British Journal of Pharmacology (1997) 121, 1665–1672

The role of cyclic nucleotides in guinea-pig bladder contractility

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1 The effects of phosphodiesterase (PDE) inhibition and forskolin pretreatment on the contractile responses of guinea-pig urinary bladder strips to electrical field stimulation, carbachol, ATP and KCl were studied.

2 Inhibition of cyclic AMP-specific PDE4 isozymes by rolipram significantly reduced the contractile response of bladder strips to field stimulation. Rolipram also suppressed the contractile response to low concentrations of carbachol, but potentiated the response to high concentrations. The contractile response to ATP was significantly reduced by rolipram treatment, but that to KCl was unaltered.

3 Inhibition of cyclic GMP-specific PDE5 isozymes by zaprinast had no effects on the contractile response of bladder strips to field stimulation, ATP or KCl. Zaprinast suppressed the contractile responses to 1 μM carbachol and potentiated the response to high concentrations.

4 Contractile responses to field stimulation and to carbachol after pretreatment with the adenylyl cyclase activator, forskolin, were qualitatively similar to those caused by rolipram treatment. β-Adrenoceptor blockade with propranolol partially reversed the inhibitory effects of rolipram on the response to field stimulation.

5 Rolipram significantly reduced the contractile response of bladder strips from sensitized guinea-pigs to ovalbumin challenge, but zaprinast was ineffective. PDE inhibition had similar effects on the responsiveness of control and of sensitized guinea-pig bladder strips to field stimulation, carbachol, ATP and KCl.

6 The data suggest that the contractile response of guinea-pig bladder strips can be modified by increases in cyclic AMP levels.

Keywords: Bladder; sensitization; rolipram; zaprinast; field stimulation; carbachol; ovalbumin; forskolin; propranolol

Introduction

The cyclic nucleotides, adenosine 3′:5′-cyclic monophosphate (cyclic AMP) and guanosine 3′:5′-cyclic monophosphate (cyclic GMP), regulate a number of different cellular processes. The interaction of a number of neurotransmitter substances with the enzymes adenylyl and guanylyl cyclase, stimulates the formation of cyclic AMP or cyclic GMP from adenosine 5′-triphosphate (ATP) and GTP. Cyclic nucleotide phosphodiesterases (PDE) catalyze the degradation of cyclic AMP and cyclic GMP (Beavo, 1995; Conti *et al*., 1995). Cellular levels of cyclic AMP and cyclic GMP are determined, in part, by the rate of degradation. At present, seven different families of PDE are known, which differ in their specificity for cyclic AMP and cyclic GMP, cofactor requirements and kinetic properties.

The regulation of smooth muscle tone and contraction by cyclic AMP and cyclic GMP is well established in the respiratory (Shahid *et al*., 1991; Torphy *et al*., 1993; Turner *et al*., 1994) and gastrointestinal tracts (Barnette *et al*., 1993; Williams & Parsons, 1995; Tomkinson & Raeburn, 1996), but is less well understood in the lower urinary tract. Most of the known PDE isozymes have been identified in pig and human bladder (Truss *et al*., 1995; 1996b). However, although rolipram, a cyclic AMP-specific PDE4 inhibitor, and zaprinast, a cyclic GMP-specific PDE5 inhibitor, were able to increase cyclic AMP and cyclic GMP levels *in vitro*, they were largely ineffective at reducing the contractile response to 1 μM carbachol, suggesting a dissociation of increases in cyclic nucleotides from the functional response, or intracellular compartmentation of cyclic nucleotides may occur in bladder smooth muscle (Truss *et al*., 1996a,c).

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The second messenger systems involved in bladder contraction are still unclear. The primary neurotransmitter responsible for neurogenic contraction of the bladder is acetylcholine, although noradrenaline and a number of non-adrenergic, non-cholinergic (NANC) transmitters are also released and produce contractile or relaxant effects (Andersson, 1993). The predominant muscarinic receptor in the bladder of all species studied is the M_2-subtype, stimulation of which inhibits adenylyl cyclase. However, the receptor that is responsible for bladder contraction in response to muscarinic stimulation is the M_3-subtype, which is coupled to phosphatidylinositol (PI) hydrolysis (Longhurst *et al*., 1995; Wang *et al*., 1995). Evaluation of the role of the M_2 muscarinic receptor in the bladder has been complicated by the lack of muscarinic agonists with selectivity for one subtype over the other. Furthermore, it is difficult to demonstrate functional effects of an agonist that decreases cyclic AMP levels unless cyclic AMP is first increased in some way (Ehlert & Thomas, 1995). Recent studies suggest that inhibition of cyclic AMP accumulation by M_2-receptor stimulation reverses the relaxation of rat bladder caused by the stimulation of cyclic AMP synthesis by β-adrenoceptor activation (Hegde *et al*., 1997).

In addition to influencing contraction and relaxation of cardiac and smooth muscles, cyclic AMP and cyclic GMP may play a role in inflammatory processes. Rolipram, a selective inhibitor of cyclic AMP-specific PDE4, and zardaverine, a dual inhibitor of cyclic GMP-inhibited PDE3 and PDE4, inhibit the response of isolated trachea from sensitized guinea-pigs to ovalbumin challenge (Underwood *et al*., 1993; 1994). Zardaverine or a combination of rolipram and siguazadon, an inhibitor of cyclic GMP-inhibited PDE3, reduced ovalbumin-induced bronchospasm in conscious guinea-pigs, as well as the resultant antigen-induced eosinophilia (Underwood *et al*., 1994). The role of PDE isozymes in antigen-

induced responsiveness in the trachea therefore seems to be established. However, the involvement of PDE isozymes in antigen-induced changes in bladder function has not been studied.

Interstitial cystitis (IC) is a crippling disease of the urinary bladder which afflicts primarily women (Messing & Stamey, 1978). Its aetiology and pathogenesis are obscure, but the general characteristics are those of an inflammatory state. Bladder wall mast cell numbers are often increased, and mast cell degranulation has been observed (Sant & Theoharides, 1994; Theoharides *et al*., 1995). The findings of immunoglobulin and complement deposits in bladders from IC patients, and the association of IC with other allergies, have suggested that it has an autoimmune aetiology (Oravisto, 1980; Denman, 1991; Ratliff *et al*., 1995). Sensitization of guinea-pigs with ovalbumin (Ova) followed by *in vivo* instillation or *in vitro* challenge of the bladder causes mast cell degranulation, release of inflammatory mediators, disturbances in micturition and bladder contraction; changes similar to those found in IC (Christensen *et al*., 1990; Kim *et al*., 1991; 1992; Bjorling *et al*., 1994). Pretreatment of guinea-pigs or of isolated bladder stips with a combination of cyclo-oxygenase inhibitors, histamine H_1-antagonists, lipoxygenase inhibitors, or leukotriene receptor antagonists reduces the response to Ova challenge, and further supports the premise that inflammatory mediators are involved (Christensen *et al*., 1990; Saban *et al*., 1994; Lee *et al*., 1995).

The objective of this study was to evaluate the role of cyclic nucleotides in regulating bladder contractility by examining the influence of inhibition of cyclic AMP-specific PDE4 by rolipram and cyclic GMP-specific PDE5 by zaprinast, and the effects of the adenylyl cyclase activator, forskolin, on the contractile response of guinea-pig isolated bladder strips to field stimulation, carbachol, ATP and KCl. In addition, we investigated the effects of rolipram and zaprinast on the responsiveness of bladder strips from Ova-sensitized guinea-pigs to Ova challenge.

Methods

Animals and sensitization

Female Hartley guinea-pigs (350–400 g) obtained from Ace Animals Inc. (Boyertown, PA) were randomly assigned to control and sensitized groups. Guinea-pigs in the sensitized group received three injections of ovalbumin (Ova) in saline (10 mg kg^{-1}, i.p.) 48 h apart. The control group was injected with saline on the same time schedule as the sensitized group. Three to four weeks after the last injection, the bladders were removed as described below. The protocols employed for this investigation were approved by the University of Pennsylvania and Albany College of Pharmacy Institutional Animal Care and Use Committees.

Tissue preparation

Guinea-pigs were anaesthetized with sodium pentobarbitone (Nembutal; 50 mg kg^{-1}, i.p.). The urinary bladder was removed and placed in ice-cold Krebs-Henseleit buffer of the following composition (mM): NaCl 113, KCl 4.8, $CaCl_2$ 2.5, KH_2PO_4 1.2, $MgSO_4$ 1.2, $NaHCO_3$ 25 and dextrose 5.6. Four equally sized longitudinal strips of approximately 2 mm × 10 mm were cut from the bladder body, suspended on 000 sutures between a pair of platinum ring electrodes, 8 mm apart and placed in 10 ml organ baths containing Krebs-Henseleit solution equilibrated with 95% O_2, 5% CO_2 at 37°C. The sutures were connected to Grass force displacement transducers (FT03) and the resting tension was adjusted to 2 g. Responses were recorded on a Grass Model 7E polygraph. All tissues were then given a 30 min equilibration period during which they were washed and the resting tension was adjusted every 10 min.

Contractile studies

After the equilibration period, frequency-response curves were elicited by stimulating the strips for 15 s with pulses of 0.05 ms width at 100 V every 3 min with a Grass S88 stimulator. These responses have previously been shown to be sensitive to tetrodotoxin (Tammela *et al*., 1994). Then non-cumulative concentration-effect curves to carbachol were generated. Tissues were washed at least twice between each incremental concentration. Responses to 1 mM ATP and hypertonic 120 mM KCl were then measured. After washing out KCl, strips were treated as follows:

Time experiment One strip was washed with Krebs containing vehicle twice over a 30 min period. The contractile responses to field stimulation, carbachol, ATP and KCl were then measured again. In addition, the response to 1 mg ml^{-1} Ova was measured. Because of tachyphylaxis to the contractile response to Ova, comparisons between responses before and after treatments could not be made.

PDE inhibitors One strip from each guinea-pig was washed with Krebs containing either 0.01, 0.1 or 1 μM rolipram or 1, 5 or 10 μM zaprinast twice over a 30 min period. The contractile responses to field stimulation, carbachol, ATP, Ova and KCl were then measured as described above.

Forskolin One strip from each guinea-pig was washed with Krebs containing either 0.1, 1, 10 or 100 μM forskolin twice over a 30 min period. The contractile responses to field stimulation, carbachol, ATP and KCl were then measured as described above.

Propranolol One strip from each guinea-pig was washed with Krebs containing 1 μM rolipram twice over a 30 min period. The contractile responses to field stimulation, carbachol, ATP and KCl were then measured as described above. Subsequently, the strip was washed twice over a 30 min period with Krebs containing both 1 μM rolipram and 1 μM propranolol and the contractile responses to field stimulation, carbachol, ATP and KCl were measured again. Time-control experiments showed that three successive frequency-response curves could be constructed on the same strip without any change in the E_{max} or EF_{50} (data not shown).

Drugs

Adenosine 5′-triphosphate, carbamylcholine chloride (carbachol), chicken egg albumin (ovalbumin), forskolin and propranolol were obtained from Sigma Chemical Company (St. Louis, MO). Rolipram and zaprinast were a gift from SmithKline Beecham Laboratories (King of Prussia, PA). Stock solutions of rolipram and zaprinast were made in 50 : 50 (v/v) dimethylsulphoxide (DMSO): ethanol EtOH.

Statistical analysis

Data were normalized to the maximal response generated during the first (no treatment) control curve, and are expressed as means ± s.e.mean. Geometric mean EC_{50} values and their 95% confidence limits were obtained by probit analysis (Fleming *et al*., 1972). Comparisons between responses before and after incubation with PDE inhibitors, propranolol, or forskolin were done by analysis of covariance followed by Dunnett's test. A P value <0.05 was considered significant.

Results

Responses to field stimulation

There were no significant differences in the peak contractile response of bladder strips from control or sensitized guinea-

pigs to field stimulation during the first and second frequency-response curves (data not shown). For controls, the E_{max} was 59.5 ± 9.9 mN during the first curve and 56.8 ± 11.4 mN during the second curve ($n = 5$). For sensitized guinea-pigs, the E_{max} was 72.1 ± 18.5 mN during the first curve and 71.9 ± 19.6 mN during the second curve ($n = 5$).

The PDE inhibitors had no effects on basal tone of the tissues. Rolipram (0.01 – 1 μM) caused concentration-dependent decreases in the contractile responses of bladder strips from both control and sensitized guinea-pigs to electrical field stimulation (Figure 1). At concentrations of 0.1 and 1 μM, rolipram inhibited responses to all frequencies of stimulation. At the highest concentration of rolipram used (1 μM) maximal contractile responses to field stimulation were inhibited by approximately 50%. Zaprinast (1 – 10 μM) had no effects on the response of control or sensitized guinea-pig bladder strips to field stimulation (Figure 2).

Forskolin decreased basal tone to baseline levels. The tension on the strips was readjusted to 1 g before the response to stimulation was measured. Forskolin (10 and 100 μM) caused concentration-dependent decreases in the contractile responses of control bladder strips to electrical field stimulation (Figure 3).

Incubation of control guinea-pig bladder strips with propranolol (1 μM) after stimulation in the presence of 1 μM rolipram caused a small, but significant, shift of the frequency-response curve to the left (EF_{50} before rolipram: 3.9 ± 0.5 Hz; EF_{50} after rolipram: 20.8 ± 3.2 Hz; EF_{50} after rolipram and propranolol: 13.9 ± 3.8 Hz) (Figure 4). Incubation with propranolol alone had no significant effects on the E_{max} or EF_{50} values (data not shown).

Responses to carbachol

There were no significant differences in the responses of bladder strips from control or sensitized guinea-pigs to high concentrations of carbachol during the first and second concentration-response curves (data not shown). For controls, the E_{max} was 41.5 ± 3.0 mN during the first and 48.2 ± 4.8 mN during the second curve. The EC_{50} values were unchanged, at

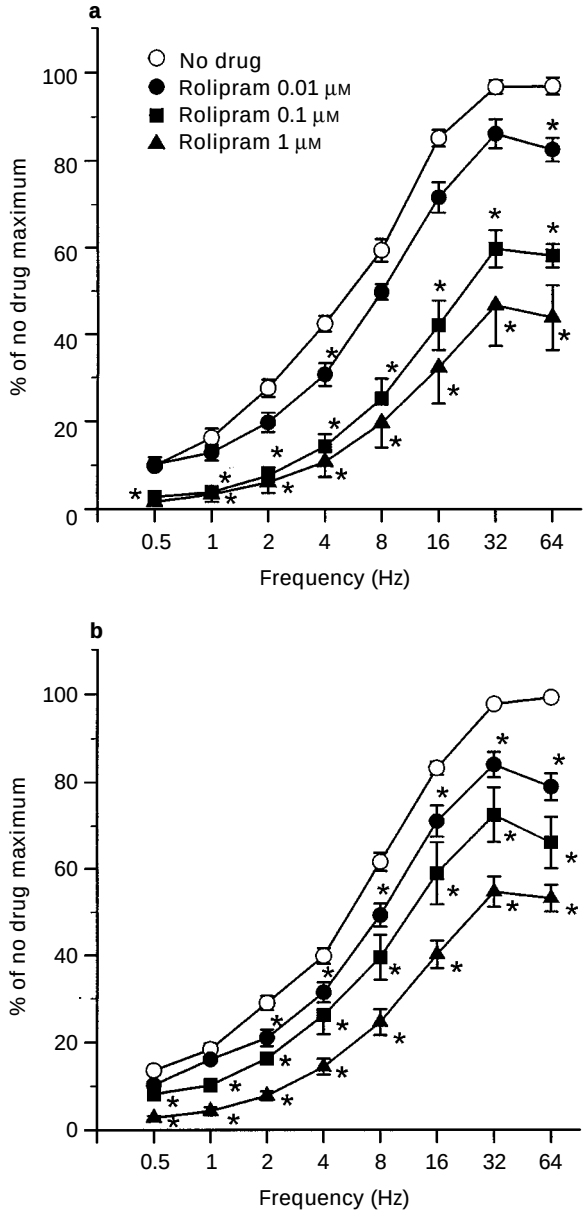

Figure 1 Effects of rolipram on the frequency-response curves of control (a) and sensitized (b) guinea-pig bladder strips. Data are expressed as percentage of the maximum no drug response. Each point represents the mean and vertical lines show s.e.mean of 3 to 5 individual observations. *$P < 0.05$ (vs no drug values).

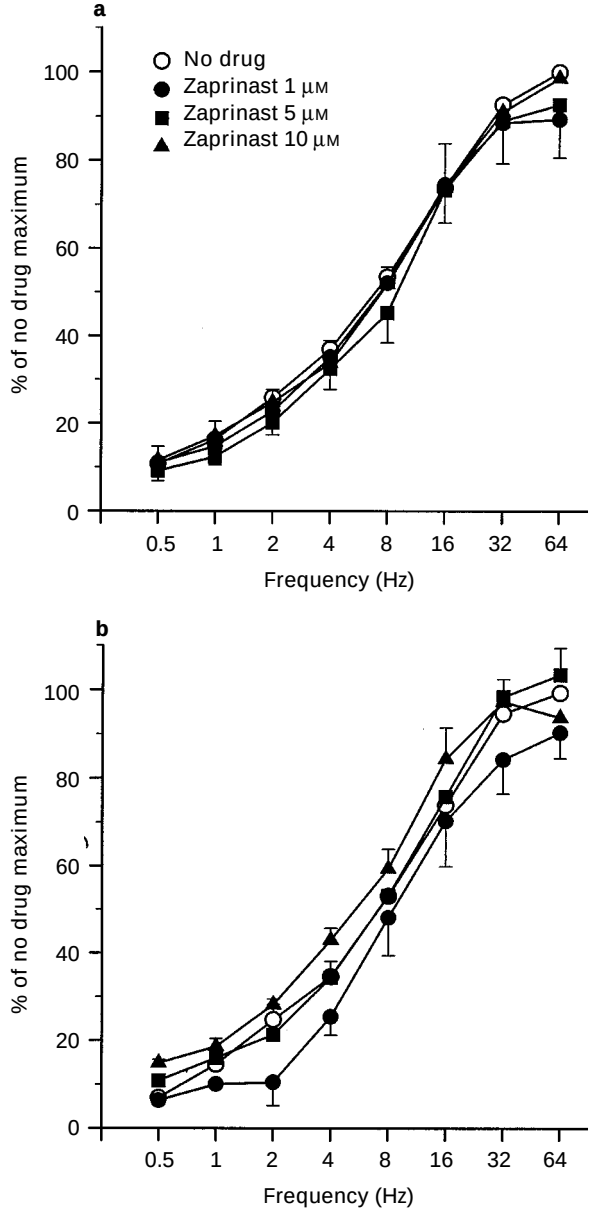

Figure 2 Effects of zaprinast on the frequency-response curves of control (a) and sensitized (b) guinea-pig bladder strips. Data are expressed as percentage of the maximum no drug response. Each point represents the mean and vertical lines show s.e.mean of 3 to 5 individual observations.

0.32 μM (95% confidence limits (CL): 0.18 ± 0.56) during the first and 0.26 μM (95% CL: 0.09–0.77) during the second curve. For strips from sensitized guinea-pigs, the E_{max} was 56.3 ± 16.6 mN during the first and 57.1 ± 17.4 mN during the second curve. EC_{50} values were 0.38 μM (95% CL: 0.19–0.76) during the first and 0.58 μM (95% CL: 0.34–1.00) during the second curve.

Rolipram caused biphasic shifts in the concentration-response curves of guinea-pig bladder strips to carbachol. In general, contractile responses to low concentrations of carbachol ($\geqslant 1$ μM) were reduced by rolipram in a concentration-dependent manner. Responses to 10 and 30 μM carbachol were increased by rolipram (Figure 5). Zaprinast had less effect on the responses to carbachol than did rolipram (Figure 6). The EC_{50} values for carbachol were increased by both PDE inhibitors (Table 1). There were no significant differences in responsiveness of strips from control or sensitized guinea-pigs to either carbachol, rolipram or zaprinast.

The effects of forskolin on the concentration-response curves of control guinea-pig bladder strips to carbachol were biphasic, similar to those seen after rolipram. Once again, contractile responses to low concentrations of carbachol ($\leqslant 1$ μM) were reduced, while responses to higher concentrations were potentiated (Figure 7). The EC_{50} values for carbachol were increased by forskolin treatment (Table 1).

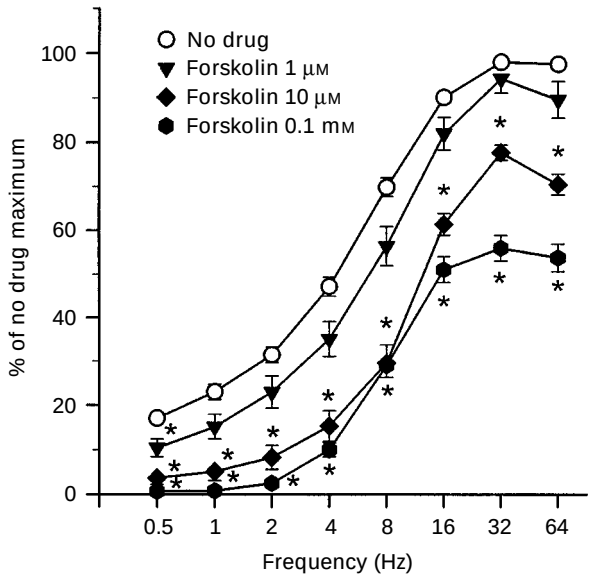

Figure 3 Effects of forskolin on the frequency-response curves of control guinea-pig bladder strips. Data are expressed as percentage of the maximum no drug response. Each point represents the mean and vertical lines show s.e.mean of 8 individual observations. $^{*}P<0.05$ (vs no drug values).

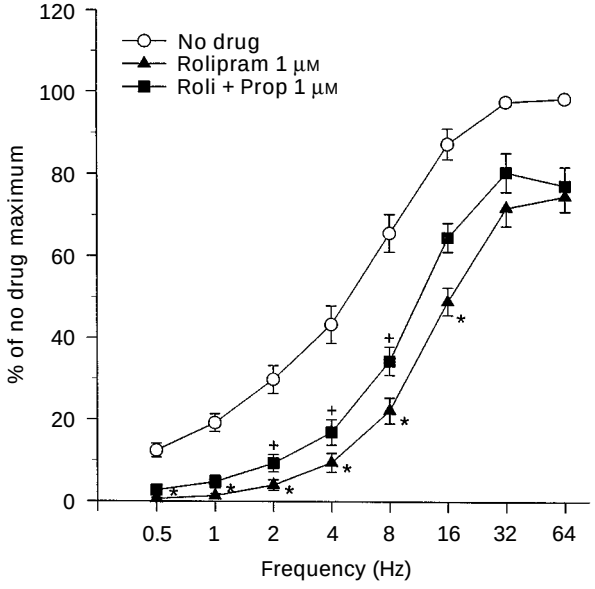

Figure 4 Effects of incubation with rolipram (1 μM) and propranolol (1 μM) on the frequency-response curves of control guinea-pig bladder strips. Data are expressed as percentage of the maximum no drug response. Each point represents the mean and vertical lines show s.e.mean of 8 individual observations. $^{*}P<0.05$ (vs no drug values), $^{+}P<0.05$ (vs rolipram alone).

Responses to Ova, ATP and KCl

Ovalbumin caused bladder strips from sensitized guinea-pigs to contract, but had no effects on strips from controls.

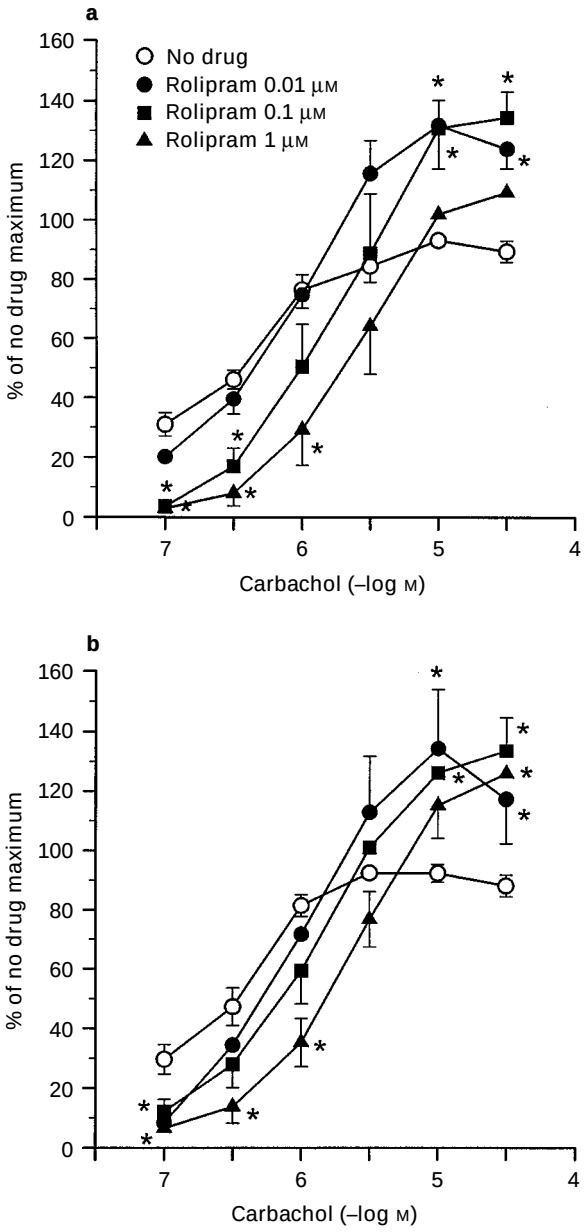

Figure 5 Effects of rolipram on the concentration-response curves to carbachol of control (a) and sensitized (b) guinea-pig bladder strips. Data are expressed as percentage of the maximum no drug response. Each point represents the mean and vertical lines show s.e.mean of 3 to 5 individual observations. $^{*}P<0.05$ (vs no drug values).

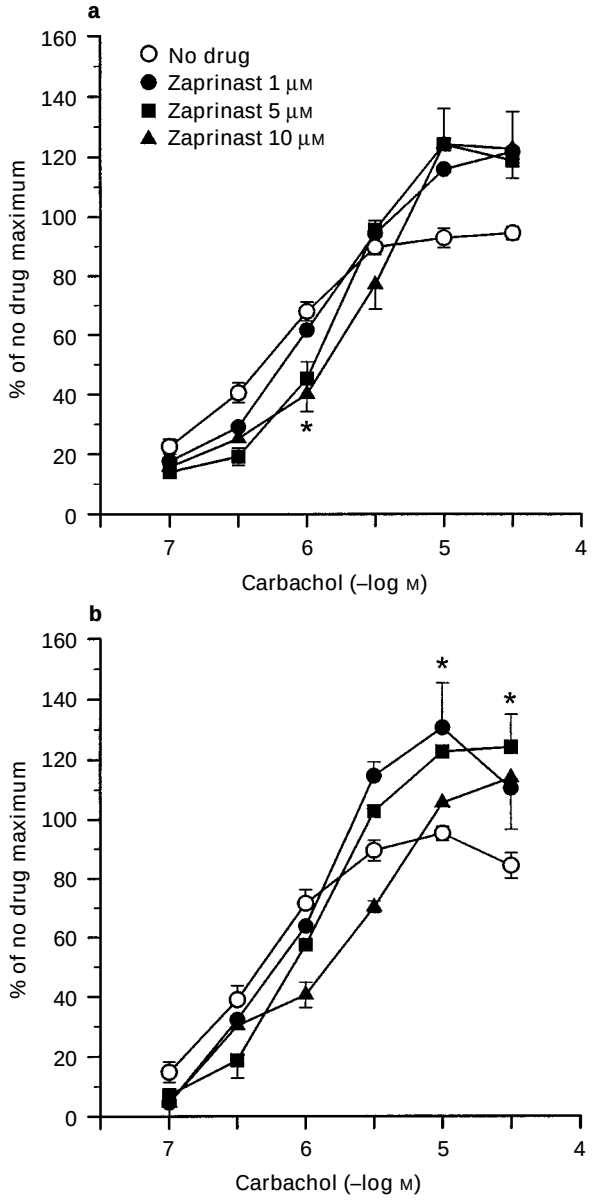

Figure 6 Effects of zaprinast on the concentration-response curves to carbachol of control (a) and sensitized (b) guinea-pig bladder strips. Data are expressed as percentage of the maximum no drug response. Each point represents the mean and vertical lines show s.e.mean of 3 to 5 individual observations. $*P<0.05$ (vs no drug values).

Table 1 Effect of treatments on EC_{50} values for carbachol in guinea-pig bladder

	Control		*Sensitized*	
Inhibitor	*Before*	*After*	*Before*	*After*
Rolipram	0.25	3.61*	0.22	1.60*
1 μM	(0.18–0.34)	(2.18–5.95)	(0.14–0.34)	(0.89–2.90)
Zaprinast	0.38	1.19	0.38	1.51*
10 μM	(0.30–0.49)	(0.79–1.79)	(0.24–0.60)	(1.22–1.86)
Forskolin	0.35	1.70*	ND	ND
10 μM	(0.22–0.55)	(1.20–2.42)		

Values indicate the geometric mean EC_{50} values (μM) of 3 to 8 individual observations made before and after each treatment. 95% confidence limits are given in parentheses. $*P<0.05$ (vs before values). ND: not determined.

Rolipram (0.1 and 1 μM) significantly reduced the response to Ova (Figure 8). Zaprinast had no effects on the contractile response to Ova (Figure 9). Rolipram significantly

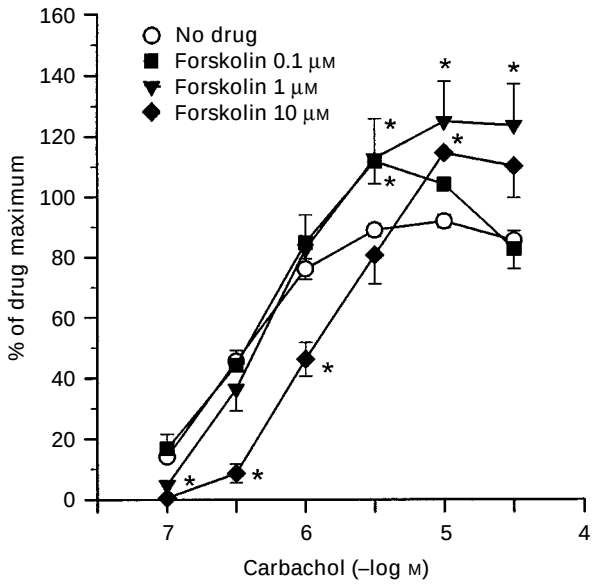

Figure 7 Effects of forskolin on the concentration-response curves to carbachol of control guinea-pig bladder strips. Data are expressed as percentage of the maximum no drug response. Each point represents the mean and vertical lines show s.e.mean of 8 individual observations. $*P<0.05$ (vs no drug values).

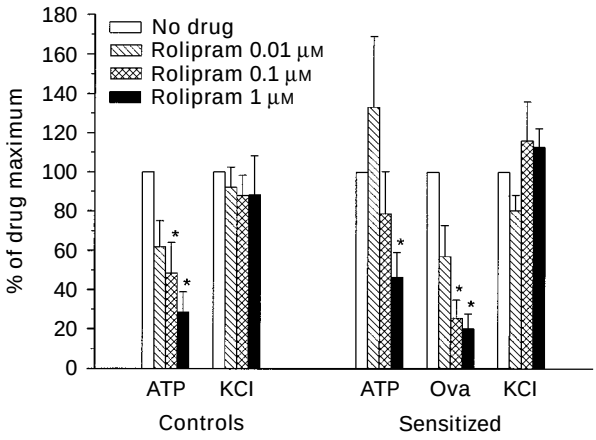

Figure 8 Effects of rolipram on contractile responses of control and sensitized guinea-pig bladder strips to 1 mg ml^{-1} ovalbumin (Ova), 1 mM ATP and 120 mM KCl. Data are expressed as percentage of the maximum no drug response. Each column represents the mean and vertical lines show s.e.mean of 3 to 5 individual observations. $*P<0.05$ (vs no drug values).

reduced the contractile responses of control and sensitized bladder strips to 1 mM ATP, but had no effects on the response to 120 mM KCl (Figure 8). Zaprinast had no effects on the contractile responses to ATP or KCl (Figure 9).

Discussion

Control responses

The contractile response of the urinary bladder to neurogenic stimulation is complex, consisting of a rapid phasic response attributed to stimulation of P2X purinoceptors by ATP and the opening of ligand-gated ion channels, and a slower tonic response which results from stimulation of M_3 receptors by acetylcholine and the concomitant hydrolysis of PI. Bladder stimulation *in vitro* also causes the release of noradrenaline (NA) from sympathetic nerve endings (Somogyi & de Groat,

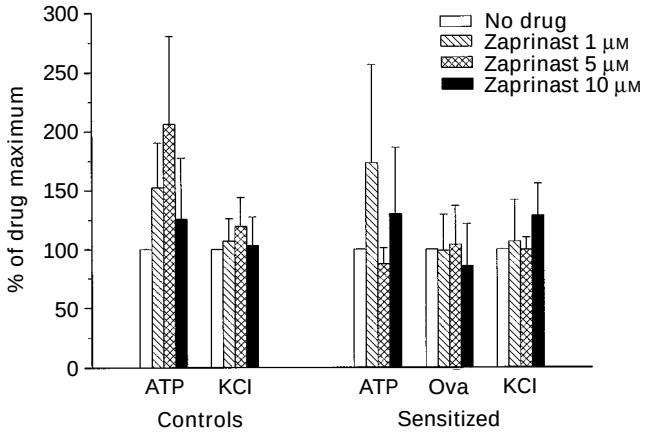

Figure 9 Effects of zaprinast on contractile responses of control and sensitized guinea-pig bladder strips to 1 mg ml^{-1} ovalbumin (Ova), 1 mM ATP and 120 mM KCl. Data are expressed as percentage of the maximum no drug response. Each column represents the mean and vertical lines show s.e.mean of 3 to 5 individual observations.

1990). β-Adrenoceptors coupled to adenylyl cyclase predominate over α-adrenoceptors in the bladder body (Levin & Wein, 1979), and exogenous NA typically causes relaxation and inhibits the contractile responses of bladder strips to field stimulation and to acetylcholine (Ambache & Zar, 1970). In addition, other NANC mechanisms may contribute to the overall response of the bladder to electrical stimulation, including the release of nitric oxide, tachykinins, neuropeptide Y, calcitonin gene-related peptide, enkephalins, bradykinin, galanin, endothelins, prostaglandins and 5-hydroxytryptamine (Andersson, 1993).

In the present study, inhibition of cyclic AMP-specific PDE4 isozymes by rolipram reduced the neurogenic response of control guinea-pig bladder strips by up to 50%. This degree of suppression was equivalent to that produced by muscarinic antagonism in the rat bladder (Longhurst *et al*., 1995), and considerably greater than that produced by atropine in the guinea-pig bladder (Brading & Mostwin, 1989). Treatment of bladder strips with the adenylyl cyclase activator, forskolin, produced a quantitatively similar inhibition of the frequency-response curve. The data therefore suggest that field stimulation of guinea-pig bladder strips produces increases in cyclic AMP which are potentiated by PDE4 inhibition with rolipram, resulting in the observed suppression of the neurogenic response. This increase in cyclic AMP probably results from stimulation of adenylyl cyclase-coupled β-adrenoceptors by NA released during field stimulation. The leftward shift of the frequency-response curve observed after pretreatment with both rolipram and propranolol lends support to this hypothesis.

Although muscarinic M_2 receptors coupled to adenylyl cyclase predominate over M_3 receptors in the bladder, until recently their functional role was unknown (Longhurst *et al*., 1995; Wang *et al*., 1995; Hegde *et al*., 1997). In trachea, intestinal and bladder smooth muscle, M_2 receptors predominate over the M_3 receptors, but the latter mediate contraction (Ford *et al*., 1991; Watson & Eglen, 1994; Hegde *et al*., 1997). It has been suggested that activation of M_2 receptors in these tissues may inhibit β-adrenoceptor-mediated relaxation by coupling to the pertusis toxin-sensitive Gi and inhibiting adenylyl cyclase, thus facilitating M_3 receptor-mediated contraction. Hegde and co-workers propose that acetylcholine released from postganglionic parasympathetic nerves, activates M_2 receptors to reverse sympathetic β-adrenoceptor-mediated relaxation of the bladder body. In concert with direct M_3-mediated contraction, this promotes more efficient bladder emptying (Hegde *et al*., 1997).

Rolipram and forskolin had biphasic effects on the responses to muscarinic stimulation by carbachol. In general, the responses to low concentrations of carbachol were inhibited, but the responses to higher concentrations of carbachol were potentiated. To our knowledge, potentiation of muscarinic agonist-induced contraction by PDE inhibitors or by forskolin has not previously been shown for any smooth muscle system. The inhibitory effects of rolipram were dose-dependent. However, the potentiation of the contractile response was more prominent in the presence of low concentrations of rolipram. Presumably rolipram and forskolin produced concentration-dependent increases in cyclic AMP. The overall contractile response is a composite of the effects of M_2 and M_3 receptor stimulation. Caulfield suggested that the different transduction mechanisms activated by M_2 or M_3 receptor stimulation had different agonist sensitivities (Caulfield, 1993). Thus, low concentrations of muscarinic agonists stimulate M_2 receptors, while M_3 receptors operate at higher agonist concentrations. Rolipram, by preventing the breakdown of basal cyclic AMP, and forskolin, by stimulating cyclic AMP formation, may increase cyclic AMP concentrations sufficiently to cause relaxation and antagonize the responses to low concentrations of carbachol. Eventually as the concentration of carbachol is increased, contraction predominates as a result of M_3-stimulated PI hydrolysis. This contribution of more than one mechanism to the carbachol concentration-response curve may explain the biphasic responses observed in this study. However, further studies comparing tissues levels of cyclic AMP and inositol phosphates with contractile responses will be required to evaluate this hypothesis.

The PDE5 inhibitor, zaprinast had no effects on the response of bladder strips to field stimulation, and was less efficaceous at inhibiting the contractile response to low concentrations of carbachol than was rolipram or forskolin. Muscarinic receptor-induced increases in cyclic GMP probably result from activation of the nitric oxide synthase pathway. The role of cyclic GMP in bladder function is unclear. Relaxation in response to field stimulation is rarely seen in pre-contracted bladder body strips, but can be demonstrated in urethral strips, where NO is thought to be a NANC transmitter (Andersson & Persson, 1994). NO donors are only moderately effective at relaxing bladder strips, even though they potently relax urethral strips. Furthermore, the density of nitric oxide synthase (NOS) immunoreactivity is significantly less in the bladder body than in the urethra (Andersson & Persson, 1994). Activation of guanylate cyclase by sodium nitroprusside, ATP and atrial natriuretic factor has been demonstrated in guinea-pig bladder homogenates (Wheeler *et al*., 1997), but whether carbachol stimulates guanylate cyclase activity in the bladder is not known. Overall, the data suggest that cyclic GMP plays little role in bladder body relaxation. Therefore, it is difficult to explain the mechanism(s) by which zaprinast altered the responses to carbachol. Inhibition of the contractile response was significant at only 1 μM carbachol in the presence of the highest concentration of zaprinast. In most systems, zaprinast is approximately 100 fold more selective for PDE5 than for the other PDE isozymes, with an IC_{50} value of about 1 μM. Therefore, inhibition of other PDEs seems unlikely at the concentrations used in this study. Like rolipram and forskolin, zaprinast potentiated the contractile response to high concentrations of carbachol, probably due to an interaction with the M_3 receptor. Further studies examining the activation of second messenger systems in the guinea-pig bladder by carbachol, as well as potential interactions between the second messenger systems, will be required to evaluate the mechanisms responsible for the observed effects of zaprinast.

Our findings differ from those of Truss and co-workers who examined the effects of various PDE inhibitors on the contractile response of pig bladder to 1 μM carbachol and on cyclic nucleotide levels (Truss *et al*., 1996c). They found significant decreases in the contractile response to carbachol and increases in cyclic AMP levels only with forskolin, the non-specific PDE inhibitor, papaverine, or the PDE1 inhibitor, vinpocetine. Neither rolipram nor zaprinast at doses up to 100 μM affected the response to carbachol or cyclic AMP and cyclic GMP levels. The latter results differ from those in the

present study. The differences between our findings and those of Truss *et al*. may be species-related, but may also result from differences in methodology. We incubated the strips with the PDE inhibitors for 30 min before doing non-cumulative concentration-response curves to carbachol. Tissues were exposed to carbachol for no longer than 5 min during which time maximal contractile responses occurred. In contrast, Truss and co-workers added increasing concentrations of the PDE inhibitors to the bath after incubating the strips with carbachol for approximately 45 min (Truss *et al*., 1996c). Although not shown in their paper, it is unlikely that the strips were incubated with the inhibitors as long as in our study. Additionally, because the effects of PDE inhibition on the concentration-response curve for carbachol may be biphasic in the pig, like in the guinea-pig, the dose of carbachol chosen by Truss *et al*. may have been in the mid to top portion of the concentration-response curve where the inhibitory effects of rolipram and zaprinast were negligible.

Our results indicate that rolipram also inhibits the contractile response to ATP, but has no effects on the response to KCl. This probably indicates that the voltage-operated calcium channels and contractile proteins are unaffected by PDE inhibition, as would be expected. The contractile response of the urinary bladder to ATP is thought to result from stimulation of ion channel-coupled P2X purinoceptors, although other excitatory and inhibitory purinoceptors coupled to different effector systems may also be involved (Ziganshin *et al*., 1993; Palea *et al*., 1994). Accumulation of basal cyclic AMP as a result of PDE4 inhibition by rolipram is probably the mechanism responsible for inhibition of the response to ATP.

Sensitized guinea-pigs

Previous studies on trachea or intestinal smooth muscle from sensitized guinea-pigs found that PDE4 inhibitors, such as rolipram, produced significant inhibition of Ova-induced bronchoconstriction and colonic smooth muscle contraction (Underwood *et al*., 1993; 1994; Grous & Barnette, 1994). Similar inhibitory effects of rolipram on Ova-induced bladder contraction were found in the present study. Our findings that zaprinast had no effect on Ova-induced bladder contraction is in agreement with data obtained with other tissues in which PDE5 inhibitors are generally ineffective (Grous & Barnette, 1994; Underwood *et al*., 1994). The mechanism of action of rolipram to inhibit the response to Ova challenge is unclear. Previous studies have suggested that the major site of action in trachea or colon is the inflammatory cell, rather than the smooth muscle cell. In the bladder, rolipram decreased responsiveness to field stimulation, carbachol and ATP, as well as Ova but not to KCl. At the concentrations used in the present study, the effects of rolipram should be specific for PDE4, but we cannot distinguish between the different sites of action.

In summary, inhibition of PDE4 isozymes by rolipram decreased the contractile responses of guinea-pig bladder strips to field stimulation, low concentrations of carbachol and ATP, as well as the response of sensitzed bladder strips to Ova challenge. The inhibitory effects of rolipram on the response to field stimulation were partially reversed by propranolol. Inhibition of PDE5 isozymes by zaprinast had no effects on the response to field stimulation, ATP or Ova. The data suggest that the contractile response of the guinea-pig bladder can be modified by changes in cyclic AMP levels.

This work was supported in part by USPHS grant DK 44801. The assistance of Professor Janice Jarvis with the statistical analyses is gratefully acknowledged.

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(Received January 3, 1997
Revised May 19, 1997
Accepted May 22, 1997)