**55**

Carly M[1], Newgreen D[2], Fry C[1]
1. University College London, 2. Pfizer R&D, Sandwich, Kent

# THE MECHANISM OF ATROPINE RESISTANCE IN HUMAN DETRUSOR MUSCLE

Hypothesis / aims of study

Nerve-mediated contractions in human detrusor from normal bladders are mediated by acetylcholine, as evidenced by their abolition with atropine. This is in contrast to detrusor from several bladder pathologies, and to that from many animals, where there is an ATP-mediated atropine-resistant fraction of contraction (1). One hypothesis for atropine-resistance in human detrusor is that neurally-released ATP is incompletely broken down at the neuromuscular junction and thus contributes to muscle activation, and is supported by reduced ectoATPase (ENTPDase) activity in human detrusor from idiopathically overactive bladders (2). If ATP breakdown is incomplete extracelluar ATP should be in excess, and ENTPDase enzyme transcription might also be reduced. We tested these hypotheses by measuring the effects of an exogenously-added ATPase, apyrase, on muscle contraction, and by measuring mRNA levels of ENTPDases in detrusor samples from stable and overactive bladders.

Study design, materials and methods

Human detrusor samples from patients with: symptomatically stable bladders undergoing cystectomy; and with urodynamically-proven detrusor overactivity, with no evidence of neurological deficits, were used. Samples from normal guinea-pig bladders were also used in some experiments. For tension recording muscle strips (1<mm diam.) were perfused in a HEPES-buffered medium (pH 7.4, 37°C) and field-stimulated (3-s trains of 0.1 ms pulses, 24Hz human, 16Hz guinea-pigs). The proportion of the atropine-resistant contraction was determined and Apyrase, 1U/ml, was added and recordings continued. Total RNA was extracted from samples, reverse transcribed to cDNA and RT-PCR performed to measure the relative quantities of ENTPDases 1,2,3 and 5 compred to the 18s housekeeping gene using primers from Applied Biosystems. Data are median (25, 75% interquartiles), differences between sets were tested with a Mann-Whitney U-test.

Results

Table 1 shows that the atropine-resistant proportion of the nerve-mediated contraction correlated with the proportional reduction of the twitch by apyrase. Thus, the greater the excess ATP in the neuromuscular junction to generate the atropine-resistant contraction the greater should be the effect of apyrase. Table 1 also shows the relative transcription of the different ENTPDases in the stable and overactive groups. The most abundant was ENTPDase1 and this was significantly reduced in the overactive bladder samples. ENTPDase3 was also significantly reduced, but the levels are were much less. The other two enzyme subtypes were not different in the two human groups.

Table 1. The proportion of atropine resistance, % reduction of the twitch by apyrase and ENTPDase transcription in human and guinea-pig detrusor. The transcription determinations are relative to the 18s gene. n= number of experiments. * overactive vs stable

| | Stable, human | Overactive, human | Guinea-pig |
|---|---|---|---|
| % atropine resistance | 0.0 (0.0, 0.0; n=28) | 11.8 (32.4,10.3;n=28) * | 42.3 (40.1,51.1; n=12) |
| % reduction by apyrase | 2.0 (1.0, 2.5; n=12) | 14.0 (8.1, 20.5; n=14) * | 23.1 (16.8, 29.4; n=12) |
| ENTPDase 1 | 33.6 (29.1, 37.1) | 27.6 (21.6, 30.7) * | |
| ENTPDase 2 | 0.42 (0.24, 0.81) | 0.51 (0.30, 0.71) | |
| ENTPDase 3 | 0.36 (0.23, 0.61) | 0.22 (0.21, 0.31) * | |
| ENTPDase 5 | 5.45 (0.80, 38.7) | 3.12 (0.50, 5.83) | |

Interpretation of results

The data confirm atropine-resistant contractions in the human detrusor from idiopathically overactive (IDO) bladders in contrast to tissue from stable bladders. If atropine resistance is due to incomplete breakdown of ATP in the neuromuscular junction then addition of an additional ATPase should reduce force selectively in the groups with atropine resistance. This was confirmed in these experiments. Furthermore, guinea-pig detrusor which shows significant atropine-resistance was also sensitive to apyrase. The incomplete breakdown of ATP in IDO may be related to the reduced expression of the predominant ATPase, ENTPDase1 as confirmed by RT-PCR.

Concluding message

Atropine-resistance in IDO results from reduced expression of the predominant ATPase, compared to normal bladders. The reduced expression results in incomplete breakdown of neurally-released ATP which generates an additional component to muscle contractility. This aberrent contractile pathway in IDO offers a target to selectively attenuate contractility in this pathology.

References

1 Journal of Urology 1999 **162**, 1833-1839.
2 Journal of Urology 2002 **168**, 1235-1239.

**FUNDING: We are grateful to the BBSRC for financial support**

**HUMAN SUBJECTS: This study was approved by the University College Hospital and followed the Declaration of Helsinki Informed consent was obtained from the patients.**