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INTERSTITIAL CELL P2Y6-RECEPTORS MODULATE DETRUSOR OVERACTIVITY FOLLOWING SPINAL CORD TRANSECTION

Hypothesis / aims of study
It is believed that detrusor overactivity may be driven by the increased pacemaker activity of bladder interstitial cells (ICs). In this study, we examined the alterations in ICs following spinal cord transection, which results in a significant degree of detrusor overactivity. We used immunohistochemistry with previously identified bladder IC markers to determine how they are changed following spinal cord transection. In addition, we correlated these findings with functional studies using selective drugs that target ICs to determine how their altered function can drive detrusor overactivity.

Study design, materials and methods
Spinal cord transection surgery
Adult female Sprague-Dawley rats were anesthetized and a laminectomy performed between T8-T10 vertebrae; the spinal cord was exposed and transected between T9-T10 spinal levels. The wound was packed with hemostatic sponge and the incision sutured closed. Transection resulted in bladder sphincter dyssynergia. Therefore, these animal's bladders required manual expression twice daily until spinal-somatic reflexes developed (10-14 days). Transected animals were used for experiments 2-3 weeks following surgery.

Immunohistochemistry
Bladders from normal adult and transected rats were removed and washed phosphate buffered saline. The bladders were filled with and embedded in OCT and frozen on dry ice. They were then sectioned 10 mm thick, placed on slides and stored at -20°C until used. Tissue sections were fixed using 4% paraformaldehyde, washed in 1x tris buffered saline (TBS) and stored in TBS overnight. Sections were blocked for an hour with serum and washed and incubated overnight at 4°C in primary antibodies against vimentin, CD34, *c-kit* or $P2Y_6$. Slides were then washed and incubated in appropriate fluorescent secondary antibodies and examined for immunofluorescence.

Optical mapping and tension measurements
Intracellular Ca^{2+} transients were recorded from the entire bladder mucosal surface using a custom-built dual-photodiode array system as described previously (1). Bladders were stained with the Ca^{2+}-sensitive dye, rhod-2-AM (10 mM, Molecular Probes) and after staining, the bladders were cut from outlet to dome along the dorsal aspect to form a sheet. The outlet was pinned to a fixed platform (with the mucosal surface facing up) in the recording chamber machined from thermally conductive, electrically insulating epoxy resin. The dome was tied with suture to a bar connected to a tension transducer. The recording chamber was placed on a Peltier block maintained at 37°C, and superfused with physiological solution at a rate of 1 $ml.min^{-1}$. Bladder sheets were stretched to 1g of resting tension and equilibrated for at least 30 min. Test drugs were added from 10 mM stocks to the perfusion solution to give working concentrations.

Results
Distinctive population of interstitial cells in the bladder
Immunohistochemical studies showed that there were distinctive populations of ICs in the rat bladder consisting of vimentin and CD34-positive cells. Both cell types were found throughout the bladder wall but did not appear to co-localize, indicating they are distinctive from one another. Vimentin-positive cells formed a dense layer along the basal aspect of the urothelium and CD34 cells formed a larger layer beneath these vimentin cells (figure 1). Also it could be seen that CD34-positive cells projected into the urothelial layer (fig 1A). Following transection, bladders became hypertrophied and showed a significant increased in the number of ICs throughout the bladder. $P2Y_6$ and *c*-kit receptor expression was also found to increase in bladders from transected animals, with $P2Y_6$-receptors selectively co-localizing to CD34-positive cells (fig 1B).

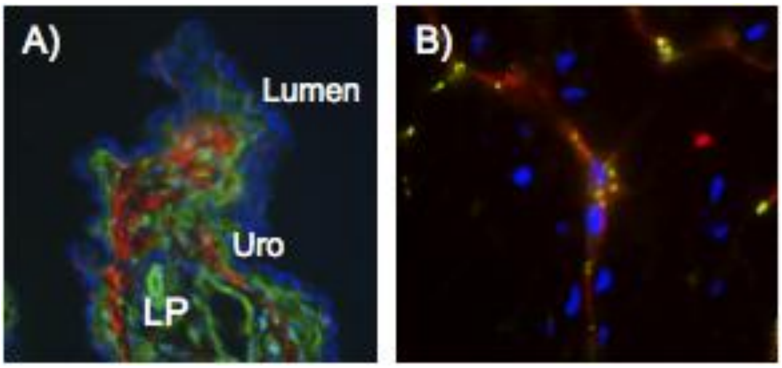

Figure 1. A) Vimentin and CD34-staining in the mucosa of a normal rat bladder. Green – vimentin, Red – CD34, blue – DAPI (x200). B) CD34 (red) and $P2Y_6$-receptor (green) staining in the transected rat bladder (x600).

Alterations in spontaneous contractile activity following spinal cord transection
Bladders from transected animals demonstrated large amplitude low frequency spontaneous contractions when compared to those from normal adult rats. The amplitude of spontaneous contractions and intracellular Ca^{2+} activity were significantly reduced by the c-kit receptor inhibitor, 30mM Gleevec, indicating ICs may be involved in driving this activity (not shown). The $P2Y_6$-selective agonist PSB0474 (1-30mM), did not reduce the amplitude of contractions but decreased their frequency. Addition of the $P2Y_6$-

selective antagonist, MRS2578 (10mM) (figure 2), following agonist application, increased the frequency back to control conditions. No effect was seen in the above-mentioned drugs on the spontaneous activity from normal adult rat bladders.

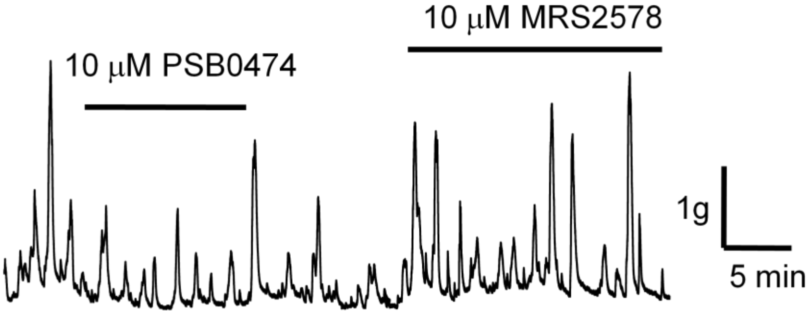

Figure 2. Tension trace from a transected rat bladder showing enhanced spontaneous activity

Interpretation of results

This study demonstrated increased expression of P2Y_6-receptors following spinal cord transection that localize to CD34-positive ICs. Additionally, it was found that activation of P2Y_6-receptors using selective agonists and antagonists altered the frequency of spontaneous contractions in the transected rat bladder. Previous studies have shown in transected rat bladders, that the gap junction proteins, connexin 43 and 26, dramatically increased in the lamina propria and urothelium, respectively. P2Y-receptors have been described in other cell types to regulate the function of gap junctions (2). It can be hypothesized that in the transected rat bladder, that activation of P2Y_6-receptors may increase the connectivity of ICs, leading to coordinated activation of the detrusor muscle.

Concluding message

P2Y_6-receptors play a role in regulating pacemaker activity of ICs in pathological bladders, potentially through regulating the connectivity of ICs *via* gap junctions. Further study into the function of these cells may uncover the intrinsic mechanism by which the bladder regulates the spontaneous activity of the detrusor and potentially lead to novel therapeutic targets.

References

1. Am J Physiol Renal Physiol., 293(4):F1018-25, 2007
2. J Physiol., 531(Pt 3):693-706, 2001

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What were the subjects in the study?	**ANIMAL**
Were guidelines for care and use of laboratory animals followed or ethical committee approval obtained?	**Yes**
Name of ethics committee	**Institutional Animal Care and Use Committee, University of Pittsburgh**