87

Fry C[1], Hussain M[2]
1. *University of Surrey,* ***2.*** *Royal Free Hospital*

DIFFERENTIAL EXPRESSION AND TRANSLATION OF ADENOSINE RECEPTOR AGONISTS IN HUMAN DETRUSOR FROM STABLE AND OVERACTIVE BLADDERS AND ITS CONSEQUENCE IN REGULATING DETRUSOR CONTRACTILITY.

Hypothesis / aims of study

Adenosine is generated from the breakdown of the excitatory neurotransmitter ATP and is an agonist for P1 receptors, of which there are four subtypes (A1, A2A, A2B, A3). It suppresses detrusor contractions in animal tissue thus offering a potential negative feedback pathway to regulate muscle contractility. We tested the hypothesis that adenosine receptor agonists modulate contraction differently in human detrusor from stable and overactive bladders and is reflected in differential adenosine receptor gene expression and translation.

Study design, materials and methods

Detrusor specimens were obtained from patients undergoing cystectomy/augmentation ileocystoplasty. Patients were grouped into two sets: symptomatically stable bladders; or those with urodynamically-proven neuropathic detrusor overactivity (NDO). Muscle strips, with the mucosa removed, were superfused with Tyrode's solution (37°C, 24 mM $NaHCO_3$, 5% CO_2). Nerve-mediated contractions were elicited by field-stimulation (0.1 ms pulses, 3s trains, 20 Hz) and were completely abolished by 1 µM tetrodotoxin. Direct-muscle stimulated contractures were generated by addition of 1 µM carbachol to the superfusate in unstimulated preparations. Responses were measured during exposure to P1 receptor agonists and antagonists and calculated as a percentage of the mean control response before and after the intervention. Portions of the biopsy were also saved in liquid N_2 for extraction of total RNA and protein. RNA was extracted, the concentrations in each sample measured, and amplified by RT-PCR against primer sequences for the four receptor subtypes (Human Genome Project website; http://www.genome.gov/10001772); GAPDH-3 was the housekeeping gene. Table 1 shows the primers used. Total protein was extracted from other samples and analysed by Western blotting using polyclonal antibodies to the four receptor subtypes (Alpha Diagnostic Chemicals, Cambridge, U.K.). Bands were analysed by densitometric analysis and normalised to those probed by the GAPDH-3 protein antibody. Data are medians [25%,75% interquartiles] and differences between samples from overactive and stable bladders were tested using Wilcoxon's rank scoring test, the null hypothesis was rejected at $p<0.05$.

Table 1. Primers used for RT-PCR of adenosine-receptor mRNA.

Primer	Sense	Antisense
A1	5'-gccacagacctacttccaca-3'	5'-ccttctcgaactcacacttg-3'
A2A	5'-aacctgcagaacgtcaccaa-3'	5'-gtcaccaagccattgtaccg-3'
A2B	5'-gatcattgctgtcctctgg-3'	5'-tcctcgagtggtccatcag-3'
A3	5'-accactcaaagaagaatatg-3'	5'-acttagctgtcttgaactcc-3'
GAPDH-3	5'-gagtcaacggatttggtcgt-3'	5'-ttgaggtcaatgaaggggtc-3'

Results

Nerve-mediated contractions were of similar magnitude in samples from patients with stable bladders and NDO (32.0 [19.7, 45.9] and 33.5 [19.6, 53.9] $mN.mm^{-2}$ respectively, n=19,19). However atropine-resistance (percentage of contractions after 1 µM atropine) was greater in the NDO preparations (2.9 [2.0, 4.6] and 36.4 [15.5, 44.4]% respectively).

Table 2 shows the effect of adenosine, as well as the A1-selective agonist CPA (N^6-cyclopentyladenosine, 10 µM) and the A2A-selective agonist CGS-21680 (3-[4-[2-[[6-amino-9-[(2R,3R,4S,5S)-5-(ethylcarbamoyl)-3,4-dihydroxy-oxolan-2-yl]purin-2-yl]amino]ethyl]phenyl] propanoic acid, 10 µM). The A3-selective agonist IB-MECA (chloro-N^6-(3-iodobenzyl)-adenosine-5′-N-methyluronamide, 10 µM) was also used but generated no specific changes in contractile responses, therefore the results are not reported here.

Adenosine reduced nerve-mediated contractions in both groups but the effect was significantly greater in the NDO group. By contrast, adenosine exerted a greater depressant action on the carbachol contracture in stable bladder samples compared to the NDO group.

CPA caused a small reduction of the nerve-mediated contraction, but the effect was significantly greater in the NDO preparations. Again, by contrast, with carbachol contractions CPA had a small depressant effect in stable bladder samples, but none on NDO preparations.

CGS-21680 had a small significant effect on nerve-mediated contractions from stable bladder samples, but had no other effects.

Table 2. Effect of adenosine, CPA and CGS-21680 on nerve-mediated and carbachol-generated contractions in human detrusor from stable and NDO bladders. Contraction, % control. * $p<0.05$ compared to control (100%); § $p<0.05$ NDO vs control

	Adenosine (1 mM)	CPA (10 µM)	CGS-21680 (10 µM)
n-m contraction (%), stable	57.3 [54.0, 67.2] * (n=16)	94.8 [94.7,97.1] * (n=6)	94.7 [94.6,97.3] * (n=6)
n-m contraction (%), NDO	43.6 [39.9, 47.8] *§ (n=10)	47.3 [42.4, 58.2] *§ (n=7)	102.0 [97,4,104.7] (n=7)

Carbachol contraction (%), stable	41.4 [36.9, 44.6] * (n=9)	88.9 [88.3, 94.3] * (n=9)	98.7 [97.4,100.0] n=9
Carbachol contraction (%), NDO	52.3 [43.5, 72.1] *§ (n=6)	95.5 [94.3, 99.7] § (n=6)	98.0 [97.3, 99.6] n=6

RT-PCR results showed that A1-receptor expression (relative to GAPDH-3 expression) was similar in stable and NDO bladder groups (0.068 [0.054, 0.099] and 0.054 [0.042, 0.067], respectively; n=6,7). However, A2A receptor expression was greatly reduced in the NDO samples (0.193 [0.157, 0.218] and 0.036 [0.034, 0.042], respectively; n=6,7; $p<0.05$). No differences in A2B and A3 expression were noted between the two groups.

Western blot analysis did not record bands to A1 protein in any sample. However, the density of A2A bands were greatly reduced in NDO samples compared to those from stable bladders; to 3.8 [1.06, 7.9] %.

Interpretation of results

Adenosine depressed nerve-mediated and agonist-induced contractions in human detrusor from stable and NDO bladders, but there were significant mechanistic differences in the mode of action between the two groups. In animal tissue A1 agonists act at a presynaptic site and A2/3 receptors on the muscle [1]. The large effect of the A1-selective agonist, CPA on depressing nerve-mediated contractions in NDO samples implies adenosine acts at a presynaptic site. By contrast the greater effect of adenosine on carbachol contractions in stable bladder samples, and the small effect of CPA implies it acts more on the muscle cell and less at a presynaptic site. The relative lack of effect of A2A and A3 agonists implies that adenosine does not act through these receptors on detrusor muscle from stable bladders. A2B agonists were not used due to the non-availability of selective agents. The greater incidence of atropine-resistant contractions in NDO bladder samples permits the postulation that adenosine has a selective presynaptic effect on suppressing non-cholinergic transmitters. The significant loss of A2A receptor expression and translation in NDO bladders should not hinder its ability to act as a presynaptic inhibitor.

Concluding message

The negative inotropic effect of adenosine on human detrusor muscle is mediated by different mechanisms in tissue from neurogenic overactive bladders and stable bladders. This offers a route to develop agents that can selectively modulate contractile function in the overactive bladder.

References

1. Acevedo et al. Br J Pharmacol 1992; 107; 120-126.

Specify source of funding or grant	**Wellcome Trust** **EU Framework 7, INComb**
Is this a clinical trial?	**No**
What were the subjects in the study?	**HUMAN**
Was this study approved by an ethics committee?	**Yes**
Specify Name of Ethics Committee	**University College London Hospitals**
Was the Declaration of Helsinki followed?	**Yes**
Was informed consent obtained from the patients?	**Yes**