A Developmental Switch in Acute Sensitization of Small Dorsal Root Ganglion (DRG) Neurons to Capsaicin or Noxious Heating by NGF

Weiguo Zhu, Sam M. Galoyan, Jeffrey C. Petruska, Gerry S. Oxford, and Lorne M. Mendell

Stark Neurosciences Research Institute, Indiana University School of Medicine, Indianapolis, Indiana 46202; and Department of Neurobiology and Behavior, State University of New York at Stony Brook, Stony Brook, New York 11794-5230

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INTRODUCTION

Nerve growth factor (NGF) plays an important role in the development and survival of primary nociceptors through transcriptional mechanisms (Ritter et al. 1991; Ruit et al. 1992). Knockout of the genes encoding NGF (Crowley et al. 1994) or the trkA receptor (Smyene et al. 1994) results in animals lacking nociception. NGF can also induce inflammatory hyperalgesia acutely (Lewin et al. 1994). Recent studies have suggested trkA-mediated sensitization of TRPV1 as the putative mechanism of NGF induced thermal hyperalgesia since NGF can acutely sensitize nociceptors (Bonington and McVicar 2003). Two capsaicin or thermal stimuli were applied to cells, separated by a 10-min interval. During this interval either SES (control) or NGF (100 ng/ml; ALomone Labs, Jerusalem, Israel) was bath-applied during this interval. The relative magnitude of the peak currents (2nd/initial) was used as indicator of sensitization or desensitization.

Report

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Address for reprint requests and other correspondence: G. S. Oxford, Stark Neurosciences Research Inst., Indiana Univ. School of Medicine, 950 W. Walnut St., Rm. 402 Research II Bldg., Indianapolis, IN 46202 (E-mail: goxford@iupui.edu).

pathways in embryonic, neonatal, and adult DRG neurons. We investigated whether there are developmental differences in acute NGF sensitization of DRG neurons by comparing responses to noxious stimuli in neurons isolated from neonatal or adult rats.

METHODS

DRG neurons from neonatal and adult rat were dissociated and cultured as described previously for adult rats (Koplas et al. 1997; Shu and Mendell 1999). In brief, DRGs isolated from lumbar segments of spinal cords of neonatal rats (P0/1, 2, 4, 6, 8, 10, or 20) and young adult rats (~150 g, 3–4 wk old) were dissociated by treatment with a dispase/collagenase or endonuclease/collagenase cocktail and mechanical disruption through a series of fire-polished glass pipettes with a decreasing inner tip diameter. The resulting suspension of single cells was plated on either poly-L-lysine–coated coverslips or polylysine-laminin–coated petri dishes (35 mm) and maintained in DMEM, Gibco, Invitrogen, Grand Island, NY supplemented with 10% fetal bovine serum (FBS, Hyclone, Logan, UT) and 100 units/ml penicillin and 100 µg/ml streptomycin for 6–24 h at 37°C under 5% CO2.

Currents were recorded from DRG neurons under voltage clamp (holding potential ~ −60 mV) using either standard whole cell patch clamp (capsaicin experiments) or perforated patch clamp (noxious heat experiments) techniques, the latter achieved using amphotericin B (125 µg/ml, Sigma, St. Louis, MO). The standard external solution (SES) contained (in mM; all from Sigma): 145 NaCl, 5 KCl, 2 CaCl2, 1 MgCl2, 10 HEPES, and 10 glucose (pH 7.3). The internal solution consisted of (in mM) 130 K-glucurate, 10 EGTA, 1 MgCl2, 1 CaCl2, 10 HEPES, and 2 Mg-ATP (pH 7.4).

Capsaicin stocks (10 mM, Sigma) were made in ethanol, diluted to 50 nM with SES, and applied locally by gravity for 40 s to a recorded cell from a small diameter (250 µm) quartz capillary at room temperature (20–22°C). Ethanol controls (0.05%) elicited no current responses. Noxious heat stimulation consisted of a thermal ramp from 38–40 to 48°C at a rate of about 1.5°C/s (Galoyan et al. 2003). Two capsaicin or thermal stimuli were applied to cells, separated by a 10-min interval. During this interval either SES (control) or NGF (100 ng/ml; Alomone Labs, Jerusalem, Israel or courtesy of Genentech) was superfused over the cell. In a few cases, bradykinin (1 µM) was bath-applied during this interval. The relative magnitude of the peak currents (2nd/initial) was used as indicator of sensitization or desensitization.

The procedures for immunocytochemistry were described previously (Galoyan et al. 2003). In brief, the recorded cells were marked with a scratch mark in the dish. They were then rinsed in 0.1 M phosphate-buffered saline (PBS) for 10–20 min followed with PBS plus 0.4% Triton X-100 (Sigma). Nonspecific binding was blocked by incubation in 1:30 normal goat serum (GS; Jackson Immunoresarch,

Zhu, Weiguo, Sam M. Galoyan, Jeffrey C. Petruska, Gerry S. Oxford, and Lorne M. Mendell. A developmental switch in acute sensitization of small dorsal root ganglion (DRG) neurons to capsaicin or noxious heating by NGF. J Neurophysiol 92: 3148–3152, 2004. Using dissociated rat dorsal root ganglion (DRG) neurons, we have explored the ability of nerve growth factor (NGF) to acutely (within minutes) sensitize responses of nociceptors to capsaicin or noxious heat during postnatal development. While robust sensitization of noxious heat or capsaicin responses by NGF is observed in adult DRG neurons, responses to such stimuli in trkA-positive neurons from early postnatal animals are not sensitized by NGF. Neurons acquire sensitivity to the hyperalgesic effects of NGF between postnatal days 4 and 10 (P4–P10). In contrast to NGF, bradykinin sensitizes responses to noxious heat in both adult and neonatal DRG neurons. These observations suggest a developmental switch in signal transduction cascades linking trkA receptors to hyperalgesia during postnatal development and differences in the signaling pathways mediating bradykinin- and NGF-induced sensitization.

1Stark Neurosciences Research Institute, Indiana University School of Medicine, Indianapolis, Indiana 46202; and 2Department of Neurobiology and Behavior, State University of New York at Stony Brook, Stony Brook, New York 11794-5230.

phosphatidylinositol-4,5-bisphosphate (PIP2) has been pro-
tively involving release of TRPV1 from tonic inhibition by

NGF can acutely sensitize nociceptors (Bonnington and Mc-

vutive mechanism of NGF induced thermal hyperalgesia since

suggested trkA-mediated sensitization of TRPV1 as the puta-

So far, almost all studies of the acute effects of NGF on

TRPV1 have been performed in adult DRG neurons. Differ-

ces in trkA signaling pathways involved in development and regen-

eration of nociceptors (Bibel and Barde 2000; Liu and

Snider 2001) suggest possible distinctions between signaling
RESULTS

In adult DRG neurons (36 ± 2 pF, n = 36), the response to the second of two capsaicin presentations was always smaller than the initial one in the absence of NGF due to desensitization triggered by calcium influx (Koplas et al. 1997; Fig. 1). If the neurons were exposed to NGF (10 min, 100 ng/ml) between stimuli, the second capsaicin response was substantially enhanced rather than desensitized in the vast majority of neurons [increased by 3.98 ± 0.98-fold (SE); n = 20] in NGF compared with 0.48 ± 0.08-fold (n = 16) in SES (Fig. 1B; P < 0.005). In a few cases, NGF elicited virtually no effect on tachyphylaxis as has been reported previously (Shu and Mendell 1999, 2001); this is likely correlated with a lack of trkA expression (Galoyan et al. 2003).

In DRG neurons from neonatal P0/P1 rats (22 ± 1 pF, n = 34), the second of two capsaicin responses in the absence of NGF was usually smaller than the initial one, as in adults. However, NGF treatment in neonates never eliminated tachyphylaxis (Fig. 1A) in contrast to its effect on the response of sensory neurons from adult rats. The magnitude of the second capsaicin response averaged 0.86 ± 0.10 (n = 15) and 0.67 ± 0.08 (n = 19) times the initial response for control and NGF treatment, respectively (Fig. 1B; P > 0.1).

A similar difference was found using noxious heat stimulation. Previously, we reported that NGF (10 min, 100 ng/ml) treatment significantly enhanced the second of two noxious heat responses in adult rat DRG neurons (Galoyan et al. 2003; and example in Fig. 2A). An inward current from a P2 neonatal DRG neuron was induced in response to the initial heat stimulus (Fig. 2B, trace 1). Following 10 min of NGF exposure (100 ng/ml), the second response to heat was diminished rather than sensitized (Fig. 2B, trace 2). Similar results were observed in six additional P2 neurons (Fig. 3). This result is consistent with the failure of NGF to sensitize capsaicin responses in P0/P1 rat DRG neurons. Since the trkA signaling pathway is critical for the NGF effect (Galoyan et al. 2003), the lack of NGF effect might reflect the absence of trkA receptors in this cell. However, double-label immunocytochemistry confirmed that this neuron expressed both trkA and TRPV1 (Fig. 2D).

To estimate the time course of development of the sensitization of noxious heat responses by NGF, the double heat stimulus protocol with NGF applied between stimuli was performed in 18 neurons isolated from rats between P2 and P10 (Fig. 3). In a further group of untreated cells between P2 and P8 (n = 9), the second response declined to an average of 0.44 of the initial response, similar to the decline to 0.51 reported previously in adults (Galoyan et al. 2003). NGF treatment of cells at P2 resulted in an average decline to 0.55 of control (n = 7), similar to the decline in untreated cells. At P4, the corresponding decline of the second response was to 0.78 of the initial response, and there was a trend for less tachyphylaxis/more sensitization as the NGF was tested on cells from older rats up to P10, at which time sensitization approached levels characteristic of adult DRG neurons (Fig. 3). This result is consistent with the failure of NGF to sensitize the response in control and NGF-treated cells in Fig. 3 suggests that P4–P10 is the critical period for establishing the ability of NGF to sensitize the response to noxious stimuli in DRG neurons.

Both bradykinin and NGF have been reported to sensitize adult DRG neurons and cells heterologously expressing TRPV1 to capsaicin, protons, and noxious heat through a mechanism thought to involve activation of phospholipase C (PLC) isoforms, hydrolysis of PIP2, and a coordinate reduction of PIP2 inhibitory binding to TRPV1 (Chuang et al. 2001). We therefore examined whether bradykinin would exhibit similar
effects in neonatal DRG neurons. In contrast to our observations with NGF, bradykinin treatment (10 min, 1 μM) in the interval between two noxious heat stimuli did enhance the second response in neonatal DRG neurons (Fig. 2C). Similar results with bradykinin were seen in 12 additional neonatal DRG neurons, with the sensitization being at levels similar on the average to that observed in adults (Fig. 3). This finding suggests that the sensitizing effects of NGF and bradykinin on DRG neurons develop independently.

**DISCUSSION**

The neurotrophin dependency of DRG neuron survival has been shown to shift during perinatal development (Acosta et al. 2001; Buchman and Davies 1993; Molliver et al. 1997), and the signaling pathways mediating neurotrophin dependent survival also differ (Salvarezza et al. 2003). This raises the possibility that the acute effects of neurotrophins may also undergo a postnatal developmental change. In support of this idea, we have observed sensitization of TRPV1 responses to both capsaicin and noxious heat by NGF in adult, but not in neonatal rat DRG neurons. It is noteworthy that these findings were very similar despite the different recording conditions (perforated or whole cell patch clamp) and the different protocols for activating the TRPV1 receptor. The acquisition of NGF sensitivity of TRPV1 occurs roughly around P4–P10.

While the mechanisms underlying this developmental change are unknown, certain possibilities can be ruled out by our observations. First, it is unlikely that TRPV1 responses of
neonatal and adult DRG neurons differ in their initial sensitivity to noxious stimuli because responses to capsaicin and thermal thresholds for noxious heat were qualitatively similar in both populations (data not shown). Furthermore, the finding of a temperature threshold around 43°C also indicates that TRPV1 rather than TRPV2, TRPV3, or TRPV4 was responsible for these responses since their temperature thresholds are much higher (TRPV2) or lower (TRPV3 and TRPV4) than the values recorded here (Benham et al. 2003). Second, the immunocytochemical findings reveal that the absence of acute NGF sensitization of TRPV1 responsiveness was not due to lack of expression of trkA in the recorded neurons (Fig. 2D). Finally, it is clear that TRPV1 in neonatal DRG neurons was neither saturated in its responsiveness nor resistant to post-translational signaling events because bradykinin was able to increase the response to noxious heat in both neonatal and adult DRG neurons (Figs. 2C and 3). Thus the inability of neonatal sensory neurons to undergo sensitization is NGF specific.

This latter observation may, however, provide an important clue as to the nature of the developmental switch. From studies combining expression of TRPV1, B2 bradykinin receptors, and trkA in mammalian and Xenopus oocyte expression systems, it has been proposed that NGF and bradykinin sensitize TRPV1 through a common mechanism involving activation of PLC (Chuang et al. 2001). If activation of PLC and subsequent alteration of TRPV1 sensitivity by either PIP2 disinhibition (Chuang et al. 2001) or PKC-mediated phosphorylation (Blave et al. 2003) is operative in rat sensory neurons, this could imply a selective impairment of trkA signaling through PLCγ versus signaling through PI-3 kinase or ERK/MAP kinase in neonatal neurons. Alternatively, it might reflect a selective change in the sensitivity of TRPV1 to signaling through trkA, but not other receptors. This would imply a mechanistic difference between modulation of the sensitivity of TRPV1 by bradykinin and NGF at least at early postnatal times. Distinct isoforms of PLC (γ for NGF and β for bradykinin) might contribute to these differences. Such differences are not unexpected since TRPV1 activation has been reported to depend on the expression system chosen (Lazar et al. 2003), and pathways other than PLCγ have been implicated in linking trkA and TRPV1 in DRG neurons (Bonnington and McNaughton 2003). Another possible contributor to change in the response to NGF is the p75 receptor. It is very unlikely that the p75 receptor rather than trkA mediates the NGF-induced sensitization of TRPV1 (Chuang et al. 2001). However, p75 is known to modulate the action of trkA (Esposito et al. 2001), and this modulation might undergo a developmental change. Evidence for developmental changes in trkA signaling has been reported for neurite outgrowth promotion in neonatal versus adult cultured DRG neurons such that a switch from MEK/PI3-K to JAK pathways underlies developmental and regenerative axon growth, respectively (Liu and Snider 2001; Markus et al. 2002). Our results show that P4–P10 is the critical period for the onset of the ability of NGF to sensitize the response of DRG neurons to noxious stimuli. This period is coincident with the end of NGF’s role as a survival factor for nociceptors (Lewin et al. 1992). It is possible that the small amount of NGF in the medium derived from the FBS required for survival of DRG neurons in neonates might have reduced the ability of NGF to sensitize the response of neonatal cells to capsaicin or noxious heat, although cells from adults cultured in precisely the same way exhibited no such deficit. A possible scenario is that in order for nociceptive afferents to make use of the NGF/trkA system both prenatally for survival and postnatally for sensitization, a signaling switch has evolved to prevent NGF-induced sensitization of nociception in utero where it might be harmful because of the long-lasting effects of sensitized nociceptive stimulation in neonates (Peng et al. 2003). Further work is required to define the exact nature of this switch for determining the action of NGF.

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