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Regulation by phosphodiesterase isoenzymes of non-adrenergic non-cholinergic contraction in guinea-pig isolated main bronchus

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1 We have investigated the role of phosphodiesterase isoenzymes in modulating electric field stimulation (EFS), substance P and capsaicin-induced contraction of the guinea-pig isolated main bronchus.

2 Non-adrenergic non-cholinergic contractile responses were elicited by EFS (3 Hz, 20 s) in the guinea-pig isolated main bronchus in the presence of the non-selective muscarinic antagonist, atropine (0.1 μM), the non-selective β-adrenoceptor antagonist, propranolol (1 μM), the neutral endopeptidase inhibitor, thiorphan (10 μM) and the cyclo-oxygenase inhibitor, indomethacin (5 μM). The type III, type III/IV, type IV and type V phosphodiesterase isoenzyme inhibitor, SKF 94836, benzafentrine, Ro-20-1724 and zaprinast respectively, significantly attenuated the contractile response to EFS. The IC_{50} (95% confidence limits) value for SKF 94836, benzafentrine, Ro-20-1724 and zaprinast was 8.3 μM (0.89-78); 0.7 μM (0.1-4.5); 0.5 μM (0.2-1.2) and 13 μM (2-87) respectively.

3 The phosphodiesterase isoenzyme inhibitors, SKF 94836, Ro-20-1724 and zaprinast, partially attenuated the contractile response to substance P (10 nM). Benzafentrine significantly inhibited the contractile response to substance P, yielding an IC_{50} value of 1.9 μM (0.9-3.8).

4 The phosphodiesterase isoenzyme inhibitor, Ro-20-1724 (0.1-100 μM) failed to reduce significantly the contractile potency of capsaicin ($P>0.05$). In contrast, SKF 94836 (1 μM), benzafentrine (10 μM) and zaprinast (100 μM) significantly reduced the contractile potency of capsaicin ($P<0.05$).

5 The selective phosphodiesterase isoenzyme inhibitors, SKF 94836, benzafentrine, Ro-20-1724 and zaprinast (0.01-100 μM) reversed in a concentration-dependent manner the contractile response to exogenously administered capsaicin (EC_{50}) yielding IC_{50} values of 3.91 μM (0.68-22); 3.37 μM (1.86-6.11); 0.366 μM (0.201-0.564) and 50.1 μM (18.6-135) respectively.

6 In conclusion, phosphodiesterase isoenzymes appear to regulate the contractile response to electrical field stimulation and our results provide circumstantial evidence for a regulatory role of phosphodiesterase type IV isoenzyme on sensory nerve function *in vitro*.

Keywords: Phosphodiesterase; main bronchus; sensory nerves; capsaicin; substance P; airway smooth muscle

Introduction

Phosphodiesterase enzymes are responsible for the breakdown of adenosine 3':5'-cyclic monophosphate (cyclic AMP) and guanosine 3':5'-cyclic monophosphate (cyclic GMP). Furthermore, at least five phosphodiesterase isoenzyme families have been identified in a number of cells and there is evidence of differences in the distribution of these isoenzymes in cells (Beavo & Reifsnyder, 1990; Torphy & Undem, 1991; Nicholson *et al.*, 1991; Nicholson & Shahid, 1994). Recently, phosphodiesterase type VI (cone and rod photoreceptor, Michaeil *et al.*, 1993), type VII (T lymphocytes, Ichimura & Kase 1993) and type VIII (rat cerebrum, Mukai *et al.*, 1994) have also been proposed. It appears that inflammatory cells contain mainly a phosphodiesterase type IV isoenzyme while airway smooth muscle contains phosphodiesterase type I-IV isoenzymes (Torphy & Undem, 1991; Nicholson & Shahid, 1994).

Recently, it has been shown that the non-selective phosphodiesterase isoenzyme inhibitors, theophylline, enprofylline and isbufylline inhibit non-adrenergic non-cholinergic contractile responses in guinea-pig isolated main bronchus. These findings suggest the possibility that phosphodiesterase isoenzymes may regulate the release of neuropeptides from sensory nerves (Aikawa *et al.*, 1992; Barlinski *et al.*, 1992; Meini *et al.*, 1993). Indeed it has been shown that the selective phosphodiesterase type IV inhibitor, rolipram but not the selective phosphodiesterase type III and type V inhibitors, siguazodan and zaprinast respectively, attenuated the non-adrenergic non-cholinergic contractile response in guinea-pig isolated main bronchus (Qian *et al.*, 1994; Undem *et al.*, 1994).

In the present study, we have investigated further the possibility that phosphodiesterase isoenzymes regulate the release of sensory neuropeptides.

Methods

Tissue preparation

Male Albino guinea-pigs (300-500 g) were killed by cervical dislocation and the lungs removed and placed in cold (4°C) Krebs-Henseleit solution, aerated with 95% O_2 and 5% CO_2. Main bronchial rings (2 mm) were suspended in 8 ml organ baths under 0.5 g tension in Krebs-Henseleit solution aerated with 95% O_2 and 5% CO_2 at 37°C and containing the cyclo-oxygenase inhibitor indomethacin (5 μM), the non-selective β-adrenoceptor antagonist, propranolol (1 μM) and the neutral endopeptidase inhibitor thiorphan (10 μM). Tissues were allowed to equilibrate for 30 min with changes in Krebs-Henseleit solution made at 10 min intervals. Methacholine (0.3 and 100 μM) was added cumulatively to the bath and after the contractile response had reached plateau, the tissues were washed 5 times over a 15 min period and allowed to equilibrate for a further 30 min.

Electrical field stimulation studies

Guinea-pig isolated main bronchi were placed between 2 platinum electrodes and electrically stimulated (1-30 Hz, 20 s, 0.5 ms pulse width, 90 V) in the presence of atropine (0.1 μM). The contractile response obtained returned to baseline after 30 min.

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In other experiments, tissues were electrically stimulated with 3 Hz and the contractile response allowed to reach baseline after 30 min. The tissues were incubated 10 min prior to stimulation with SKF 94836 (0.001 – 100 μM), benzafentrine (0.1 nM – 100 μM), Ro-20-1724 (0.01 – 100 μM) and zaprinast (0.01 – 100 μM). In each preparation, 4 increasing cumulative concentrations of the phosphodiesterase inhibitors were tested. In other bronchial preparations from the same animal, tissues were repeatedly stimulated at the appropriate times either in the absence or presence of vehicle to determine the effect of time-related fading and drug solvent on the contractile response to electrical field stimulation (EFS). Data have been expressed as a percentage of the contractile response obtained prior to the administration of the selective phosphodiesterase isoenzyme inhibitors.

Spasmogen studies

In the presence of thiorphan (10 μM), guinea-pig isolated main bronchi were contracted with a concentration of substance P (10 nM) which gave a similar increase in tension to that observed with EFS (3 Hz, 20 s). Contractile responses to substance P were elicited in the absence or presence of increasing concentrations of SKF 94836 (0.001 – 100 μM), benzafentrine (0.1 nM – 100 μM), Ro-20-1724 (0.01 – 100 μM) or zaprinast (0.01 – 100 μM). The phosphodiesterase isoenzyme inhibitors were administered 10 min prior to the addition of substance P. After the contractile response had reached a plateau, the tissues were washed and the contractile response allowed to return to baseline. The substance P contractile responses were performed at 30 min intervals. This was repeated in the presence of increasing concentrations of the phosphodiesterase isoenzyme inhibitors. Data were expressed as a percentage of the contractile response obtained prior to the administration of the selective phosphodiesterase isoenzyme inhibitors.

In other experiments, cumulative dose–response curves to capsaicin (0.001 – 1 μM) was obtained in the absence or presence of SKF 94836 (0.01 – 100 μM), benzafentrine (0.01 – 100 μM), Ro-20-1724 (0.01 – 100 μM) and zaprinast (0.1 – 100 μM). In these experiments, the neutral endopeptidase inhibitor, phosphoramidon (10 μM), was present throughout.

Relaxation studies

Guinea-pig main bronchial and distal tracheal preparations were pre-contracted to capsaicin. The concentrations chosen yielded a contractile response that was 50% of the maximal contractile response to methacholine (100 μM). Once the contractile response had reached plateau, relaxation concentration-effect curves to SKF 94836 (0.01 – 100 μM), benzafentrine (1 – 100 μM), Ro-20-1724 (0.03 – 100 μM) and zaprinast (1 – 100 μM) were superimposed on this response. In these experiments, the neutral endopeptidase inhibitor phosphoramidon (10 μM) was present throughout.

Analysis of results

The concentration of the selective phosphodiesterase isoenzyme inhibitor that inhibited the contractile response to EFS by 50% of the control response and reversed the contractile response to capsaicin to 50% of the maximum relaxation (IC_{50}) were taken as a measure of potency and expressed as the geometric mean together with 95% confidence limits. In other cases, the arithmetic mean and s.e.mean has been used. Data were analysed by analysis of covariance and differences between means were assessed by Student's paired and non-paired t test and considered significant if $P<0.05$.

Drugs

Atropine, capsaicin, dimethylsulphoxide (DMSO), indomethacin, methacholine, phosphoramidon, substance P, thiorphan (Sigma); SKF 94836 siguazodan (SmithKline Beecham); Benzafentrine (Sandoz); Ro-20-1724 [4-(3-butoxy-4-methoxybenzyl)-2-imidazlidone] (Calbiochem); Zaprinast (Rhone Poulenc Rorer). All drugs were dissolved in Krebs-Henseleit solution. Composition of Krebs-Henseleit solution was (mM): NaCl 117.6, KCl 5.4, $MgSO_4.7H_2O$ 0.57, KH_2PO_4 1.03, $NaHCO_3$ 25.0, glucose 11.1 and $CaCl_2.2H_2O$ 2.5. The stock concentration (0.01 M) of indomethacin was prepared in 0.5% Na_2CO_3. The stock concentration of capsaicin (0.01 M) was prepared in 100% ethanol. Stock concentrations of substance P (0.6 mM) were prepared in 10% acetic acid and stored at −20°C. Stock concentrations (0.01 M) of benzafentrine, SKF 94836 and Ro-20-1724 were prepared in 10% DMSO and zaprinast was prepared in 0.1 M NaOH. The appropriate dilution was then made in Krebs-Henseleit solution.

Results

Electrical field stimulation

Electrical field stimulation (3 Hz) of guinea-pig isolated main bronchus induced a contractile response of 47 ± 12% ($n=6$) of the maximum response to methacholine (100 μM). The contractile response to electrical field stimulation was tetrodotoxin-sensitive (data not shown). No significant reduction in the contractile response to repeated EFS was observed in vehicle controls ($P>0.05$).

The phosphodiesterase isoenzyme inhibitors SKF 94836 (type III; Figure 1), benzafentrine (type III/IV; Figure 2), Ro-20-1724 (type IV; Figure 3) and zaprinast (type V; Figure 4) inhibited the non-adrenergic non-cholinergic contractile response yielding IC_{50} values for SKF 94836, benzafentrine, Ro-20-1724 and zaprinast of 8.3 μM (0.78 – 78), $n=8$; 0.7 μM (0.1 – 4.5), $n=4$; 0.5 μM (0.2 – 1.2), $n=6$ and 13 μM (2 – 87), $n=6$ respectively. The maximum inhibitory response (% reversal of control EFS response) by SKF 94836, benzafentrine, Ro-20-1724 and zaprinast (100 μM) was 66 ± 6% ($n=8$), 94 ± 6% ($n=5$), 87 ± 4% ($n=6$) and 65 ± 6% ($n=5$) respectively.

Substance P

Substance P (10 nM) induced a contractile response of 52 ± 3% ($n=9$) of the maximum response to methacholine (100 μM). The maximum inhibitory response (% reversal of control

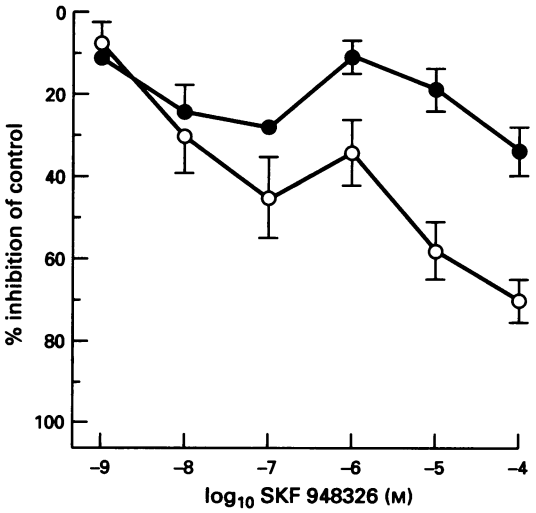

Figure 1 Inhibitory concentration-effect curves to SKF 94836 on electrical field (3 Hz, 20 s, 0.5 ms; ○; $n=8$) and substance P (●, $n=8$)-induced contraction of guinea-pig isolated bronchus. Each point represents the mean with s.e.mean. Experiments performed in the presence of indomethacin (5 μM), propranolol (1 μM), atropine (0.1 μM) and thiorphan (10 μM).

substance P response) by SKF 94836, benzafentrine, Ro-20-1724 and zaprinast (100 μM) was 34±3% (n=8), 93±2% (n=6), 41±7% (n=6) and 58±6% (n=4) respectively. Inhibitory potency values could only be obtained for benzafentrine yielding an IC_{50} value of 1.9 μM (0.9–3.8), n=6 (Figure 2). The phosphodiesterase isoenzyme inhibitor SKF 94836 (Figure 1), Ro-20-1724 (Figure 3) and zaprinast (Figure 4) reduced the contractile response to exogenously administered substance P (10 nM) at a concentration of 10 μM ($P<0.05$ cf. control, paired t test) and 100 μM ($P<0.05$ cf. control, paired t test). Both SKF 94836 and Ro 20-1724 were significantly less effective at inhibiting the contractile response to substance P than to EFS ($P<0.01$, ANCOVA). Indeed, the maximum inhibitory effect for SKF 94836 and Ro-20 1724 on substance P induced contraction was significantly less than for the effect of these drugs on EFS-induced contraction ($P<0.01$, non-paired t test).

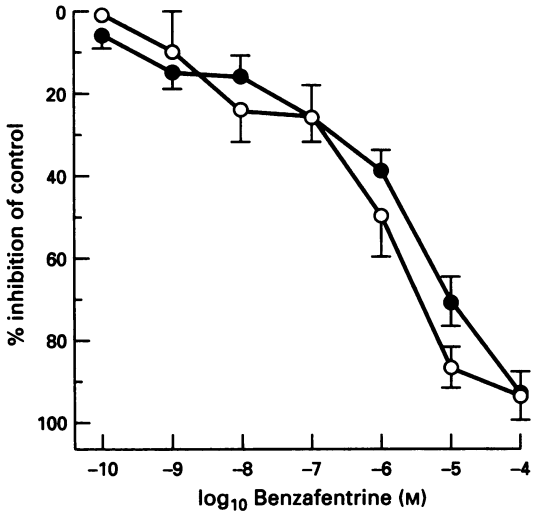

Figure 2 Inhibitory concentration-effect curves to benzafentrine on electrical field (3 Hz, 20 s, 0.5 ms; ○; n=5) and substance P (●, n=3–6)-induced contraction of guinea-pig isolated bronchus. Each point represents the mean and vertical lines represent s.e.mean observations. Experiments performed in the presence of indomethacin (5 μM), propranolol (1 μM), atropine (0.1 μM) and thiorphan (10 μM).

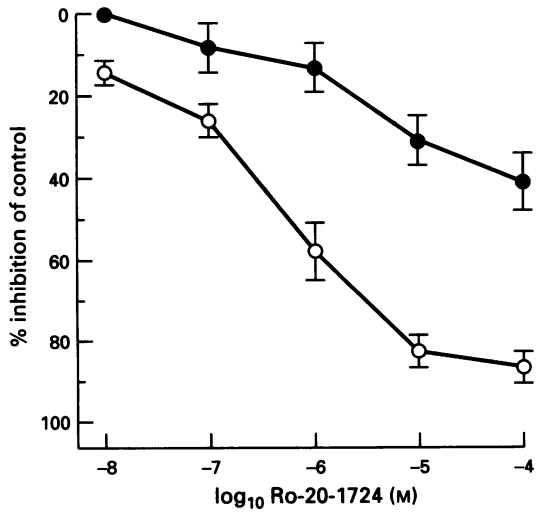

Figure 3 Inhibitory concentration-effect curves to Ro-20-1724 on electrical field (3 Hz, 20 s, 0.5 ms; ○; n=3–6) and substance P (●, n=5–6)-induced contraction of guinea-pig isolated bronchus. Each point represents the mean with s.e.mean. Experiments performed in the presence of indomethacin (5 μM), propranolol (1 μM), atropine (0.1 μM) and thiorphan (10 μM).

Capsaicin

Capsaicin induced a concentration-dependent contractile response in guinea-pig isolated main bronchus and distal trachea with a potency (−log EC_{50}) of 7.63±0.05 (n=20) and 7.53±0.04 (n=19) respectively. The phosphodiesterase isoenzyme inhibitors SKF 98436 (1 μM; Figure 5), benzafentrine (10 and 100 μM; Figure 6) and zaprinast (100 μM; Figure 7) significantly reduced the contractile potency to capsaicin (Figures 3–5; Table 1, $P<0.05$). In contrast, although at high concentrations, Ro-20-1724 attenuated the contractile response to capsaicin this failed to achieve statistical significance (Figure 8, Table 1, $P>0.05$).

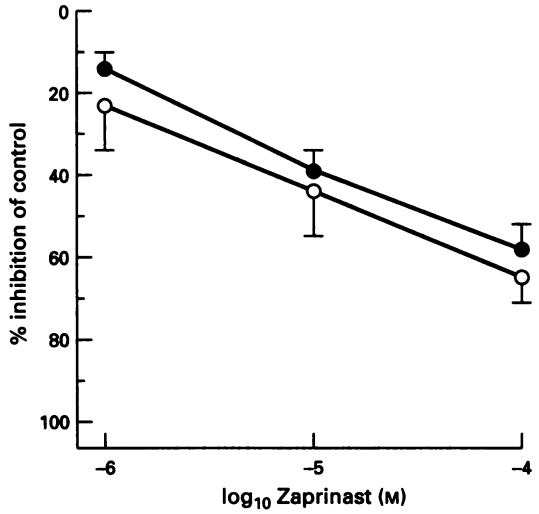

Figure 4 Inhibitory concentration-effect curves to zaprinast on electrical field (3 Hz, 20 s, 0.5 ms; ○; n=5–6) and substance P (●, n=3–4)-induced contraction of guinea-pig isolated bronchus. Each point represents the mean with s.e.mean. Experiments performed in the presence of indomethacin (5 μM), propranolol (1 μM), atropine (0.1 μM) and thiorphan (10 μM).

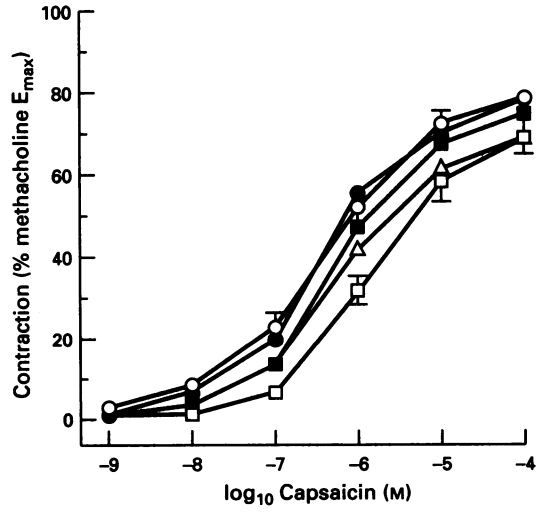

Figure 5 Concentration-effect curves to capsaicin in the absence (○) or presence of the phosphodiesterase type III isozyme inhibitor SKF 94836 0.1 μM (●), 1 μM (□), 10 μM (■), 100 μM (△). Each point represents the mean with s.e.mean of (5–12) observations. Experiments performed in the presence of indomethacin (5 μM), propranolol (1 μM), and phosphoramidon (10 μM). For the sake of clarity error bars have been omitted on some curves obtained in the presence of SKF 94836.

Relaxant responses

The phosphodiesterase isoenzyme inhibitors SKF 94836, benzafentrine, Ro-20-1724 and zaprinast reversed capsaicin-induced contraction yielding IC_{50} values of 3.91 μM (0.68–22), $n=6$; 3.37 μM (1.86–6.11), $n=5$; 0.37 μM (0.20–0.56), $n=6$ and 50.1 μM (18.6–135), $n=3$ respectively (Figure 9). The maximum relaxant response (% reversal capsaicin-induced tone) mediated by SKF 94836 (100 μM), benzafentrine (100 μM), Ro-20-1724 (10 μM) and zaprinast (100 μM) was 79.6±8.5% ($n=6$), 112.4±3.3% ($n=5$), 90.1±9.2% ($n=6$) and 61.4±9.6% ($n=4$) respectively.

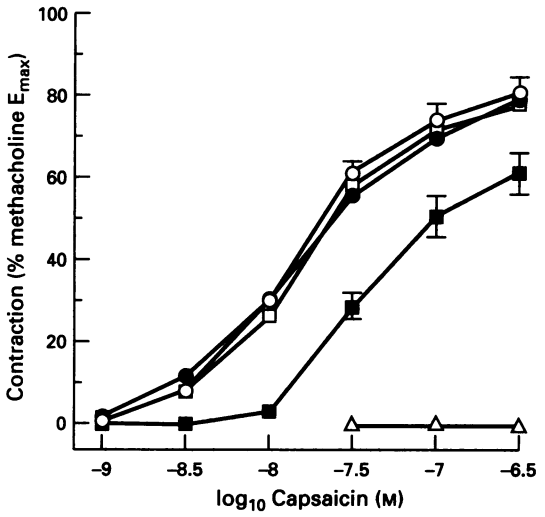

Figure 6 Concentration-effect curves to capsaicin in the absence (○) or presence of the phosphodiesterase type III/IV isozyme inhibitor, benzafentrine 0.1 μM (●), 1 μM (□), 10 μM (■), 100 μM (△). Each point represents the mean with s.e.mean of 3–6 observations. Experiments performed in the presence of indomethacin (5 μM), propranolol (1 μM), and phosphoramidon (10 μM). For the sake of clarity, error bars have been omitted on some curves obtained in the presence of benzafentrine.

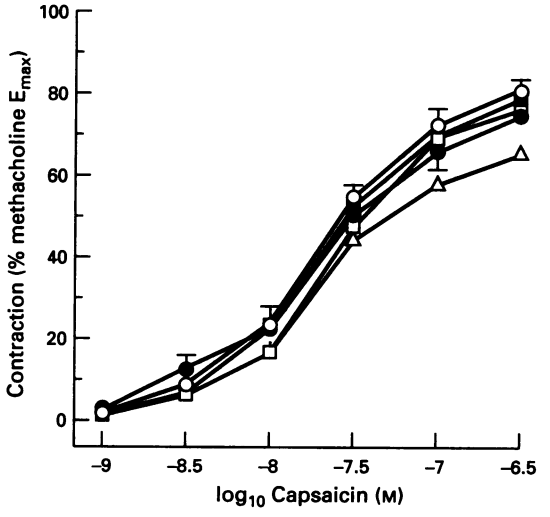

Figure 7 Concentration-effect curves to capsaicin in the absence (○) or presence of the phosphodiesterase type IV isozyme inhibitor, Ro-20-1724 0.1 μM (●), 1 μM (□), 10 μM (■), 100 μM (△). Each point represents the mean and vertical lines represent s.e.mean of 4–10 observations. Experiments performed in the presence of indomethacin (5 μM), propranolol (1 μM), and phosphoramidon (10 μM). For the sake of clarity, error bars have been omitted on the curves obtained in the presence of Ro-20-1724.

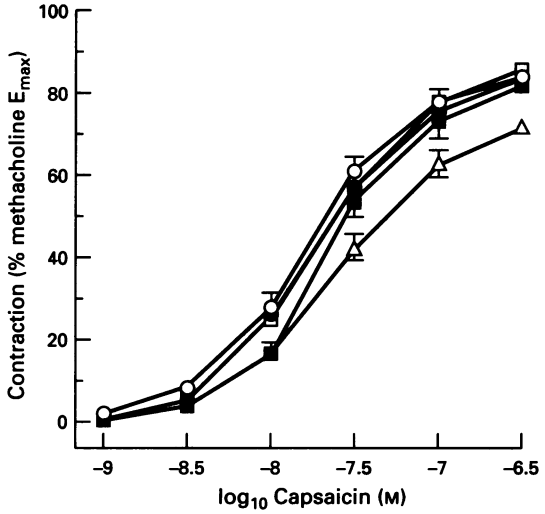

Figure 8 Concentration-effect curves to capsaicin in the absence (○) or presence of the phosphodiesterase type V isozyme inhibitor, zaprinast 0.1 μM (●), 1 μM (□), 10 μM (■), 100 μM (△). Each point represents the mean with s.e.mean of 5–11 observations. Experiments performed in the presence of indomethacin (5 μM), propranolol (1 μM), and phosphoramidon (10 μM). For the sake of clarity, error bars have been omitted on the curves obtained in the presence of zaprinast.

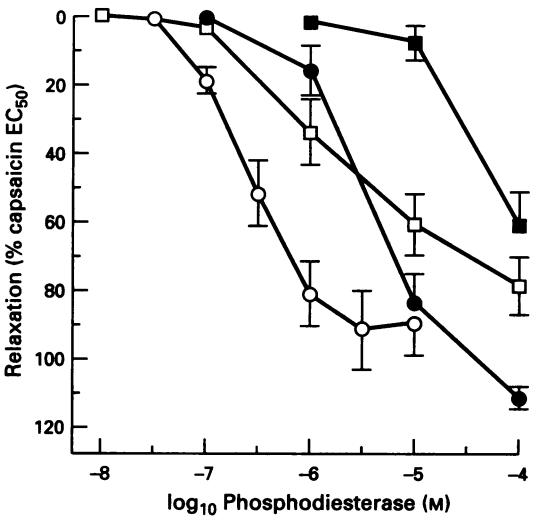

Figure 9 Inhibitory concentration-effect curves for the phosphodiesterase isozyme inhibitors Ro-20-1724 (○, $n=5$), benzafentrine (●, $n=5$), SKF 94836 (□, $n=6$) and zaprinast (■, $n=4$) on guinea-pig isolated bronchus pre-contracted with capsaicin (EC_{50}). Each point represents the mean with s.e.mean. Experiments performed in the presence of indomethacin (5 μM), propranolol (1 μM), and phosphoramidon (10 μM).

Discussion

We have demonstrated that the type IV phosphodiesterase isoenzyme inhibitor, Ro-20-1724 preferentially inhibits the contractile response elicited by EFS rather than that to exogenously administered substance P in guinea-pig isolated bronchus. In contrast, Ro 20-1724 failed to alter the contractile response to exogenously administered capsaicin. These findings suggest a differential role for type IV phosphodiesterase isoenzymes in modulating electrical rather than capsaicin-induced release of sensory neuropeptides from guinea-pig isolated airways.

The non-selective phosphodiesterase inhibitors, isbufylline, enprofylline and theophylline have previously been shown to inhibit the non-adrenergic non-cholinergic contractile response

Table 1 Contractile potency (pD_2) and maximum contractile response (% methacholine E_{max}) values for capsaicin in the absence and presence of various selective phosphodiesterase isoenzyme inhibitors

	pD_2	% E_{max}	n
Control	7.52±0.6	78±2	12
SKF 94836 (0.1 μM)	7.54±0.08	78±2	6
SKF 94836 (1 μM)	7.11±0.11*	69±4	5
SKF 94386 (10 μM)	7.42±0.04	75±3	6
SKF 94836 (100 μM)	7.39±0.08	69±4	6
Control	7.57±0.08	80±4	6
Benzafentrine (0.1 μM)	7.63±0.23	79±5	4
Benzafentrine (1 μM)	7.58±0.13	78±4	4
Benzafentrine (10 μM)	6.89±0.16*	61±5*	4
Benzafentrine (100 μM)	NR		3
Control	7.53±0.08	80±3	10
Ro-20-1742 (0.1 μM)	7.46±0.11	74±2	5
Ro-20-1742 (1 μM)	7.43±0.09	75±4	5
Ro-20-1742 (10 μM)	7.44±0.16	78±6	5
Ro-20-1742 (100 μM)	7.20±0.325	65±7	4
Control	7.64±0.08	84±2	11
Zaprinast (0.1 μM)	7.61±0.06	83±3	5
Zaprinast (1 μM)	7.60±0.12	85±1	5
Zaprinast (10 μM)	7.52±0.06	82±2	5
Zaprinast (100 μM)	7.30±0.09*	72±2*	7

Values are shown as mean±s.e.mean, *n* represents the number of preparations. NR: no response obtained to capsaicin. *$P<0.05$ of control (non-paired Student's t test with Bonferroni Correction).

in guinea-pig isolated main bronchus (Barlinski *et al.*, 1992; Meini *et al.*, 1993). The inhibitory effect of these xanthines was attributed to a preferential effect on the prejunctional release of neuropeptides since these xanthines failed to influence the contractile response to exogenously administered substance P and to the cholinergic component of the contractile response to electrical field stimulation (Aikawa *et al.*, 1992; Barlinski *et al.*, 1992; Meini *et al.*, 1993). The mechanism of this preferential action on sensory nerves is not clear. However, enprofylline was more effective than theophylline at inhibiting non-adrenergic non-cholinergic contractile responses (Barlinski *et al.*, 1992) suggesting that adenosine antagonism does not play a role in this effect (Persson, 1986). Furthermore, adenosine has been demonstrated to inhibit non-adrenergic non-cholinergic contractile responses in guinea-pig isolated bronchus (Kamikawa & Shimo, 1989). It has recently been demonstrated that the type IV phosphodiesterase inhibitor, rolipram, selectively attenuated the non-adrenergic non-cholinergic contractile response to EFS in guinea-pig isolated main bronchus (Qian *et al.*, 1994; Undem *et al.*, 1994). The prejunctional effect of rolipram was confirmed by the inability of rolipram to attenuate cholinergic- and neuropeptide-induced contractile responses (Qian *et al.*, 1994; Undem *et al.*, 1994).

These data are therefore consistent with the findings that elevation of the intracellular level of the second messenger, cyclic AMP can modulate the release of sensory neuropeptides from guinea-pig airway sensory nerves as assessed by measuring the effect of various agents on non-adrenergic non-cholinergic contractile responses *in vitro*. Such a conclusion is supported by the observations that prostaglandin E_1 (Aikawa *et al.*, 1990), prostaglandin E_2 (Johansson-Rydberg *et al.*, 1992), forskolin (Aikawa *et al.*, 1992), the β_2-selective agonists, procaterol, salbutamol and aformoterol (Aikawa *et al.*, 1992; Verleden *et al.*, 1993) and the β_3-selective agonists SR 58611A and BRL 37344 (Itabashi *et al.*, 1992; Martin *et al.*, 1993) also inhibit non-adrenergic non-cholinergic contractile responses *in vitro*. The inhibitory effect of these agents at low concentrations demonstrated a selectivity toward a prejunctional rather than a postjunctional mechanism of action; thus, the inhibitory effect of these agonists could not be solely attributed to functional antagonism of airway smooth muscle. In contrast to the effect of cyclic AMP on sensory neuropeptide release in the airways, a number of studies have demonstrated that elevation in the intracellular levels of cyclic AMP in sensory neurones leads to an increase in sensory nerve function. Thus, prostaglandin E_2 (Ferreira & Nakamura, 1979; Taiwo & Levine, 1991) and forskolin (Taiwo & Levine 1991) decreases the nociceptive threshold in rat hind paw. This effect was unrelated to protein kinase C activation (Taiwo & Levine, 1991). In contrast, the excitation of sensory nerves is dependent on protein kinase C activation (Rang & Ritchie, 1988). These studies demonstrate that cyclic AMP has different effects on sensory nerve activation and sensitization.

In the present study, we have demonstrated the ability of the type IV selective phosphodiesterase isoenzyme inhibitor to attenuate non-adrenergic non-cholinergic contractile responses in guinea-pig isolated main bronchus. Thus, Ro-20-1724 was more effective against EFS than substance P-induced contraction. Furthermore, while benzafentrine, and zaprinast inhibited the contractile response to electrical field stimulation, the roles of type III and type V phosphodiesterase isoenzymes on sensory nerve function could not be discriminated from the effect of these isoenzyme inhibitors on airway smooth muscle function. Furthermore, while SKF 94836 appeared to inhibit the response to EFS this was achieved at high concentrations only. Qian *et al.* (1994) previously demonstrated that low concentrations of SKF 94836 have a minimal effect on the atropine-resistant EFS induced contraction which we have confirmed in the present study.

In contrast to the effect of Ro-20-1724 on the non-adrenergic, non-cholinergic contractile response, Ro-20-1724 was without significant effect on the capsaicin-induced contractile response. Our observation is also consistent with the finding that neuropeptide Y (Giuliani *et al.*, 1989b), μ-opioids (Bartho *et al.*, 1987), galanin (Giuliani *et al.*, 1989a), ω-conotoxin (Maggi *et al.*, 1988a,b) and the type IV phosphodiesterase isoenzyme inhibitor, rolipram (Undem *et al.*, 1994) selectively inhibit the activation of sensory nerves following electrical but not capsaicin stimulation. It has been suggested that neuropeptide Y may inhibit the activation of voltage-sensitive calcium channels (Walker *et al.*, 1988; Maggi *et al.*, 1988b; Giuliani *et al.*, 1989a) possibly by a protein kinase C dependent mechanism (Ewald *et al.*, 1988).

It has also been shown that μ-opioids and NPY are functionally linked to calcium activated potassium channels (Stretton *et al.*, 1992). Isoprenaline causes membrane hyperpolarization of rabbit isolated tracheal cells as a consequence of increasing the open probability time of calcium-activated potassium channels (Kume *et al.*, 1989) by both cyclic AMP-dependent (Kume *et al.*, 1989) and cyclic AMP-independent mechanisms (Kume *et al.*, 1992; 1994). Thus, the possibility exists that Ro-20-1724 may also attenuate sensory nerve function by elevating cyclic AMP which subsequently increases the open probability time of calcium activated potassium channels. Indeed, the high conductance calcium-activated potassium channel blockers, charybdotoxin and iberiotoxin antagonized aminophylline-induced relaxation of guinea-pig isolated trachea (Jones *et al.*, 1990; 1993). It is not clear whether differences in the ability of electrical field stimulation and capsaicin to activate calcium-activated potassium channels can account for the selective effect of Ro-20-1724. This would seem unlikely given that capsaicin appears to activate this channel (Marsh *et al.*, 1987; Wood *et al.*, 1988).

In contrast to the inability of Ro-20-1724 to antagonize the capsaicin concentration-effect curve, Ro-20-1724 was effective in reversing the contractile response to capsaicin. This differential ability of Ro-20-1724 to reverse capsaicin-induced contractile responses compared with its effect on the capsaicin concentration-effect curve is analogous to the inability of salbutamol to shift the concentration-effect curves to acetylcholine in human bronchus at concentrations that reversed acetylcholine-induced contractile responses (Advenier *et al.*,

1991). These findings can be explained in terms of functional antagonism (Van de Brink, 1973a,b). Thus, if the receptor-subeffect response relationship to Ro-20-1724 has a much lower reserve than for capsaicin, then one may expect only minimal antagonism of the concentration-effect curve to capsaicin. In contrast, the reserve for benzafentrine must be greater than that for Ro-20-1724, since the former significantly shifted the concentration-effect curve to capsaicin. Indeed, this may be related to that fact that unlike Ro-20-1724, benzafentrine is an inhibitor of both type III and type IV phosphodiesterase isoenzymes (Nicholson & Shahid, 1994). However, despite the lower reserve for Ro-20-1724 compared with capsaicin, Ro-20-1724 possesses sufficient stimulus to reverse the contractile response to capsaicin. In contrast, the reserve for benzafentrine is relatively greater than for Ro-20-1724 since benzafentrine produced a much greater reversal of capsaicin-induced tone compared with Ro-20-1724.

In conclusion our results demonstrate the ability of type IV phosphodiesterase isoenzyme to modulate the non-adrenergic non-cholinergic contractile responses to electrical field stimulation. However, this isoenzyme does not appear to modulate the release of sensory neuropeptides mediated via activation of the capsaicin-receptor on airway sensory nerves.

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