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EFFECTS OF 4-DAMP ON CHOLINERGIC MECHANISMS ACTIVATING PHASIC ACTIVITY IN THE ISOLATED BLADDER.

Hypothesis / aims of study

The isolated guinea pig bladder generates spontaneous, co-ordinated activity resulting in transient rises in intra-vesical pressure which can be augmented by the muscarinic agonist arecaidine [1]. Our aim is to examine the effects of 4-DAMP (a relatively selective M_3 antagonist) on this activity in an attempt to investigate different components of the complex responses that may represent different physiological systems operating in the bladder wall.

Study design, materials and methods

Bladders were isolated from female guinea pigs (n=6), cannulated per urethra and placed in Tyrodes solution. Drugs were added were added directly to the solution bathing the bladder to achieve final dilution. 4-DAMP was added followed by 1000nM arecaidine. Initial maximal frequency (IF_{max}) following addition of arecaidine, frequency at steady state (f_{ss}), maximal underlying contraction (uc_{max}) and underlying contraction at steady state (uc_s) were assessed.

Results

Immediately after application of arecaidine a burst of activity is seen comprising of high frequency, low amplitude transients associated with an increase in underlying basal pressure. Following prolonged exposure the frequency and basal pressure decrease to a 'steady state'. With pre-exposure of the bladder to 4-DAMP maximal underlying contraction and initial maximal frequency were significantly reduced by small doses of 4-DAMP: 0.3nM 4-DAMP reduced the initial maximal frequency by 50%. However, underlying contraction and frequency at steady state required larger doses to have affect (10nM). (table; mean (1 s.d.)), *p<0.05).

	Control	0.3nM	1nM	3nM	10nM
IF_{max} (Hz)	0.14 (0.01)	0.11* (0.01)	0.10* (0.007)	0.08* (0.02)	0.06* (0.01)
uc_{max} (cmH20)	4.45 (1.05)	2.80* (0.49)	2.24* (1.03)	1.81* (1.55)	0.19* (0.08)
f_{ss} (Hz)	0.04 (0.008)	0.04 (0.004)	0.04 (0.006)	0.04 (0.008)	0.03* (0.008)
uc_{ss} (cmH2O)	0.87 (0.18)	0.72 (0.46)	0.56 (0.41)	0.41 (0.41)	0.03* (0.044)

Interpretation of results

This analysis suggests that distinct cholinergic mechanisms exist contributing to complex contractile activity within the isolated bladder. Two components are seen consisting of an 'initial' response associated with a burst of activity and a 'latent' response as the preparation approaches a steady state. Mechanisms involved in the initial response to muscarinic stimulation are sensitive to 4-DAMP, suggesting this component acts through M_3 receptor mediation. Those involved at steady state are either less sensitive or mediated via alternative receptors (such as M_2).

Concluding message

Such experiments may give insight into new relative roles for M_3 receptors within the bladder as well as highlighting the role of integrative physiology in the identification of further potential targets for antimuscarinic drugs.

1. Exp Physiol 2003; 88: 343-57.

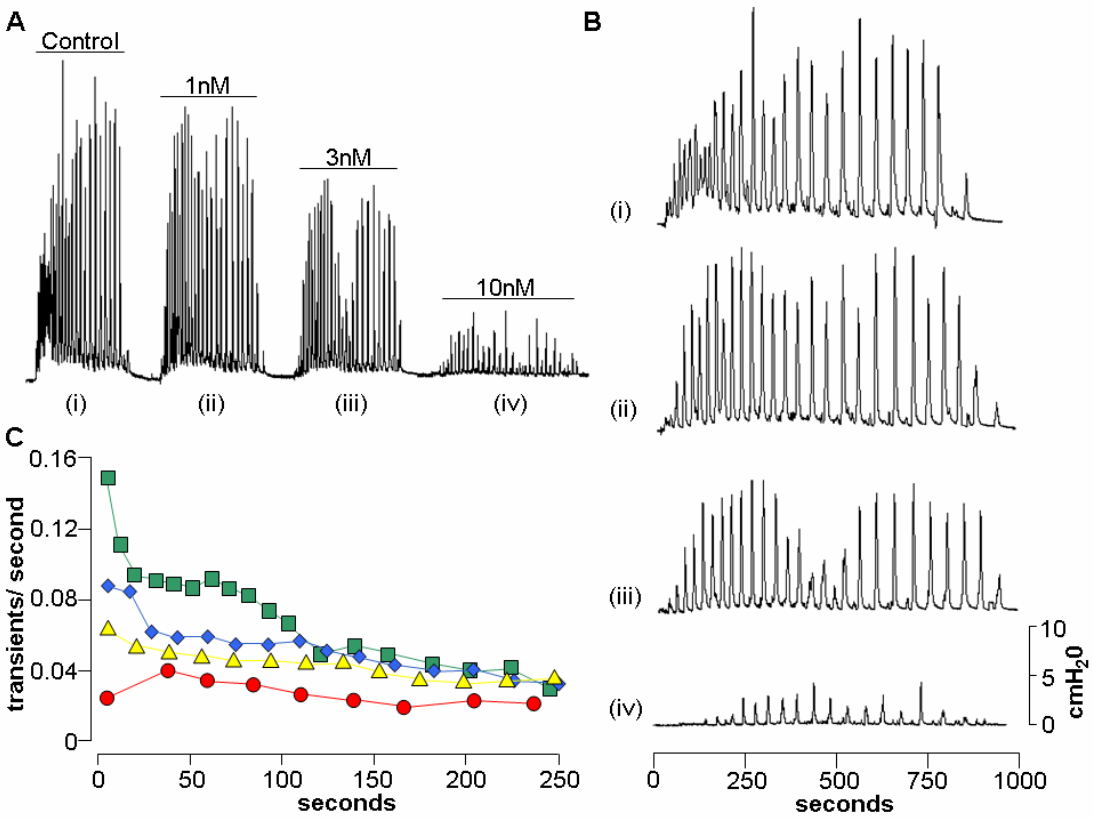

Figure 1: Dose dependent actions of the M_3 specific antagonist 4-DAMP on an isolated guinea pig bladder in the presence of 1000nM arecaidine. A shows the effects resulting from adding 1000nM arecaidine to the bathing solution in the presence of different concentrations of 4-DAMP. Between arecaidine applications the bladder was washed in Tyrode's solution containing the next incrementally increased dose of 4-DAMP. B shows the records illustrated in A for each concentration of 4-DAMP on an expanded time scale. C shows the instantaneous frequency plot for the first 250 seconds following addition of arecaidine in the presence of doses 0 - 10nM 4-DAMP, (control (■), 1 nM (♦), 3 nM (▲) and 10 nM (●)). These illustrate the different components of the responses. At low doses, 1nM, the most obvious action is the reduction in frequency during the initial phase. At higher concentrations, 3nM and 10nM, there is a reduction in amplitude and underlying pressure in both the initial phase and at steady state. In panels A and B the ordinates show pressure in cm H_2O and abscissae time in seconds. Panel C ordinate, frequency (transients/ second) and abscissa, time (seconds).

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