

## EXPRESSION AND FUNCTION OF BETA3-ADRENOCEPTORS ON MOUSE BLADDER AFFERENTS

### Hypothesis / aims of study

Beta3-adrenoceptors are highly expressed in the urinary bladder of various species (1). These receptors are believed to play a major role in mediating detrusor relaxation and gained much interest as a target for the treatment of overactive bladder symptoms. However, whether there is a direct effect of beta3-adrenoceptor agonists on sensory nerve function has yet to be fully evaluated, particularly on mechanosensitive afferents. Effects on afferent nerves during filling can be difficult to evaluate as beta3-agonists relax smooth muscle and alters the tissue compliance, making it difficult to determine if reduced afferent activity is due to direct action on the nerves or indirectly through muscle relaxation. To determine the possibility of a direct action on afferent nerves, we examined for the expression of beta3-receptors on bladder afferents and direct action on afferent firing using *in vitro* approaches.

### Study design, materials and methods

**Bladder fast blue (FB) injections:** Normal adult female C57BL/10 mice (n=5) were anesthetized with 2.5% isoflurane and a lower midline incision was performed to expose the bladder. Approximately 10 µl of 1% FB solution was injecting into the bladder wall using a 32G-syringe into 2-3 sites. The incision was sutured and animals were allowed to recover for 3-5 days on prophylactic antibiotics and analgesics.

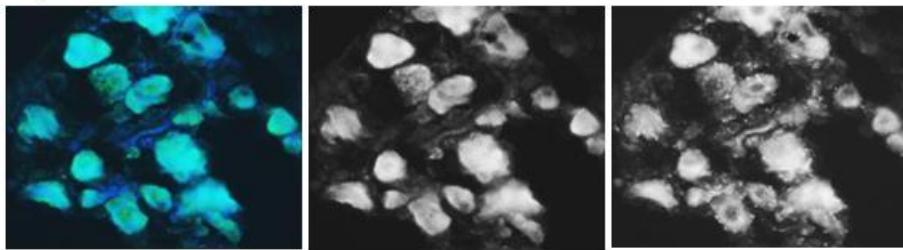
**Immunohistochemistry:** Mice were perfused with 4% paraformaldehyde, a laminectomy performed and L<sub>6</sub>-S<sub>1</sub> DRGs excised. The DRGs were set into cryomolds and covered in optimal cutting temperature (OCT) medium and frozen on dry ice. DRGs were sectioned 8 µm thick on a cryostat, placed onto slides and stored at -20°C until use. Tissue sections were washed in 1x tris buffered saline (TBS) and blocked for an hour with 10% donkey serum/1x TBS, washed in TBS and incubated (overnight at 4°C) in primary antibodies against the mouse beta3-adrenoceptor (Lifespan Bioscience). Slides were washed in TBS and incubated for an hour in a fluorescent secondary antibody. Immunolabelled sections were examined with an Olympus BX62 microscope and images were captured using a Hamamatsu ORCA-ER digital camera. Images were acquired and analysed using Hamamatsu HCimage software.

**In vitro single-unit bladder afferent nerve recordings:** Methodology for these afferent nerve recordings have been previously described (2). Briefly, bladders with associated pelvic (L<sub>6</sub>-S<sub>2</sub>) nerves were excised and placed in a recording chamber with oxygenated Krebs. Organs were cut from outlet to dome along the midline ventral aspect to form sheets. The bladder base was pinned to a fixed platform with the mucosal surface facing up and the dome connected to a tension transducer. The associated spinal nerves were placed into adjacent oil chambers and electrical signals recorded through platinum-iridium electrodes. Preparations were stretched to optimal resting length (L<sub>0</sub>) and allowed to equilibrate for 30 min. Longitudinal stretch was applied via a tension transducer and computer-controlled programmable stepper motor.

### Results

**Localization of beta3-adrenoceptors to bladder afferent DRGs:** Beta3-adrenoceptor labeling was found in mouse L<sub>6</sub> and S<sub>1</sub> DRGs. Using FB tracing from the bladder, there was co-localization of beta3-adrenoceptor labeling with the FB-labeled neurons (figure 1).

Figure 1



L6 DRG x400 mag

Blue = FB

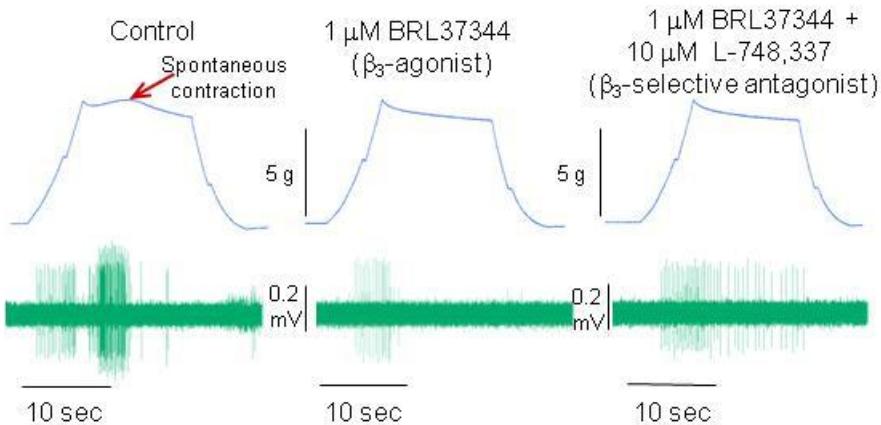
Green = Beta3

Beta3

FB

**Effect of beta3-adrenoceptor agonist on afferent nerve firing:** *In vitro* afferent recordings were obtained from mouse bladder preparations where firing was elicited in response to controlled stretches (figure 2) (3). Using this approach, detrusor relaxation by beta3-adrenoceptor activation and effects on tissue compliance can be determined from tension profiles. At the drug doses utilized, there was no alteration in compliance so the tension profiles were unchanged. In the presence of a beta3-selective agonist, 1 µM BRL37344, there was a marked decrease in stretch-evoked firing rates as compared to controls. The beta3-selective antagonist, 10 µM L-748,337, reversed the effects of BRL37344 and enhanced firing rates in response to stretch, presumably by blocking the action of endogenous noradrenalin.

Figure 2



#### Interpretation of results

Beta3-adrenoceptor agonists decreased afferent firing in response to mechanical distention. As there was also expression of beta3-adrenoceptors on bladder afferent DRGs, it adds further evidence for a direct effect of beta3-agonists on bladder sensory function and that these agents may have multiple sites-of-action to ameliorate overactive bladder symptoms.

#### Concluding message

Beta3-adrenoceptors have shown great promise as a therapeutic agent; however, the mode of action appears to be more complex than just relaxation of detrusor smooth muscle. Further studies of alternative target sites for beta3-agonist should result in a better understanding of the adrenergic regulation of sensory function in the urinary bladder.

#### References

1. Michel M., β-Adrenergic Receptor Subtypes in the Urinary Tract, *Handb Exp Pharmacol.*;202:307-18, 2011
2. McCarthy C., Zabbarova I., Brumovsky P., Roppolo J., Gebhart G., Kanai A., *J Urol.*; 181(3):1459–1466, 2009
3. Kanai A., Wyndaele JJ., Andersson KE., Fry C., Ikeda Y., Zabbarova I., De Wachter S., *Neurorouro Urodyn.*; 2011 (in press)

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<b>Is this a clinical trial?</b>	No
<b>What were the subjects in the study?</b>	ANIMAL
<b>Were guidelines for care and use of laboratory animals followed or ethical committee approval obtained?</b>	Yes
<b>Name of ethics committee</b>	University of Pittsburgh Institutional Animal Care and Use Committee