos Board B207

Molecular Basis of Calmodulin and Ca^{2+} interaction with thermoTRP channels

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The TRP (transient receptor potential) family of proteins are relatively non-selective cation channels, most of which are highly permeable to Ca^{2+}. They participate in many sensory and physiological processes, and subdivided into seven major subfamilies: TRPV, TRPA, TRPC, TRPM, TRPP, TRPML, and TRPN. They are predicted to have six transmembrane domains per subunit, intracellular N- and C-termini, and form tetrameric assemblies. Several TRP channels have been proposed to be regulated by intracellular Ca^{2+}, and/or Calmodulin (CaM). Immunoprecipitation of TRP channels expressed in cultured cells suggests that CaM forms complexes with some of them, but questions remain about whether full-length TRP channels bind CaM directly or through some intermediary protein(s), and about the affinity and kinetics of complex formation. We have used fluorescence emission anisotropy of Alexa 488-labeled CaM to measure binding of CaM and Ca^{2+}-CaM to full-length TRP channels expressed in yeast and purified to homogeneity in detergent solution. At nanomolar concentrations, CaM bound rapidly to TRPA1 and to purified ryanodine receptor used as a positive control. CaM binding was enhanced by Ca^{2+}, but not strictly dependent on it, and binding of labeled CaM was antagonized by addition of unlabeled CaM. Dissociation of the complex occurred much more slowly than association. Under conditions in which TRPA1 and ryanodine receptor bound Ca^{2+}-CaM with high affinity, no binding of Ca^{2+}-CaM was observed for TRPV1 or TRPV2.

1364-Pos Board B208

Rapid Temperature Jump by Low Cost Laser Diode Irradiation

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Thermal TRP ion channels play important functions in somatosensation and pain. Aside from activation by agonist or voltage, they are directly gated by temperature, a novel property unique to these channels. However, the underlying mechanism of the abnormally high thermal sensitivity has remained elusive. Studies on thermal activation of these channels have been challenging, partly because of the difficulty for rapid alteration of ambient temperature in live cells. Existing approaches mostly employ resistive or thermal electric heating/cooling, and have a response time far slower than channel activation. As a result, they only allow for measurement of steady-state properties of the channel. We report here an alternative approach for rapid perturbation of temperature. The approach employs an infra-red laser diode as a heat source, and by restricting laser irradiation around a single cell, it can produce constant temperature jumps over 50oC in sub-milliseconds. Experiments with several heat-gated channels (TRPV1-3) demonstrate its applicability as a general tool for local temperature stimulation of single cells. Compared to other laser heating approaches such as those based on Raman-shifting of the Nd:YAG fundamentals, it is more cost-effective while providing adequate resolution for detection of ion channel kinetics.

1365-Pos Board B209

Heat Activation of Temperature-Gated Ion channels Studied by Fast Temperature Jumps

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Temperature-gated ion channels are distinct for their extraordinarily high thermal sensitivity. Activation by temperature is made possible with large changes in enthalpy and entropy and appropriate compensation between them, yet the source of the energy and the molecular basis of temperature sensing have remained elusive. The present knowledge on the thermal gating of these channels has resulted largely from equilibrium measurements of temperature responses. Nonsteady-state properties of the gating have been lacking because of the difficulty in rapid perturbation of ambient temperature. To address these issues, we have developed a laser-based fast-heating approach and used it to investigate the heat activation of the vanilloid receptor TRPV1. In response to a temperature step, the channel was activated with an exponential time course, and the responses were sustained at hyperpolarization but became slowly inactivated at depolarization. The rate of activation was variable depending on cell conditions, but had a time constant generally on the order of milliseconds. The activation showed saturation in both kinetics and steady-state currents at temperatures above 50oC. Strong activation occurred in saturating temperatures independent of membrane potentials. Nonlinear relaxation behavior was observed especially during the deactivation process under extreme hyperpolarization. Our data indicate that, despite large energetic changes, the temperature activation can occur rapidly, and consists of multiple states involving both temperature-dependent and independent transitions. The results provide further constraint on the locality of the energetic distributions and novel insights for understanding the mechanism of temperature sensing.

1366-Pos Board B210

Controlling Temperature and Chemical Environment for Patch Clamp Studies of TRP Channels

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A microfluidic device fabricated in PDMS, which permits control of local temperature and rapid exchange of solution around a single cell, has recently been developed. The system allows an experimental design where a single cell can be exposed to a large set of temperatures and concentrations of chemicals in a short time.

Transient receptor potential (TRP) channels are activated by both ligands and temperature, and synergetic effects exist between temperature and concentration of ligand. Therefore, together with patch clamp recording, the microfluidic system is used for studying the response and cooperative effects of temperature and chemicals in TRP channels.

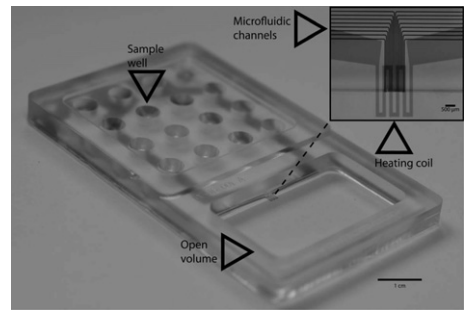

Figure 1. The microfluidic device contains 16 wells connected to separate channels, with separate outlets in the open volume. In the immediate vicinity of the outlet, there is no mixing between the flows from the different channels due to laminar flow. A thin-film Cr/Au-structure is aligned beneath the channel outlets, which yields resistive heating when a voltage is applied.

1367-Pos Board B211

Single-Cell RT-PCR Analysis of TRPC Channels Expressed in Rat Cholinergic, Dopaminergic, Noradrenergic, and Serotonergic Neurons in the Brain

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The canonical transient receptor potential (TRPC) channels comprise a family of nonselective cation channels composed of seven members (TRPC1-7). TRPC channels are widely distributed in the nervous system and contribute to neuronal excitation. In the present study, using the single-cell RT-PCR method, the distribution of TRPC channel mRNA is analyzed in cholinergic neurons in the nucleus basalis (NB), serotonergic neurons in the dorsal raphe nucleus (DRN), noradrenergic neurons in the locus coeruleus (LC), dopaminergic neurons in the substantia nigra (SN), and dopaminergic neurons in the ventral tegmental area (VTA). NB, LC, SN and VTA neurons are cultured from 3-5 day-old and DRN from 10-12 day-old Long Evans rats. Single-cell RT-PCR was performed on these cultured neurons using the previously described method (Kawano et al., 2004, Neuroscience letters, 358:63). Tyrosine hydroxylase primers were used to identify noradrenergic neurons in LC

and dopaminergic neurons in SN and VTA. Choline acetyltransferase primers were used to identify cholinergic neurons in NB, and tryptophan hydroxylase primers were used to identify serotonergic neurons in DRN. TRPC1 mRNA was most frequently detected in all neuron types. TRPC2, involved in pheromone sensing, was not present in any neuron. TRPC3, 4 and 5 existed most frequently in cholinergic neurons in NB (80-90%). TRPC6 was relatively more frequent in dopaminergic neurons in VTA (58%) and SN (46%). TRPC7 was most frequently found in noradrenergic neurons (80%) in LC and cholinergic neurons (80%) in the NB. Interestingly, cholinergic neurons in the NB show the highest frequency of TRPC mRNA expression. Present results demonstrate that each type of neuron expresses specific combination of TRPCs, suggesting that the specific TRPCs are responsible for excitation of each type of neurons.

1368-Pos Board B212

Capsaicin Protects Mouse Neuromuscular Junctions From The Paralytic Effects Of Botulinum Neurotoxin A

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Botulinum neurotoxins (BoNTs) are the most toxic naturally occurring proteins. Botulinum serotype A (BoNT/A) selectively cleaves SNAP-25 and inhibits acetylcholine release from motor nerve endings resulting in life threatening paralysis of skeletal muscles. Here we report that capsaicin (8-methyl-N-vanillyl-6-nonenamide), an irritant active principle of chili peppers, protects against the paralytic effects of BoNT/A in mouse. In *Triangularis sterini* nerve muscle preparations, capsaicin pretreatment *in vitro*, significantly reduced fluorescent labeled BoNT/A uptake mediated by depolarization with 40 mM KCl or neural stimulations. *In vivo* injection of capsaicin (3 µl of a 1 mM stock solution) either coinjected or injected 4 or 8 hours before BoNT/A injection protected the mice from the inhibitory effects of BoNT/A measured by toe spread reflex. In controls the toe spreading reflex was inhibited within 24 hrs of poisoning with BoNT/A. Also wortmannin, a PIP5K inhibitor, injected prior to BoNT/A exposure, protected the mice from the paralytic effect of BoNT/A. *In vitro* muscle tension measurements demonstrate that capsaicin pretreatment partially protected the functions of the mouse *Extensor digitorum longus* nerve muscle preparations. Also pretreatment of cultured cholinergic neuroblastoma (N2a) cells with 10 µM capsaicin for 10 minutes prior to exposure of 10 pM BoNT/A significantly preserved labeling of synaptic vesicle pools by FM1-43. Our data collectively demonstrate that capsaicin offers protection from the paralyzing effect of BoNT/A by significantly reducing the uptake of the toxin in mouse neuromuscular junction probably by interfering with endocytosis of the toxin.

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1369-Pos Board B213

Stretch-induced Up-regulation of Caveolae Formation and SOC Activities in HUVEC

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We have studied about molecular identities of stretch-activated (SA) channels and the mechanism of Ca^{2+} influx evoked by mechanical stretch in human umbilical vein endothelial cells (HUVECs). Previously, we showed that a targeting suppression of transient receptor potential 2 (TRPV2) protein expression in HUVEC using a TRPV2-specific morphorino-oligo completely blocked a transient increase of intracellular Ca^{2+} in response to stretch through the activation of SA channels. Furthermore, after these morphant HUVECs were subjected to 20% uni-axial cyclic stretch at 1 Hz for 1 h, neither a stretch-enhanced stress fiber formation nor a shift in the cell orientation transverse to the strain direction could not be observed. From these results, we concluded that TRPV2 would be a key component of SA channel complex and stretch-induced reorganization of cytoskeletons in HUVEC. Here, we examined the remodeling of Ca^{2+} responses evoked by uni-axial cyclic stretch in HUVEC. Before and after the cyclic stretch, a magnitude of single stretch-evoked Ca^{2+} transient did not change. However, the Ca^{2+} influx through the store-operated Ca^{2+} channels (SOCs) was significantly increased after stretch stimulation. Recent studies have demonstrated that caveolae are microdomains in the plasma membrane and contain functionally organized signaling molecules, including Ca^{2+} signaling. Immunohistochemistry revealed accumulation of caveolin-1 and TRPCs, some of which serve as SOCs, in caveolae after the cyclic stretch to HUVEC. Electron microscopy confirmed that the incidence of caveolae in HUVEC was increased after the stretch. On the other hands, TRPV2-knocked down HUVECs suppressed the increased SOC activities and caveolae formation after cyclic stretch. Such the up-regulation of SOC activities through stretch-dependent TRPV2 activation might contribute to sustained intracellular Ca^{2+} increase, which is thought to be a primary etiology of the vascular remodeling, and a potent risk factor of pressure-dependent hypertrophic diseases.

1370-Pos Board B214

Regulatory Elements of TRPA1 Function

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Transient Receptor Potential Ankyrin 1 channel, TRPA1, is a member of the TRP family and has been shown to be expressed in dorsal root ganglions, trigeminal ganglion neurons and hair cells. TRPA1 is proposed to play an important role in pain perception and temperature sensing, and might be involved as transduction channel in mechanosensation essential for the auditory response in mammals. Pungent chemicals and environmental irritants (e.g. mustard oil) are substances activating this ion channel. Specifically, activation of TRPA1 by allyl isothiocyanates occurs via covalent modification of cysteine residues within the cytoplasmic N terminus of the channel. Here we have focused on the role of TRPA1 C-terminus employing Ca^{2+} imaging, patch-clamp and confocal fluorescence resonance energy transfer (FRET) microscopy. A TRPA1 C-terminal deletion mutant failed to activate upon allyl isothiocyanate application despite a similar homomerization potential as the wild-type form evident from both functional assays and FRET measurements, respectively. The TRPA1 C-terminus alone exhibited multimerization potential in FRET microscopy, yet failed to function as a dominant negative species when co-expressed with wild-type TRPA1 suggesting a minor role for channel assembly. Further, a single amino acid mutation in TRPA1 C-terminus led to a substantial reduction in TRPA1 currents. With regard to a potential regulatory role of calmodulin (CaM), FRET experiments suggested its association with TRPA1 upon elevation of intracellular calcium concentrations either by allyl isothiocyanate induced stimulation of TRPA1 or ionomycin. Consistently, electrophysiological experiments revealed faster inactivation of TRPA1 in presence of over-expressed CaM. In summary our experiments point to TRPA1 C-terminus a crucial element for channel function that is additionally regulated by CaM. (supported by FWF P18169)