British Journal of Pharmacology (2008) 153, 1438–1444
 0007–1188/08 $30.00
www.brjpharmacol.org

RESEARCH PAPER

Cholinergic nitric oxide release from the urinary bladder mucosa in cyclophosphamide-induced cystitis of the anaesthetized rat

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Background and purpose: Previous reports have suggested that nitric oxide (NO) may be released by cholinergic stimuli in the rat bladder in cyclophosphamide-induced cystitis, affecting bladder function. In the current study, we evaluated the effects of cyclophosphamide-induced cystitis on muscarinic whole bladder contractile responses *in vivo*, and further, if NO might be released from the mucosa by cholinergic stimuli.
Experimental approach: Male rats were pre-treated either with cyclophosphamide (100 mg kg^{-1}; to induce cystitis) or saline (serving as controls). 60 h later, rats were anaesthetized and bladder pressure monitored.
Key results: The muscarinic receptor agonist methacholine (MeCh; 0.5–5 μg kg^{-1} i.v.) induced similar contractions (i.e. bladder pressure increases) in inflamed bladders as in controls, which were attenuated dose-dependently by the muscarinic $M_1/M_3/M_5$ antagonist 4-diphenylacetoxy-*N*-methylpiperidine (4-DAMP; 0.1–1000 μg kg^{-1} i.v.). In inflamed bladders, the cholinergic bladder contractions were enhanced after removing the mucosa, while cholinergic contractions were similar in intact and urothelium-denuded inflamed bladders in the presence of the NO synthase inhibitor N^{ω}-nitro-L-arginine methyl ester (L-NAME; 30 mg kg^{-1} i.v.). L-NAME attenuated the antagonistic effect of 4-DAMP on MeCh-induced contractions in intact inflamed bladders. However L-NAME did not affect the antagonism by 4-DAMP of MeCh-induced contractions of urothelium-denuded bladders, under control conditions or with cyclophosphamide-induced cystitis.
Conclusions and implications: In cyclophosphamide-induced cystitis, the cholinergic function of the bladder is altered. In the inflamed bladder, NO seems to be released via cholinergic stimuli through mucosal muscarinic M_3/M_5 receptors, presumably on urothelial cells, affecting bladder function.
British Journal of Pharmacology (2008) **153,** 1438–1444; doi:10.1038/bjp.2008.6; published online 4 February 2008

Keywords: urinary bladder; urothelium; cystitis; cyclophosphamide; muscarinic receptor; nitric oxide

Abbreviations: 4-DAMP, diphenylacetoxy-*N*-methylpiperidine methiodide; eNOS, endothelial NOS; IC, interstitial cystitis L-NAME, N^{ω}-nitro-L-arginine methyl ester HCl; MeCh, acetyl-β-methylcholine chloride, methacholine

Introduction

Nitric oxide (NO) plays a role in both physiological and pathological conditions of the urinary bladder. Studies have revealed the presence of nitric oxide synthase (NOS) in nerve fibres in the normal bladder, and NO has therefore been suggested to influence efferent and afferent neurotransmission in the bladder (Vizzard *et al*., 1993; Andersson and Arner, 2004; Masuda *et al*., 2007). Interstitial cystitis (IC) is a chronic inflammatory bladder disease, affecting predominantly women, and the disease is characterized by urinary frequency, urgency and bladder/pelvic pain (Chancellor and Yoshimura, 2004; Selo-Ojeme and Onwude, 2004). In IC patients, it has been reported that afferent pathways are sensitized and the release of several substances such as ATP, prostanoids and NO are associated with the condition (Andersson, 2002; Logadottir *et al*., 2004; Sun and Chai, 2006). Of these mediators, NO was proposed to have a pivotal role as a signalling molecule in IC (Logadottir *et al*., 2004). It has also been suggested that the increased activity of NO during IC leads to changes in tight junction protein dynamics leading to the observed disrupted barrier function of the urothelium in IC (Han *et al*., 2004; Birder *et al*., 2005; Parsons, 2007). In studies in animal models for IC, it has been reported that changes in the inflamed urinary bladder also occur in the efferent innervation and in the detrusor function (Mok *et al*., 2000; Giglio *et al*., 2005, 2007). Studies performed in our group revealed that during cyclophosphamide-induced cystitis in the rat, an upregulation of muscarinic M_5 receptors occurs, particularly in the

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Received 16 October 2007; revised 30 November 2007; accepted 14 December 2007; published online 4 February 2008

urothelium, together with an increase in endothelial NOS (eNOS) expression in the submucosa/mucosa (Giglio *et al*., 2005). Functional studies showed an altered cholinergic function of the inflamed bladder, which was proposed to depend on the cholinergic release of NO.

In view of the changed cholinergic function of the bladder in cyclophosphamide-induced cystitis *in vitro* (Giglio *et al*., 2005, 2007), we wondered whether or not muscarinic whole bladder contractile responses are affected by cyclophosphamide-induced cystitis *in vivo*. It was also possible that NO might be released from the mucosa by cholinergic stimuli, affecting bladder function. For this purpose, we used a cholinergic agonist methacholine (acetyl-β-methylcholine chloride (MeCh)) with low effect on nicotinic receptors and a muscarinic antagonist 4-diphenylacetoxy-*N*-methylpiperidine methiodide (4-DAMP) that has been shown *in vitro* to potently inhibit muscarinic M_3 receptor contractions and to antagonize muscarinic receptors that induce the release of NO during cystitis (Tobin and Sjogren, 1995; Hegde *et al*., 1997; Andersson and Arner, 2004; Giglio *et al*., 2005). To block the synthesis of NO, the non-selective NOS inhibitor N^{ω}-nitro-L-arginine methyl ester HCl (L-NAME) was used (Dubbin *et al*., 1990). To induce experimental cystitis, rats were currently pretreated with cyclophosphamide, a cytostatic agent that is used in the treatment of neoplastic diseases. The metabolite of cyclophosphamide, acrolein, has been attributed as being responsible for inducing haemorrhagic cystitis after cyclophosphamide treatment (Cox, 1979; Batista *et al*., 2006). Furthermore, by removing the urothelium, its role as a NO generator was examined.

Methods

Animals and pretreatments

All animal procedures and the study design were approved by the Ethical Committee of Göteborg University. A total of 40 adult male rats (300–350 g) of the Sprague–Dawley strain were used. Sixty hours prior to experiments, rats were given either a single dose of cyclophosphamide (100 mg kg^{-1} i.p.) to induce a pronounced cystitis at the time of the experiment, as previously described (Giglio *et al*., 2005), or saline (9 mg ml^{-1} i.p.) serving as control. The administration of both cyclophosphamide and saline was conducted in the presence of the analgesic buprenorphine (10 μg kg^{-1} i.m.).

Surgical procedures

On the day of the experiments, the animals were anaesthetized with pentobarbitone (45 mg kg^{-1} i.p.) followed by supplementary doses injected intravenously as required during the experiments. For maintaining a free airway, a cannula was placed in the trachea of the rat after tracheotomy. Body temperature was maintained at about 38 °C by means of a thermostatically controlled blanket connected to a thermister inserted into the rectum. The blood pressure was monitored continuously via a catheter placed into the femoral artery. A cannula placed in the femoral vein was used for all drug administrations. To block adrenergic effects, rats were administered propranolol (1 mg kg^{-1}) and phentolamine (1 mg kg^{-1}). The urinary bladder pressure was measured continuously via a catheter inserted through a small incision in the bladder and fixed with a ligature at the top of the bladder, as previously described (Modiri *et al*., 2002). An additional catheter was inserted through the same incision and fixed next to the other catheter. By the injection of small volumes of saline (0.05–0.20 ml), the bladder pressure was maintained at 10–15 mm Hg; in a state of stable anaesthesia, the variation of basal bladder pressure was minute.

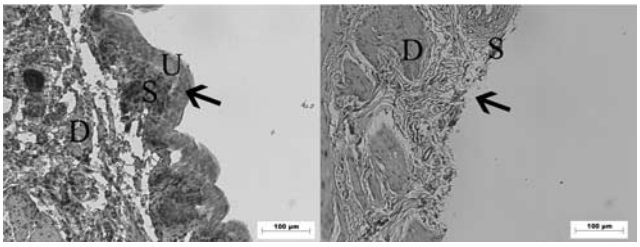

Figure 1 Photomicrographs of representative sections of an intact normal bladder (left) and a urothelium-denuded normal bladder (right). The sections are stained with haematoxylin. Arrows denote the intact urothelium and the absent urothelium in the left and right panel, respectively. *U* denotes the urothelium; *S* denotes the submucosa; *D* denotes the detrusor (smooth muscle). The horizontal scale bar indicates 100 μM.

Denudation of bladder mucosa

In one group of rats pretreated with cyclophosphamide and in one group of normal rats, collagenase I (0.1% in Hanks' balanced salt solution) was injected via one of the catheters into the urinary bladder in order to remove the bladder mucosa (referred to in the text as urothelium-denuded inflamed bladders and urothelium-denuded normal bladders, respectively). The inside of the bladder was filled with and exposed to collagenase for 30 min. Subsequently, this was followed by gently rubbing the serosal side of the bladder to detach urothelial cells on the inside of the bladder. Collagenase and loose urothelial cells were then removed from the bladder through one of the catheters by rinsing the bladder with saline. Microscopical examinations of the preparations revealed that the bladder mucosa was removed by the pretreatment (Figure 1).

Experimental protocol

In all experiments, MeCh was first injected intravenously in successively increasing doses (0.5–5 μg kg^{-1}). In one group of normal rats and in one group of cyclophosphamide pretreated rats, the bladder mucosa was then removed (see above) followed by an additional dose–response series of MeCh (0.5–5 μg kg^{-1} i.v.). In another experimental series, MeCh responses (0.5–5 μg kg^{-1}) in the presence of L-NAME (30 mg kg^{-1} i.v.) before and after denudation of the bladder mucosa were assessed in cyclophosphamide pretreated rats.

In the experimental series examining the effect of NOS blockade, L-NAME (30 mg kg^{-1} i.v.) or saline (0.9% i.v.) was administered to normal rats, cyclophosphamide pretreated rats, and cyclophosphamide and collagenase pretreated rats

(that are urothelium-denuded) followed by repeated administrations of MeCh (2 μg kg^{-1} i.v.) challenged with increasing doses of 4-DAMP (0.1–1000 μg kg^{-1} i.v.).

After the experiments, the rats were killed with an overdose of pentobarbitone and the urinary bladders were removed and examined, macroscopically as well as microscopically. Sixty hours after cyclophosphamide pretreatment, all examined bladders displayed signs of cystitis (that is bladder wall thickening, oedema and haemorrhages). The period of cyclophosphamide pretreatment was chosen, because the inflammatory signs were previously observed to be the most prominent at 60 h (Souza-Fiho *et al*., 1997; Giglio *et al*., 2005). All pressure measurements were recorded using an MP100WSW data acquisition system and Acquire software (Biopac, Goleta, CA, USA).

Statistics and calculations

Statistical significance was determined by Student's *t*-test for unpaired and paired data. *P*-values of 0.05 or less were regarded as statistically significant. Values are presented in the form of means ± s.e.mean. Data were computed and graphs were generated using the GraphPad Prism program (GraphPad Software Inc., San Diego, CA, USA). Calculations and statistics are performed on increases in bladder pressures (Δ bladder pressure) as the result of contractions, that is, bladder pressure immediately prior to contraction to peak pressure response. Statistics are calculated on raw data or on relative data when comparing the effect of 4-DAMP on responses to MeCh between groups.

Drugs

The drugs employed were buprenorphine hydrochloride, collagenase I, cyclophosphamide monohydrate, 4-DAMP, MeCh, L-NAME, phentolamine methansulphate and propranolol hydrochloride (ICI Pharmaceuticals, London, UK). All drugs were purchased from Sigma-Aldrich (St Louis, MO, USA) if not mentioned otherwise.

Results

Small spontaneous bladder contractions (< ± 3 mm Hg) occurred throughout the experiments but did not normally induce any micturition. The spontaneous contractions tended to be more pronounced in cystitis than in normal bladders but still without eliciting a micturition. Occasionally, however, a drop of urine appeared when the largest dose of MeCh was injected (5 μg kg^{-1} i.v.). The basal bladder pressure (10–15 mm Hg), induced by bladder filling with small volumes of saline (0.05–0.2 ml), was well maintained over the whole experimental period (2.5 h). In normal and cyclophosphamide pretreated rats, the mean arterial blood pressure was maintained throughout the experiments (102 ± 16 to 102 ± 13 mm Hg and 72 ± 9 to 84 ± 10 mm Hg, respectively; $n = 9$–11). Rats pretreated with cyclophosphamide displayed tendencies towards a lower blood pressure at the start of experiments compared to normal rats; however, no significance was attained (that is 73 ± 5 mm Hg compared to 95 ± 11 mm Hg, respectively; $P = 0.07$; $n = 13$–20). L-NAME (30 mg kg^{-1}) enhanced the blood pressure of both normal and cyclophosphamide pretreated rats from 89 ± 12 to 163 ± 27 mm Hg and from 73 ± 4 to 119 ± 8 mm Hg, respectively ($P < 0.05$–0.001; $n = 4$–9).

Responses to MeCh in the presence and absence of the mucosa

Intravenous injections of MeCh (0.5–5 μg kg^{-1}) evoked dose-dependent and equipotent bladder contractions in intact normal and inflamed bladders ($n = 12$–13; Figure 2a). In inflamed bladders, MeCh gave rise to larger contractions after removing the mucosa; at the highest dose (5 μg kg^{-1}), the contractions in the absence of the mucosa were 56 ± 19% larger than in its presence ($P < 0.05$; $n = 5$; Figure 2c). No differences in responses were observed between urothelium-denuded and intact normal bladders ($n = 4$; Figure 2b). In the presence of L-NAME (30 mg kg^{-1}), the contractions evoked by MeCh (0.5–5 μg kg^{-1}) were similar in intact and urothelium-denuded inflamed bladders (Figure 4a).

Effects of 4-DAMP on responses to MeCh in the presence and absence of the mucosa

Intravenous injections of 4-DAMP (0.1–1000 μg kg^{-1}) dose dependently and invariably inhibited MeCh-evoked contractions at 2 μg kg^{-1}, both in intact normal and inflamed bladders (pIC_{50} 5.1 ± 0.3 and 5.4 ± 0.2, respectively; Figures 3a and b; $n = 6$). This dose-dependent antagonism by 4-DAMP of MeCh-evoked responses was unaffected by L-NAME (30 mg kg^{-1}) in intact normal bladders (Figure 3a). However, such antagonism was less effective in the presence of L-NAME in intact inflamed bladders ($P < 0.05$; $n = 6$; Figure 3b). In urothelium-denuded normal bladders, 4-DAMP still potently antagonized the contractions evoked by MeCh (2 μg kg^{-1}; pIC_{50} 5.4 ± 0.2; data not shown). In contrast to intact inflamed bladders, L-NAME had no effect on the dose-dependent inhibitory effect of 4-DAMP on MeCh-evoked responses in urothelium-denuded inflamed bladders (Figure 4b).

Discussion

The present results show that during cystitis, the mucosa may exert an inhibitory effect on cholinergic bladder contractions. To our knowledge, this is the first *in vivo* study comparing bladder contractions in the presence and absence of the urothelium. Similar to previous reports (Hegde *et al*., 1997; Andersson and Arner, 2004; Giglio *et al*., 2005), the present results support the consensus that the cholinergic bladder contraction is mediated primarily by the stimulation of muscarinic receptors of the M_3 subtype, both in normal and inflamed bladders. Furthermore, evidence for a cholinergic-induced release of NO from the submucosa/mucosa during cystitis suggested from the *in vitro* experiments (Giglio *et al*., 2005) was presently also observed *in vivo*; that is after removing the mucosa, the MeCh-evoked contraction was enhanced in inflamed bladders, whereas this did not

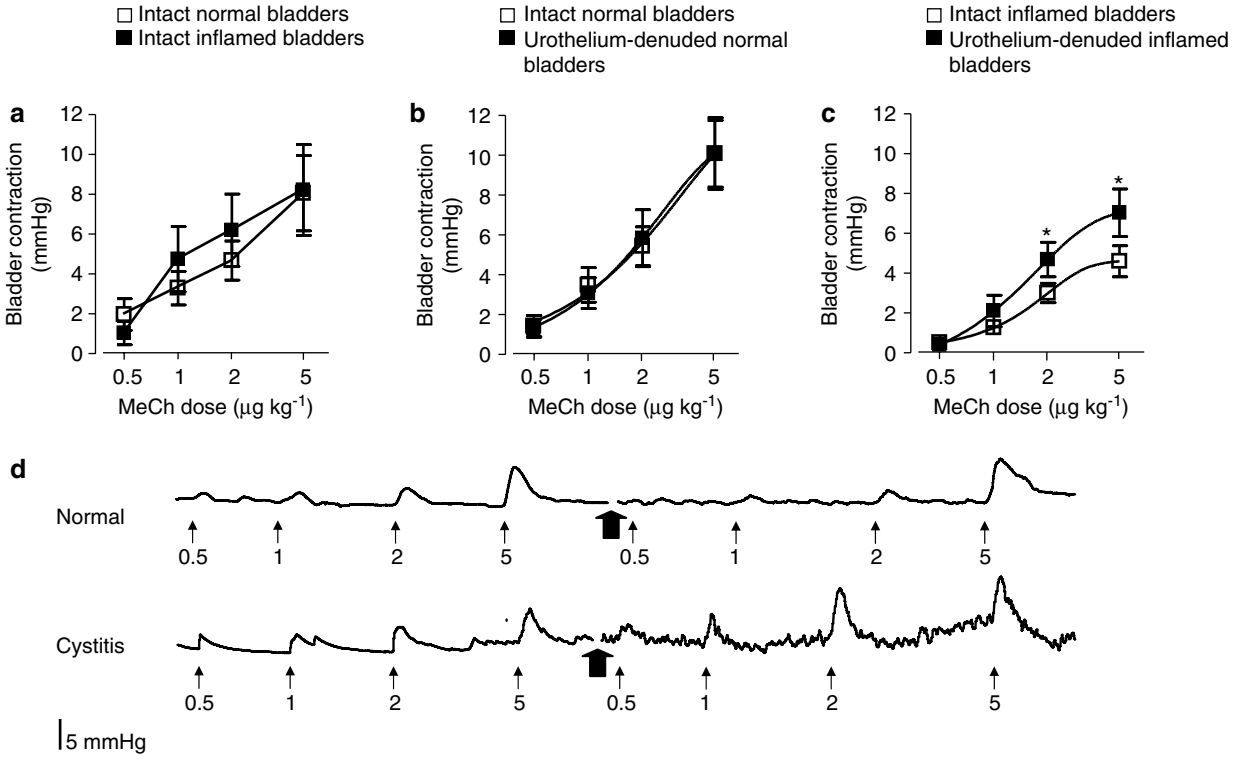

Figure 2 Mean contractile responses to i.v. injections of MeCh (0.5–5 μg kg^{-1}) in (**a**) intact normal rat bladders ($n=12$) and intact inflamed rat bladders ($n=13$), (**b**) intact normal rat bladders ($n=4$) and urothelium-denuded normal rat bladders ($n=4$) and (**c**) intact inflamed rat bladders ($n=5$) and urothelium-denuded inflamed rat bladders ($n=5$). Vertical bars represent s.e.mean. *$P<0.05$. (**d**) Representative recordings of contractions evoked by i.v. injections of MeCh (0.5–5 μg kg^{-1}) in an intact normal rat bladder and after removing the mucosa (upper recording) and in an intact inflamed rat bladder and after removing the mucosa (lower recording). Thin arrows indicate administration of MeCh (μg kg^{-1}) and thick arrows indicate removal of the mucosa.

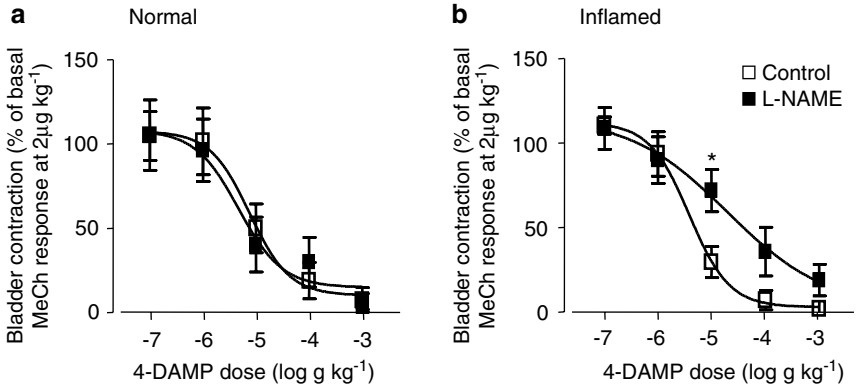

Figure 3 Mean contractile responses to i.v. injections of MeCh (2 μg kg^{-1}) in (**a**) intact normal rat bladders and in (**b**) intact inflamed rat bladders after administration of successively larger doses of 4-DAMP (0.1–1000 μg kg^{-1} i.v.) in the absence ($n=6$) and presence of L-NAME (30 mg kg^{-1} i.v.; $n=6$). Expressed as percentages of basal MeCh (2 μg kg^{-1}) response. Vertical bars represent s.e.mean. *$P<0.05$. 4-DAMP, diphenylacetoxy-*N*-methylpiperidine methiodide; L-NAME, N^{ω}-nitro-L-arginine methyl ester HCl.

occur in normal bladders. The former *in vitro* observations as well as the current *in vivo* findings support the well-established but relatively small relaxatory effect of NO on the bladder smooth muscle (Persson *et al*., 1992; Meulemans *et al*., 1995; Chung *et al*., 1996; Mothet *et al*., 1996; Masuda *et al*., 2007). However, recent studies have shown that NO donors may enhance detrusor contractions (Weng *et al*., 2006; Yanai *et al*., 2007). The mechanism of the contractile-enhancing effect involving intracellular Ca^{2+} is not fully clarified, but may involve other cell types than smooth muscle cells, for instance, interstitial cells. In the intestine, NO has been shown to modulate the function of the corresponding Cajal cells (Publicover *et al*., 1993; Daniel, 2001). In view of L-NAME enhancing the cholinergic

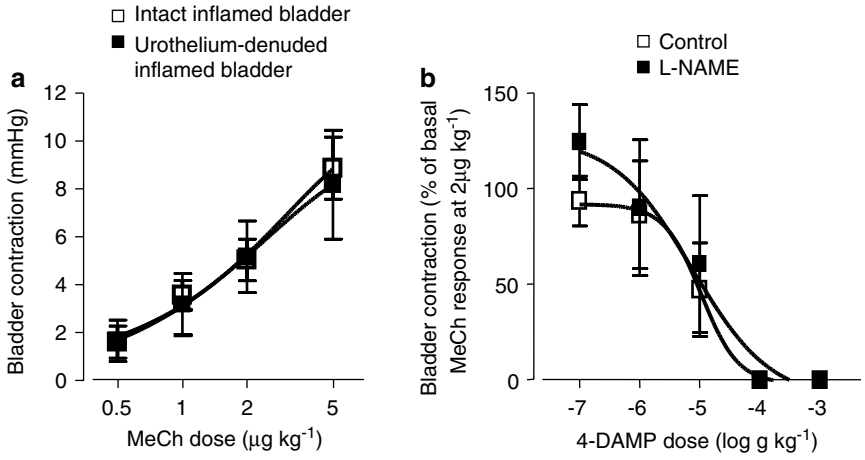

Figure 4 Mean contractile responses to i.v. injections of MeCh (0.5–5 μg kg^{-1}) in (**a**) intact inflamed rat bladders ($n=5$) and urothelium-denuded inflamed rat bladders ($n=5$) in the presence of L-NAME (30 mg kg^{-1} i.v.) and (**b**) mean contractile responses to i.v. injections of MeCh (2 μg kg^{-1}) in urothelium-denuded inflamed rat bladders after administration of successively larger doses of 4-DAMP (0.1–1000 μg kg^{-1} i.v.) in the absence ($n=5$) and presence of L-NAME (30 mg kg^{-1} i.v.; $n=5$). Expressed as percentages of basal MeCh (2 μg kg^{-1}) response. Vertical bars represent s.e.mean. 4-DAMP, diphenylacetoxy-*N*-methylpiperidine methiodide; L-NAME, N^{ω}-nitro-L-arginine methyl ester HCl.

contractions in the current study, and further, as the NO contractile effect was shown to be transient (Yanai *et al*., 2007), the current NO effect is likely to be exerted via cGMP-mediated relaxation.

Compelling evidence for NO being synthesized largely by the mucosa in the inflamed bladders is provided by the following observations. First, by blocking the synthesis of NO, the enhanced cholinergic contraction was absent in urothelium-denuded inflamed bladders. Vessels in the submucosa acting as the source for NO cannot be excluded, but the observations from the immunohistochemistry reveal that the submucosa is intact. Thus, any effect of the urothelium-removal procedure on the submucosa seems to be too small to have had any substantial influence on the bladder responses. Secondly, the NOS inhibitor L-NAME attenuated the 4-DAMP antagonizing potency on cholinergic contractions in inflamed intact bladders, whereas this effect of L-NAME was absent after removing the mucosa. Furthermore, L-NAME did not affect the potency of 4-DAMP as an antagonist of cholinergic contractions either in intact or in urothelium-denuded normal bladders.

In previous findings from our laboratory (Giglio *et al*., 2005), a pronounced upregulation of muscarinic receptors (that is, M_5) occurred and 4-DAMP showed dual effects on muscarinic responses of strip preparations of inflamed bladders *in vitro*; in addition to its inhibition of contractions, low concentrations induced a leftward shift of the concentration–response curve to carbachol. This shift could not be reproduced by the administration of the muscarinic M_1 receptor antagonist pirenzepine and, therefore, it was concluded that the muscarinic receptor responsible for the leftward shift presumably was of the M_3 and/or M_5 subtype. One plausible explanation for the apparent potentiation by 4-DAMP of the carbachol concentration–response curve of inflamed strip preparations *in vitro* could be that it inhibits muscarinic receptor-induced NO synthesis (Giglio *et al*., 2005). Regarding the anticholinergic characteristics of 4-DAMP, it possesses high affinity for muscarinic M_1, M_3 and M_5 receptors and 10 times lower affinity for the muscarinic inhibitory M_2 and M_4 receptors (Eglen and Nahorski, 2000). In fact, 4-DAMP even tends to have greater affinity for muscarinic M_5 receptors than for muscarinic M_3 receptors (Eglen and Nahorski, 2000). A corresponding leftward shift was, however, presently not observed *in vivo*; 4-DAMP had the same inhibitory potency on cholinergic bladder contractions both in inflamed and normal bladders.

Besides an upregulation of muscarinic M_5 receptors in the mucosa, immunohistochemical analyses have revealed that eNOS, and not inducible NOS or neuronal NOS, expression is increased during cyclophosphamide-induced cystitis, particularly in the submucosal and mucosal layers (Giglio *et al*., 2005). Therefore, it is postulated that through stimulation of urothelial muscarinic M_5 receptors, the urothelium releases NO affecting the cholinergic bladder contraction during inflammation. Previous studies have revealed that NO release during cystitis may initiate an afferent nerve reflex in the bladder (Vizzard *et al*., 1996). NO release may also have effects on the bladder barrier function, as it has been suggested that the augmented NO activity in IC leads to changes in tight junction protein dynamics and to disrupted barrier function of the urothelium observed in the disease (Han *et al*., 2004; Parsons, 2007).

Other effects besides stimulation of muscarinic receptors are probably not of any major importance in the current study, particularly in view of the experiments being carried out in the presence of adrenoceptor antagonists. Enzymatically and mechanically removing the mucosa could have triggered an afferent bladder reflex, thereby sensitizing bladder contractions. However, as rats were under deep anaesthesia, any of these possible effects are most likely blocked. On the other hand, removing the mucosa could have decreased the smooth muscle function of the detrusor but still MeCh elicited similar or larger contractions in urothelium-denuded bladders compared to intact bladders. Furthermore, microscopical examinations revealed an intact detrusor of the bladder after enzymatically and mechanically

removing the mucosa. Needless to say, even though the treatment removed the major part of the mucosa, it was unsuccessful in removing the mucosa in the bladder outlet. Therefore, it may only be postulated that the cholinergic release of NO during cystitis occurs from urothelial cells in the bladder body even if it is likely to occur in the bladder outlet as well.

The muscarinic receptor agonist MeCh evoked contractions of the whole bladder that were of equal magnitude in intact normal bladders and intact inflamed bladders. This is in contrast to observations made *in vitro*. Namely, *in vitro* inflammation markedly hampered bladder contractions (Giglio *et al*., 2005). One reason for the discrepancy could be that there is a limit to the rise in bladder pressure in the whole bladder preparation as the rat urinates when the bladder pressure exceeds the sphincter tonus. Also, variation in blood pressure may affect bladder pressure and the contractile responses to MeCh may have been influenced indirectly (Andersson *et al*., 1990); at the larger doses, there was a dramatic blood pressure fall. Nevertheless, when MeCh is administered intravenously, it reaches the biophase from blood vessels and is not likely to occur in high concentrations in the urothelium. *In vitro*, on the other hand, the cholinergic agonist reaches the smooth muscle and the urothelium in equal amounts. Therefore, a cholinergic release of NO from the mucosa may be more easily revealed *in vitro* than *in vivo*.

Tentatively, muscarinic receptors may also stimulate the release of some factor, besides NO, that inhibits contraction and this effect is not activated *in vivo*. Hence, the stimulation of muscarinic receptors in the mucosa *in vitro* has been suggested to induce the release of a hitherto unidentified, potent, relaxing factor, which presumably is neither NO nor a product of the COX pathway, affecting the contractile response of the pig and human bladder (Hawthorn *et al*., 2000; Chaiyaprasithi *et al*., 2003). In *in vitro* studies of the rat bladder, a cholinergic-induced release of a relaxing factor has also been demonstrated, but it seems not to be released from the mucosa (Fovaeus *et al*., 1999; Inci *et al*., 2003). In line with these studies, a relaxing factor released from the mucosa after cholinergic stimuli in the normal rat bladder could not presently be demonstrated *in vivo*, neither did removal of the mucosa alter the carbachol-evoked contractions of normal bladders *in vitro*, as studies performed at the laboratory indicate (unpublished data).

Cyclophosphamide-induced cystitis has been a model for IC in many previous reports and the two conditions resemble each other in the following aspects: both cyclophosphamide-induced cystitis and IC are non-infectious, NO has been reported as an important key factor in the pathogenesis (Alfieri *et al*., 2001; Logadottir *et al*., 2004), the urothelial barrier is disrupted (Themann *et al*., 1987; Parsons, 2007) and the conditions may display similar symptoms (Chancellor and Yoshimura, 2004; Wantuch *et al*., 2007). At present, the cholinergic function of urinary bladders affected by IC has not been assessed extensively. In view of the absence of clinical effects of anticholinergic drugs in IC (Minaglia *et al*., 2005), discrepancies in the cholinergic control of bladder contractions in IC versus the idiopathic overactive bladder may exist.

In conclusion, in cyclophosphamide-induced cystitis in the rat, the cholinergic function of the bladder is altered. In cystitis, the mucosa exerts an inhibitory role in the bladder function, presumably by releasing NO through the stimulation of mucosal muscarinic receptors of the M_3/M_5 subtype. The difference in 4-DAMP inhibitory potency on MeCh-evoked contractions (M_3) and NO release favours the assumption that the latter effect may be exerted via muscarinic M_5 receptors.

Acknowledgements

The study was supported by grants from the Lars Hierta Foundation, the Council for Medical Tobacco Research, the Wilhelm and Martina Lundgren's Foundation and the Åke Wiberg Foundation.

Conflict of interest

The authors state no conflict of interest.

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