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1. Neuroscience and physiology

NITRIC OXIDE AND ANTICHOLINERGIC EFFECTS IN NORMAL AND INFLAMED URINARY BLADDERS IN ANAESTHETIZED RATS

Hypothesis / aims of study

In the current study it was wondered if muscarinic whole bladder contractile responses are affected by cyclophosphamide-induced cystitis in vivo, and further, if nitric oxide influences cholinergic responses differently in the inflamed than in the normal urinary bladder of anaesthetized rats.

Study design, materials and methods

22 male Sprague-Dawley rats (300-350 g) were used. They were anaesthetized with pentobarbitone (45 mg.kg -1 I.P.) followed by supplementary I.V doses when required. The trachea was cannulated and the body temperature was maintained at about 38°C using a thermostatically controlled blanket connected to a rectal thermister. The blood pressure was measured continuously via a femoral arterial catheter. A femoral venous cannula was used for all drug administrations. The urinary bladder pressure was measured continuously via a catheter inserted through a small incision and fixed at the top of the bladder. By injection of small saline volumes the pressure was kept at 10 to 15 mmHg; during stable anaesthesia, the variation of basal bladder pressure was minute.

Methacholine was injected intravenously in successively increasing doses (1 – 5 μg.kg -1 I.V.). Two different protocols were then used. Either a standard dose of methacholine (2 μg.kg -1 I.V.) was given repeatedly before and after administration of the muscarinic antagonist 4-DAMP (4-diphenylacetoxy-N-methylpiperidine) at increasing doses (0.1 – 1000 μg.kg -1 I.V.). Or, the same procedure was performed but in the presence of L-NAME (100 μg.kg -1 I.V.; Nω-nitro-L-arginine methyl ester). To exclude adrenergic effects, α- and β-adrenergic antagonists were given to the rats (phentolamine and propranolol at 1.0 mg.kg -1 I.V.).

Statistical significance was determined by Student's t-test for unpaired and paired data. P values of 0.05 or less were regarded as statistically significant.

Results

Small spontaneous bladder contractions (< ±3 mmHg) occurred throughout the experiments but did not normally induce any micturition. The spontaneous contractions tended to be more pronounced in cystitis than in normal bladders but without eliciting micturition.The bladder pressure was well-maintained over the whole experimental period (2.5 h), except in cyclophosphamide-pretreated animals given L-NAME intravenously (100 μg.kg-1). In the latter animals, the bladder pressure rose steadily (up to 25 mmHg).

Intravenous injections of methacholine evoked dose-dependent contractions in both groups and the increase in bladder pressure was almost identical in cyclophosphamide-pretreated animals and in controls. The time period to peak response was, however, substantially longer in the former group. At 1 μg.kg-1 I.V. of methacholine, the peak was reached within 10 s (5-10 s; n=12) in controls, while it took invariably more than 10 s (10 – 20 s; n=10) in the cyclophosphamide-pretreated animals.

Intravenous injection of 4-DAMP dose-dependently inhibited methacholine-evoked contractions in cyclophosphamide-pretreated animals, irrespective of the presence of L-NAME or not. In controls 4-DAMP inhibited responses only at doses larger than 1 μg.kg-1 I.V. in the absence of L-NAME and only above 10 μg.kg-1 I.V. in its presence. At lower doses, the methacholine-evoked contractions were even increased.

Interpretation of results

The 4-DAMP left-shift of the concentration-response curve of inflamed strip preparations thst has been seen in vitro [1] could be because 4-DAMP inhibits muscarinic receptor induced NO synthesis. In the in vivo experiments, 4-DAMP did not have this effect on methacholine-elicited responses in the cyclophosphamide pretreated animals. On the contrary, a 4-DAMP opposite effect occurred. In the normal rats methacholine-evoked responses were increased in the presence of a low dose of 4-DAMP. One explanation may be that there is an ongoing high production of NO in the inflamed bladder and that the muscarinic receptor-evoked induction is minute and therefore this effect is absent in the cyclophosphamide-pretreated rats. In normal rats, no production normally occurs, except when the muscarinic receptor is stimulated, and consequently, the methacholine-evoked response is increased in the presence of a low 4-DAMP dose. This low dose thus primarily affects the NOS (nitric oxide synthesis) coupled receptor, while a higher dose is required for affecting the muscarinic M3 contractile receptor. The observation that the bladder pressure rose steadily in cystitis, and not in normal bladders, after NOS blockade supports this tentative assumption.

Concluding message

In conclusion, the bladder pressure increase in response to muscarinic stimulation seems to remain in the state of cystitis. However, the muscarinic M1/M3/M5 receptor antagonist 4-DAMP shows a different inhibitory pattern in the cyclophosphamide-pretreated rats than in normal rats.

Reference

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