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AGE-RELATED DECREASE IN PURINERGIC P2X1 RECEPTOR MRNA IN THE MALE DETRUSOR MUSCLE

Hypothesis / aims of study

In young adults (<60 years), bladder contraction is almost entirely due to activation of muscarinic receptors by acetylcholine released from parasympathetic nerves. However, with ageing (1), and obstruction (2), the atropine resistant component of bladder contraction, ascribed to ATP, becomes increasingly important. Urodynamic tests have demonstrated an age-related reduction in bladder capacity and increased incidence of detrusor overactivity as well as decreased flow rate and poor bladder emptying (3). The preliminary aim of this study was to determine whether the expression of $P2X_1$ purinergic receptors was altered in ageing, in control patients and also in patients with bladder outlet obstruction. Our secondary aim was examine the expression of $P2X_1$ receptors in detrusor from control compared to obstructed patients.

Study design, materials and methods

Biopsies of normal human detrusor were collected from 31 male patients (age range 30 to 84 years) undergoing cystoscopy due to a history of bladder cancer or to asymptomatic haematuria. All patients displayed normal micturition frequency, with no symptoms of urge incontinence. In addition, bladder biopsies were collected from 20 patients with bladder outlet obstruction (age range 34 to 81). Biopsies taken from macroscopically normal areas of the bladder were collected into RNA later, dissected into detrusor muscle and mucosa and stored at -80°C until RNA extraction.

The expression of $P2X_1$ receptor mRNA was quantified using quantitative competitive RT-PCR (QCRT-PCR) (4). Standard cRNA (stdRNA) was generated by RT-PCR using the human $P2X_1$ receptor sequence with sense primer incorporated with T7 promoter. After gel purification, the PCR product was used as a template to synthesise stdRNA by T7 RNA polymerase (Epicentre). After treatment with RNase-free DNase for removal of the DNA template, stdRNA concentration was quantified by spectrophotometer, the concentration adjusted to 100ng/ l and the RNA stored at -80°C. The internal deleted cRNA (idRNA) for $P2X_1$ receptor was generated in the same way as stdRNA except a for ~30% internal deletion in the sequence. The standard curve was constructed with a range of P2X1 stdRNA (3000-10 fg) co-amplified with 300 fg idRNA by RT-PCR.

Expression of $P2X_1$ receptor mRNA in human bladder was determined by co-amplifying 100ng bladder total RNA with 300 fg idRNA. The PCR products (492bp and 342bp for stdRNA and idRNA, respectively) were then separated by gel electrophoresis (2.5% agarose) and quantified by densitometry. The amount of sample mRNA was determined by plotting the relative intensity of DNA bands compared to the relative intensity of bands in the standard curve.

The housekeeping gene, GAPDH, was used as an internal control to monitor RNA quality. GAPDH mRNA in human bladder detrusor was quantified in the same way as $P2X_1$ receptor mRNA. The QCRT-PCR data for $P2X_1$ receptor expression in detrusor muscle were then normalised for the expression of GAPDH in the same sample.

Results

The expression of $P2X_1$ receptor mRNA in the human detrusor showed an age-related decrease in both control ($p = 0.0003$) and obstructed ($p = 0.01$) patients (see Fig 1A). This was not due to a decrease in the quality of RNA extracted from the ageing patients as expression of the internal control GAPDH did not vary with age in either patient groups (Fig 1B).

No change was seen when comparing detrusor $P2X_1$ receptor mRNA expression in control compared to obstructed bladder (Fig 2, p = 0.88). In addition, no change was seen in the expression of GAPDH in control and obstructed patients (p = 0.25, data not shown).

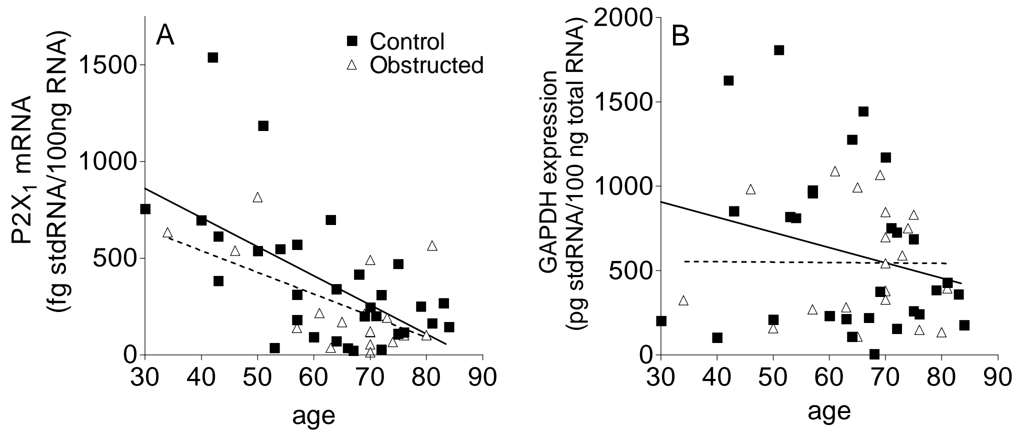

Figure 1. *Changes in expression of mRNA in human bladder detrusor. (A) shows a significant decrease in $P2X_1$ receptor expression with age in control* (n = 31, p = 0.0003, r^2 = 0.35) *and obstructed bladder* (n = 19, p = 0.01, r^2 = 0.33)*. (B) shows no correlation of GAPDH expression with age* (control: p = 0.19 and obstructed: p = 0.97).

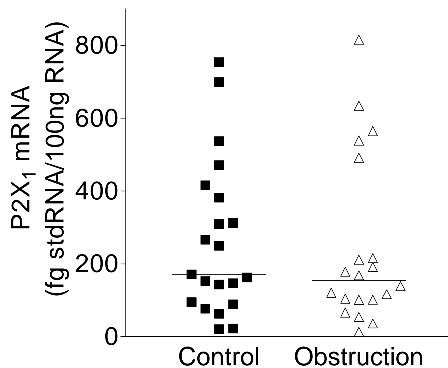

Figure 2. *There was no change in P2X1 mRNA expression in control compared to obstructed bladder(p = 0.88, median control = 171 fg stdRNA/ 100ng bladder RNA, median obstruction 154 fg stdRNA/ 100ng bladder RNA).*

Interpretation of results

There was an age-related decrease in $P2X_1$ receptor mRNA expression in both control and obstructed detrusor muscle. This result is in contrast to reports of an increased role of atropine-resistant (ATP mediated) contraction in ageing human bladder strips (1). This highlights the difficulties in relating changes in receptor mRNA levels to alterations in receptor protein levels or functional responses.

In this study, QCRT-PCR data were normalized to expression of an internal control (GAPDH). It is important that expression of any internal control does not vary with age or disease, and here we show that GAPDH is a suitable choice for studies in detrusor muscle.

Concluding message

We have described an age related decrease in $P2X_1$ receptor mRNA expression in the human bladder detrusor, which has not previously been reported.

References

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