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Effects of combination drug therapy against OAB on micturition parameters and release of nitric oxide in the healthy and inflamed rat bladder

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Introduction and Aims

Overactive bladder (OAB) is a diagnosis which encompasses bladder hyperactivity and hypersensitivity and affects a large number of patients worldwide. The symptoms experienced by patients vary, especially when comparing OAB patients with an otherwise healthy bladder to those with concomitant inflammation. Further, it is well-known that the most common treatment against OAB, namely antimuscarinics, is less effective in patients with concomitant cystitis. Not long ago, mirabegron, a selective β3-adrenoceptor agonist, was approved for the treatment of OAB and studies have indicated that one benefit of mirabegron is its efficacy also during cystitis. While the most common treatment against OAB worldwide is still monotherapy, a combination therapy including an antimuscarinic and mirabegron has been suggested as the new golden standard treatment. While a direct effect on the detrusor is assumed, some reports on mirabegron have suggested that part of its relaxatory effect is exerted via release of nitric oxide (NO).

The aim of the current study was to compare how micturition patterns are affected by combination drug therapy against OAB in health and cystitis and if the efficacy of the drugs can be linked to altered release of NO.

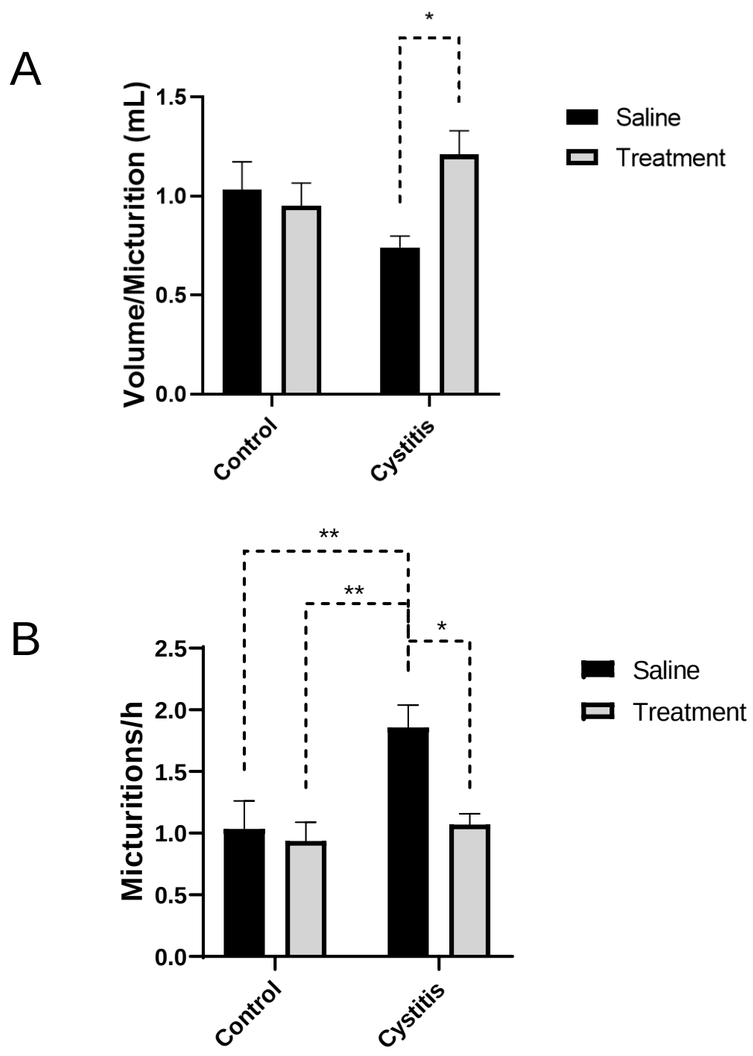

Figure 2. Volume per micturition and voiding frequency in healthy (control) rats and rats treated with CYP in order to induce cystitis. In both groups, animals were pre-treated for 10 days with either saline (■; 1 mL/kg/day s.c.) or a combination drug treatment (□) against OAB consisting of the muscarinic antagonist tolterodine (0.05 mg/kg/day s.c) and the β_3-adrenoceptor agonist mirabegron (0.6 mg/kg/day s.c.). Induction of cystitis caused (A) a decrease in micturition volume and (B) an increase in frequency which was normalized by OAB drug treatment. Statistical comparisons were made by two-way ANOVA followed by Tukey's correction for multiple comparisons. n = 8 in all groups. * p<0.05

Methods

A total of 32 adult male Sprague-Dawley rats were used in the current study, which followed national guidelines for the care and use of laboratory animals and was approved by the local ethics committee (permit #196/2013). Each rat was pre-treated twice daily with either saline (1 mL/kg s.c.) or a combination of the antimuscarinic drug tolterodine (0.05 mg/kg/day s.c.) and the β3-adrenoceptor agonist mirabegron (0.6 mg/kg/day s.c.) for a period of 10 days. Sixty hours prior to spending 16 h in a metabolism cage, the rats were treated with either cyclophosphamide (CYP; 100 mg/kg i.p.) to induce experimental cystitis or saline (4 mL/kg i.p.), serving as controls. This yielded four groups (saline pre-treated controls, drug pre-treated controls, saline pre-treated inflamed and drug pre-treated inflamed) with n = 8 in each group (Fig 1).

During the time in the metabolism cage, the micturition pattern was recorded and the total volume of urine was collected. The collected urine was rapidly frozen at -60 C and used for voltammetric measurement of nitric oxide content.

All data analysis and statistical comparisons were performed using GraphPad Prism 8.01 (GraphPad Software Inc., San Diego, USA). Two-way ANOVA followed by Tukey's correction for multiple comparisons was used to compare micturition parameters and data collected from the urine samples. All data are expressed as mean±SEM.

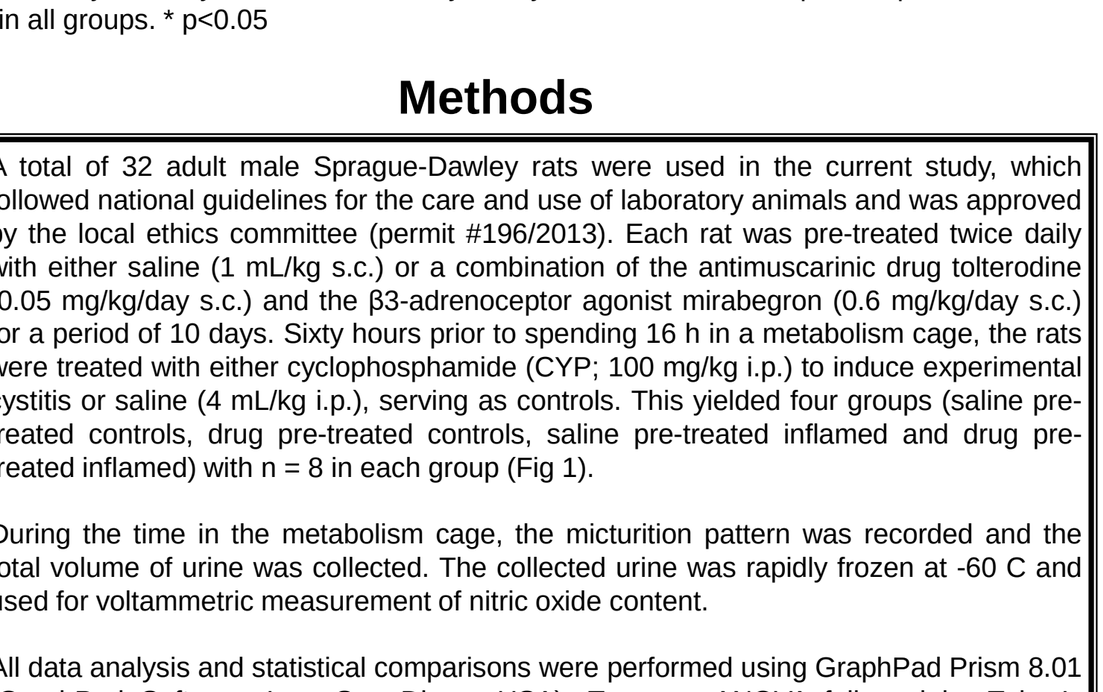

Figure 1. Experimental design. Rats were treated for 10 days with either vehicle (A, C; saline in 6% DMSO; 1 mL/kg/day i.p.) or a drug combination (B, D; mirabegron 0.6 mg/kg/day i.p. and tolterodine 0.05 mg/kg/day i.p.). Forty-eight hours prior to spending 16 h in a metabolic cage, the rats received an intraperitoneal injection with either saline (A, B; 4 mL/kg) or cyclophosphamide (C, D; CYP; 100 mg/kg).

Results

In control rats, the volume/micturition was not affected by the combination drug therapy (1.03±0.14 mL and 0.95±0.11 mL in saline pre-treated and drug pre-treated control rats, respectively; Fig 2A). Likewise, no difference could be seen when comparing number of micturitions per hour (1.03±0.23 and 0.94±0.15 mict/h in saline pre-treated and drug pre-treated control rats, respectively; Fig 2B). However, induction of cystitis with CYP caused a non-significant decrease in volume/micturition (from 1.03±0.23 to 0.74±0.06 mL; Fig 2A) and a significant increase in the number of micturitions per hour (from 1.03±0.23 to 1.86±0.18 mict/h; Fig 2B). When pre-treating the CYP-treated (inflamed) rats with the combination of tolterodine and mirabegron, the micturition pattern was normalized and no significant differences as compared to control rats were seen (1.21±0.12 mL vol/mict and 1.07±0.09 mict/h, respectively; Fig 2). Similarly, the micturition parameters in the inflamed rats which were pre-treated with the combination of drugs was significantly different as compared to saline pre-treated inflamed rats (Fig 2). Release of NO, measured by voltammetry as amount of nitrite in urine, increased significantly upon treatment with the drug combination, both in controls and rats with CYP-induced cystitis (from 25.86±1.96 in saline pre-treated controls to 48.54±2.88 and 53.42±4.28 µM in drug pre-treated controls and drug pre-treated inflamed, respectively; Fig 3). Further, induction of cystitis with CYP *per se* increased the release of NO (from 25.86±1.96 in saline pre-treated controls to 42.99±5.51 µM in saline pre-treated inflamed; Fig 3).

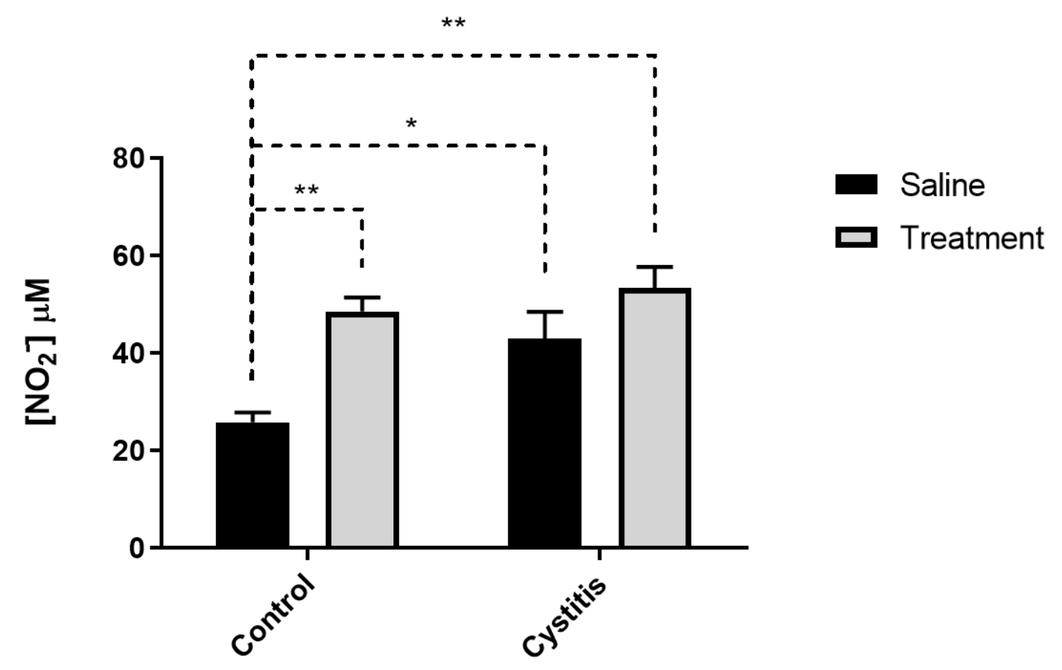

Figure 3. Levels of nitric oxide (NO) in urine from healthy (control) rats and rats treated with CYP in order to induce cystitis. In both groups, animals were pre-treated for 10 days with either saline (■; 1 mL/kg/day s.c.) or a combination drug treatment ( ) against OAB consisting of the muscarinic antagonist tolterodine (0.05 mg/kg/day s.c) and the β_3-adrenoceptor agonist mirabegron (0.6 mg/kg/day s.c.). Both combination drug treatment and cystitis caused a significant increase in the levels of NO. Measurements were conducted by voltammetry and concentration of nitrite corresponds to levels of NO in urine. Statistical comparisons are made by two-way ANOVA followed by Tukey's correction for multiple comparisons. n = 8 in all groups. * p<0.05, ** p<0.01,

Discussion

Induction of cystitis with CYP alters the micturition pattern, causing a state of overactivity. This is in line with previous studies[1,2]. The current data show that the altered micturition parameters were normalized by a combination therapy which is suggested as the golden standard drug therapy against OAB. The drug combination does not, however, affect healthy bladder parameters. This study does not take side effects into account, but clearly shows the beneficial effects of the combination therapy during cystitis. It is evident that treatment with a combination of tolterodine and mirabegron increases the release of nitric oxide. This needs to be argued to be mainly caused by mirabegron, since muscarinic antagonism most likely inhibits release of nitric oxide, if anything[3]. This is highly interesting in the context of understanding the mechanism of effect of mirabegron. However, it should be noted that inflammation per se increases the levels of NO and the level of NO is not significantly higher when comparing levels of NO during cystitis with and without drug pre-treatment. Tentatively, this is an indication of maximum NO synthase activity.

Conclusions

Induction of inflammation by cyclophosphamide alters micturition parameters. Combination drug therapy against OAB can normalize the altered parameters. Further, treatment with the combination therapy increases release of nitric oxide in the urinary bladder. However, it remains to be concluded if this significantly contributes to the beneficial effects of, mainly, mirabegron.

References

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