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Phosphodiesterase inhibitors suppress α_2-adrenoceptor-mediated 5-hydroxytryptamine release from tracheae of newborn rabbits

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Abstract

The outflow of 5-hydroxytryptamine (5-HT) from isolated tracheae of newborn rabbits was determined by high pressure liquid chromatography with electrochemical detection. This 5-HT outflow reflects release from neuroendocrine epithelial cells of the airway mucosa, as previously shown. Phenylephrine, via α_{2B}-adrenoceptors, caused a transient increase in 5-HT outflow, maximally by about 250%, an effect mediated by liberation of intracellular Ca^{2+}, as previously shown. The non-selective phosphodiesterase inhibitor 2-isobutyl-1-methylxanthine (IBMX) concentration-dependently inhibited phenylephrine-induced 5-HT release (completely at 100 μM, IC_{50}: 1.3 μM). Likewise, benzafentrine (inhibitor of phosphodiesterase 3 and 4) and siguazodan (inhibitor of phosphodiesterase 3) also almost completely inhibited phenylephrine-induced 5-HT release with IC_{50} values of 1.7 and 4.2 μM, respectively. Rolipram (inhibitor of phosphodiesterase 4), in a concentration of 10 μM, which exceeds more than 10-fold the reported IC_{50} for phosphodiesterase 4, did not significantly affect phenylephrine-induced 5-HT release. 5-HT release induced by depolarizing concentrations of K^+ (45 mM), which largely depends on extracellular Ca^{2+}, was not affected by IBMX. In conclusion, phosphodiesterases, with characteristics of phosphodiesterase 3, appear to play an important role in the control of cyclic nucleotide mediated inhibition of 5-HT release from neuroendocrine epithelial cells.

Keywords: Airway; 5-HT (5-hydroxytryptamine, serotonin) secretion; Neuroendocrine epithelial cell; Phosphodiesterase inhibitor; α-Adrenoceptor

1. Introduction

Neuroendocrine epithelial cells are a population of specialized epithelial cells which express several neuroendocrine markers and which are distributed all over the airway mucosa (see Scheuermann, 1987; Adriaensen and Scheuermann, 1993). Several biological amines and neuropeptides have been demonstrated in neuroendocrine epithelial cells and 5-hydroxytryptamine (5-HT) is one of the mediators which appears to be expressed in all neuroendocrine epithelial cells. 5-HT is stored in secretory granules and morphological evidence suggests that it is released at the basal and basolateral cell membrane (Lauweryns et al., 1982; Dey et al., 1981).

5-HT and the different mediators of the neuroendocrine epithelial cells can exert multiple effects in the airways and there is increasing evidence suggesting a role of the neuroendocrine epithelial cells in the development of airway hyperresponsiveness (see Belvisi et al., 1995; Cazzola et al., 1995; Sunday, 1996). Nevertheless, there is limited knowledge about the mechanisms controlling the secretory activity of neuroendocrine epithelial cells.

Recently, we showed that 5-HT release from isolated rabbit tracheae reflects secretion from neuroendocrine epithelial cells (Freitag et al., 1995). Due to the high density of neuroendocrine epithelial cells during the perinatal period (Redick and Hung, 1984; Cho et al., 1989), tracheae of newborn animals showed a relatively high in vitro-release of 5-HT (Freitag et al., 1995), and proved to be an in vitro-preparation useful for pharmacological studies (Freitag et al., 1995, 1996, 1997). Thus, using this preparation it was shown that 5-HT release from neuroendocrine epithelial cells is stimulated via α-adrenoceptors which were further characterized as α_{2B} (Freitag et al., 1996). β-Adrenoceptors were found to mediate inhibitory effects as did direct activation of adenylyl cyclase by forskolin (Freitag et al., 1996). Finally, nitric oxide (NO) was also

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shown to exert inhibitory effects on 5-HT release (Freitag et al., 1997), and by the use of ODQ, (1*H*-[1,2,4]oxadiazolo[4,3-a]quinoxalin-1-one), an inhibitor of soluble guanylyl cyclase (Garthwaite et al., 1995), evidence was obtained that NO may act via increase in cGMP formation (Freitag et al., 1997). Thus, receptor-mediated mechanisms appear to mediate stimulatory as well as inhibitory effects on the secretory activity of neuroendocrine epithelial and both, cAMP and cGMP appear to be inhibitory intracellular signals. The intracellular action of both nucleotides is terminated by phosphodiesterases and because of their direct and indirect inhibitory effects on broncho-constriction and inflammatory responses (e.g., Nicholson et al., 1995; Spina et al., 1995; Ortiz et al., 1996), phosphodiesterase inhibitors gain increasing interest for the treatment of obstructive and inflammatory airway diseases (Nicholson and Shahid, 1994; Teixeira et al., 1997; Torphy, 1998). Therefore, it was of interest to test whether phosphodiesterase inhibitors might also affect 5-HT release from neuroendocrine epithelial cells in the airways.

2. Materials and methods

2.1. Materials

Benzafentrine (AH 21-132, Novartis, Basal, Switzerland); IBMX (2-isobutyl-1-methylxanthine, Biomol, Hamburg, Germany); L-phenylephrine hydrochloride (all Sigma, Deisenhofen, Germany); ODQ (1*H*-[1,2,4]oxadiazolo[4,3-a]quinoxalin-1-one), (Tocris Cookson, Bristol, UK); rolipram (Schering, Berlin, Germany); siguazodan (SKF 94 836, SmithKline Beecham Pharmaceuticals, Worthing, UK).

Drugs were dissolved in distilled water, 1 mM HCl (phenylephrine), dimethyl sulfoxide (IBMX, ODQ, siguazodan) or ethanol (rolipram). Dimethyl sulfoxide or ethanol alone at the maximum concentrations (1% and 0.1%, respectively) did not affect spontaneous or phenylephrine-evoked 5-HT outflow (not shown).

2.2. Preparation and incubation of the tracheae

Newborn mongrel rabbits (1 day old) of either sex (Lammers, Euskirchen, Germany) were used. The animals were killed by stunning followed by exsanguination. Whole tracheae were dissected and incubated as described in detail previously (Freitag et al., 1995, 1996). Briefly, single preparations were fixed in a glass organ bath and then incubated in 1.4 ml Krebs–HEPES solution of the following composition (mM): NaCl 118.5; KCl 5.7; $CaCl_2$ 1.25; $MgCl_2$ 1.2; Na_2EDTA 0.03; (+)ascorbic acid 0.06; HEPES 20.0 (adjusted to pH 7.4 using NaOH) and D-glucose 11.1, kept at 37°C and saturated with 100% O_2. The bath fluid was changed every 10 min. The medium of the first 30 min was discarded (= equilibration period). Then it was collected in plastic tubes which contained 50 μl of 57 mM (+)ascorbic acid, 50 μl of 10 mM EDTA and 100 μl of 1 M perchloric acid (suprapure®) to protect released 5-HT from spontaneous non-enzymatic degradation. When a high K^+-medium was used, KCl was increased to 45 mM and NaCl correspondingly reduced to maintain osmolarity.

At the end of the incubation each preparation was blotted, weighed and extracted in 2 ml of 0.4 M $HClO_4$ for 2 h at 0–4°C. The supernatants were stored at 0–4°C until analysed later the same day.

2.3. Measurement of 5-HT

5-HT was measured by high pressure liquid chromatography (HPLC) with electrochemical detection as described previously (Freitag et al., 1995). HPLC separation was performed by a reverse phase column (length 250 mm, inner diameter 4.6 mm, prepacked with Shadon ODS-Hypersil, 5 μm) using a mobile phase of 0.1 M phosphate buffer (adjusted to pH 3.0), containing octane sulfonic acid sodium salt (160 mg/l), sodium EDTA (0.3 mM) and methanol (12%, v/v). 5-HT eluted with a retention time of about 21 min. Quantitation was achieved with an electrochemical detector (Gynkothek M20) equipped with glass carbon working electrodes and Ag/Ag reference electrodes. The potential was set to +0.62 V. Portions of 200 μl of the incubation media or tissue extracts were directly injected into the HPLC column. The limit of detection was between 20 and 60 fmol 5-HT per injection.

2.4. Calculations and statistical analysis

The outflow of 5-HT is expressed as pmol per gram wet weight of tissue and per collection period (pmol/g/10 min) or as a percentage of the mean outflow observed from 40 to 50 min of incubation of the individual experiments ('initial outflow'). The stimulation evoked release of 5-HT was calculated by summing the 5-HT outflow that, during the respective stimulus, exceeded the basal outflow. The respective basal outflow was extrapolated from the individual initial outflow assuming that the fractional decline would be similar to that observed in control experiments. Calculations of EC_{50} values was carried out by the use of a computer programm (Tallarida and Murray, 1988). Mean values are given ±S.E.M. of *n* experiments. The significance of differences was evaluated by analysis of variance (ANOVA) followed by Student's *t*-test with Bonferoni's correction, using the computer program InStat®.

3. Results

The mean spontaneous outflow of 5-HT from mucosa-containing tracheae of newborn rabbits in the absence of drugs (determined between 40 and 50 min of incubation)

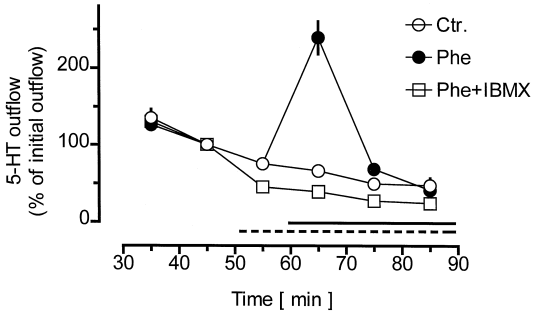

Fig. 1. Effect of 10 μM phenylephrine, in the absence (Phe, $n = 51$, filled circles) or presence of IBMX (100 μM, $n = 3$, open squares), on the outflow of 5-HT from isolated tracheae of newborn rabbits. Phenylephrine was present from 60 to 90 min of incubation as indicated by the horizontal bar. IBMX was present from 50 to 90 min of incubation as indicated by the horizontal dotted line. The open circles show the spontaneous outflow in the absence of any drug (Ctr., $n = 7$). Ordinate: outflow of 5-HT, expressed as percent of the initial outflow (40–50 min of incubation) in the respective individual experiment, means ± S.E.M. of n experiments (S.E.M. often smaller than the symbols).

was 21.4 ± 1.6 pmol/g/10 min ($n = 197$). Similar to previous observations (Freitag et al., 1995, 1996, 1997), the outflow of 5-HT continuously declined during the observation period in control experiments (Fig. 1). IBMX, benzafentrine and siguazodan at the highest concentration tested (100 μM) tended to reduce spontaneous 5-HT outflow (see Fig. 1 for IBMX and data not shown). However, as the spontaneous outflow of 5-HT was sometimes very close to the detection limit, effects on spontaneous 5-HT outflow were not quantitatively described and therefore statistical analysis was not performed.

Tissue 5-HT determined at the end of the incubation experiments was 746 ± 48 pmol/g ($n = 184$). A pooled value is given, as none of the treatments had a significant effect on the tissue 5-HT levels, except of the high K^+-stimulus which reduced tissue 5-HT (Freitag et al., 1995)

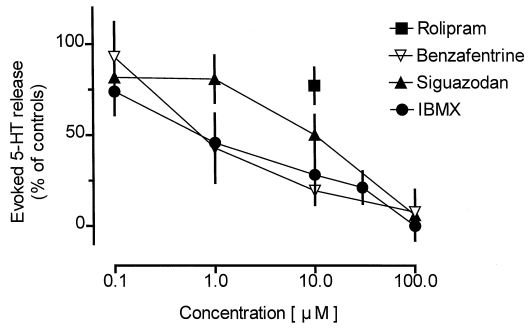

Fig. 2. Effects of different phosphodiesterase inhibitors on 5-HT release from isolated tracheae of newborn rabbits evoked by 10 μM phenylephrine. Phenylephrine and the phosphodiesterase inhibitors were added as shown in Fig. 1. Ordinate: 5-HT release, expressed as percent of the mean increase in 5-HT outflow (peak outflow, 60–70 min of incubation, see Fig. 1) evoked by 10 μM phenylephrine alone. Given are means ± S.E.M. of 3–9 experiments. The phenylephrine controls ($n = 51$) are shown in Fig. 1.

Table 1
Comparison of the inhibitory potency (IC_{50} values, μM) of the phosphodiesterase (PDE) inhibitors IBMX, benzafentrine, siguazodan and rolipram on α-adrenoceptor-mediated 5-HT release from neuroendocrine epithelial cells (see Fig. 2) with their reported inhibitory potency on different phosphodiesterase isoenzymes (see Nicholson and Shahid, 1994)

	IC_{50} (μM)					
	5-HT release	PDE 1	PDE 2	PDE 3	PDE 4	PDE 5
IBMX	1.3	19	50	18	13	32
Benzafentrine	1.7	> 100	35	0.3	0.5	–
Siguazodan	4.2	> 100	> 100	3	> 100	> 100
Rolipram	> 10	> 100	> 100	> 100	0.6	> 100

As in previous studies (Freitag et al., 1996, 1997), phenylephrine (10 μM, the maximally effective concentration) induced a clear, although transient increase in the outflow of 5-HT (Fig. 1); compared with the respective outflow in controls, 5-HT outflow was enhanced by 251 ± 22% ($n = 51$). The total phenylephrine-induced 5-HT release amounted to 30 ± 1.6 pmol/g.

IBMX (0.1–100 μM), when added 10 min before the phenylephrine-stimulus, inhibited the phenylephrine-evoked 5-HT release in a concentration-dependent manner. At 100 μM, IBMX completely suppressed the evoked 5-HT release (Figs. 1 and 2) and an IC_{50} of 1.3 μM was determined (Fig. 2 and Table 1). Benzafentrine, an inhibitor of phosphodiesterase 3 and 4, showed a similar inhibitory effect with almost equal potency (Fig. 2 and Table 1). Siguazodan, a selective inhibitor of phosphodiesterase 3 inhibited the phenylephrine-evoked 5-HT release with an IC_{50} of 4.2 μM, a value which corresponds well with the reported inhibitory potency of siguazodan at phosphodiesterase 3 (Table 1). Finally, the phosphodiesterase 4 selective inhibitor rolipram, in a concentration of 10 μM which exceeds more than 10-fold its reported IC_{50} concentration for phosphodiesterase 4, showed no significant inhibitory effect (Fig. 2 and Table 1).

ODQ (1 μM), a selective inhibitor of the soluble guanylyl cyclase, had no effect on the inhibitory action of 30 μM IBMX, an inhibition by 79 ± 8% ($n = 7$) and 74 ± 10% ($n = 3$) was observed in the absence and presence of ODQ, respectively.

As in previous studies (Freitag et al., 1995, 1997), incubation of the tracheae for 30 min with depolarizing concentrations of K^+ (45 mM) induced a large release of 5-HT (405 ± 51 pmol/g, $n = 9$). IBMX, 100 μM, present already 10 min before and during the high K^+-stimulus, did not affect the evoked 5-HT release (388 ± 62 pmol/g, $n = 4$).

4. Discussion

As shown by several recent studies the isolated trachea of newborn rabbits is a useful preparation to study regula-

tory mechanisms involved in the control of 5-HT release from neuroendocrine epithelial cells of the upper airways (Freitag et al., 1995, 1996, 1997). As outlined in the Introduction, using this model it was shown that α_{2B}-adrenoceptors mediate stimulation of 5-HT release via liberation of Ca^{2+} from intracellular stores, whereas 5-HT release evoked by depolarizing concentrations of K^+ depends on extracellular Ca^{2+} (Freitag et al., 1997). Activation of β-adrenoceptors as well as direct stimulation of adenylyl cyclase by forskolin opposed the α-adrenoceptor-mediated increase in 5-HT release suggesting that a rise in intracellular cAMP can mediate inhibitory effects on 5-HT secretion from neuroendocrine epithelial (Freitag et al., 1996). Furthermore, NO via activation of soluble guanylyl cyclase also inhibited α-adrenoceptor-mediated 5-HT release indicating that, in addition to cAMP, cGMP may also exert inhibitory effects on receptor-mediated 5-HT release from neuroendocrine epithelial cells (Freitag et al., 1997).

The present experiments showed that several inhibitors of phosphodiesterase greatly inhibited the α-adrenoceptor-mediated 5-HT release further supporting the conclusion that cyclic nucleotides play an important role as inhibitory cellular signals in neuroendocrine epithelial cells. Similar to observations on NO-mediated inhibition of 5-HT secretion, the inhibitory effect of phosphodiesterase inhibitors appears to depend on the stimulus by which 5-HT secretion is induced. Thus, IBMX suppressed the α-adrenoceptor-mediated 5-HT release, but did not affect 5-HT release evoked by depolarizing concentrations of K^+. As already mentioned, there is one major difference between both stimuli: the high K^+-evoked 5-HT release is triggered by Ca^{2+} influx through voltage regulated channels whereas the α-adrenoceptor-mediated 5-HT release involves liberation of Ca^{2+} from intracellular stores. Thus, it appears that 5-HT release triggered by voltage dependent influx of Ca^{2+} is not affected by an increase in intracellular cyclic nucleotides whereas that induced by receptor-mediated liberation of intracellular Ca^{2+} is effectively suppressed.

The present observations do not allow a final conclusion whether elevation of cAMP or cGMP is responsible for the inhibition of 5-HT release caused by the phosphodiesterase inhibitors, as both, cAMP and cGMP can mediate inhibitory effects (Freitag et al., 1996, 1997). Since ODQ has been shown to abolish NO-mediated inhibition of 5-HT release in this preparation (Freitag et al., 1997), the lack of effect of ODQ on the inhibition produced by IBMX excludes that potentiation of subthreshold formation of cGMP by the NO activated soluble guanylyl cyclase plays a significant role in the action of phosphodiesterase inhibitors under the present in vitro conditions. However, a role of cGMP cannot be completely excluded as other sources for cGMP appear still possible.

On the other hand, there are at least 7 phosphodiesterase families and the phosphodiesterases 1–5 appear to be widely distributed in the mammalian organism. Phosphodiesterase 6 is a photoreceptor enzyme and little is known at present about phosphodiesterase 7 (see Beavo, 1995). The phosphodiesterases 1–5 can well be discriminated by pharmacological tools (see Table 1). Phosphodiesterase 3 and particularly phosphodiesterase 4 have been shown to be involved in the control of broncho-constriction and inflammatory response in the airways (e.g., Nicholson et al., 1995; Spina et al., 1995; Ortiz et al., 1996; see also Nicholson and Shahid, 1994; Teixeira et al., 1997; Torphy, 1998). The potency of the different isoenzyme selective phosphodiesterase inhibitors as observed in the present experiments (see Table 1) suggest that inhibition of phosphodiesterase 3 may be mainly responsible for the inhibitory effects on 5-HT release. Contributions of other phosphodiesterases cannot be ruled out completely, particularly as the potency of the non-selective inhibitor IBMX was higher than expected from its known inhibitory potency at phosphodiesterase 3 (see Table 1). Phosphodiesterase 3 shows a functional selectivity for cAMP, as it hydrolysis cAMP at a much higher rate than cGMP (see Torphy, 1998), supporting the assumption that cAMP may be the primary nucleotide involved in the present effects. However, cGMP is an effective inhibitor of phosphodiesterase 3 (see Nicholson and Shahid, 1994; Beavo, 1995; Torphy, 1998) and therefore it appears possible that the inhibitory effects of cGMP on 5-HT release are caused indirectly by inhibition of phosphodiesterase 3. The resulting increase in cAMP may then be the next step in the chain of events causing the inhibition of 5-HT release from the neuroendocrine epithelial cells.

5. Conclusion

Phosphodiesterase inhibitors suppress 5-HT release from neuroendocrine epithelial cells in a stimulus-dependent manner, indicating that cyclic nucleotides are important inhibitory cellular signals in neuroendocrine epithelial cells. Thus, the neuroendocrine epithelial cells of the airways should be considered as potential targets for phosphodiesterase inhibitors used in the treatment of inflammatory and obstructive airway diseases. However, if the same phosphodiesterase family as observed in the rabbit is of significance in humans, only phosphodiesterase inhibitors acting on phosphodiesterase 3 (including non-selective or partially selective inhibitors such as phosphodiesterase 3/4 inhibitors) are expected to affect 5-HT release from the neuroendocrine epithelial cells, whereas the currently favoured selective phosphodiesterase 4 inhibitors (see Teixeira et al., 1997; Torphy, 1998) would be ineffective.

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References

Adriaensen, D., Scheuermann, D.W., 1993. Neuroendocrine cells and nerves of the lung. Anat. Rec. 236, 70–85.

Beavo, J.A., 1995. Cyclic nucleotide phosphodiesterases: functional implications of multiple isoforms. Physiol. Rev. 75, 725–748.

Belvisi, M.G., Ward, J.K., Fox, A.J., 1995. 5-Hydroxytryptamine. In: Reaburn, D., Giembycz, M.A. (Eds.) Airway Smooth Muscle: Neurotransmission, Amines, Lipid Mediators and Signal Transduction. Birkenhäuser, Basel, pp. 157–180.

Cazzola, M., Matera, M.G., D'Amato, G., Rossi, F., 1995. Effects of serotonin on airways: recent developments. Allergy 50, 1–10.

Cho, T., Chan, W., Cutz, E., 1989. Distribution and frequency of neuro-epithelial bodies in post-natal rabbit lung: quantitative study with monoclonal antibody against serotonin. Cell Tissue Res. 255, 353–362.

Dey, R.D., Echt, R., Dinerstein, R.J., 1981. Morphology, histochemistry and distribution of serotonin-containing cells in tracheal epithelium of adult rabbit. Anat. Rec. 199, 23–91.

Freitag, A., Wessler, I., Racké, K., 1995. Characterization of 5-hydroxytryptamine release from isolated rabbit and rat trachea: the role of neuroendocrine epithelial cells and mast cells. Naunyn-Schmiedeberg's Arch. Pharmacol. 353, 55–63.

Freitag, A., Wessler, I., Racké, K., 1996. Adrenoceptor- and cholinoceptor-mediated mechanisms in the regulation of 5-hydroxytryptamine release from isolated tracheae of newborn rabbits. Br. J. Pharmacol. 119, 91–98.

Freitag, A., Wessler, I., Racké, K., 1997. Nitric oxide, via activation of guanylyl cyclase, suppresses α_2-adrenoceptor-mediated 5-hydroxytryptamine release from neuroendocrine epithelial cells of rabbit tracheae. Naunyn-Schmiedeberg's Arch. Pharmacol. 356, 856–859.

Garthwaite, J., Southam, E., Boulton, C.L., Nielsen, E.B., Schmidt, K., Mayer, B., 1995. Potent and selective inhibition of nitric oxide-sensitive guanylyl cyclase by 1*H*-[1,2, 4]oxadiazolo[4,3-a]quinoxalin-1-one. Mol. Pharmacol. 48, 184–188.

Lauweryns, J.M., de Bock, V., Verhofstad, A.A.J., Steinbusch, H.W.M., 1982. Immunohistochemical localization of serotonin in intrapulmonary neuro-epithelial bodies. Cell Tissue Res. 226, 215–223.

Nicholson, C.D., Shahid, M., 1994. Inhibitors of cyclic phosphodiesterase isoenzymes—their potential utility in the therapy of asthma. Pulm. Pharmacol. 7, 1–7.

Nicholson, C.D., Shahid, M., Bruin, J., Barron, E., Spiers, I., De Boer, J., Van Amsterdam, R.G.M., Zaagsma, J., Kelly, J.J., Dent, G., Giembycz, M.A., Barnes, P.J., 1995. Characterization of ORG 20241, a combined phosphodiesterase IV/III cyclic nucleotide phosphodiesterase inhibitor for asthma. J. Pharmacol. Exp. Ther. 274, 678–687.

Ortiz, J.L., Valles, J.M., Marticabrera, M., Cortijo, J., Morcillo, E.J., 1996. Effects of selective phosphodiesterase inhibitors on platelet-activating factor- and antigen-induced airway hyperreactivity, eosinophil accumulation, and microvascular leakage in guinea-pigs. Naunyn-Schmiedeberg's Arch. Pharmacol. 353, 200–206.

Redick, M.L., Hung, K.-S., 1984. Quantitation of pulmonary neuroepithelial bodies in pre- and postnatal rabbits. Cell Tissue Res. 238, 583–587.

Scheuermann, D.W., 1987. Morphology and cytochemistry of the endocrine epithelial system in the lung. Int. Rev. Cytol. 106, 35–88.

Spina, D., Harrison, S., Page, C.P., 1995. Regulation by phosphodiesterase isoenzymes of non-adrenergic non-cholinergic contraction in guinea-pig isolated main bronchus. Br. J. Pharmacol. 116, 2334–2340.

Sunday, M.E., 1996. Pulmonary neuroendocrine cells and lung development. Endocrine Pathol. 7, 173–201.

Tallarida, R.J., Murray, R.B., 1988. Manual of Pharmacological Calculations. Springer, Berlin.

Teixeira, M.M., Gristwood, R.W., Cooper, N., Hellewell, P.G., 1997. Phosphodiesterase (PDE)4 inhibitors: anti-inflammatory drugs of the future?. Trends Pharmacol. Sci. 18, 164–170.

Torphy, J.T., 1998. Phosphodiesterase isoenzymes. Molecular targets for novel antiasthmatic agents. Am. J. Respir. Crit. Care Med. 157, 351–370.