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INVOLVEMENT OF 5-HT RECEPTOR SUBTYPES IN PUDENDAL NERVE INHIBITION OF NOCICEPTIVE AND NON-NOCICEPTIVE BLADDER ACTIVITY IN ANESTHETIZED CATS

Hypothesis / aims of study

Understanding the neurotransmitter mechanisms involved in the inhibition of bladder activity induced by neuromodulation could lead to identify new pharmacological targets for overactive bladder (OAB). This study used methysergide (ME; non-selective 5-HT_2 receptor antagonist), ondansetron (OD; 5-HT_3 receptor antagonist) or WAY100635 (WAY; 5-HT_{1A} receptor antagonist) to examine the involvement of these receptors in the micturition reflex and pudendal nerve stimulation (PNS)-induced inhibition of bladder activity.

Study design, materials and methods

The effects of ME, OD or WAY on bladder capacity (BC) and PNS-induced inhibition were investigated using a total of 48 cats under α-chloralose anesthesia. Urine from ureters was continuously drained. The bladder was cannulated through the urethra with a double lumen catheter to infuse (1-2 ml/min) saline or 0.25% acetic acid (AA) via one lumen and measure bladder pressure via another lumen. A ligature was tied around the urethra to prevent leakage. An electrical stimulation (5 Hz frequency, 0.2 ms pulse width) was applied to the right pudendal nerve via a tripolar cuff electrode during cystometrogram (CMG) with saline infusion for assessing non-nociceptive bladder activity or AA infusion for assessing nociceptive bladder activity. Intensity threshold (T) was defined as the minimal intensity to induce observable anal sphincter twitch. Pharmacological studies were separately performed in two experimental groups; i.e. AA CMG and saline CMG group. In both experimental groups, BC was measured during four CMGs: (1) control CMG without stimulation, (2) CMG with PNS at 1-2T, (3) CMG with PNS at 3-4T, (4) control CMG without stimulation to determine the post-stimulation effect. The cumulative doses of ME (0.01, 0.03, 0.1, 0.3 and 1 mg/kg), OD (0.003, 0.01, 0.03, 0.1, 0.3, 1 and 3 mg/kg) or single dose of WAY (0.5 mg/kg) were administered via the right cephalic vein. The vehicle control experiments were conducted in a separate group of cats during repeated saline CMGs or AA CMGs using the same stimulation.

Results

AA significantly ($p < 0.001$) reduced BC to 20.7 ± 3.7 % of control capacity. PNS at 1-2T and 3-4T suppressed AA-induced bladder overactivity and significantly increased BC to 73.2 ± 8.4 at 1-2T ($p < 0.001$) and 96.5 ± 10.8 % at 3-4T ($p < 0.001$) of control BC. ME significantly increased BC at the doses of 0.3-1 mg/kg ($p < 0.05$) (Figure 1A). ME (0.03-1 mg/kg) suppressed PNS-induced inhibition of bladder overactivity at low intensity (1-2T) but not at high intensity (3-4T) (Figure 1A). OD significantly increased BC at the doses of 0.1-3 mg/kg ($p < 0.05$), but BC during PNS was not changed (Figure 2A). OD (1-3 mg/kg) suppressed PNS-induced inhibition of bladder overactivity at low intensity (1-2T) but not at high intensity (3-4T) (Figure 2A). During saline CMG PNS increased BC to 160.4 ± 12.5 at 1-2T ($p < 0.001$) and 185.9 ± 13.1 % at 3-4T ($p < 0.001$) of BC before PNS. ME significantly increased BC at the doses of 0.1-1 mg/kg ($p < 0.05$) and suppressed PNS-induced inhibition at the doses of 0.03-1 mg/kg (Figure 1B). OD significantly increased BC at the doses of 0.1-3 mg/kg ($p < 0.05$), but did not alter PNS-induced inhibition (Figure 2B). The post-stimulation effect was not observed in both groups. WAY (0.5 mg/kg) did not change BC during AA or saline CMG. BC of vehicle controls was not changed significantly during the entire period of experiment.

Interpretation of results

Present study revealed that intravenous administration of ME or OD significantly increased BC during both AA and saline infusion CMGs. It was also shown that ME completely eliminated the PNS-induced increase in BC during saline CMGs and partially eliminated this effect in AA irritated bladders, suggesting a possible role of 5-HT_2 receptors in PNS inhibition. Regarding OD, it eliminated the PNS-induced increase in BC at lower intensity of PNS during AA infusion and did not affect PNS inhibition during saline CMGs, indicating a relatively minor role of 5-HT_3 receptor. The descending 5-HT pathway from raphe nuclei in the brainstem has been known to inhibit nociceptive afferent inputs via the activation of inhibitory interneurons in the spinal cord [1, 2]. Therefore, it is possible that the pudendal afferent firing might be transmitted to the brain and activates raphe nuclei that drive the descending 5-HT pathway to activate 5-HT_2 and 5-HT_3 excitatory receptors on inhibitory spinal interneurons that in turn suppress the micturition reflex. This possibility is supported by Radhakrishnan's report which indicates the involvement of spinal 5-HT_{2A} and 5-HT_3 receptors in the antinociceptive effect induced by transcutaneous electrical nerve stimulation [3]. Present results suggest that the partial involvement of 5-HT_2 and 5-HT_3 receptors in PNS-induced inhibition on micturition reflex. We have already shown in cats that naloxone, an opioid receptor antagonist, only has a minor role in PNS-induced inhibition. Taking our previous reports into consideration, no single neurotransmitter plays dominant role in this inhibitory mechanism. Thus, it can be assumed that PNS-induced inhibition of the bladder is derived from multiple neurotransmitters in complicated mechanisms.

Concluding message

These results indicate that 5-HT_2 and 5-HT_3 receptors play an excitatory role in both nociceptive and non-nociceptive bladder activity, but both receptors are partially involved in the mechanisms of PNS-induced bladder inhibition. Although antagonistic agents for 5-HT_2 or 5-HT_3 receptor might be useful for treating OAB, their agonist potentially enhance PNS inhibition on bladder activity.

Figure 1

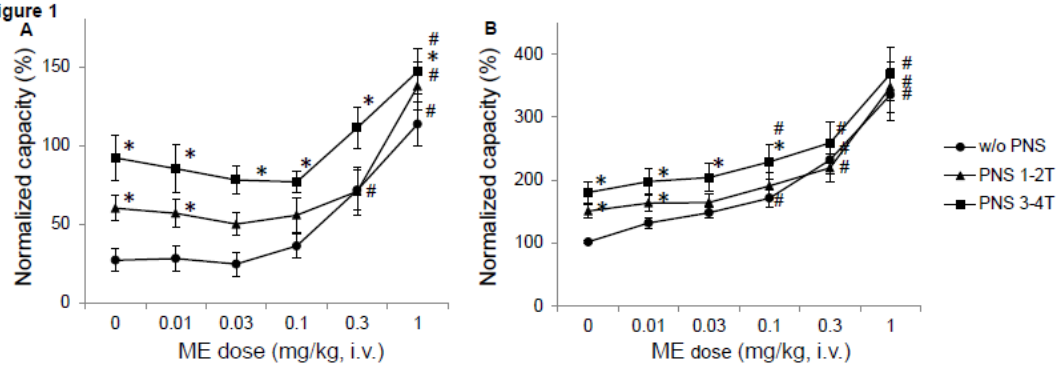
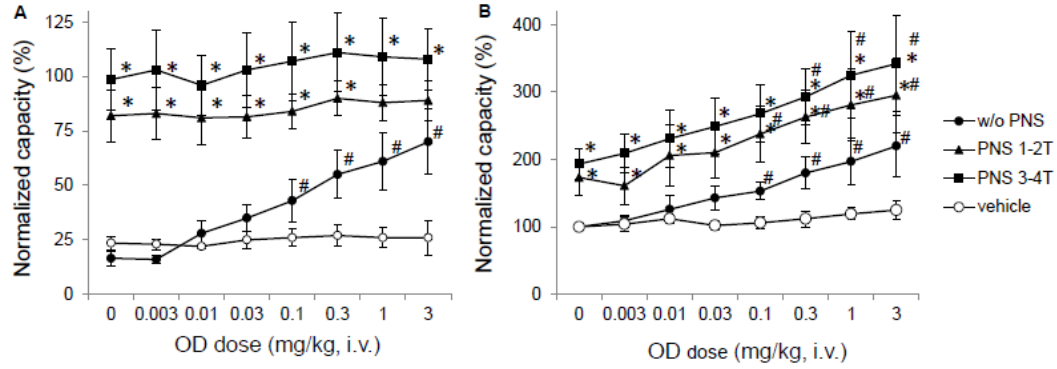


Figure 2



* Indicates significantly ($p < 0.05$) different from BC without PNS. # indicates significantly ($p < 0.05$) different from BC measured before drug administration.

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Disclosures

Funding: This study was supported by the National Institutes of Health under Grants DK-068566, DK-090006 and DK-091253. None of the authors have any conflicts of interest associated with this study. **Clinical Trial:** No **Subjects:** ANIMAL **Species:** Cat **Ethics Committee:** the Animal Care and Use Committee of the University of Pittsburgh