zolantidine (H2 receptor antagonist), but not with pyrilamine (H1 receptor antagonist), reversed the antihypersensitivity effect of histamine. Zolantidine or pyrilamine alone in LC failed to influence pain behavior. The antihypersensitivity effect induced by histamine in LC was reduced also by spinal administration of atipamezole (an α_2-adrenoceptor antagonist).

Conclusions: The results indicate that histamine acting on H2 receptors in the LC attenuates mechanical hypersensitivity in peripheral neuropathy. The histamine-induced descending antihypersensitivity effect is at least partly mediated by noradrenergic pathways acting on the spinal α_2-adrenoceptor.

http://dx.doi.org/10.1016/j.sjpain.2013.07.015

B9

Pronociceptive effects of a TRPA1 channel agonist methylglyoxal in healthy control and diabetic animals

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Aims: Methylglyoxal (MG), a reactive carbonyl compound generated in diabetes mellitus (DM), activates the TRPA1 ion channel. Here we studied whether MG induces mechanical hypersensitivity or ongoing pain and whether the pronociceptive effect of MG is changed following its sustained endogenous release in DM.

Methods: DM was induced by streptozotocin (50–60 mg/kg i.p.) in the rat. MG and Chembridge-5861528 (CHEM), a selective TRPA1 channel antagonist, were administered intraplantarly (i.pl.) in control and diabetic animals. Limb withdrawal to monofilaments was used as an index of hypersensitivity, and observation of sustained pain-like behavior and conditioned place-avoidance test were used to assess ongoing pain. *In vitro* calcium imaging was used to study whether MG induces sustained activation of dorsal root ganglion (DRG) neurons of diabetic as well as control animals.

Results: MG produced mechanical hypersensitivity and ongoing pain behavior in control animals, which effects were reduced in diabetic animals. CHEM treatment at a dose suppressing the MG-induced mechanical hypersensitivity failed to suppress the MG-induced ongoing pain behavior. MG was able to produce sustained calcium inflow in DRG neurons of DM as well as control animals.

Conclusions: The results suggest that MG induces hypersensitivity and ongoing pain that are reduced in diabetes mellitus, possibly due to changes caused by the DM-induced sustained endogenous release of MG. Moreover, the MG-induced mechanical hypersensitivity can be more effectively reversed by a TRPA1 antagonist than the MG-induced ongoing pain behavior.

http://dx.doi.org/10.1016/j.sjpain.2013.07.016

B10

Human inducible pluripotent stem cell-derived sensory neurons express multiple functional ion channels and GPCRs

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Aims: Aim of the study was to characterize functional ion channel and GPCR responses by using selective pharmacological tools and intracellular calcium imaging from human inducible pluripotent stem cell-derived sensory neurons.

Methods: Sensory neurons were generated from human keratinocytes that were reprogrammed to inducible pluripotent stem cells by using standard Yamanaka factors. Inducible pluripotent stem cells were differentiated into sensory neurons by using 2 differentiation protocols (small molecule and PA6 co-culture). Sensory neurons were loaded with intracellular calcium dye Fluo-4. Single-cell calcium imaging was performed with Photometrics Evolve EM-CCD camera at physiological temperature. Cells were perfused with a Ringer solution at 2–3 ml/min into which pharmacological compounds were dissolved. Data was analyzed with Till Photonics Offline Analysis program.

Results: Most of the results were obtained from PA6 differentiated neurons. 50 s application of 50 mM KCl solution was used as diagnostic tool to activate voltage-gated calcium channels and thereby evoke intracellular calcium elevation. Functional ASIC, NMDA, kainate and TRPA1 ion channels were present in a subset of sensory neurons. Majority of sensory neurons showed robust responses to purinergic stimulation with ATP and histaminergic stimulation with histamine, but not to subtype selective histamine H1, H2 or H4 stimulation suggesting the presence of H3 receptor subtype All cells responded strongly to protease-activated receptor stimulation with a low dose of trypsin. Interestingly, at single-cell level notable heterogeneity of ion channel and GPCR responses was observed.

Conclusions: Our results suggest that iPS-derived sensory neurons will be valuable in further pharmacological studies as well as sensory neuropathy disease modeling.

http://dx.doi.org/10.1016/j.sjpain.2013.07.017