NOVEL APPROACH IN UROLOGICAL RESEARCH REVEALS DIFFERENTIAL ION CHANNEL SUBCELLULAR LOCALIZATION IN HUMAN URINARY BLADDER SMOOTH MUSCLE

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INTRODUCTION

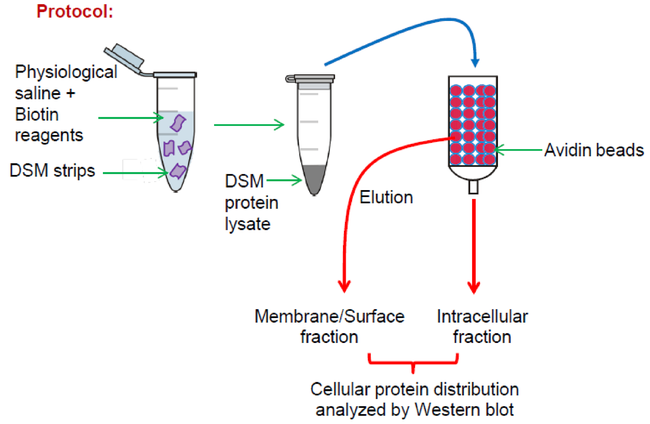
Contraction and relaxation of detrusor smooth muscle (DSM) control micturition. DSM cell excitability and contractility depend on synchronized activity of multiple ion channels types. Our P20 Center group, in collaboration with urologists, has the unique advantage of studying the expression, function, and regulation of human DSM ion channels. Here, we have focused on key DSM channel families including, the TRPM (activation of which causes membrane depolarization and contraction), voltage-gated Kv7 and K_{Ca} channels (the activation of which causes hyperpolarization and relaxation). To exert their regulatory role, these channels would be expected localize to the DSM plasma membrane (surface) but so far investigations on this concept are lacking. Additionally, molecular and cellular mechanisms that control the expression and trafficking of ion channel subunits are unknown. To test the surface expression hypothesis, we selected the four most important family member representatives, the TRPM4, Kv7.4 and Kv7.5 and BK/K_{Ca}1.1 channels. We also examined trafficking mechanisms of the pore-forming α subunit of BK/K_{Ca}1.1 channel and their regulation by muscarinic and protein kinase-A (PKA) signaling pathways in DSM.

OBJECTIVE

To examine the overall plasma membrane versus intracellular localization and regulation of cell surface expression of important regulatory ion channels in the DSM using the novel technique called **'surface biotinylation'** in conjunction with immunocytochemistry.

METHODS

The reagents and protocol we employed for performing '***surface biotinylation***' are listed in the diagram below. DSM tissue strips were incubated with non-cell permeable biotin tagged reagents that specifically bind to cysteine and lysine protein residues. Biotinylated surface proteins were then separated using avidin beads, eluted and Western blotting performed to determine the overall surface to intracellular localization of these channel proteins. Immunocytochemistry analyses were also performed on freshly isolated DSM cells. Immunohistochemistry analyses for biotin-tagged proteins was performed on biotinylated and PFA-fixed DSM strips.



RESULTS

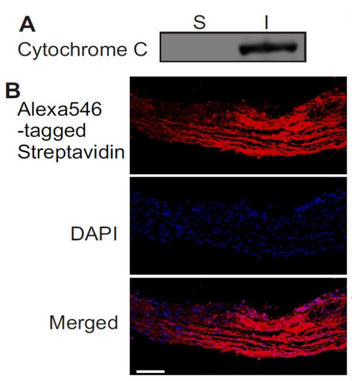


Fig. 1. Validation of the DSM surface biotinylation protocol ***(A)*** Representative Western blot after surface biotinylation from DSM showing localization of the mitochondrial protein, cytochrome-C, which indicates that intracellular proteins are not biotinylated. *S- Surface fraction, I- Intracellular fraction.* ***(B)*** Immunofluorescence of whole DSM showing biotin tagged protein throughout all DSM layers. Scale = 100 µm.

RESULTS

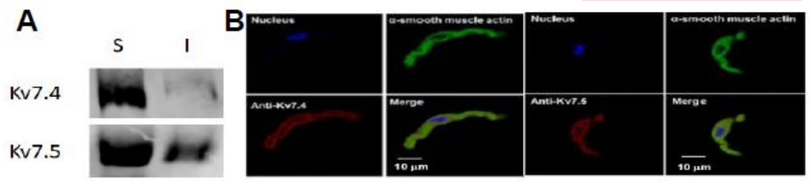


Fig. 2. ***(A)*** Western blot detection of surface plasma membrane (S) and intracellular (I) fractions, obtained using biotinylation assay, for Kv7.4 and Kv7.5 channel proteins in **human** DSM tissue. >82% of total Kv7.4 and ~66% of total Kv7.5 proteins were also localized on the surface of DSM cells. Interestingly, the Kv7.5 distribution surface/intracellular (~66%/34%) ratio was the lowest among the three channels studied. ***(B)*** Immunocytochemistry data analyses revealed that Kv7.4 channels were predominantly surface localized while Kv7.5 showed both membrane and intracellular staining.

Fig. 3. Western blot detection of surface plasma membrane (S) and intracellular (I) fractions, obtained using biotinylation assay, for TRPM4 in **human** DSM tissue. >85% of total TRPM4 protein was localized to the surface of **human** DSM cells, with only ~15% appearing in the intracellular fraction.

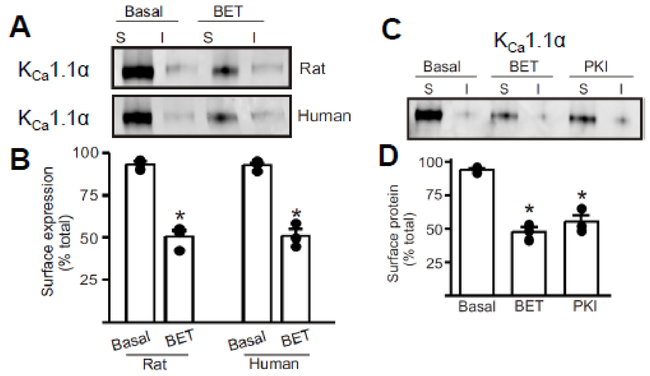


Fig. 4. ***(A)*** Representative Western blots after surface biotinylation from rat and **human** DSM showing localization of BK/K_{Ca}1.1α subunits under basal conditions and after treatment with bethanechol (BET). ***(B)*** mean surface protein (S) levels. *P<0.05 vs basal. ***(C)*** Representative Western blots after surface biotinylation of DSM showing the effects of BET and Protein Kinase A Inhibitor (PKI). ***(D)*** mean surface protein levels. *P<0.05 vs basal.

SUMMARY AND CONCLUSIONS

By employing ***surface biotinylation***, a novel approach in urological research, along with immunocytochemistry, we revealed differential expression of ion channel subunits in human DSM. We also revealed that in rat and human DSM, BK/K_{Ca}1.1α subunits are primarily surface localized and that muscarinic receptor and PKA signaling pathways differentially modulate the DSM BK/K_{Ca}1.1α subcellular localization. These exciting new data offer vital clues to the relative importance of the TRPM4 and Kv7 channels and reveal paradigm-shifting novel regulatory pathways of BK/K_{Ca}1.1α ion channel subunit trafficking mechanisms that regulate human urinary bladder function.

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