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## DISTRIBUTION AND FUNCTION OF CELLS EXPRESSING SEROTONIN IN THE LOWER URINARY TRACT OF NORMAL AND INFLAMED RATS

### Hypothesis / aims of study

It is well recognized that serotonin (5HT) pathways in the central nervous system (CNS) play an important role in the control of bladder and urethral function (1). In contrast, the role of 5HT at the periphery is less well characterized. However, cells expressing 5HT have been described in the urethral epithelium of several mammals, including humans (2) suggesting that locally released 5HT may also influence urethral and bladder function through a paracrine mechanism. However, this hypothetical role has never been extensively investigated, neither in normal nor in pathological conditions.

Hypothesis: To describe the distribution of 5HT cells in the lower urinary tract of normal and inflamed rats as well as the functional effect of exogenous 5-HT administration during continuous cystometry.

### Study design, materials and methods

All experimental procedures were carried out according to the European Community Directives and ARRIVE guidelines. Female Wistar rats (n=24) were used and divided in two groups. The first group received an intravesical instillation of 0,5 ml of lipopolysaccharide (LPS; 2mg/ml) for one hour to induce lower urinary tract inflammation. The LPS solution was replaced 30 minutes after the beginning of instillation to avoid dilution with urine. The second group received intravesical instillation of saline and served as controls. Twenty-four hours after LPS or saline instillation 16 animals (8 saline; 8 LPS) were sacrificed, bladder and urethra were collected and processed for immunohistochemistry or 5-HT quantification by HPLC. For immunohistochemistry, antibodies against 5-HT, the pan-neuronal marker  $\beta$ 3-Tubulin, calcitonin gene-related peptide (CGRP), vesicular acetylcholine transporter (VACHT), synaptic vesicle 2 (SV2) and tryptophan hydroxylase (TPH) were used. The number of cells expressing 5-HT was quantified and averaged in at least 5 sagittal slices per animal. The total amount of 5-HT in the entire bladder and entire urethra was quantified by HPLC and expressed as pmol per mg of tissue. The remaining 8 animals (4 saline; 4 LPS) underwent cystometry under urethane anaesthesia for evaluation of bladder reflex activity. Bladder recordings were acquired during the continuous infusion of saline during 1 hour followed by the continuous infusion of 5-HT (100  $\mu$ M) for another hour. Urodynamic parameters were analysed, quantified and averaged before and after 5-HT infusion. Statistical analysis was performed in the GraphPad prism software using an unpaired t-test.

### Results

Immunoreactivity for 5-HT was found in dispersed cells along the entire length of the urethra although more abundant in the proximal and middle parts, particularly at the level of the external urethral sphincter (EUS). No 5HT immunoreactivity was detected in the urinary bladder. Positive 5HT cells exhibited a round cell body commonly positioned in the basal layers of the epithelium and emitted slender processes reaching either the lumen or the lamina propria of the urethra. Positive 5HT cells also expressed  $\beta$ 3-Tubulin (pan-neuronal marker), SV2 (synaptic vesicle marker) and TPH (tryptophan hydroxylase, rate-limiting enzyme for the production of 5HT). Below the urethral epithelium, a dense neuronal network was found, containing cholinergic (VACHT<sup>+</sup>) and sensory (CGRP<sup>+</sup>) fibers frequently showing appositions with the basal aspect of 5HT positive cells.

In the urethra of control animals, the mean number of cells expressing 5HT was 181,3 $\pm$ 7,80 cells/slice which significantly decreased to 117,1 $\pm$ 10,35 cells/slice ( $p \leq 0.01$ ) in animals with LPS inflammation.

Tissue levels of 5HT measured by HPLC were below sensitivity (1.5 pmol/mg of tissue) in the bladder. In the urethra of control rats 5HT levels were 15,36 $\pm$ 2,81 pmol/mg tissue. After inflammation, 5HT levels decreased to 7,45 $\pm$ 0,42 pmol/mg tissue ( $p \leq 0.05$ ). Regarding cystometry, the normal micturition pattern observed during the continuous infusion of saline in control animals was significantly altered by the continuous infusion of 5HT (100  $\mu$ M). The urinary frequency was significantly decreased (0.6 $\pm$ 0.093 to 0.28 $\pm$ 0.037;  $p < 0.05$ ) and the maximal detrusor pressure and the amplitude of voiding contractions increased (44.71 $\pm$ 1.458 to 53.49 $\pm$ 1.176 and 34.95 $\pm$ 0.804 to 46.24 $\pm$ 1.011, respectively  $p \leq 0.001$ ). In inflamed animals, a typical high frequency of voiding contractions was observed during saline infusion. This pattern was immediately altered by the infusion of 5-HT (100  $\mu$ M), with a significant decrease of the frequency of voiding contractions to values similar to controls (1.4 $\pm$ 0.10 to 0.53 $\pm$ 0.025;  $p < 0.05$ ). Interestingly contractions appeared in bursts.

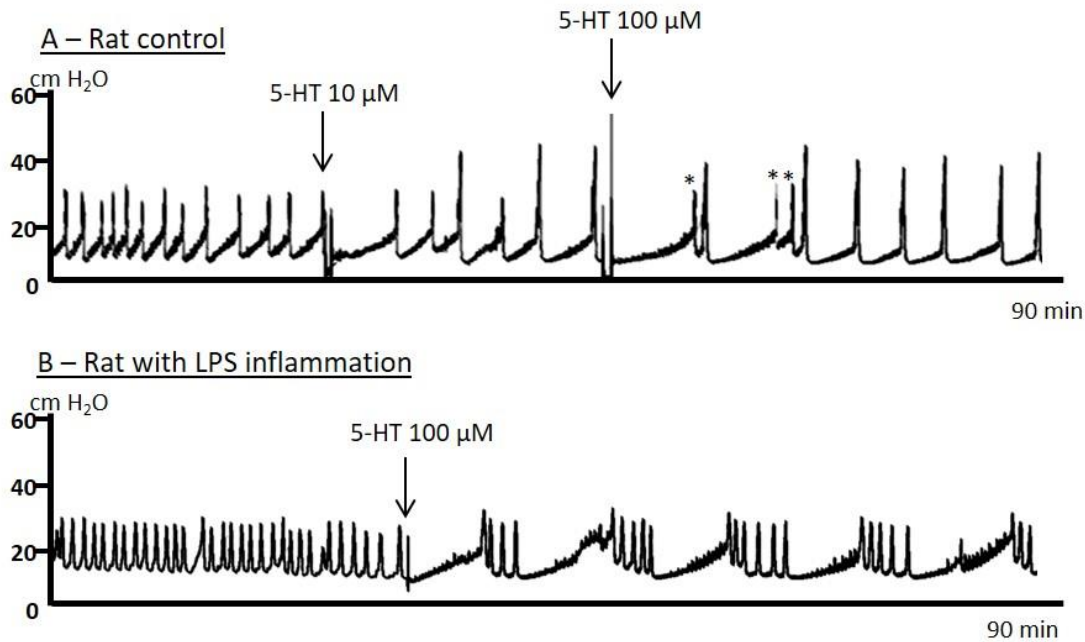


Figure 1: Cistometrograms depicting the bladder reflex activity of a control rat (A) and a rat with LPS inflammation (B). The infusion of a 10 $\mu$ M 5-HT solution did not change micturition pattern (A) in contrast to a 100 $\mu$ M 5-HT solution (\* indicate non-voiding contractions).

#### Interpretation of results

5HT cells occurring in the urethral urothelium, in close proximity with sensory and cholinergic suburothelial nerve fibers exhibit neuronal-like characteristics (paraneurons) and may regulate the frequency and amplitude of bladder voiding contractions through the release of 5HT. Therefore we suggest that urethral 5HT cells may act as local sensors of external stimuli, similarly to what has been shown to occur with acetylcholine-containing cells in the same epithelium (3). 5HT cells decrease during inflammation.

#### Concluding message

5HT produced and released by urethral paraneurons regulate the frequency and amplitude of bladder voiding contractions in normal and inflammatory conditions.

#### References

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#### Disclosures

**Funding:** Ana Coelho is supported by an individual post-doc fellowship reference SFRH/BPD/108468/2015 from FCT under the POCH programme – Programa Operacional Capital Humano partially funded by Fundo Social Europeu and national funds from MCTES. Raquel Oliveira is supported by an individual PhD fellowship from International Programme in Neurosciences (NORTE-08-5369-FSC-000026) under CCDRN funds. **Clinical Trial:** No **Subjects:** ANIMAL **Species:** Rat **Ethics Committee:** Ethics Committee from Faculty of Medicine, University of Porto