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 sterni nerve muscle preparations, capsaicin pretreatment in vitro, significantly reduced fluorescence labeled BoNT/A uptake mediated by depolarization with 40 mM KC1 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 Ca2+ 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 Ca2+ 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 Ca2+ responses evoked by uni-axial cyclic stretch in HUVEC. Before and after the cyclic stretch, a magnitude of single stretch-evoked Ca2+ transient did not change. However, the Ca2+ influx through the store-operated Ca2+ 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 Ca2+ 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 Ca2+ 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 ganglia, 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 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 Ca2+ 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. The TRPA1 C-terminal deletion mutant failed to activate upon allyl isothiocyanate application despite a similar homomerization potential as the wild-type form. The TRPA1 C-terminal deletion mutant failed to activate upon allyl isothiocyanate application despite a similar homomerization potential as the wild-type form. The TRPA1 C-terminal deletion mutant failed to activate upon allyl isothiocyanate application despite a similar homomerization potential as the wild-type form.