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# Effects of nicotinic receptor agonists on bladder afferent nerve activity in an in vitro bladder–pelvic nerve preparation

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ABSTRACT

Effects of nicotinic receptor agonists (epibatidine and nicotine) on mechano-sensitive bladder afferent nerve (MS-BAN) activity were studied in an in vitro bladder–pelvic afferent nerve preparation. MS-BAN activity was induced by isotonic distention of the bladder at pressures of 10–40 cm$H_2O$. The effect of epibatidine varied according to the concentration, route of administration and the intravesical pressure stimulus. Epibatidine (300–500 nM) administered in the perfusate to the serosal surface of the bladder decreased distension evoked afferent firing by 30–50% depending on the bladder pressure. However these concentrations also produced an immediate increase in tonic afferent firing in the empty bladder. Lower concentrations (50–100 nM) elicited weaker and more variable effects. The inhibitory effects were blocked by bath application of mecamylamine (150 μM) a nicotinic receptor antagonist. Bath application of nicotine (20 μM) elicited similar effects. Intravesical administration of epibatidine (500 nM) significantly increased MS-BAN firing by 15–30%; while lower concentrations (200–300 nM) were ineffective. This facilitatory effect of epibatidine was blocked by intravesical administration of mecamylamine (250 μM). Electrical stimulation on the surface of the bladder elicited action potentials (AP) in BAN. Bath application of epibatidine (300 nM) or nicotine (20 μM) did not change either the voltage threshold or the area of evoked AP. These results indicate that nicotinic agonists: (1) enhance MS-BAN activity originating at afferent receptors near the urothelium, (2) inhibit MS-BAN activity originating at afferent receptors located at other sites in the bladder, (3) directly excite unidentified afferents, (4) do not alter afferent axonal excitability.



*Abbreviations*: ACh, acetylcholine; AMN, atropine methyl nitrate; AP, action potential; EPI, epibatidine; BAN, bladder afferent nerve; MEC, mecamylamine; NNR, neuronal nicotinic receptors; NO, nitric oxide; NOS, nitric oxide synthase; PPADS, pyridoxal-phosphate-6-azophenyl-2′,4′-disulfonate

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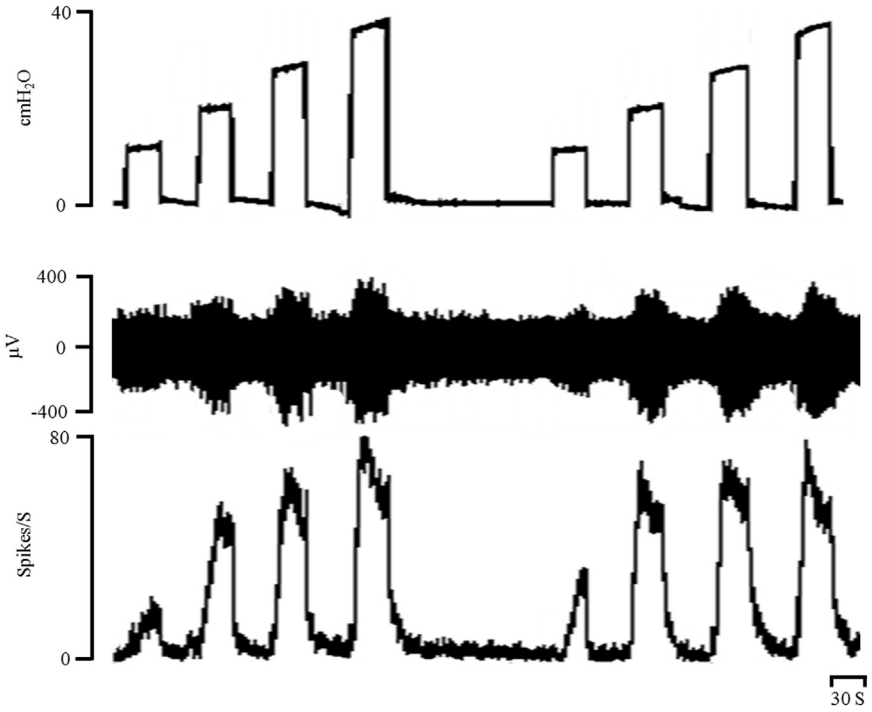


**Fig. 1 – Increases in bladder afferent firing evoked by 10, 20, 30 and 40 cm$H_2O$ intravesical pressures applied for 30 s at 30 s intervals. Top trace: intravesical pressure (cm$H_2O$), middle trace afferent firing (μV), bottom trace rate meter recording of afferent firing (spikes/s). Horizontal calibration bar represents 30 s.**

## 1. Introduction

Intravesical administration of nicotinic (Beckel et al., 2006; Beckel and Birder, 2012; Kontani et al., 2009; Masuda et al., 2006) or muscarinic (Kullmann et al., 2008b; Matsumoto et al., 2010, 2012) cholinergic receptor agonists increases or decreases the frequency of reflex voiding in rats depending on the concentration of the agonist and/or the type of receptor activated (de Groat et al., 2015). These observations raise the possibility that sensory pathways in the urinary bladder are sensitive to cholinergic modulatory mechanisms. The modulation may occur as a result of a direct action on afferent nerves which express nicotinic and muscarinic receptors (Kontani et al., 2009; Masuda et al., 2006; Nandigama et al., 2010, 2013; Yu and de Groat, 2010) or indirectly via an action on urothelial cells which also express these receptors (Beckel and Birder, 2012; Kullmann et al., 2008a) and release neurotransmitters such as ATP, ACh and NO (Birder et al., 1998; Ferguson et al., 1997; Hanna-Mitchell et al., 2007; Lips et al., 2007; Silva et al., 2015; Yoshida et al., 2010) that can influence the excitability of adjacent afferent nerves (Birder and Andersson, 2013; Cockayne et al., 2000; de Groat and Yoshimura, 2009; Ford et al., 2015; Kullmann et al., 2008b).

In anesthetized rats intravesical administration of oxotremorine, a non-selective muscarinic receptor agonist, reduces voiding frequency in low concentrations and increases voiding frequency in high concentrations (Kullmann et al., 2008b). The former is suppressed by a NOS inhibitor and the latter is partially reduced by a purinergic receptor antagonist (PPADS) indicating that NO and ATP contribute to the inhibitory and facilitatory effects, respectively.

Intravesical administration of neuronal nicotinic receptor (NNR) agonists elicits similar mixed effects on voiding frequency (Beckel et al., 2006). Nicotine or choline reduce voiding frequency; an effect blocked by a specific α7 NNR antagonist (methyllycaconitine); whereas cytisine, an α3 NNR agonist increases voiding frequency; an effect blocked by PPADS (Beckel and Birder, 2012). Cytisine increases release of ATP from cultured urothelial cells, whereas choline suppresses release suggesting that the effects of the NNR agonists on voiding are mediated in part by activation of receptors in the urothelium leading to changes in urothelial–afferent interactions and in turn changes in bladder afferent input to the spinal cord (Beckel and Birder, 2012).

Combined immunohistochemical and RT-PCR analysis has revealed that bladder afferent neurons in mouse lumbosacral dorsal root ganglia express multiple subtypes of NNRs (Nandigama et al., 2013). The majority of afferent neurons express α3 NNRs which are present in at least two distinct populations of bladder afferents: (1) mucosal high-threshold mechanoreceptors with chemosensitivity and (2) fast-conducting mechanosensors.

In the present experiments we used an in vitro bladder–pelvic afferent nerve preparation (Yu and de Groat, 2008) to examine the effects of nicotinic receptor agonists (epibatidine or nicotine) and antagonists (mecamylamine or hexamethonium) on the activity of mechano-sensitive bladder afferents induced by bladder distension. The results indicate that activation of nicotinic receptors enhances the activity of mechano-sensitive afferents with receptors near the urothelium, inhibits the activity of mechano-sensitive afferents with