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Original article

A new method for producing urinary bladder hyperactivity using a non-invasive transient intravesical infusion of acetic acid in conscious rats

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Abstract

Introduction: Animal models that closely resemble the pathophysiology of human overactive bladder are important for evaluating novel therapeutics to treat the disorder. We established a non-invasive hyperactive bladder model that is sensitive to anti-muscarinic drugs and without bladder inflammation. **Methods:** Acetic acid solution was infused into the bladder for 5 min via the urethral orifice without any surgical procedures under isoflurane anaesthesia. After washing the bladder with saline, voiding frequency (VF) and total urine volume were determined for 9 h under conscious conditions. **Results:** Infusion of a 0.5% acetic acid solution caused a significant increase in VF, without influencing total urine volume or inducing significant histopathological inflammatory alterations in the bladder urothelium. Oral administration of oxybutynin (3 and 10 mg/kg) significantly ameliorated increases in VF induced by 0.5% acetic acid. Infusion of 0.75% acetic acid induced intensive urinary inflammation and a decrease in total urine volume as well as an increase in VF. Oral treatment with oxybutynin (10 mg/kg) did not significantly improve the increased VF due to 0.75% acetic acid. Acetic acid (0.5%) infusion evoked bladder hyper-responsiveness whether applied at night or during the day. However, VF was increased more by the nighttime application of acetic acid, while there were no significant differences in basal levels of VF between daytime and nighttime. **Discussion:** In this study, the non-invasive rat urinary hyperactive bladder model indicated minimizes the secondary effects of experimental procedures such as surgical operations and anesthesia on bladder function and is sensitive to oxybutynin. Thus, the model may be useful for investigating novel therapeutics for OAB treatment.

Keywords: Overactive bladder; Hyperactive bladder; Acetic acid; Cystitis; Oxybutynin; Rat

1. Introduction

Overactive bladder (OAB) is characterized by urinary urgency, with or without urge urinary incontinence, usually with frequency and nocturia and the absence of obvious pathology to account for these symptoms (Abrams et al., 2002). Although the pathogenic mechanisms of OAB are not fully understood, both neurogenic and myogenic theories have been proposed (Wein & Rackley, 2006; Fowler, 2002; Andersson, 2004; Brading, 1997). Increased afferent activity, decreased capacity to process afferent information, decreased suprapointine inhibition, increased sensitivity to contraction-mediating transmitters, and changes in detrusor smooth muscle structure or function may all contribute to OAB. Therefore, pharmacological treatment of OAB is directed toward neuronal pathways and/or the detrusor muscle itself (Wein, 2001).

Animal models that closely resemble the pathophysiology of human OAB are critical for the development of pharmacological therapies to treat the disorder. One of the most commonly utilized methods of investigating the effects of compounds on bladder function is cystometry in either conscious or anaesthetized rats with or without the infusion of irritative agents. Irritative cystometry (produced by the infusion of chemical agents such as acetic acid or citric acid leading to the hypersensitization of nociceptive afferent fibres), has been used to investigate new

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therapies for OAB (Wein, 2001). However, while irritative rat cystometry models display some characteristics of human OAB (such as increased frequency and decreased void volume) the similarity to human OAB pathophysiology has been questioned in several reports (McMurray, Casey & Naylor, 2006). The implantation of cannulas and intravesical infusion of chemical irritants may produce inflammatory damage in the bladder urothelium. As inflammation is not believed to be an underlying pathology in OAB, results from such models may be misleading (McMurray, Casey & Naylor, 2006; Morikawa et al., 1989). In addition, the anti-muscarinics (gold standard treatment for OAB) do not appear to be efficacious in these types of models (Angelico et al., 2005). It is likely that surgical operation, anaesthetic treatment and the continuous infusion of chemical irritants alter both myogenic and neural activities influencing urinary micturition.

In the present study, we investigated the effect of a transient intravesical infusion of acetic acid (under isoflurane anesthesia without surgical procedures) on micturition patterns and bladder morphology in female rats to establish a novel non-invasive hyperactive bladder model with a significant sensitivity to anti-muscarinic drug and without bladder inflammation.

2. Methods

2.1. Animals

Female Sprague–Dawley rats at 9-week age weighting 180 to 250 g (Charles River Japan Inc., Japan) were used in all experiments. Rats were housed in a plastic cage (3 per cage) with wood chip bedding, allowed access to food and water ad libitum, and were maintained on a 12-hour dark: 12-hour light (7:00 to 17:00) cycle in a temperature (23 ± 2 °C)- and humidity (55 ± 10%)-controlled room. All protocols used in the present studies were reviewed and approved by the Experimental Animal Ethical Committee of GlaxoSmithKline according to *the Guide for the Care and Use of Laboratory Animals* published by US Institutes of Health (NIH Publication No. 85-23, revised 1996).

2.2. Drugs

Acetic acid, oxybutynin chloride, mineral oil, hydroxypropyl methyl cellulose (HPMC) and Tween 80 were purchased from Sigma Chemical Co. (St. Louis, MO, USA). Isoflurane (Escain) was obtained from Merck HOEI (Japan). Acetic acid was diluted to 0.2, 0.5 and 0.75% with saline. Oxybutynin chloride was dissolved in a volume of 10 mL/kg with 0.5% HPMC solution containing 0.1% Tween 80.

2.3. Acetic acid-induced hyperactivity

Each rat was placed in a metabolic cage (3700 M071, Tecniplast, USA) with free access to food and water to acclimatize for 3 days prior to the induction of OAB. The following procedures were performed under isoflurane anesthesia (concentration 4%, flow 3 mL/min). The bladder was catheterized (I.D. 0.5 mm/O.D. 0.8 mm) from the external urethral orifice without any surgical operation, and then this intravesical catheter was connected via a 3-way stopcock to a transducer (P23XL, Becton Deckinson, USA) for recording intravesical bladder pressure. After the residual urine was removed from the bladder, acetic acid solution or saline at room temperature was injected into the bladder via the inserted catheter until the bladder pressure reached at 10 cmH_2O. Five minutes later, the acetic acid solution was removed from the bladder and the inside of the bladder was gently washed twice with saline, and the rat was placed in the metabolic cage with free access to water and food during the recovery from anesthesia.

2.4. Measurement of micturition pattern

Micturition pattern was determined according to the modified method reported by Ozawa et al. (1999). One hour after the treatment with acetic acid solution, voided urine was collected for 9 h per the following procedures. Urine was collected in a polypropylene beaker containing 3 mL of mineral oil on an electronic scale (GX-200, AND, USA), connected to a computer for recording of micturition frequency and volume. Data were recorded and stored using data acquisition and analysis software (Notocord-hem evolution. 4.1.0.69., HEM systems, USA). Micturition parameters, voiding frequency (VF) per 9 h, total urine volume per 9 h and micturition volume (MV) was determined.

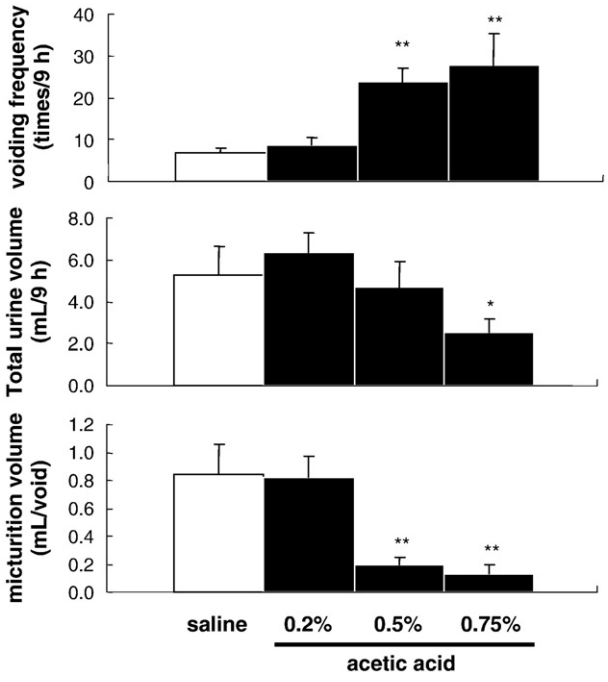

Fig. 1. Effect of a transient intravesical application of acetic acid on voiding patterns in conscious rats. Under isoflurane anesthesia, acetic acid solutions (0.2, 0.5 and 0.75%) were infused into the bladder for 5 min at 17:00. After recovery from anesthesia, the micturition patterns were monitored for 9 h from 18:00 to 3:00. Each bar represents the mean ± S.E.M ($n=4$). *$p<0.05$ and **$p<0.01$, compared with the saline-treated group by the Dunnett's test.