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THE FIRST INDICATION OF PHOSPHODIESTERASE TYPE 2 PRESENCE IN THE GUINEA PIG BLADDER

Hypothesis / aims of study

Phosphodiesterase inhibitors type 5 (PDE5i), have recently been suggested to have positive effects on the treatment of LUTS and DO [1,2]. Besides the well known PDE5 inhibitors, PDE2 selective inhibitors (PDE2i) have recently become available for clinical studies, showing positive effects on memory function. Provided that the PDE2 enzyme is expressed in the bladder, the study of the effect of PDE2i on bladder function would be very interesting. Antibodies against PDE2 are not available and the distribution of PDE2 in the bladder has never been demonstrated prior to this study.

Study design, materials and methods

Since antibodies to PDE2 enzyme are not available, we used a novel indirect technique, to study the localisation of PDE2, by visualizing the product of inhibition of PDE2, namely cGMP. Bladders of 12 guinea pigs, were dissected, and treated in wells containing 2 ml Krebs' solution and 1 µM of the PDE-2 selective inhibitor Bay 60-7550, at 36°C for 30 min. Tissues were then stimulated with 100 µM of the Nitric oxide (NO) donor (diethylamine-NONOate) for 10 min. The tissues were then snap frozen and 10 µm sections cut. Sections were examined for cGMP immuno-reactivity and co-stained for a non-specific nerve marker (PGP9,5).

Results

Comparing bladder sections of NO stimulated guinea pig bladders and bladders treated with a PDE2i after NO stimulation, we were able to study the site of action of the PDE2i in the guinea pig lateral wall.
As shown in Figure 1A, PDE2i inhibits cGMP breakdown the most in the, urothelial and suburothelial layers as well as the nerve fibers. In the outer muscle layers of lateral wall, cGMP is mainly expressed in the intermuscle interstitial cells and the nerve fibers, indicating the presence of PDE 2 enzyme activity (fig. 1B).

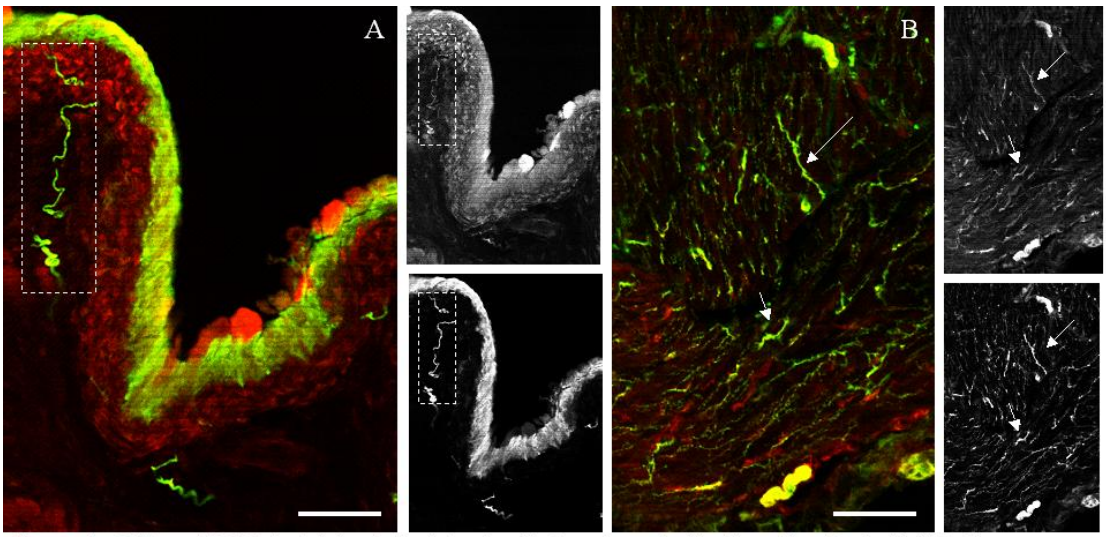

Figure 1. cGMP and PGP 9,5 staining in the lateral wall of a guinea pig bladder, stimulated with NO after pre-incubation with a 10^{-6} concentration of a selective PDE-2 inhibitor (Bay 60-7550).

In panel A, The urothelium and suburothelial layers are shown. cGMP is stained in red. cGMP is mainly expressed in the umbrella cells of the urothelium. But also in the basal and intermediate layers of the urothelium, the suburothelial interstitial cells and a nerve fibre indicated by the dotted box, indicating the presence of PDE2i.
A non-specific nerve marker PGP 9.5 is stained in green. The basal and intermediate urothelial level as well as the indicated nerve fibre are PGP9.5 positive. The yellow colour is caused by the co-localisation of cGMP (red) and PGP 9.5 (green).The black and white images show the individual cGMP (top panel) and PGP 9.5 stainings (Bottom panel). Note that the nerve fibre indicated by the dotted box is both cGMP and PGP9.5 positive. Calibration bar 50 µm.

In panel B, the outer muscle layer is presented. PGP 9.5 is stained in green and cGMP in red. The arrows point to the nerve fibres co-localising cGMP and PGP9.5, indicating that the nerve fibres express the PDE2i. The black and white images show the individual cGMP (top panel) and PGP 9.5 stainings (Bottom pane) Calibration bar 50 µm.

Interpretation of results

Our study shows the distribution of PDE2 in the bladder. PDE2 is shown to be present in the urothelium and bladder muscle layers of the guinea pig. PDE2 activity is mainly located on umbrella cells and interstitial cells of the suburothelium. In the outer muscle, Both the interstitial cells and some nerve fibers show PDE2 activity. This observation suggests the involvement of the NO-mediated cGMP system in the regulation of bladder activity, both through interstitial cells as well as through the nervous system.

Concluding message

Knowledge of the presence and the location of the PDE2 activity in the bladder is the first step towards physiological experiments required for studying the possible role of PDE2i in modulating bladder activity and possibly in the patho-physiology of urgency symptoms as in the overactive bladder syndrome.

References

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Specify source of funding or grant	**1. Mozaïek research grant to M.S. Rahnama'i M.D. by the Netherlands Organisation for Scientific Research (NWO). 2. The BJU International Collaborative Research award 2007**
Is this a clinical trial?	**No**
What were the subjects in the study?	**ANIMAL**
Were guidelines for care and use of laboratory animals followed or ethical committee approval obtained?	**Yes**
Name of ethics committee	**Maastricht University Animal Ethical Committee.**