

## STREPTOZOTOCIN-INDUCED DIABETES INCREASES INTRAVESICAL ATP RELEASE AND NERVE-EVOKED CONTRACTIONS IN THE RAT BLADDER

### Hypothesis / aims of study

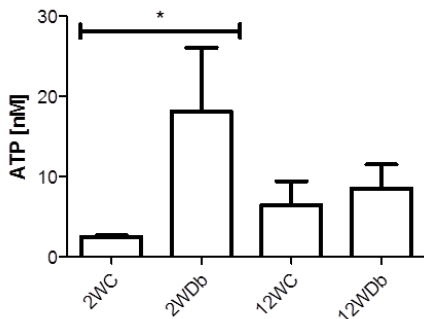
Diabetes mellitus is associated with a number of lower urinary tract complications, with approximately 50% of patients diagnosed with diabetes affected by diabetic bladder dysfunction (DBD), characterized by symptoms ranging from bladder hypercontractility to hypocontractility (1). The variability of symptoms has been attributed to time-dependent changes in bladder function, which maybe at the level of the smooth, innervation and/or urothelium. The urothelium influences bladder function via the release of mediators including ATP and ACh during bladder filling (2), and previous studies have shown time-dependent changes in the receptor expression within the urothelium in diabetes, indicating that urothelial function may be altered (3). The aim of the study was therefore to investigate urothelial mediator release from isolated whole bladders and contractility of bladder strips in the streptozotocin-induced (STZ) diabetic rat model.

### Study design, materials and methods

8 week old Male Wistar-Hannover rats were injected with STZ (65mg/kg, *i.p.*, in 0.01M citrate buffer) to induce diabetes. Controls were age-matched. Bladders were harvested at 2 and 12 weeks following induction of diabetes. Isolated whole bladders were cannulated with a two-way cannula via the urethra and mounted in customised tissue baths containing gassed Krebs bicarbonate solution (95% O<sub>2</sub>/5% CO<sub>2</sub>) at 37°C. Bladders were distended by intravesical infusion of isotonic saline (150 µl/min) and subjected to low (275µl volume) and maximal distension (1.25ml volume). Intravesical contents were collected for measurement of Ach and ATP using commercially available kits (Amplex Red and Luciferin-Luciferase, Molecular Probes). Isolated bladder strips were mounted in tissue baths in Krebs bicarbonate solution. Contractile responses to carbachol and electrical field stimulation (EFS) (1-40Hz, 0.1ms duration, 40V for 5s every 100s) were measured in strips in the presence and absence of urothelium, where half of the strips were denuded by careful dissection under microscopy. All data are expressed as mean ± SEM. Data was compared via ANOVA with Bonferroni post hoc test; P<0.05 was considered significant.

### Results

Intravesical ATP release following maximal distension of isolated whole bladders was significantly greater in bladders from 2 week diabetic animals compared to controls, whilst ATP release was similar from bladders from the 12 week animals (*Fig. 1*). In contrast, intraluminal ACh release was not significantly altered by diabetes of 2 or 12 week duration.



*Fig. 2. Intravesical ATP release in isolated whole bladders from 2 week (2WDb) and 12 week diabetic animals (12WDb) vs controls (2WC, 12WC)(n=5-7, \*p<0.05 2WDb vs. 2WC)*

Isolated bladder strips from 2 week diabetic animals showed a significantly greater contractile response to EFS when compared to controls (*Fig. 2*), whilst there was no significant difference between responses in strips from 12 week diabetic animals and their

controls. Maximum contractions and potency of the muscarinic agonist carbachol were similar between bladder strips from all control and diabetic animals, and contractile responses to carbachol were not significantly affected by removal of the urothelium.

Nerve-evoked contractions to EFS were however reduced by removal of the urothelium, although responses remained significantly elevated in bladder strips from 2 week diabetic animals versus controls at the lower frequencies (<2Hz).

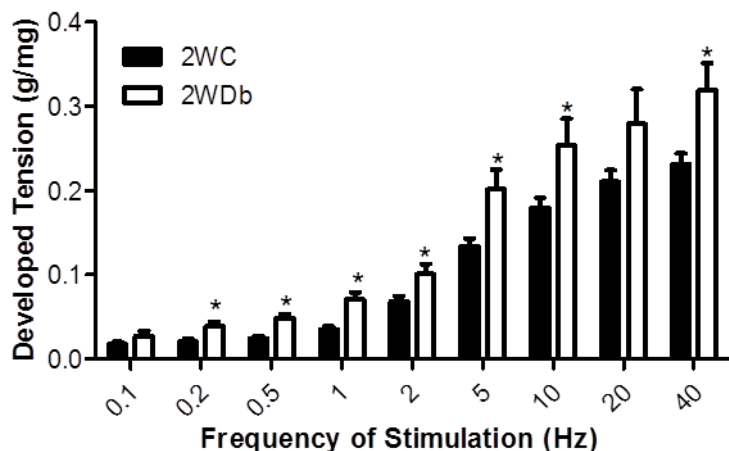


Fig. 3. Nerve-evoked contractions of isolated tissue strips from 2 week control (2WC) and diabetic (2WDb) animals. Frequency response curve (n=5-9), \*p<0.05 2WDb vs. 2WC

#### Interpretation of results

STZ-induced diabetes resulted in increased intravesical ATP release from whole rat bladders at the 2 week time point, but not at 12 weeks. Similarly, nerve-evoked contractile responses to EFS were greater in bladder strips from 2 week diabetic animals, though not 12 week diabetic animals. Cholinergic contractions to carbachol and intravesical release of ACh were not altered by diabetes. In the absence of the urothelium, nerve-evoked responses of bladder strips from diabetic animals remained significantly greater only at lower frequencies of stimulation, which are thought to activate purinergic nerves.

#### Concluding message

Diabetes of 2 weeks duration increased ATP release in the rat bladder and enhanced nerve-evoked contractions, although these changes were not observed at 12 weeks of diabetes. This data supports the evidence for time-dependent changes in bladder function with diabetes, and indicate that altered ATP release from the urothelium may be involved in hypercontractility observed at early time points of diabetes, along with enhanced purinergic mediated responses.

#### References

1. Daneshgari F et al. (2009) J. Urol. 182(6 Suppl), S18-26.
2. Birder L & Andersson KE (2013) Physiol. Rev. 93(2), 653-680.
3. Hanna-Mitchell AT et al. (2013). Am. J. Physiol. Reg. Int. Comp. Physiol. 304(2), R84-93

#### Disclosures

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