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Changes in Cholinergic and Purinergic Neurotransmission in the Diabetic Rabbit Bladder

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Abstract. *Background: Diabetes mellitus (DM)-associated alterations in bladder function have been attributed to changes in autonomic receptors and alterations in detrusor structure and function. The changes in cholinergic and purinergic neurotransmission in the DM rabbit bladder were evaluated. Materials and Methods: DM was induced with alloxan in adult male New Zealand White rabbits. At 6 months, detrusor and bladder neck muscle strips were obtained and mounted in organ baths. Transmural electrical field stimulation (EFS: supramaximal voltage, 0.1 ms duration, 10 s trains) was performed in the presence of atropine (10^{-6} M) or alpha, beta-methylene ATP (10^{-6} M), and after adding tetrodotoxin10^{-6} M. Purinergic, alpha, beta-methylene ATP-sensitive, and cholinergic, atropine-sensitive, components were calculated independently and compared with those from controls. Results: Both normal and DM detrusor and bladder neck strips contracted in a frequency-dependent fashion in response to transmural EFS. A plot of EFS vs. detrusor contractility showed a decrease (ANOVA <0.001) in the cholinergic nerve-mediated component, whereas the purinergic nerve-mediated component was increased (ANOVA <0.001) in the DM detrusor compared to the control. The total EFS- and KCl-induced responses were unaltered in the DM group compared to the controls. There was no difference in purinergic, alpha, beta-methylene ATP-sensitive, and cholinergic, atropine-sensitive, components in strips from the bladder neck for both normal and DM rabbits. Conclusion: These results suggest that an enhancement of purinergic and a reduction of cholinergic neurotransmission occur in the detrusor muscle of the diabetic rabbit. These changes may contribute to the pathophysiology of diabetic cystopathy.*

Alterations in bladder function are well recognized in diabetes mellitus (DM), with an overall reported prevalence of 40% to 90% (1). The changes have been attributed to autonomic neuropathy, changes in autonomic receptors and alterations in detrusor muscle structure and function. The changes result in a large bladder capacity, increased residual urine, detrusor areflexia, low flow rate, detrusor instability and, hence, more risk of urinary infection (1, 2).

The postganglionic neurotransmission in the mammalian urinary bladder appears to be dual in nature; an atropine-sensitive (cholinergic) and atropine-resistant (non-cholinergic) component. The proportion and importance of each in the micturition process is species-dependent and is influenced by the functional integrity of the bladder (3). In 1972, Burnstock *et al.* proposed that ATP was the key neurotransmitter released from purinergic nerves (4). It is now well established that lower mammals have a significant purinergic component in their nerve-mediated detrusor contraction (3). In contrast, in the healthy human bladder, the purinergic component was less than 5% (3).

Our group demonstrated an increase in the purinergic nerve-mediated detrusor contraction in the early stages of bladder outlet obstruction (BOO) in a rabbit model (5). The morphological and functional responses of the bladder to stresses induced by polyuria secondary to DM and BOO showed some similar characteristics (6). Hence, the purpose of the present study was to examine whether alterations in cholinergic and purinergic signalling are evident in the DM bladder.

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Materials and Methods

α,β-methylene ATP, KCl and tetrodotoxin were supplied from Sigma Chemical Co. (Poole, UK). Atropine sulphate was obtained from Antigen Pharmaceuticals Ltd. (Roscrea, Ireland).

Our study was approved by the U.K. Home Office. DM was induced by a single intravenous injection of alloxan monohydrate (65 mg/kg) into an ear vein of adult male New Zealand white rabbits. Control animals were injected with the vehicle alone (normal saline). There was no significant mortality from the alloxan treatment. The rabbits were classified as diabetic and only accepted for our study if their serum glucose concentrations were 16 mM/l or greater throughout the study. At 6 months, the animals (6 control and 6 diabetic) were killed by cervical dislocation. Six strips were used from each animal.

Muscle strips, measuring approximately 1 x 1 x 5 mm, were excised from the dome and neck of the bladders. One end was attached to a fixed hook, while the other was attached to a force transducer (FT-03C, Grass Instruments, Quincy, Massachusetts, USA, using a Grass Polygraph, model 79). The strips were bathed in Tyrode's solution maintained at 37˚C by a thermo-regulated circuit and bubbled with a mixture of 95% O_2 and 5% CO_2 at pH 7.4. The Tyrode's solution had a composition (mM) of NaCl 118, $NaHCO_3$ 24.0, KC1 4.0, $CaCl_2$ 1.8, NaH_2PO_4 0.4, $MgCl_2$ 1.0, glucose 6.1 and Na pyruvate 5.0. An initial tension of 2 g (detrusor) and 1 g (bladder neck) was applied and the strips were allowed to equilibrate for 30 min, after which contractions were induced by electrical field stimulation (EFS). The field was generated between two circular electrodes surrounding each end of the tissue in 10 s trains of 0.1 ms width pulses. In each experiment, the contractions were measured at 0.5, 1, 2, 5, 10, 20 Hz. This was repeated after the addition of atropine (10 mM) or α,β-methylene ATP (10 μM) and finally after the addition of tetrodotoxin (10 μM) following incubation for 30 min each. Contractions induced by potassium chloride (KCl, 124 mM) were also measured for each tissue at the end of each experiment.

The tissue used in the organ bath studies was weighed and contractile responses expressed as tension g/g of tissue by dividing the amount of contraction occurring on exposure to an agent by the weight of the tissue.

The "purinergic" or "cholinergic" component was independently calculated for each frequency as the proportion of the total contraction inhibited by α,β-methylene ATP (10 μM) or atropine (10 μM), respectively, divided by the proportion of the total contraction inhibited by tetrodotoxin to allow for any directly stimulated component of the muscle contraction.

Statistical analysis. All contractile responses for the EFS studies are expressed as mean±SEM. PRISM (Graph Pad Inc., USA) software was used for statistical analysis. Comparisons were made using a 2-way analysis of variance (ANOVA) with statistical significance accepted at $p<0.05$.

Results

Response to KCl. The mean contraction in response to KCl was 88.4±4.5 g tension/ g tissue (controls and DM, n=12 each). There was no significant difference between the response of muscle strips to KCl from the neck and dome (n=12 each). There was no significant difference between the response to KCl of the detrusor from the control and DM rabbits (n=12).

Cholinergic component of EFS-induced contraction. The component of the EFS-induced contraction that was inhibited by atropine was independently calculated for each muscle strip, at each frequency. A plot of "cholinergic component" against EFS-frequency for both the control and DM groups' detrusor strips is shown in Figure 1. At 0.5 Hz the cholinergic component was 34% in the control and 28% in the DM group, while at 20 Hz it was 70% in the control and 49% in the DM group. The cholinergic component was significantly smaller in the DM muscle strips than in the control muscle strips (2-way ANOVA, $p<0.0001$, n=12). There was no difference in the cholinergic component of muscle strips from the neck compared with those from the dome in both groups (n=12).

Purinergic component of EFS-induced contraction. The component of EFS-induced contraction that was inhibited by α,β-methylene ATP (10 μM) was also independently calculated for each muscle strip, at each frequency (Figure 2). At 0.5 Hz the mean purinergic component was 40% in the control group and 62% in the DM group, while at 20 Hz the mean purinergic component was 23% in the control group compared to 34% in the DM group. The purinergic component was significantly greater in the muscle strips from the DM bladder detrusor compared with those from the control bladder (2-way ANOVA, $p<0.0001$, n=12). There was no significant difference between the purinergic components of muscle strips from the bladder neck compared with those from the dome from the two groups (n=12 each).

Discussion

Our results demonstrated that the cholinergic component of the nerve-mediated (tetrodotoxin-sensitive) detrusor contraction decreases in the DM rabbit bladder. Conversely, the purinergic component, mediated by ATP (α,β-methylene ATP -sensitive), was increased. Our findings are supported by Moss *et al.* (7), who demonstrated a significant increase in the sensitivity of the detrusor to ATP and a decreased response to acetylcholine in bladders from 8-week-old diabetic rats.

There is evidence of a similar pattern of altered purinergic and cholinergic bioactivities in human interstitial cystitis (IC). Palea *et al.* (8) demonstrated the presence of the purinergic contraction (not detected in normal detrusor muscle) in addition to a decreased cholinergic activity in detrusor muscle specimens obtained from patients with IC. Our group demonstrated that the purinergic component of

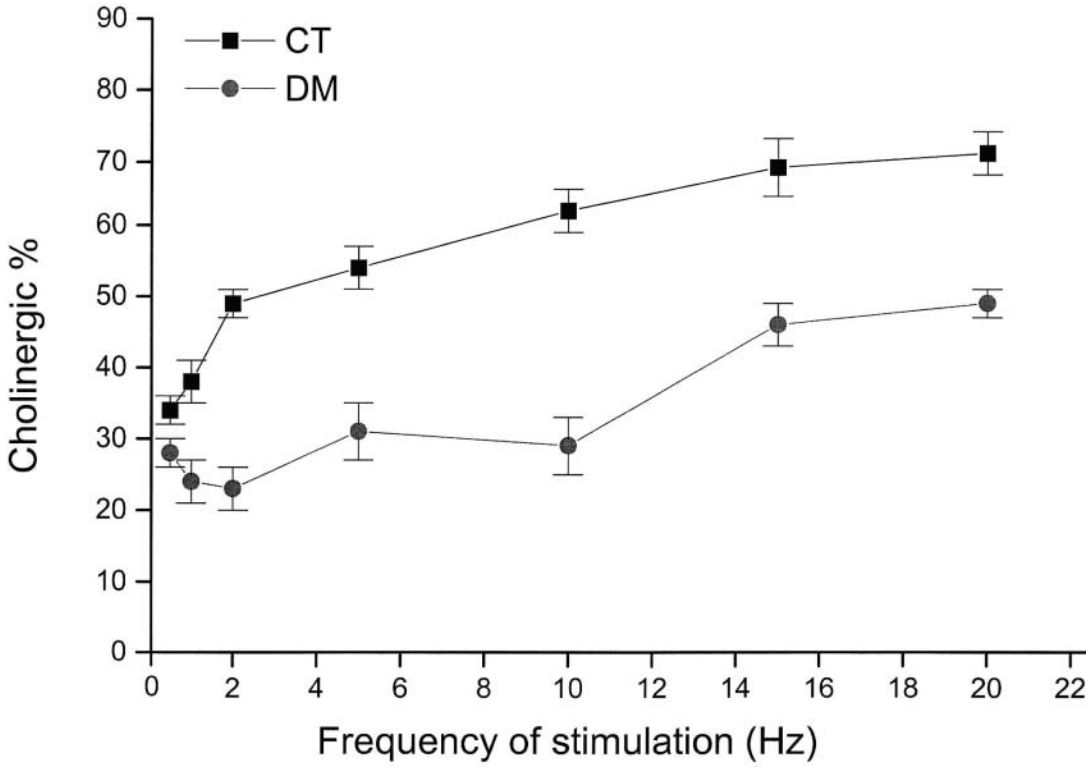

Figure 1. *Cholinergic component of nerve-mediated detrusor contraction at various electrical stimulation frequencies in controls and rabbits with DM (mean ± SEM).*

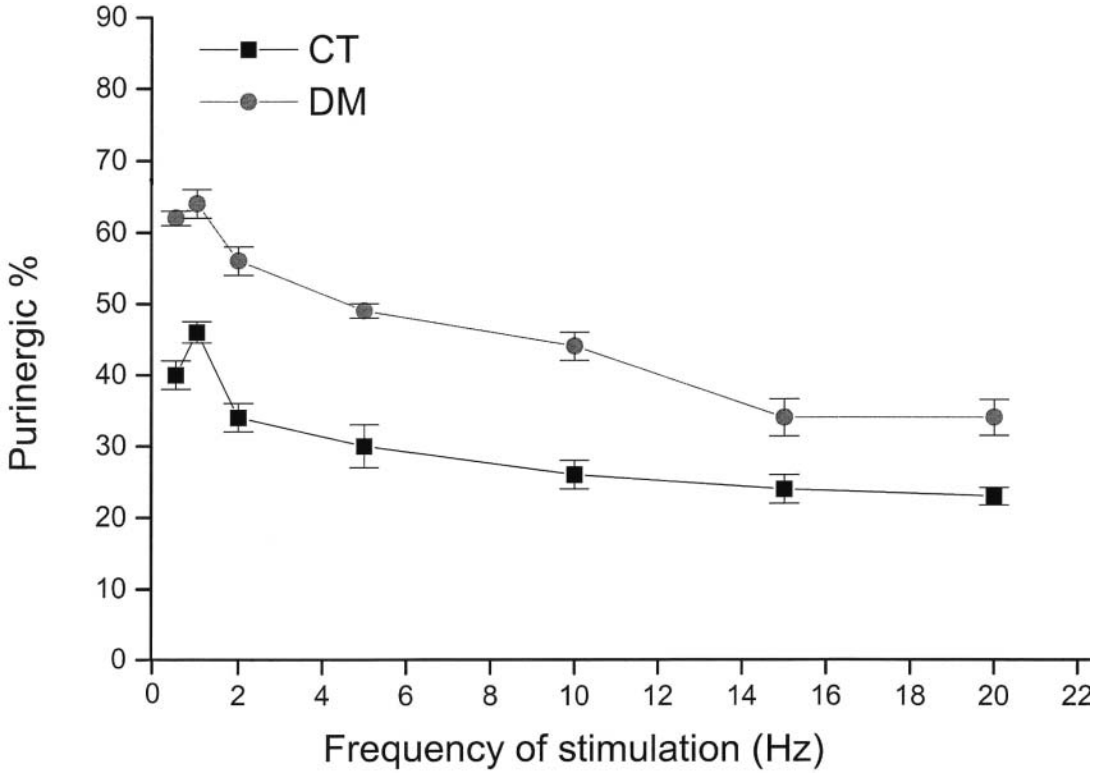

Figure 2. *Purinergic component of nerve-mediated detrusor contraction at various electrical stimulation frequencies in controls and rabbits with DM (mean ± SEM).*

nerve-mediated detrusor contraction is increased and the cholinergic component is decreased in the early stages of BOO in rabbits (5). It is not surprising, therefore, that the three pathological conditions mentioned (DM, IC and BOO) share some similar clinical features of bladder dysfunction.

Previous studies indicated the presence of compensatory mechanisms affecting the parasympathetic pathway in DM detrusor. These included the up-regulation of the muscarinic receptors and an increased 'turnover' of acetylcholine in rat DM detrusor (9, 10). Latifpour *et al.* (9) and Wahba *et al.* (10) showed an up-regulation of muscarinic receptors in the bladder dome (rat) using receptor binding studies with [^{3}H]quinuclidinyl benzilate and significant increases in both choline acetyltransferase (ChAT) and acetyl-cholinesterase (AChE) activities in DM detrusor (rat) compared to the control animals, respectively. Here, we showed a functional decrease of the cholinergic pathway in the detrusor muscle in a rabbit model of DM. It is possible that, if the muscarinic receptor in the detrusor muscle is up-regulated in rabbit DM, it may be compensating for decreased receptor sensitivity to acetylcholine. The increase in the purinergic component shown in this study is possibly another compensatory response to a reduction in cholinergic bioactivity in the DM bladder.

The purinergic pathway is also involved in urothelial mechanoafferent transduction (11). Released ATP from the urothelium during bladder distension (12) acts on P2X2/3 receptors on subepithelial sensory nerves (nociceptive) (13) to provide mechanosensory feedback affecting bladder pain and the micturition reflex (14). It is possible that enhanced neuronal ATP release may act additively/synergistically with that derived from the urothelium which contributes to the clinical features of bladder dysfunction including detrusor instability, which is often associated with DM.

In conclusion, our findings support the evidence that purinergic signalling plays a pathophysiological role in the DM bladder. Therefore, targeting the purinergic pathway might be useful in the treatment of detrusor instability in DM.

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