ALTERED CAMP-MEDIATED POTASSIUM CHANNEL CURRENTS IN DETRUSOR MYOCYTES FROM DIABETIC RATS

Hypothesis / aims of study
More than 50% of diabetic patients have bladder dysfunction that is associated with a range of cystopathic (i.e., bladder) conditions. Detrusor overactivity is a frequent urodynamic finding, with a reported prevalence of 39% to 76%. The goal of these studies was to further explore the myogenic basis for diabetes (DM)-related bladder dysfunction. In this regard, potassium (K⁺) channels regulate smooth muscle excitability by maintaining the resting membrane potential and promoting repolarization of the cell membrane.[2,3] The second messenger signalling molecule cyclic AMP (cAMP) has been implicated in the modulation of potassium channel activity in smooth muscle cells. Our hypothesis is that alterations of K⁺ channel function play an important role in the pathogenesis of a number of smooth muscle-related diseases including the urologic complications of diabetes. Therefore, the aim of this study was to investigate cAMP-mediated regulation of K⁺ channel function in detrusor myocytes in diabetic, insulin-treated and age-matched control rats using patch clamp techniques.

Study design, materials and methods
We evaluated K⁺ channel function and cAMP-mediated regulation in short term cultures of detrusor myocytes derived from urotheilal denuded bladders of 2-month streptozotocin (STZ)-diabetic (DM) and age-matched control rats. Cell cultures (passages 1-3) were derived from male F344 rats in the following 3 groups, DM (n=4 rats), insulin (IN)-treated DM (n=4 rats), and an age-matched control group (AMC; n=4 rats). Whole-cell membrane currents were recorded using a conventional patch clamp technique. Cells were voltage clamped at -70 mV and I-V curves were performed in 10 mV increments ranging from -60 mV to +100 mV. Cells were subjected to 6 consecutive I-V curves. Following a control I-V curve, cells were exposed to the following drugs at 10-15 minute intervals: 1) 1 mM 8-Br-cAMP, 2) 100 nM IBTX (BK block), 3) 10 μM glybenclamide (K_ATP block), 4) 100 μM 4-AP (K_v block), and 5) 100 nM apamin (SK block). The voltage protocol was generated by pclamp9 (Axon Instruments). The recordings were stored as pclamp9 files with a four-channel digitizing unit, and analyzed with clampfit9.2.

Results
We observed a statistically significant DM-related, insulin (IN)-reversible, increase in resting membrane potential (from -45 ± 3 (AMC) to -33 ± 2 mV (DM) to -40 ±5 (IN)) and a corresponding decrease in cAMP-induced whole cell K⁺ current and current density (p<0.05, 2-Way ANOVA for repeated measures) (Table 1; Figure 1). A significant DM-related decline in the IBTX-sensitive portion of the whole cell K⁺ current, and a significant increase in the 4-AP sensitive portion of the whole cell outward K⁺ currents (p<0.05) was also noted.

Table 1. Impact of cAMP on whole cell outward K currents in detrusor myocytes.

<table>
<thead>
<tr>
<th></th>
<th>Current (pA)</th>
<th>Capacitance (pF)</th>
<th>Current density (pA/pF)</th>
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</thead>
<tbody>
<tr>
<td></td>
<td>Before cAMP</td>
<td>After cAMP</td>
<td>Before cAMP</td>
</tr>
<tr>
<td>AMC (n=11)</td>
<td>359±52</td>
<td>721±100</td>
<td>26±2.6</td>
</tr>
<tr>
<td>DM (n=14)</td>
<td>260±34</td>
<td>400±45</td>
<td>29±2</td>
</tr>
<tr>
<td>IN (n=11)</td>
<td>332±52</td>
<td>555±53</td>
<td>33±2.5</td>
</tr>
</tbody>
</table>

a: Significantly different from control in the same group (Two Way RM ANOVA).
b: Significantly different from same condition in AMC (Two Way RM ANOVA).

Figure 1. Mean whole cell outward currents observed in short term cultures of detrusor myocytes from STZ-diabetic and age-matched control rats. As indicated, there was a quite dramatic decrease in the maximal amplitude of both the baseline outward K⁺ currents and the cAMP-mediated K⁺ currents. "-" denotes a statistically significant increase over baseline.
Interpretation of results
There are at least 4 distinct K channel subtypes found in detrusor myocytes that are apparently differentially impacted by STZ-diabetes. Of note, these studies document significant DM-related alterations in the resting potential and whole cell cAMP-stimulated K+ currents and current density. Such observations are consistent with increased detrusor myocyte excitability. These data provide novel mechanistic insight into diabetes-related changes in detrusor myocytes that may be of therapeutic value.

Concluding message
These studies indicate the presence of a significant diabetes-related K channelopathy in detrusor myocytes that may contribute to increased detrusor myocyte excitability, and thus explain, at least in part, diabetes-related detrusor overactivity.

References

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Is this a clinical trial? No

What were the subjects in the study? ANIMAL

Were guidelines for care and use of laboratory animals followed or ethical committee approval obtained? Yes

Name of ethics committee Wake Forest University ACUC