Journal of Pharmacological and Toxicological Methods 74 (2015) 7–16

Contents lists available at ScienceDirect

Journal of Pharmacological and Toxicological Methods

journal homepage: www.elsevier.com/locate/jpharmtox

Original article

A new model of detrusor overactivity in conscious rats induced by retinyl acetate instillation

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ARTICLE INFO

Article history:
Received 11 November 2014
Received in revised form 19 March 2015
Accepted 22 April 2015
Available online 6 May 2015

Keywords:
Bladder hyperactivity
Cystometry
Methods
Rats
Retinyl acetate

ABSTRACT

Introduction: A credible animal overactive bladder model used in basic research is an indispensable harbinger of safe and ethical clinical trials on human subjects. Our objective was to develop a new animal model of a hyperactive bladder that will be void of inflammatory urothelium lesions and display significant sensitivity to muscarinic receptor antagonists.
Methods: To examine the influence of 0.75% retinyl acetate solution on cystometric parameters, it was infused into the bladder for 5 min. Cystometric studies with physiological saline were performed in conscious unrestrained rats 3 days later. To examine the influence of retinyl acetate, acetic acid or cyclophosphamide on morphology of urinary bladders, the bladders were subjected to histopathological examination.
Results: We demonstrated that in rats subject to previous 5-minute bladder instillations with retinyl acetate, an increase of basal pressure, threshold pressure, micturition voiding pressure, bladder contraction duration, relaxation time, detrusor overactivity index, nonvoiding contraction frequency and amplitude occurs. On the other hand, a decrease in voided volume, post-void residual, volume threshold, voiding efficiency, intercontraction interval, bladder compliance and volume threshold to elicit nonvoiding contractions was observed. Administration of oxybutynin chloride (0.5 mg/kg, i.v.) reversed changes of cystometric parameters evoked by retinyl acetate. Contrary to acetic acid and cyclophosphamide, bladders subjected to retinyl acetate infusion had no signs of bladder inflammation.
Discussion: The results obtained indicate that transient infusion of 0.75% retinyl acetate can induce detrusor overactivity, which is often observed in patients with overactive bladder syndrome (OAB). In addition, it was demonstrated that stimulating afferent C-fibres using retinyl acetate did not induce evident histopathological inflammatory lesions in the urinary bladder wall. It appears that in the future this model can prove useful in gaining more knowledge on the pathophysiology of OAB, and contribute to the preparation of new, more effective options of OAB pharmacotherapy.

1. Introduction

Overactive bladder (OAB) is a symptom-based diagnosis defined by the International Continence Society (ICS) as urinary urgency with or without urinary incontinence, usually with frequency (usually small amounts) and nocturia (>2 acts of micturition during nightly rest) with the absence of a local underlying pathological condition (Abrams et al., 2002). It is a social disease which, though not life-threatening, influences the quality of life, resulting in physical, social, psychological and sexual limitations, as well as the degradation of the quality of one's sleep. The most frequent and most characteristic symptom of OAB is polyuria, which is reported by 85% of patients. Furthermore, OAB has a detrimental effect on the self-esteem of those suffering from it. It can also cause depression and often leads to withdrawal from social activities. As reported in various studies, the incidence of this condition ranges from 14% to 40% and increases with age. These data demonstrate that the incidence of OAB is almost equal to arterial hypertension, cardiovascular diseases and asthma (Semins & Chancellor, 2004).

The causes of OAB include poor impulse inhibition in the CNS, the reorganisation of the spinal reflex, afferent fibre hyperactivity, an increase in the sensitivity of the detrusor muscle to efferent stimulation, or a combination of the aforementioned causes (Chancellor & Yoshimura, 2004).

The effectiveness of OAB treatments is conditional on the precise identification of potential targets for a pharmacological intervention. Urinary bladder pathophysiology is still largely unknown when compared to other systems of the human body.

Antimuscarinic drugs are currently the first-line therapy for OAB. However, their clinical use is restricted by well-known side effects, and not-always-satisfactory clinical effects (Radomski & Barkin, 2012).

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http://dx.doi.org/10.1016/j.vascn.2015.04.006
1056-8719/

Antimuscarinic drugs are currently the first-line therapy for OAB. They reduce the urgency, stabilise hyperactivity of the detrusor muscle, and increase the capacity of the urinary bladder. However, their clinical use is restricted by well-known side effects — constipation, nausea, dry mouth, dizziness, headache, drowsiness, confusion, blurred vision and not-always-satisfactory clinical effects (Radomski & Barkin, 2012). The urinary bladder was found to contain the receptors M_1, M_2 and M_3. Despite the fact that, in terms of density, M_2 (M_2:M_3 = 4:1) is the dominant receptor, it is M_3 that acts as an agent in detrusor-muscle contraction (Eglen, Hegde, & Watson, 1996). An important aspect of the muscarinic-receptor antagonist operation is the adverse effects on the part of CUN, where M_1 receptors act as agents. Up to 70% of patients discontinue their therapies because of them. Due to anticholinergic symptoms, which accompany the use of muscarinic-receptor antagonists, new drugs are being sought which would be organ-specific and not only selective in terms of a specific muscarinic-receptor subtype.

The restrictions in the use of the currently used drugs in OAB treatment have become an impulse for the search for new directions for the pharmacological treatment of this condition, and also for further studies on physiological foundations and lower urinary tract dysfunctions. The possibility of affecting the afferent mechanism regulating the micturition reflex is especially interesting.

The many years of research have not yet brought us the direct cause of OAB. Currently used medications yield unsatisfactory therapeutic results, which, in turn, cause not only substantial social and mental effects in the patients themselves, but also a financial burden for the State. Therefore, there is a massive need for modern and effective therapeutic methods that will solve this problem. One of the most important fundamental objectives is to discover an animal model that would mirror an overactive bladder in humans to the greatest possible degree. This, in turn, will make it possible to better understand the aetiology and pathophysiology of OAB, and also to work out an effective pharmacotherapy. Animal models allow us to fully verify the results of experiments in "intact" biological systems, and with no need for interference in the human body. On the other hand, *in vitro* research on tissues does not always reflect those conducted under *in vivo* conditions. Hence, animal-model experiments are significantly superior to *in vitro* studies utilising tissue fragments or cells (Boucher et al., 2000). Cystometry is the most frequent examination in human OAB diagnostics. It allows us to measure the interrelation between the volume of liquid emptied from the bladder and intravesical pressure. The animal models used so far have utilised irritative cystometry, consisting of the intravesical infusion of an irritative agent, such as acetic acid, citric acid, hydrochloric acid, capsaicin, protamine sulphate, xylene and turpentine, which lead to the hypersensitisation of nociceptive afferent C-fibres within the bladder wall. This results in increased sensory activity, which is considered to be the potential cause of urgency (Fry et al., 2010). However, despite the fact that the symptoms induced by these substances display some characteristics of human OAB (such as increased micturition frequency and decreased void volume), in many studies their similarity to OAB was called into question, *i.e* due to inflammation and damage to the urinary bladder wall caused by these compounds. As urothelium inflammation does not underlie human OAB pathophysiology, the results of research utilising these models can be misleading (McMurray, Casey, & Naylor, 2006). This can be also corroborated by the fact that muscarinic receptor antagonists, which are considered the gold standard of OAB treatment in humans, display no effectiveness in these models (Angelico et al., 2005).

The research described below utilises retinyl acetate (RA) – a vitamin A derivative – as the irritative agent. It has been proven that the retinoids play a vital role in the division and differentiation of cells in such processes as apoptosis, morphogenesis, proliferation regulation and cellular differentiation. Retinol derivatives demonstrate very gentle irritative and exfoliating properties, stimulate the production of hyaluronic acid, and also have anti-inflammatory properties, which are conducive to wound healing (Fernández-García et al., 2012).

Retinoids are derivatives of vitamin A. They have found clinical applications in the treatment of many dermatological and oncological diseases. Nevertheless, they tend to induce irritating side effects in the form of hypersensitivity. *In vivo* research has shown that these compounds cause the sensitisation of nociceptive pathways (Alique, Lucio, & Herrero, 2006; Romero-Sandoval et al., 2004). It has been proven that both natural and synthetic retinoids activate transient receptor potential channel vanilloid subtype 1 ($TRPV_1$), which leads to the stimulation of nociceptive sensory neurons, and, consequently, to sensory hypersensitivity (Alique et al., 2006; Shijin et al., 2013). These effects can be reduced by the genetic ablation of the $TRPV_1$ function, or the application of AMG9810, a selective antagonist of $TRPV_1$ receptor (Shijin et al., 2013). The results of these studies show that $TRPV_1$ is an ionotropic retinoid receptor that mediates sensory hypersensitivity in primary sensory neurons induced by retinoids. $TRPV_1$ receptors are located on afferent neurons, mainly type C, but also type A δ (Ost, Roskams, Van Der Aa, & De Ridder, 2002). In addition to the direct activation of $TRPV_1$, retinoids can also regulate its function indirectly. It has been found that they enhance its function and expression in neuroblastoma cells (Andaloussi-Lilja, Lundqvist, & Forsby, 2009). Retinoids can also modulate pain sensation through influencing the levels of proinflammatory mediators, such as prostaglandins and NGF (Devaux et al., 2001; Hernández-Pedro et al., 2008). It is interesting to note that vitamin A deficiency has been found to reduce acetylcholine release in rat hippocampus (Carta et al., 2006; Cocco et al., 2002). This is particularly noteworthy given the fact that the primary receptor of the detrusor urinae muscle, which is responsible for its contraction and participates in inducing OAB symptoms, is M_3. Therefore, it seems that the irritation or mild pain observed following the application of exogenous retinoids are the results of $TRPV_1$ receptor stimulation (Shijin et al., 2013; Steinhoff ebt al., 2003).

In the present study, we investigated the effect of a transient intravesical infusion of RA on the micturition cycle, which was assessed by way of continuous cystometry with physiological saline. At the same time, we conducted a histopathological examination of a urinary bladder wall treated with RA and two substances most often used in animal OAB models — acetic acid (AA) and cyclophosphamide (CYP). Our objective was to develop a new animal model of a hyperactive bladder that will be void of inflammatory urothelium lesions and display significant sensitivity to muscarinic receptor antagonists.

2. Materials and methods

All procedures were conducted according to NIH Animal Care and Use Committee guidelines, and approved by the Ethics Committee of the Medical University of Lublin.

2.1. Animals

The study was conducted on female Wistar rats (weighting initially 200–225 g). A natural light/dark cycle, temperature + 22 °C and humidity 60% were maintained. Food and water were provided *ad libitum*. All experimental procedures were carried out between 8 a.m. and 1 p.m. Rats were experimentally naive and tested once.

A total of 45 female Wistar rats were used in cystometric studies and divided into three groups of 15 animals each. All the surgical procedures were performed under anaesthesia with intraperitoneal injection of 75 mg/kg of ketamine hydrochloride (Ketanest, Pfizer) and 15 mg/kg of xylazine (Sedazin, Biowet). Rats were placed supine on a warming mattress (37 °C). Ketamine is often used in animal studies because it causes a dissociative type anaesthesia with minimal cardiac and respiratory depression. It is reported that ketamine in combination with xylazine does not abolish the micturition reflex in female rats (Cannon & Damaser, 2001). Lack of spontaneous movement and lack of withdrawal response to noxious toe pinch were taken to indicate an adequate depth of anaesthesia.