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IN VIVO CHARACTERIZATION OF COLORECTAL AND CUTANEOUS INPUTS TO LUMBOSACRAL DORSAL HORN NEURONS IN THE MOUSE SPINAL CORD

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Abstract—Chronic abdominal pain is a common symptom of inflammatory bowel disease and often persists in the absence of gut inflammation. Although the mechanisms responsible for ongoing pain are unknown, clinical and preclinical evidence suggests lumbosacral spinal cord dorsal horn neurons contribute to these symptoms. At present, we know little about the intrinsic and synaptic properties of this population of neurons in either normal or inflammed conditions. Therefore, we developed an *in vivo* preparation to make patch-clamp recordings from superficial dorsal horn (SDH) neurons receiving colonic inputs in naïve male mice. Recordings were made in the lumbosacral spinal cord (L6–S1) under isoflurane anesthesia. Noxious colorectal distension (CRD) was used to determine whether SDH neurons received inputs from mechanical stimulation/distension of the colon. Responses to hind paw/tail cutaneous stimulation and intrinsic and synaptic properties were also assessed, as well as action potential discharge properties. Approximately 11% of lumbosacral SDH neurons in the cohort of neurons sampled responded to CRD and a majority of these responses were subthreshold. Most CRD-responsive neurons (80%) also responded to cutaneous stimuli, compared with <50% of CRD-non-responsive neurons. Furthermore, CRD-responsive neurons had more hyperpolarized resting membrane potentials, larger rheobase currents, and reduced levels of excitatory drive, compared to CRD-non-responsive neurons. Our results demonstrate that CRD-responsive neurons can be distinguished from CRD-non-responsive neurons by several differences in their membrane properties and excitatory synaptic inputs. We also demonstrate that SDH neurons with colonic inputs show predominately subthreshold responses to CRD and exhibit a high degree of viscerosomatic convergence.

Key words: patch-clamp, viscera, synaptic current, pain, colorectal distension.

INTRODUCTION

Persistent abdominal pain and visceral hypersensitivity are debilitating symptoms of inflammatory bowel disease (IBD), a group of chronic inflammatory conditions of the gastrointestinal tract (Wagtmans et al., 1998; Schirbel, 2010). While pain most commonly occurs during disease flare-ups, upto 30–50% of IBD patients report ongoing pain in the absence of active inflammation (Minderhoud et al., 2004; Farrokhyar et al., 2006; Siegel and MacDermott, 2009). Due to a limited number of effective pain therapies, long-term use of narcotics is prevalent, despite a variety of negative side effects (Edwards et al., 2001; Cross et al., 2005; Makharia, 2011; Farrell et al., 2014a). This lack of adequate pain management results in significantly decreased health-related quality of life scores, and increased anxiety and depression in IBD patients (Farrokhyar et al., 2006; Schirbel, 2010). Therefore, to improve patient outcomes, a greater understanding of pain signaling in IBD is required.

Clinically, there is evidence that neuronal remodeling or *plasticity* within the central nervous system (CNS) plays a significant role in the development of abnormal pain processing in IBD. For example, in addition to chronic pain, patients with IBD experience "referred" pain, where pain from the gut is felt in cutaneous or other visceral sites (Bernstein et al., 1996; Minderhoud et al., 2004). These common IBD symptoms are thought to be the result of neuronal plasticity in the CNS, particularly where somatic and visceral pathways overlap. Moreover, animal models of visceral hypersensitivity have demonstrated behavioral, anatomical, molecular and physiological evidence that gastrointestinal inflammation can cause CNS plasticity (Farrell et al., 2014b). Importantly, most of these changes have been shown to occur in the spinal cord dorsal horn (Harrington et al., 2012; Farrell et al., 2014b).

Both painful and non-painful sensations from the distal colon are transmitted to the spinal cord via primary afferents in the splanchnic and pelvic nerves. These

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Abbreviations: AHP, afterhyperpolarization; AP, action potential; CNS, central nervous system; CRD, colorectal distension; CRD-NR, CRD-non-responsive; CRD-R, CRD-responsive; DF, delayed firing; DRG, dorsal root ganglion; IB, initial bursting; IBD, inflammatory bowel disease; SDH, superficial dorsal horn; sEPSPs, spontaneous excitatory postsynaptic potentials; sIPSPs, spontaneous inhibitory postsynaptic potentials; SS, single spiking; TF, tonic firing; U, unclassified.

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afferents synapse within the dorsal horn in the thoracolumbar (T10–L1) and lumbosacral (L6–S1) spinal cord, respectively (Cervero, 1994). Incoming sensory information is then processed by dorsal horn interneurons before ascending to various brain structures (Todd, 2010). The role of the dorsal horn in sensory processing is complex, as local-circuit excitatory and inhibitory interneurons receive and integrate inputs from peripheral structures like viscera and skin, higher brain centers, and local interneurons (Graham et al., 2007). Therefore, interneurons are crucial for determining the overall excitability within the dorsal horn and hence its output. Despite this, we know little about the intricacies of sensory processing in these interneuron networks under both normal and pathological conditions like inflammation (Todd, 2010). This is especially so for dorsal horn neurons which receive input from the gut.

In an attempt to understand the function of dorsal horn neurons that receive inputs from the colon, their physiology has been studied using *in vivo* recording techniques. Dorsal horn neurons have been classified based on their responses to colorectal distension (CRD), convergence with somatic regions or other viscera, and chemical sensitivity (Ness and Gebhart, 1987a; Katter et al., 1996; Andrew and Blackshaw, 2001). Collectively these studies have shown dorsal horn neurons typically respond to CRD with excitation, although inhibition of spontaneous activity has been reported. The thresholds for excitation/inhibition appear to be varied, with many neurons responding to CRD across a wide range of pressures (i.e. a wide dynamic range) or exclusively to innocuous or noxious stimulation (Ness and Gebhart, 1987a; Katter et al., 1996; Andrew and Blackshaw, 2001). Dorsal horn neurons also show a high degree of convergence with somatic regions, such as skin (Honda, 1985; Ness and Gebhart, 1987a; Katter et al., 1996), as well as other viscera (Honda, 1985; Andrew and Blackshaw, 2001).

Notwithstanding the valuable insights these studies provide on how dorsal horn neurons respond to visceral and cutaneous simulation, these experiments were only able to monitor suprathreshold (action potential (AP) discharge responses) because of the recording techniques employed. Furthermore, these approaches do not permit assessment of subthreshold membrane potential fluctuations, and/or responses that reflect a neuron's intrinsic or synaptic properties and ultimately mediate neuronal function. This is important, as the intrinsic and synaptic properties of neurons are critical determinants of overall excitability in both normal and inflammed conditions. Therefore, the aim of this study was to develop a mouse *in vivo* preparation that permits whole-cell electrophysiological recordings from superficial dorsal horn (SDH) neurons that receive inputs from the colon. We used naïve mice, as the intrinsic and synaptic properties of SDH neurons that receive inputs from the colon have not been characterized under normal or inflammed conditions. Our data demonstrate that SDH neurons with colonic inputs show predominately subthreshold responses to colon distension and have a high degree of viscerosomatic convergence. We also show these neurons are less excitable than neurons without colonic inputs due to several differences in their membrane properties and excitatory synaptic inputs. These data highlight that in naïve mice, lumbosacral SDH neurons that receive colonic inputs are distinguishable from those that do not. This is likely a reflection on the differential central processing of visceral *versus* cutaneous afferent signaling.

EXPERIMENTAL PROCEDURES

Surgery

The University of Newcastle Animal Care and Ethics Committee approved all procedures used in this study (protocol # A-2012-223). Experiments used male mice (C57BL/6, aged 6–8 weeks, body weight 16.9–23.6 g). Preparation of the adult mouse for *in vivo* patch-clamp recording from spinal neurons was adapted from work previously described by our group (Graham et al., 2004a,b; Jobling et al., 2010).

Animals were anesthetized with isoflurane (2–3%, 2 L/min O_2 induction; 1–2% maintenance). Isoflurane was adjusted throughout the experiment to maintain surgical anesthesia, confirmed by the absence of hind limb and corneal reflexes. Once mice were anesthetized, the skin over the thoracolumbar vertebral segments was shaved and the animal was immobilized on a stereotaxic frame (Narishige Corp., Tokyo, Japan). The head was stabilized with custom ear bars and anesthetic was delivered continuously via a nose cone (Fig. 1A). An electric heat mat, with a feedback circuit, was placed under the animal to maintain the body temperature at 37 °C.

Surgeries were performed with the aid of a dissecting microscope (Leica Microsystems, Nuslock, Germany). A midline incision was made to expose the thoracolumbar vertebrae. The skin was retracted and the paravertebral musculature was reflected to expose the spinous processes and vertebral arches. Clamps were applied to the T13 and L2 vertebral bodies to stabilize the spinal column and the remaining musculature and connective tissue overlying the vertebral arches were cleared. A laminectomy at L1 exposed the underlying L6–S1 spinal cord segments (Harrison et al., 2013). These segmental levels were selected because the majority of afferents from the colon enter the spinal cord via the pelvic nerve at this level (Blackshaw et al., 2007). The dura was opened and reflected, and a small incision was made in the pia to allow recording pipettes to penetrate the spinal cord. Throughout the experiment, the surface of the spinal cord was irrigated with artificial cerebrospinal fluid to maintain tissue health, which contained (in mM): 118 NaCl, 25 $NaHCO_3$, 11 glucose, 2.5 KCl, 1 NaH_2PO_4, 1 $MgCl_2$, and 2.5 $CaCl_2$, equilibrated with 95% O_2–5% CO_2 to achieve a final pH of 7.3. At the completion of the experiment animals were overdosed with pentobarbital (200 mg/kg i.p.).

Electrophysiology

Recording pipettes (4–7 M Ω) were made from thin-walled filamented borosilicate glass capillaries (1.5 mm o.d.,