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THERMAL RECEPTORS IN GU TRACT: CHANGES IN NEUROGENIC BLADDER MODELS

Hypothesis / aims of study

Overactive bladder symptoms due to various etiologies have been successfully treated with agents such as capsaicin or resiniferatoxin, which target a temperature sensitive receptor, transient receptor potential vanilloid 1 (TRPV1). Recently, another member of the transient receptor potential (TRP) channels, TRPM8, which is activated by menthol and cool temperature (10-25 °C), has been described. This receptor is likely the proposed "cool" receptor targeted in the ice water test, i.e. bladder contraction in response to cold water for patients with upper motor neuron lesions Studies in spinalized and non-spinalized cats have noted c-fiber activity in response to intravesically infused cold water or menthol, but the receptor mediating this activity had not yet been elucidated.

The aim of our study was to define the sites of mRNA and protein expression of TRPM8 and TRPV1 in the rat and human genitourinary (GU) tract, and to evaluate the quantitative expression of TRPM8 and TRPV1 mRNA in the bladders of rats with spinal cord injury (SCI) and diabetes mellitus (DM).

Study design, materials and methods

Prostate, testicular, penile, bladder and dorsal root ganglia (DRG L6-S1) tissue was obtained from male and female Sprague-Dawley rats. Tissue samples from the prostate, testicle, seminiferous tubules, corpus cavernosum, glans, overlying glans skin, scrotal skin, and bladder were obtained from human patients undergoing TURP, radical retropubic prostatectomy, and gender reassignment. Most human bladder specimens were obtained from the posterior bladder neck and separation of urothelium from detrusor layers was performed using microdissection techniques. Good separation of bladder layers was assured by H&E histochemistry.

Twelve female Sprague-Dawley rats underwent spinal cord transection at the T 8-9 level and three were sacrificed at each time point (3 days, 7 days, 14 days, and 21-28 days post-spinalization). Their bladders were collected as well as bladders from three normal controls. Four rats had DM (blood glucose ≥ 350 mg/dl) induced by a single intra-peritoneal injection of streptozotocin (65 mg/kg) and were sacrificed either at 26 days or 10 weeks. Their bladders were also compared to normal control bladders (n=3).

Total RNA was extracted from every tissue and subsequent reverse transcription-polymerase chain reaction (RT-PCR) was carried out using species-specific primers for TRPM8, TRPV1, and GAPDH as an internal loading control. cDNA amount and cycle number were adjusted to render submaximal amplification for all three genes analyzed.

Rat tissues were fixed in 4% paraformaldehyde, cryopreserved in sucrose gradient and cryosectioned to 10 µm. TRPM8 was detected by standard immunofluorescence.

Results

TRPM8 and TRPV1 mRNA were detected in the rat prostate, testicle, penis, bladder and DRG. Human samples demonstrated TRPM8 mRNA in prostate, testicle, seminiferous tubules, scrotal skin, and bladder. No TRPM8 mRNA was identified in human corpus cavernosum, glans, or overlying glans skin. Location of TRPM8 transcripts in the human bladder was further defined by separation of layers and demonstrated mRNA only in the urothelium and not in the detrusor. TRPV1 mRNA was detected in all human GU tract tissues. Clearly positive staining was noted in most DRG neurons, in scattered prostatic stroma cells, and in the most luminal side of bladder urothelium. Other rat tissues analyzed (penis and testicle) showed inconclusive staining results inconsistant with RT-PCR data.

An increase in TRPM8 transcripts in the SCI rats as a whole versus normal controls was appreciated ($p=0.04$). Diabetic rats, on the other hand, demonstrated no change in TRPM8 mRNA vs. control rats ($p=0.84$). TRPV1 mRNA was also noted in all bladders studied. No change in TRPV1 mRNA expression was noted in either DM ($p=0.78$) or SCI rats ($p=0.81$).

Interpretation of results

Prior to this study, in the GU tract TRPM8 had only been investigated and detected in human prostate. Now that a new role for this protein as a temperature-sensitive “cool” receptor has been elucidated, it has been our goal to define the extent of expression of this protein throughout the GU tract. As demonstrated by our results, mRNA for this receptor exists in multiple GU organs in the rat and human, and with the concentration of its transcripts in the urothelium, the “cool” receptor may be involved in various temperature-specific functions including the positive ice water test

An increase in receptor transcripts suggests that TRPM8 receptor expression is upregulated in SCI bladders. This likely contributes to the response seen with ice water provocation in patients with UMN lesions and may be a target for neurogenic bladder therapy.

Concluding message

These results demonstrate that mRNA and protein for TRPM8 exist in multiple GU organs in the rat and human, and it may be considered as a possible new therapeutic target, as is TRPV1, for pharmacologic treatment of detrusor overactivity or other urologic disorders.