

## PHOSPHODIESTERASE TYPE 2 (PDE2) EXPRESSION ON NERVES AND INTERSTITIAL CELLS OF THE GUINEA PIG URINARY BLADDER

### Hypothesis / aims of study

Detrusor overactivity (DO) is a prevalent condition for which truly effective drug therapy is lacking. The study of PDE2 enzyme expression in the bladder, could offer new perspective in treatment of DO.

### Study design, materials and methods

Six male guinea pig bladders were dissected and treated in 2 ml Krebs' solution and 10  $\mu$ M of the specific PDE2 inhibitor Bay-60-7550 at 36°C for 30 minutes. After stimulating tissues with 100  $\mu$ M of the NO donor diethylamine-NONOate for 10 min, the tissues were snap frozen and cut in 10  $\mu$ m sections. Sections were examined for cGMP immune-reactivity, co-stained with either vimentine, synaptic vesicle protein 2 (SV2), calcitonin gene related protein (cGRP) and protein gene product 9.5 (PGP9.5), using the two-step indirect immunohistochemistry technique.

### Results

Comparison of NO stimulated, guinea pig bladder sections with bladders treated with a PDE2i after NO stimulation, showed that PDE2 inhibits cGMP breakdown the most in urothelial and suburothelial layers as well as on nerve fibres. In the outermuscle layers of lateral wall, cGMP is mainly expressed in the intermuscle interstitial cells and the nerve fibres, indicating the presence of PDE 2 enzyme activity. In figure 1, the coloured panel shows a high power image of the urothelium of the lateral wall of a guinea pig bladder stained with the antibody for cGMP (red) and vimentin (green). The bladder lumen (LU), the urothelium (UR) and the suburothelial layer (SU) are marked. The different layers of the urothelium can be noted. The outer layer stains positive for cGMP. The black and white panels show the individual images of cGMP, and vimentin respectively.

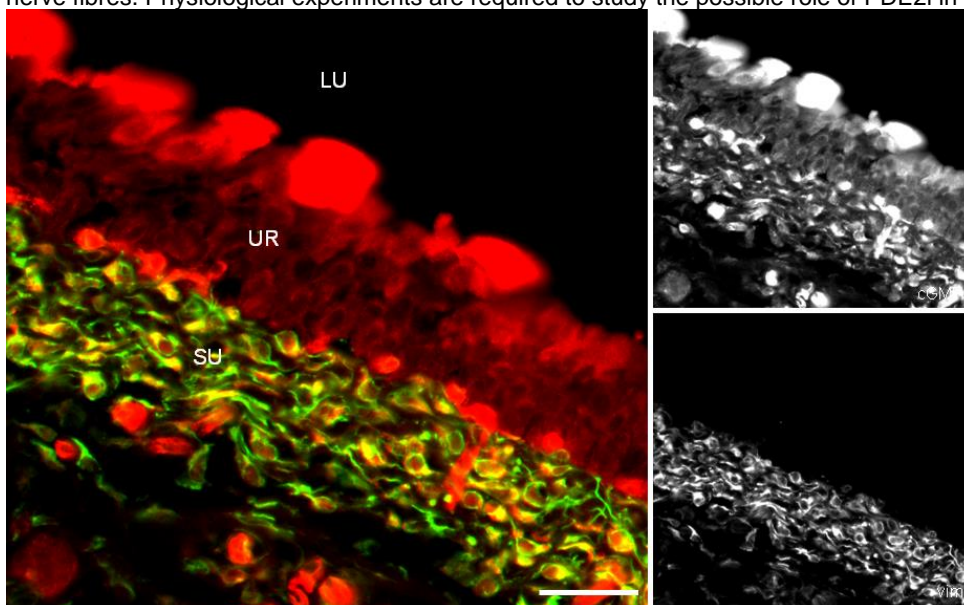
Figure 2 shows a high power image of nerves of the lateral wall of a guinea pig bladder stained with the antibody for cGMP (red), cGRP (green) and Hoechst (blue). In Panel A, a nerve of the urothelium is shown, staining for both, cGRP and cGMP. Panel B shows a nerve of the urothelium which is cGRP positive but doesn't stain for cGMP. In Panel C, a cGMP and cGRP positive nerve is shown, which is located in the outer muscle. Panel D shows a cGRP positive nerve of the outer muscle which is cGMP negative. However, a subpopulation of cGRP positive nerves are cGMP negative, most of them are cGMP positive. The black and white panels show the individual images of cGMP and cGRP, respectively.

### Interpretation of results

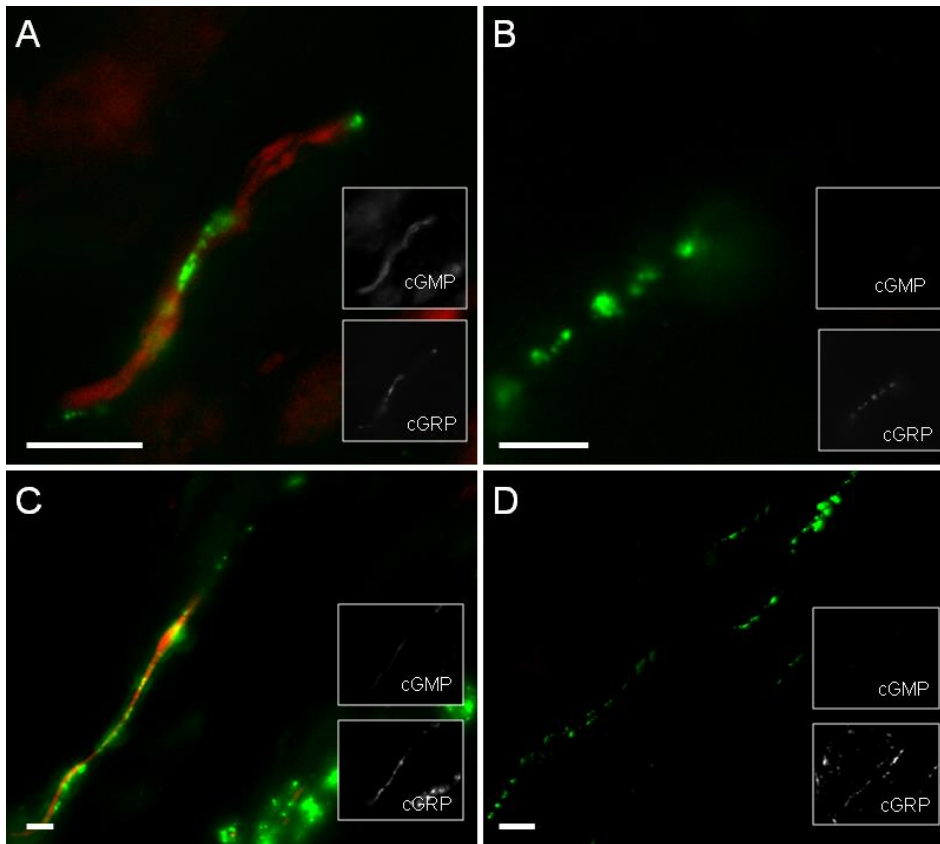
PDE2 is present at several sites. In the urothelium PDE2 is shown to be present in the umbrella cells and the basal layer of the urothelium. The suburothelial layer also contains PDE2. It is located in the interstitial cells and the endothelium of blood vessels. In the inner and outer muscle PDE2 has been shown in the muscle bundles and between them. Furthermore, several nerve types contain PDE2.

### Concluding message

This study is the first to show the distribution of PDE2 in the bladder. PDE2 is present in the urothelium and bladder muscle layers of the guinea pig, mainly located on umbrella cells, interstitial cells of the suburothelium and the outermuscle, as well as in the nerve fibres. Physiological experiments are required to study the possible role of PDE2i in modulating bladder activity.



**Figure 1.** The distribution of cGMP and vimentin after PDE2 inhibition, in the urothelium of the lateral wall of the guinea pig bladder at a higher magnification. Calibration bar: 25  $\mu$ m



**Figure 2. The distribution of cGMP and cGRP after PDE2 inhibition, in the nerves of the urothelium en outer muscle of the lateral wall of the guinea pig bladder**  
 Calibration bars: 10  $\mu$ m

Disclosures

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