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 accommodation weakness and obstipation amongst others. Direct application of anticholinergics 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 urodynamical 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.

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 % after 405 min and 6.1 ± 2.0 % after 270 min, respectively (both n=3).

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

<table>
<thead>
<tr>
<th>Specify source of funding or grant</th>
<th>Granted by Arbeitsgemeinschaft für industrielle Forschungsförderung (AiF), Germany: grant no. ZN 09397/05</th>
</tr>
</thead>
<tbody>
<tr>
<td>Is this a clinical trial?</td>
<td>No</td>
</tr>
<tr>
<td>What were the subjects in the study?</td>
<td>ANIMAL</td>
</tr>
<tr>
<td>Were guidelines for care and use of laboratory animals followed or ethical committee approval obtained?</td>
<td>No</td>
</tr>
<tr>
<td>Statement that no ethical approval was needed</td>
<td>pig bladders were obtained from a slaughterhouse, no laboratory animals</td>
</tr>
</tbody>
</table>