

## Best in Category Prize - Pharmacology

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### AGE IS ASSOCIATED WITH AN ALTERED ROLE OF THE HYPERPOLARIZATION ACTIVATED, CYCLIC NUCLEOTIDE GATED (HCN) ION CHANNEL IN ADRENERGIC DETRUSOR RELAXATION

#### Hypothesis / aims of study

The Hyperpolarization activated Cyclic Nucleotide gated (HCN) ion channel is the molecular analog of an inward, depolarizing current,  $I_h$ . This current is known in several tissues including neural, cardiac, and gut, and has recently been identified in human and rat bladders. It serves variously as the feedback current in neural oscillators, membrane potential stabilization, and regulator of susceptibility to excitatory potentials. It is activated by hyperpolarization, and its dynamics enhanced by intracellular cyclic nucleotides. Mouse cystometric evidence suggests enhanced sympathetic-mediated detrusor muscle relaxation with advancing age. This could underpin the loss of bladder volume sensitivity in older humans, associated with an increased risk of urinary dysfunction. We hypothesized that changes in HCN expression would be associated with changes in detrusor relaxation to adrenergic stimulation, in the mouse model.

#### Study design, materials and methods

Expression of HCN mRNA and the impact of aging was tested with qRT-PCR using bladder tissue from WT 2-3month (Young, YWT) and 21-22 month old (Old, OWT) C57Bl/6 female mice. HCN1 protein was confirmed by Western Blot from Young WT female mice. The dependence of adrenergic detrusor relaxation on HCN was tested in bladder strips from YWT and OWT mice and young HCN1 KO mice (YKO). In these studies, 1 mm mucosa-intact strips taken transversely from the mid-bladder were stabilized at 8-10 mN tension in a  $Ca^{++}$ -contained buffer, and loss of tension measured in response to adrenergic stimulation using 1 microM isoproterenol. After re-tensioning the strip with carbachol, the degree of isoproterenol-induced relaxation was again measured in the presence of an HCN blocker, either CsCl or ZD7288. Strip integrity was confirmed at experiment end with carbachol-induced contraction. Tension and spectral power (0.01-0.05 Hz) were compared with 2-way ANOVA across groups and conditions.

#### Results

YWT mouse bladders express HCN1>HCN2. OWT bladders express significantly less HCN1 but HCN2 levels are similar to YWT. The presence of HCN1 protein was confirmed. Strip tension studies demonstrated isoproterenol-induced relaxation in YWT and YKO strips, significantly inhibited by HCN blockade only in YWT. OWT bladders conversely showed minimal relaxation to isoproterenol in the absence of HCN blockade but significant relaxation in the presence of HCN blockade. Paralleling these findings, maximum spectral power was increased by isoproterenol in YWT in the absence of HCN blockade, and in OWT in the presence of HCN blockade.

#### Interpretation of results

Aging and/or maturation is associated with a change in HCN expression, away from HCN1 dominance. HCN1 partially mediates adrenergic detrusor muscle relaxation in young mouse bladders, however altered isoform distribution in old bladders may inhibit adrenergic relaxation. Enhanced isoproterenol-induced relaxation is marked by increased spectral power suggesting myocyte coordination and is age- and HCN-status dependent. HCN is an age-sensitive determinant of bladder responses to sympathetic stimulation with advancing age.

#### Concluding message

We conclude that altered bladder HCN function with advancing age could contribute to loss of range of response to sympathetic input, diminished volume sensitivity, and impaired detrusor preparation for voiding contraction. As symptom complexes of overactive/underactive bladder and associated incontinence can be considered disorders of bladder volume sensitivity, these changes contribute to an increased prevalence of disorders of urine storage and voiding in later life.

#### Disclosures

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