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THE TrKA RECEPTOR MEDIATES EXPERIMENTAL THERMAL HYPERALGESIA PRODUCED BY NERVE GROWTH FACTOR: MODULATION BY THE p75 NEUROTROPHIN RECEPTOR

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Abstract—The p75 neurotrophin receptor (p75^{NTR}) and its activation of the sphingomyelin signaling cascade are essential for mechanical hypersensitivity resulting from locally injected nerve growth factor (NGF). Here the roles of the same effectors, and of the tropomyosin receptor kinase A (TrkA) receptor, are evaluated for thermal hyperalgesia from NGF. Sensitivity of rat hind paw plantar skin to thermal stimulation after local sub-cutaneous injection of NGF (500 ng) was measured by the latency for paw withdrawal (PWL) from a radiant heat source. PWL was reduced from baseline values at 0.5–22 h by ~40% from that in naïve or vehicle-injected rats, and recovered to pre-injection levels by 48 h. Local pre-injection with a p75^{NTR} blocking antibody did not affect the acute thermal hyperalgesia (0.5–3.5 h) but hastened its recovery so that it had reversed to baseline by 22 h. In addition, GW4869 (2 mM), an inhibitor of the neutral sphingomyelinase (nSMase) that is an enzyme in the p75^{NTR} pathway, also failed to prevent thermal hyperalgesia. However, C2-ceramide, an analog of the ceramide produced by sphingomyelinase, did cause thermal hyperalgesia. Injection of an anti-TrkA antibody known to promote dimerization and activation of that receptor, independent of NGF, also caused thermal hyperalgesia, and prevented the further reduction of PWL from subsequently injected NGF. A non-specific inhibitor of tropomyosin receptor kinases, K252a, prevented thermal hyperalgesia from NGF, but not that from the anti-TrkA antibody. These findings suggest that the TrkA receptor has a predominant role in thermal hypersensitivity induced by NGF, while p75^{NTR} and its pathway intermediates serve a modulatory role.

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Abbreviations: aPKC, atypical protein kinase C; C2-ceramide, N-acetyl-D-sphingosine; CLP, contralateral paw; IgG, immunoglobin; NGF, nerve growth factor; nSMase, neutral sphingomyelinase; p75^{NTR}, p75 neurotrophin receptor; PBS, phosphate buffered saline; PI3K, phosphatidyl inositol 3 kinase; PKMζ, protein kinase M zeta; PWL, paw withdrawal latency; S1P, sphingosine 1-phosphate; S1PR, sphingosine 1-phosphate receptor; SphK, sphingosine kinase; TrkA, tropomyosin receptor kinase A; TRPV1, transient receptor potential vanilloid 1.

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INTRODUCTION

In addition to influencing the development, survival and differentiation of peripheral sensory and sympathetic neurons, nerve growth factor (NGF), induces thermal and mechanical sensitization of these neurons in adult humans (Dyck et al.,1997; Bennett et al., 1998; Svensson et al., 2003; Rukwied et al., 2010) and decreases both mechanical and thermal nociceptive thresholds in rodent models of pain (Lewin et al., 1993; Woolf et al., 1994; Amann et al., 1995; McMahon et al., 1995a,b; Woolf 1996; Hathway and Fitzgerald, 2006; Mills et al., 2013). The initiation and maintenance of nociceptor hypersensitivity as a part of the inflammatory response after tissue injury manifests as acute and chronic pain (see Pezet and McMahon, 2006). Results of recent clinical trials using NGF-sequestering antibodies attests to the ongoing role of this neurotrophin in chronic pain (Wild et al., 2007; Cattaneo, 2010; Shelton, 2014; Schnitzer et al., 2015), although pre-clinical findings show a different contribution of NGF between inflammatory and neuropathic pain (Djouhri, 2016).

Both the trophic actions and the hyperalgesic effects of NGF have been attributed to tropomyosin receptor kinase A (TrkA) that is expressed on peripheral and central neurons and is distinguished by its high affinity for NGF (Meakin and Shooter, 1992; Barker and Murphy, 1992; Fundin et al., 1997). A lower affinity NGF receptor, the p75 neurotrophin receptor (p75^{NTR}), activates a different intracellular pathway than that of TrkA. Traditionally, p75^{NTR} was thought to be exclusively involved in the development and survival aspects of NGF, and hyperalgesia in the developed, adult peripheral nervous system was attributed to TrkA (Bergmann et al., 1998; Watanabe et al., 2008; Mantyh et al., 2011). However, the NGF enhancement of excitability of isolated sensory neurons relies on activation of p75^{NTR}, which triggers the sphingomyelin signaling cascade (for a review see Nicol and Vasko, 2007; Zhang et al., 2012). Neutral sphingomyelinase(s) (nSMase), its metabolic product, ceramide, and the atypical PKC (*aPKC*), protein kinase M zeta (PKMζ), are important effector molecules of this intracellular pathway. We recently reported that mechanical sensitivity was rapidly enhanced following NGF injection into the plantar surface of the rat paw and that the p75^{NTR} pathway

played a key role, since the hypersensitivity was prevented by antibody blockade of that receptor and by inhibition of either nSMase or an atypical protein kinase C (aPKC) (Khodorova et al., 2013). In addition, NGF-induced mechanical hypersensitivity was recapitulated by a membrane permeant homolog of ceramide. These findings on mechano–hypersensitivity are all consistent with signaling through the $p75^{NTR}$-nSMase-aPKC pathway. However, changes in thermal sensitivity caused by NGF, and the involvement of the TrkA pathway were not explored in this preceding work.

Therefore, the present work expands these studies by determining the contributions of the $p75^{NTR}$- and of the TrkA- coupled pathways to NGF-induced thermal hypersensitivity in (male) rats. The results show that TrkA is essential for this thermal response and that $p75^{NTR}$ plays a modulatory role in shaping the duration, but does not affect the acute phase, of thermal hypersensitivity.

EXPERIMENTAL PROCEDURES

Experiments were conducted on 118 adult male Sprague–Dawley rats (230–300 g). Rats were housed 2 per cage under a 12:12-h dark–light cycle and were provided with food and water *ad libitum*. Animals were experimentally treated and cared for in accordance with the Guide for the Care and Use of Laboratory Animals (Guide, 1996) as reviewed and approved by the Harvard Committee on Animals. For most tests, eight rats were assigned to each control or treatment group, unless otherwise noted.

Thermal testing

The sensitivity of the plantar paw to noxious radiant heat was determined by the method of Hargreaves et al. (1988) (IITC, Life Science, Inc., Woodland Hills, CA, USA). The animals were habituated and tested on a raised glass platform over 5–7 days before each experiment in order to achieve a consistent paw withdrawal latency (PWL), as free of stress-related effects as possible. A series of 3–4 withdrawal latencies was determined, within each test session, alternately on left and right paws (more than four tests were applied in the case of a high variability in the behavioral responses); the first paw tested was assigned randomly. A 20-s cut-off time was set to avoid overt thermal sensitization from testing per se, and tests of the same paw were separated by 3–4-min intervals. Three to four measurements for each intact hind paw, performed on the two days (including the day of the experiment) preceding any injections, were averaged and the mean value taken as the baseline nociceptive PWL. Following any treatment, withdrawal latency measurements were carried out alternately on the NGF (or vehicle)-injected (ipsilateral) paw and the uninjected contralateral, paw.

Injection procedures

All injections were made subcutaneously (s.c.) into the mid-plantar surface of the hind paw, 1 cm distal from the heel using a 30-G needle attached to a 10 μl Hamilton microsyringe (Hamilton Co., Reno, NV, USA). The NGF-injected paw was identified as the Ipsilateral Paw (ILP) and the opposite paw as the contralateral paw (CLP). Injections occurred under brief general anesthesia caused by inhalation of the rapidly reversible agent sevoflurane (Abbott Labs, N. Chicago, IL, USA). Recovery of the righting reflex occurred in < 30 s after anesthesia inhalation was discontinued; 5–10 min later "normal" nocifensive responses to paw pinch could be assessed in control animals.

NGF, N-acetyl-D-sphingosine (C2-ceramide), GW4869, K252a or their vehicles, each were injected in 10 μl volumes, and antibodies to $p75^{NTR}$ or the TrkA receptor were injected in 20 μl volumes. The non-selective myristoylated pseudosubstrate inhibitor of atypical PKCs (mPSI- or "ZIP"; Standaert et al., 1997), or its inactive scrambled peptide homolog (scrambled ZIP), both at 40 μg/20 μl, were similarly injected, before NGF.

Drugs

NGF-β (rat, recombinant) (Cat No.N-2513, Sigma–Aldrich, St. Louis MO, USA), or NGF-β/CF (rat) (R&D Systems, Inc., MN, USA) was dissolved in phosphate buffered saline (PBS; In Vitrogen) as a stock solution (1000 ng/10 μL) and stored in small aliquots at −80 °C. Prior to the injection, NGF stock aliquots were diluted in PBS (pH 7.4) to the noted final concentration of 50 ng/μl, serving to deliver 500 ng per 10 μL injection. C2-ceramide (Cat No. BML-SL 100-005; Enzo Life Sciences, Inc., NY, USA) was dissolved initially to 250 μg/10 μl in pure DMSO, and stored in aliquots at -20 °C, then diluted to 20 μg/10 μl DMSO before the injection. GW4869 (Cat No. D1692; Sigma–Aldrich) was dissolved in DMSO as a 2 mM stock solution, and aliquots were prepared under a stream of N_2 before freezing, to avoid atmospheric oxidation. A wide spectrum inhibitor of tropomyosin receptor kinases, K252a (Cat No.K1639, Sigma–Aldrich), was dissolved in DMSO as a 2 mM stock solution and used either at that concentration or further diluted in PBS to 200 μM or 20 μM before injection. An inhibitor of atypical PKCs, including PKMζ, mPSI (Cat No. ALX-260-155-M001; Enzo Life Sciences, Inc.), was dissolved in PBS as a 50 μg/10 μl stock solution and then diluted in PBS to its final concentration. All aliquots were stored at −80 °C. The immunoglobin (IgG) antibodies to $p75^{NTR}$ and TrkA (Clary et al., 1994), generously given by Professor L. Reichardt, of UCSF and the Simon Foundation, New York, were kept at +4 °C for 2–3 days, at most, before injection. The anti-$p75^{NTR}$ antibody blocks the extracellular domain of this receptor and prevents agonist binding and receptor activation (Weskamp and Reichardt, 1991). In contrast, the anti-TrkA antibody blocks the neurotrophin binding site but also activates this receptor, independently of NGF (Clary et al., 1994).

Experimental design

For all experiments, rats were allowed to rest quietly in an isolated behavioral testing room for 30 min before any