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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%.[1] 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 urothelial 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.

	Current (pA)		**Capacitance (pF)**	**Current density (pA/pF)**	
	Before cAMP	After cAMP		Before cAMP	After cAMP
AMC (n=11)	359±52	721±100[a]	26±2.6	15.3±3	32±7.2[a]
DM (n=14)	260±34	400±45[a,b]	29±2	9.4±1.5	14±1.8[b]
IN (n=11)	332±52	555±53[a]	33±2.5	11.3±2.2	18.9±3[a,b]

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).

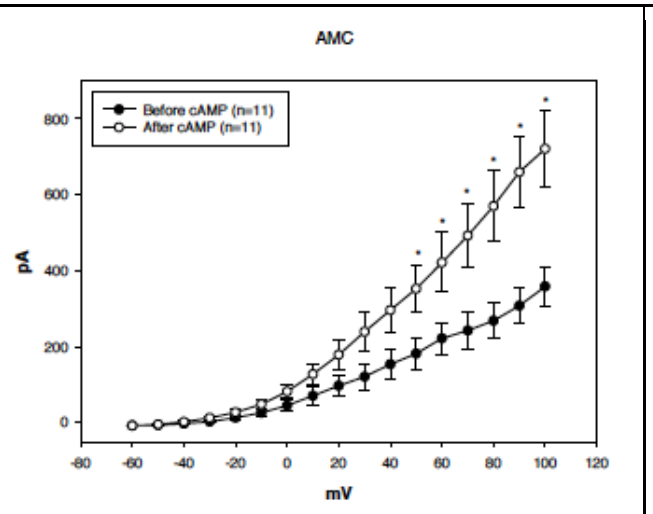
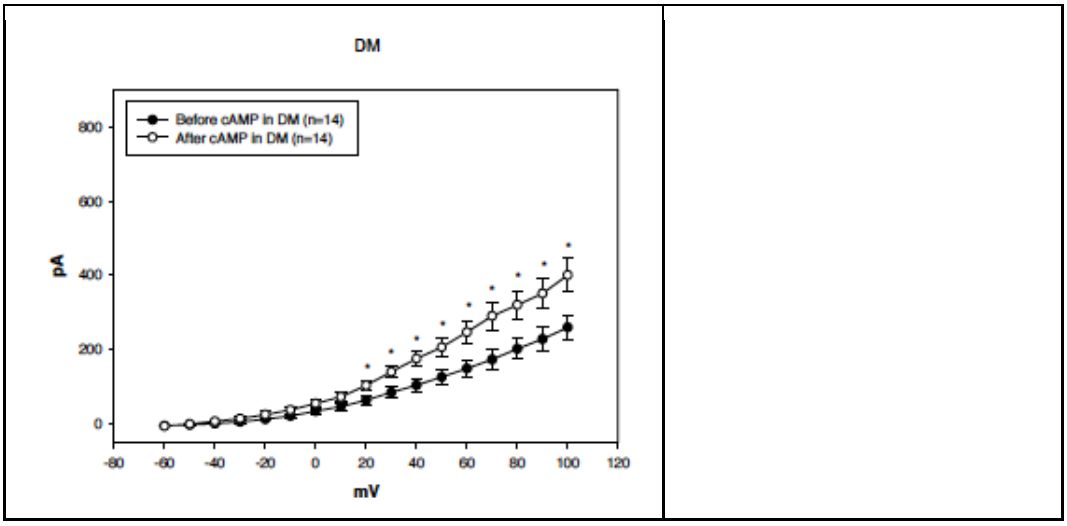


Figure 1. Mean whole cell outward currents observed in short term cultures of detrusor myocytes from STZ-diabetic and age-matched control rats. As indicated, there was a quite dramatic decrease in the maximal amplitude of both the baseline outward K^+ currents and the cAMP-mediated K^+ currents. *-denotes 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

1. Sarma AV, Kanaya AM, Nyberg LM, Kusek JW, Vittinghoff E, Rutledge B, Cleary PA, Gatcomb P, Brown JS. Urinary incontinence among women with type 1 diabetes--how common is it? J Urol. 181(3):1224-30, 2009
2. Thorneloe KS, Nelson MT. Ion channels in smooth muscle: regulators of intracellular calcium and contractility. Can J Physiol Pharmacol. 83(3):215-42, 2005
3. Hristov KL, Cui X, Brown SM, Liu L, Kellett WF, Petkov GV. Stimulation of beta3-adrenoceptors relaxes rat urinary bladder smooth muscle via activation of the large-conductance Ca2+-activated K+ channels. Am J Physiol Cell Physiol. 295(5):C1344-53, 2008

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Name of ethics committee	**Wake Forest University ACUC**