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The effect of isoenzyme-selective PDE inhibitors on methacholine-induced contraction of guinea-pig and rat ileum

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1 We have examined the effects of the isoenzyme-selective phosphodiesterase (PDE) inhibitors, vinpocetine (type 1), siguazodan (type 3), rolipram (type 4) and zaprinast (type 5) and the non-selective PDE inhibitor enprofylline on methacholine (MCh) contractile concentration-response curves on guinea-pig and rat isolated ileum.

2 In guinea-pig ileum, vinpocetine (10-300 μM), zaprinast (1-300 μM) and enprofylline (100-1000 μM) produced a concentration-dependent depression of the maximum response (E_{max}) to MCh only without effect on the MCh EC_{50} values (rank order of potency: zaprinast > vinpocetine > enprofylline). In contrast, siguazodan (10-300 μM) and rolipram (10-300 μM) produced a rightward displacement of the MCh concentration-response curve (increase in EC_{50}: rank order: rolipram > siguazodan), with effects on the MCh maximum seen only at higher concentrations.

3 In the rat ileum, vinpocetine (10-300 μM), zaprinast (0.1-300 μM) and enprofylline (100-1000 μM) caused depression of the MCh maximum contraction (rank order: zaprinast > vinpocetine > enprofylline). Low concentrations of rolipram and siguazodan had no significant effect on the MCh maximum. In the presence of higher concentrations (> 100 μM) of rolipram and siguazodan, a maximum response was not achieved at the highest concentration of MCh tested. As in the guinea-pig ileum, only rolipram (10-300 μM) and siguazodan (10-300 μM) produced a significant, concentration-dependent, rightward displacement of the MCh concentration-response curve (increase in EC_{50}: rank order: rolipram > siguazodan).

4 In the guinea-pig ileum, isoprenaline (0.1 μM) produced a rightward displacement (~3 fold) of the MCh concentration-response curve, accompanied by a significant depression of the maximum response. Increasing the isoprenaline concentration (1 μM) had no further effect on either parameter. Sodium nitroprusside (SNP, $\geq$10 μM) produced a concentration-dependent depression of the MCh maximum without an effect on the EC_{50}.

5 In the rat ileum, isoprenaline (1 μM) produced a concentration-dependent rightward displacment (~2.8 fold) of the MCh concentration-response curve with depression of the MCh maximum at higher ($\geq$100 μM) concentrations. SNP produced depression of the MCh maximum at a concentration of 10 μM and above. Effects on the MCh EC_{50} were seen only at 100 and 300 μM.

6 In guinea-pig ileum, isoprenaline (0.1 μM) in combination with rolipram (10 μM) further increased the MCh EC_{50} and reduced the MCh maximum. The combination of SNP (10 μM) with zaprinast (0.1 μM) produced no further significant effect than SNP alone.

7 In rat ileum, isoprenaline (1 μM) in combination with rolipram (10 μM) further increased the EC_{50} and reduced the maximum. SNP (10 μM) had no significant effect on either the MCh maximum or EC_{50}. A combination with zaprinast (1 μM) had no further effect.

8 In conclusion, all the PDE inhibitors tested produced a concentration-dependent inhibition of the MCh concentration-response curve, indicating a modulator role for the PDE isoenzymes in gastrointestinal smooth muscle contractility. The PDE inhibitors that elevate cyclic GMP produced a depression of the MCh maximum response only, whilst those that elevate cyclic AMP produced a rightward displacement of the MCh concentration-response curve. This was confirmed by the use of isoprenaline and SNP. This difference in the type of inhibition produced by these PDE isoenzyme inhibitors may reflect a different intracellular site/mechanism by which the cyclic AMP- and cyclic GMP-activated kinases act functionally to antagonize the contractile response.

Keywords: PDE inhibitors; rolipram; siguazodan; vinpocetine; zaprinast; enprofylline; cyclic AMP; cyclic GMP; gastrointestinal smooth muscle; ileum

Introduction

Elevation of cellular adenosine 3':5'-cyclic monophosphate (cyclic AMP) and guanosine 3':5'-cyclic monophosphate (cyclic GMP) has been associated with smooth muscle relaxation in several regions of the gastrointestinal tract including the lower oesophageal sphincter, ileum, proximal colon, taenia coli, and internal anal sphincter (Barnette *et al.*, 1988; 1989; 1993; Joslyn *et al.*, 1988; 1990: Small *et al.*, 1989b; Grouse *et al.*, 1991). The cellular levels of the cyclic nucleotides reflect a balance between their synthesis and catabolism, the latter being regulated by the phosphodiesterase (PDE) family of enzymes. At least five families, designated PDE 1-5, have been classified according to their substrate preference, cofactor requirements, and sensitivity to endogenous inhibitors and activators (Beavo & Reifsnyder 1990; Nicholson & Challiss, 1991; Thompson, 1991). In addition, molecular biology techniques have revealed the existence of at least three other isoenzymes, 6, 7 and 8 (Nicholson & Shahid, 1994).

Differences in the tissue distribution of these PDE isoenzymes has lead to a resurgence of interest in the development of selective inhibitors of the PDE isoenzymes and a number of

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PDE inhibitors with selectivity for a specific isoenzyme have been developed (Karlsson *et al.*, 1993; Raeburn *et al.*, 1993; Nicholson & Shahid, 1994; Dent *et al.*, 1994). These include several type 3 inhibitors, a smaller but growing number of type 4 inhibitors, a limited number of type 5 and type 1 inhibitors and the recent description of a putative type 2 inhibitor, MEP-1 (Podzuweit & Muller, 1993).

With the development of such isoenzyme-selective PDE inhibitors, the functional role of the different PDE isoenzymes in both airways and vascular smooth muscle has been extensively investigated. In contrast the functional role of PDE isoenzymes in the gastrointestinal tract smooth muscle is much less well defined. In the canine lower oesophageal sphincter, both type 3 (SKF 94120) and type 5 (M&B 22948, zaprinast) inhibitors have been shown to produce a concentration-dependent relaxation of basal tone (Rattan & Moummi, 1989; Barnette *et al.*, 1990), the type 3 PDE inhibitor being the more potent. In another study, the type 3/4 mixed PDE inhibitor AH 21-132 exhibited a non-specific relaxant effect on guinea-pig ileum (Small *et al.*, 1989a). A more complete study of canine colon (Barnette *et al.*, 1993) indicated the presence of at least five different PDE isoenzymes. By use of selective PDE isoenzyme inhibitors for types 3, 4 and 5, they demonstrated the type 4 isoenzyme as the most important functionally. More recently, the type 3, 4 and 5 PDE isoenzymes have been shown to be involved in the regulation of tone in the cat gastric fundus (Barbier & Lefebvre, 1995). As in the canine colon, the type 4 PDE was the most important functionally.

In the present study we have used the isoenzyme-selective PDE inhibitors vinpocetine (type 1), siguazodan (type 3), rolipram (type 4) and zaprinast (type 5) and the non-selective PDE inhibitor, enprofylline, to examine the functional role of PDE isoenzymes in regulating contractility of guinea-pig and rat ileum. In addition, we have also investigated the effect of isoprenaline and sodium nitroprusside (SNP), compounds which stimulate the synthesis of cyclic AMP and cyclic GMP respectively, and their possible interaction with PDE inhibitors on ileum contractility.

A preliminary account of some of these findings has been presented previously (Tomkinson & Raeburn, 1995).

Methods

Tissue preparation

Guinea-pigs (male, Dunkin Hartley, 400–500 g) or rats (male, Sprague Dawley, 300–450 g) were killed by cervical dislocation/stunning and exsanguination. The ileum were removed and placed in Tyrode solution and cleaned. Lengths (15–20 mm) of ileum were cut and suspended in Tyrode under an applied load (1 g). The Tyrode was maintained at 35°C and gassed with 5% CO_2 in O_2, pH was 7.4. The composition of the Tyrode solution was (mM): NaCl 137, KCl 2.7, $MgCl_2$ 1.0, $CaCl_2$ 1.8, NaH_2PO_4 0.4, $NaHCO_3$ 11.9, glucose 5.5. Changes in the force of contraction were measured by an isometric force-transducer (Grass FT03C) connected to a pen recorder (Lectromed, Multitrace 8). Tissues were allowed to equilibrate for 30 min in Tyrode with washing at 10 min intervals.

Effect of PDE inhibitors on methacholine-induced contractions

After equilibration, a methacholine (MCh) cumulative (1 nM–30 μM, 3 min time cycle) concentration-response curve was performed on all tissues. Preparations were then washed three times over the next 10 min before the tissues were allowed to re-equilibrate for a further 10 min. At the end of this time period MCh concentration-response curves were repeated a second time (control curve). Tissues were again washed during the subsequent 10 min period at the end of which tissues were dosed with either vehicle or a single PDE inhibitor (10 μM: vinpocetine, siguazodan, rolipram, zaprinast; 100 μM: enprofylline). Following a further 10 min equilibration, cumulative concentration-response curves to MCh were then performed. Tissues were again washed and the procedure repeated a further two times with increasing concentrations of the PDE inhibitors (100 μM and 300 μM; enprofylline, 300 μM and 1 mM). In addition, subsequent experiments for zaprinast (0.1 and 1 μM) were performed.

Effect of isoprenaline and sodium nitroprusside on methacholine-induced contractions

Experiments were performed as described above. Tissues were incubated sequentially with increasing concentrations of either isoprenaline or sodium nitroprusside (SNP, 0.1, 1, 10, 100, 300 μM) or vehicle.

Combination studies

The effect of rolipram and zaprinast on the isoprenaline and SNP inhibition of methacholine-induced contraction of guinea-pig and rat ileum was investigated. Tissues were prepared and equilibrated as described above, then a MCh cumulative concentration-response curve was produced. Preparations were then washed three times over the next 10 min before the tissues were allowed to re-equilibrate for a further 10 min. At the end of this time period MCh concentration-response curves were repeated a second time (control curve). Tissues were again washed during the subsequent 10 min period at the end of which tissues were dosed with either vehicle, rolipram (10 μM) or zaprinast (guinea-pig: 0.1 μM; rat: 1 μM), as determined in our initial experiments. Tissues were allowed to incubate for 10 min before being dosed with either isoprenaline (guinea-pig: 0.1 μM; rat: 1 μM; vehicle and rolipram pretreated tissues) or SNP (10 μM; vehicle and zaprinast pretreated tissues). Following a further 10 min, MCh concentration-response curves were repeated.

Drugs and solutions

The following substances were used: dimethyl sulphoxide (DMSO), (−)-isoprenaline (+)-bitartrate, methacholine chloride (all Sigma); enprofylline (Research Biochemicals inc., U.S.A.), rolipram, zaprinast and sodium nitroprusside (synthesized by Rhône-Poulenc Rorer, Dagenham); siguazodan (SKF 94836, Smithkline Beecham, Welwyn); vinpocetine (Gedeon Richter Ltd., Budapest).

MCh was dissolved and diluted in Tyrode solution. Siguazodan, rolipram, zaprinast and enprofylline were dissolved and diluted in DMSO. Vinpocetine was dissolved in 0.1 N HCl and diluted in distilled water. Sodium nitroprusside was dissolved and diluted in distilled water. Isoprenaline was dissolved and diluted in ascorbic acid (0.57 μM) to prevent oxidative degradation.

Statistical analysis

Responses to methacholine were standardised as a percentage of the maximum methacholine response of the control concentration-response curve. MCh EC_{50} (concentration of MCh producing half maximal response for each respective curve) values in the presence and absence of inhibitor were calculated by regression analysis. Values are expressed as the geometric mean (95% confidence intervals) from n animals. E_{max} values represent the maximum contractile response to methacholine expressed as a percentage of the control methacholine response curve and are given as the mean ± s.e.mean from n animals. Results were examined by one-way analysis of variance (ANOVA). Where a difference was found across groups, a multiple comparison test (Dunnett's) was performed to assess significance of difference ($P<0.05$ was accepted as significant).

Results

Effect of PDE inhibitors on methacholine-induced contractions

In guinea-pig ileum, vinpocetine (10–300 μM), siguazodan (10–300 μM), rolipram (10–300 μM), zaprinast (1–300 μM) and enprofylline (100–1000 μM), produced concentration-dependent inhibition of the MCh concentration-response curve (Figure 1a, b, c, d, e). The effect of the PDE isoenzyme inhibitors on the MCh E_{max} and EC_{50} values are shown for guinea-pig ileum in Tables 1 and 2. Vinpocetine and zaprinast produced a concentration-dependent depression of the maximum response to MCh (E_{max}, rank order: zaprinast > vinpocetine) without significant effect on the MCh EC_{50} values. In contrast, siguazodan and rolipram produced a rightward displacement of the MCh concentration-response curve indicated by the increase in MCh EC_{50} values (rank order: rolipram > siguazodan). In addition, rolipram produced some depression of the MCh maximum but only at higher (≥100 μM) concentrations. The non-selective PDE inhibitor, enprofylline, produced a concentration-dependent inhibition of the MCh maximum with some effect on the MCh EC_{50} at the highest concentration tested (1 mM).

In the rat ileum as in the guinea-pig ileum all PDE inhibitors produced inhibition of the MCh-induced contraction (Figure 2a, b, c, d, e). The effect of the PDE isoenzyme inhibitors on the MCh E_{max} and EC_{50} values are shown for rat ileum in Tables 3 and 4 respectively. Vinpocetine (10–300 μM) and zaprinast (0.1–300 μM) caused depression of the MCh maximum contraction (rank order: zaprinast > vinpocetine). Low concentrations of rolipram (10 μM) and siguazodan (10–100 μM) had no significant effect on the MCh maximum. In the presence of higher concentrations (≥100 μM) of rolipram and siguazodan, a maximum response could not be obtained with the highest concentration of MCh tested. Similar to the guinea-pig ileum, rolipram and siguazodan produced a significant, concentration-dependent, rightward displacement of the MCh

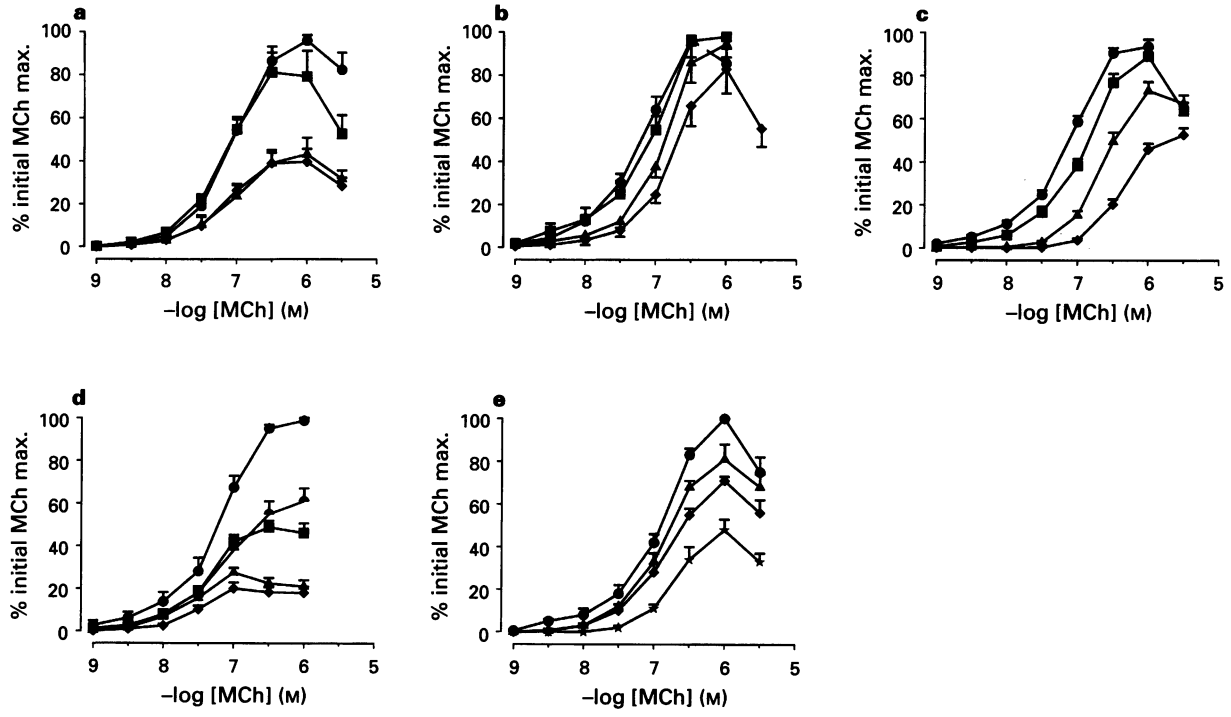

Figure 1 The effect of vinpocetine (a, $n=5$), siguazodan (b, $n=8$), rolipram (c, $n=19$), zaprinast (d, $n=4-7$) and enprofylline (e, $n=4$) on MCh-induced contraction of guinea-pig ileum. Ordinate scales: % control MCh maximum. Abscissa scales: – log molar concentration of MCh. Concentration-response curves to MCh are shown for control (●) and following incubation (10 min) with and in the presence of 1 μM (▲), 10 μM (■), 100 μM (▲), 300 μM (◆) and 1 mM (★) PDE inhibitor. Values represent the mean and vertical lines show s.e.mean.

Table 1 The effect of PDE inhibitors on the MCh-induced maximum contraction of guinea-pig ileum

		MCh E_{max} (% control MCh maximum)					
PDE inhibitor	n	*Control*	*1 μM*	*10 μM*	*100 μM*	*300 μM*	*1 mM*
Vinpocetine	5	96±3 [2.3±0.7]	ND	81±12	43±8*	40±6*	ND
Siguazodan	8	97±2 [2.1±0.3]	ND	98±9	95±11	82±11	ND
Rolipram	19	93±3 [2.2±0.2]	ND	89±4	73±4*	53±3*	ND
Zaprinast	7	99±1 [2.9±0.4]	61±6*†	49±3*	28±2*	20±3*	ND
Enprofylline	4	100±0 [1.8±0.2]	ND	ND	81±7*	71±2*	48±5*

E_{max} is expressed as a percentage of the initial MCh control concentration-response curve maximum. Values in parentheses represent the maximum force [g] produced by MCh for control curves. *$P<0.05$ from control. ND, not determined †$n=4$.

Table 2 The effect of PDE inhibitors on MCh EC_{50} values for guinea-pig ileum

		MCh EC_{50} (nM)					
PDE inhibitor	n	*Control*	*1 μM*	*10 μM*	*100 μM*	*300 μM*	*1 mM*
Vinpocetine	5	86 (59–126)	ND	52 (25–110)	69 (21–231)	54 (22–129)	ND
Siguazodan	8	63 (51–81)	ND	67 (30–153)	115* (81–170)	136* (89–208)	ND
Rolipram	19	75 (62–92)	ND	114* (98–134)	231* (179–300)	381* (313–465)	ND
Zaprinast	7	57 (36–91)	64† (48–85)	47 (32–67)	26* (18–37)	34 (21–56)	ND
Enprofylline	4	123 (87–173)	ND	ND	134 (87–208)	132 (84–207)	200 (136–292)

Ec_{50} represents the concentration of MCh producing 50% of the maximum contraction for each respective concentration response curve. *$P<0.05$ from control; ND, not determined; †$n=4$.

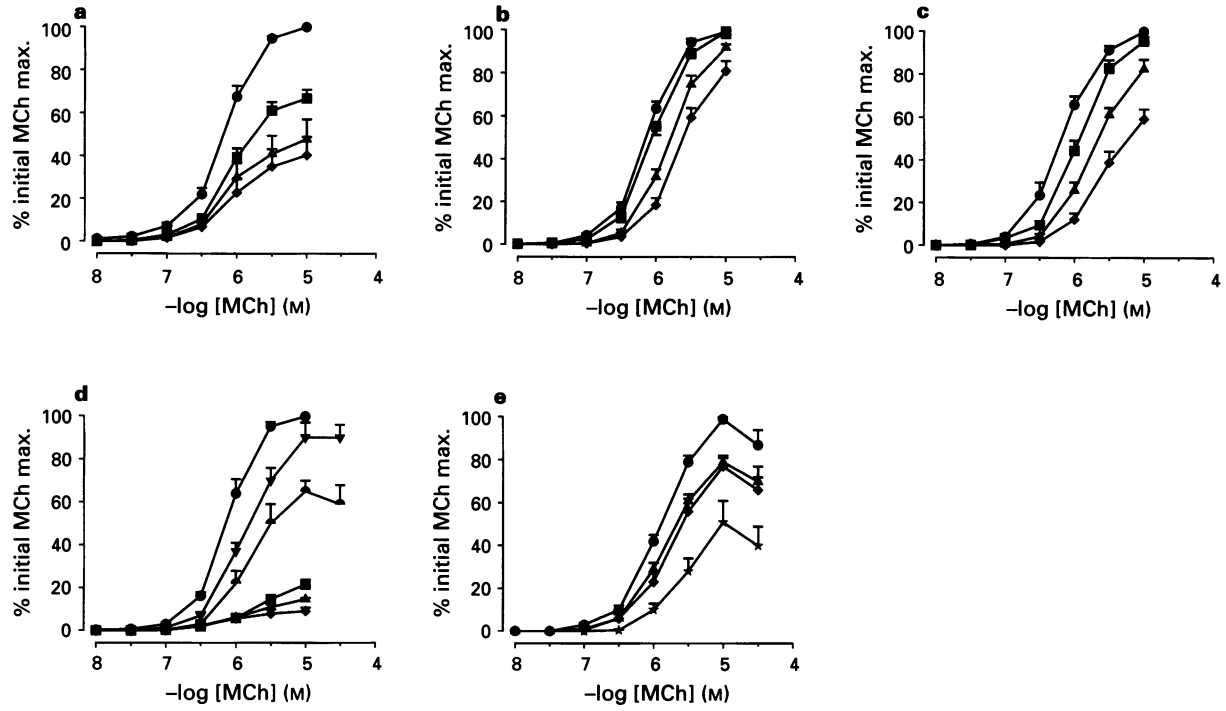

Figure 2 The effect of vinpocetine (a, $n=8$), siguazodan (b, $n=7$), rolipram (c, $n=7$), zaprinast (d, $n=4-6$) and enprofylline (e, $n=5$) on MCh-induced contraction of rat ileum. Ordinate scales: % control MCh maximum. Abscissa Scales: −log molar concentration of MCh. Concentration-response curves to MCh are shown for control (●) and following incubation (10 min) with and in the presence of 0.1 μM (▼), 1 μM (◢), 10 μM (■), 100 μM (▲), 300 μM (◆) and 1 mM (★) PDE inhibitor. Values represent the mean and vertical lines show s.e.mean.

Table 3 The effect of PDE inhibitors on the MCh-induced maximum contraction of rat ileum

		MCh E_{max} (% control MCh maximum)						
PDE inhibitor	n	*control*	*0.1 μM*	*1 μM*	*10 μM*	*100μM*	*300 μM*	*1 mM*
Vinoceptine	8	100±0 [2.5±0.2]	ND	ND	67±4*	48±9*	40±9*	ND
Siguazodine	7	99±1 [2.3±0.3]	ND	ND	99±3	92±2	⩾81±5*	ND
Rolipram	7	100±0 [2.4±0.3]	ND	ND	96±2	⩾83±4*	⩾59±5*	ND
Zaprinast	6	100±0 [2.6±0.2]	90±6*†	59±9*†	22±2*	15±1*	9±2*	ND
Enprofylline	5	99±1 [1.4±0.2	ND	ND	ND	79±2*	77±5*	51±10*

E_{max} is expressed as a percentage of the initial MCh control concentration-response curve maximum. Values in parentheses represent the maximum force [g] produced by MCh for control curves. ⩾ indicates MCh E_{max} was not achieved. *$P<0.05$ from control; ND, not determined; †$n=4$.

concentration-response curve (rank order: rolipram > siguazodan) indicated by the increase in MCh EC_{50} value (Table 4). Vinpocetine and zaprinast had no significant concentration-related effect on the MCh EC_{50}. As in guinea-pig ileum, enprofylline produced a depression of the MCh maximum with an effect on the MCh EC_{50} only at the highest concentration tested (1 mM).

Effect of isoprenaline and sodium nitroprusside on methacholine-induced contractions

The effects of isoprenaline and SNP on the maximum response to MCh and MCh EC_{50} value in guinea-pig ileum are shown in Table 5 and Figure 3 (a and b). Isoprenaline (0.1 μM) produced a rightward displacement of the MCh concentration-response curve, indicated by a significant increase in MCh EC_{50} from 56 (50–62) nM to 158 (73–345) nM ($n=4$). In addition, a significant depression of the maximum response to 79±5% of the control response ($n=4$) was also seen. Increasing the isoprenaline concentration had no further effect on either parameter. SNP (1–300 μM) produced a concentration-dependent depression of the MCh maximum without an effect on the EC_{50} (Table 5, Figure 3 a and b).

In the rat ileum, isoprenaline (0.1–300 μM) produced a concentration-dependent rightward displacement of the MCh concentration-response curve as indicated by the increase in MCh EC_{50} with depression of the MCh maximum at higher (≥100 μM) concentrations (Table 6, Figure 3 c and d). SNP (1–300 μM) produced depression of the MCh maximum at a concentration of 10 μM and above. Effects on the MCh EC_{50} were seen only at 100 and 300 μM (Table 6, Figure 3 c and d).

Combination studies

In a third series of experiments on guinea-pig ileum, isoprenaline (0.1 μM) produced an increase in the MCh EC_{50} from 76 (62–94) nM to 131 (101–169) nM ($n=6$) without an effect on the MCh maximum (Figure 4a). In combination with rolipram (10 μM) the EC_{50} was increased to 626 (497–788) nM ($n=6$) and the maximum reduced to 42±3% ($n=6$). SNP (10 μM) produced a depression of the MCh maximum from 97±2% to 79±4% ($n=7$) without significant effect on the MCh EC_{50} (Figure 4b). Combination with zaprinast (0.1 μM) produced no further significant effect on either parameter.

In rat ileum, isoprenaline (1 μM) produced an increase in the MCh EC_{50} from 1158 (689–1949) nM to 3429 (1615–7282) nM ($n=4$) but also produced a reduction (60±13% of control) of the MCh maximum (Figure 4c). In combination with rolipram (10 μM) the EC_{50} was further increased to 8548 (3741–19532) nM ($n=4$) and the maximum reduced to 27±5% ($n=4$). SNP (10 μM) had no significant effect on either the MCh maximum (reduced from 100±0% to 79±4%; $n=4$) or EC_{50} (Figure 4d). Combination with zaprinast (1 μM) had no further effect.

Discussion

In both the guinea-pig and rat ileum, pretreatment with the isoenzyme-selective PDE inhibitors, vinpocetine (type 1), siguazodan (type 3), rolipram (type 4) and zaprinast (type 5) all produced a concentration-dependent inhibition of the MCh concentration contractile-response curves. This suggests the presence of the type 3, 4 and 5 PDE isoenzymes and their possible modulator role, to varying degrees, in the contraction of guinea-pig and rat ileum, at least to MCh. Whilst a modulator role for the type 1 PDE isoenzyme is also suggested, interpretation of the vinpocetine results is complicated by the multiple mechanisms of action of this compound at higher concentrations (Nicholson, 1990).

The non-selective PDE inhibitor, enprofylline, also produced inhibition of the MCh concentration-response curves in both guinea-pig and rat ileum. The concentrations of enprofylline (>100 μM) were higher than those required for the isoenzyme selective PDE inhibitors. Similarly, the concentrations were higher than those required to relax guinea-pig trachealis (Takagi *et al.*, 1992).

In the present study, rolipram at concentrations of 10 μM and above produced similar inhibition of the MCh concentration-response curves for both guinea-pig and rat ileum. Siguazodan, was less potent producing effects at concentra-

Table 5 The effect of isoprenaline and SNP on the MCh-induced maximum contraction and MCh EC_{50} values for guinea-pig ileum

	Isoprenaline ($n=6$)		*SNP* ($n=4$)	
Conc. (μM)	EC_{50} (nM)	E_{max} (% control)	EC_{50} (nM)	E_{max} (% control)
Control	56 (50–62)	100±0 [1.8±0.2]	70 (38–130)	95±5 [1.5±0.1]
0.1	158* (73–345)	79±5*	ND	ND
1	166* (77–356)	78±5*	106 (65–174)	87±6
10	159* (92–279)	75±3*	91 (48–174)	77±4*
100	157* (79–311)	80±2*	93 (74–116)	67±5*
300	235* (155–356)	77±6*	126 (76–209)	66±7*

E_{max} is expressed as a percentage of the initial MCh control concentration-response curve maximum. EC_{50} represents the concentration of MCh producing 50% of the maximum contraction for the respective concentration-response curve. Values in square parentheses represent the maximum force [g] produced by MCh for control curves. *$P<0.05$ from control; NE, not determined.

Table 4 The effect of PDE inhibitors on MCh EC_{50} values for rat ileum

		MCh EC_{50} (nM)						
PDE inhibitor	n	*control*	*0.1 μM*	*1 μM*	*10 μM*	*100μM*	*300 μM*	*1 mM*
Vinpocetine	8	596 (396–898)	ND	ND	816 (651–1026)	775 (338–1773)	1097 (695–1733)	ND
Siguazodan	7	710 (590–855)	ND	ND	861 (701–1058)	1440* (1193–1738	≥1815* (1515–2176)	ND
Rolipram	7	619 (427–898)	ND	ND	1112* (875–1413)	≥1540* (1282–1852)	≥2282* (1656–3155)	ND
Zaprinast	6	691 (463–1031)	1366† (1196–1560)	1619† (674–3893)	1917* (1281–2869)	1379 (748–2544)	980 (472–2034)	ND
Enprofylline	5	1229 (976–1547)	ND	ND	ND	1462 (1041–2055)	1755 (1336–2306)	2508* (1639–3837)

EC_{50} represents the concentration of methacholine producing 50% of the maximum contraction for each respective concentration-response curve. ≥ indicates MCh E_{max} was not achieved and hence EC_{50} values are underestimated. *$P<0.05$ from control; ND, not determined; †$n=4$.

tions of 100 μM and above, suggesting a more important role for the type 4 PDE isoenzyme in regulating contraction. These observations confirm and extend those of Small *et al.* (1989) who evaluated the effect of AH 21–132, a type 3/4 mixed PDE inhibitor, on spasmogen-induced concentration-response curves in the guinea-pig ileum. AH 21–132 produced a sup-

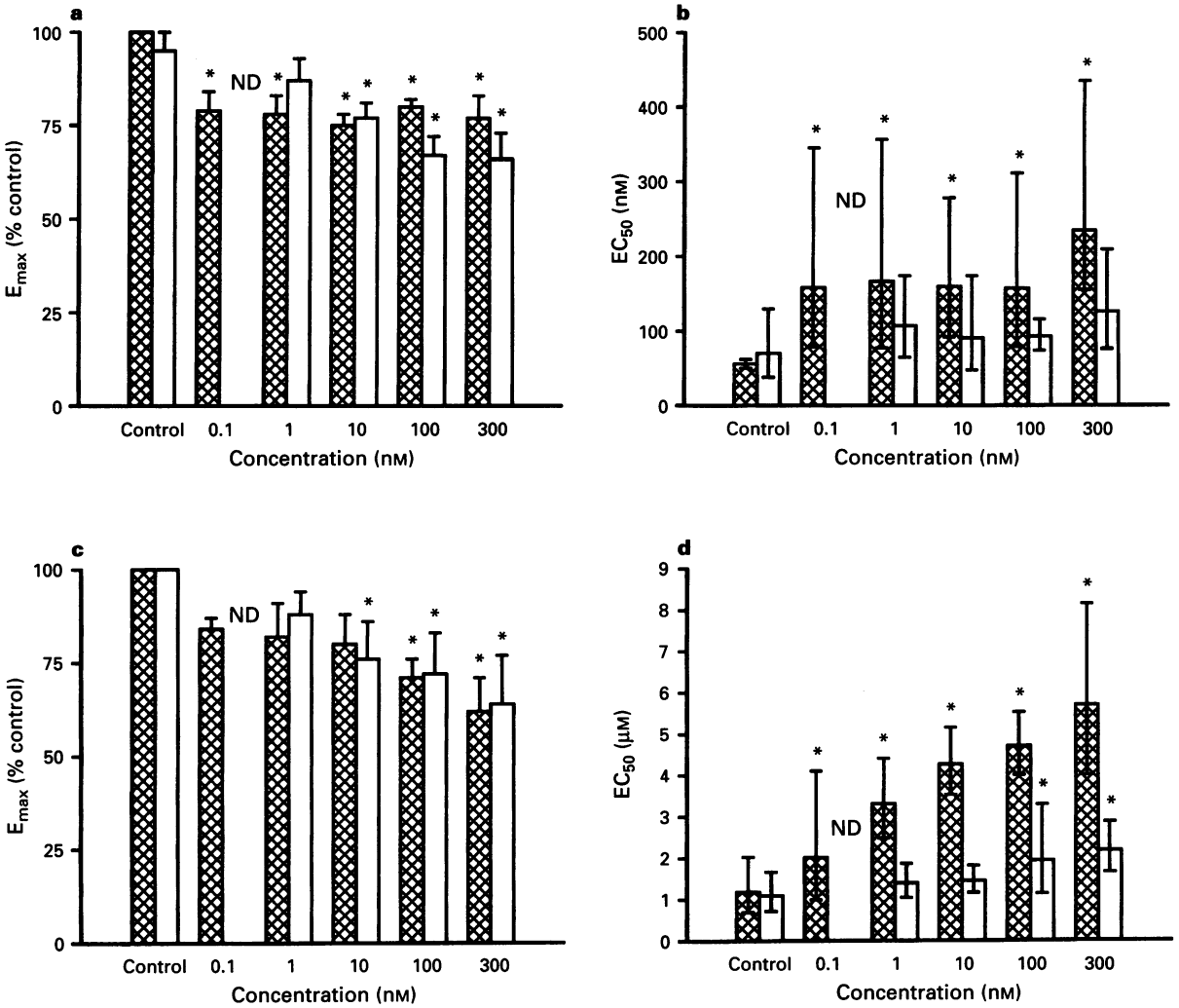

Figure 3 The effect of isoprenaline (hatched columns) and sodium nitroprusside (SNP; open columns) on the MCh-induced maximum contraction (E_{max}, a), and EC_{50} values (b) for guinea-pig ileum; and the MCh-induced maximum contraction (E_{max}, c) and EC_{50} values (d) for rat ileum. E_{max} is expressed as a percentage of the initial MCh control concentration-response curve maximum. EC_{50} represents the concentration of MCh producing 50% of the maximum contraction for the respective concentration-response curve. E_{max} values are the mean ± s.e.mean. EC_{50} values are the geometric mean and 95% confidence intervals. ND, not determined.

Table 6 The effect of isoprenaline and SNP on the MCh-induced maximum contraction and MCh EC_{50} values for rat ileum

	Isoprenaline (n = 6)		*SNP* (n = 6)	
Conc. (*μM*)	*EC_{50}* (*nM*)	*E_{max}* (*% control*)	*EC_{50}* (*nM*)	*E_{max}* (*% control*)
Control	1184 (692–2029)	100 ± 0 [2.0 ± 0.7]	1091 (715–1667)	100 ± 0 [1.4 ± 0.2]
0.1	2021 (993–4116)	84 ± 3	ND	ND
1	3313* (2489–4410)	82 ± 9	1397 (1046–1866)	88 ± 6
10	4270* (3531–5163)	80 ± 8	1454 (1161–1821)	76 ± 10*
100	4714* (4016–5534)	71 ± 5*	1935* (1135–3297)	72 ± 11*
300	5706* (4001–8138)	62 ± 9*	2180* (1653–2875)	64 ± 13*

E_{max} expressed as a percentage of the initial MCh control concentration-response curve maximum. EC_{50} represents the concentration of MCh producing 50% of the maximum contraction for the respective concentration-response curve. Values in square parentheses represent the maximum tension [g] induced by MCh for control curves. *$P < 0.05$ from control; ND, not determined.

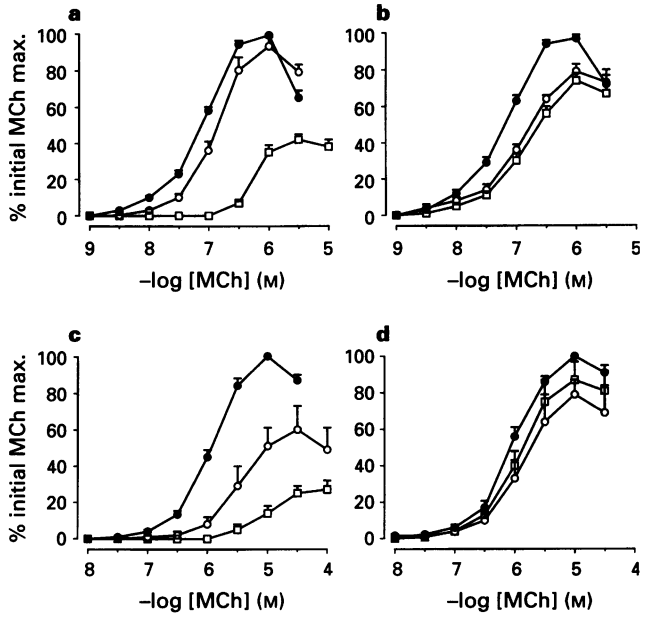

Figure 4 The effect of: (a) isoprenaline (0.1 μM, $n=6$, ○) alone and isoprenaline (0.1 μM) in combination with rolipram (10 μM, $n=6$, □) on the control (●, $n=6$) MCh concentration-response curve; (b) sodium nitroprusside (SNP; 10 μM, $n=7$, ○) alone and SNP (10 μM) in combination with zaprinast (0.1 μM, $n=7$, □) on the control (●, $n=7$) MCh concentration-response curve of guinea-pig ileum. The effect of: (c) isoprenaline (1 μM, $n=4$, ○) alone and isoprenaline (1 μM) in combination with rolipram (10 μM, $n=4$, □) on the control (●, $n=4$) MCh concentration-response curve; (d) SNP (10 μM, $n=3$, ○) alone and SNP (10 μM) in combination with zaprinast (1 μM, $n=4$, □) on the control (●, $n=4$) MCh concentration-response curve of rat ileum. Ordinate scales: % control MCh maximum. Abscissa scales: −log molar concentration of MCh. Values represent the mean and vertical lines show s.e.mean.

pression of concentration-response curves to a variety of spasmogens including ACh, histamine and KCl indicating a functional role for cyclic AMP PDE. These effects of AH 21–132 were only seen at concentrations of 10 μM and above. The high concentrations of the type 3 and type 4 PDE inhibitors, siguazodan and rolipram in the present study and for AH 21–132 contrast with those required to exert a spasmolytic effect on guinea-pig trachealis (Bewley & Chapman, 1988; Harris *et al.*, 1989; Small *et al.*, 1989a; Tomkinson *et al.*, 1993). More recently, Underwood *et al.* (1994) were unable to demonstrate a significant antispasmogenic effect of either rolipram or siguazodan (10 μM) on either histamine or leukotriene D_4 (LTD_4)-induced contraction of guinea-pig trachea suggesting the difference to be a consequence of the nature of the studies. Indeed, it seems unlikely that the difference in the functional potency of the PDE inhibitors reflects differences in their potency to inhibit gastrointestinal and airways smooth muscle PDE type 3 and 4, since Small *et al.* (1989b) obtained an IC_{50} value for AH 21–132 as an inhibitor of cyclic AMP PDE derived from guinea-pig ileum similar to that for cyclic AMP PDE derived from guinea-pig trachealis (Small *et al.*, 1989a). However, differences may exist in the abundance of the PDE isoenzymes present in gastrointestinal and airways smooth muscle.

As we have demonstrated functionally the presence of the PDE isoenzymes types 1, 3, 4 and 5, biochemical isolation and purification techniques have demonstrated the presence of five different isoenzymes in canine colon (Barnette *et al.*, 1993). Additional, functional studies on canine colon (Barnette *et al.*, 1993) and functional studies on cat gastric fundus (Barbier & Lefebvre 1995), using inhibitors of the type 3 and 4 PDE isoenzymes, indicated the type 4 isoenzyme as the most important of the two in modulating tone, consistent with our own findings in the present study. These observations, however, contrast with studies of canine lower oesophageal sphincter where the predominant isoenzyme appears to be the type 3 (Barnette *et al.*, 1989), suggesting regional differences in the distribution and functional importance of the PDE isoenzymes in the gastrointestinal smooth muscle. In the present study in guinea-pig and rat ileum and the previous study of Small *et al.* (1989b) inhibition of the spasmogen-induced responses was produced by PDE inhibitor alone. This differs from studies performed on canine colon where inhibition of the carbachol contractions was only seen in the presence of the adenylyl cyclase stimulant, forskolin, suggesting a difference in the basal turnover of cyclic AMP.

Using rolipram and siguazodan which hydrolyse cyclic AMP, we have demonstrated a role for cyclic AMP and the importance of the type 4 PDE in regulating contraction in guinea-pig and rat ileum. In addition, we have evaluated the effect of vinpocetine and zaprinast which hydrolyse cyclic GMP. Both produced concentration-dependent inhibition of the MCh concentration-response curves in both guinea-pig and rat ileum suggesting a role for cyclic GMP in regulating contraction. Of these cyclic GMP PDE inhibitors zaprinast was the more effective, producing inhibition at concentrations as low as 0.1 μM in rat ileum. Using the cat gastric fundus, Barbier & Lefebvre (1995) were, similarly, able to demonstrate a relaxation of basal tone with zaprinast. In contrast, vinpocetine had no relaxant effect. In canine colon zaprinast also produced inhibition of carbachol-induced contractions but at higher concentrations than in our studies (Barnette *et al.*, 1993).

A direct comparison of the relative importance of the PDE isoenzymes based on our functional studies has been complicated by the type of inhibition of the MCh concentration-response curve produced by the different PDE inhibitors. Interestingly, the results of this study indicate a difference in the type of inhibition produced by the different PDE inhibitors. The type 1 and 5 PDE inhibitors, vinpocetine and zaprinast, which elevate cyclic GMP, produced largely a depression of the MCh maximum response only, indicative of a classical non-competitive or insurmountable antagonism. In contrast the type 3 and 4 PDE inhibitors, siguazodan and rolipram, which elevate cyclic AMP, produced predominantly a rightward displacement of the MCh concentration-response curve with little or no effect on the MCh maximum response, more indicative of a competitive or surmountable antagonism. The non-selective PDE inhibitor, enprofylline, produced both depression of the MCh maximum response and a rightward displacement of the MCh concentration-response curve, although effects were more apparent on the former. The reason for this is not clear given the relative potency of enprofylline against the different PDE isoenzymes (Ukena *et al.*, 1993), but may indicate a tighter regulation of the contractile process by cyclic GMP.

Our observations with the isoenzyme-selective PDE inhibitors were confirmed by the use of isoprenaline which increases intracellular cyclic AMP through β_1-adrenoceptor-mediated activation of adenylyl cyclase (Grassby & Broadley, 1984; Murray, 1990) and by the use of SNP which increases cyclic GMP by activation of soluble guanylyl cyclase (Waldman & Murad, 1987). In both guinea-pig and rat ileum the effects of SNP on the MCh concentration-response curve were seen as a depression of the maximum response. In contrast to SNP, the effect of isoprenaline on the MCh concentration-response curve in the rat ileum was seen as an initial rightward displacement of the MCh concentration-response curve, with effects on the MCh maximum response at higher concentrations only. Although the effects of isoprenaline on the MCh concentration-response curve on guinea-pig ileum were less apparent, these data support our observations on the different types of functional inhibition produced with the cyclic AMP and cyclic GMP PDE inhibitors. The regulation of contraction associated with an elevation of intracellular cyclic AMP and cyclic GMP is thought to be mediated through the activation of A- and G-kinase respectively. However, in both airways and

vascular smooth muscle, it has been demonstrated that cyclic AMP may activate G-kinase (Lincoln *et al.*, 1990; Landgraf *et al.*, 1992). A similar cross-activation of G-kinase by cyclic AMP in gastrointestinal smooth muscle may explain the depression of the maximum response to MCh seen at higher concentrations of rolipram and isoprenaline and with the combination of rolipram and isoprenaline. The activated A- and G-kinases may subsequently modulate contraction by their action on several possible substrate protein sites involved in the contractile process, including: (1) the synthesis and metabolism of inositol (1, 4, 5) trisphosphate (IP_3); (2) the IP_3-induced release of calcium from intracellular stores; (3) the level of intracellular free calcium through possible interactions with ion exchange processes; (4) the interaction of the contractile proteins actin and myosin (see Giembycz & Raeburn, 1991 and references therein). Differences in the site/mechanism at which these two kinases interact may explain the difference in the type of inhibition produced by the PDE inhibitors elevating cyclic AMP and cyclic GMP. Alternatively, the difference in the type of functional inhibition produced could also reflect differences in the way in which the A- and G-kinases interact at the same protein phosphorylation site in the contractile mechanism.

In conclusion, the present study demonstrates a modulatory role for the PDE isoenzymes in guinea-pig and rat gastrointestinal smooth muscle contractility. More interestingly, the results indicate a difference in the type of inhibition produced by the type 1 and 5 PDE isoenzymes, which elevate cyclic GMP and the type 3 and 4 inhibitors, which elevate cyclic AMP. This difference in the type of inhibition produced by these PDE isoenzyme inhibitors may reflect a different intracellular site/mechanism by which the cyclic AMP- and cyclic GMP- activated kinases act to antagonize functionally the contractile response.

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