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Chronic Administration of Anticholinergics in Rats Induces a Shift from Muscarinic to Purinergic Transmission in the Bladder Wall

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Abstract

Background: First-line pharmacotherapy for overactive bladder consists of anticholinergics. However, patient compliance is exceptionally low, which may be due to progressive loss of effectiveness.
Objective: To decipher the involved molecular mechanisms and to evaluate the effects of chronic systemic administration of anticholinergics on bladder function and on muscarinic and purinergic receptors expression in rats.
Design, setting, and participants: Female Wistar rats were implanted with an osmotic pump that chronically administered vehicle (Veh_c), 0.36 mg/kg per day oxybutynin (Oxy_c), or 0.19 mg/kg per day fesoterodine ($Feso_c$) for 28 d.
Interventions: For cystometry experiments, a small catheter was implanted in the bladder.
Outcome measurements and statistical analysis: Urologic phenotype was evaluated by the analysis of the micturition pattern and urodynamics. Expression of muscarinic and purinergic receptors was assessed by Western blot analysis of detrusor membrane protein. Functional responses to carbachol and adenosine triphosphate (ATP) were evaluated using muscle-strip contractility experiments.
Results and limitations: The number of voided spots was transiently decreased in Oxy_c rats. In Oxy_c rats, the effect of an acute high dose of oxybutynin (1 mg/kg intraperitoneally [IP]) on the intermicturition interval was abolished. Expression experiments revealed a decrease of muscarinic acetylcholine receptors M2 (mAChR2) and M3 (mAChR3), whereas the purinergic receptor P2X, ligand-gated ion channel, 1 (P2X1) was enhanced in Oxy_c and $Feso_c$ rats compared to Veh_c rats. In concordance with the modification of the expression pattern in Oxy_c rats, the force generated by carbachol and ATP in muscle-strip contractility experiments was, respectively, lower and higher. Urodynamics revealed that the effects of systemic administration of the purinergic blocker pyridoxalphosphate-6-azophenyl-2',4'-disulphonic acid (50 mg/kg IP) were enhanced in Oxy_c rats. As rat bladder physiology is different from that of humans, it is difficult to directly extrapolate our findings to human patients.
Conclusions: Chronic administration of anticholinergics in rats induces receptor loss of efficiency and a shift from muscarinic to purinergic transmission.

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1. Introduction

Anticholinergics such as oxybutynin (Oxy_c) and fesoterodine ($Feso_c$) are currently the first-line pharmacotherapy for the treatment of patients with overactive bladder (OAB), about 90% of whom suffer from idiopathic OAB [1,2]. By blocking the muscarinic receptors on the detrusor muscle, the neuromuscular junction is inhibited. A potential action at the urothelial and suburothelial level has also been suggested [2], with a possible effect on C-fiber activity [3]. However, the compliance rate of patients taking anticholinergics is notoriously low. Indeed, Yeaw et al. found a 6-mo compliance rate of only 28%, significantly lower than other chronic therapeutic areas [4]. The reason for discontinuing this medication class has not been clearly determined, but it may be due to a combination of side effects, financial costs, and loss of drug efficiency [5]. Oxy_c and $Feso_c$ are more frequently discontinued than other anticholinergic agents [6]. Understanding the molecular mechanism of the failure of anticholinergics will help physicians treat patients more efficiently.

Bladder physiology is mediated by multiple signaling molecules [7]. Excitation of parasympathetic efferents causes release of acetylcholine, which is generally seen as the main neurotransmitter in the voiding cycle, and of adenosine triphosphate (ATP) at the nerve endings [8] These transmitters act respectively on muscarinic acetylcholine receptors (mainly mAChR2 and mAChR3) and purinergic receptors (mainly purinergic receptor P2X, ligand-gated ion channel, 1 [P2X1]) to cause detrusor smooth-muscle contraction [9,10]. The relative importance of both signaling molecules is highly dependent on the species [11]. In rats, ATP plays a substantial role in the initiation of the voiding contraction, whereas its role seems to be much less important in humans [11,12].

The chronic, systemic administration of anticholinergics in animal models induces urodynamic changes after long-term administration of a low dose of Oxy_c [13]. In this study, we evaluated the effects of such administration on molecular and in vivo characteristics of the bladder physiology. Long-term Oxy_c administration exerts a bimodal action on muscarinic and purinergic receptors. The functional expression of mAChR is decreased, while the relative importance of purinergic system is enhanced. These results may help explain the loss of efficiency of anticholinergic-based treatment on patients with OAB.

2. Material and methods

The timeline of experiments is shown in Figure 1.

2.1. Animals

All experiments were approved by the local ethics committee of the Katholieke Universiteit Leuven. The rats were obtained from Janvier (Le Genest-Saint-Isle, France).

2.2. Drug delivery

Osmotic pumps (Alzet 2004; Durect, Cupertino, CA, USA) delivered distilled water (vehicle) (Veh_c), Oxy_c (0.36 mg/kg per day), or $Feso_c$ (0.19 mg/kg per day) for 28 d [13]. These two doses are in the therapeutic range in human patients (20 mg Oxy_c and 10 mg $Feso_c$ per day for a person of 55 kg).

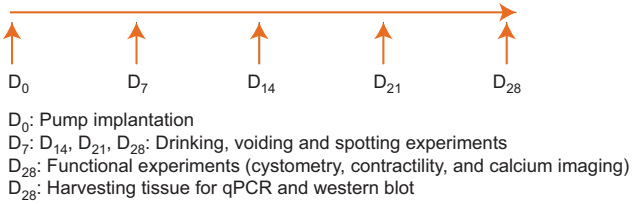

Fig. 1 – Timeline of experiments. The pumps were implanted at day 0. In total, we implanted 36, 40, and 12 pumps filled with water, oxybutynin, and fesoteridine, respectively, in female Wistar rats. The spotting, drinking, and voiding experiments were conducted with two groups of six rats at day 7 (D_7), day 14 (D_{14}), day 21 (D_{21}), and day 28 (D_{28}). We performed cystometry on those animals to decrease the number of tested rats. For the expression experiments (quantitative polymerase chain reaction [qPCR; three groups of six animals] and Western blot [three groups of six animals]), the bladders were harvested at D_{28} and frozen immediately at −80 °C until the tissues were processed. For the functional experiments (calcium imaging [vehicle (Veh_c), n = 8; oxybutynin (Oxy_c), n = 9], contractility [Veh_c, n = 6; Oxy_c, n = 7], and cystometry [Veh_c, n = 9; Oxy_c, n = 10; and Veh_c, n = 7; Oxy_c, n = 8]), the rats were euthanized at D_{28}, and the experiments were performed immediately after the death of the rats.

2.3. Micturition pattern analysis

Estimation of the voided volume of urine was determined as previously described [14].

2.4. Drinking and diuresis

Rats were placed individually in metabolic cages. The voided urine and water uptake were measured over 24 h.

2.5. Cystometry

Cystometry experiments were done as previously described [15]. To evaluate the effects of an acute administration of a high dose of Oxy_c on the urodynamic parameters following a chronic Oxy_c treatment, Oxy_c (Sigma-Aldrich Co, St. Louis, MO, USA) was dissolved in saline and injected at an intraperitoneal (IP) dose of 1 mg/kg in Veh_c rats and in Oxy_c rats 30 min after the start of the cystometry recordings. To evaluate the effects of an acute administration of a purinergic blocker following a chronic Oxy_c treatment, pyridoxalphosphate-6-azophenyl-2',4'-disulphonic acid (PPADS) (Sigma-Aldrich Co, St. Louis, MO, USA) was dissolved in saline and injected intraperitoneally, 50 mg/kg, in Veh_c rats and in Oxy_c rats 30 min after the start of cystometry recordings.

2.6. Quantitative real-time polymerase chain reaction

Frozen bladders were sliced (25 8-μm slices per bladder), homogenized in a QIAshredder (Qiagen, Venlo, The Netherlands), and RNA was extracted using the RN Easy Plus Micro Kit (Qiagen, Venlo, The Netherlands). Quantitative polymerase chain reaction (qPCR) was performed in a 7500 Fast Real-Time PCR System (Life Technologies Corp, Glasgow, UK) using specific, rat, Taqman gene-expression assays for mAChR2, mAChR3, and P2X1. The two gene reference genes were eukaryotic translation initiation factor 4A3 (Eif4A3) and Pol2J.