250

McMurray G[1], Piper-Brown S[1], Lee A[2]
1. Pfizer Global Research and Development, 2. Kings College, London

ALPHA ADRENOCEPTOR INDUCED POTENTIATION OF PUDENDAL NERVE EVOKED INTRAURETHRAL PRESSURE RISES IN AN IN VITRO WHOLE URETHRAL PRESSURE MODEL

Hypothesis / aims of study

Although many investigations have highlighted the importance of alpha1 adrenoceptors in the contractile activity of urethral smooth muscle, little work has focussed on the possible influence of adrenergic agonists on urethral striated muscle activity. The present study aimed to investigate the functional role of alpha-adrenoceptor agonism on the contractile activity of both smooth and striated urethral muscle, utilising a novel *in vitro* rat whole urethra with intact pudendal nerve preparation.

Study design, materials and methods

Female Sprague Dawley (CD) rats weighing 200g-250g were euthanased (Schedule1), the abdominal cavity was opened through a midline incision and the whole lower urinary tract, from the ureters to the external urethral meatus, was removed en bloc with intact, full-length pudendal nerves. The urethra was cannulated proximally through an incision in the bladder base and distally at the urethral meatus. The tissue preparation was mounted in a 40ml jacketed organ bath perfused with Krebs' Ringer solution (2.5 mM Ca^{2+}, 37^0C) at a flow rate of 5 ml/min. Both cannulae were attached to a pressure transducer for isovolumetric recording of intraurethral pressure. Tension along the length of the urethra was maintained by attaching the proximal cannula to an isometric tension transducer and securing the distal cannula to the base of the organ bath. The urethra was tensioned to 0.5g and filled with Krebs' solution via the proximal cannula until intraurethral pressure of the closed system was maintained at $\approx$ 100 mmH_2O and left to equilibrate for 1hr. Electrical field stimulation (EFS) was delivered to the pudendal nerves via 2 platinum ring electrodes in the form of single pulses (0.1ms pulse width, 30V, 0.25Hz). The contractile activity of the urethral striated muscle in response to pudendal nerve stimulation, and of circular smooth muscle in response to drug application, was represented as phasic intraurethral pressure rises and changes in baseline pressure respectively. For the purposes of data analysis, pudendal nerve evoked stimulation was taken as the average peak pressure response over 5 stimulations. For baseline pressure changes the average baseline pressure was recorded over a 2sec period and the mean of 5 readings within a 20sec period calculated. Agonists and antagonists were added cumulatively to the bath with the bath closed to the pump, responses being allowed to stabilise before subsequent addition. Data is expressed as the mean $\pm$ SEM, n represents sample size. For each individual tissue responses were expressed as a percentage of the maximum (100%) agonist-induced response in that tissue, sigmoid curves were then fitted and EC_{50}s derived. For control experiments or experiments where little effect with agonist was achieved, changes were tested for significance using Students t test (paired).

Results

The α2 adrenoceptor agonist UK14304 had no effect on either baseline pressure or pudendal nerve evoked responses (n=4). In contrast the alpha1 adrenoceptor agonist phenylephrine caused a concentration dependent (10nM-100μM) increase in both baseline intraurethral pressure and pudendal nerve evoked phasic pressure rises by 49.0 ± 9.7% and 108.8 ± 15.4% of control values with EC_{50} values of 2.03 ± 0.25μM and 1.54 ± 0.23μM respectively (n=8). The $alpha1_{A/L}$ selective agonist A-61603 caused a concentration dependent (0.3nM-3μM) increase in both baseline urethral and pudendal nerve evoked pressure of a similar magnitude to phenylephrine with EC_{50} values of 52.5 ± 2.6nM and 20.0 ± 3.1nM respectively (n=6). Application of a sub-maximal concentration of A-61603 (300nM) induced a sustained increase in both baseline pressure and pudendal nerve evoked responses, subsequent application of the $alpha1_{A/L}$ selective antagonist 5-methyl-urapidil (0.3nM-3μM) in the continued presence of A-61603 caused a concentration dependent decrease in both baseline and pudendal nerve evoked activity to control values. The IC_{50} values for these effects were

6.3 ± 1.4nM and 9.7 ± 2.1nM respectively (n=5). A-61603 induced responses were also inhibited by the alpha1$_{A/L}$ selective antagonist RS100329 (n=1).

Interpretation of results

In the present study phenylephrine and A-61603 caused concentration-dependent increases in neuronally evoked contractions of urethral striated muscle and induced contraction of smooth muscle. 5-methyl-urapidil and RS 100329 inhibited these agonist-induced responses. The high potency of A-61603 (100-fold) relative to phenylephrine, and its EC_{50} recorded in the present work suggests the presence of alpha1$_{A/L}$ adrenoceptors. Similarly the antagonist potencies measured in this study lead to the conclusion that the alpha1$_{A/L}$ adrenoceptor is the predominant subtype involved in the responses examined in this study.

Concluding message

The present results suggest that in the rat urethra, alpha1$_{A/L}$ adrenoceptors are involved not only in maintaining/increasing urethral tone through activation of urethral smooth muscle, but that alpha1$_{A/L}$ adrenoceptor activation may also increase the activity of urethral striated muscle. Whether this effect is due to activation of the muscle itself, e.g. through some form of calcium sensitisation, or is a result of presynaptic modulation of acetylcholine release is a matter for further investigation. These results suggest a further mechanism of action and greater potential for the use of alpha1$_{A/L}$ adrenoceptor agonists in the treatment of stress urinary incontinence.