

Abstract 4.5

Functional expression of the multimodal extracellular calcium-sensing receptor in the complex pulmonary neuroepithelial body microenvironment

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The Ca^{2+} -sensing receptor (CaSR) is the master regulator of whole body extracellular free ionized Ca^{2+} (Ca^{2+}_o). In addition to sensing Ca^{2+}_o , the CaSR integrates inputs from a variety of different physiological stimuli such as nutrient availability, redox status, pH and ionic strength. Owing to this multimodal ability, the CaSR is expressed in many regions outside of the Ca^{2+} homeostatic system, including the fetal lung where it plays a crucial role in lung development. Here we show that neuroepithelial bodies (NEBs) of the postnatal mouse lung express a functional CaSR. NEBs are densely innervated groups of pulmonary neuroepithelial endocrine cells, representing complex sensory receptors in the airways and exhibiting stem cell characteristics. qRT-PCR performed on laser microdissected samples from GAD67-GFP mouse lung cryosections revealed exclusive expression of the CaSR in the NEB microenvironment. NEBs show CaSR immunoreactivity from postnatal day 14 onwards. Confocal imaging of live lung slices revealed that NEB cells respond to an increase in Ca^{2+}_o concentration with a rise in intracellular Ca^{2+} concentration ($[\text{Ca}^{2+}]_i$); an effect mimicked by several membrane-impermeant CaSR agonists (e.g. the calcimimetic R-568) and that was blocked by the calcilytic, Calhex-231. We further aimed at exploring potential roles for the CaSR in pulmonary NEB physiology. Our results demonstrate that the CaSR regulates baseline $[\text{Ca}^{2+}]_i$ in NEBs and, via paracrine signaling from Clara-like cells, coordinates intercellular communication in the NEB microenvironment. By integrating multiple physio(patho)logical signals and coordinating intra- and extracellular Ca^{2+} signaling, the CaSR appears to serve key roles for the NEB microenvironment as a complex chemosensory receptor and potential regulator of airway stem cell niche.

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Abstract 4.6

Regulation of TRPA1 and TRPV1 in C-fibers: Opposite effects of RET activation in jugular and nodose neurons and its role in allergic asthma

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Glial cell-line derived growth factor (GDNF) and artemin are neurotrophic factors of the GDNF family of ligands. GDNF binds to its co-receptor GDNF family receptor alpha1 (GFR α 1) and artemin to GFR α 3. Both signal through the receptor tyrosine kinase RET.

Previously, we have shown that GFL-receptors and channels of the transient receptor potential (TRP) family, TRPV1 and TRPA1, are co-expressed in lung-labelled jugular-nodose C-fibers. Even though RET was constitutively expressed in C-fibers, GFR α 1 was predominantly expressed in nodose C-fibers (placode-derived), whereas GFR α 3 expression was restricted to jugular C-fibers (neural crest-derived). The aim of the study was to compare the effects of GDNF- and artemin-induced RET activation on TRPV1- and TRPA1-function in jugular and nodose C-fibers and to speculate about their role in allergic asthma, which is associated with TRP-channel dysfunction.

Calcium-imaging was performed in murine jugular-nodose neurons which were pre-incubated with GDNF, artemin or control medium after stimulation with capsaicin (CPS, TRPV1-agonist) and cinnamaldehyde (CA, TRPA1-Agonist).

The response to CPS (defined as percentage of responsive neurons or the maximal 340/380 peak ratio) was down-regulated in α, β -mATP $^{+}$ (nodose) C-fibers by GDNF, but was up-regulated in α, β -mATP $^{-}$ (jugular) C-fibers by artemin. While GDNF-stimulation had no effect on the CA response in α, β -mATP $^{+}$ (nodose) C-fibers, artemin leads to a significant increase of the CA-response α, β -mATP $^{-}$ (jugular) neurons.

To investigate if GDNF and artemin might be involved in TRPV1 and TRPA1 regulation in allergic asthma, we performed quantitative RT-PCR in lung samples from animals with an OVA-induced airway inflammation and their respective controls. GDNF was significantly down-regulated in mice exhibiting an inflammation, while artemin was slightly up-regulated ($p = 0.198$).

Our results show that RET activation in nodose and jugular C-fibers by GDNF and Artemin, respectively, may have opposite effects on TRPV1 and TRPA1 regulation. Due to the opposite allergen-dependent regulation of GDNF and artemin in asthma, we speculate that both factors might contribute to TRP-channel dysfunction via RET.

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Abstract 5.2

Sympathetic neuroplasticity following T5 spinal cord transection increases the susceptibility to ischemia-induced sustained ventricular tachycardia

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Spinal cord injury-induced neuroplasticity within sympathetic pathways causes cardiac dysfunction and increases the susceptibility to life-threatening ventricular arrhythmias. For example, using coronary artery occlusion we documented a dramatic increase in the susceptibility to ventricular tachy-arrhythmias in conscious rats with mid-thoracic spinal cord injury (T₅X). Furthermore, using injection of cholera toxin B into the left and right stellate ganglia, as well as pericardial sac, and using the Sholl analyses, we documented that stellate-projecting sympathetic pre-ganglionic neurons within spinal segments T₁–T₅ as well as cardiac projecting sympathetic post-ganglionic neurons within the stellate ganglia from T₅X rats have larger dendritic trees than uninjured rats. The hearts of rats with T₅X are also hyper-innervated by tyrosine hydroxylase (TH)-immunoreactive sympathetic axons. These neuroplastic changes are associated with an increased nerve growth factor content within the heart and stellate ganglia. Thus, by using a combination of techniques and lines of evidence, we documented that mid-thoracic spinal cord injury results in cardiac sympathetic hyper-innervation and increased susceptibility to life-threatening ventricular arrhythmias. These results have important implications for understanding the mechanisms responsible for the high mortality rates and