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CHANGES IN PURINERGIC AND NITRERGIC SENSORY SIGNALS IN FEMALE RATS DURING EARLY DIABETES

Hypothesis / aims of study

Hypoosmotic or chemical stimulation of the bladder urothelium triggers the release of ATP and nitric oxide (NO) that may activate bladder sensory pathways. Early stage diabetes is characterized by bladder overactivity, suggesting that the afferent limb of the micturition reflex is sensitized. The purpose of this project was to evaluate whether bladder overactivity seen in early stages of diabetes is accompanied by alterations in urothelial release of ATP and NO.

Study design, materials and methods
All animal experiments were carried out in accordance with the National Institute of Health Guidelines for the Care and Use of Laboratory Animals, and were approved by the Institutional Animal Care and Use Committee. Diabetes was induced in female Sprague-Dawley rats with a single 65 mg/kg streptozocin injection. Four weeks later the bladders were isolated and mounted in a perfused organ bath. For chemical stimulation, the stable purinergic agonist alpha-beta methyleneATP (ab-MeATP; 50μM) or the cholinergic agonist carbachol (CCh, 50μM) was applied in the perfusate for 1 min, while for hypoosmotic stimulation the osmolarity of the Krebs solution was decreased by 40 %. The released amount of ATP was determined with a luminometer for ab-MeATP and with HPLC for carbachol and hypoosmotic stimulation. In addition, cystometric measurements were performed in urethane-anesthetized rats to evaluate for bladder overactivity.

Results
Purinergic or cholinergic receptor activation evoked a greater release of ATP in the diabetic than in control strips (370 % and 230 %, respectively, $p<0.05$). However, the release of NO after activation of purinergic (ab-MeATP) or cholinergic (CCh) receptors was similar in diabetic and control conditions. In contrast, hypoosmotic stimulation of the urothelium induced a higher release of ATP (170 %) and a lower release of NO (60%) in diabetic compared to control bladders. During urodynamic studies, diabetic rats exhibited an increase in bladder contraction frequency and decreased compliance as compared to control rats.

Interpretation of results
Purinergic or cholinergic receptor activation triggers a significantly greater release of ATP but not NO in diabetic compared to control bladders. In addition, early diabetes is associated with bladder overactivity during urodynamic studies.

Concluding message
These observations underscore the importance of the purinergic system in bladder overactivity and imply that targeting sensory purinergic receptors with selective purinergic antagonists may ameliorate diabetic bladder symptoms.

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Is this a clinical trial?	**No**
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	**Baylor College of Medicine/Institutional Animal Care and Use Committee**