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Short communication

Leukemia inhibitory factor differentially regulates capsaicin and heat sensitivity in cultured rat dorsal root ganglion neurons

Sabine Engert [a], Jens R. Wendland [b], Albrecht Schwab [c], Marlen Petersen [d,*]

[a] Department of Obstetrics and Gynecology, University of Würzburg, 97080 Würzburg, Germany
[b] Laboratory of Clinical Science, National Institute of Mental Health, Bethesda, MD, USA
[c] Institute of Physiology II, University of Münster, 48149 Münster, Germany
[d] Department of Anesthesiology and Intensive Care Medicine, University of Heidelberg, Theodor-Kutzer-Ufer 1-3, 68167 Mannheim, Germany

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Abstract

Thermal hyperalgesia is one hallmark of neuropathic pain conditions. Although the exact pathophysiological mechanisms remain elusive, nerve growth factor (NGF) and leukemia inhibitory factor (LIF) are considered key mediators. Their local availability or synthesis are altered by nerve damage and, in turn, they entail changes in phenotype of affected neurons. We examined the effects of LIF on capsaicin sensitivity, heat responsiveness, and galanin immunoreactivity in rat dorsal root ganglion neurons cultured for up to 6 days without supplemented NGF. Using double labeling, the proportions of heat-sensitive/galanin-immunoreactive (GAL-IR) and capsaicin-sensitive/GAL-IR neurons were compared over time in culture with galanin immunoreactivity being a marker for nociceptive neurons.

The time course of the proportions of neurons responding to heat (44 °C) or capsaicin (1 μM) which also were GAL-IR was differently affected by LIF. In the absence of LIF, within the population of heat-sensitive neurons, the proportion of neurons also GAL-IR increased from 17% to 32% between 6 h and 1 day in culture to stay at this level. For the capsaicin-sensitive neurons, the proportion of neurons also GAL-IR increased from 10% after 6 h to 18% at day 2 and then decreased to 4% at day 4. In contrast, LIF prevented the increase in the proportion of heat-sensitive/GAL-IR neurons and the decrease of capsaicin-sensitive/GAL-IR neurons.

The results suggest that LIF partially prevents TRPV-1 downregulation in NGF-deprived nociceptive galaninergic DRG neurons. Furthermore, there is evidence that LIF regulates the expression of a heat receptor distinct from TRPV-1.

Keywords: TRPV-1; Nerve growth factor (NGF); LIF; Dorsal root ganglion (DRG); Heat receptors; Galanin

There is evidence that for the development of thermal hyperalgesia under pathophysiological conditions the TRPV-1 is not, or not only, the heat transducing receptor involved. Experiments with TRPV-1-lacking mice showed that under inflammatory conditions induced by mustard oil or complete Freunds adjuvant these animals did not develop thermal hyperalgesia (Caterina et al., 2000; Davis et al., 2000), after nerve injury, however, the thermal hyperalgesia was similar to wild-type mice (Caterina et al., 2000). Clinically, differences in heat-transducing receptors under both conditions would imply different therapeutic targets.

Peripheral nerve injury impedes retrograde NGF transport, resulting in changes in the phenotype of dorsal root ganglion (DRG) neurons: There is a decrease of the neuropeptides CGRP and SP (Hökfelt et al., 1994; Landry et al., 2000) and of TRPV-1 (Bevan and Winter,

* Corresponding author. Tel.: +49 (0) 621 383 5616; fax: +49 (0) 621 383 1463.
E-mail address: marlen.petersen@anaes.ma.uni-heidelberg.de (M. Petersen).

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1995; Winter et al., 1988) and on the other hand, an increase of the neuropeptide galanin, presumably in the population of nociceptive neurons (Hökfelt et al., 1987, 1994; Kerr et al., 2000; Ma and Bisby, 1997; Shadiack et al., 2001; Skofitsch and Jacobowitz, 1985; Verge et al., 1995; Wendland et al., 2003). We showed that there are only very few neurons that exhibit both galanin immunoreactivity and capsaicin sensitivity under condition of NGF deprivation (Wendland et al., 2003). This led us to ask whether galanin-immunoreactive (GAL-IR) and capsaicin-insensitive neurons are nonetheless heat-sensitive. The reduced TRPV-1 expression observed in isolated DRG neurons after nerve injury together with the fact of heat sensitivity maintained under in vivo conditions suggests a heat-transducing receptor distinct from TRPV-1.

Galanin expression after nerve injury is triggered not only by NGF deprivation but also by *de novo* synthesis of the cytokine leukemia inhibitory factor (LIF) by Schwann cells at the lesion site (Corness et al., 1996, 1998; Curtis et al., 1994; Dowsing et al., 1999; Sun and Zigmond, 1996). In situ, LIF is retrogradely transported to the DRG and signals via a heterodimeric receptor complex (Gardiner et al., 2002; Thompson et al., 1997).

To investigate heat sensitivity of nociceptive neurons under conditions similar to nerve injury (NGF deprivation and presence of LIF) in isolated DRG neurons, we used galanin immunoreactivity as a marker to identify nociceptive neurons. By double labeling experiments, we tested whether LIF added to the culture medium modifies the proportions of capsaicin-sensitive/GAL-IR and heat-sensitive/GAL-IR neurons.

Cell dissociation and culture: Male Sprague-Dawley rats (180–250 g; Charles River, Sulzfeld, Germany) were sacrificed by an i.p. lethal dose of sodium pentobarbital. Procedures were performed according to recommendation of IASP (Zimmermann, 1983). DRG from all segments of the spinal cord were excised and neurons were isolated enzymatically and mechanically as described previously (Petersen et al., 1998). Briefly, ganglia were incubated in Dulbecco's modified Eagle's medium (D-MEM) containing collagenase type CLS IV (260 U/mg; Biochrom, Berlin, Germany) at 37 °C for 90–135 min followed by an incubation for 11 min in D-MEM containing trypsin (9820 U/ml; Sigma, Neu-Ulm, Germany). The ganglia were suspended in D-MEM containing gentamicin (25 μg/ml; Gibco, Karlsruhe, Germany) and mechanically dissociated. Cells were centrifuged using Percoll® as density gradient to minimize the amount of non-neuronal cells and debris. Then the pellet was suspended in Ham's F12 medium supplemented with 10% heat-inactivated horse serum (Gibco), 2 mM L-glutamine, 100 U/ml penicillin, and 100 mg/ml streptomycin (Gibco). Cells were plated on glass coverslips coated with poly-L-lysine (Sigma) and maintained in supplemented Ham's F12 medium. LIF (30 ng/ml; Chemicon, Hampshire, UK) and NGF-7S (100 ng/ml; Calbiochem, Bad Soden, Germany) were added as indicated. In the following, "no NGF" or "no LIF" are meant to indicate that no growth factors were added to the culture medium. Neurons were kept in culture for 6 h–6 days at 37 °C and 5% CO_2. Half of the medium was replaced daily.

Double labeling experiments: Heat- or capsaicin-sensitive neurons were first identified using the cobalt-uptake method (Hogan et al., 1983; Winter, 1987). Briefly, after washing, neurons were stimulated with either capsaicin (1 μM) or heat (44 °C) for 8 min in assay buffer containing 5 mM $CoCl_2$. In neurons expressing the TRPV-1, capsaicin- or heat-induced activation of the receptor leads to an influx of Co^{2+}. Coverslips were placed 2 min in 1% ammonium sulfide yielding a brownish cobalt precipitate. Then, coverslips were washed, and fixed with 4% paraformaldehyde.

For subsequent immunocytochemical staining, coverslips were washed in 0.1 M phosphate buffered saline with 0.1% triton X-100 (PBS-Tx) (Sigma). Coverslips were preincubated 15 min with 10% donkey serum (NDS; Jackson ImmunoResearch, West Grove, PA, USA) diluted in 0.1 M PBS-Tx and incubated 12–16 h at 4 °C with rabbit anti-rat galanin antibody (Peninsula Laboratories, San Carlos, CA, USA) diluted 1/1000 in 0.1 M PBS-Tx with 1% NDS. Then, coverslips were washed in 0.1 M PBS-Tx and incubated for 2 h at room temperature with Cy3-labeled donkey anti-rabbit antibody diluted 1/300 in 0.1 M PBS-Tx. Finally, coverslips were washed with 0.1 M PBS-Tx, dipped in distilled water, and mounted with Vectashield® (Vector Laboratories, Burlingame, CA, USA). In each experiment one coverslip underwent the same procedure as above, except for the heat or capsaicin stimulation and omitting the primary antibody, to ensure the specificity of the staining. All neurons on these coverslips were unstained. For the cobalt-uptake experiments, the criterion for a positive neuron was a distinct brown staining due to the cobalt sulfide precipitation. This was determined by visual inspection. The data analysis was performed with the investigator blinded to the identity of the coverslips.

Statistical tests were performed using the Tukey test within the analysis of variance, ANOVA. All calculations were done using STATISTICA software package (StatSoft Inc., Tulsa, OK, USA). Data are given as mean ± SEM. A probability of $p < 0.05$ was regarded as significant.

Double labeling experiments were performed for (i) heat sensitivity and GAL-IR and (ii) capsaicin sensitivity and GAL-IR. Neurons were NGF-deprived for up to 6 days without LIF (Fig. 1 left) and with LIF (Fig. 1 right). First, the proportions of heat- or capsaicin-sensitive neurons and the proportions of GAL-IR neurons were determined separately, i.e., not regarding