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HYPERALGESIC PRIMING IS RESTRICTED TO ISOLECTIN B4-POSITIVE NOCICEPTORS

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Abstract—We have previously described a rat model for the contribution of neuroplastic changes in nociceptors to the transition from acute to chronic pain. In this model a prior injury activates protein kinase C epsilon (PKCε), inducing a chronic state characterized by marked prolongation of the hyperalgesia induced by inflammatory cytokines, prototypically prostaglandin E_2 (PGE_2), referred to as hyperalgesic priming. In this study we evaluated the population of nociceptors involved in priming, by lesioning isolectin B4-positive (IB4(+)) nociceptors with intrathecal administration of a selective neurotoxin, IB4-saporin. To confirm that the remaining, TrkA(+)/IB4(−), nociceptors are still functional, we evaluated if nerve growth factor (NGF) induced hyperalgesia. While pretreatment with IB4-saporin eliminated the acute mechanical hyperalgesia induced by glia-derived neurotrophic factor (GDNF), NGF and $\Psi\varepsilon$RACK, a highly selective activator of PKCε, induced robust hyperalgesia. After injection of NGF, GDNF or $\Psi\varepsilon$RACK, at a time at which hyperalgesia induced by PGE_2 is markedly prolonged (hyperalgesic priming) in control rats, in IB4-saporin-pretreated rats PGE_2 failed to produce this prolonged hyperalgesia. Thus, while PKCε is present in most dorsal root ganglion neurons, where it can contribute to acute mechanical hyperalgesia, priming is restricted to IB4(+)-nociceptors, including those that are TrkA(+). While PKCε activation can induce acute hyperalgesia in the IB4(+) population, it fails to induce priming. We suggest that hyperalgesic priming occurs only in IB4(+) nociceptors, and that in the peripheral terminals of nociceptors separate intracellular pools of PKCε mediate nociceptor sensitization and the induction of hyperalgesic priming.

Key words: chronic pain, protein kinase C epsilon, dorsal root ganglion neurons, isolectin B4, NGF, GDNF.

In studies designed to detect transition from acute to chronic pain, we demonstrated that inflammation produces a long-lasting neuroplastic change in the signaling pathway mediating inflammatory cytokine-induced nociceptor sensitization and mechanical hyperalgesia, at a previously inflamed site. We have referred to this phenomenon as hyperalgesic priming (Aley et al., 2000; Reichling and Levine, 2009). The induction of priming is mediated by activation of protein kinase C epsilon (PKCε) in the peripheral terminal of the nociceptor (Aley et al., 2000; Parada et al., 2003). While PKCε is found in almost all dorsal root ganglion neurons (Cesare et al., 1999; Hundle et al., 1995; Khasar et al., 1999; Numazaki et al., 2002; Summer et al., 2006; Yamamoto et al., 2006), its G-protein coupled receptor activation-induced translocation from the cytoplasm to the plasma membrane is restricted to the isolectin B4-positive (IB4(+)) population of nociceptors (Hucho et al., 2005). While we have recently shown that both nerve growth factor (NGF) and glia-derived neurotrophic growth factor (GDNF) induce hyperalgesic priming (Ferrari et al., 2010), roughly a third of TrkA(+) neurons are IB4(+) (Fang et al., 2006; Kashiba et al., 2001). Since PKCε is translocated to the plasma membrane in IB4(+) but not IB4(−) neurons (Hucho et al., 2005), we evaluated if NGF-induced *priming* like that induced by GDNF is mediated by its action on the TrkA(+) subpopulation of IB4(+) neurons, by lesioning the IB4(+) population of dorsal root ganglion neurons with the selective neurotoxin, IB4-saporin (Joseph et al., 2008).

EXPERIMENTAL PROCEDURES

Animals

Experiments were performed on adult male Sprague–Dawley rats (n=27; 250–350 g; Charles River, Hollister, CA, USA). Animals were housed three per cage, under a 12-h light/dark cycle, in a temperature and humidity controlled environment at the University of California, San Francisco (UCSF) animal care facility. Food and water were available *ad libitum*. All nociceptive testing was done between 10:00 AM and 4:00 PM. All experimental protocols were approved by the UCSF Committee on Animal Research and conformed to National Institutes of Health Guidelines for the Care and Use of Laboratory Animals.

Nociceptive testing

The nociceptive flexion reflex was quantified with an Ugo Basile Analgesymeter (Stoelting, Chicago, IL, USA), which applies a linearly increasing mechanical force to the dorsum of a rat's hind paw. Nociceptive threshold, defined as the force in grams at which the rat withdrew its paw, was the mean of three readings taken at 5 min intervals. Rats were lightly restrained in cylindrical transparent acrylic restrainers designed to allow extension of the hind leg from the restrainer for nociceptive threshold testing. All rats were acclimatized to the testing procedures to reduce variability and produce a more stable baseline of the paw-withdrawal threshold. The mechanical paw withdrawal threshold was determined before and after administration of test agents. Each paw was treated as an independent measure and each experiment performed on separate groups of rats. The results are expressed as percentage change from baseline mechanical nociceptive threshold.

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Abbreviations: GDNF, glia-derived neurotrophic factor; IB4(+), isolectin B4-positive; NGF, nerve growth factor; PGE_2, prostaglandin E_2; PKCε, protein kinase C epsilon; SEM, standard error of the mean.

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Drugs

Drugs employed in this study were prostaglandin E_2 (PGE_2) (a direct-acting hyperalgesic agent) and NGF from Sigma (St. Louis, MO, USA), GDNF from EMD Biosciences (La Jolla, CA, USA), and ΨεRACK, a selective activator of PKCε (Dorn and Mochly-Rosen, 2002) prepared by SynPep (Dublin, CA, USA). Drugs were applied by intradermal injection on the dorsum of the hind paw. A stock solution of NGF (1 μg/μl in 0.9% NaCl containing 0.5% bovine serum albumin) was diluted in 0.9% NaCl at the time of injection (dose 1 μg) (Mallik-Hall et al., 2005). GDNF was similarly prepared. (Bogen et al., 2008) ΨεRACK was dissolved in saline (Joseph et al., 2007). The stock solution of prostaglandin E_2 (1 μg/μl) was prepared in absolute ethanol and additional dilutions made with physiological saline; the final concentration of ethanol was ≤2%. IB4-saporin, which consists of isolectin B4 coupled to the neurotoxin saporin, was purchased from Advanced Targeting Systems (San Diego, CA, USA). All drugs, except IB4-saporin, which was administered by the spinal intrathecal route, were administered intradermally in a volume of 5 μl using a 30-gauge hypodermic needle attached to a microsyringe (Hamilton, Reno, NV, USA). The selection of the drug doses used in this study was based on dose–response curves determined during previous studies (Joseph et al., 2007; Khasar et al., 1993; Malik-Hall et al., 2005).

Intrathecal administration of IB4-saporin

IB4-saporin was diluted with saline and a dose of 3.2 μg/20 μL administered intrathecally 10 days prior to experiments (Bogen et al., 2008; Joseph et al., 2008). With the use of an insulin syringe, IB4-saporin was injected into the subarachnoid space on the midline between the L4 and L5 vertebrae. For this procedure, rats were anesthetized with 2.5% isoflurane (Phoenix Pharmaceuticals, St. Joseph, MO, USA) (97.5% O_2).

Statistical analysis

In all experiments, the dependent variable was change in paw withdrawal threshold represented as percentage change from baseline paw withdrawal threshold. Group data are presented as mean±standard error of the mean (SEM). Statistical comparisons were done by ANOVA or Student's *t*-test, as appropriate. *P*-values <0.05 were considered statistically significant.

RESULTS

Effect of IB4-saporin on hyperalgesia

Ten days after the intrathecal administration of IB4-saporin, a time at which IB4(+) terminals in the dorsal horn of the spinal cord have degenerated, GDNF (10 ng), NGF (1 μg) or ΨεRACK (1 μg), was injected intradermally, on the dorsum of the hind paw, in separate groups of rats. Pretreatment with IB4-saporin almost completely eliminated the mechanical hyperalgesia induced by GDNF. However, NGF and ΨεRACK induced robust hyperalgesia in IB4-saporin treated rats (Fig. 1B). Thus, the remaining TrkA(+) nociceptors, which are IB4(−), remain functional as NGF and PKCε activation produces mechanical hyperalgesia. These findings confirm the role of PKCε in nociceptor sensitization and mechanical hyperalgesia, as well as demonstrating that this function can be distinguished from its role in hyperalgesic priming. In control animals, not treated with IB4-saporin, GDNF, NGF and ΨεRACK all produced mechanical hyperalgesia (Fig. 1A).

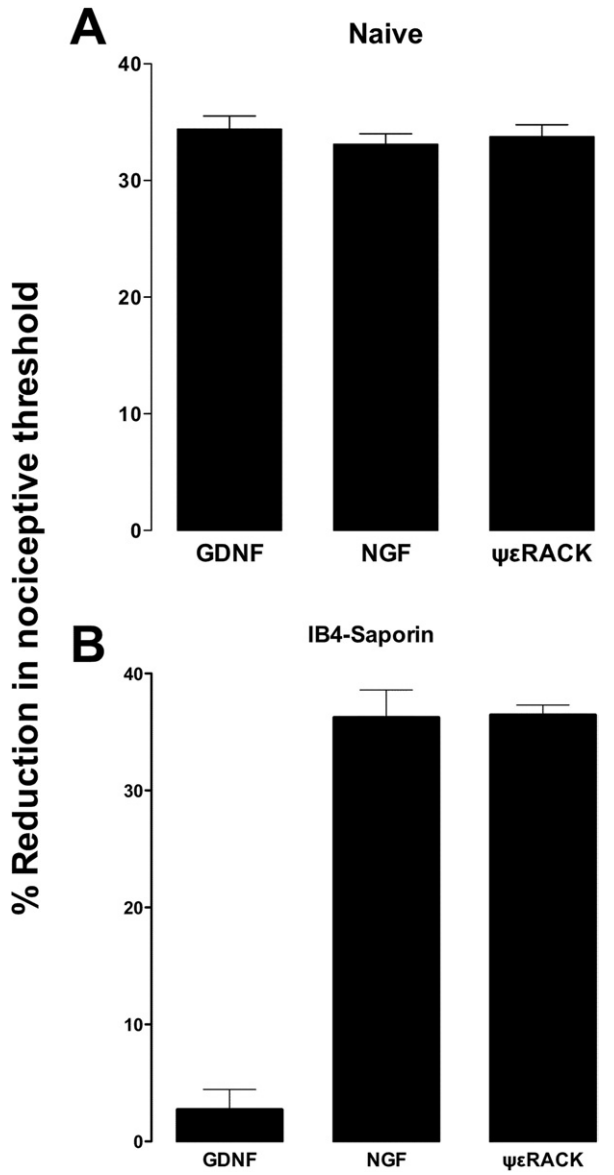

Fig. 1. Changes in mechanical hyperalgesia in IB4-saporin pretreated rats. (A) Hyperalgesia induced by GDNF, NGF and ΨεRACK. Intradermal administration of GDNF (10 ng), NGF (1 μg) and ΨεRACK (1 μg) produced mechanical hyperalgesia in naive rats. All drugs were injected in a volume of 5 μl and the paw withdrawal thresholds measured 30 min after each injection (n=6/group, P<0.001). (B) Changes in mechanical hyperalgesia in IB4-saporin pretreated rats. Intradermal injection of glia-derived neurotrophic factor (GDNF) in rats that were pretreated with the neurotoxin isolectin B4 (IB4)-saporin failed to produce mechanical hyperalgesia (n=6), while in groups of rats similarly pretreated with IB4-saporin, nerve growth factor (NGF) and ΨεRACK produced robust hyperalgesia (both n=6; P<0.001).

Effect of IB4-saporin on priming

Intradermal injection of PGE_2 (100 ng) in naive rats produces a robust, short-lived hyperalgesia, in which mechanical nociceptive threshold has returned to baseline by 4 h (Aley et al., 2000). When PGE_2 is administered to rats previously treated with GDNF, NGF or ΨεRACK—when paw withdrawal threshold had returned to baseline—PGE_2