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Ion channel and receptor mechanisms of bladder afferent nerve sensitivity

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ABSTRACT

Sensory nerves of the urinary bladder consist of small diameter A_δ and C fibers running in the hypogastic and pelvic nerves. Neuroanatomical studies have revealed a complex neuronal network within the bladder wall. Electrophysiological recordings *in vitro* and *in vivo* have revealed several distinct classes of afferent fibers that may signal a wide range of bladder stimulations including physiological bladder filling, noxious distension, cold, chemical irritation and inflammation. The exact mechanisms that underline mechanosensory transduction in bladder afferent terminals remain ambiguous; however, a wide range of ion channels (e.g., TTX-resistant Na^+ channels, Kv channels and hyperpolarization-activated cyclic nucleotide-gated cation channels) and receptors (e.g., TRPV1, TRPM8, TRPA1, $P2X_{2/3}$, etc) have been identified at bladder afferent terminals and implicated in the generation and modulation of afferent signals. Experimental investigations have revealed that expression and/or function of these ion channels and receptors may be altered in animal models and patients with overactive and painful bladder disorders. Some of these ion channels and receptors may be potential therapeutic targets for bladder diseases.

1. Introduction

The primary functions of the urinary bladder to store and to evacuate urine are controlled by a hierarchy of neuronal circuits located in the brain, the spinal cord and the periphery (de Groat, 2006). Adequate afferent input from the urinary bladder is required for the central neuronal circuits to generate efferent nerve activity that drives coordinated activity of bladder contraction and urethral sphincter relaxation. Dysfunction of the bladder afferent nerves has been implicated in disturbances in bladder function and pain. In recent years, extensive efforts have been devoted to investigate the mechanisms that control bladder afferent activity. A cohort of ion channels and receptors has been identified in bladder afferent neurons. In this paper, we will briefly review the anatomical features and functional properties of bladder afferent nerves followed by a discussion on some of the ion channels and receptors that have been implicated in the transduction, transmission and modulation of bladder afferent signals.

2. Anatomical features and functional properties of bladder afferents

2.1. Afferent pathways

Afferent nerve fibers travel to the urinary bladder within pelvic and hypogastric nerves, which also contain parasympathetic and sympathetic efferent fibers. Retrograde tracing studies have established L_{1-2} (hypogastric) and L_6–S_3 (pelvic) dorsal root ganglia (DRG) as the primary source of afferent innervation of the urinary bladder (Pascual et al., 1993; Keast and De Groat, 1992; Downie et al., 1984; Applebaum et al., 1980).

Distribution of afferent fibers within the urinary bladder has been examined by means of immunohistochemical staining of calcitonin gene-related peptide (CGRP), substance P (SP), TRPV1 and other sensory neuronal markers. Afferent fibers are abundant within the muscle and in the suburothelial layer. Gabella and Davis (1998) demonstrated that CGRP-positive afferent axons were distributed over four distinct targets: at the base of the epithelium, inside the epithelium, on blood vessels (both arteries and veins) and along muscle bundles (Gabella and Davis, 1998). In the mucosa, afferent axons lay either inside the epithelium or in a subepithelial plexus very close to the basal surface of the epithelium. Most of the axons in the subepithelial plexus were terminal axons with conspicuous varicosities arranged in very long chains. Afferent fibers in the muscle layer ran in parallel to the muscles, submucosa, and epithelium.

Gillespie and colleagues identified two types of afferent nerve fibers in the guinea pig bladder, fibers positive for CGRP and fibers positive for choline acetyltransferase (ChAT) (Gillespie et al., 2006). $ChAT^+$ fibers were found to originate close to the urothelium, to transit the sub-urothelial interstitial cell layer and to pass into the lamina propria. $CGRP^+$ fibers were seen to intertwine with the $ChAT^+$ fibers in the lamina propria. Both types of afferent fibers were seen in contact with intramural ganglionic cells. The intramural ganglia appear to have multiple inputs not only from the extrinsic afferent fibers but also from adjacent ganglia and they send axons to the

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muscle. Thus, within the bladder, a complex neuronal network may represent the elements generating and modulating bladder sensations. The morphology of mechanosensory transduction sites within the bladder is also discussed elsewhere in this issue.

Evidence suggests that the hypogastric and the pelvic bladder afferent nerve terminals may not distribute uniformly in the bladder. In a series of studies in the cat, Uemura and colleagues reported that the pelvic bladder afferent axons were distributed equally to all areas of the bladder, whereas axons from the lumbar innervation were most abundant in the trigone region and the ventral neck of the bladder. Floyd et al., made single unit recordings of hypogastric bladder afferents; they found punctate receptive fields along the blood vessels near the bladder and at the base of the bladder (Floyd et al., 1976). More recently, Xu and Gebhart mapped the distribution of mecahnosensitive receptive endings of hypogastric and pelvic nerve fibers using a flat-sheet mouse bladder preparation and they found that the pelvic receptive endings were distributed throughout the bladder whereas those of hypogastric nerves were concentrated at the base of the bladder and that a major proportion (97%) of them were serosal and muscular receptors (Xu and Gebhart, 2008). It seems reasonable to assume that hypogastric bladder afferents project preferentially to serosal and muscular layers of the base of the bladder.

2.2. Functional properties of bladder afferents

Electrophysiological properties of pelvic bladder afferents have been studied extensively in different species both *in vivo* and *in vitro*. The vast majority of bladder afferents are small diameter A_δ and C fibers with a conduction velocity of less than 2.5 m/s (Vera and Nadelhaft, 1990). Few bladder afferents exhibit spontaneous discharge when the bladder is empty in the normal condition. This is in contrast to the extrinsic afferents of the GI tract, which exhibited considerable level of spontaneous activity. Three major functional populations of bladder afferents, namely, mechanoreceptors, chemoreceptors and silent receptors, have been reported. In a single unit study in the rat *in vivo*, Shea et al. identified 100 bladder afferent units by electrical stimulation of both pelvic nerve and bladder nerves (Shea et al., 2000). The receptive characteristics were studied by direct mechanical stimulation, filling of the bladder with 0.9% NaCl or with solutions containing capsaicin, potassium, or turpentine oil. They found that the majority (61%) of bladder afferents were mechanoreceptors responding to bladder filling with 0.9% NaCl. Mechanoreceptors with receptive fields on the body of the bladder had low pressure thresholds ($\leq$10 mm Hg); receptive fields of units with higher thresholds were near the ureterovesical junction, on the base of the bladder or could not be found. In the thirty-nine units (39%) that were not responsive to bladder filling, 8 units were chemoreceptors responding to intravesical potassium or capsaicin. The remaining 31 units were not excited by any stimulus tested. These units presumably were "silent receptors", which could be sensitized by inflammatory mediators (Habler et al., 1988).

Two types of *in vitro* preparations have been used to examine the electrophysiological properties of bladder afferents. Rong et al. described an *en bloc* mouse bladder/pelvic nerve preparation which enabled recording of bladder afferent activity in response to bladder filling and chemical stimulations (Rong et al., 2002; Vlaskovska et al., 2001). Through single unit discrimination, they described low threshold and high threshold mechanoreceptors, chemorecetpors which responded to chemical stimulation and "silent receptors" which became mechano-sensitive only after chemical exposure. A similar *en bloc* rat bladder preparation was described more recently (Yu and de Groat, 2008). Bladder afferents have also been studied using a flat-sheet bladder preparation, which enabled recording of afferent activity in response to stretch, probing and chemicals (Zagorodnyuk et al., 2007; Zagorodnyuk et al., 2006). Five functionally distinct populations of afferent fibers were distinguished by these stimuli in the guinea pig bladder, which are discussed in detail elsewhere in this issue.

Information about the functional properties of hypogastric bladder afferents has been relatively scarce; but available evidence suggests that they may differ from the pelvic afferents. Moss et al. compared the activity of bladder afferents in pelvic and hypogastric nerves of rats in vivo (Moss et al., 1997). They found that a large proportion of hypogastric bladder afferents were chemosensitive receptors since they were activated by bladder filling with KCl in a concentration-dependent fashion but were insensitive to bladder filling with NaCl. A smaller proportion of hypogastric bladder afferent fibers were mechanoreceptors activated to a similar extent by NaCl and KCl solutions. In contrast, chemoreceptive bladder afferent fibers were rare in the pelvic nerve (1 of 15 units). Shankar, et al found that serosal but not mucosal application of chemical irritants to the urinary bladder resulted in an increase in heart rate, rise of blood pressure and increase in respiratory rate and depth in anesthetized dogs (Shankar et al., 1993). These cardio-respiratory responses were abolished by section of the hypogastric nerves, whereas bilateral pelvic nerve section did not modify the responses. Mitui et al. studied the role of bladder afferent fibers in the hypogastric nerves in modulation of the micturition reflex induced by chemical bladder irritation in unanesthetized conscious rats (Mitsui et al., 2001). Hypogastric nerve transection prevented the increase in urinary frequency following intravesical application of acetic acid and decreased acetic acid-induced c-fos expression at L_1 spinal cord. Dang et al. compared the chemical sensitivity of lumbosacral (pelvic) and thoracolumbar (hypogastric) DRG neurons that innervate the rat urinary bladder by whole-cell patch-clamp recordings. They found that pelvic and hypogastric neurons differ in the responsiveness to α,β-methyleneATP, capsacin and protons (Dang et al., 2005). These data suggest that hypogastric afferents are functionally distinct from pelvic afferents and may signal noxious overdistension and/or chemical irritation of the bladder. However, it should be noted that other studies indicate that both hypogastric and pelvic afferents signal physiological filling, noxious distension and chemical irritation of the bladder. For examples, Cruz and Downie found that in awake rats, abdominal muscle activity during intravesical saline or acetic acid infusion was modified but not reversed by hypogastric neurectomy, suggesting that the afferents activating the visceromotor reflex during normal voiding and the increased reflex caused by acetic acid are both carried by the pelvic as well as the hypogastric nerves (Cruz and Downie, 2006).

3. Mechanisms of sensory transduction and modulation

At the present, the mechanisms that underline mechanosensory activation of pelvic and hypogastric bladder afferents remain poorly understood. Presumably, two main types of mechanosensory transduction may mediate activation of bladder afferents in response to bladder filling. The direct mechanism of mechanosensory transduction relies on mechanically-gated ion channels expressed on afferent terminals; but the molecular identity of mechanically-gated ion channels is still illusive. Possible candidates may include the ENaC/ASIC/degenerin Na^+ channels and transient receptor potential (TRP) cation channels (see below). The indirect mechanism of mechanosensory transduction relies on interaction of chemical mediators (such as ATP) released by non-neuronal (urothelial and detrusor muscle) cells with neuronal ionotropic receptors (such as $P2X_3$ receptor). There has been ample evidence that the urothelium express a range of "sensory molecules" such as TRPV1, TRPV4 and ENaC/ASIC/degenerin Na^+ channels and may have a sensory function by releasing chemical mediators such as ATP, NO and prostanoids in response to mechanical and chemical stimulations (Birder, 2006; Birder, 2005; Gevaert et al., 2007). However, the significance of such indirect mechanism of mechanosensory transduction has been contested since