

ALTERATIONS TO THE NETWORK OF CYCLIC GUANOSINE MONOPHOSPHATE RESPONSIVE INTERSTITIAL CELLS INDUCED BY OUTLET OBSTRUCTION IN THE GUINEA PIG BLADDER.

Hypothesis / aims of study

Bladder outflow obstruction can result in symptoms of urgency and frequency. Little is known about the origins of pathological bladder activity or altered sensation. Interstitial cells (ICs) may play a role in regulating normal bladder activity (1,2). Alterations to the number and distribution of ICs may contribute to the development of the overactive bladder syndrome (1). The purpose of this study was to explore the possibility that the sub-urothelial and muscle networks of cyclic guanosine monophosphate (cGMP) responsive ICs are altered in animals with a bladder outlet obstruction (BOO).

Study design, materials and methods

In total 14 male guinea pigs (266-299g) were used. In 8 animals a silver ring (1.8 internal diameter) was placed around the proximal urethra and 6 animals underwent a sham operation. Four weeks after the operation the animals were sacrificed. The bladder weights of sham operated and obstructed animals were 400 ± 73 mg and 1568 ± 500 mg, respectively. Sections of the lateral wall of the bladder were incubated in a Krebs solution (36°C, gassed with 95% O₂ and 5% CO₂), containing the non-specific phosphodiesterase inhibitor isobutyl-methyl-xanthine (1mM) and the nitric oxide donor NONOate (0.1mM) to stimulate cGMP formation. Tissues were then fixed in 4% paraformaldehyde and processed for immunohistochemistry. cGMP, neuronal nitric oxide synthase (nNOS) and the non-specific nerve marker PGP 9.5 were visualised using appropriate primary and secondary antibodies. The method for detecting cGMP in tissue sections has been described extensively (3).

Results

The structural components of the bladder wall of the sham operated animals were similar to those described previously in un-operated animals (2). Briefly, the urothelium comprised NO stimulated cGMP positive (cGMP⁺) umbrella cells, an intermediate urothelial layer 2-3 cells thick and a basal urothelial layer which stained for nNOS. Below the urothelium, within the lamina propria, were regions of cGMP⁺ sub-urothelial interstitial cells. Different cGMP⁺ ICs were also associated with the outer muscle layers: cells on the serosal surface, on the surface of the muscle bundles and within the muscle bundles (Figure 1). In tissues from obstructed animals the number of cGMP⁺ ICs in the muscle layer was dramatically increased (Figure 1). Bundles of cGMP⁺ ICs were seen to penetrate the muscle bundles and cGMP⁺ ICs were also seen within the bundles. Nodes of cGMP⁺ ICs were located in the space between the muscle bundles. The number of cells forming the muscle coat (serosa) was increased. Regions of the lateral wall were apparent where cGMP⁺ cells were associated with the inner muscle layer.

The number of cGMP⁺ umbrella cells and intensity of staining associated was reduced. The intermediate layer of the urothelium consisted of multiple cell layers. The number of cGMP⁺ ICs in the sub-urothelial layer was increased in some regions with cells dispersed through out the lamina propria.

There was a patchy denervation in the muscle layer and a few nerves were associated with nodes of ICs. Nerves were found in the lamina propria but rarely associated with the urothelium.

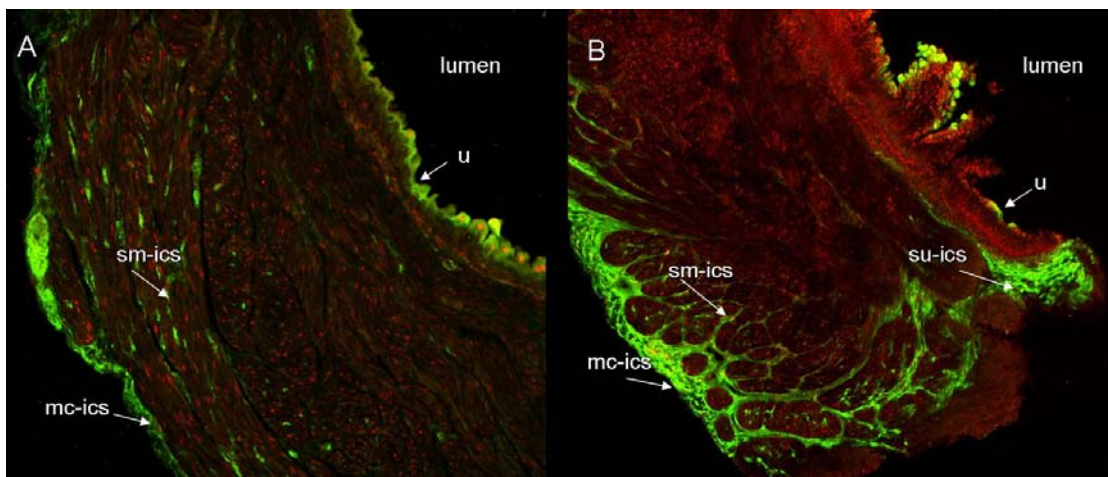


Figure 1 illustrates sections of the entire bladder wall (from urothelium to the outer muscle coat) stained for cGMP (green) in sham operated (A) and obstructed (B) guinea pig bladders. In the urothelial layer there are cGMP positive

umbrella cells (u). In the lamina propria there are regions of cGMP positive sub-urothelial interstitial cells (su-ics). Muscle coat interstitial cells (mc-ics) and superficial muscle interstitial cells (sm-ics) can be detected in the muscle layer.

Interpretation of results

Our data demonstrate clear differences in the number and distribution of cGMP positive interstitial cells in obstructed bladders compared to sham operated bladders of guinea pigs. There are major alterations to the cGMP positive interstitial cells in the muscle layer and the urothelium. These structural changes likely play a role in the altered functioning of the obstructed bladder. Such major changes may have important implications for unravelling the nature of the clinical problem of lower urinary tract symptoms seen in patients with outflow obstruction.

Concluding message

There are changes in the network of cGMP responsive interstitial cells following bladder outflow obstruction, which may play a role in the pathophysiology of the overactive bladder syndrome.

References

1. Nat Clin Pract Urol (2005) 2; 546-554.
2. BJU Int (2004) 94; 1114-1124.
3. Neuroscience (1998) 87; 207-241.

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