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# COMPLEX SIGNAL INTERACTIONS BETWEEN THE DIFFERENT LAYERS OF THE UROTHELIUM OF THE RAT

Hypothesis / aims of study

It is widely accepted that the urothelium acts as part of a sensory system, releasing acetylcholine (ACh), nor-adrenaline (NA), adenosine tris-phosphate (ATP), prostaglandins (PG) and nitric oxide (NO) when stretched. When released, these signals are thought to act upon sub-urothelial afferent nerves to signal to the CNS information related to bladder volume. Although this appears to be a simple and elegant idea, it is now apparent that the multitude of signals emanating from the urothelium can interact such that the output of the urothelium is the result of complex signal interactions. Also, it has recently been recognised that there are major regions of the bladder wall (the lateral wall and dome) that are not innervated with sub-urothelial sensory fibres but which retain urothelial signalling systems. In order to explore this evolving complex system it is essential to know where different signals originate within the urothelial layers in different regions of the bladder and where they are targeted. The present study was done, using immuno-histochemical techniques, to explore the origins and site of action of a number of the urothelial signals present in the rat bladder.

Study design, materials and methods

24 rat bladders were used in this study. Each bladder was isolated immediately after cervical dislocation of the animal and prepared for immuno-histochemistry by either (i) fixation in 4% paraformaldehyde in PBS (2 hours) and then frozen in isopentane or (ii) snap frozen in isopentane. Frozen section (7□m) were cut and fixed in methanol (-20$^{o}$C). Sections were then labelled with primary antibodies (24 hrs at 4$^{o}$C), washed and labelled with appropriate secondary antibodies for immuno-fluorescence. The following panel of antibodies were found to be reliable and specific for the designated epitopes: (COX I (cyclo-oxygenase 1), EP1 and EP2 (prostaglandin receptors type 1 and 2), P2X3 and P2X7 (purinergic receptors), synaptic vesicle protein (SV2), □1 adrenergic receptor (□1), nitric oxide synthase (NOS), muscarinic acetylcholine receptor type 3 ($M_3$), vimentin (vim), calcitonin gene related peptide (CGRP) and the enzymes Na/K ATPase (Na/K) and adenylate cyclase (AC). Other antibodies were explored but if non-specific staining was evident or inconsistent they were not included.

Results

The bladder wall was divided into three regions, base, lateral wall and dome. The gross structure of the urothelium was the same in all regions. Three layers were easily identifiable: an outer layer of large cells (the umbrella cell layer), an intermediate layer that was typically 1-2 cell layers thick and a basal cell layer consisting of a single layer of small densely packed cells (see Figure 1 A). Immediately below the urothelium was a dense layer of vimentin positive actin negative cells (sub-urothelial interstitial cells (su-ics)). Between this layer and the muscle layer was a diffuse layer of a second type of interstitial cell (lamina propria interstitial cells (lp-ics)). CGRP positive nerves were found in the sub-urothelial layers in the bladder base. Few CGRP fibres were found in the lateral wall and no fibres in the dome.

In all regions, the basal urothelial layer was positive for COX I suggesting that it was a source of prostaglandin. EP2 receptor reactivity was seen in the intermediate and umbrella cells suggests that this COX I positive layer may be signalling to the outer layers. The su-ics expressed EP1 receptors suggesting that the same PG signal is being transmitted into the lamina propria. The intermediate layer expressed NOS. Activation of this layer will almost certainly cause the production of nitric oxide that must be affecting adjacent cell layers. □1 receptors ware found in the intermediate and umbrella cell layers suggesting that these layers can respond to adrenergic stimuli. The enzyme choline acetyl transferase (ChAT) was detected primarily in the intermediate cell layers, but the outer margins of the umbrella cells also stained. $M_3$ receptor immune-reactivity was detected in the basal and intermediate layers suggesting that the ACh synthesised in these umbrella cells and intermediate cells target these intermediate and basal cells. SV2 positive synaptic vesicles were seen primarily in the umbrella cells. Interestingly, SV2 immuno-signal was seen in the membranes of umbrella cells facing the intermediate cell layer suggesting the possible exocytosis of SV2 positive vesicles into the inner layers of the urothelium. P2X7 antibody binding was found on the intermediate layer while P2X3 was associated with the COX I positive cells of the basal layer.

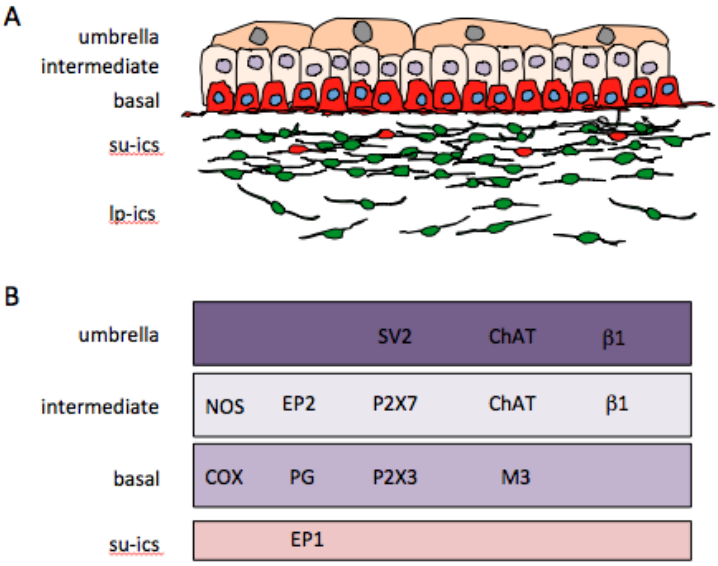


Figure 1. The general structure of the rat urothelium and the signals, receptors and mechanisms detected in the different layers. A shows a basic cartoon illustrating the different layers observed in the rat urothelium. The layer facing the lumen consists of large umbrella cells. Below this layer is the intermediate cell layer that is located above a basal layer. Immediately below the basal layer is a dense region of interstitial cells (sub-urothelial interstitial layers (su-ics)). Below this layer is a sparsely populated layer of interstitial cells of the lamina propria (lp-ics). B illustrates the signalling components detected in the different layers (COX I (cyclooxygenase 1), EP1 and EP2 (prostaglandin receptors type 1 and 2), P2X3 and P2X7 (purinergic receptors), synaptic vesicle associated protein (SV2), nitric oxide synthase (NOS), choline acetyl transferase (ChAT) and muscarinic acetylcholine receptor type 3 ($M_3$).
Highly localised regions 'hot spots' were also detected in the bladder base that stained intensely for P2X3 or ChAT. These appeared at apparently random sites, adjacent to the general regions described above.
These patterns of urothelial staining were seen in all regions. However, CGRP positive nerve fibres, associated with the urothelium, were only seen in the bladder base. This observation highlights the difficulty that the complex signal output of the urothelium cannot be targeted to nerve fibres exclusively and suggests that the complex mechanisms within the urothelium must operate on other systems within the wall.

Interpretation of results

These observations demonstrate the complexity of the urothelium and its component layers. The clear separation of signals and receptors between the different layers suggests directionality to the signalling processes. Also, the separation but close proximity of the different signalling systems suggest the possibility of interactions between systems.

Concluding message

The urothelium is a highly organised but complex structure expressing multiple signalling components involved in the production and response to signals. The absence of nerves in regions where these signals are being generated suggests that these systems must underpin functional mechanisms not involved directly in afferent signalling.

Disclosures

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