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THE OVERATIVE BLADDER AND THE ROLE OF P2Y AGONISTS

Hypothesis / aims of study

Spinal cord injury (SCI) generates an overactive bladder phenotype, but with an unknown pathophysiology. In a rat model of SCI an increase of suburothelial interstitial cell number has been measured [1], and an intact mucosa (urothelium and suburothelium) is associated with augmented overactive behaviour [2]. Several lines of evidence implicate a role for P2Y-receptor agonists such as ADP in this overactivity: ADP is a potent excitatory agent on suburothelial interstitial cells [3]; and ATP, a precourser of ADP, is generated by urothelium in response to physiological stimuli such as bladder stretch. We hypothesised that ADP and other P2Y agonists augment bladder overactivity with a crucial role for the mucosa. We used a rat model of SCI in the presence of P2Y agonists by recording contractile activity, as well as electrical and Ca^{2+}-dependent signalling between different regions of the bladder wall.

Study design, materials and methods

Rats underwent spinal (T8/T9) cord transection under isoflurane/O_2 (2%:98%) anaethesia and recovered with prophylactic antibiotics (ampicillin, 100 mg/day im). For the first two weeks bladders were emptied by manual compression until micturition reflexes recovered. Animals were killed at week four by inhaling CO_2. Bladders were loaded with a Ca^{2+}- (Rhod-2) and a membrane potential (Em, Di-4-ANEPPS) fluorochome [1]. Bladder sheets were prepared by cutting the ventral surface from the neck to the dome with the mucosa uppermost. Mucosa was removed from one half by blunt dissection. Bladder wall sections were cut with a fine razor and the cut-end positioned face-up. Preparations were tied to an isometric force tranducer and intracellular Ca^{2+} and Em monitored with an optical imaging system [1] that collected 256 separate images within the field of view collected over 60 s. Isochronal maps were constructed from analysis of the relative delays of separate images and the signal with the shortest delay taken as the signal source (lightest shaded area, Figure 2). Data are means±SD and difference between data sets tested by Student's t-test; the null hypothesis was rejected at $p<0.05$.

Results

SCI bladder preparations exhibited regular spontaneous contractions, increased by P2Y agonists such as ADP or UTP, Figure 1A. The effect was reversible, but only after a transient suppression of activity upon removal of the agonist.

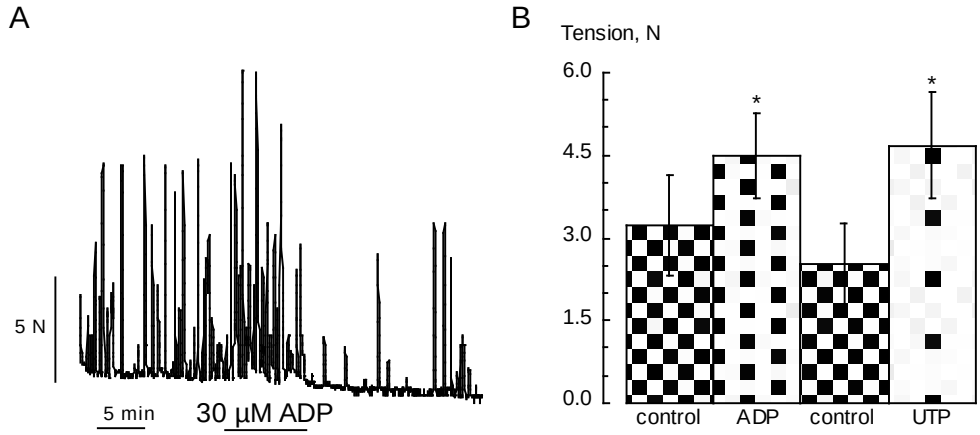

Figure 1. A: Effect of ADP on spontaneous activity in a bladder sheet from a spinal cord-injured (SCI) rat. The effect of ADP (30 µM) and UTP (10 µM) on the magnitide of mean spontaneous contraction magnitude in SCI rat bladder sheets. *$p<0.05$ vs control.

Group data showed that 30 µM ADP increased the mean tension of spontaneous contractions (3.25±0.92 vs 4.49±0.78 N, $p<0.01$, n=7) whereas the frequency was not significantly altered (2.81±0.38 vs 3.14±0.46 per minute). Similar effects were observed with UTP (10 µM); average tension was increased (2.52±0.74 vs 5.12±1.11 N, $p<0.005$, n=8), and frequency was unaltered (2.64±0.43 vs 2.68±0.24 per minute). Tension data are shown in Figure 1B.

Optical imaging experiments to monitor spontaneous changes to intracellular [Ca^{2+}] and membrane potential in synchrony with tension changes were carried out on the two types of preparation: i) a whole bladder sheet, urothelium facing up and with mucosa removed from half the sheet; ii) transverse sections through the bladder wall. Figure 2A shows a bladder sheet pinned out and part B a Ca^{2+} wave during application of 30 µM ADP and corresponding to a spontaneous contraction. The mucosa remained intact in the upper part of the bladder sheet and on application of ADP activity was first generated in this fraction of the preparation. The lighter regions show where activity was first observed propagating to other, darker-shaded regions of the preparation.

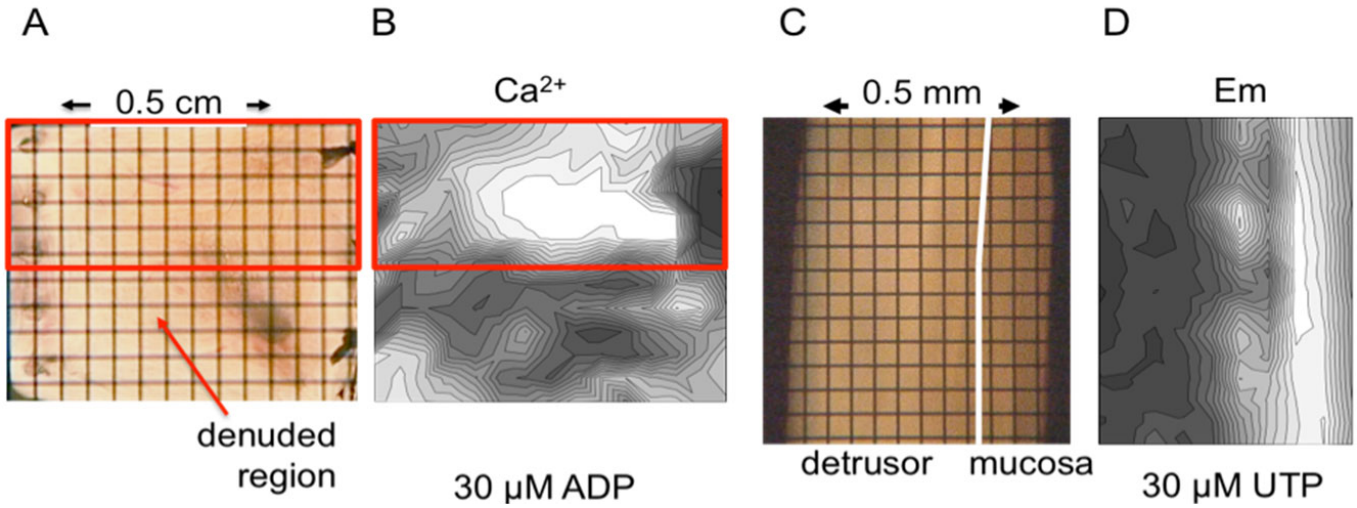

Figure 2. Optical imaging experiments of a bladder sheet (parts A,B) and a transverse section of the bladder wall (parts C,D). Parts A and C are photographs of the preparation and show: (part A) the region of the sheet with intact mucosa (red box); part C the mucosal and detrusor layers of the bladder wall. Parts B and D are contour maps of the spread of Ca^{2+} or Em waves upon application of ADP or UTP to the whole preparation; signals were initiated in the white shaded areas, propagating with increasing delay to successivelty darker regions.

Figure 2C,D shows a similar experiment, this time on a bladder cross section. In this example application of 30 µM UTP generated signals, in this case an Em wave is illustrated, first in the mucosa section of the preparation, which then propagated to the detrusor layer. Similar phenomena were observed in five bladder sheets and four transverse sections using either UTP or ADP.

Interpretation of results

The data show that P2Y agonists such as ADP and UTP increase spontaneous contractile activity in a spinal cord injury model of bladder overactivity. This activity was accompanied by waves of intracellular Ca^{2+} and Em waves propagating across the surface of the preparation. The bladder sheet experiments showed that these signals required an intract mucosa for initiation of a response and the tranverse section preparations also showed that activity originated in the mucosa before propagation to the detrusor layer

Concluding message

We conclude that P2Y agonists augment spontaneous activity in the overactive bladder and that this increase of response is initiated from the mucosal regin of the bladder wall. We hypothesise that such activity originates from the layer in which are located suburothelial interstitial cells. Modulation of P2Y-receptor activity thus represents a novel target to modulate the overactive bladder.

References

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Name of ethics committee	**University of Pittsburgh animal care committee**