

## CONTRACTILE EFFECTS AND RECEPTOR ANALYSIS OF ADENOSINE-RECEPTOR IN STABLE AND NEUROPATHIC DETRUSOR OVERACTIVE BLADDERS.

### Hypothesis / aims of study

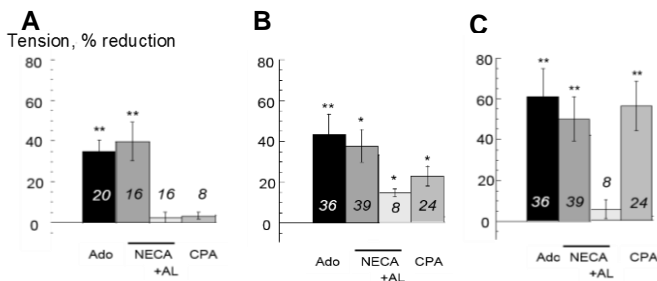
To measure the relative transcription of adenosine receptor subtypes and the contractile effects of adenosine receptor ligands on detrusor smooth muscle (DSM) from patients with neuropathic overactive (NDO) and stable bladders. We tested the hypothesis, 'adenosine causes relaxation of DSM from normal bladders but greater in NDO due to an increased ATP contractile component.' Preliminary contraction experiments were done with guinea-pig tissue to help interpret the action of adenosine receptor ligands on human detrusor.

### Study design, materials and methods

Human bladder biopsies were obtained from patients who had: i) stable bladder; bladder carcinoma undergoing cystectomy ( $n=16$ : 9 female; 7 male; age  $56\pm 14$  yr); ii) NDO; spinal cord injury or multiple sclerosis undergoing clam ileocystoplasty ( $n=18$ : 8 female; 10 male; age  $33\pm 7$  yr). Animal experiments used guinea-pig (Dunkin-Hartley, males, 350-450 g) bladders. All bladder tissue prior to use was stored in ice-cold fresh, gassed (95% O<sub>2</sub>: 5% CO<sub>2</sub>) Tyrode's solution. *In vitro* isometric contractions were elicited by carbachol (CCh, 1  $\mu$ M) added to the Tyrode's or electrical field stimulation (0.1 ms pulses, 3 s stimulation every 90 s, TTX-sensitive, 37°C; pH  $7.4\pm 0.02$ ). The effects of adenosine receptor ligands were measured as the mean percentage change to peak contracture magnitude normalised to the pre-intervention control, or changes to the contraction at low (2 or 4 Hz) and high (20 or 40 Hz) frequency EFS-stimulated responses. Changes to the ratios of low and high frequencies were guinea-pig: T<sub>2/20</sub> and human: T<sub>4/40</sub>. All adenosine receptor agonists and antagonists were dissolved in DMSO. Expression profiles of adenosine-receptor (A<sub>1</sub>, A<sub>2A</sub>, A<sub>2B</sub>, A<sub>3</sub>) transcripts between stable and NDO patients were done by RT-PCR. All data are mean $\pm$ SD, differences between data sets were examined with paired or unpaired Student's *t*-tests; the null hypothesis was rejected at  $p<0.05$ . *n*-values refer to the number of preparations.

### Results

With guinea-pig bladders, adenosine attenuated CCh contractures ( $34.7\pm 5.7\%$ ), which was mirrored by the A<sub>1</sub>/A<sub>2</sub> agonist NECA (10  $\mu$ M), in turn blocked by the A<sub>2B</sub> antagonist, alloxazine; AL (1  $\mu$ M, Figure 1A). The selective A<sub>1</sub> agonist CPA (10  $\mu$ M) had no effect.



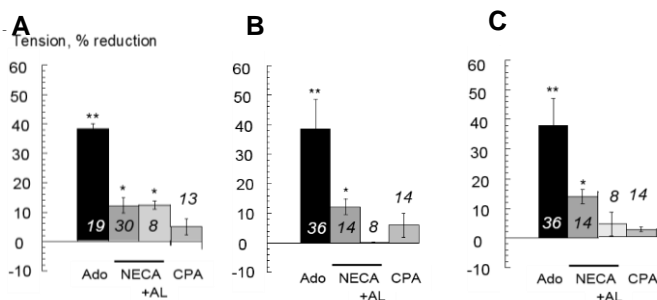
**Figure 1. The effect of adenosine and selective A-receptor subtype ligands on detrusor from guinea-pig bladders.**

Values are % reduction of tension from control. Three sets of data refer to actions on the carbachol contracture (A); T<sub>20</sub> Hz (B), and T<sub>2</sub> Hz (C). \* $p<0.05$ , \*\* $p<0.01$  vs control. Values in or above bars are number of preparations.

Adenosine also attenuated nerve-mediated responses, being more effective at lower frequencies (20 Hz:  $43.4\pm 10.0\%$ ; 2 Hz:  $61.2\pm 14.0\%$ ,  $p<0.01$ ; Figure 1B,C). NECA also reduced nerve-mediated responses, an action again reversed by AL. In contrast with its effect on carbachol responses, CPA also

reduced nerve-mediated responses, more effective at 2 Hz compared to 20 Hz stimulation. There were no effects from A<sub>2</sub> and A<sub>3</sub> antagonists alone on all tissues (data not shown).

With human detrusor from stable bladders adenosine reduced the carbachol response by a similar magnitude ( $38.1\pm 2.0\%$ ) as guinea-pig tissue (Figure 2A). However, the effect of NECA ( $12.2\pm 2.6\%$ ) was much smaller than adenosine in contrast to the effect on guinea-pig detrusor. This residual effect blocked by AL and CPA, as above, had no significant effect.

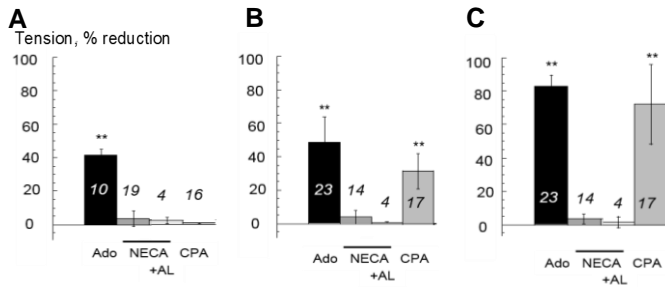


**Figure 2. The effect of adenosine and selective A-receptor subtype ligands on detrusor from human stable bladders.**

Values are % reduction of tension from control. Three sets of data refer to actions on the carbachol contracture (A); T<sub>40</sub> Hz (B), and T<sub>4</sub> Hz (C). \* $p<0.05$ , \*\* $p<0.01$  vs control. Values in or above bars are number of preparations.

The effects of adenosine, NECA ( $\pm$ AL) and CPA on T<sub>4</sub> and T<sub>40</sub> were similar in magnitude to those on the carbachol contracture (Figure 2B,C).

With human detrusor from NDO bladders adenosine blocked the carbachol contracture by a similar amount ( $41.5\pm 3.7\%$ ) to that of human stable and guinea-pig detrusor. However, NECA and CPA had no significant effects (Figure 3A).



**Figure 3. The effect of adenosine and selective A-receptor subtype ligands on detrusor from human NDO bladders.** Values are % reduction of tension from control. Three sets of data refer to actions on the carbachol contracture (A);  $T_{40}$  Hz (B), and  $T_4$  Hz (C). \* $p < 0.05$ , \*\* $p < 0.01$  vs control. Values in or above bars are number of preparations.

With  $T_{40}$  contractions adenosine and NECA had similar actions to those on the carbachol contracture. However, CPA had a significant action. With  $T_4$  contractions adenosine and CPA had significantly greater effects than that on the  $T_{40}$  contraction,

whilst NECA again had no effect.

Adenosine receptor subtype transcription was measured in human detrusor and was similar in both groups, except reduced  $A_{2A}$  levels in NDO bladders.

#### Interpretation of results

Adenosine reduced carbachol contracture magnitude in guinea-pig and human detrusor, mediated by  $A_{2B}$  receptors only in guinea-pig tissue. Nerve-mediated responses are due to acetylcholine and ATP release in human NDO and guinea-pig detrusor, with ATP dominant at low frequencies, but only acetylcholine in stable human tissue. Adenosine had greater effects, and CPA only had actions, when ATP release was more dominant. We interpret this as adenosine selectively reducing ATP release from efferent nerves.

#### Concluding message

With human detrusor neurotransmitter ATP release is associated with overactive bladder pathologies. Adenosine ( $A_1$ ) receptor activation is suggested a drug target to block release of a pathological neurotransmitter.

#### Disclosures

**Funding:** Wellcome Trust **Clinical Trial:** No **Subjects:** HUMAN **Ethics Committee:** All human procedures were in accordance with ethical committee approval of University College London Hospitals, and with the 1964 Helsinki declaration. Guinea-pigs were euthanised by cervical dislocation in accordance with UK Home Office procedures (UK Animals (Scientific Procedures) Act, 1986). **Helsinki:** Yes **Informed Consent:** Yes