Characterisation of P2 receptor-mediated Ca^{2+} signalling in isolated smooth muscle of rat bladder

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INTRODUCTION

To help maintain continence; the filling (relaxation) and emptying (contraction) of the bladder muscle is tightly regulated. Control of bladder muscle relaxation and contraction is mediated via a number of different receptors[1], which interact with neurotransmitters released from the brain, to decode their signals. This process is partially mediated by purinergic (e.g. ATP) signalling through either P2X or P2Y receptors (see Fig 1), whose activation leads to rise in intracellular calcium and muscle contraction[2]. Overstimulation of these signalling pathways can lead to inappropriate bladder contractions termed overactive bladder which is a major cause of incontinence. Indeed, presently up to 9 million people in the UK[3] are incontinent, and by 2018 this figure is estimated to rise to 546 million worldwide[4] , which suggests that understanding more about how purinergic signalling regulates bladder contraction is important in terms of understanding this disease.

AIM OF STUDY: To characterise which purinergic receptors are present in rat bladder and to determine their relative contributions to global calcium signalling and thus bladder contraction.

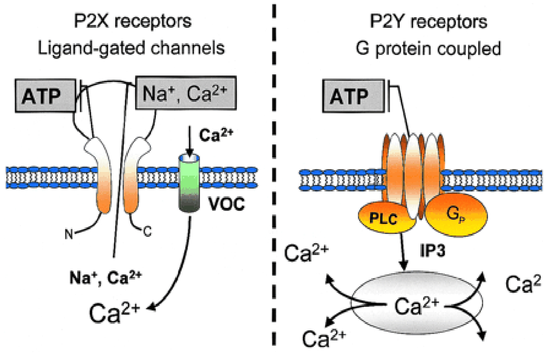

Figure 1. P2 receptor subtypes and the calcium signalling mechanisms

METHODOLOGY

Detrusor smooth muscle cells (DSMC) were isolated from male Wistar rats via enzymatic digestion using papain and collagenase. DSMC were cultured in 199 media supplemented with 10% foetal calf serum, penicillin and streptomycin. Two days later DSMC were loaded with the Ca^{2+}-sensitive dye Fluo4-AM (4μM) at room temperature for 60 min. Changes in intracellular Ca^{2+} ($[Ca^{2+}]_i$) were measured in single cells using confocal imaging in the presence of various P2 receptor agonists or antagonists.

RESULTS

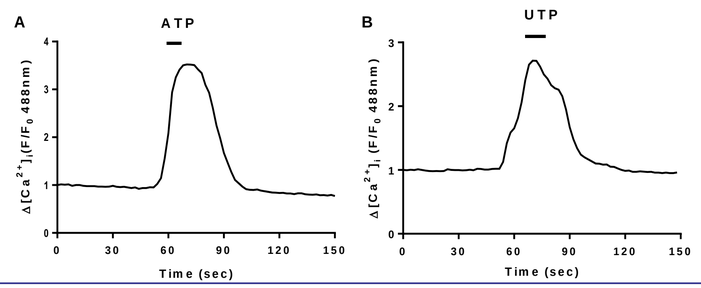

Figure 2. ***Identification of P2 receptor signalling in rat DSMC***
DSMC were loaded with Ca^{2+}-sensitive dye Flou4-AM (4 μM), before stimulation with ATP (100 μM) **(A)**, or UTP (100 μM) **(B)**, for 30 sec. ATP and UTP produced transient elevations in $[Ca^{2+}]_i$, suggesting the presence of P2Y and/or P2X receptors in DMSC (n=164 cells from 3 different bladder preparations).

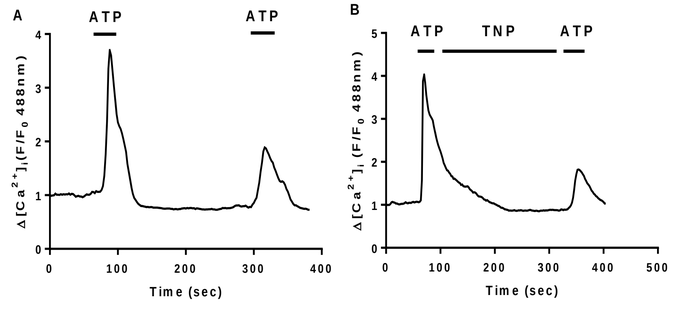

Figure 3. **Do** ***P2X receptors contribute to purinergic calcium signals in cultured DSM C ?***
To investigate whether P2X receptors contributed to the ATP generated Ca^{2+} signalling in DSMC, we used the non-specific P2X receptor antagonist, 2', 3'-O-trinitrophenyl-ATP (TNP-ATP) to block P2X activity. DSMC were challenged with ATP (100 μM) for 30 sec and then either incubated with buffer or TNP (10 nM) for 5 min, followed by ATP (100 μM) for 30sec. The profiles of ATP-stimulated $[Ca^{2+}]_i$ changes in the presence or absence of TNP were similar (n=50-148 cells from >3 different bladder preparations).

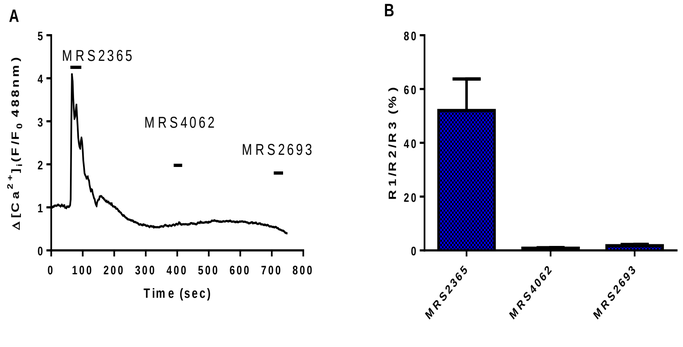

Figure 4. ***Identification of which P2Y receptor subtypes mediate Ca^{2+} signalling in isolated DSMC***
A) DSMC were challenged with the either a $P2Y_1$ (MRS2365), $P2Y_4$ (MRS4062 100 nM) or $P2Y_6$ (MRS2693, 10 nM) selective agonist for 30 sec as shown by the black bars. **B)** Cumulative data showing the peak intracellular Ca^{2+} changes following agonist addition. (n= 20-38 cells from 3 separate bladder preparations).

Our results show that the purinergic agonists ATP and UTP produced transient elevations in $[Ca^{2+}]_i$, (Figure 2), suggesting the presence of P2Y and/or P2X receptors in DMSC. In Figure 3 ATP-stimulated $[Ca^{2+}]_i$ changes in the presence or absence of TNP were the similar, suggesting that cultured DMSC may not express P2X receptors. Ca^{2+} signals were evoked by the $P2Y_1$ selective agonist MRS2365, but not $P2Y_4$ or $P2Y_6$-selective agonist, suggesting the presence of $P2Y_1$ receptors (Figure 4). Finally, as UTP does not activate $P2Y_1$ receptors, but does activate $P2Y_2$ receptor signalling this suggests the presence of $P2Y_2$ receptors in DSMC.

DISCUSSION

Our data confirm that P2Y purinergic receptors promote increased $[Ca^{2+}]_i$ in DSMC, and thus are likely to induce contraction of the urinary bladder. Since the co-ordinated activity of receptor function is vital in regulating the contractile state of the bladder, regulation of receptor function may play an important role in the maintenance of continence. As little is known about how P2Y receptor activity is regulated in DSMC we will identify the mechanisms mediating this process, which should highlight processes that might be dis-regulated during incontinence.

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