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THE EFFECTS OF EXOGENOUS PROSTAGLANDINS AND THE IDENTIFICATION OF CYCLO-OXYGENASE IMMUNO- REACTIVE CELLS IN THE NORMAL GUINEA PIG BLADDER.

Hypothesis / aims of study

The bladder is capable of synthesising prostaglandins (PGs). Both the mucosa and muscle layers of the bladder are reported to be capable of synthesising and releasing PGs in response to distension and to inflammation (1-2). It is now known that there are two enzymes responsible for PG production: cyclo-oxygenase I (COX I) and cyclo-oxygenase II (COX II). The purpose of this study was to investigate the effects of exogenous prostaglandins on small transient rises in pressure (autonomous activity) of the isolated whole bladder and to determine by immuno-localisation of COX I and COX II which cells are producing endogenous prostaglandins.

Study design, materials and methods

In total 15 guinea pigs were used. 7 animals were used for immuno-histochemistry and 8 for physiological measurement.

The physiological experiments were done on whole isolated bladders. A cannula was inserted into the urethra to monitor intra-vesicle pressure and the bladder was suspended in a heated chamber containing carboxygenated Krebs solution at 33-36^{o}C. Prostaglandins (PGE_1, PGE_2, $PGF_{2\alpha}$ and arachadonic acid) were added to the solution on the ablumenal bladder surface.

For the structural studies, sections of the lateral wall of the bladder were incubated in a Krebs solution (36^{o}C) containing isobutyl-methyl-xanthene (1mM) and the nitric oxide donor NONOate (0.1mM) to stimulate cyclic guanosine monophosphate (cGMP) formation. Tissues were then fixed in 4% paraformaldehyde and processed for immunohistochemistry. cGMP, COX1 and COX2 were visualised using appropriate primary and secondary antibodies.

Results

In the resting bladder small transient rises in pressure can be recorded: autonomous activity
(Figure 1). Exposure to PGE_2 (3 nM to 300 nM) resulted in an increase in basal pressure and an increase in the amplitude and frequency of the autonomous activity (Figure 1). The changes in autonomous activity were dependent on the concentration of PGE_2. After a brief exposure (240 sec) to PGE_2 the augmentation of the autonomous activity continued for over 60 minutes despite regular washing. Similar responses were seen with PGE_1 but $PGF_{2\alpha}$ and arachadonic acid evoked smaller effects. The augmented activity was reduced by the EP1/EP2 receptor blocking agent (AH6809 10uM)

Using a polyclonal antibody to the 70 kD constitutive active form of the enzyme cyclo-oxygenase (COX I), COX I immuno-reactivity (COX I-IR) was located to cells in the basal urothelium, in lamina propria and on the surface of the inner muscle bundles. There were few COX I-IR cells associated with the outer muscle bundles which were surrounded by cGMP positive cells. The COX I-IR cells lying within the lamina propria were distinct from the sub-urothelial cells which respond to nitric oxide with a rise in cGMP. The lamina propria COX I-IR cells appeared to form a network surrounding muscle trabeculae within the inner muscle layer. COX II-IR was associated with the nuclei of cells in the urothelium, lamina propria and muscle. Both COX I-IR and COX II-IR were removed by pre-incubation of the antibody with the peptides against which they were raised.

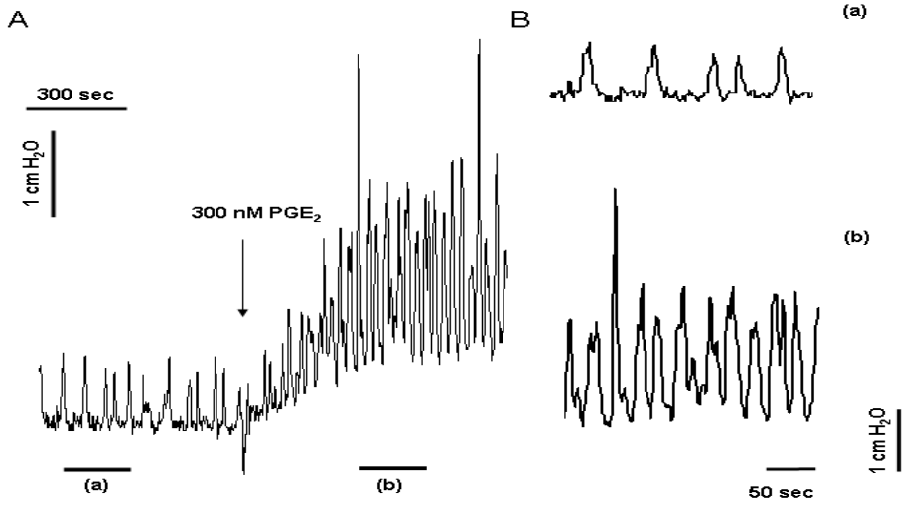

Figure 1. The effect of PGE_2 (A) on autonomous activity in the isolated whole bladder of the guinea pig. Two sections of this record are also shown in B. B (a) shows autonomous activity before application of PGE_2 while (b) is during the application of the agent.

Interpretation of results

These data demonstrate that prostaglandins play a role in regulating autonomous activity in the isolated bladder. Potential sources of endogenous prostaglandin are identified. It is not clear how the prostaglandins produced by these cells alter autonomous activity. There may be a direct activation of the muscle by prostaglandins released by the network of interstitial cells. It is also possible that prostaglandin released from the urothelium may influence phasic contractile activity via the networks of COX I-IR interstitial cells.

Concluding message

Our results suggest that endogenous prostaglandins play a role in regulating autonomous activity in the isolated bladder. The possible roles and importance of this system for the physiology and patho-physiology of the bladder remain to be elucidated.

References

1. Br. J. Urol (1987) 59; 36-39.
2. J. Urol (1987) 137; 577-582.

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