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CHOLINERGIC FUNCTIONAL CHANGES IN THE CYCLOPHOSPHAMIDE-INDUCED CYSTITIS OF THE RAT URINARY BLADDER

Hypothesis / aims of study

The contraction of the bladder is predominantly mediated by the stimulation of the muscarinic receptors distributed over the detrusor. Of the expressed muscarinic receptors (M_1-M_5) the muscarinic M_3 receptor has been attributed the major contractile role. A plasticity of the bladder muscarinic receptor population during pathology has been described in several reports. Previous studies have demonstrated that the cholinergic contractile response is also reduced during inflammation. The present study was undertaken to characterise the cholinergic bladder contractions assessed to electrical field stimulation (EFS) and whether changes in the cholinergic function occur during cystitis.

Study design, materials and methods

Male rats of the Sprague-Dawley strain were used. In order to induce cystitis, rats were pre-treated with cyclophosphamide, which is metabolised to acrolein causing a severe inflammation of the bladder wall. The rats were either given cyclophosphamide (100 mg/kg) in combination with the anaesthetic buprenorphinum (10 μg/kg IM) or saline (9 mg/ml serving as controls). 60 hours later the rats were anaesthetised with CO_2 followed by the removal of the urinary bladders. The rats were subsequently killed by CO_2 asphyxiation. Administration of cyclophosphamide resulted 60 hours later in a macroscopically apparent haemorrhagic cystitis in all treated rats. Whole thickness urinary bladder strips (6x2 mm) were excised above the trigonum and proximal to the ureters. The detrusor strip was then mounted between two steel rods where one was fixed and the other adjustable and connected to an isometric force transducer in 25 ml organ baths. The organ baths contained Krebs solution gassed with 5 % CO_2 in O_2 and kept at 37^0 C by a thermo-regulated water circuit and at pH 7.4. The detrusor strip was thereafter pre-stretched which resulted in gradual tension relaxation and was then stretched repeatedly so that a stable tension of around 5 mN was achieved after 60 minutes of equilibrium. In the experimental protocol, electrical field stimulation (EFS) was assessed on the detrusor strip preparations at varying frequencies (between 2 and 60 Hz; 0.8 ms) at supramaximal voltage continuously, until the peak response was obtained. The phasic and tonic component of the EFS-generated contraction were analysed in the absence and presence of muscarinic receptor antagonists with different muscarinic receptor profiles; *i.e.* the non-selective atropine, the "M1-selective" pirenzepine, the "M2-selective" methoctramine and the "M_3/M_5/M_1-selective" 4-DAMP.

Results

The phasic component of the EFS-evoked contraction (2-60 Hz) lasted 0.5-0.9 sec and 0.4-1.2 sec, in which the contraction amounted to 31-49 % and 40-50 % of the composite contraction (phasic + tonic) in inflamed bladder preparations (n=23) and controls (n=22), respectively. The remaining tonic component of the contraction (2-60 Hz) lasted 5.8-9.2 sec in inflamed bladder preparations and 6.7-12.6 sec in controls. In controls, the muscarinic receptor antagonists attenuated concentration-dependently and more potently the tonic than the phasic component of the EFS-generated contractions. On the tonic component, 4-DAMP was the most potent antagonist (pIC_{50} values between 9.1-9.4; EFS 2-60 Hz) compared to methoctramine (pIC_{50}<7.3) and pirenzepine (pIC_{50}<6.7). The phasic part of the EFS-evoked contractions (2-60 Hz) was reduced by the muscarinic receptor antagonists but concentration-dependence was not attained.

In inflamed bladder preparations, the muscarinic receptor antagonists did not affect the phasic component of the EFS-evoked contractions (2-60 Hz) in contrast to controls and were markedly less efficient in inhibiting the tonic component compared to controls. Antagonism on the tonic component was, furthermore, not lineally correlated to concentration. 4-DAMP (0.1 μM) only reduced the contractions at high frequencies (>10 Hz) by no more than 24-33 % ($p<0.01$-0.05; n=8), while pirenzepine (0.1 μM) did not affect the responses at all. In contrast

to controls, methoctramine tended to increase instead of decrease the contractions of the inflamed bladder preparations. However, atropine at a high concentration (5 μM) reduced the composite EFS-evoked response (phasic + tonic) similarly in inflamed bladder preparations and controls by 30-51 % and 37-49 %, respectively.

Interpretation of results

The present results show that the muscarinic M_3 receptors principally mediate the tonic component of the EFS-evoked contraction of the normal bladder. The muscarinic receptors seem, however, to modulate the rapid phasic response as well but to a less extent. During cystitis, the cholinergic function of the urinary bladder is changed. An increased effect on the bladder contraction by prejunctional inhibitory muscarinic receptors possibly of M2/M4 subtype seems to occur in response to inflammation.

Concluding message

The current study shows that during inflammation, besides changes in the afferent nervous input, manifest changes occur as well in the bladder efferent nervous control. The changed parasympathetic control during cystitis occurs principally prejunctionally.

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