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Immunoglobulinfree light chains reduce in an antigen-specific manner the rate of rise of action potentials of mouse non-nociceptive dorsal root ganglion neurons

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ABSTRACT

Plasma B cells secrete immunoglobulinfree light chains (IgLC) which by binding to mast cells can mediate hypersensitivity responses and are involved in several immunological disorders. To investigate the effects of antigen-specific IgLC activation, intracellular recordings were made from cultured murine dorsal root ganglion (DRG) neurons, which can specifically bind IgLC. The neurons were sensitized with IgLC for 90 min and subsequently activated by application of the corresponding antigen (DNP-HSA). Antigen application induced a decrease in the rate of rise of the action potentials of non-nociceptive neurons (MANOVA, $p = 2.10^{-6}$), without affecting the resting membrane potential or firing threshold. The action potentials of the nociceptive neurons ($p = 0.57$) and the electrical excitability of both types of neurons ($p > 0.35$) were not affected. We conclude that IgLC can mediate antigen-specific responses by reducing the rate of rise of action potentials in non-nociceptive murine DRG neurons. We suggest that antigen-specific activation of IgLC-sensitized non-nociceptive DRG neurons may contribute to immunological hypersensitivity responses and neuroinflammation.

1. Introduction

During the production of immunoglobulins by plasma B cells, light chains are produced in excess over heavy chains (Redegeld et al., 2002). Increased serum levels of immunoglobulinfree light chains (IgLC) have been reported in several immunological disorders, like allergic asthma (Kraneveld et al., 2005), inflammatory bowel diseases (Rijnierse et al., 2010), pneumonitis and idiopathic pulmonary fibrosis (Groot Kormelink et al., 2011), food allergy (Schouten et al., 2011) and rhinitis (Powe et al., 2010). In rheumatoid arthritis (Groot Kormelink et al., 2010), the levels of IgLC are positively correlated with disease activity. In 2002, Redegeld et al. demonstrated that IgLC mediate antigen-specific degranulation of mast cells, thereby contributing to hypersensitivity responses in the skin. More recently, IgLC were also shown to be involved in mast cell-mediated hypersensitivity responses in airways and the gastro-intestinal tract (Kraneveld et al., 2005; Rijnierse et al., 2010). In these tissues, mast cells communicate bidirectionally with nearby nerve endings of sensory origin (Suzuki et al., 1999, 2001). This bidirectional communication has been extensively described (De Jonge et al., 2004; Furuno et al., 2004; Buhner and Schemann, 2012) and is thought to have an important role in neurogenic inflammation (Richardson and Vasko, 2002; Buhner and Schemann, 2012). Such bidirectional communication also plays a role in the central nervous system (CNS) where activation of other cell types (e.g. microglia and astrocytes) and their subsequent production of inflammatory mediators are involved in the development of neuroinflammation, e.g. following cerebral ischemia (Ceulemans et al., 2010).

Recently, it was shown by immunohistochemistry and pharmacological binding studies that IgLC can bind specifically with murine primary sensory dorsal root ganglion (DRG) neurons (Rijnierse et al., 2009). Moreover, a slowly developing increase of the intracellular Ca^{2+} concentration could be detected in a subpopulation of the primary cultured IgLC-sensitized DRG neurons in response to antigen-specific (DNP-HSA) stimulation (Rijnierse et al., 2009). This neural response indicated that complexes of IgLC and antigens affect the functional properties of DRG neurons and therefore can contribute to neurogenic

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inflammation (Mayer et al., 1988; Rijnierse et al., 2007; Ben-Horin and Chowers, 2008) and to centrally-mediated sensations such as pain and itch. That not all DRG neurons responded to sensitization with IgLC and subsequent activation by antigen is thought to reflect the fact that DRG neurons exhibit different phenotypes with different sensory function (Scott, 1992).

DRG contain the soma of sensory afferents that are depolarized by nociceptive, mechanical, chemical or thermal stimuli. This depolarization activates voltage-gated Na^+ (Na_v) and K^+ (K_v) channels leading to action potential (AP) generation. The AP not only transfer sensory information to the CNS (Scott, 1992) but can also induce release of neuropeptides such as substance P and calcitonin gene-related peptide from varicosities in the periphery (Mayer et al., 1988; Beyak and Vanner, 2005; Rijnierse et al., 2007; Ben-Horin and Chowers, 2008). DRG neurons can, in vivo (Ritter and Mendell, 1992; Fang et al., 2005) as well as in vitro (Harper and Lawson, 1985b; Heyman and Rang, 1985; McLean et al., 1988; Moore et al., 2002; De Jonge et al., 2004), be distinguished into two groups by using as criterion the presence or absence of a shoulder on the falling phase of the AP. The difference in waveform of the AP between these two groups of neurons results from the different subtypes of Na_v channels these neurons possess (Beyak et al., 2004; Krafte and Bannon, 2008; Momin and Wood, 2008; Docherty and Farmer, 2009). Of the 9 different types of Na_v channels that have been identified in the nervous system (Lampert et al., 2010) based on differences in their α-subunits, several types are abundantly found in DRG neurons. These Na_v channels, based on their response to tetrodotoxin (TTX), are divided in tetrodotoxin-sensitive (TTXS) and tetrodotoxin-resistant (TTXR) channels (Docherty and Farmer, 2009). Non-nociceptive neurons express the TTXS Na_v 1.7 channel (Djouhri et al., 2003a; Beyak and Vanner, 2005), usually together with TTXS Na_v 1.1, 1.3 and 1.6 channels (Beyak and Vanner, 2005). Most of the nociceptive neurons express the Na_v 1.7 channel (Djouhri et al., 2003a) and also the TTXR Na_v 1.8 (Djouhri et al., 2003b) and Na_v 1.9 channels (Fang et al., 2002, 2006). The TTXR Na_v 1.8 and 1.9 channels are a fairly unique property of nociceptive neurons and have been shown to influence membrane properties and to dominate AP generation in these neurons (Renganathan et al., 2001; Fang et al., 2002; Djouhri et al., 2003a, 2003b; Beyak and Vanner, 2005). TTXS Na_v channels are characterized by an inactivation which is much faster than that of the TTXR channels (Roy and Narahashi, 1992; Beyak et al., 2004). As a result, the AP of nociceptive neurons show in comparison to non-nociceptive neurons, a relatively long duration (Fang et al., 2002; Djouhri et al., 2003a,b; Fang et al., 2006) and a shoulder on the falling phase (Moore et al., 2002; Djouhri et al., 2003a, 2003b; De Jonge et al., 2004; Fang et al., 2006). This division of nociceptive and non-nociceptive neurons based on the presence or absence of a shoulder on the AP, although not under all conditions absolute (see f.i. Renganathan et al., 2001), has been identified in many species (see Ritter and Mendell, 1992), including mice (McLean et al., 1988; De Jonge et al., 2004).

In the gastrointestinal tract, chronic inflammation is known to induce hyperexcitability of nociceptive DRG neurons that innervate the site of inflammation (review Yoshimura and De Groat, 1999; Moore et al., 2002; Beyak et al., 2004; Beyak and Vanner, 2005), causing increased nociceptive signaling to the CNS (Moore et al., 2002; Beyak et al., 2004; Beyak and Vanner, 2005). This hyperexcitability results from alterations in the properties (Moore et al., 2002; Beyak and Vanner, 2005) and expression (King et al., 2009) of the Na_v 1.8 channel. Although the precise signaling pathways involved in modulation of the Na_v 1.8 channel are not known, it is evident that these may involve the actions of many pro-inflammatory mediators (e.g. serotonin, PGE_2 and NGF; (Beyak and Vanner, 2005)). The previously observed increase in intracellular Ca^{2+} in DRG neurons following sensitization (SE) with antigen specific IgLC and subsequent activation (AC) with the corresponding antigen (Rijnierse et al., 2009) may under chronic conditions potentially induce hyperexcitability of these neurons, since Ca^{2+} is a key regulator of neural excitability (Smith et al., 2003). Therefore, the aim of the present study was to determine if sensitization with IgLC followed by antigen-specific activation (SEAC) affects the AP waveform or increases the electrical excitability of nociceptive and non-nociceptive DRG neurons.

2. Methods

2.1. Animals

Male BALB/c mice (Harlan, Horst, The Netherlands) were housed in groups with a maximum of eight mice per cage. Tap water, chow food and cage enrichment were ad libitum present and a 12–12 h day–night cycle was maintained. All experiments were approved by the local committee on Animal Bioethics of Utrecht University.

2.2. Culture of DRG neurons

Single DRG neurons were cultured as described previously (De Jonge et al., 2004; Rijnierse et al., 2009), with small modifications. Mice (age 6–8 weeks) were sacrificed by suffocation with carbon dioxide. The spinal column was taken out by a dorsal approach and cut sagittally into two parts. About 20 dorsal root ganglia (DRG; range T1–L3) were taken out by cutting through the nerve bundles close to the ganglion and stored in ice-cold L-15 medium (Invitrogen, Breda, The Netherlands) until the end of the dissection. DRG obtained from two mice were enzymatically digested with 10 mg/ml collagenase A (Roche Diagnostics, Almere, The Netherlands) in L-15 medium at 37 °C, 5% CO_2/95% O_2 during 30 min. The reaction was stopped by adding an equal volume of fetal calf serum (Perbio, Ettenleur, The Netherlands) for 2 min. The ganglia were suspended in DRG culture medium (see 2.3) by mechanic dissociation using a pipette. The cell suspension was placed on a serum-coated Petri dish (30 min at 37 °C) to allow non-neuronal cells (De Jonge et al., 2004) to adhere for 90 min at 37 °C, 5% CO_2/95% O_2. The neuronal cells were collected from the dish and placed in DRG culture medium onto the laminine-coated (Roche Diagnostics, Almere, The Netherlands) glass bottom (diameter = 10 mm) of 6 to 8 culture dishes (diameter =35 mm; MatTek, Ashland, MA, USA). Cultures were maintained at 37 °C, 5% CO_2/95% O_2 and studied at days 4–6 of culture. The medium was refreshed at day 4. The culture of mouse DRG neurons consisted of a heterogeneous population with respect to soma size and shape. It should be noted that the mean soma diameter of the nociceptive neurons is in vivo generally smaller than that of the non-nociceptive neurons (Harper and Lawson, 1985a; Moore et al., 2002; Ho and O'Leary, 2011), but this difference is not expressed in culture. After 2 days, the DRG neurons exhibited usually one and sometimes two axons (DRG neurons being pseudounipolar) and full-size neurite outgrowths contacting with other neurites and neuronal cell bodies (Fig. 1A; cf [11, 15]). A few fibroblasts were present in the culture dishes, which were identified by their appearance through the microscope. In Fig. 1B, a representative picture of a DRG neuron is shown.

2.3. Solutions

DRG culture medium: DMEM high glucose without glutamine and DMEM F-12 medium at a 1:1 ratio, enriched with 1% N2 human serum supplement, 1.2% Glutamax, 500 μg/ml gentamicine (all from Invitrogen, Breda, The Netherlands) and 2 μM cytosine β-D-arabinoside (Sigma Chemical Co., St. Louis, MO, USA). During the electrophysiological measurements (see 2.4), the dishes containing DRG neurons were perfused with Artificial CerebroSpinal Fluid (ACSF), containing (in mM) 127 NaCl, 1.9 KCl, 1.2 KH_2PO_4, 1.3 $MgSO_4$, 20 $NaHCO_3$, 10 D-glucose and 2.4 $CaCl_2$, aerated with 5% CO_2/95% O_2, pH 7.4.