#531 Prejunctional M1 receptor – A Therapeutic target for underactive bladder

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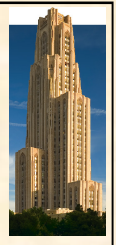


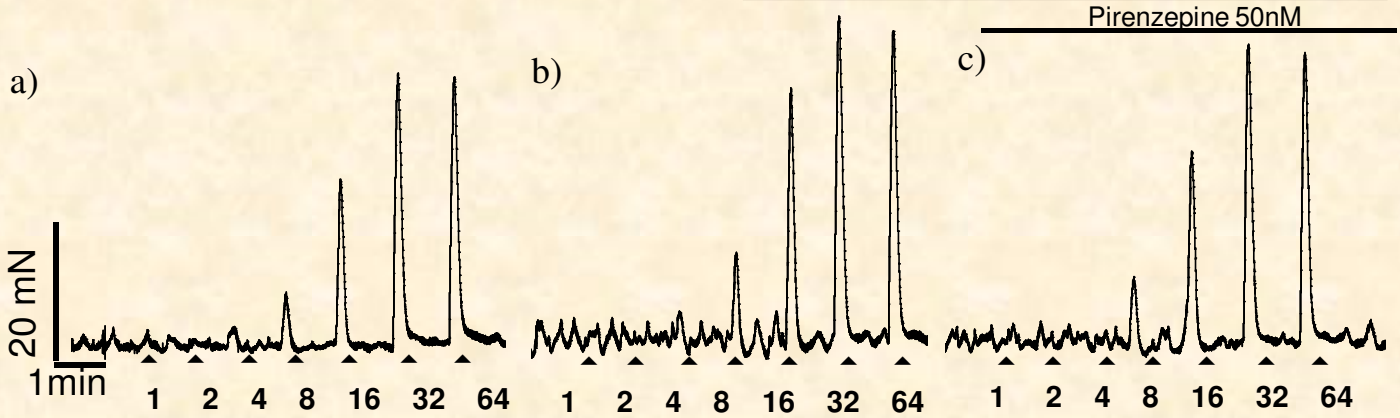
Introduction

Underactive bladder (UAB) is the clinical manifestation of detrusor underactivity (DU), which is defined as a contraction of reduced strength and/or duration resulting in prolonged bladder emptying and/or a failure to achieve complete bladder emptying within a normal time span. Available treatments for UAB include, Bethanechol, which mimics the action of acetylcholine (ACh) in bladder, but its poor M1-M4 subtype selectivity, off-target side effects can reduce its effectiveness. A recent pilot study on DU patients demonstrated the clinical potential of an alternative pharmacological approach, which involves facilitated release of endogenous ACh through blockade of the negative auto-feedback of muscarinic M4 receptors[1]. We postulate that similar clinical outcomes in UAB can also be achieved through activation of pre-junctional muscarinic M1 receptors, which are known to exert a positive auto-feedback in ACh release[2]. Cevimeline is a muscarinic agonist, FDA approved for dry mouth with reported >8 fold higher selectivity for M1 over M3 muscarinic receptors. Because the expression and function of M1 receptors in rat bladder is very well characterized[2], a comprehensive description of how cevimeline increases the contractile activity of rat bladder should provide insight into the potential therapeutic role of M1 agonists in UAB.

METHODS

Longitudinal, urothelium intact bladder strips were removed from euthanized Sprague-Dawley rats of either sex (10-12 weeks old) and mounted in 37°C organ bath constantly gassed with 95% oxygen-5% carbon dioxide for isometric tension studies. Strips were stretched to 1 g of tension for eliciting spontaneous phasic contractions. Nerve-mediated contractions (tetrodotoxin-sensitive) were generated by electrical field stimulation of strips (EFS: 5 ms pulses, 1-64Hz, 2s train at 20V) in presence or absence of Cevimeline with or without an M1 receptor antagonist Pirenzepine [50nM]. EFS frequency response curve were generated by stimulating at 1, 2, 4,8, 6, 32 and 64 Hz (one stimulation at each frequency) with 1-min intervals between stimulations. EFS evoked contraction amplitude was normalized by the response to 120mM KCl. α,βme-ATP (10 μM), an ATP analogue, was used to desensitize purinergic receptors and leave only ACh-mediated EFS contractions. Effect of Cevimeline on spontaneous phasic contractions was assessed on strips not exposed to EFS.

RESULTS



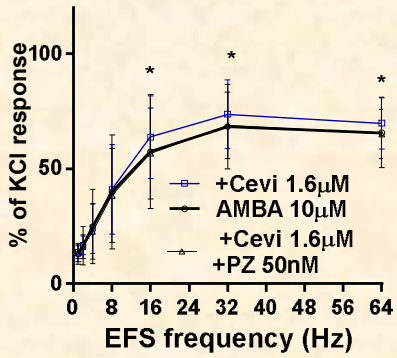
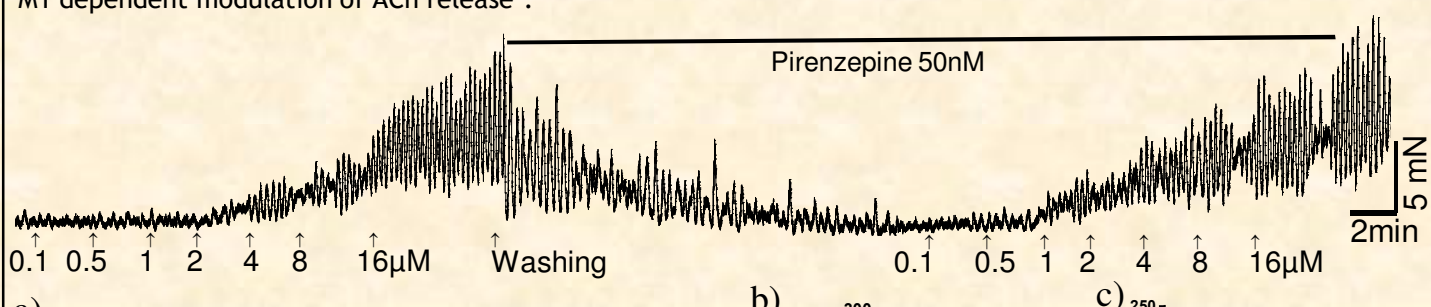


Figure 1: Effect of Cevimeline on EFS (5 ms pulses, 1-64Hz, 2s train at 20V, 60s interval) evoked contractions in α,βme-ATP (10 μM) pretreated urothelium intact rat bladder strips. Traces are shown for (a) control, (b) 1.6μM Cevimeline and 1.6μM Cevimeline + Pirenzepine 50nM (d) Force-frequency relationships in the absence and presence of Cevimeline with or without Pirenzepine PZ(50nM). Two -way ANOVA followed Tukey's multiple comparison; *p<0.01. TTX sensitive contractions elicited by EFS at <8Hz frequencies are dominated by ATP release, whereas contractions evoked by ≥8Hz involves M1 dependent ACh release from post-ganglionic cholinergic nerves. Increased magnitudeof nerve evoked contraction in presence of Cevimeline implicates M1 dependent modulation of ACh release[2].



a)

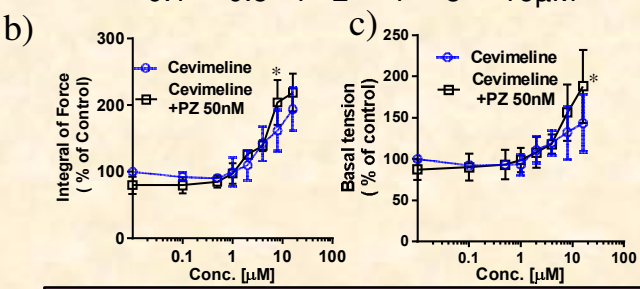
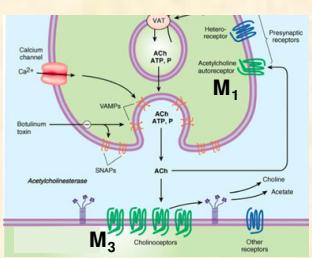


Figure 2: Concentration dependent increase (panel a) in spontaneous contractions (panel b) and basal tension (panel c) of urothelium intact strips by Cevimeline involves M1 and M3 activation at low and higher concentrations, respectively. M3 activation at higher concentrations of Cevimeline is potentiated by the blockade of M1 binding by Pirenzepine (PZ). PZ 50nM *per se* reduces the basal tension and spontaneous contractions. (*p=0.01; Two-way ANOVA and Sidak's test). Activation of M1 receptors in urothelium can contribute to the increased basal tone and spontaneous contractions presumably via enhanced ATP release. *Ex vivo* findings shown here are consistent with the increased frequency of non-voiding contractions in normal young rats following acute intravenous administration of Cevimeline at M1 selective dose of 0.3-5mg/kg[3]. Animal and clinical studies on UAB report a deficient sensation of bladder filling[4], which can potentially be restored by enhancing the spontaneous contractions.

CONCLUSIONS



M_1 selective pharmacological effects of Cevimeline in bladder can be the basis for repurposing it as a novel therapy for UAB and underlying DU.

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