The elusive electromyogram in the overactive bladder: a spark of understanding

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ABSTRACT

It has been said that a technique capable of recording a urinary bladder electromyogram could be useful in the clinical evaluation of the detrusor neuropathies and myopathies implicated in the generation of lower urinary tract symptoms. However, in contrast to electromyography of skeletal and cardiac muscle, detrusor smooth muscle electromyography has remained in its infancy despite 50 years of scientific effort. The principal problems appear to be isolation of the real signal from artefacts, and the doubtful existence of electromyographic activity during cholinergic muscle contraction. The discovery of purinergic neuromuscular transmission in the overactive human bladder has renewed interest in detrusor electromyography as, in contrast to cholinergic mechanisms, purinergic mechanisms can generate extracellular electrical activity. In this paper, the development and validation of a novel technique for recording electrical activity from neurologically intact guinea-pig and human detrusor *in vitro* is described. A purinergic electromyographic signal is characterised and it is shown that detrusor taken from overactive human bladders has a greater propensity to generate electromyographic activity than normal by virtue of an aberrant purinergic mechanism.

KEYWORDS

Electromyogram – Overactive bladder – Purinergic signalling – Neuromuscular transmission – Detrusor

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The importance of purines such as adenosine triphosphate (ATP) in neurotransmission is increasingly recognised. In addition to its role as an energy substrate, ATP is a ubiquitous signalling molecule central to the regulation of many biological systems. Purinergic signalling has also been implicated in the genesis of disease, including detrusor overactivity in the human bladder. Detrusor overactivity is characterised by involuntary bladder contractions, urinary urgency and incontinence and is one of the most prevalent causes of morbidity in the community.¹ However, the aetiology is poorly understood, and although anticholinergic drugs are effective in attenuating detrusor contractions, their side effects can be intolerable. The development of a targeted drug model is, therefore, highly desirable.

Contractions of human detrusor elicited by electrical stimulation of motor nerves *in vitro* and of the bladder when stimulated through its nerves *in situ* are reliably abolished by atropine.^{2,3} This indicates that they are generated exclusively by activation of cholinergic muscarinic receptors during neurotransmission. However, as well as responding to neurally released acetylcholine, detrusor

smooth muscle contracts vigorously in the organ bath in response to applications of ATP,² and P_{2x}-purinoceptors have been found widely distributed in detrusor samples.⁴ The human bladder, therefore, appears equipped with the cellular apparatus needed to generate purinergically-mediated contractions, but which is redundant under normal conditions.

New insight into bladder dysfunction has emerged from the observation that detrusor samples taken from idiopathically overactive and obstructed (but not neurogenically overactive or functionally normal bladders) generate nerve-mediated, atropine-resistant contractions. Indirect evidence suggests that these are mediated by neurally released ATP, as they are abolished by α,β -methylene ATP (α,β -mATP), an agent that desensitises detrusor purinoceptors to subsequently administered purinergic agonists.^{2,5} Overactive detrusor, therefore, appears to express dual transmission with acetylcholine and ATP, and these two agents appear to be co-released. In this respect, detrusor from overactive bladders appears analogous to that of many small animals in which purinergic transmission generates a short, sharp contraction, said to be useful during territorial marking behaviour, with cholinergic mechanisms mediating a

Figure 1 Schematic diagram illustrating the mechanisms of contractile activation and associated extracellular electrical activity by acetylcholine and adenosine triphosphate in the detrusor muscle cell.

longer lasting, more tonic, contraction to achieve bladder emptying.⁶ Interestingly, the expression of atropine-resistant activity is lost in more evolutionary advanced mammals, perhaps in association with their tendency to practice continence and more civilised methods of territorial marking behaviour.⁷ The emergence of effective purinergic transmission in overactive human bladders may, therefore, represent a vestigial mechanism unmasked.

Adenosine triphosphate is co-transmitted along with acetylcholine in many signalling systems, and is normally rapidly inactivated in the synaptic cleft by ATPases. Ecto-ATPases are highly sensitive to hypoxia and intracellular acidosis, and exhibit reduced activity in samples of overactive bladders allowing ATP to activate its effector mechanisms.⁸ Overactive detrusor also exhibits structural changes compared with normal detrusor characterised by a patchy infiltration of connective tissue in-between muscle bundles.⁹ These changes render detrusor hypoxic, and this is likely to be particularly evident during bladder filling.¹⁰ There may, therefore, be an association between the structural and functional changes seen in overactive detrusor, however, evidence for expression of purinergic neurotransmission in the overactive bladder is currently indirect, and the role of purinergic mechanisms in the generation of overactive bladder contractions can only be postulated on in the absence of a technique with which to detect them directly in the intact bladder *in situ*.

Electrophysiological studies suggest such a method. In the guinea-pig, cholinergic transmission generates detrusor contractions by mobilising the calcium ions needed to activate the contractile apparatus from intracellular stores using a secondary messenger system, and this occurs independently of changes to membrane potential. In contrast, activation of purinoceptors causes the influx of calcium ions through voltage-gated channels and this depolarises the cell membrane.¹¹ In the guinea-pig, at least, these membrane potential changes cause extracellular electrical activity and this can be recorded from a detrusor strip preparation using the double sucrose gap technique (Fig. 1).¹²

No extracellular electrical recordings have been made from intact human detrusor. However, intracellular recordings from isolated human detrusor cells using micro-electrodes show that the same differential electrical response as in the guinea-pig occurs with cholinergic and purinergic agonists.¹¹ If the same is true of intact human detrusor, overactive detrusor should generate extracellular electrical activity more readily than normal. Recordings of this activity could provide direct evidence for the presence of pathological purinergic transmission in overactive detrusor, and a technique to enable clinical evaluation of the myopathic process by bladder electromyography.

In contrast to electromyography of cardiac and skeletal muscle, which have been developed over many years into advanced clinical sciences, detrusor electromyography has

remained in its infancy. No extracellular electrical activity has been recorded from human detrusor and, despite over 50 years of recording attempts, no electromyographic recordings from animal or human bladders have been verified as real. In fact, no electromyographic recordings from any smooth-muscle containing organ have been validated sufficiently for clinical use. Early studies of bladder electrical activity used steel electrodes embedded in the bladders of conscious volunteers during voluntary voiding. The first published recordings reported a 'bioelectrical bladder wave';¹⁵ however, subsequent studies showed that an identical signal could be recorded in response to bladder wall movement and was, therefore, an artefact. Later studies showed that the striated muscle EMG and electrocardiogram could also contaminate the signal.

In 1976, a careful study in cat bladders stimulated by sacral nerves identified a signal using electrical filters that preceded the onset of bladder contraction and might be real. The investigators found that the most serious contaminant to this signal was electromechanical artefact generated at the tissue electrode interface by minute fluid and tissue movements, as it could be replicated in the dead bladder.¹⁴ Subsequent attempts to record a bladder electromyogram employing sacral nerve stimulation in animals verified this artefact, but the detrusor electromyogram remained elusive.

Detrusor electromyography was developed in the hope that the technique could facilitate the clinical assessment of

detrusor function, and enable direct evaluation of the primary detrusor myopathies and motor neuropathies implicated in the generation of lower urinary tract symptoms. However, human detrusor had only been presumed to generate electromyographic activity by analogy with striated muscle, which, in accordance with its more excitable action and the need for contractions to be under voluntary control, contracts by a different mechanism. The striated muscle EMG is a large extracellular signal reflecting the net potentials generated by transmembrane sodium ion currents in geometrically aligned, synchronously activated muscle fibres. In contrast, the detrusor smooth muscle action potential is calcium ion dependent. If action potentials are generated during detrusor contractions, the associated extracellular currents would be expected to be much smaller than those generated in striated muscle, as the detrusor cell membrane expresses a much lower population of calcium channels than striated muscle does sodium channels.¹⁵ Furthermore, as detrusor fibres appear to be arranged randomly and activated asynchronously rather than as discreet motor units, any extracellular currents would be less likely to summate, and the net potential, therefore, likely to be smaller and more susceptible to contamination by the relatively large electromechanical artefact generated at the tissue electrode interface as the muscle contracts.

The difficulties associated with smooth muscle electromyography could theoretically be overcome by the use of electrically reversible suction electrodes. These allow elec-

Figure 3 Representative traces of electrical and mechanical activity recorded from an isolated whole guinea-pig bladder showing the electrical signal (circled) unchanged by repetitive stimulated contractions (A) and reversibly sensitive to α,β -mATP (B).

trical recordings to be made from a bleb of tissue immobilised within a suction field, thereby minimising mechanical artefact while tension measurements are made from the remainder of the contracting bladder. In addition, the use of electrically reversible electrodes would be likely to optimise recordings of a real signal by reducing their impedance to low-frequency signals thereby minimising their contamination by electromechanical artefact.

We first set out to determine whether the extracellular electrical activity known to be generated by purinergic neurotransmission in guinea-pig muscle strips could be recorded electromyographically from the whole contracting organ using suction electrodes. We designed and built a reversible bi-polar suction electrode with a recording aperture of 1.5 mm, and a novel organ bath apparatus to enable simultaneous electrical and mechanical recordings from whole, excised guinea-pig bladders during electrical stimulation by an isolated bladder neck stimulator (Fig. 2).

Experiments were conducted to test the hypothesis that any signal recorded conformed to previously published criteria designed to validate the signals as real reflections of transmembrane electrical activity associated with contractile activation.¹⁶ We recorded a predominantly bi-phasic electrical signal from six guinea-pig bladders.¹⁷ The signal preceded the onset of contraction and was clearly separate from the stimulus artefact and abolished by neurotoxins.

The signal was not affected by repetitive stimulation resulting in summated contractions, as would be expected were there a contribution to it from bladder movement, and the signal was sensitive to graded reduction in the calcium chloride content of the superfusate indicating it was biological in origin (Fig. 3). The signal was also abolished by α,β -mATP but not by atropine; however, the precise relationship between the signal and neuromuscular blocking agents and tension generation could not be determined in this relatively crude preparation as the bladder could only be superfused on its serosal aspect, and adequate penetration of pharmacological agents into the detrusor wall was uncertain.¹⁷

To quantify more precisely the relationship of the signal to tension generation and neuromuscular blocking agents, and to validate an experimental set-up for recording from human detrusor *in vitro*, the recording apparatus was adapted to recording from urothelially denuded detrusor strips. In the subsequent experiments, a very similar electrical signal to that recorded from the whole bladder was recorded from 37 detrusor strips, and was shown to be sensitive to graded reduction in calcium ion content of the perfusate and abolished by calcium-ion channel blocking agents.¹⁸ The signal was not affected by atropine although the contraction was reduced, and was reliably abolished by purinoceptor desensitisation and neurotoxins indicating that it originated from a nerve-mediated purinergic mechanism.¹⁸

We then tested the hypothesis that purinergically mediated contractions of overactive human detrusor generated extracellular electrical activity using the validated detrusor strip and suction electrode recording apparatus. We obtained bladder samples from clinically characterised human bladders during augmentation cystoplasty and cystectomy with local ethical committee approval. We recorded simultaneous extracellular electrical and mechanical activity from detrusor strips during electrical stimulation. The degree of nerve-mediated, atropine-resistant activity generated by each sample was recorded by a second investigator from smaller strips taken from the same bladder samples using a previously validated experimental set-up.¹⁹

We recorded a bi-phasic electrical signal that was similar in morphology, but much smaller, than that recorded from guinea-pig from samples of five human bladders. We characterised the signal as before, and showed that it was separate from stimulus and movement artefact, and abolished by neurotoxins. In all cases, the signal could be abolished by α,β -mATP in association with attenuation of the contraction, but was not affected by atropine, indicating that it was generated by a purinergic mechanism (Fig. 4).

The bladder samples from which an electrical signal was recorded generated significantly greater atropine-resistant activity than those from which no signal could be recorded, suggesting that the signal was coupled to atropine-resistant

tension generation, and atropine-resistant activity was significantly greater in samples of overactive detrusor compared with controls.

Figure 5 shows the proportion of the total contraction that was resistant to atropine for bladder samples taken

from clinically normal and overactive bladders, and its relation to the expression of an electrical signal. An electrical signal was only recorded from samples of overactive bladders in association with the expression of atropine-resistant contractions, and from those samples expressing the greatest atropine-resistant activity. In most overactive samples not expressing a signal, very little real atropine resistance was found. No signal was recorded from any completely atropine-sensitive tissue, although these samples generated contractions of a similar magnitude as those generating a signal. These experiments showed that a purinergically mediated extracellular electromyographic signal coupled to nerve-mediated, atropine-resistant tension generation could be recorded from overactive but not functionally normal human bladder.

Conclusions

Normal human detrusor smooth muscle appears electromyographically inert. In contrast, the functional emergence of pathological purinergic neurotransmission in overactive detrusor generates recordable extracellular electrical activity. These studies demonstrate for the first time recordings of biological electrical activity from contracting human detrusor smooth muscle. Moreover they provide direct evidence supporting the presence of a functional purinergic myopathy in the overactive human bladder, and suggest that extracellular electrical recording methods, if appropriately developed to enable clinical bladder electromyography, could enable clinical detection of such a myopathy and the evaluation of novel drugs targeted against it.

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