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GENE EXPRESSION OF PURINERGIC, MUSCARINIC AND TACHYKININ RECEPTORS IN PORCINE BLADDER: COMPARISON IN FRESH TISSUE AND CULTURED UROTHELIUM, MYOFIBROBLAST AND DETRUSOR MUSCLE CELLS

Hypothesis / aims of study

In the mammalian bladder, urothelial, myofibroblast, and detrusor muscle cells are known to express multiple receptors, neurochemicals and enzymes which participate in communication between different cell layers, and in bladder function. Expression levels of these mediators can be affected by disease and by experimental inflammation [1]. The pig bladder is an excellent model for human bladder. Previously, it has been shown that three distinct cell types, urothelial, myofibroblast, and detrusor muscle, can be cultured from porcine bladder, and distinguished on the basis of morphological, immunological and pharmacological characterisation [2]; however no gene expression studies have carried out. Our aim was to determine the pattern of expression of a range of genes known to be in involved in bladder function [3], in these three cell types. In addition, we compared expression between the cultured homogeneous cells and the fresh tissue, to establish changes during cell proliferation and differentiation, and determine the reliability of cultured porcine bladder cells.

Study design, materials and methods

Female pig bladders were collected from local abattoir, and transported on ice to the laboratory. Any damaged or inflamed bladders were discarded. Fat tissue was removed; bladders were washed in PBS several times, and dissected immediately. One half of bladder was used as fresh tissue; urothelial tissue (UT) was scraped off, and the remaining bladder dissected into mucosa (minus urothelium) (MucT) and detrusor (DT). Each dissected tissue was submerged in RNALater and kept at 4˚C overnight. The other half bladder was cultured into three cell populations of urothelial (UC), suburothelial mucosal (myofibroblast) cells (MyC) and detrusor muscle cells (DC), as described [2], with cells harvested at 2 weeks.
Total RNA was extracted from the dissected tissues and the confluent cultured cells by TRIZOL reagent and treated with DNase to remove any cellular DNA. The genes under investigation encoded for purinergic receptors (P2X1R, P2X3R, and P2Y6R), muscarinic receptors (M3 and M5), tachykinin receptors (TACR1 and TACR2), and the transient receptor potential vanilloid ion channel (TRPV1) [3]. P2X1, P2X3 and P2Y6 are associated with detrusor muscle, afferent nerves and myofibroblasts, respectively. M3 receptors are found principally on smooth muscle whereas M5 is associated with the urothelium. TACR1 and TACR2 encode for the NK1 and NK2 receptors which respond to substance P and neurokinin A, involved in sensory and motor actions in the bladder [3].
Primers were designed based on porcine cDNA sequences, using Primer 3 software. The primer for TACR1 was not available from the porcine genome and was derived from sequences conserved across a broad range of mammalian species. Gene (mRNA) expression was measured by quantitative real-time polymerase chain reaction (qRT-PCR). GAPDH was used as the housekeeping gene. The C_t value, which is the cycle at which the fluorescent signal of the PCR reaction reaches threshold, was recorded for each gene. The authenticity of the PCR products was confirmed by the expected sizes in 2% agarose gels.
The results were expressed as percent expression compared with GAPDH. Data were compared using the Kruskal-Wallis test followed by Dunn's multiple comparison test.

Results

Fig 1 shows that, in general, there was no difference in gene expression between detrusor (DT) and mucosal tissue (MucT). Expression of transcripts appeared generally lower in cultured cells than in fresh tissue. No significant differences were seen for expression of any gene between the UC, MyC and DC cultures.
Compared with GAPDH, expression of P2X1 (Fig 1A) was very high in both mucosal (MucT, 12.2%) and detrusor tissue (DT, 29.4%). TACR2 (Fig 1F) was moderately highly expressed in mucosal (MucT, 1.56%) and detrusor (DT, 0.68%) tissue. M3 appeared more highly expressed than M5 (DT, 0.32% vs. 0.0015%) in the same tissues. Both P2X3 (DT, 0.0014%) and P2Y6 (DT, 0.0026%) were comparatively weakly expressed in detrusor and mucosa. Notably, no expression of TACR1 was observed in any group. TRPV1 was very weakly expressed in all tissues and cultured cells (range 0.0001 – 0.01% of GAPDH), with no significant changes observed in the cultured cells compared with their "parent" tissue.
For some transcripts, there was a significant decrease in expression in cultured myofibroblast cells and cultured detrusor smooth muscle cells, compared with that in the corresponding fresh tissue. For example, in detrusor, P2X1 and M3 expression was reduced by several orders of magnitude; TACR2 expression was also substantially reduced.
In fresh and cultured urothelium, all genes (except TACR1) were expressed, to varying extents. P2X3 and P2Y6 were present in UT, approximately tenfold greater than the other transcripts. Only P2Y6 was significantly ($P<0.05$) less expressed in cultured UC compared with UT, but there was a trend for some other transcripts to appear reduced in UC.

Interpretation of results

To our knowledge, this is first report of gene expression for these receptors in the porcine bladder. In the urinary bladder, the genes for M3, P2X1 and TACR2 are highly expressed in detrusor muscle, where their corresponding receptor proteins have been demonstrated to have contractile functions [3]. If confirmed in future experiments, the lack of TACR1 expression would be quite remarkable, given the prominent role of the NK1 receptor in micturition in other mammalian bladders [3].
In detrusor and myofibroblasts, the significant decrease in expression after culture, particularly notable for M3, M5, P2X1 and TACR2 transcripts, suggests that these genes were originally expressed on the "parent tissue" cells but were lost or deregulated after 2 weeks in primary culture. However, the detrusor and the (urothelially-denuded) mucosa would contain various cell types (e.g. nerves, blood vessels, etc) in addition to smooth muscle and myofibroblasts, respectively. Thus,

alternatively, the transcripts might have been originally associated with a variety of cell types that did not grow in culture. Note that the expression level of a transcript may not be related to the expression level of its associated protein.

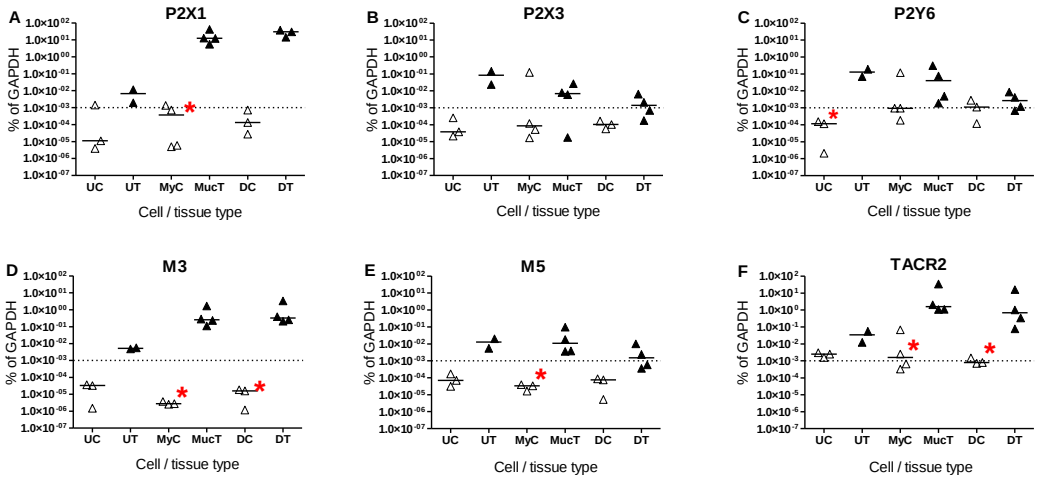

Figure 1. *Expression of mRNA for purinergic (A,B,C), muscarinic (D,E) and tachykinin (F) receptors in (▲) porcine bladder tissue and (∆) cultured cells (n=2-4). Data are expressed as percent of GAPDH expression and are shown on a* $\log_{10}$ *scale; the bar represents the median. The dotted line is drawn at 1 x* 10^{-3}*%. Significant differences (Kruskal-Wallis test) between groups were seen, as follows: A, P=0.0114; B, not significant; C, P=0.0341; D, P=0.0099; E, P=0.011; F, P=0.0079. Significant decreases in expression in cultured cells compared with corresponding native tissue are indicated by * (P<0.05, Dunn's test).*

Concluding message

This novel study employed qRT-PCR to demonstrate the expression of some functionally important receptor transcripts in porcine bladder. The pattern of expression for each receptor transcript was very similar between fresh mucosal and fresh detrusor tissue, but different for urothelium. For P2X1, M3 and TACR2, very marked decreases of transcript expression were found in cultured detrusor cells, compared with fresh detrusor tissue.

References

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Disclosures

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