Epithelium-dependent potentiation of anaphylactic contractions by β -endorphin in tracheae isolated from actively sensitized guinea-pigs

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1 It has been shown that opioid peptides modulate airway function. In the present study, the effect of β -endorphin on antigen-induced contractions of isolated tracheal rings from actively sensitized guinea-pigs has been studied.

2 β -Endorphin had a concentration-dependent bimodal effect on anaphylactic contractions of the trachea. Low concentrations of β -endorphin (10^{-10} and 10^{-8} M) significantly potentiated anaphylactic contractions, whereas higher concentrations (10^{-7} and 10^{-6} M) significantly suppressed anaphylactic contractions of guinea-pig trachea.

3 β -Endorphin in concentrations of 10^{-8} M and 10^{-7} M did not affect the responsiveness of the tracheal rings to histamine or leukotriene D₄. This indicates that β -endorphin does not influence the responsiveness of tracheal smooth muscle to anaphylactic mediators.

4 In the presence of the non-selective opioid receptor antagonist naloxone, 10^{-8} M β -endorphin still potentiated the anaphylactic contractions of the trachea. In addition, an equimolar concentration of des-Tyr¹- β -endorphin, a fragment of β -endorphin without opioid-like activity, also potentiated anaphylactic contractions. The potentiation of anaphylactic contraction by 10^{-8} M β -endorphin is not therefore mediated by classical opioid-receptors.

5 In the presence of naloxone, 10^{-7} M, β -endorphin did not suppress anaphylactic contractions of the trachea. Thus, the suppression of anaphylactic contraction is mediated via a classical opioid-receptor.

6 In epithelium-denuded tracheae, both 10^{-8} and 10^{-7} M β -endorphin suppressed the anaphylactic contractions, whereas 10^{-8} and 10^{-7} M des-Tyr¹- β -endorphin did not affect anaphylactic contractions. It is concluded that the potentiation of the anaphylactic contraction in intact trachea is epithelium-dependent whereas the suppression of the anaphylactic contraction is epithelium-independent.

Keywords: β -Endorphin; naloxone; anaphylaxis; trachea; mast cell; epithelium

Introduction

Opioid peptides play a role in the modulation of airway function. It has been shown that opioid peptides inhibit non-adrenergic, non-cholinergic neural control of mucus secretion (Rogers & Barnes, 1989a,b), inhibit cough and reflex bronchoconstriction (Karlsson *et al.*, 1990) and inhibit non-adrenergic, non-cholinergic-induced bronchoconstriction (Barnes *et al.*, 1990). Besides beneficial effects, opioid peptides may also have harmful effects on airway function. Popa & Rients (1989) demonstrated that exercise-induced bronchoconstriction in asthmatic patients could be partially prevented by the opiate receptor antagonist naloxone. Furthermore, opiate antagonists have been found to protect animals against anaphylactic shock (Amir, 1982).

Opioid peptides may affect airway function after release of these peptides from the anterior pituitary into the blood or after local release from nerves (Fischer, 1988; Olson *et al.*, 1989; Shimosegawa *et al.*, 1989; Barnes *et al.*, 1990). In the present study, we investigated the effect of the opioid peptide β -endorphin on ovalbumin-induced contractions in tracheae isolated from actively sensitized guinea-pigs. Effects of β -endorphin on cell functions are either mediated by binding to classical opioid receptors or via a non-opioid receptor-mediated mechanism (De Wied & Jolles, 1982; Fischer, 1988; Sibinga & Goldstein, 1988). Binding of β -endorphin to classical opioid receptors requires an intact NH₂-terminal tyrosine and is sensitive to blockade by naloxone (De Wied &

Jolles, 1982; Sibinga & Goldstein, 1988). To investigate whether the effects of β -endorphin on anaphylactic contractions of guinea-pig trachea are mediated by classical opioid receptors or via a non-opioid receptor-mediated mechanism, the non-opioid derivative, des-Tyr¹- β -endorphin and the non-selective opioid receptor antagonist, naloxone, have been used. Furthermore, the effect of β -endorphin and des-Tyr¹- β -endorphin on anaphylactic contractions in epithelium-denuded tracheae was examined. Some preliminary results of this study have been presented at the European Histamine Research Society (1989, Breda, The Netherlands).

Methods

Male guinea-pigs weighing 300–350 g (Olac Ltd., Bicester, England) were sensitized by intraperitoneal injection of ovalbumin (2 mg) in aluminium hydroxide adjuvant (Van Oosterhout & Nijkamp, 1986). Three weeks after sensitization the animals were killed by an i.p. injection of 180 mg pentobarbitone sodium (Nembutal), the tracheae removed and dissected free of connective tissue and blood vessels. In one series of experiments, epithelium denudation of the tracheae was performed with a cotton swab. The effectiveness of this procedure was examined by light microscopy (hematoxylin and eosin staining). One trachea was divided in 4 to 6 parts each of three cartilage rings. The tracheal rings were mounted in organ baths filled with Krebs bicarbonate buffer (37°C) of the following composition (mmol l⁻¹): NaCl 118.1, KCl 4.7, CaCl₂·2H₂O 2.5, MgSO₄·7H₂O 1.2, NaHCO₃ 25.0, KH₂PO₄ 1.2 and glucose 8.3. The organ baths were siliconized to prevent adherence of the peptides to the glass. Krebs solution was

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continuously gassed with a 5% CO₂ and 95% O₂ gas mixture. Changes in tension were measured by an isometric transducer (Harvard Bioscience) and displayed on a two-channel pen recorder (Servogor, type 220). Before the experiment, tracheal rings were equilibrated for at least 60 min under a resting load of 2 g. β-Endorphin (10⁻¹² M to 10⁻⁶ M) or des-Tyr¹-β-endorphin (10⁻⁸ M) was introduced into the organ bath 1 min before the first ovalbumin or histamine addition. Naloxone (10⁻⁶ M) was administered 5 min prior to the addition of β-endorphin. A single (10⁻³ mg ml⁻¹) or cumulative concentration(s) of ovalbumin (10⁻⁸ to 10⁻¹ mg ml⁻¹), histamine (10⁻⁹ to 3 × 10⁻⁴ M) or leukotriene D₄ (10⁻¹¹ to 10⁻⁷ M) was added to the organ bath in the absence or presence of drugs. Different parts of one trachea were alternately used in each group to avoid possible differences between proximal and distal parts. Results are expressed as a percentage of the maximal response of the tracheal ring to ovalbumin in the absence of drugs. Results of histamine and leukotriene D₄ concentration-response curves are expressed as absolute value's. Levels of significance were calculated by the paired *t* test. A value of *P* < 0.05 was considered to be significant. Responses are presented as mean ± s.e.mean.

Materials

Nembutal, containing pentobarbitone sodium 60 mg ml⁻¹ was from Abbot Laboratories, North Chicago, IL, U.S.A. Histamine phosphate was obtained from the Onderlinge Pharmaceutische Groothandel, Utrecht, The Netherlands. Leukotriene D₄ was a gift from J. Rokach (Merck-Frosst, Point Clair-Dorval, Canada). β-Endorphin and des-Tyr¹-β-endorphin were kindly donated by Organon International B.V., Oss, The Netherlands. Naloxone was obtained from DuPont Pharmaceuticals, Wilmington, Delaware, U.S.A.

Results

Addition of β-endorphin in concentrations of 10⁻¹² M to 10⁻⁶ M to the organ bath did not affect initial tension of the tracheae (results not shown). The mean anaphylactic contraction of tracheal rings induced by ovalbumin 10⁻³ mg ml⁻¹ in the absence of β-endorphin was 1.78 ± 0.24 g (*n* = 7). β-Endorphin had a concentration-dependent effect on ovalbumin-induced contractions. At 10⁻¹² M, β-endorphin did not affect these contractions, whereas at 10⁻¹⁰ and 10⁻⁸ M β-endorphin significantly potentiated anaphylactic contractions in tracheal rings induced by 10⁻³ mg ml⁻¹ ovalbumin as compared to the contractions in the absence of β-endorphin (Figure 1). The potentiation of contractions induced by 10⁻³ mg ml⁻¹ ovalbumin amounted to 27% and 22% in the presence of respectively 10⁻¹⁰ and 10⁻⁸ M β-endorphin, as compared to contractions in the absence of β-endorphin. Higher concentrations of β-endorphin (10⁻⁷ and 10⁻⁶ M) significantly suppressed ovalbumin induced contractions of tracheal rings as compared to responses in the absence of β-endorphin (Figure 1).

In Figure 2, the contractions of the tracheal rings to cumulative concentrations of ovalbumin are presented. The maximal response of tracheal rings to ovalbumin (10⁻¹ mg ml⁻¹) in the absence of drugs amounted to 1.55 ± 0.21 g (*n* = 6). At 10⁻⁸ M, β-endorphin significantly enhanced anaphylactic contractions induced by 10⁻⁴ to 10⁻¹ mg ml⁻¹ ovalbumin, but not contractions induced by 10⁻² mg ml⁻¹ (*P* = 0.15). The potentiation varied between 69% at 10⁻⁴ mg ml⁻¹ and 39% at 10⁻¹ mg ml⁻¹ ovalbumin. In the presence of the non-selective opioid receptor antagonist naloxone (10⁻⁶ M), 10⁻⁸ M β-endorphin significantly enhanced the contractions of the tracheal rings induced by 10⁻³, 10⁻² and 10⁻¹ mg ml⁻¹ ovalbumin by 115%, 82% and 64%, respectively. Naloxone, 10⁻⁶ M, itself however, significantly suppressed the anaphylactic contractions of tracheal rings

Figure 1 The effect of different concentrations of β-endorphin on ovalbumin (10⁻³ mg ml⁻¹)-induced anaphylactic contractions in tracheal rings isolated from actively sensitized guinea-pigs. The results are expressed as the percentage potentiation or suppression as compared to the contractions of tracheal rings in the absence of β-endorphin (*n* = 7). The number of experiments is indicated in parentheses. **P* < 0.05 and ***P* < 0.01 as compared to contractions of tracheal rings in the absence of β-endorphin.

induced by 10⁻⁴ to 10⁻¹ mg ml⁻¹ ovalbumin by about 30% as compared to contractions in the absence of naloxone.

Additionally, an equimolar concentration of des-Tyr¹-β-endorphin, the non-opioid fragment of β-endorphin, also significantly potentiated anaphylactic contractions induced by 10⁻³, 10⁻² and 10⁻¹ mg ml⁻¹ ovalbumin by about 40% (Table 1). In this experiment the maximal contraction of tracheae to ovalbumin in the absence of drugs was 0.83 ± 0.09 g (*n* = 4).

The effect of 10⁻⁷ M β-endorphin on cumulative ovalbumin-induced contractions is presented in Figure 3. The mean anaphylactic contraction of tracheae in the absence of drugs was 1.98 ± 0.24 g (*n* = 8). Suppression of ovalbumin-induced contractions in the presence of 10⁻⁷ M β-endorphin was significant at 10⁻² and 10⁻¹ mg ml⁻¹ ovalbumin and was 28% and 24% respectively. Naloxone itself significantly decreased anaphylactic contractions of the tracheal rings induced by 10⁻¹ mg ml⁻¹ ovalbumin by 30%. In the presence of naloxone (10⁻⁶ M), 10⁻⁷ M β-endorphin did not further suppress ovalbumin-induced contractions. The effects of β-endorphin

Figure 2 The effect of 10⁻⁸ M β-endorphin in the absence (filled columns) or presence (hatched columns) of 10⁻⁶ M naloxone on the anaphylactic contractions in tracheal rings isolated from actively sensitized guinea-pigs induced by cumulative concentrations of ovalbumin. The results (*n* = 6) are expressed as a percentage of the maximum ovalbumin-induced (10⁻¹ mg ml⁻¹) contraction in the absence of β-endorphin and naloxone (open columns). **P* < 0.05 and ***P* < 0.01 as compared to contractions of tracheal rings in the absence of β-endorphin and naloxone. †*P* < 0.05 and ††*P* < 0.01 as compared to contractions of tracheal rings in the absence of β-endorphin and in the presence of naloxone (cross-hatched columns).

Table 1 The effect of 10^{-8} M des-Tyr¹- β -endorphin on the anaphylactic contractions of tracheal rings induced by cumulative concentrations of ovalbumin

Ovalbumin (mg ml ⁻¹)	Control	des-Tyr ¹ - β -Endorphin
10^{-5}	1 $\pm$ 1	1 $\pm$ 1
10^{-4}	9 $\pm$ 2	13 $\pm$ 3
10^{-3}	36 $\pm$ 10	53 $\pm$ 8*
10^{-2}	66 $\pm$ 11	94 $\pm$ 14**
10^{-1}	100 $\pm$ 11	141 $\pm$ 7*

The results ($n = 4$) are expressed as a percentage of the maximum ovalbumin-induced (10^{-1} mg ml⁻¹) contraction in the absence of des-Tyr- β -endorphin (control).

* $P < 0.05$ and ** $P < 0.01$ as compared to control.

and des-Tyr¹- β -endorphin (10^{-8} and 10^{-7} M) on ovalbumin-induced contractions of epithelium-denuded tracheae are summarized in Table 2. Ovalbumin-induced (10^{-1} mg ml⁻¹) contractions of tracheae in the absence of drugs (control) were 1.37 ± 0.18 g ($n = 7$) and 1.36 ± 0.11 g ($n = 5$) in experiments with β -endorphin and des-Tyr¹- β -endorphin respectively. In epithelium-denuded tracheal rings, β -endorphin (10^{-8} and 10^{-7} M) significantly suppressed anaphylactic contractions by about 20–25% (Table 2). des-Tyr¹- β -endorphin (10^{-8} and 10^{-7} M) did not affect ovalbumin-induced contractions in epithelium denuded tracheae.

Figure 3 The effect of 10^{-7} M β -endorphin in the absence (filled columns) or presence (hatched columns) of 10^{-6} M naloxone on the anaphylactic contractions in tracheal rings isolated from actively sensitized guinea-pigs induced by cumulative concentrations of ovalbumin. The results ($n = 8$) are expressed as a percentage of the maximum ovalbumin-induced (10^{-1} mg ml⁻¹) contraction in the absence of β -endorphin and naloxone (open columns). * $P < 0.05$ and ** $P < 0.01$ as compared to contractions of tracheal rings in the absence of β -endorphin and naloxone. Cross-hatched columns represent effects of naloxone alone.**Table 2** The effect of β -endorphin (β -End) and des-Tyr¹- β -endorphin (dT β -End, 10^{-7} and 10^{-8} M) on the anaphylactic contractions of epithelium-denuded tracheal rings induced by 10^{-1} mg ml⁻¹ of ovalbumin

Treatment	n	Contraction (% of control)
Control	7	100 $\pm$ 6
10^{-7} M β -End	7	78 $\pm$ 14*
10^{-8} M β -End	7	77 $\pm$ 3*
Control	5	100 $\pm$ 6
10^{-7} M dT β -End	5	110 $\pm$ 10
10^{-8} M dT β -End	5	102 $\pm$ 7

The results are expressed as a percentage of the ovalbumin-induced contraction in the absence of drugs (control).

* $P < 0.01$ as compared to control.

Table 3 The effect of β -endorphin (β -End, 10^{-8} and 10^{-7} M) on histamine- and leukotriene D₄-induced contraction of guinea-pig tracheal rings

Treatment	n	Maximal response (g)	EC ₅₀
Histamine			($\times 10^{-6}$ M)
Control	4	1.76 $\pm$ 0.17	15.69 $\pm$ 2.27
10^{-8} M β -End	4	1.79 $\pm$ 0.11	15.75 $\pm$ 1.91
Control	3	2.13 $\pm$ 0.24	18.03 $\pm$ 3.25
10^{-7} M β -End	3	2.20 $\pm$ 0.25	17.33 $\pm$ 1.69
Leukotriene D ₄ :			($\times 10^{-8}$ M)
Control	4	0.55 $\pm$ 0.10	1.95 $\pm$ 0.70
10^{-8} M β -End	4	0.58 $\pm$ 0.06	1.82 $\pm$ 0.26
10^{-7} M β -End	4	0.64 $\pm$ 0.10	1.88 $\pm$ 0.45

The maximal response and the EC₅₀ values of the histamine concentration-response curves are given. Leukotriene D₄ concentration-response curves were established with 10^{-7} M being the highest concentration. This concentration induces a near maximal contraction. The EC₅₀ values for leukotriene D₄ are the concentrations that induce 50% of the contraction by 10^{-7} M. The results are expressed as the mean value of 3 or 4 experiments (n) performed in duplicate.

β -Endorphin (10^{-8} and 10^{-7} M) had no effect on the sensitivity or reactivity of the tracheal rings to histamine or leukotriene D₄ (Table 3).

Discussion

β -Endorphin, in the concentrations tested, while not affecting the tension of the tracheae when used alone, had a concentration-dependent bimodal influence on ovalbumin-induced contractions. At low concentrations (less than 10^{-7} M), β -endorphin potentiated anaphylactic contractions of the tracheae whereas higher concentrations suppressed contractions. Effects of β -endorphin on cell functions are either mediated by binding to classical opioid receptors or via a non-opioid receptor-mediated mechanism (De Wied & Jolles, 1982; Fischer, 1988; Sibinga & Goldstein, 1988). Binding of β -endorphin to classical opioid receptors requires an intact NH₂-terminal tyrosine and is sensitive to blockade by naloxone (De Wied & Jolles, 1982; Fischer, 1988; Sibinga & Goldstein, 1988). In our experiments, the non-selective opioid receptor antagonist naloxone did not inhibit the potentiation of the anaphylactic contraction induced by 10^{-8} M β -endorphin. Additionally, the non-opioid fragment des-Tyr¹- β -endorphin, derived from β -endorphin, appeared equally potent in enhancing anaphylactic contractions of the tracheae. Thus, potentiation of anaphylactic contractions by β -endorphin (10^{-8} M) is not mediated by classical opioid receptors. Furthermore, 10^{-8} M β -endorphin also seems to inhibit anaphylactic contractions through opioid receptors since the potentiation of the anaphylactic contraction with this concentration of β -endorphin is enhanced in the presence of naloxone. The suppression of the anaphylactic contraction by β -endorphin becomes predominant at 10^{-7} M β -endorphin and can be antagonized by naloxone. It can be concluded that β -endorphin potentiates anaphylactic contractions via a non-opioid receptor-mediated mechanism and at the same time suppresses anaphylactic contractions via classical opioid receptors.

In the presence of naloxone, to prevent the suppressive influence of β -endorphin, the anaphylactic contractions of the tracheae were not enhanced by 10^{-7} M β -endorphin. Thus, the potentiation of anaphylactic contractions with β -endorphin disappears at concentrations higher than 10^{-8} M. This indicates a bell-shaped concentration-response relationship for the potentiation of the anaphylactic contractions with β -endorphin. This agrees with our unpublished observations that 10^{-7} M des-Tyr¹- β -endorphin does not enhance anaphylactic contractions of guinea-pig isolated tracheae. A bell-

shaped concentration-response relationship for the effects of β -endorphin on immune cells has been described by several other groups (Sibinga & Goldstein, 1988). The reason for this phenomenon remains unclear.

Our data show that naloxone itself is also able to suppress anaphylactic contractions of the tracheae. The inhibition of anaphylactic contractions with naloxone does not necessarily imply that endogenous opioid peptides are involved, because naloxone, in high concentrations ($>10^{-4}$ M), has apparent opioid receptor agonistic activity and can modify the effects of a variety of non-opioid drugs (Badawy *et al.*, 1983; Swaynok *et al.*, 1979). Although we cannot rule out similar effects of naloxone in our experiments, it is unlikely since 10^{-6} M naloxone was used.

The bimodal influence of β -endorphin on the anaphylactic contractions in our study, may be due to an interaction with mast cells, smooth muscle cells or other cells present in tracheal rings. We demonstrated that 10^{-8} and 10^{-7} M β -endorphin do not affect the sensitivity and reactivity of tracheal rings to histamine or leukotriene D_4 . Thus, an effect of β -endorphin on the responsiveness of smooth muscle to anaphylactic mediators seems unlikely. However, epithelial cells are involved in the β -endorphin-induced potentiation of anaphylactic contractions. This suggests that β -endorphin induces the production of a factor by epithelial cells that influences anaphylactic contractions, possibly through an interaction with mast cells. At present, nothing is known about epithelial-derived factors that can potentiate anaphylactic reactions. Another explanation for the epithelium-dependent potentiation of anaphylactic contractions could be that β -endorphin is cleaved into fragments by peptidases. One of these fragments may then be responsible for the potentiation of anaphylactic contractions. Aminopeptidases, endopeptidases and enkephalinases can degrade β -endorphin into fragments such as α - and γ -endorphin and the des-Tyr¹ fragments of these endorphins (De Wied & Jolles, 1982). Interestingly, the cell membrane-bound enzyme enkephalinase also called neutral endopeptidase is largely present in epithelial cells of the airways of many species, including guinea-pigs (Dusser *et al.*,

1989; Erdos & Skidgel, 1989). Besides epithelial cells, other cells in the airways also contain the enzyme enkephalinase such as nerves, smooth muscle cells and glands (Dusser *et al.*, 1989), which makes this possibility less likely.

It has been shown that opioid peptides like β -endorphin can affect mediator release of peritoneal mast cells (Mediratta *et al.*, 1988; Sydbom, 1988). However, these data cannot be directly extrapolated to the airways due to tissue-related mast cell heterogeneity (Shanahan *et al.*, 1984; Bienenstock, 1988). In rodents, the mast cells present in the airways are of the mucosal type (Bienenstock, 1988; Kitamura, 1989). Shanahan *et al.* (1984) compared endorphin-induced histamine release from peritoneal and mucosal mast cells in the rat and demonstrated that only peritoneal mast cells responded to endorphins. Recently, Miadonna *et al.* (1988) demonstrated that opioid peptides neither elicited histamine release from human lung mast cells nor influenced IgE-mediated histamine release. In our experiments, tracheal mast cell mediator release was not studied. Therefore, no conclusion can be drawn as to whether β -endorphin directly or indirectly affects mast cell mediator release.

The opioid peptide β -endorphin is released from the anterior pituitary into the blood depending on autonomous rhythms and psychophysiological state (Fischer, 1988; Sibinga & Goldstein, 1988). The level of circulating β -endorphin is increased after stress, exercise, or endotoxin injection (Fischer, 1988; Sibinga & Goldstein, 1988). Opioid peptides may play a role in the pathophysiology of asthma. Firstly, Popa & Rients (1989) recently demonstrated that exercise-induced bronchoconstriction in asthmatic patients could be partially prevented by the opiate-receptor antagonist naloxone. Secondly, opioid peptides can be produced by immune cells such as lymphocytes and monocytes or macrophages (Smith *et al.*, 1985; Lolait *et al.*, 1986; Sibinga & Goldstein, 1988). Furthermore, it has recently been demonstrated that preproenkephalin mRNA is present in a mast cell line (Martin *et al.*, 1987). These data may point to a local production of opioid peptides in airway inflammation, which is common in asthma.

References

- AMIR, S. (1982). Opiate antagonists improve survival in anaphylactic shock. *Eur. J. Pharmacol.* **80**, 161–162.
- BADAWY, A.A.-B., EVANS, M., PUNJANI, N.F. & MORGAN, C.J. (1983). Does naloxone always act as an opiate antagonist? *Life Sci.* **33**, Suppl. I, 739–742.
- BARNES, P.M., BELVISI, M.G. & ROGERS, D.F. (1990). Modulation of neurogenic inflammation: novel approaches to inflammatory disease. *Trends Pharmacol. Sci.*, **11**, 185–189.
- BIENENSTOCK, J. (1988). An update on mast cell heterogeneity. *J. Allergy Clin. Immunol.* **81**, 763–769.
- DE WIED, D. & JOLLES, J. (1982). Neuropeptides derived from pro-opiocortin: behavioral, physiological, and neurochemical effects. *Physiol. Rev.* **62**, 976–1059.
- DUSSER, D.J., JACOBY, D.B., DJOKIC, T.D., RUBINSTEIN, I., BORSON, D.B. & NADEL, J.A. (1989). Virus induces airway hyper-responsiveness to tachykinins — role of neutral endopeptidase. *J. Appl. Physiol.* **67**, 1504–1511.
- ERDOS, E.G. & SKIDGEL, R.A. (1989). Neutral endopeptidase 24.11 (enkephalinase) and related regulators of peptide hormones. *FASEB J.* **3**, 145–151.
- FISCHER, E.G. (1988). Opioid peptides modulate immune functions. A review. *Immunopharmacol. Immunotoxicol.* **10**, 265–326.
- KARLSSON, J.A., LANNER, A.S. & PERSSON, C.G.A. (1990). Airway opioid receptors mediate inhibition of cough and reflex bronchoconstriction in guinea pigs. *J. Pharmacol. Exp. Ther.* **252**, 863–868.
- KITAMURA, Y. (1989). Heterogeneity of mast cells and phenotypic change between subpopulations. *Annu. Rev. Immunol.* **7**, 59–76.
- LOLAIT, S.J., CLEMENTS, J.A., MARKWICK, A.J., CHENG, C., McNALLY, M., SMITH, A.I. & FUNDER, J.W. (1986). Pro-opiomelanocortin messenger ribonucleic acid and posttranslational processing of beta endorphin in spleen macrophages. *J. Clin. Invest.* **77**, 1776–1779.
- MARTIN, J., PRYSTOWSKI, M.B. & ANGELETTI, R.H. (1987). Pre-proenkephalin mRNA in T-cells, macrophages, and mast cells. *J. Neurosci. Res.* **18**, 82–87.
- MEDIRATTA, P.K., DAS, N., GUPTA, V.S. & SEN, P. (1988). Modulation of humoral immune responses by endogenous opioids. *J. Allergy Clin. Immunol.* **81**, 27–32.
- MIADONNA, A., LEGGIERI, E., TEDESCHI, A., LORINI, M., FROLDI, M. & ZANUSSI, C. (1988). Study of the effect of some neuropeptides and endogenous opioid peptides on in vitro histamine release from human lung mast cells and peripheral blood basophils. *Agents Actions*, **25**, 11–16.
- OLSON, G.A., OLSON, R.D. & KASTIN, A.J. (1989). Endogenous opiates: 1987. *Peptides*, **10**, 205–236.
- POPA, V. & RIENTS, P. (1989). The effect of inhaled naloxone on resting bronchial tone and exercise-induced asthma. *Am. Rev. Respir. Dis.* **139**, 702–709.
- ROGERS, D.F. & BARNES, P.J. (1989a). Opioid inhibition of neurally mediated mucus secretion in human bronchi. *Lancet*, **i**, 930–932.
- ROGERS, D.F. & BARNES, P.J. (1989b). Opioid inhibition of non-adrenergic, non-cholinergic neural control of mucus secretion in human bronchi in vitro. *Am. Rev. Respir. Dis.* **139**, A411.
- SHANAHAN, F., LEE, T.D.G., BIENENSTOCK, J. & BEFUS, A.D. (1984). The influence of endorphins on peritoneal and mucosal mast cell secretion. *J. Allergy Clin. Immunol.* **74**, 499–504.
- SHIMOSEGAWA, T., FODA, H.D. & SAID, S.I. (1989). Opioid peptides in guinea pig and rat lungs: their sources and colocalization with VIP and PHI. *Am. Rev. Respir. Dis.* **139**, Suppl., A470.
- SIBINGA, N.E.S. & GOLDSTEIN, A. (1988). Opioid peptides and opioid receptors in cells of the immune system. *Annu. Rev. Immunol.* **6**, 219–249.
- SMITH, E. M., HARBOUR-MCMENAMIN, D. & BLALOCK, J.E. (1985). Lymphocyte production of endorphins and endorphin-mediated immunoregulatory activity. *J. Immunol.* **135**, 779–782.

- SWAYNOK, J., PINSKY, C. & LABELLA, F.S. (1979). On the specificity of naloxone as an opiate antagonist. *Life Sci.*, **25**, 1621–1632.
- SYDBOM, A. (1988). Characteristics of beta-endorphin-induced histamine release from rat serosal mast cells. Comparison with neurotensin, dynorphin and compound 48/80. *Naunyn Schmiedeberg's Arch. Pharmacol.*, **338**, 567–572.

- VAN OOSTERHOUT, A.J.M. & NIJKAMP, F.P. (1986). Anterior hypothalamic lesions decrease anaphylactic contractions in guinea pig trachea in vitro by reducing histamine and LTC₄ reactivity. *Int. J. Immunopharmacol.*, **8**, 975–983.

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