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INFLUENCE OF INTRAVESICAL TROSPIUM CHLORIDE ON THE CHOLINERGIC ACTIVITY OF THE ISOLATED PIG URINARY BLADDER

Hypothesis / aims of study

Overactive Bladder (OAB) is a highly prevalent urological condition which includes the typical symptoms of urinary urgency, increased frequency, incontinence and nocturia [1]. Oral administration of anticholinergic drugs is the common therapy of OAB, which is associated with side effects like mouth dryness, eye accomodation weakness and obstipation amongst others. Direct application of anticholinergica into the bladder is an alternative to reduce these systemic side effects [2]. While instillation of drugs like oxybutinin and trospium chloride has been described [3], no detailed and effective dosage regime for intravesical treatment has been elaborated yet. Moreover, little is known about the detailed fate of the drugs within the bladder tissue when applied intravesically. Our aim was to establish an ex vivo bladder model which can be used to investigate cholinergic action of the bladder in dependence of intravesically (*ivs.*) applied anticholinergic drugs. Here, we show the effects of *ivs.* trospium chloride (TrCl) on the cholinergic activity of isolated pig bladders.

Study design, materials and methods

Pig bladders were obtained from the slaughterhouse and transported in RPMI culture medium at 4°C. Serosal tissue was removed and the uretra were ligated close to the bladder body. The bladders were fitted with a transurethral adapter and an urodynamic 2-channel catheter / pressure transducer to measure *ivs.* pressure differences. They were incubated in an organ bath with carbogen-gassed Krebs buffer (Kb) at 37°C and filled with artificial urine (Griffith) without excess pressure. The organs were stimulated in intervals of 45 min using carbachol extravesically (*evs.*) and relaxed in fresh Kb 10 min after each stimulation. TrCl was applied *ivs.* after stabilization of the responses (3 intervals), followed by up to 10 further stimulation intervals to record the drug-dependent change of the cholinergic response over time until equilibration.

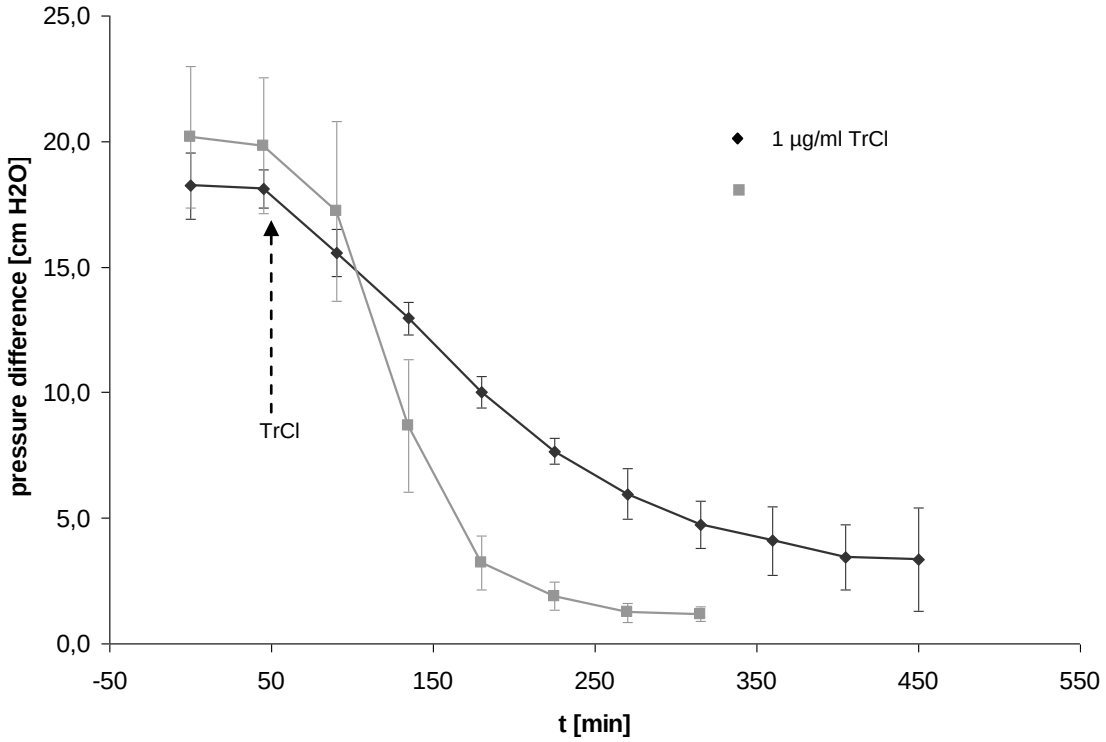

Fig. 1: Effect of *ivs.* TrCl on cholinergic response of isolated pig bladders. The bladders were stimulated with carbachol in intervals of 45 min. The graph shows the *ivs.* pressure differences at each stimulation. TrCl was applied *ivs.* where indicated (arrow). After application of 1 and 5 µg/ml TrCl, the response was reduced to 18.8 ± 7.2 % after 405 min and 6.1± 2.0 % after 270 min, respectively (both n=3).

Results

First, pig bladders were subjected to fatigue experiments. The organs were repeatedly stimulated without any *ivs.* anticholinergic treatment. A slight decrease (approx. 11% of the initial response) was monitored over 9 stimulations (6 hours 45 min in total). In dose/response experiments using carbachol concentrations ranging from 0.16 to 16.00 µM, our model showed a saturating dose-dependent response to carbachol stimulation as expected. For all further experiments, 8 µM carbachol was chosen as suitable stimulation dose. To investigate the influence of *ivs.* TrCl on the cholinergic response, TrCl was applied *ivs.* at concentrations ranging from 1 to 100 µg/ml. A dose-dependent and retarded anticholinergic effect was monitored. At 1 and 5 µg/ml, *ivs.* TrCl reduced the cholinergic response to 18.8 ± 7.2 % (405 min until equilibrium, n=3) and 6.1 ± 2.0 % (270 min until equilibrium, n=3) of the last non-treated signal before drug application, respectively (Fig. 1). As a control, 100 ng/ml of TrCl was applied *evs.* in one of the experiments, which led to an immediate and complete suppression of cholinergic response. A subsequent incubation in Kb containing 80 mM potassium resulted in a signal with approx. 76% of the non-treated cholinergic response.

Interpretation of results

The fatigue experiment showed that our model had a sufficient endurance for experiments lasting several hours. The dose/response assay proved that the model was functional and showed that 8 µM of carbachol was appropriate as working concentration for all further experiments. When TrCl was applied *ivs.*, a dose-dependent and retarded anticholinergic effect was observed. After the drug effect reached equilibrium, there was no further change of cholinergic responses, although the *ivs.* TrCl doses were in a range which would lead to a complete suppression when in direct contact with the detrusor (see control with *evs.* TrCl). We assume that *ivs.* TrCl has a local effect on the urothelium itself, possibly combined with active transport of the drug and secondary signalling leading to inhibition of the detrusor. Compared to clinical studies in which TrCl concentrations of approx. 1 mg/ml were instilled [3], TrCl doses applied in our study were much lower while being clearly effective, supposing that dosages for *ivs.* treatment of OAB might have to be reconsidered.

Concluding message

Our study proves anticholinergic effectiveness of *ivs.* TrCl in isolated whole pig bladder at concentrations ranging down to 1 µg/ml and further shows that isolated - and therefore denervated and non-perfused - bladder is a helpful model for investigating the influence of *ivs.* drugs on cholinergic activity of the bladder. The focus of this model is on local effects within the bladder tissue, because the influence of spinal reflexes is excluded.

References

[1] Urology 2004; 64(6 Suppl 1):2-6
[2] Curr Urol Rep. 2005 Nov;6(6):429-33
[3] Neurourol Urodyn 1999; 18(5) :447-53

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Were guidelines for care and use of laboratory animals followed or ethical committee approval obtained?	**No**
Statement that no ethical approval was needed	**pig bladders were obtained from a slaughterhouse, no laboratory animals**