When control is lost: Studying the interactions of ATP and acetylcholine in the urinary bladder

Research Objectives
Prof Tobin developed a split bladder model to examine the interactions of ATP and acetylcholine in the rat urinary bladder.

Detail
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Bio
Gunnar Tobin is Professor at the Department of Pharmacology, Sahlgrenska Academy. Dr Tobin has over the years worked with drug development both at pharmaceutical companies and at the university, and has been an assessor at the Swedish Medical Products Agency. Dr Tobin has authored 120 peer-reviewed publications and more than 100 abstracts.

Funding
Wilhelm & Martina Lundgren foundation

Collaborators
• Dr Michael Winder
• Dr Patrik Aronsson
• Dr Thomas Carlsson
• Dr Johanna Stenqvist

References


Personal Response
Based on your studies, how long do you think it will take for the development of a pharmacological solution?

“Pharmaceutical companies have, for many years, searched for M3-selective drugs. And there exist some drugs which are claimed to have such a selectivity. But the selectivity window is very narrow. However, changing the focus towards another type of selectivity may open up for new possibilities. If the same mechanisms occur in humans as in rats, which has to be established, the development of a solution would be possible within a decade.”
The inner lining of the bladder, known as the urothelium, not only serves as a passive barrier but also triggers the reflex controlling urination (the micturition reflex). The neurotransmitter ATP released from the stretched urothelium activates the bladder afferent nerves and evokes the release of acetylcholine that substantially contributes to the contractile response. Prof Gunnar Tobin from the University of Gothenburg, Sweden, developed a split bladder model to examine the interactions of ATP and acetylcholine in order to optimise the design of new drugs that control the activity of the lower urinary tract.

Urinary incontinence is a condition where urine is unintentionally passed out of the body. It is a common problem that affects millions of people worldwide. Urine leaks when the muscles of the bladder override the strong sphincter muscles of the urethra, the duct through which urine is let out of the body. Age, pregnancy, obesity and the consumption of caffeinated or alcoholic drinks can all have an influence on the development of urinary incontinence.

The urinary system has a particularly important role in the context of excretion, the removal of waste from the body. Among the organs making up the urinary system, the bladder has two functions: the storage and release of urine. When full, the stretching of the inner lining of the bladder, known as the urothelium, causes the release of several substances that in turn activate a response from the central nervous system (CNS). Among these substances, adenosine triphosphate (ATP) is the main activator of the bladder afferent nerve fibres. These fibres communicate with the CNS which controls the response leading to the contraction of the bladder and the expulsion of urine from the body. Prof Gunnar Tobin from the Sahlgrenska Academy, University of Gothenburg, developed an in vivo model to further investigate how the two neurotransmitters ATP and acetylcholine interact, coordinating the response that controls urinary bladder function.

**TWO PROCEDURES TO INVESTIGATE BLADDER FUNCTION**

Prof Tobin previously showed through in vitro studies that ATP can initiate a cascade which results in the release of acetylcholine from the urothelium, indicating that acetylcholine can act as a hormone within the bladder. However, those studies did not address the question of whether acetylcholine can have an effect on afferent nerve fibres as well.

Two types of in vivo procedures on rats consisted of a set of whole bladder experiments and another set of split bladder experiments. In the whole bladder experiments, a cannula was placed into the femoral vein for drug administration, and the bladder pressure was monitored continuously via a catheter inserted through a small incision at the top of the bladder. In the split bladder experiments, threads were ligated at the top, sides and below the entrance of the pelvic nerve on each side of the bladder. The bladder was divided into two separated compartments along the midline from the top of the bladder all the way to the urethra. In the latter model, the levels of anaesthesia were kept as low as reasonably possible, to avoid interfering with the micturition reflex – the reflex controlling urination.

**ATP, ACETYLCHOLINE AND THE ROLE OF THE UROTHELUM**

The study confirmed that ATP controls the response of the micturition reflex in the rat urinary bladder. The induction of the reflex involves specifically the ATP in the urothelium. As expected, the sensory nerves of the bladder are modulated by an interplay involving different receptors. The study also confirmed the physiological significance of the stretching of the urothelium, since in bladders stripped of their inner layer, the ATP- and the acetylcholine-mediated responses were minimal or absent.

In the split bladder preparation, drugs were administered on one half so that contractile responses could be observed in the other half. This model can only work if the reflex response is first relayed via the CNS so that the impulse can be sent to the other half of the bladder. The reflex relay, induced by bladder stretching and ATP, was confirmed by its absence during deep anaesthesia and its disappearance after the pelvic nerve was cut. After the disruption of the pelvic innervation, the reflex-induced ATP effects were abolished, and only the direct effects of ATP on the urothelium remained. The further addition of atropine, an inhibitor of the action of acetylcholine, indicated that approximately 40–50% of the ATP response in the absence of the reflex depends on urothelial acetylcholine.

**AN OVERVIEW OF THE ROLE OF MUSCARINIC RECEPTORS**

The research conducted by Prof Tobin and his team shows that muscarinic receptors (responsible for muscle contraction) are present in the urothelium. ATP acts via its receptors, ATP-muscarinic M2 and/or M4 receptors, to cause inhibition of the release of acetylcholine. This results in no muscle being expelled. When the signalling increases as a result of a full bladder, more acetylcholine is released, acting on neuronal M1 receptors. The M1 receptor stimulates further release of acetylcholine, resulting in an amplification of the signal. Eventually, acetylcholine acts on smooth muscle muscarinic M3 receptors, which causes the muscle to contract and to expel urine.

**IMPLICATIONS FOR THE TREATMENT OF URINARY INCONTINENCE**

Urinary incontinence is normally treated with lifestyle changes, such as losing weight or cutting down on alcohol and caffeine. Other management strategies include pelvic floor exercises and using incontinence products, such as absorbent pads. Sometimes surgical procedures such as bladder enlargement are considered. Blockade of muscarinic receptors has been a common way to treat incontinence pharmacologically. Prof Tobin aims to corroborate existing research on muscarinic receptors to find new strategies for the pharmacological treatment of urge incontinence.

**The best candidates for the development of effective drugs to treat incontinence should be identified among blockers of M1 receptors and low-affinity inhibitors of M3 receptors.**
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