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Research Paper

Enhanced spinal neuronal responses as a mechanism for the increased nociceptive sensitivity of interleukin-4 deficient mice

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ABSTRACT

Lack of the anti-inflammatory and analgesic cytokine interleukin-4 (IL-4) is associated with mechanical hypersensitivity in mice, and low systemic levels of IL-4 are associated with pain in humans. We investigated whether the firing properties of murine nociceptive neurons in the spinal dorsal horn are affected by IL-4 deficiency. Single unit recordings from lumbar dorsal horn wide-dynamic-range (WDR) neurons were performed in IL-4 knock out (ko) mice and wild type (WT) littermates (3, 9, and 22 months old). We measured neuronal responses to innocuous (1 g) and noxious (26 g) von Frey mechanical stimulation at the plantar hind paw. Additionally, we induced secondary hyperalgesia by intraplantar injection of capsaicin and recorded discharges before and 5 and 10 min after injection. Baseline activity, activity upon innocuous stimulation, and postdischarges after noxious stimulation were not different between genotypes and ages. Responses to the noxious von Frey filament in 3 (34.5, 26.6–41.1 Hz) and 9 month old (49.7, 21.7–108.2 Hz) IL-4 ko mice were greater than in WT littermates (3 months, 18.1, 16.3–27.2 Hz, n.s.; 9 months, 33.6, 10.4–69.7 Hz; $p < 0.05$). In contrast, 22 month IL-4 ko mice had lower discharges (22.4, 16.8–28.9 Hz) than 3 and 9 month IL-4 ko mice ($p < 0.01$ each) and age-matched WT littermates (36.6, 10.4–59.4 Hz; n.s.). This pattern was also found 5 and 10 min after capsaicin injection. An enhanced excitability in the first segment of the nociceptive pathway may contribute to the increased behavioral responsiveness to painful stimuli of young IL-4 ko mice.

1. Introduction

Neuro-immune interactions via immune cells, neurotrophic factors, microRNAs, chemokines, and cytokines play a major role in the induction and maintenance of neuropathic pain (Austin and Moalem-Taylor, 2010; Calvo et al., 2012; Kress et al., 2013). As shown in animal models, the balanced expression of pro- and anti-inflammatory cytokines that have algesic and analgesic effects seems to be the basis for a physiological pain homeostasis. Altered behavior has been observed in animal models of cytokine or immune component deficiency (Üçeyler et al., 2010a; Üçeyler and Sommer, 2008).

While the role of the pro-inflammatory cytokines in pain has been investigated extensively, research on anti-inflammatory cytokines is scarce. The most frequently assessed candidates are interleukin-4 (IL-4), IL-10, and IL-13, which have analgesic effects in neuropathic and inflammatory pain models (Cunha et al., 1999; Hao et al., 2006; Karam et al., 2011; Ledeboer et al., 2007; Milligan et al., 2005; Vale et al., 2003). IL-4 connects the immune and opioid systems by inducing μ and δ opioid receptor transcription (Börner et al., 2004b; Kraus et al., 2001), which makes this cytokine an even more interesting candidate for pain research.

In previous studies we and others reported a reduced systemic expression of IL-4 in patients with chronic pain syndromes (Alexander et al., 2007; Üçeyler et al., 2006, 2007). Correspondingly, we found that genetically modified IL-4 knock out (ko) mice have enhanced nociceptive reflexes upon mechanical stimulation with von Frey filaments (Üçeyler et al., 2011). In the present study we investigated whether an increased responsiveness of nociceptive neurons in the spinal dorsal horn underlies this increased pain sensitivity of IL-4 deficient mice. Single unit recordings were made from wide-dynamic-range (WDR) neurons in the lumbar dorsal horn of IL-4 ko mice and wild type (WT) littermates, and their discharges upon innocuous and noxious mechanical stimulation of a hind paw were compared. Additionally, we investigated the presence and extent of capsaicin-induced secondary hyperalgesia in genotypes and age groups. We found that spinal nociceptive neuronal responses were greater in young IL-4 ko mice than in WT mice. This might represent one of the reasons for the enhanced nociception of IL-4 deficient mice and humans. Unexpectedly, we also found that in old IL-4 ko mice the spinal nociceptive neuronal responses were lower than in WT mice.

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2. Methods

2.1. Mice

Electrophysiological experiments were carried out in 81 male IL-4 ko mice (age 3 months: n = 20; 9 months: n = 42; 22 months: n = 19) and 62 WT littermates of C57BL/6J background (3 months: n = 12, 9 months: n = 32; 22 months: n = 18). For behavioral tests we additionally investigated 21 IL-4 ko mice and 16 WT littermates at the ages of 3 (n = 5 each), 10 (n = 5 each), and 18 (n = 11 IL-4 ko, n = 6 WT) months. IL-4 ko mice breeder pairs on C57BL/6J background were purchased from Jackson Laboratories (Maine, USA). WT C57Bl/6J mice were purchased from Charles River Laboratories (Sulzfeld, Germany). WT littermates were generated by cross-breeding IL-4 ko and WT mice to the third generation. Experiments were approved by the Bavarian State authorities (Regierung von Unterfranken, #02/08; #3/12). All mice were held at the animal facilities of the Department of Neurology, University of Würzburg, under standard conditions with food and water access ad libitum. Animal use and care were in accordance with the institutional guidelines.

2.2. Anesthesia and surgery

Mice (median body weight 32 g, range 25–40 g) were deeply anesthetized with intraperitoneal injection of ketamine/xylazine (ketamine 100 mg/kg/xylazine 10 mg/kg; Pfizer, Berlin, Germany/CP-Pharma, Burgdorf, Germany) and fixed in a stereotaxic frame with spinal clamps (Stoelting Spinal Cord Surgery Adaptor, Dublin, Ireland). Additional anesthetic doses were given if necessary. Rectal temperature was kept at 38 °C by means of a feedback-controlled thermoelectric pad. A laminectomy was performed and the dura mater was carefully opened over the lumbar enlargement of the spinal cord, which was then covered with mineral oil to avoid drying (Cuellar et al., 2004; Urch and Dickenson, 2003). The right hind paw was affixed to the frame's base plate, plantar side up for mechanical stimulation.

2.3. Behavioral testing

Paw withdrawal latencies to heat were determined following the method of Hargreaves (Hargreaves et al., 1988) and applying a standard Ugo Basile Algesiometer (Comerio, Italy). Mice were placed on a glass surface and a radiant heat source was positioned under one hind paw. The time until paw withdrawal was recorded automatically. To avoid tissue damage a time limit for heat application of 15 s was observed. Each hind paw was tested three times.

Paw withdrawal thresholds to mechanical stimulation were investigated with the von Frey test based on the up-and-down-method (Chaplan et al., 1994). Animals were placed in plexiglass cages on a wire mesh. The plantar surface of the hind paws was touched with a von Frey filament starting at 0.07 g. When the mouse withdrew its hind paw upon administration of mild pressure the next thinner von Frey filament was used. If the animal did not react to this stimulation, the next thicker von Frey filament was applied. Each hind paw was tested three times. The 50% withdrawal threshold (i.e., force of the von Frey hair to which an animal reacts in 50% of the administrations) was recorded. Tests were performed by an investigator blinded for mouse genotype.

2.4. In vivo single unit recordings

At a depth of 200–500 μm from the spinal cord dorsum, individual single unit action potentials with amplitudes well above noise level were recorded (Suppl. Figs. 1 and 2) by means of tungsten microelectrodes (9–12 M-Ohm, FHC, Bowdoin, USA) mounted on a micromanipulator (ROE-200, Sutter Instrument Company, Novato, USA). Signals were amplified (ELC-03XS, NPI Electronic GmbH, Tamm, Germany), displayed, discriminated, and stored with the aid of the Patchmaster software (HEKA Elektronik Dr. Schulze GmbH, 67466 Lambrecht, Germany).

Neurons were characterized as WDR if they responded to both light touch (brush) and noxious (pinching with a forceps) stimulation. The neurons chosen for study had a receptive field in the plantar surface of the right hind paw and discharged upon application of a nylon von Frey filament (Healthcare Products, Lehi, USA). Two different von Frey filaments were applied: the von Frey filament with a bending force of 1 g (filament size 4.08, innocuous) never elicited paw withdrawal reflexes, and the one with a 26 g (filament size 5.4, noxious) bending force always elicited paw withdrawal, when applied to the hind paw in unanesthetized, freely moving IL-4 ko or WT mice (Üçeyler et al., 2011). Each von Frey filament was perpendicularly applied to the neuron's receptive field with bending force during 15 s. Once stable responses from a single neuron were reliably obtained, the following stimulation cycle was carried out (Suppl. Fig. 1): 15 s without stimulation (baseline); 15 s stimulation with 1 g; 15 s without stimulation; 15 s stimulation with 26 g; and 15 s without stimulation (postdischarge; Suppl. Fig. 1). Examples of spike recordings for the different conditions are illustrated in Suppl. Fig. 2. The number of action potentials discharged during each step of 15 s in a stimulation cycle was expressed as firing frequency (Hz).

In order to elicit secondary hyperalgesia (Kim et al., 2009; Lee et al., 2007), capsaicin (0.05%, 5 μl, Torcan Chemical, Aurora, Canada) was injected intraplantarly (i.pl.) >10 mm away from the neuron's receptive field (Suppl. Fig. 3). Stimulation cycles were carried out before, and 5 and 10 min after capsaicin injection.

2.5. Statistical analysis

Statistical analysis and graphs were prepared using SPSS software version 22 (Ehningen, Germany). Data were tested for their pattern of distribution using histograms and the Kolmogorov–Smirnov-test. Since the data did not show a normal distribution we applied the non-parametric Mann–Whitney-*U*-test for pairwise comparison. To correct for multiple comparisons we applied the Bonferroni–Holm procedure as appropriate. Differences with a $p < 0.05$ were considered as statistically significant. Data are expressed as median (and range in parenthesis) and illustrated as box-and-whisker plots showing the median, the upper 75% and lower 25% percentiles and the minimum and maximum values.

3. Results

3.1. Baseline discharges

At baseline, i.e., during the 15 s period before the stimulus applications were begun, neuronal discharges reached a median of 0.2, 0.4, and 0.1 Hz in IL-4 ko, and of 1.6, 0.7, and 0.7 Hz in WT mice, of the three age groups, respectively (3, 9, and 22 months) without differences between genotypes or between age groups (Table 1).

3.2. Responses to mechanical stimulation

WDR neuronal responses to the 1 g von Frey filament reached a median of 3.6, 3.4, and 2.4 Hz for IL-4 ko, and 3.2, 1.3, and 1.1 Hz for WT mice of the three age groups, respectively, without differences between genotypes or between age groups (Table 1).

In contrast, neuronal discharges elicited by the noxious 26 g von Frey filament were 34.5 Hz for IL-4 ko versus 18.1 for WT 3 month old mice ($p < 0.05$, n.s. after Bonferroni–Holm correction), and 49.7 versus 33.6 for 9 month old mice ($p < 0.05$; Table 1). As Fig. 1 shows, at these two ages, responses of nociceptive spinal neurons were greater for IL-4 ko than for WT mice, and nociceptive responses increased from 3 months to 9 months of age. Interestingly, in WT mice the responses to the