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The mineralocorticoid receptor antagonist spironolactone enhances morphine antinociception

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Aims: Spironolactone, an antimineralocorticoid, has been reported to potentiate the cataleptic effect of morphine in the rat. Since no previous research exists on the matter and the interaction might be clinically significant, the effects of spironolactone on morphine antinociception and pharmacokinetics in the rat were investigated.

Methods: Male SD rats were used to assess the effects of spironolactone on acute morphine-induced antinociception, development of morphine tolerance, and established morphine tolerance in the tail-flick and hot plate tests. Spironolactone was also administered with loperamide to assess whether spironolactone enhances the brain distribution of the acknowledged P-glycoprotein substrate across the blood–brain barrier.

Results: Spironolactone had no antinociceptive effects of its own but when co-administrated with morphine the antinociceptive effect of morphine was greatly enhanced. Morphine concentrations in the brain were increased fourfold in the spironolactone co-administrated group. Spironolactone did not inhibit the formation of pro-nociceptive morphine-3-glucuronide, nor did inhibit the development of tolerance. The peripherally restricted opioid, loperamide, had no antinociceptive effects by itself, but co-administration with spironolactone produced a clear change in the hot plate test.

Conclusions: Although mineralocorticoids have been proposed to take part in pain signaling, in our setting spironolactone did not have antinociceptive properties of its own. The increased antinociceptive effect of morphine is apparently caused by the increased morphine brain concentrations. We suggest this to be due to P-glycoprotein inhibition, as indicated by the loperamide assay. The clinical relevance of P-glycoprotein inhibition by spironolactone should be studied.

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B7

Expression of calcium/calmodulin-dependent protein kinase II in dorsal root ganglia in diabetic rats 6 months and 1 year after diabetes induction

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Aim: The aim of this study was to compare expression of total calcium/calmodulin-dependent protein kinase II (tCaMKII) and its α, β, γ and δ isoforms in dorsal root ganglion (DRG) in rat models of diabetes mellitus type I (DM1), 6 months and 1 year after diabetes induction.

Methods: A total of 45 male Sprague-Dawley rats weighing 160–200 g were assigned into four experimental groups: 6-months DM1 and its control group, 1-year and its control group. For the induction of DM1, after overnight fasting animals were injected intraperitonealy with 55 mg/kg of the streptozotocine (STZ). Rats were sacrificed 6 months and 1 year after the diabetes induction. The L4 and L5 ganglions were removed, fixed, embedded in freezing medium and sectioned on a cryostat. Immunofluorescence analysis was performed for detection of tCaMKII and its α, β, γ and δ isoforms. Image J software was used for analysis of immunofluorescence.

Results: The diabetes was successfully induced as confirmed by measurement of glucose levels and weight increase. Analysis of tCaMKII expression in DRGs revealed no differences between DM1 and control animals after 6 and 12 months. In diabetic animals, the expression of α and β isoforms decreased significantly after 6 months, compared to the controls, while decrease of γ and δ was observed after one year of diabetes in diabetic animals.

Conclusions: The observed changes in the expression of CaMKII isoforms reveal plastic changes of this enzyme during the chronic diabetic state and may be involved in the chronic neuropathic pain development.

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Histamine in the locus coeruleus attenuates neuropathic hypersensitivity

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Aims: Among brain structures receiving efferent projections from the histaminergic tuberomammillary nucleus is the pontine locus coeruleus (LC), a structure involved in descending noradrenergic control of pain. Here we studied whether histamine in the LC is involved in descending regulation of neuropathic hypersensitivity.

Methods: Peripheral neuropathy was induced by unilateral spinal nerve ligation (SNL) in the rat with a chronic intracerebral and intrathecal catheter for drug administrations. Mechanical hypersensitivity in the injured limb was assessed by monofilaments. Heat nociception was assessed by determining radiant heat-induced paw flick.

Results: Histamine in the LC (ipsilateral to nerve injury) produced a dose-related (1–10 μg) mechanical antihypersensitivity effect (maximum effect at 15 min and duration of effect 30 min), without influence on heat nociception. Pretreatment of LC with

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.

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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.

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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.

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