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STUDY OF THE CONTRACTILE AND RELAXANT ALTERATIONS OF DETRUSOR SMOOTH MUSCLE IN A BOO MICE MODEL: INVESTIGATION OF THE NITRIC OXIDE – CYCLIC GMP – PDE-5 SIGNALING PATHWAY

Hypothesis / aims of study
Benign prostatic obstruction (BPO) represents a clinically significant cause of bladder outflow obstruction in men. Clinical manifestations are related to obstruction of the urethra and bladder dysfunction. Bladder outlet obstruction (BOO) results in an impaired ability of the urinary bladder to store and empty urine. In animal models, BOO results in bladder dysfunction and alterations of the contractile machinery in detrusor smooth muscle (DSM), similar to those found in men with BPO. However, the alterations in the relaxant mechanisms in DSM undergoing BOO have been poorly investigated. Nitric oxide (NO) has been recognized as an important neurotransmitter in the lower urinary tract, where it exerts its actions mainly through activation of soluble guanylyl cyclase leading to accumulation of cGMP levels. Intracellular cGMP is rapidly inactivated to GMP by the activity of phosphodiesterase-5 (PDE-5). The present study aimed to investigate the in vivo (cystometry), as well as the in vitro contractile and relaxant alterations of DSM in a mice model of BOO.

Study design, materials and methods
All animal procedures were approved by the Ethical Principles in Animal Research. C57BL/6 male mice (28-32 g) were anaesthetized with xilazine (30 mg/kg i.p.) and ketamine (2 mg/kg i.p.) for surgical procedure. BOO was performed using nylon ligature in the bladder neck, and animals were used after 5 weeks. Continuous cystometry was carried out by infusing saline into the bladder at a rate of 0.6 mL/h for 50 min in anaesthetized mice. In separate experimental groups, in vitro concentration-response curves to both contractile (carbachol) and relaxant agents (BAY-412272, sodium nitroprussiate and tadalafil) were carried out in DSM with intact urothelium. Contractions to electrical-field stimulation (EFS; 1-32 Hz) were also obtained.

Results
An increased bladder weight was observed in BOO group (122±24 mg, $P<0.05$) compared with Sham mice (51±24 mg). The cystometric showed that BOO mice exhibited an increase in capacity and non-voiding contractions (NVCs) compared with sham animals. The *in vitro* contractile responses to carbachol were significantly lower ($P<0.005$) in BOO compared with Sham group (Emax: 0.61±0.05 and 1.01±0.07, respectively). Similarly, EFS-induced DSM contractions were lower ($P<0.05$) in group BOO compared to Sham, and this difference was maintained even after preparation treatment with atropine (10 □M). DSM relaxations to the sodium nitroprusside (NO donor) and BAY-412272 (NO-independent soluble guanylyl cyclase activator) did not significantly differ between BOO and Sham groups. However, tadalafil-induced DSM relaxations were higher in BOO compared with Sham mice (Emax: 65.5±5.2 and 52.7±4.3, respectively; $P<0.05$).

Interpretation of results
BOO animals had bladder hypertrophy and increase in detrusor activity and instability, characterized by increased NVCs in the cystometry. Functional studies in vitro showed reduced muscarinic- and EFS-induced contractions. No differences for the NO-cGMP signalling pathway in DSM of BOO mice were found. However, the higher relaxant responses to tadalafil may indicate that the cGMP breakdown by PDE-5 is affected by BOO.

Concluding message

The reduced muscarinic-induced and neurogenic-induced DSM contractions in BOO mice suggest an impaired cholinergic and/or purinergic activity in DSM. The greater DSM relaxant responses to tadalafil in BOO mice indicates a potential value for the PDE-5 inhibitors for treatment of BOO complications.

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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	**Brazilian College for Animal Experimentation (COBEA)**