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THE PHYSIOLOGICAL ROLE OF THE DIACYLGLYCEROL INTRACELLULAR SIGNALLING PATHWAY IN HUMAN DETRUSOR MYOCYTES.

Aims of Study

Muscarinic (M3) receptor activation in human detrusor muscle results in contraction via the production of inositol trisphosphate from membrane-bound phosphatidyl 4,5 bisphosphate (PIP-2). However, PIP-2 breakdown also produces diacylglycerol (DAG) which remains in the vicinity of the cell membrane. In other cell types DAG influences the generation of cAMP (1,2) which itself may have a modulatory effects on intracellular Ca^{2+} and contraction. This accessory pathway has not been investigated in detrusor but offers another potential route for the therapeutic manipulation of detrusor function. We have investigated this intracellular pathway by the use of forskolin (a surrogate for DAG) and directly increased intracellular cAMP using the permeant analogue dibutyryl cyclic AMP (db-cAMP).

Methods

Human bladder biopsies were obtained with Local Regional Ethical Committee approval and informed patient consent. Fresh human detrusor myocytes were dissociated using collagenase digestion. Cultured human detrusor smooth muscle cells from human bladder biopsies were cultured as described previously (3). Experiments were carried out at 37°C in a HCO_3^-/CO_2-buffered Tyrode's solution. Intracellular Ca^{2+} was measured by epifluorescence microscopy with the fluorochrome Fura-2. Intracellular Ca^{2+}-transients were evoked by the addition of 30 µM carbachol to the superfusate. The effect of 1 nM forskolin or 1 nM db-cAMP on the resting intracellular Ca^{2+} and the magnitude of the carbachol-evoked Ca^{2+}-transients were measured. Data are mean±s.d., n=number of interventions, significance between sets ($p<0.05$) was evaluated with Student's *t*-test. . $[Ca^{2+}]$ are expressed as pCa ($=-\log_{10}[Ca^{2+}]$) values throughout.

Results

The resting pCa ($-\log_{10}[Ca^{2+}]$) was 6.97±0.11 (n=28) in freshly isolated cells and 6.91±0.08 (n=35) in cultured cells; these mean values were not significantly different. Both forskolin and db-cAMP significantly increased the resting intracellular Ca^{2+} (decreased the pCa) and the changes of pCa (?pCa) are shown in table 1. In freshly isolated cells both db-cAMP and forskolin had no significant effect on the magnitude of the carbachol-induced Ca^{2+}-transient (±dbcAMP; ?pCa 0.89±0.37 vs 0.78±0.14 units, n=10: ±forskolin; ?pCa 1.29±0.30 vs 1.19±0.61 units, n=9). However, in cultured cells dbcAMP increased the Ca^{2+}-transient magnitude (?pCa 1.04±0.20 vs 1.43±0.14 units, n=17), whilst forskolin had an opposite effect (?pCa 0.73±0.14 *vs* 0.45±0.09 units, n=14).

Table 1 The change of resting Ca^{2+} (?pCa) generated by forskolin and db-cAMP

Intervention	**Freshly isolated cells ?pCa**	**Cultured cells ?pCa**
1 nM Forskolin	-0.59±0.31	-0.55±0.16
1 nM dbcAMP	-0.29±0.12	-0.29±0.17

Conclusions

Both interventions led to a significant rise in the resting intracellular $[Ca^{2+}]$. In freshly isolated cells regulation of the intracellular cAMP pathway had no effect on the magnitude of the carbachol-induced Ca^{2+} transient indicating that this route exerts no net effect on store-mediated intracellular Ca^{2+} release. However, in cultured cells db-cAMP increased the transient magnitude possibly due to an enhanced loading of intracellular stores in these cells. The reduction of the Ca^{2+} transient magnitude by forskolin may represent an additional, as yet unrecognized, effect on these cells.

References

1. Burt et al *Br J Pharmacol* 1998; 123: 317-25
2. Pfeiffer et al *Am.J.Physiol* 1998; 274: C663-72
3 Sui *et al J Urol* 2001; 165: 627-632