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Research report

Electrophysiological properties of lumbosacral primary afferent neurons innervating urothelial and non-urothelial layers of mouse urinary bladder

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ABSTRACT

Pelvic nerve (PN) bladder primary afferent neurons were retrogradely labeled by intraparenchymal (IPar) microinjection of fluorescent tracer or intravesical (IVes) infusion of tracer into the bladder lumen. IPar and IVes techniques labeled two distinct populations of PN bladder neurons differentiated on the basis of dorsal root ganglion (DRG) soma labeling, dye distribution within the bladder, and intrinsic electrophysiological properties. IPar (Fast blue)- and IVes (DiI)-labeled neurons accounted for 91.5% (378.3 ± 32.3) and 8% (33.0 ± 26.0) of all labeled neurons, respectively ($p < 0.01$), with only 2.0 ± 1.2 neurons labeled by both techniques. When dyes were switched, IPar (DiI)- and IVes (Fast blue) labeled neurons accounted for 77.6% (103.0 ± 25.8) and 22.4% (29.8 ± 10.5), respectively ($P < 0.05$), with 6.0 ± 1.5 double-labeled neurons. Following IPar labeling, DiI was distributed throughout non-urothelial layers of the bladder. In contrast, dye was contained within the urothelium and occasionally the submucosa after IVes labeling. Electrophysiological properties of DiI-labeled IPar and IVes DRG neurons were characterized by whole-mount, in situ patch-clamp recordings. IPar- and IVes-labeled neurons differed significantly with respect to rheobase, input resistance, membrane capacitance, amplitude of inactivating and sustained K^+ currents, and rebound action potential firing, suggesting that the IVes population is more excitable. This study is the first to demonstrate that IVes labeling is a minimally invasive approach for retrograde labeling of PN bladder afferent neurons, to selectively identify urothelial versus non-urothelial bladder DRG neurons, and to elucidate electrophysiological properties of urothelial and non-urothelial afferents in an intact DRG soma preparation.

1. Introduction

The urinary bladder stores and evacuates urine via complex reflexes involving coordination between the efferent and afferent branches of the peripheral nervous system. Intravesical pressure during bladder filling is encoded by in-series, low threshold mechanosensitive bladder afferents with receptive fields in the bladder wall that convey information along the pelvic nerve (PN) (Janig and Morrison, 1986). Sensitization of these afferents, along with activity in and/or acquisition of mechanosensitivity by other types of afferents, is thought to contribute to bladder hypersensitivity associated with various pathological conditions (e.g., overactive bladder, interstitial cystitis/painful bladder syndrome; see (Nickel et al., 2012)).

Four functionally distinct classes of mechanosensitive PN bladder afferents have been characterized in mouse based upon extracellular recordings of compound action potential firing patterns in response to mechanical stimuli applied to the bladder (Xu and Gebhart, 2008). All PN bladder afferents responded to blunt probing of their receptive field and were distinguished by their sensitivity to fine urothelial stroking and stretch. Functional classes were described in terms of their putative terminal ending distribution pattern as muscular, urothelial, muscular/mucosal, and serosal (Xu and Gebhart, 2008; Brierley et al., 2004). Muscular afferents responded to stretch, urothelial afferents responded to fine stroking, muscular/urothelial fibers responded to both urothelial stroking and graded intensities of bladder stretch, and serosal afferents responded only to probing of their receptive field. Although mechanical stimuli were directed at specific tissue

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layers, the actual location within the bladder wall of the afferent endings in each class was not determined. Furthermore, extracellular recordings provide little information on the ion channel basis that regulates the intrinsic membrane excitability of afferent neurons. Such mechanisms have been the focus of various other studies that used whole cell patch-clamp recordings of bladder afferent neurons from rat and mouse following acute dissociation or growth in culture conditions (Yoshimura et al., 2001, 2003; Yoshimura and Groat, 1996; Sculptoreanu et al., 2004; Takahashi et al., 2013; Masuda et al., 2006; Hayashi et al., 2009; Black et al., 2003; Waterman, 1996; Yunoki et al., 2014; Yoshimura and de Groat, 1997; Hougaard et al., 2009; Lei and Malykhina, 2012; Malykhina et al., 2004; Yoshimura et al., 2001). A disadvantage of these techniques is that the electrophysiological properties of many neurons may be significantly altered by dissociation, or may vary based on culture medium. The electrophysiological properties of intact bladder afferent neurons have not been reported, presumably because of the technical difficulty associated with performing patch-clamp recordings in intact DRG soma preparation.

To begin to address some of the aforementioned issues, the present study utilized two strategies to retrogradely label bladder primary afferent neurons within lumbosacral dorsal root ganglia (DRG), where the cell bodies of PN afferents are located. Bladder neurons were labeled either by intraparynchemal (IPar) injection or intravesical (IVes) infusion of fluorescent dye into the bladder, and were anatomically differentiated on the basis of dye distribution in the bladder wall and neuron soma labeling in the DRG. For simplicity, we will refer to neurons labeled by IPar injection as "non-urothelial" and the periurothelial afferents labeled by IVes infusion as "urothelial." *In situ* patch-clamp recordings from non-urothelial and urothelial bladder afferent neurons were performed using a whole-mount DRG preparation that more closely approximates the in vivo condition than previously used methods. The results of these studies indicate that non-urothelial and urothelial subpopulations of bladder-innervating neurons exhibit differences in electrophysiological characteristics that reflect neuronal excitability.

2. Results

2.1. Anatomical properties of urothelial and non-urothelial bladder afferents

In two groups of mice, we determined the number of bladder primary afferent DRG neurons that were retrogradely labeled by IPar injection and IVes infusion of dye (Fig. 1). There was a profound effect of the route of dye administration on the number of labeled neurons ($P < 0.0001$). When Fast blue (FB) was delivered IPar and 1,1′-dioctadecyl-3,3,3′,3′-tetramethylindocarbocyanine perchlorate (DiI) was delivered IVes (Fig. 1A-C), FB-positive neurons accounted for 91.5% (378.3 ± 32.3 neurons per mouse) of labeled L6 neurons, whereas DiI-positive neurons accounted for only 8% (33.0 ± 26.0 neurons per mouse) of labeled L6 neurons ($P < 0.01$, $n = 3$; Fig. 1D,E). The degree of overlap between subsets of labeled neurons was virtually non-existent, with only 2.0 ± 1.2 neurons per mouse expressing both FB and DiI, corresponding to 6.06% of DiI-positive co-labeled with FB and 0.53% of FB-positive co-labeled with DiI. A similar pattern of labeling was observed with IPar DiI and IVes FB, although the relative proportions of IVes- and double-labeled afferents were greater, and

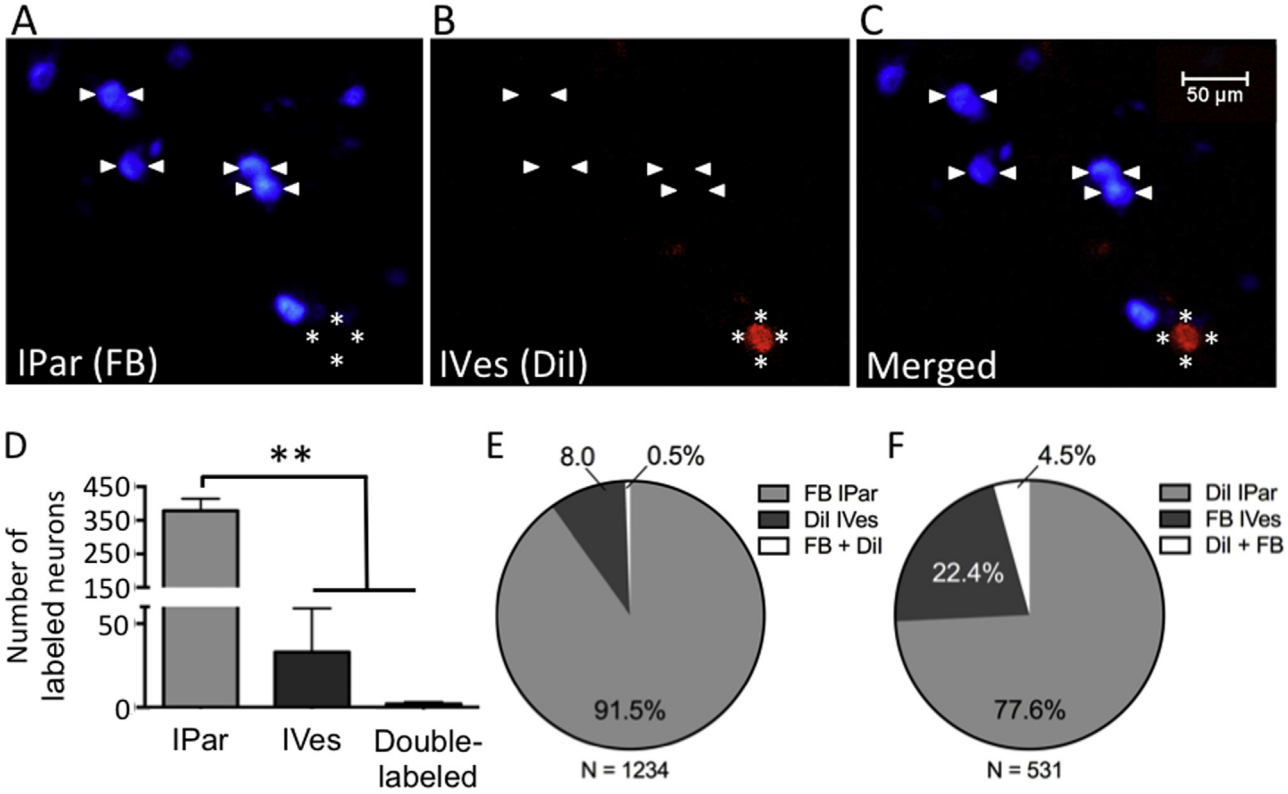

Fig. 1. IPar- and IVes-labeled neurons are anatomically distinct subsets of PN bladder afferents. Intraparynchemal (IPar) injection of FB and intravesical (IVes) infusion of DiI retrogradely labeled two subpopulations of bladder-innervating L6 DRG neurons (**A-C**). Arrowheads indicate IPar neurons labeled by FB and asterisks indicate a DiI-positive, IVes neuron. One wk after dye administration, the total number of L6 DRG neurons labeled by IPar FB was significantly higher than the number labeled by IVes DiI or by both FB and DiI (**D**). Of a total of 1234 labeled L6 DRG neurons, 91.5% were positive for FB, 8% were positive for DiI, and 0.5% were double-labeled (**E**). When dyes were reversed, 77.6% of IPar-labeled neurons were positive for DiI, 22.4% IVes-labeled neurons were positive for FB, and 4.5% were double-labeled out of a total of 531 labeled neurons (**F**). ** indicates $P < 0.01$.