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Pharmacological modulation of the micturition pattern in normal and cyclophosphamide pre-treated conscious rats

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ABSTRACT

In the current study, we wanted to assess the influence of muscarinic receptors, nitric oxide and purinoceptors on the micturition pattern of conscious normal and cyclophosphamide (CYP) pre-treated rats. The micturition parameters were assessed using a metabolic cage. Rats were pre-treated with either saline or CYP, to induce cystitis, followed by treatment with either the muscarinic M1/M3/M5 receptor antagonist 4-diphenylacetoxy-N-methylpiperidine (4-DAMP), the nitric oxide synthase blocker N^{ω}-nitro-L-arginine methyl (L-NAME), the P2 purinoceptor antagonist pyridoxalphosphate-6-azophenyl-2′,4′-disulfonic acid (PPADS) or a combination of 4-DAMP with PPADS or L-NAME. Voiding volumes per micturition event were significantly lower in CYP pre-treated than in saline pre-treated rats. Neither 4-DAMP nor L-NAME had any effect in the normal rats, whereas PPADS reduced the micturition volume per event. In CYP pre-treated rats, 4-DAMP and L-NAME significantly increased voiding volumes per event and micturition frequency, respectively. 4-DAMP dose-dependently reduced the differences in micturition activity between saline and CYP pre-treated rats.

We show that cystitis changes the urodynamics in conscious rats and that this change seems to depend on the production of NO and on altered muscarinic receptor effects. The altered muscarinic receptor responses are likely to per se involve NO-mediated mechanisms.

1. Introduction

The peripheral action of muscarinic receptor antagonists on detrusor receptors has generally been considered to be the primary therapeutic mechanism when pharmacologically treating the overactive bladder (Andersson, 2004). At therapeutic doses, however, anticholinergic drugs do not seem to inhibit the detrusor contractility (Finney et al., 2006). Therefore, it has been hypothesized that the antagonists act by blocking urothelial muscarinic receptors. By such a blockade they would affect the release of other urothelial substances such as adenosine triphosphate (ATP; (Smith et al., 2005)) and nitric oxide (NO; (Andersson et al., 2008)). Since purinoceptors mediate sensory functions in the lower urinary tract, tentatively via P2X purinoceptors, (Ford et al., 2006), inhibition of ATP release has been suggested to cause a reduction of bladder sensory activity (Finney et al., 2006). Furthermore, hyper-excitability of afferent pathways contributes to bladder overactivity and pain in interstitial cystitis (Yoshimura et al., 2002). In patients with interstitial cystitis, afferent pathways are sensitized, and the release of substances such as ATP and NO are associated to the condition (Logadottir et al., 2004). Rats with CYP-induced cystitis exhibit bladder overactivity (Borvendeg et al., 2003) and an enhanced urothelial ATP release (Smith et al., 2005). Also, nitric oxide synthase (NOS) as well as muscarinic receptors are up-regulated in the urothelium (Giglio et al., 2005). In *in vitro* and *in vivo* studies, the blockade of muscarinic receptors with 4-DAMP reversed cholinergic responses in inflamed bladders (Giglio et al., 2005; Andersson et al., 2008). In the same study, by removal of the bladder mucosa, it was shown that muscarinic bladder responses include a down-stream involvement of NO.

In the current study, we wondered whether or not a muscarinic, possibly NO-coupled, mechanism affects the micturition pattern in the urinary bladder of conscious rats in the same way as it does *in vitro* and *in vivo* (Giglio et al., 2005; Andersson et al., 2008). We also wondered whether or not the blockade of purinergic receptors affects the bladder function differently in inflamed and normal bladders, and further, if interactions concerning pharmacological influences on voiding occur between PPADS and 4-DAMP. In order to induce experimental cystitis, we pre-treated rats with CYP. CYP is a bladder toxic cytostatic drug widely used in the treatment of neoplastic diseases, and is subsequently often used to induce cystitis in a rat urinary bladder model (Devries and Freiha, 1990). For the examination of cholinergic effects, in control and in CYP pre-treated rats, 4-DAMP, a muscarinic antagonist that potently inhibits muscarinic M3 receptor-mediated contractions *in vitro*, was employed (Tobin and

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Sjogren, 1995). For inhibiting the synthesis of NO and for antagonizing P2 purinoceptor effects, L-NAME (Persson et al., 1992) and PPADS (Lambrecht et al., 1992) were used, respectively.

2. Methods

2.1. Ethical approval

The animal ethics committee at the University of Gothenburg approved the present study.

2.2. Experimental design

Male Sprague Dawley rats (310–400 g; n = 100; average weight 344 ± 16 g and 355 ± 16 g for saline- and CYP pre-treated rats, respectively) were used in the study and were given food and water *ad libitum* before experiments. Rats were administered intraperitoneally either saline (9 mg/ml; serving as controls) or CYP (100 mg/kg; in order to induce cystitis), both in combination with the analgesic buprenorphinum (10 μg/kg). Forty-eight hours after the treatment, the rats (both saline and CYP pre-treated rats) received either saline, 4-DAMP, L-NAME, PPADS or the combinations of 4-DAMP and PPADS or 4-DAMP and L-NAME. All administrations were given in a volume of 1 mL/kg intraperitoneally (IP).

The study included three different protocols (Fig. 1): a) saline and CYP pre-treated rats were administered either saline (n = 10 and 6; saline and CYP pre-treated, respectively), 4-DAMP (1 mg/kg IP; n = 6 in both groups), L-NAME (30 mg/kg IP; n = 6 in both groups) or PPADS (10 mg/kg IP; n = 6 in both groups). In the second protocol (b) saline pre-treated rats received either 4-DAMP (1 mg/kg; n = 5), L-NAME (30 mg/kg; n = 5) or PPADS (10 mg/kg; n = 5) alone or, L-NAME or PPADS (30 mg/kg and 10 mg/kg, respectively) in combination with 4-DAMP (1 mg/kg; n = 5 in both groups). After a two week wash-out period, in order for the rats urodynamics to be normalized and drugs to be completely washed out, they were CYP pre-treated and the protocol was repeated. In the third protocol (c) rats (n = 20) were administered saline or 4-DAMP at different doses (10, 100 or 1000 μg/kg IP; n = 5 for each group) after saline pre-treatment. After a two week wash-out period they were pre-treated with CYP and thereafter the saline and 4-DAMP protocol was repeated. The first and third protocols (a and c) included control groups (1 mL/kg of saline IP) for both the saline and the CYP pre-treated rats (saline–saline and CYP-saline). In the second protocol (b), the comparisons were made between the groups given either 4-DAMP, L-NAME or PPADS alone and the groups given the respective combination (4-DAMP + PPADS or 4-DAMP + L-NAME). The doses were chosen based on previous studies (Andersson et al., 2008; Tobin et al., 2002; Koganezawa et al., 2006).

After pre-treatment with either saline or CYP, and the following drug administration (saline, antagonists or NOS inhibitor), the rats were placed in a metabolic cage with free supply of water and were kept in a light–dark–light cycle of 6 and 12 and 6 h, respectively. Since only small volumes of urine were produced during the last 6 h of the cycle, when comparing data gathered at several time points results from this period have generally been disregarded. The evaluation of drug effects was therefore focused on the dark cycle due to rats diurnal micturition pattern, i.e. they are active during the night and sleep during the day. However, to give a proper view of water intake, a comparison was made between data from the whole 24 h observation period.

Urine was collected in a graded bottle below the cage, and expelled urine drops from the rats were registered by a laser Doppler coupled to a transducer. Micturition parameters including urine output, number of micturitions per hour and voided volume per micturition event were measured continuously, and assessed after 6, 18 and 24 h. Water consumption was solely measured after 24 h. Data were recorded using an MP100WSW data acquisition system and Acquire Software (BioPac, Goleta, CA, US).

2.2.1. Data analysis and statistical procedure

All data values are expressed as mean ± SEM. Statistical significance was determined by one-way or two-way analysis of variance

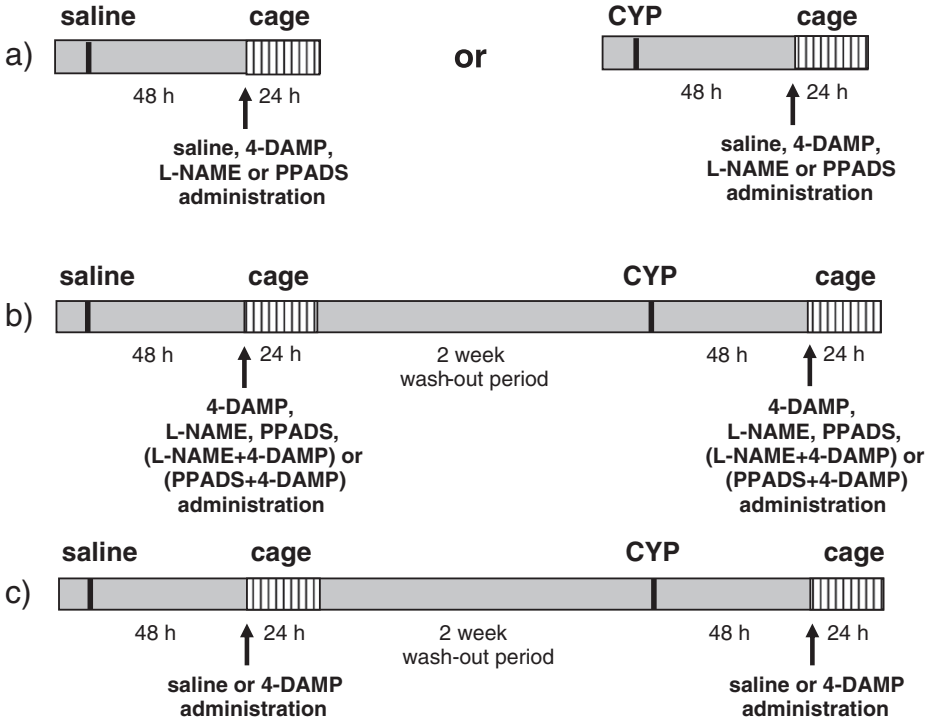

Fig. 1. Scheme of the design of the three different protocols used in the study. Saline and cyclophosphamide (CYP) pre-treatments are indicated by vertical thick bars in the time lines, while the following treatments at the onset of the observation period are indicated by arrows.