

Functional studies of purinergic and cholinergic interactions in the rat urinary bladder

Characterization of ATP-evoked urothelial release of acetylcholine

Akademisk avhandling

Som för avläggande av farmacie doktorsexamen vid Sahlgrenska akademien, Göteborgs universitet kommer att offentligen försvaras i hörsal Arvid Carlsson, Academicum, Medicinargatan 3, den 17 april 2020, klockan 13:00

av

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Fakultetsopponent:
Professor Bengt-Olof Nilsson,
Lund University, Sweden

Avhandlingen baseras på följande delarbeten

- I. **Stenqvist J**, Winder M, Carlsson T, Aronsson P & Tobin G. Urothelial acetylcholine involvement in ATP-induced contractile responses of the rat urinary bladder. *Eur J Pharmacol.* 2017, 809:253-260; doi: 10.1016/j.ejphar.2017.05.023
- II. **Stenqvist J**, Carlsson T, Winder M & Aronsson P. Effects of caveolae depletion and urothelial denudation on purinergic and cholinergic signaling in healthy and cyclophosphamide-induced cystitis in the rat bladder. *Auton Neurosci.* 2018, 213:60-70; doi: 10.1016/j.autneu.2018.06.001
- III. **Stenqvist J**, Carlsson T, Winder M & Aronsson P. Functional atropine sensitive purinergic responses in the healthy rat bladder. *Submitted*
- IV. **Stenqvist J**, Aronsson P, Carlsson T, Winder M & Tobin G *In vivo* paracrine effects of ATP-induced urothelial release of acetylcholine in the rat urinary bladder. *Submitted*

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Characterization of ATP-evoked urothelial release of acetylcholine

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Abstract

Functional interplays between different transmitter systems are known to affect the signalling of the micturition reflex arc. The functional implications of many of these interactions, such as between ATP and acetylcholine, have yet to be unravelled. This thesis aimed to identify and characterize the functional importance of a proposed atropine-sensitive component in purinergic responses. Furthermore, its impact on afferent signalling and changes during experimental (cyclophosphamide-induced) cystitis were to be studied *in vitro* and *in vivo*.

The existence of an atropine-sensitive part of purinergic responses important for direct detrusor contractions was proven. Furthermore, this purinergic-cholinergic link was shown to be dependent on an intact urothelium as well as caveolae. This suggests a pronounced interaction between these structures, tentatively indicating the caveolae in the urothelium to be of importance for the ATP-evoked release of acetylcholine. Blockade of neuronal transmission by tetrodotoxin did not affect this release, suggesting it to be non-neuronal. Cystitis altered the urothelial signalling, diminishing the purinergic atropine-sensitivity, showing this link to be important for healthy bladder signalling and to be affected during cystitis. Also, atropine significantly decreased contractions induced by the stable ATP-analogue α - β -Me-ATP, demonstrating the P2X-purinoreceptors (likely P2X1 and/or P2X3) to be involved in purinergic release of acetylcholine in the healthy rat urinary bladder. Notably, *in vivo* the purinergic release of acetylcholine was able to activate the micturition reflex arc and trigger afferent signalling. The cholinergic afferent modulation depends on the activation of muscarinic receptors of the M₂/M₄ (inhibitory) and M₃-subtypes (facilitatory).

Thus, a cholinergic part of purinergic signalling exists in the rat urinary bladder, with important function both *in vitro* (e.g. direct contractile responses) and *in vivo* (e.g. modulation of afferent signalling). Cystitis hinders the purinergic-cholinergic signalling in the urothelium, presumably mediated via caveolae, showing changes in urothelial signalling to be of outmost importance in inflammatory lower urinary tract disorders. These results increase the knowledge of healthy bladder signalling and may provide new theories for future pharmacotherapies.

Keywords: *ATP, acetylcholine, urothelium, caveolae, afferents, cystitis*

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