72

Granato C[1], Korstanje C[1], Rouget C[2], Lluel P[2], Palea S[2], Gillespie J[3]
1. *Astellas Pharma Europe B.V.,* ***2.*** *UROsphere S.A.S.,* ***3.*** *Urophysiology Research Group, The Medical and Dental School, Newcastle University*

MODULATION OF PGE2-AUGMENTED MICRO-CONTRACTIONS BY THE NON-SELECTIVE B-ADRENOCEPTOR AGONIST ISOPRENALINE IN ISOLATED STRIPS OF RAT URINARY BLADDER

Hypothesis / aims of study

It has long been recognized that prostaglandins are involved in the physiology and pathology of the urinary bladder. Particularly prostaglandin E_2 (PGE_2) is thought to play a role in detrusor overactivity (DO) and it has been hypothesized that the key symptoms of overactive bladder (OAB) such as frequency and urgency often related to DO, may be correlated with the PGE_2 presence into the bladder. Indeed, outlet obstruction is associated with increased levels of PGE_2 production [1], while the intra-vesical instillation of PGE_2 increases micturition frequency [2]. How PGE_2 mediates its actions in the bladder still remain unclear and there is a growing interest to its potential in the regulation of micro-contractions as the motor-sensory component that drives the afferent branch from the bladder.

β-adrenoceptor (AR) agonists contribute to urine storage by relaxing the detrusor muscle [3] and mirabegron, a selective β_3-AR agonist, has recently been approved for treatment of OAB. However, this might not be the single mode of action of this drug since at therapeutic doses mirabegron has a modest relaxant effect on detrusor contractility. In search of alternative mechanisms of action, it has been proposed that β_3-AR agonists may influence bladder sensation via a direct effect on the motor component of motor/sensory noise.

The present study was designed (i) to explore the actions of PGE_2 on micro-contractions of isolated strips of rat bladder and (ii) to investigate the modulation of PGE_2 effects by the non-selective β-adrenoceptor agonist isoprenaline.

Study design, materials and methods

Female Sprague-Dawley rats were sacrificed by carbon dioxide asphyxia. Longitudinal bladder strips (n=12) were suspended in organ baths, equilibrated under a resting tension of 5-10 mN for at least 60 min and washed with oxygenated Krebs solution (5% CO_2/95%O_2 at 37^{0}C) every 10 min. Strips were then subjected to EFS single stimulation (5 sec, 15 Hz) to confirm their viability. PGE_2 (10 µM) was added to the bathing solution to determine its effects on motor noise, then a cumulative concentration-response curve to isoprenaline (0.01-10 µM) was then carried out by adding the agonist to the organ bath in the presence of PGE_2.

Results

PGE_2 induced a concentration-dependent smooth muscle contraction as well as an increase of amplitude and frequency of micro-contractions (motor 'noise'). The maximal effect was obtained at 10 µM.

Figure 1A illustrates the effects of PGE_2 (10 µM) on a rat bladder strip. On application of PGE_2 there is an initial transient rise in basal tension. This is accompanied by a rise in the frequency of micro-contractions of motor 'noise'. After approximately 100 seconds the pattern of the 'noise' changes with a progressive increase in the amplitude and decrease in frequency. Figure 1B illustrates the PGE_2-augumented 'noise' complexity. On close examination the recording appears to be made up of large slow contractions superimposed by rapid small contractions.

Figure 1C illustrates the cumulative effects of isoprenaline on the micro-contractions increased by 10 µM PGE_2. As the concentration of agonist is increased (10^{-8} - 10^{-5} M) the 'noise' is reduced.

Figure 2 shows the slow waves and the transients rapid contractions induced by PGE_2 (panel A). At 10^{-7} M isoprenaline the slow waves are reduced in amplitude possibly as a result of a reduction in the number of small events contributing to the slow events (panel B). At the highest concentration of isoprenaline used (10^{-6} M, panel C) the waves are abolished and the frequency of the rapid transients dramatically slowed.

Figure 1

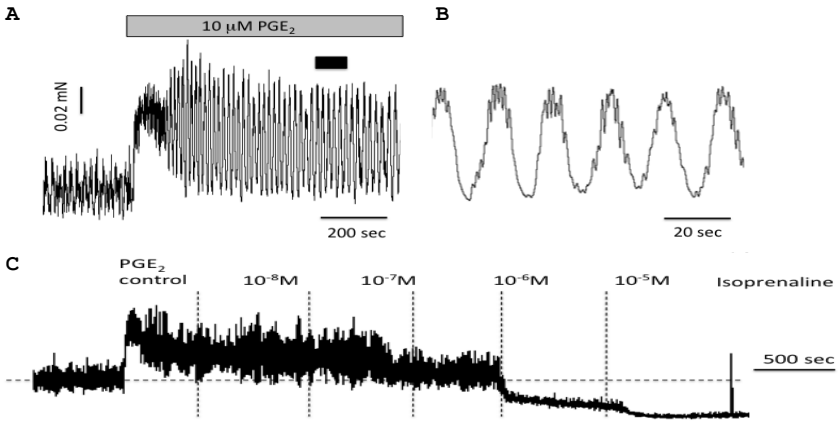
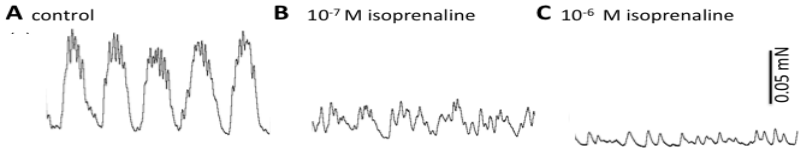


Figure 2



Interpretation of results

PGE_2 has shown to modulate the motor activity in urinary bladder strips thereby generating complex micro-contraction responses. The nature of the processes generating this complexity is, as yet, poorly understood. PGE_2-augmented 'noise' apparently is sensitive to β-adrenergic inhibitory stimulation. This may point at PGE_2 as an augmentor of motor 'noise' in DO and β-AR stimulated inhibition as a mechanism to reduce DO. It is tempting to speculate on the relevance of these results in the light of new treatment paradigm of OAB with mirabegron. Further studies are needed in the rat isolated urinary bladder to investigate which β-adrenoceptor subtype (β_1, β_2 and β_3) mediates the effect of isoprenaline on the motor 'noise'.

Concluding message

This study suggests that one possible mechanism of action of PGE_2 and non-selective β-AR agonist isoprenaline is the modulation of the motor-sensory system that contributes to the afferent outflow from the bladder. The inhibition of PGE_2-augmented motor 'noise' by isoprenaline may have consequences for the understanding of the therapeutic site of action of β_3-AR agonists for the treatment of OAB in man, but clearly requires additional focussed mode of action studies.

References

1. Gillespie JI et al., (2012). Modulation of non-voiding activity by the muscarinergic antagonist tolterodine and the ß3 agonist mirabegron in conscious rats with partial outflow obstruction. BJU 110, E132-E142.
2. Ishizuka et al., (1995). Prostaglandin E2-induced bladder hyperactivity in normal conscious rat: involvement of tachykinins? J urol 153: 2034.
3. Andersson KE (1993). Pharmacology of the lower urinary tract smooth muscle and penile erectile tissues. Pharmacol Rev 45:253-308.

Disclosures

Funding: FP7 TRUST funding (Research fellowship to Granato C.) **Clinical Trial:** No **Subjects:** ANIMAL **Species:** Rat **Ethics Committee:** Midy Pyrenees Ethical Committee