URINARY INCONTINENCE

Pirt inhibits overactive bladder

Overactive bladder (OAB) can lead to urinary incontinence owing to uncontrolled, spontaneous detrusor contractions during the filling phase. Now, newly published research in the journal *Nature Communications* demonstrates that phosphoinositide-interacting protein (pirt), a transmembrane protein that is predominantly expressed in peripheral neurons, is a promising novel treatment target for a range of urinary tract dysfunctions including OAB and interstitial cystitis.

Explaining the rationale for this research, lead author Cheng He explains “antimuscarinic drugs are the standard-of-care treatment of patients with OAB, although the adverse effects can cause poor patient compliance.” thus, “modifying afferent nerve activity and sensation in the bladder wall might be an alternative therapeutic strategy.”

Investigators initially demonstrated a significant increase in the frequency of voiding contractions, and a decrease in voided volume in *PIRT*^{-/-} mice compared with wild-type mice, reflecting similar symptoms to those of humans with OAB. Following this finding, pirt expression patterns were investigated: pirt expression was detected in bladder nerve fibres but not smooth muscle, urothelial or other cell types, and was strongly co-localized with P2X₃ receptors. This association was confirmed in dorsal-root ganglion neurons using co-immunoprecipitation experiments, which suggests a physical interaction between pirt and P2X₃.

Functional data on this interaction were provided using an electrophysiological approach in pirt/P2X₃ co-transfected HEK293 cells. Transient applications of the P2X₃ receptor agonist α,β -meATP resulted in inward currents in single P2X₃-transfected cells. These currents were also detected in pirt/P2X₃ co-transfected cells, although the current density was significantly reduced, suggesting that pirt is a negative regulator of P2X₃ receptor function. Using a range of truncated mutant pirt/P2X₃ transfected cells, binding of pirt to a sequence of 14 amino acids in the N-terminal of the P2X₃ receptor was proven to mediate this inhibition of P2X₃ currents.

Based on this finding, an inhibitory peptide, pirt^{N14} was produced, which targets this peptide sequence. The inhibitory influence of pirt was confirmed by intravesical instillation of pirt^{N14} in *PIRT*^{-/-} mice, which significantly reduced the frequency of voiding contractions and significantly increased the voided volume.

Demonstrating the translational potential of this research, pirt^{N14} was tested in a mouse model of cyclophosphamide-induced cystitis. Intravesical instillations of pirt^{N14} reduced the frequency of voiding contractions in this model, and increased the voided volume. Furthermore, mice with cyclophosphamide-induced cystitis had a significant reduction in pirt expression, suggesting a central role of pirt in the control of bladder activity.

These findings provide evidence that sensitization of afferent nerve fibres might contribute to the symptoms of urinary urgency and increased micturition frequency reported by many patients with lower urinary tract dysfunctions. When asked about the conclusions of this research, He states “The most significant finding is that pirt can interact with and inhibit P2X₃ through the first 14 amino acids of the N-terminal. This expands our view of pirt functions.” adding that, “pirt should no longer be considered an endogenous regulator of TRP channels only.”

These findings provide some hope for the many patients with lower urinary tract dysfunctions, including OAB and interstitial cystitis, whose symptoms are often refractory to noninvasive treatment or management. Commenting on future directions of this work, He summarizes: “a small-molecule inhibitor that mimics the function of pirt^{N14} would be extremely useful in providing relief from bladder overactivity” and that “a systematic structure–function analysis and screening is greatly needed in order to identify such a molecule.”

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Original article Gao, X.-F. *et al.* Pirt reduces bladder overactivity by inhibiting purinergic receptor P2X₃. *Nat. Commun.* 6, 7650