

ROLE OF 5-HYDROXYTRYPTAMINE IN URINARY BLADDER AFFERENT SIGNALLING

Hypothesis / aims of study

Interstitial cystitis or painful bladder syndrome (PBS) patients develop pain, urgency and frequency due to hypersensitivity of the afferent nerves innervating the bladder. A previous study has shown 5-hydroxytryptamine (5-HT, serotonin) plays a role in hypersensitivity of the bowel⁽¹⁾. However, little is known about the peripheral roles of 5-HT on bladder afferent activity. 5-HT receptors are classified into 7 subtypes (5-HT₁₋₇), however, the expression of 5-HT receptors in the urothelium has yet to be determined. The aim of this study was to examine mRNA expression of 5-HT receptors in mouse urothelium and investigate the effects of 5-HT on peripheral bladder afferent nerves in a mouse model.

Study design, materials and methods

Adult C57/BL6 male mice (13-16 weeks old) were sacrificed humanely. For the mRNA expression study, RNA was extracted from urothelial cells by gently scraping the urothelial layers and dissociating cells with 0.025% trypsin EDTA. cDNA was synthesized. RT-PCR was performed for 5-HT receptors 1-7, 5-HT-producing enzymes, TPH1, TPH2, and the serotonin reuptake transporter, SERT. For functional studies, the pelvic region of the mice was placed in a recording chamber which was continually perfused with oxygenated (95% O₂ and 5% CO₂) Krebs-bicarbonate solution at 35°C. The afferent nerve bundle (mixture of hypogastric and pelvic nerves) was identified, dissected and placed into a recording electrode, connected to a NeuroLog headstage. Signals were amplified, filtered, and captured by the computer via Spike2 (version 7.13, Cambridge Electronic Design, UK). The following pharmacological agents were used to investigate the effects of 5-HT on bladder afferent firing:

- (i) 5-HT (100 μ M)
- (ii) 5-HT 3 receptor agonist, 2-methyl-hydroxytryptamine (2-Me-5-HT, 100 μ M)
- (iii) 5-HT 1, 2, 4-7 receptor agonist, 5-methoxytryptamine (5-MT, 100 μ M)
- (iv) 5-HT 3 receptor antagonist (granisetron, 1 μ M)
- (v) Rho kinase inhibitor (Y27632, 10 μ M) and myosin light chain kinase inhibitor (ML-9, 10 μ M) to block detrusor contractions

In order to investigate the effects of 5-HT on baseline afferent firing, bladders were maintained under isovolumetric conditions at 10 mmHg for 30 minutes and pharmacological agents were continually applied into the bath. The effect of 5-HT on bladder mechanosensitivity was determined by measuring afferent firing in response to bladder filling with isotonic saline with/without pharmacological agents to a maximal intravesical pressure 50 mmHg. Bladder compliance was gauged by the pressure-volume relationship. To examine the role of endogenous 5-HT on bladder afferent firing, citalopram (selective 5-HT reuptake inhibitor, 1 μ M) was perfused into the bladder and the bath. Baseline firing and afferent firing in response bladder distension were measured at 30, 60, 90, and 120 minutes post application.

Results

mRNA expression of 5-HT_{1A}, 1B, 1D, 2A, 2B, 4, 6, 7 mRNA expression were detected in the urothelium but not 5-HT_{1F}, 3A, 3B, 5A, 5B. In addition, TPH1, TPH2, and SERT were also expressed in mouse urothelial cells (N=3). Bath application of 5-HT and 2-Me-5-HT significantly increased baseline firing of afferent nerves (14.68 \pm 5.43 imp/sec vs. 61.49 \pm 12.68 imp/sec, n=6, P<0.05 and 18.98 \pm 7.24 imp/sec vs. 79.21 \pm 20.03 imp/sec, P<0.05, n=6 Student's t-test respectively). Application of a Rho-kinase inhibitor had no effect on the afferent response to 2-ME-5-HT (n=6). 5-MT also significantly increased baseline firing (25.39 \pm 7.93 imp/sec vs. 46.22 \pm 10.96 imp/sec n=8, P<0.05 Student's t-test) but this effect was significantly abolished by pre-incubated with Y27632 and ML-9 (12.36 \pm 4.18 imp/sec n=6, P<0.05 Student's t-test). The afferent response to 5-HT was significantly inhibited by application of the 5-HT₃ antagonist, granisetron and by application of Y27632 and ML-9 (see Figure 1). Similarly, The afferent response to 5-MT significantly decreased compared to 5-HT and the application of Y27632 and ML-9 further attenuated these effects (see Figure 1). Conversely 5-HT attenuated the afferent response to distension and decreased bladder compliance (n=6, P<0.01, 2-Way ANOVA). Pre-incubation with granisetron significantly reversed this inhibition (n=5, P<0.01, 2-Way ANOVA). In contrast, 5-MT application had no effect on distension induced firing. Interestingly, application of the 5-HT reuptake inhibitor citalopram increased baseline firing in a time-independent manner and significantly attenuated the afferent response to distension after 60 mins.

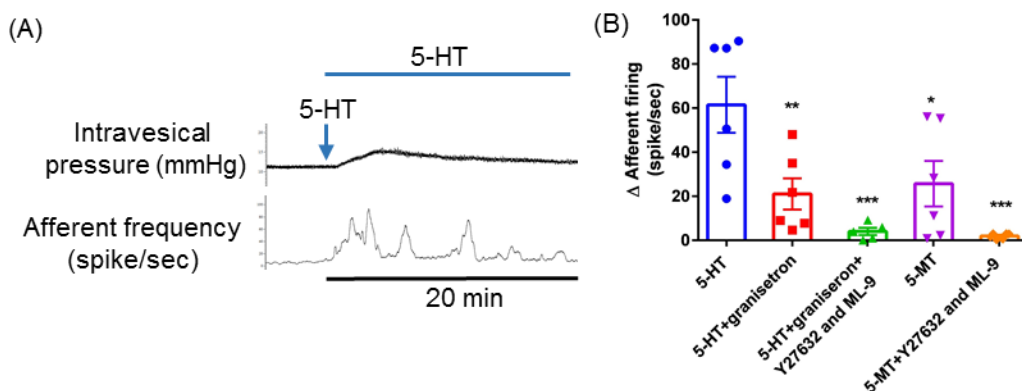


Figure 1 (A) Sample trace showing the action of 5-HT on bladder afferent firing. (B) Peak firing in response to 5-HT agonists and antagonists (*P<0.05, **P<0.01, ***P<0.001 vs.5-HT One-way ANOVA with Dunnett's multiple comparison).

Interpretation of results

5-HT receptor stimulation evoked an increase in baseline afferent firing but attenuated the afferent response to bladder distension. This was mainly driven through the 5-HT₃ receptor. 5-HT₃ receptors were not detected in urothelium, however previous reports have found 5-HT₃ receptors in the detrusor muscle and dorsal root ganglion neurons⁽²⁾. 5-HT₃ receptors are also reported to play a role in bladder hypersensitivity and somatic analgesia after 5-HTP (5-HT precursor) administration⁽³⁾. 5-HT could directly bind to receptors (5-HT₃ and non 5-HT₃) on the nerve endings to evoke afferent signals. In addition, 5-HT could also act on the non-5HT₃ receptors expressed in the detrusor muscle layer to potentiate bladder contraction or on the urothelium, evoking the release of inhibitory or excitatory neurotransmitters (Figure2). Interestingly, the citalopram experiments suggest that they maybe an endogenous source of 5-HT in the bladder wall.

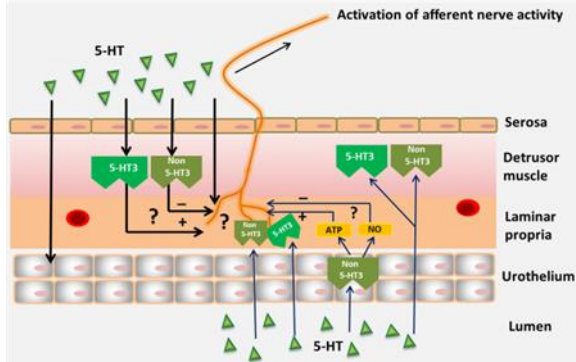


Figure 2 Schematic diagrams of proposed mechanisms of 5-HT actions on bladder afferent nerve activity.

Concluding message

5-HT receptors have a modulatory action on peripheral bladder afferents. These mechanisms may underlie the hypersensitivity associated with a number of urological disorders. In addition, this is the first study to show that blocking 5-HT reuptake with citalopram, an anti-depressant, also peripherally affects bladder afferent function. Further studies are required to investigate the mechanisms involved.

References

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Disclosures

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