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INFLAMMATION-INDUCED INCREASE IN NICOTINIC ACETYLCHOLINE RECEPTOR CURRENT IN CUTANEOUS NOCICEPTIVE DRG NEURONS FROM THE ADULT RAT

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Abstract—The goals of the present study were to determine (1) the properties of the nicotinic acetylcholine receptor (nAChR) currents in rat cutaneous dorsal root ganglion (DRG) neurons; (2) the impact of nAChR activation on the excitability of cutaneous DRG neurons; and (3) the impact of inflammation on the density and distribution of nAChR currents among cutaneous DRG neurons. Whole-cell patch-clamp techniques were used to study retrogradely labeled DRG neurons from naïve and complete Freund's adjuvant inflamed rats. Nicotine-evoked currents were detectable in ~70% of the cutaneous DRG neurons, where only one of two current types, fast or slow currents based on rates of activation and inactivation, was present in each neuron. The biophysical and pharmacological properties of the fast current were consistent with nAChRs containing an α7 subunit while those of the slow current were consistent with nAChRs containing α3/β4 subunits. The majority of small diameter neurons with fast current were IB4− while the majority of small diameter neurons with slow current were IB4+. Preincubation with nicotine (1 μM) produced a transient (1 min) depolarization and increase in the excitability of neurons with fast current and a decrease in the amplitude of capsaicin-evoked current in neurons with slow current. Inflammation increased the current density of both slow and fast currents in small diameter neurons and increased the percentage of neurons with the fast current. With the relatively selective distribution of nAChR currents in putative nociceptive cutaneous DRG neurons, our results suggest that the role of these receptors in inflammatory hyperalgesia is likely to be complex and dependent on the concentration and timing of acetylcholine release in the periphery. **© 2014 IBRO. Published by Elsevier Ltd. All rights reserved.**

Key words: nociceptor sensitization, inflammatory pain, ligand-gated ion channel, voltage-clamp, current clamp.

INTRODUCTION

Despite a number of studies on the subject, there is still no consensus on the role of nicotinic acetylcholine receptor (nAChR) signaling in sensory neurons. Activation of nAChRs on afferent terminals was initially assumed to be pro-nociceptive, since activation of cationic channels by acetylcholine drives membrane depolarization (Steen and Reeh, 1993; Lang et al., 2003). Consistent with this assumption, there is evidence for nicotine-induced activation and sensitization of nociceptive afferents (Steen and Reeh, 1993; Bernardini et al., 2001). More recently, however, there is evidence that low concentrations of agonist not only desensitize neurons to subsequent nAChR activation, but also desensitize nociceptive neurons to the prototypical algogen, capsaicin (Cap) (Fucile et al., 2005). Further, there is evidence that α7-selective agonists are analgesic, at least in part via a mechanism in the periphery (Loram et al., 2012). There is also evidence that the algesic effects of nicotine may be mediated via the activation of transient receptor potential ankyrin type 1 (TRPA1) rather than nAChRs (Talavera et al., 2009), raising doubt as to whether any of the nociceptive actions of nicotine are mediated through nAChRs. However, this last study relied on the use of heterologous expression systems and mouse dorsal root ganglion (DRG) neurons, and it is becoming increasingly clear that there are species differences in the density and distribution of nAChRs, where, for example, currents are detected in >60% of rat DRG neurons (Genzen et al., 2001; Dube et al., 2005; Hone et al., 2012) but less than 20% of mouse DRG neurons (Talavera et al., 2009; Albers et al., 2014). Thus, it remains to be determined whether nAChRs underlie the algesic vs analgesic actions of nicotine on cutaneous afferents.

There is also uncertainty as to the role of nAChR signaling in the presence of tissue injury. While not

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Abbreviations: AITC, allyl isothiocyanate; ANOVA, analysis of variance; Cap, capsaicin; CFA, complete Freund's adjuvant; DiI, 1,1′-dioctadecyl-3,3,3′,3′-tetramethylindocarbocyanine perchlorate; DMSO, dimethyl sulfoxide; DRG, dorsal root ganglion; EGTA, ethylene glycol tetraacetic acid; HEPES, 4-(2-hydroxyethyl)-1-piperazineethanesulfonic acid; Hex, hexamethonium; IB4, isolectin B4; Mec, mecamylamine; MLA, methyllycaconitine citrate; nAChR, nicotinic acetylcholine receptor; TRPA1, transient receptor potential ankyrin type 1.

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extensively studied, the majority of published studies have focused on changes in the presence of nerve injury. In these studies, there appears to be a decrease in nAChR current (Dube et al., 2005). Unless this is a feedback-inhibitory mechanism that counters a number of well-described changes that contribute to an increase in afferent excitability, for a decrease in nAChRs to contribute to neuropathic pain, one would have to conclude that nAChRs normally function to decrease afferent excitability. However, we have recently observed in the mouse that the neurotrophic factor artemin drives an increase in nAChR subunit expression which is associated with an increase in both the density and distribution of nAChR currents in mouse sensory neurons (Albers et al., 2014). Furthermore, given evidence that artemin is increased in peripheral tissue in the presence of inflammation, and artemin-induced hyperalgesia can be attenuated with peripheral administration of the nAChR antagonist hexamethonium (Hex) (Albers et al., 2014), our results suggest that in the presence of inflammation, nAChR signaling may be pro-nociceptive. Given recent evidence that nicotine attenuates the increase in the excitability of colonic afferents following inflammation of the colon (Abdrakhmanova et al., 2010), this may also be an issue of target of innervation.

The purpose of the present study was therefore to further clarify the role of nAChR activation in nociceptive signaling in sensory neurons in the absence and presence of inflammation. Nicotine-evoked currents were recorded and the impact of nicotine on excitability was assessed using patch-clamp methodology in 1,1′-dioctadecyl-3,3,3′,3′-tetramethylindocarbocyanine perchlorate (DiI)-labeled cutaneous DRG neurons from naïve and complete Freund's adjuvant (CFA) inflamed rats.

EXPERIMENTAL PROCEDURES

Animals

Adult male Sprague–Dawley rats (Harlan-Sprague Dawley, Indianapolis, IN, USA) weighing between 250 and 350 g were used for all experiments. Rats were housed two per cage in the University of Pittsburgh AAALAC approved animal facility on a 12:12 light:dark schedule with food and water *ad libitum*. All procedures were approved by the University of Pittsburgh Institutional Animal Care and Use Committee and performed in accordance with the National Institutes of Health Guide for the Care and Use of Laboratory Animals. All efforts were made to minimize the number of animals used and their suffering.

Labeling and inflammation

DRG neurons that innervate glabrous skin of the rat hind paw were retrogradely labeled with DiI (17 mg/ml in dimethyl sulfoxide (DMSO) and saline), which was injected (3–5 sites at 3–2 μL/site) with a 30-g needle 14–17 days prior to electrophysiological recording (Lu and Gold, 2008). Complete Freund's adjuvant (CFA, Sigma–Aldrich, St. Louis, MO, USA; mixed 1:1 with saline), was injected (100 μL) into the site previously injected with DiI. Inflamed DRG neurons were studied 72 h after CFA injection. Both DiI and CFA were injected under isoflurane-induced anesthesia.

Preparation of isolated DRG neurons

Prior to tissue harvest, rats were deeply anesthetized with a subcutaneous injection (1 ml/kg) of a cocktail containing ketamine (55 mg/ml), xylazine (20 mg/ml) and acepromazine (5.5 mg/ml). L4 and L5 DRG were harvested bilaterally. DRG were trimmed of connective tissue, enzymatically treated and mechanically dispersed as previously described (Lu et al., 2006). Isolated neurons were plated on poly-lysine coated coverslips and electrophysiology experiments were performed 2–8 h after plating.

Electrophysiology

Prior to study, neurons were incubated for 10 min in the bath solution used for electrophysiological recording. Whole-cell current or voltage data were recorded in a bath solution containing (in mM): NaCl 130, KCl 3, $CaCl_2$ 2.5, $MgCl_2$ 0.6, HEPES 10, glucose 10; pH 7.4 (adjusted with Tris-base), 325 mOsm (adjusted with sucrose), to which fluorescein isothiocyanate (FITC)-conjugated isolectin B4 (IB4) had been added to a final concentration of 5 μg/ml. Neurons were then placed in a recording chamber continuously superfused with bath solution at room temperature. Retrogradely labeled neurons were identified under epifluorescence illumination. The cell body diameter was determined with a calibrated eyepiece reticle. Neurons were considered IB4+ if a clear ring of IB4 staining was visible on the plasma membrane (Stucky and Lewin, 1999). At the end of each experiment, Cap sensitivity was assessed with a 4-s application of Cap (500 nM) (Lopshire and Nicol, 1997). Neurons were considered Cap responsive (Cap+), if the application of Cap resulted in an inward current >20% above the greatest deflection in holding current observed with application of Cap vehicle (~10 pA). A neuron was considered a putative nociceptor if it had a small cell body diameter (<30 μm, (Lawson, 2002)), was IB4+ (Fang et al., 2006) and/or Cap+ (Schmelz et al., 2000). Of note, while IB4 is generally used to identify a subpopulation of nociceptive afferents devoid of the neuropeptides substance P and calcitonin gene-related peptide (non-peptidergic neurons), we (Lu et al., 2006) and others (Petruska et al., 2000) have previously observed that there is a majority of both IB4+ and IB4− small diameter neurons that are Cap+. Thus, while these appear to be distinct subpopulations of nociceptive afferents, we do not distinguish among them with our use of the term "putative nociceptor".

Voltage- and current-clamp data were acquired using conventional whole-cell patch-clamp techniques with an Axopatch 200B amplifier (Molecular Devices, Sunnyvale, CA, USA) controlled with a PC running pClamp Software (V 10.3, Molecular Devices). Current and voltage data were sampled at 5–10 kHz and filtered at 1–2 kHz. Patch electrodes were pulled from borosilicate glass (WPI, Sarasota, FL, USA) on a horizontal puller (Sutter Inst.,

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