Prevention by phosphodiesterase inhibitors of antigen-induced contraction of guinea-pig colonic smooth muscle

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1 The ability of various phosphodiesterase (PDE) inhibitors to reduce the initial and/or late response to ovalbumin (OVA) in isolated strips of guinea-pig colonic smooth muscle from sensitized animals was examined.

2 Both the initial and late responses to OVA (0.05 mg ml⁻¹) were inhibited by the non-selective PDE inhibitor, 3-isobutyl-1-methyl-xanthine (IBMX, EC₅₀ = 26.0 and 6.1 µM, respectively), and the selective inhibitors of low K_m cyclic AMP specific PDE (PDE IV), (R)- and (S)- rolipram. The (S)- isomer (EC₅₀ = approximately 1.0 µM for both responses) was about 10 fold less potent than the (R)- isomer (EC₅₀ = approximately 0.1 µM for both responses).

3 Zaprinast, a selective inhibitor of the cyclic GMP-specific PDE (PDE V) inhibited only the late response (EC₅₀ = 1.4 µM).

4 SK&F 94120, a selective inhibitor of the cyclic GMP-inhibited low K_m cyclic AMP PDE (PDE III), inhibited the initial response (45.9 ± 11.9%, *P* < 0.05) at the highest concentration tested (100 µM), and had no effect on the late response.

5 The results suggest that PDE inhibitors, especially PDE IV inhibitors, can attenuate the contractile response of guinea-pig colon to OVA.

Keywords: Phosphodiesterase inhibitors; antigen-induced contraction; guinea-pig colon; zaprinast; rolipram; SK&F 94120; 3-isobutyl-1-methyl-xanthine

Introduction

The release of inflammatory mediators such as histamine or the peptidoleukotrienes can change gastrointestinal motility (Collins *et al.*, 1989). For example, addition of antigen to isolated strips of gastrointestinal smooth muscle obtained from sensitized rat or guinea-pig, contracts both small intestinal and colonic tissue (Vermillion *et al.*, 1988; Barnette & Grous, 1992). In previous studies using sensitized guinea-pig colon, we found that antigen addition produced a biphasic contraction consisting of an initial rapid response that is blocked by the H₁-histamine receptor antagonist mepyramine, and a slower developing late contraction that is reduced by a peptidoleukotriene (LTD₄) receptor antagonist, Wy 48252, (Barnette & Grous, 1992). The combination of both an H₁-histamine receptor antagonist and an LTD₄ receptor antagonist eliminated both responses to antigen. Thus, these findings strongly suggest that antigen addition is causing the activation of an inflammatory cell, most likely the mast cell, and the release of histamine and peptidoleukotrienes (Barnette & Grous, 1992).

Although PDE isozymes have been characterized by several investigators in cardiac muscle, airway and arterial smooth muscles (Silver *et al.*, 1988; Torphy & Cieslinski, 1990; Shadid *et al.*, 1991), only recently have a limited number of studies characterizing the profile in gastrointestinal smooth muscle been reported (Silver *et al.*, 1990; Barnette *et al.*, 1993). PDE inhibitors potentiate forskolin-induced relaxation of rabbit intestinal smooth muscle (Muller & Baer, 1983) and enhance the increase in adenosine 3':5'-cyclic monophosphate (cyclic AMP) produced by forskolin in canine colonic smooth muscle (Barnette *et al.*, 1993). However, it is not known what influence PDE inhibitors have on the response to antigen in gastrointestinal smooth muscle.

The existence of at least five families of PDE isozymes is widely accepted (Torphy & Cieslinski, 1990; Nicholson *et al.*, 1991; Shahid *et al.*, 1991). Although non-selective PDE

inhibitors, such as 3-isobutyl-1-methylxanthine (IBMX) have been known for some time, only recently have selective PDE inhibitors been identified, thus, giving us the pharmacological tools needed to study the effects of PDE inhibition on tissue function. The selective inhibitors for individual PDE isozymes used in this study are: (1) SK&F 94120, a selective inhibitor of cyclic GMP-inhibited PDE (PDE III); (2) (R)- and (S)- rolipram, selective inhibitors of low K_m cyclic AMP specific PDE (PDE IV); (3) zaprinast, a selective inhibitor of cyclic GMP specific PDE (PDE V).

Increases in cyclic nucleotide content produced by PDE inhibition will inhibit the release of mediators from inflammatory cells (Torphy & Undem, 1991). Activators of adenylate cyclase such as isoprenaline have been shown to inhibit the release of mast cell mediators induced by IgE stimulation (Undem *et al.*, 1988) as well as the production of oxygen free radicals from neutrophils (Nielson *et al.*, 1990). Increasing cyclic AMP via PDE inhibition will inhibit the respiratory bursts from eosinophils and neutrophils (Nielson *et al.*, 1990; Dent *et al.*, 1991) and inhibit mediator release from rat mast cells (Frossard *et al.*, 1981), human basophils (Frossard *et al.*, 1981; Peachell *et al.*, 1992) and human neutrophils (Schudt *et al.*, 1991). With their combined ability to relax smooth muscle and to inhibit inflammatory cell function, PDE inhibitors could exert a profound action on gastrointestinal motility. Therefore, the aim of this study was to examine the ability of selective PDE inhibitors to alter the response of guinea-pig colonic smooth muscle to antigen.

Methods

Tissue preparation

Male Hartley guinea-pigs (250–300 g) were sensitized to OVA by either an intramuscular injection (0.35 ml of OVA in 0.9% NaCl injected into each hindlimb), or an intraperitoneal injection of 0.5 ml of OVA (10 µg) in aluminium

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hydroxide (100 mg). No differences in tissue responsiveness to antigen between either sensitization procedure were noted. After a minimum of 14 days the sensitized animals were killed by cervical dislocation. A segment of distal colon (10–15 cm) was removed approximately 10 cm above the anus and immediately placed in oxygenated saline on ice. The segment of colon was cut along the longitudinal axis, pinned flat to a dissecting board and the mucosa removed. Eight strips of circular smooth muscle (2–3 mm by 10–15 mm) were tied with suture at either end. One end was attached to a stationary rod and the other to a Grass force-displacement transducer (FT-03) to measure isometric contractions using a multi-channel Grass polygraph (Grass Instrument Co., Quincy, MA, U.S.A.). The strips were suspended in 10 ml water jacketed tissue baths containing oxygenated Krebs buffer which was maintained at 37°C. The composition of Krebs buffer was (mM): NaCl 117.9, KCl 4.7, CaCl₂ 2.5, KH₂PO₄ 1.2, MgSO₄ 1.2, NaHCO₃ 25.0 and dextrose 11.1. Two grams of tension were applied to each strip which was equilibrated for a minimum of 1.5 h. The tension was readjusted during equilibration until the tissues maintained this level of tone.

Experimental protocol

After equilibration the tissues were contracted with carbachol (1.0 µM). When the carbachol-induced contractions reached a plateau, the tissues were washed for 25–30 min; no adjustments to the resting tension were made after the carbachol-induced contraction. The colonic segments were then pretreated for 20 min with either vehicle (control) or the various PDE inhibitors prior to challenge with OVA (0.05 mg ml⁻¹). After OVA challenge, a biphasic response was observed (Barnette & Grous, 1992). The initial response occurred within 2–3 min after the addition of OVA. Later responses were sustained and were calculated 15 min after the initial response. Both responses were calculated in relation to the baseline after the addition of the PDE inhibitors because addition of the PDE inhibitors decreased basal tone and phasic activity. Time-matched control tissues were run in parallel with drug-treated preparations, and control responses were calculated at the same time points as those of the PDE-treated tissues.

At the end of each experiment, tissues were contracted to carbachol (1.0 µM) and the response obtained compared to the initial carbachol contraction.

Data analysis

Both the initial and late responses to OVA were expressed as a percentage of the initial contraction produced by carbachol. The data are given as the mean ± s.e.mean ($n = 5-6$). Results from the control and treated tissues were analysed for statistical differences by a Student's paired *t* test with a probability value, *P*, less than 0.05 regarded as significant. IC₅₀ values for individual compounds were calculated as 50% inhibition of the control response to antigen by linear regression.

Materials

Ovalbumin, carbachol and IBMX (3-isobutyl-1-methylxanthine) were obtained from Sigma Chemical Corp. (St. Louis, MO, U.S.A.). SK&F 94120 [5-(4-acetamidophenyl)-pyrazin-2-(1H)-one] one zaprinast (M&B 22,948) were synthesized by Dr William Coates and colleagues (SmithKline Beecham Pharmaceuticals, Welwyn, England). (R)- and (S)-rolipram were synthesized by Dr Sigfried Christensen and colleagues at SmithKline Beecham Pharmaceuticals (King of Prussia, PA, U.S.A.) and aluminium hydroxide was obtained from Aldrich Chemical Co. (Milwaukee, WI, U.S.A.).

Results

Characteristics of antigen-induced contractions in guinea-pig colon have been described previously (Barnette & Grous, 1992). The initial response was blocked by an H₁-histamine receptor antagonist, while the late response was reduced by a peptidoleukotriene receptor antagonist.

All of the PDE inhibitors tested produced a concentration-dependent reduction in both basal tone and phasic activity. Pretreatment of colonic smooth muscle strips with IBMX (1.0–100 µM), a non-selective PDE inhibitor, blocked both the initial and late response to antigen challenge (0.05 mg ml⁻¹ OVA, Figure 1a) with EC₅₀ values of 26.0 µM and 6.1 µM, respectively. At 100 µM, IBMX significantly

Figure 1 Effect of 3-isobutyl-1-methylxanthine (IBMX) (a) zaprinast (b) or SK&F 94120 (c) on the initial (○) and late (●) phases of ovalbumin-induced contraction of guinea-pig colonic smooth muscle. The data are the mean ± s.e.mean of 6 experiments; **P* < 0.05.

decreased ($P < 0.05$) the initial ($95.5 \pm 2.5\%$, $n = 6$) and late ($84.6 \pm 10.1\%$, $n = 6$) responses to OVA.

Pretreatment with zaprinast ($1\text{--}100\text{ }\mu\text{M}$), a PDE V inhibitor, did not inhibit the initial response to antigen (Figure 1b); however, it did produce a concentration-related inhibition of the late response with an EC_{50} of $1.4\text{ }\mu\text{M}$ and a maximum decrease of $88.0 \pm 7.7\%$ from the control at $10\text{ }\mu\text{M}$ ($P < 0.05$).

Addition of SK&F 94120 ($1.0\text{--}100\text{ }\mu\text{M}$), a selective PDE III inhibitor, did not significantly reduce the late response to antigen (Figure 1c), and although, at a high concentration of $100\text{ }\mu\text{M}$, SK&F 94120 significantly decreased ($P < 0.05$) the initial response ($45.9 \pm 11.9\%$, $n = 6$), an EC_{50} value could not be determined.

Rolipram, a PDE IV inhibitor, exists as a mixture of two enantiomers. The (R)- isomer of rolipram ($0.01\text{--}3\text{ }\mu\text{M}$) produced a concentration-dependent reduction of both the initial and late response to antigen with an EC_{50} of about $0.1\text{ }\mu\text{M}$ for both responses (Figure 2). Maximal decreases for inhibition of the initial and late responses occurred at 1 and $3\text{ }\mu\text{M}$, respectively, and were $79.3 \pm 1.9\%$, $n = 5$ and $77.8 \pm 9.8\%$, $n = 5$, respectively. (R)-rolipram significantly decreased ($P < 0.05$) the initial response at $0.1\text{--}1.0\text{ }\mu\text{M}$, and significantly reduced ($P < 0.05$) the late response at $0.1\text{--}3.0\text{ }\mu\text{M}$. In contrast, the (S)- isomer of rolipram was approximately 10 fold less potent with respective EC_{50} 's of about $1.1\text{ }\mu\text{M}$ and $1.0\text{ }\mu\text{M}$, for the initial and the late responses. At 1.0 and $3.0\text{ }\mu\text{M}$, the initial response was significantly decreased ($P < 0.05$), while the late response was significantly reduced ($P < 0.05$) at $3.0\text{ }\mu\text{M}$. Maximal decreases

from the control were 73.1 ± 2.7 , $n = 5$ and $82.4 \pm 11.1\%$, $n = 5$ for the initial and late responses, respectively, both of which occurred at $3.0\text{ }\mu\text{M}$.

To determine any non-specific relaxant effects of PDE inhibitors, all tissues were contracted to carbachol ($1.0\text{ }\mu\text{M}$) at the end of each experiment. This contraction was compared to the initial carbachol ($1.0\text{ }\mu\text{M}$) response by a paired t test and it was noted that none of the compounds examined affected the carbachol-induced contractions (data not shown).

Discussion

Since PDE inhibitors, especially PDE IV inhibitors, have been shown to inhibit the production and release of mediators from inflammatory cells (Torphy & Undem, 1991), we were interested in determining their effects on an antigen-induced contraction of gastrointestinal smooth muscle.

In previous studies in colonic strips obtained from guinea-pigs sensitized to OVA, antigen addition produced a biphasic contraction. Moreover, the different phases of the antigen-induced response were differentially inhibited by either an H_1 -histamine receptor antagonist or an LTD_4 -receptor antagonist. These results suggest that the initial and late responses were caused by the release of two different inflammatory mediators, namely histamine and the peptidoleukotrienes, respectively (Barnette & Grous, 1992).

Pretreatment of colonic smooth muscle strips with IBMX, which non-selectively inhibits all PDE isozymes, inhibited both the initial and late response to antigen challenge with EC_{50} values of $26.0\text{ }\mu\text{M}$ and $6.1\text{ }\mu\text{M}$, respectively. Although there is little information with regard to the effects of PDE inhibitors on gastrointestinal smooth muscle, IBMX was shown to potentiate the concentration-response curve to forskolin in non-sensitized rabbit small intestine, with an EC_{50} of $0.41\text{ }\mu\text{M}$ (Muller & Baer, 1983). More recently, in non-sensitized canine colonic smooth muscle in the presence of forskolin, IBMX inhibited carbachol-induced contractions with an EC_{50} of $4.0\text{ }\mu\text{M}$ (Barnette *et al.*, 1993). These values are in agreement with the ability of IBMX to inhibit all five PDE isozymes found in the canine trachealis where IC_{50} values range from 1 to $30\text{ }\mu\text{M}$ (Torphy & Cieslinski, 1990). They are also similar to results obtained with antigen-sensitized mouse mast cells (PT-18) in the presence of $0.1\text{ }\mu\text{M}$ forskolin (Torphy *et al.*, 1992a), where $5\text{ }\mu\text{M}$ IBMX produced 45 to 55% inhibition of antigen-induced LTC_4 release, and are consistent with the potency of IBMX for inhibition of PDE activity in mouse mast cells (Torphy *et al.*, 1992a).

Unlike IBMX, pretreatment with zaprinast, a PDE V inhibitor, did not inhibit the initial response to antigen; however, it did produce a concentration-related inhibition of the late response with an EC_{50} of $1.4\text{ }\mu\text{M}$. When PDE isozymes were characterized in cardiac, arterial and airway smooth muscle in several different species the IC_{50} values for zaprinast range from 0.5 to $4.2\text{ }\mu\text{M}$ (Silver *et al.*, 1988). Zaprinast has also been reported to reduce histamine release from rat mast cells (Frossard *et al.*, 1981) but not to inhibit the release of histamine or LTC_4 from human basophils (Peachell *et al.*, 1992). Although, the PDE isozymes present in guinea-pig mast cells have not yet been characterized, guinea-pig eosinophil cyclic AMP PDE activity has been reported to be only weakly inhibited by zaprinast ($\text{IC}_{50} = 35\text{ }\mu\text{M}$), and in these cells zaprinast is probably not acting on the PDE V isozyme (Souness *et al.*, 1991). Likewise, in non-sensitized canine colonic smooth muscle strips, the potency of zaprinast ($\text{EC}_{50} = 23.1\text{ }\mu\text{M}$) at relaxing carbachol-induced contractions is also weak and the authors conclude that it is not acting on the PDE V isozyme (Barnette *et al.*, 1993). Overall, in the guinea-pig colon, these data suggest that zaprinast decreases or modulates antigen-induced contractions of the late response via PDE V inhibition.

Figure 2 Effect of (R)-rolipram (●) or (S)-rolipram (○) on the initial (a) and late (b) phases of ovalbumin-induced contraction of guinea-pig colonic smooth muscle. The data are the mean $\pm$ s.e. mean of 5 experiments; * $P < 0.05$.

Pretreatment with SK&F 94120, a selective PDE III inhibitor, did not reduce the late response to antigen. Although, the initial response was significantly decreased at the highest concentration tested (100 μ M), the effects at this concentration are likely to be non-selective and not due to inhibition of PDE III. This finding is in agreement with the lack of effects of PDE III inhibitors on inflammatory cell function (Nielson *et al.*, 1990; Dent *et al.*, 1991; Souness *et al.*, 1991; Peachell *et al.*, 1992) as well as the lack of effect on bovine tracheal smooth muscle (Nicholson *et al.*, 1990) and gastrointestinal motility (Silver *et al.*, 1990). However it contrasts with results in canine colon where SK&F 94120 decreased carbachol-induced contractions (Barnette *et al.*, 1993), and also canine trachea (Torphy *et al.*, 1991b), where PDE III plays a significant role in regulating cyclic AMP hydrolysis and smooth muscle tone.

Rolipram exists as a mixture of two enantiomers. (R)-rolipram is 15 to 20 fold more potent than (S)-rolipram at binding to a site in rat brain (Schneider *et al.*, 1986), whereas, it is about 3 fold more potent at inhibiting human recombinant PDE IV catalytic activity (Torphy *et al.*, 1992b). In this study it was observed that (R)-rolipram was about 10 fold more potent than (S)-rolipram in inhibiting both the initial and late response to antigen. This effect is similar to the action of these enantiomers at the binding site in rat brain but is in marked contrast to their effect against antigen-induced contractions of guinea-pig airways, where their anti-allergic activity is associated with PDE IV catalytic activity and they lack enantiomeric selectivity (Underwood *et al.*, 1993). Although the anti-allergic activity of these enantiomers is believed to be associated with PDE IV catalytic activity, other investigators have observed a significant correlation between the displacement of rolipram binding and gastrointestinal transit (Silver *et al.*, 1990). Taken together, the results of our study suggest that the inhibitory activity of rolipram in the guinea-pig colon is associated with the rolipram binding site.

Although the site of action for these compounds is unknown, PDE inhibitors could be acting either at the level of smooth muscle or the inflammatory cell. In this study, however, PDE inhibition did not affect carbachol-induced contractions suggesting that smooth muscle is probably not the

site of action. Studies in non-sensitized canine colon also support this hypothesis, since PDE inhibitors, in the absence of forskolin, did not reduce carbachol-induced contractions (Barnette *et al.*, 1993).

An alternate site of action might be the inflammatory cell itself, possibly a tissue mast cell. Inhibition of PDE, especially PDE IV, has been reported to inhibit LTC₄ and histamine release from human basophils (Peachell *et al.*, 1992), as well as superoxide anion production in human neutrophils (Schudt *et al.*, 1991) and guinea-pig eosinophils (Dent *et al.*, 1991). However, inhibition of histamine release from rat peritoneal mast cells was not affected by rolipram but was inhibited by zaprinast (Frossard *et al.*, 1981). In guinea-pig isolated airways, rolipram inhibited both antigen-induced contractions and the release of prostaglandin D₂ (PGD₂) and histamine (Underwood *et al.*, 1993). In the same study, contractions of OVA-sensitized guinea-pig tracheal smooth muscle rings produced by histamine or LTD₄ were not inhibited by rolipram suggesting it was more effective at inhibiting mediator release than smooth muscle contraction *per se*. We have previously demonstrated a biphasic response in colonic strips obtained from OVA-sensitized guinea-pigs. The initial response is mimicked by histamine and blocked by an H₁ receptor antagonist, and the late response is mimicked by LTD₄ and blocked by an LTD₄-receptor antagonist (Barnette & Grous, 1992). Since the PDE inhibitors used in this study were effective against the initial and late response to antigen in OVA-sensitized guinea-pigs, these results suggest that the compounds could be acting on inflammatory cells to reduce the release of histamine and leukotriene.

In conclusion, our findings indicate that PDE inhibitors inhibit antigen-induced contractions in guinea-pig colon, perhaps by decreasing the release of inflammatory mediators. This suggests that selective PDE inhibition may decrease the responsiveness of the gastrointestinal tract seen during inflammation, and may have a therapeutic benefit in the treatment of altered gastrointestinal motility associated with inflammatory diseases of the gastrointestinal tract.

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References

- BARNETTE, M.S. & GROUS, M. (1992). Characterization of the antigen-induced contraction of colonic smooth muscle from sensitized guinea pigs. *Am. J. Physiol.*, **262**, G144–G149.
- BARNETTE, M.S., MANNING, C.D., PRICE, W.J. & BARONE, F.C. (1993). Initial biochemical and functional characterization of cyclic nucleotide phosphodiesterase isozymes in canine colonic smooth muscle. *J. Pharmacol. Exp. Ther.*, **264**, 801–812.
- COLLINS, S.M., HUIZINGA, J.D. & VERMILLION, D.L. (1989). Smooth muscle function in the inflamed bowel. In *Inflammatory Bowel Disease*. ed. Freeman, H.J. vol. 1, pp. 59–75. Boca Raton, FA, USA: CRC Press Inc.
- DENT, G., GIEMBYCZ, M.A., RABE, K.F. & BARNES, P.J. (1991). Inhibition of eosinophil cyclic nucleotide PDE activity and opsonized zymozan-stimulated respiratory burst by 'type IV' selective PDE inhibitors. *Br. J. Pharmacol.*, **103**, 1339–1346.
- FROSSARD, N., LANDRY, Y., PAULI, G. & RUCKSTUHL, M. (1981). Effects of cyclic AMP- and cyclic GMP-phosphodiesterase inhibitors on immunological release of histamine and on lung contraction. *Br. J. Pharmacol.*, **73**, 933–938.
- MULLER, M.J. & BAER, H.P. (1983). Relaxant effects of forskolin in smooth muscle. *Naunyn-Schmied. Arch. Pharmacol.*, **322**, 78–82.
- NICHOLSON, C.D., CHALLISS, R.A.J. & SHAHID, M. (1991). Differential modulation of tissue function and therapeutic potential of selective inhibitors of cyclic nucleotide phosphodiesterase isozymes. *Trends Pharmacol. Sci.*, **12**, 19–27.
- NICHOLSON, C.D., SHAHID, M., VAN AMSTERDAM, R.G.M. & ZAAGSMA, J. (1990). Cyclic nucleotide phosphodiesterase (PDE) isozymes in bovine tracheal smooth muscle, their inhibition and the ability of isozyme inhibitors to relax recontracted preparations. *Eur. J. Pharmacol.*, **183**, 1097–1098.
- NIELSEN, C.P., VESTLA, R.E., STURM, R.J. & HEASLIP, R. (1990). Effects of selective phosphodiesterase inhibitors on the polymorphonuclear leukocyte respiratory burst. *J. Allergy Clin. Immunol.*, **86**, 801–808.
- PEACHELL, P.T., UNDEM, B.J., SCHLEIMER, R.P., MACGLASHAN, D.W., LICHTENSTEIN, L.M., CIESLINSKI, L.B. & TORPHY, T.J. (1992). Preliminary identification and role of phosphodiesterase isozymes in human basophils. *J. Immunol.*, **148**, 2503–2510.
- SCHNEIDER, H.H., SCHMIECHEN, R., BREZINSKI, M. & SEIDLER, J. (1986). Stereospecific binding of the antidepressant rolipram to brain protein structures. *Eur. J. Pharmacol.*, **127**, 105–115.
- SCHUDT, C., WINDER, S., FORDERKUNZ, S., HATZELMANN, A. & ULLRICH, V. (1991). Influence of selective phosphodiesterase inhibitors on human neutrophil functions and levels of cAMP and Ca. *Naunyn-Schmied. Arch. Pharmacol.*, **344**, 682–690.
- SHAHID, M., VAN AMSTERDAM, R.G.M., DE BOER, J., TEN BERGE, R.E., NICHOLSON, C.D. & ZAAGSMA, J. (1991). The presence of five cyclic nucleotide phosphodiesterase isozyme activities in bovine tracheal smooth muscle and the functional effects of selective inhibitors. *Br. J. Pharmacol.*, **104**, 471–477.
- SILVER, J.P., HAMEL, L.T., PERRONE, M.H., BENTLEY, R.G., BUSHOVER, C.R. & EVANS, D.B. (1988). Differential pharmacological sensitivity of cyclic nucleotide phosphodiesterase isozymes isolated from cardiac muscle, arterial and airway smooth muscle. *Eur. J. Pharmacol.*, **150**, 85–94.

- SILVER, P.J., HARRIS, A.L., BUCHHOLZ, R.A., MILLER, M.S., GORDON, R.J., DUNDORE, R.L. & PAGANI, E.D. (1990). Low K_m cAMP phosphodiesterase isozymes and modulation of tone in vascular, airway and gastrointestinal smooth muscle. In *Purines in Cellular Signaling, Targets for New Drugs*. ed. Jackson, K.A., Daley, J.W. & Manganiello, V. pp. 358–364. New York: Springer-Verlag.
- SOUNESS, J.E., CARTER, C.M., DIOCEE, B.K., HASSALL, G.A., WOOD, L.J. & TURNER, N.C. (1991). Characterization of guinea-pig eosinophil phosphodiesterase activity. *Biochem. Pharmacol.*, **42**, 937–945.
- TORPHY, T.J. & CIESLINSKI, L.B. (1990). Characterization and selective inhibition of cyclic nucleotide phosphodiesterase isozymes in canine tracheal smooth muscle. *Mol. Pharmacol.*, **37**, 206–214.
- TORPHY, T.J., LIVI, G.P., BALCAREK, J.M., WHITE, J.R., CHILTON, F.H. & UNDEM, B.J. (1992a). Therapeutic potential of isozyme-selective phosphodiesterase inhibitors in the treatment of asthma. *Adv. Sec. Messenger Phosphoprotein Res.*, **25**, 289–305.
- TORPHY, T.J., STADEL, J.M., BURMAN, M., CIESLINSKI, L.B., MCLAUGHLIN, M.M., WHITE, J.R. & LIVI, G.P. (1992b). Co-expression of human cAMP-specific phosphodiesterase activity and high affinity rolipram binding in yeast. *J. Biol. Chem.*, **267**, 1798–1804.
- TORPHY, T.J. & UNDEM, B.J. (1991a). Phosphodiesterase inhibitors: new opportunities for the treatment of asthma. *Thorax*, **46**, 512–523.
- TORPHY, T.J., ZHOU, H.-L., BURMAN, M. & HUANG, L.B.F. (1991b). Role of cyclic nucleotide phosphodiesterase isozymes in intact canine trachealis. *Mol. Pharmacol.*, **39**, 376–384.
- UNDEM, B.J., PEACHELL, P.T. & LICHTENSTEIN, L.M. (1988). Isoproterenol-induced inhibition of immunoglobulin E-mediated release of histamine and arachidonic acid metabolites from human lung mast cells. *J. Pharmacol. Exp. Ther.*, **247**, 209–217.
- UNDERWOOD, D.C., OSBORN, R.R., NOVAK, L.B., MATTHEWS, J.K., NEWSHOLME, S.J., UNDEM, B.J., HAND, J.M. & TORPHY, T.J. (1993). Inhibition of antigen-induced bronchoconstriction and eosinophil infiltration in the guinea-pig by cyclic AMP-specific phosphodiesterase inhibitor, rolipram. *J. Pharmacol. Exp. Ther.*, **266**, 306–313.
- VERMILLION, D.L., ERNST, P.B., SCICCHITANO, R. & COLLINS, S.M. (1988). Antigen-induced contraction of jejunal smooth muscle in the sensitized rat. *Am. J. Physiol.*, **255**, G701–G708.

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