Neurokinin A-induced contraction of guinea-pig isolated trachea: potentiation by hepoxilins

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1 Hepoxilin A₃ (8R and 8S isomers) (HxA₃), hepoxilin A₃-C (8R and 8S isomers) (HxA₃-C) and trioxilin A₃ (8S isomer) (TrXA₃, the stable derivative of HxA₃) were tested for their effects on helicoidal strips of guinea-pig isolated tracheae.

2 None of the compounds (10^{-9} – 10^{-6} M) tested had a direct effect on resting tension of trachea.

3 HxA₃ (8S) and HxA₃-C (8R) (10^{-8} M) produced a significant leftward shift of the log concentration-response curves to neurokinin A (NKA) (EC₅₀ (nM), control = 29.0 ± 2.8 , HxA₃ (8S) = 21.7 ± 3.7 , HxA₃-C (8R) = 13.8 ± 3.8 , $n = 6$ for each). Also the maximal response to NKA was significantly increased when the tissues were exposed to these hepoxilins (% of the maximal response to NKA, control = 100, HxA₃ (8S) = 114.5 ± 5.3 , HxA₃-C (8R) = 139.0 ± 6.2 , $n = 6$ for each). The threshold concentrations for both hepoxilins was 10^{-8} M and their effects were dose-related.

4 Stereochemical specificity was observed. The 8S-isomer of HxA₃ was active in potentiating the NKA-induced contraction of the trachea while the 8R isomer was inactive. In contrast, the 8R isomer of HxA₃-C was active while the 8S isomer was inactive. The trihydroxy metabolite of the active isomer of HxA₃ (8S), i.e. TrXA₃ (8S) (10^{-6} M), was inactive in potentiating the NKA-induced contraction of the trachea.

5 It is concluded that hepoxilins sensitize the guinea-pig isolated trachea to the potent bronchoconstrictor, NKA.

Keywords: Hepoxilin A₃; hepoxilin A₃-C; contraction; guinea-pig trachea; potentiation; neurokinin A

Introduction

Neurokinin A (NKA) belongs to a family of peptides called tachykinins. Three distinct tachykinin receptors denoted as neurokinin₁ (NK₁), NK₂ and NK₃ have been characterized by pharmacological and radioligand binding studies. These receptors are widely distributed in mammalian tissues and interact differently with the natural tachykinins, substance P (SP) being the preferential agonist for the NK₁ receptor, NKA for the NK₂ receptor and neurokinin B (NKB) for the NK₃ receptor (Regoli *et al.*, 1988; Helke *et al.*, 1990).

Tachykinins have been implicated in the non-adrenergic and non-cholinergic nervous system which exerts potent control of airways (Naline *et al.*, 1989). SP and NKA have been co-localized in a population of primary sensory C-fibres which have peripheral branches in various tissues including the respiratory tract (Hua *et al.*, 1985; Lundberg & Saria, 1987). Local release of SP and/or NKA from sensory fibres seems to play an important role in neurogenic inflammation (vasodilatation and plasma protein extravasation), airway oedema and bronchoconstriction (Hua *et al.*, 1984; 1985; Lundberg *et al.*, 1983a,b). NKA was more potent than SP and other tachykinins in causing bronchoconstriction *in vivo* in the guinea-pig (Hua *et al.*, 1984; Martling *et al.*, 1987a), rat (Joos *et al.*, 1986), sheep (Abraham *et al.*, 1991) and man (Evans *et al.*, 1988). Similarly, NKA appears to be particularly active on human isolated bronchi (Martling *et al.*, 1987b; Naline *et al.*, 1989) and on the guinea-pig isolated trachea which has been particularly useful in understanding the mechanisms that could control airway narrowing (Uchida *et al.*, 1987; Devillier *et al.*, 1988).

The enhancement of vascular permeability and stimulation of mucus secretion are also important physiological effects that contribute to airway narrowing. A variety of mediators such as eicosanoids are released during these physiological processes that lead to an inflammatory reaction. Among arachidonic acid metabolites studied, leukotrienes have received a lot of attention because they cause airway contraction both *in vivo* and *in vitro* (Hedqvist *et al.*, 1980). Also leukotrienes C₄ and D₄ promote plasma leakage (Dahlén *et al.*, 1981) and leukotriene B₄ is a potent chemokinetic and chemotactic agent released from polymorphonuclear leukocytes (Ford-Hutchinson *et al.*, 1980) all contributing to the process of inflammation. Our recent studies have demonstrated that a particular group of lipoxygenase metabolites, hepoxilins, also play a significant role in the modulation of the physiological events that take place during the process of inflammation (Dho *et al.*, 1990; Laneuville *et al.*, 1991a,b; 1992). Two hepoxilins have been isolated, i.e. 8-hydroxy-11,12-epoxy eicosa-5Z, 10E, 14Z-trienoic acid (hepoxilin A₃) and 10-hydroxy-11,12-epoxy eicosa-5Z, 8Z, 14Z-trienoic acid (hepoxilin B₃); these metabolites are formed from the 12-lipoxygenase product, 12-HPETE (Pace-Asciak *et al.*, 1983; Pace-Asciak & Martin, 1984). Biological activities of the hepoxilins relate to the modulation of vascular tone, the increase of vascular permeability in the skin and activation of human neutrophils. Based on these studies we investigated their possible role in the modulation of muscular tone in the guinea-pig isolated trachea.

Methods

Preparation of tracheal smooth muscle

Male guinea pigs (purchased from Charles River, St-Constant, Quebec, Canada) weighing 300–450 g were killed

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by stunning and exsanguination through sectioning of the carotid arteries. The trachea was rapidly removed and placed in Krebs solution (composition in mM: NaCl 118.1, KCl 4.7, KH_2PO_4 1.2, NaHCO_3 25.0, MgSO_4 1.2, CaCl_2 2.5, D-glucose 5.5) kept at 37°C and continuously gassed with 95% O_2 , 5% CO_2 mixture. Helicoidal strips were cut from the trachea according to Constantine (1965), and mounted in organ baths filled with 10 ml Krebs solution maintained at 37°C and oxygenated. The lower end of the tracheal strip was attached to a glass hook at the base of the chamber by a loop of silk thread. The upper end was attached in the same manner to a force transducer (Grass FT.03C) for continuous recording of isometric tension by a Grass polygraph (model 79D). Each trachea was cut into four strips of equal dimensions; 4 mm wide by 25 mm long. Tissues were stretched at a tension of 3.0 g and equilibrated for 90 min during which time the bath solution was replaced every 15 min.

Effects of hepoxilins on neurokinin A-induced contraction of the trachea

Preliminary experiments showed that the addition of hepoxilins to the chamber at a final concentration of up to 10^{-6} M failed to cause muscle contraction or to relax NKA precontracted guinea-pig tracheal strips. To determine whether hepoxilins alter the NKA-mediated contraction, the response to NKA was first tested and its stability and reproducibility was established through three consecutive concentration-response curves. A period of 10 min was left between each dose-response curve which was constructed by cumulative addition of NKA. As the dose-response curves were not significantly different from each other, the third curve was used as the preadministration (control) value. Hepoxilins were then added and 45 min later, a concentration-response curve to NKA was obtained. Cumulative dose-responses to each hepoxilin (10^{-9} , 10^{-8} , 10^{-7} , and 10^{-6} M) were carried out on the same preparations. No measurements were made of reversibility of effect on wash-out of hepoxilins. Only one hepoxilin was tested on each preparation. In control experiments, the effect of the vehicle, dimethyl sulphoxide (DMSO, 13 mM) on NKA-induced contraction was assessed.

Expression of results and statistical analysis

Contractions are expressed as the percentage of the maximal response to NKA obtained before the addition of hepoxilins. Sensitivity to NKA in the trachea was expressed as EC_{20} and EC_{50} values, EC_{20} being the concentration of agonist (NKA) required to give 20% of maximal response of the trachea, EC_{50} representing half-maximal response of agonist. EC_{50} values were obtained by logit/log regression analysis of each individual preparation and averaged. Results are expressed as mean $\pm$ s.e.mean of six separate experiments. The differences between the values, obtained in the same strip, were tested for significance by use of Student's *t* test for paired observations. Probabilities (*P*) smaller than 0.05 were considered to be significant.

Drugs

Neurokinin A (Institut Armand Frappier, Laval, Canada) was dissolved in saline (NaCl 0.9%) to give a stock solution of 13 nM to 13 μM . NKA doses were added in a volume of 100 μl to the bath. Authentic hepoxilins prepared chemically were kindly provided by Prof. E.J. Corey (Harvard University, Cambridge, U.S.A.); HxA_3 and TrXA_3 were used as the methyl ester forms and dissolved in DMSO (Calbiochem, Toronto, Canada) and $\text{HxA}_3\text{-C}$ was dissolved in a mixture of phosphate buffer and ethanol (4:1) (Corey & Su, 1984; 1990). Purity was greater than 95% as judged by thin layer chromatography and high performance liquid chromatography. Hepoxilins were diluted with Krebs solution to a final concentration of DMSO or ethanol not higher than 0.02%.

Results

Potentialiation by hepoxilins of the neurokinin A-induced contraction of the trachea

The contraction of the isolated trachea to NKA consisted of slow increase in tension which persisted at a plateau level as long as the peptide was kept in contact with the tissue (Figure 1). The addition of NKA caused concentration-dependent contraction of the guinea-pig trachea (Figure 2). The minimum concentration of NKA needed to evoke a detectable increase in tension was >0.13 nM (Figure 1) and the sensitivity of the tissues as calculated by the EC_{50} varied between 15.9–35.9 nM (Table 1) which were comparable to earlier values obtained on the same preparation (Dion *et al.*, 1987). Tension of the trachea returned to the resting level when the bath fluid was replaced with fresh Krebs buffer. The level of contraction of the trachea obtained by the addition of NKA remained unaltered both in its sensitivity and in its maximal response to NKA in the presence of the vehicle of hepoxilins, DMSO (Table 1). The dose-response curves to NKA were quite reproducible throughout the experiments. A series of experiments was conducted over a period of 4 h where a dose-response curve to NKA was performed every 65 min. The EC_{50} for the 5 curves generated and the maximal contraction to NKA were not significantly different from each other reflecting the stability of the preparation in its response to NKA throughout the experiment (Table 1). Typical variations around the maximal response to NKA within a series of dose-responses on one preparation were 101.1 ± 2.0 ($n = 6$).

Figure 1 shows tracings of cumulative dose-dependent contractions of the trachea to NKA before and after the administration of vehicle (DMSO) and 10^{-8} M concentration of HxA_3 (8S). The 8S isomer of HxA_3 significantly reduced the threshold concentration at which NKA caused a contraction, while the tissue was slightly contracted by the addition of 1.43 nM NKA, it contracted much more when the same dose of NKA (1.43 nM) was added in the presence of HxA_3 (8S) 10^{-8} M (Table 1). It should also be noted that the maximal contraction of the trachea obtained with 366.5 nM NKA reached 114.5 to 121.9% of control in the presence of 10^{-8} to 10^{-6} M (8S) (Table 1). The cumulative dose-response curve to NKA was significantly shifted to the left in the presence of HxA_3 (8S) (Figure 2a) and the sensitivity of the tissues (EC_{50} values) was markedly enhanced (Table 1). EC_{20} values are also shown giving a measure of threshold concentrations of NKA.

The stable trihydroxy metabolite of HxA_3 (8S), i.e. TrXA_3 (8S), added at a concentration where HxA_3 (8S) significantly potentiated the contraction of the trachea to NKA (10^{-6} M) was inactive in modifying the NKA response (Table 1). This suggests that the effects of HxA_3 (8S) are not mediated by its trihydroxy metabolite, TrXA_3 (8S).

The effects of the glutathionyl conjugate of hepoxilin A_3 , $\text{HxA}_3\text{-C}$, on the potentiation of NKA-induced contraction of the guinea-pig trachea were more pronounced than those of its precursor HxA_3 . Figure 1 compares the effects of an equal dose of both HxA_3 (8S) and $\text{HxA}_3\text{-C}$ (8R) at 10^{-8} M concentration on the contraction of the trachea exposed to NKA. Note that the contraction of the trachea obtained with 1.43 nM NKA was much larger in the presence of $\text{HxA}_3\text{-C}$ (8R) ($38.9 \pm 7.5\%$) than it was in the presence of HxA_3 (8S) ($11.3 \pm 3.5\%$). The maximal response to NKA was increased to a greater extent when the trachea was exposed to $\text{HxA}_3\text{-C}$ (8R) 10^{-8} M ($134.0 \pm 6.2\%$) as compared to HxA_3 (8S) 10^{-8} M ($114.5 \pm 5.3\%$) (Table 1). Higher concentrations (10^{-7} and 10^{-6} M) of hepoxilins $\text{A}_3\text{-C}$ were also tested and the effects measured were proportionately greater from the preadministration values. The dose-response curve to NKA was shifted further to the left, as reflected by a lower EC_{50} value, when higher concentrations of $\text{HxA}_3\text{-C}$ (8R) were added (Table 1).

Figure 1 Effect of hepixilins on contractions of strips of guinea-pig trachea induced by cumulative doses of neurokinin A (NKA). Three successive complete dose-response curves to NKA (0.13 to 366.54 nM) were obtained (the last one is shown here in each panel) followed by tissue washout. Subsequent application was made of dimethylsulphoxide vehicle (a), hepixilin A₃ (8S) (HxA₃ (8S)) (b) or HxA₃-C (8R) (c) followed 45 min later by another cumulative dose-response curve to NKA. Note that in the presence of HxA₃ (8S) (b) or HxA₃-C (8R) (c), the contraction of the tissue to NKA was enhanced so that significant contraction takes place at 1.43 nM NKA.

Table 1 EC₂₀ and EC₅₀ values for neurokinin A (NKA) in the presence and absence of dimethylsulphoxide (DMSO, 13 mM) and of the 8-enantiomers of HxA₃, TrXA₃ and HxA₃-C in the guinea-pig trachea

Treatment	EC ₂₀ (nM)	EC ₅₀ (nM)	Maximal response to NKA
Preadministration			
DMSO after 20 min	8.6 ± 2.6	29.0 ± 2.8	100
after 85 min	10.0 ± 3.7	31.4 ± 3.7	103.5 ± 4.1
after 150 min	9.6 ± 2.8	28.7 ± 3.0	104.2 ± 3.3
after 215 min	9.1 ± 4.8	29.9 ± 2.5	101.6 ± 2.8
Preadministration	9.3 ± 5.2	31.9 ± 5.5	105.4 ± 7.2
HxA ₃ (8S) Me 10 ⁻⁹ M	7.5 ± 3.0	30.4 ± 4.0	100
10 ⁻⁸ M	6.3 ± 3.5	21.8 ± 3.9*	107.7 ± 2.3
10 ⁻⁷ M	4.8 ± 2.9	21.7 ± 3.7*	114.5 ± 5.3*
10 ⁻⁶ M	3.8 ± 2.7*	14.0 ± 4.9**	120.5 ± 7.6**
Preadministration	4.0 ± 2.2*	13.5 ± 4.6***	121.9 ± 10.5**
HxA ₃ (8R) Me 10 ⁻⁸ M	8.8 ± 3.4	35.9 ± 2.8	100
10 ⁻⁶ M	8.8 ± 2.9	36.1 ± 6.7	94.7 ± 4.3
Preadministration	7.0 ± 3.4	32.6 ± 3.4	93.1 ± 5.0
TrXA ₃ (8S) Me 10 ⁻⁶ M	6.9 ± 3.5	33.2 ± 5.3	100
10 ⁻⁶ M	8.4 ± 4.0	31.1 ± 6.5	105.1 ± 6.4
Preadministration	4.0 ± 2.9	15.9 ± 5.7	100
HxA ₃ -C (8S) 10 ⁻⁶ M	6.5 ± 3.1	20.6 ± 4.8	106.5 ± 10.7
Preadministration	5.0 ± 3.5	26.4 ± 6.3	100
HxA ₃ -C (8R) 10 ⁻⁹ M	10.0 ± 3.6*	21.4 ± 5.0	99.7 ± 6.6
10 ⁻⁸ M	3.6 ± 2.0	13.8 ± 3.8**	134.0 ± 6.2***
10 ⁻⁷ M	3.0 ± 3.0*	11.4 ± 4.1***	142.7 ± 7.1***
10 ⁻⁶ M	2.2 ± 1.8**	6.3 ± 4.8***	170.6 ± 7.6***

HxA₃: hepixilin A₃; TrXA₃: trioxilin A₃

Values represent the mean ± s.e.mean for six separate tissue preparations from six different animals. Each treatment was compared to the preadministration value for each tissue by use of Student's *t* test for paired samples.

P* < 0.05; *P* < 0.01 and ****P* < 0.005 with respect to corresponding control values.

Figure 2 Dose-response curves to neurokinin A (NKA, $n = 6$) in the presence of (a) hepoxinil A_3 (8S) (HxA_3 (8S)), (b) HxA_3 (8R), (c) HxA_3 -C (8S), (d) HxA_3 -C (8R). Note the leftward shift to NKA in the presence of 10^{-6} M concentration of HxA_3 (8S) or HxA_3 -C (8R). Also note the increase of the maximal response to NKA in the presence of HxA_3 (8S) or HxA_3 -C (8R). In (a): ($\diamond$) HxA_3 (8S) Me, 10^{-7} M; ($\circ$) HxA_3 (8S) Me, 10^{-9} M; ($\blacksquare$) control (preadministration). In (b): ($\square$) HxA_3 (8R) Me, 10^{-6} M; ($\blacksquare$) control (preadministration). In (c): ($\circ$) HxA_3 -C (8S), 10^{-6} M; ($\blacksquare$) control (preadministration). In (d): HxA_3 -C (8R), ($\circ$) 10^{-6} M; ($\diamond$) 10^{-8} M; ($\square$) 10^{-9} M; ($\blacksquare$) control (preadministration).

Stereospecificity of the hepoxinil-induced potentiation in the contraction of the trachea to neurokinin A

In contrast to the 8S isomer, the 8R isomer of HxA_3 was essentially inactive in potentiating the NKA-induced contraction of the trachea at a concentration as high as 10^{-6} M (Figure 2a,b, Table 1). Conversely, the 8R isomer of the glutathione conjugate of HxA_3 , i.e. HxA_3 -C, was active but not the 8S isomer (Figure 2c,d, Table 1).

Discussion

As reported by Dion *et al.* (1987), the tracheal smooth muscle contracted in a concentration-dependent manner to NKA and is a classical pharmacological preparation used to study the NK_2 receptor. In contrast to the human trachea where only the NK_2 receptor is present (Naline *et al.*, 1989), in guinea-pig trachea, contractile responses to tachykinins can be induced by activation of both NK_1 and NK_2 receptors (Devillier *et al.*, 1988; Maggi *et al.*, 1991; Ireland *et al.*, 1991).

From the results described here, we conclude that (1) hepoxilins are not active on their own in changing the muscular tone of the trachea; (2) NKA-induced contraction of the guinea-pig trachea is potentiated by hepoxilins; (3) HxA_3 and HxA_3 -C decrease both the EC_{20} and EC_{50} and increase the maximal contraction to NKA; (4) the glutathione conjugate of hepoxinil A_3 , HxA_3 -C, is more active than its precursor, HxA_3 , and finally; (5) hepoxinil-induced potentiation is stereospecific.

We have recently shown that hepoxilins potentiate the contractile effects of noradrenaline on the rat isolated thoracic aorta and portal vein (Laneuville *et al.*, 1992). Although the threshold doses of hepoxilins described here are identical, 10^{-8} M, the following differences were observed. Firstly, the maximal contraction to the constrictor agent was unchanged in the blood vessels while it was significantly increased in the tracheal preparation, and secondly, the potentiation of contractions by hepoxilins in blood vessels showed a narrow dose-response (one log dose) in comparison to the wider response range seen for the trachea. Whether these differences can be explained by the fact that a different preparation of smooth muscles was used or by the fact that a different constrictor agent was used remains to be investigated.

The stereospecificity of action of hepoxilins described here is similar to that previously reported for the vascular preparation, i.e. the 8S isomer of HxA_3 is active while the 8R isomer is inactive and the 8R isomer of HxA_3 -C is active while the 8S isomer is inactive (Laneuville *et al.*, 1992). It has been shown in human neutrophils that the hepoxinil-mediated actions on intracellular calcium and second messenger release are inhibited by pertussis toxin, suggesting that hepoxinil action may be under receptor control coupled to G-protein activation (Dho *et al.*, 1990; Nigam *et al.*, 1990).

Hepoxilins are recent messengers identified in cellular communication. The present data suggest that in addition to the 5-lipoxygenase products, leukotrienes, the 12-lipoxygenase pathway may also be important in modulating the control of inflammation and contraction in the trachea through formation of the hepoxilins. However, we do not know whether the hepoxinil effect is general to other bronchoconstrictors or whether the hepoxilins affect only the NKA-induced contraction.

No information is currently available on the metabolism of hepoxilins by the trachea. The present experiments showing stereospecificity of action of the hepoxilins suggest that the hepoxilins are either stable during the 45 min exposure of the tissue or that the hepoxilins induced a time-dependent cellular change which facilitates contraction by NKA. The fact that considerable time (longer than 30 min) is required to elicit a potentiation of the NKA-induced contractile response

suggests that hepoxilins may have an effect on protein synthesis. Further studies are needed to define the mechanism involved. An overproduction of hepoxilins may sensitize airways to substance P and neurokinin A (NKA) and other bronchoconstrictors. Thus, elucidation of this mechanism may provide new insight in the control of airway

function and into the pathophysiology of asthma and bronchial hyperreactivity.

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References

- ABRAHAM, W.M., AHMED, A., CORTES, A., SOLER, M., FARMER, S.G., BAUGH, L.E. & HARBESON, S.L. (1991). Airway effects of inhaled bradykinin, substance P, and neurokinin A in sheep. *J. Allergy Clin. Immunol.*, **87**, 557–564.
- CONSTANTINE, J.W. (1965). The spirally cut tracheal strip preparation. *J. Pharm. Pharmacol.*, **17**, 384–385.
- COREY, E.J. & SU, W.-G. (1984). Total synthesis of biologically active metabolites of arachidonic acid. Two 8-hydroxy-11,12(S,S)-epoxy-eicosa-5(Z),9(E),14(Z)-trienoic acids. *Tetrahedr. Lett.*, **25**, 5119–5122.
- COREY, E.J. & SU, W.-G. (1990). (8R)- and (8S)-hepoxilin A₃. Assignment of configuration and conversion to biologically active conjugates with glutathione. *Tetrahedr. Lett.*, **31**, 2113–2116.
- DAHLÉN, S.E., BJÖRK, J., HEDQVIST, P., ARFORS, K.E., HAMMARSTRÖM, S., LINDGREN, J.A. & SAMUELSSON, B. (1981). Leukotrienes promote plasma leakage and leukocyte adhesion in postcapillary venules: *in vivo* effects with relevance to the acute inflammatory response. *Proc. Natl. Acad. Sci. U.S.A.*, **78**, 3887–3891.
- DEVILLIER, P., ADVENIER, C., DRAPEAU, G., MARSAC, J. & REGOLI, D. (1988). Comparison of the effects of epithelium removal and of an enkephalinase inhibitor on the neurokinin-induced contractions of guinea-pig isolated trachea. *Br. J. Pharmacol.*, **94**, 675–684.
- DHO, S., GRINSTEIN, S., COREY, E.J., SU, W.-G. & PACE-ASCIAC, C.R. (1990). Hepoxilin A₃ induces changes in cytosolic calcium, intracellular pH and membrane potential in human neutrophils. *Biochem. J.*, **266**, 63–68.
- DION, S., D'ORLÉANS-JUSTE, P., DRAPEAU, G., RHALEB, N.E., ROUISSI, N., TOUSIGNANT, C. & REGOLI, D. (1987). Characterization of neurokinin receptors in various isolated organs by the use of selective agonists. *Life Sci.*, **41**, 2269–2278.
- EVANS, T.W., DIXON, C.M., CLARKE, B., CONRADSON, T.-B. & BARNES, P.J. (1988). Comparison of neurokinin A and Substance P on cardiovascular and airway function in man. *Br. J. Clin. Pharmacol.*, **25**, 273–275.
- FORD-HUTCHINSON, A.W., BRAY, M.A., DOIG, M.V., SHIPLEY, M.E. & SMITH, M.J.H. (1980). Leukotriene B₄, a potent chemokinetic and aggregating substance released from polymorphonuclear leukocytes. *Nature*, **286**, 264–265.
- HEDQVIST, P., DAHLÉN, S.E., GUSTAFSSON, L., HAMMARSTRÖM, S. & SAMUELSSON, B. (1980). Biological profile of leukotrienes C₄ and D₄. *Acta Physiol. Scand.*, **110**, 331–333.
- HELKE, C.J., KRAUSE, J.E., MANTYH, P.W., COUTURE, R. & BANNON, M.J. (1990). Diversity in mammalian tachykinin peptidergic neurons: multiple peptides, receptors, and regulatory mechanisms. *FASEB J.*, **4**, 1606–1615.
- HUA, X.-Y., LUNDBERG, J.M., THEODORSSON-NORHEIM, E. & BRODIN, E. (1984). Comparison of cardiovascular and bronchoconstrictor effects of substance P, substance K and other tachykinins. *Naunyn Schmiedeberg's Arch. Pharmacol.*, **328**, 196–201.
- HUA, X.-Y., THEODORSSON-NORHEIM, E., BRODIN, E., LUNDBERG, J.M. & HOCKFELT, T. (1985). Multiple tachykinins (neurokinin A, neuropeptide K and substance P) in capsaicin-sensitive sensory neurons in the guinea pig. *Regul. Pept.*, **13**, 1–19.
- IRELAND, S.J., BAILEY, F., COOK, A., HAGAN, R.M., JORDAN, C.C. & STEPHENS-SMITH, M.L. (1991). Receptors mediating tachykinin-induced contractile responses in guinea-pig trachea. *Br. J. Pharmacol.*, **103**, 1463–1469.
- JOOS, G., KIPS, J., PAUWELS, R. & VAN DER STRAETEN, M. (1986). The effect of tachykinins on the conducting airways of the rat. *Arch. Int. Pharmacodyn.*, **280**, 176–190.
- LANEUVILLE, O., COREY, E.J., COUTURE, R. & PACE-ASCIAC, C.R. (1991a). Hepoxilin A₃ increases vascular permeability in the rat skin. *Eicosanoids*, **4**, 95–97.
- LANEUVILLE, O., COREY, E.J., COUTURE, R. & PACE-ASCIAC, C.R. (1991b). Hepoxilin A₃ (HxA₃) is formed by the rat aorta and is metabolized into HxA₃-C, a glutathione conjugate. *Biochim. Biophys. Acta*, **1084**, 60–68.
- LANEUVILLE, O., COUTURE, R. & PACE-ASCIAC, C.R. (1992). Hepoxilins sensitize blood vessels to noradrenaline - stereospecificity of action. *Br. J. Pharmacol.*, **105**, 297–304.
- LUNDBERG, J.M., BRODIN, E. & SARIA, A. (1983a). Effects and distribution of vagal capsaicin-sensitive substance P neurons with special reference to the trachea and lungs. *Acta Physiol. Scand.*, **119**, 243–252.
- LUNDBERG, J.M., MARTLING, C.R., SARIA, A., FOLKERS, K. & ROSELL, S. (1983b). Cigarette smoke-induced airway oedema due to activation of capsaicin-sensitive vagal afferents and substance P release. *Neuroscience*, **10**, 1361–1368.
- LUNDBERG, J.M. & SARIA, A. (1987). Polypeptide-containing neurons in airway smooth muscle. *Annu. Rev. Physiol.*, **49**, 557–572.
- MAGGI, C.A., GIULIANI, S., BALLATI, L., LECCI, A., MANZINI, S., PATACCHINI, R., RENZETTI, A.R., ROVERO, P., QUARTARA, L. & GIACHETTI, A. (1991). *In vivo* evidence for tachykinergic transmission using a new NK-2 receptor-selective antagonist, MEN 10,376. *J. Pharmacol. Exp. Ther.*, **257**, 1172–1178.
- MARTLING, C.R., THEODORSSON-NORHEIM, E., NORHEIM, I. & LUNDBERG, J.M. (1987a). Bronchoconstrictor and hypotensive effects in relation to pharmacokinetics of tachykinins in the guinea pig - evidence for extraneuronal cleavage of neuropeptide K to neurokinin A. *Naunyn Schmiedeberg's Arch. Pharmacol.*, **336**, 183–189.
- MARTLING, C.R., THEODORSSON-NORHEIM, E. & LUNDBERG, J.M. (1987b). Occurrence and effects of multiple tachykinins; substance P, neurokinin A and neuropeptide K in human lower airways. *Life Sci.*, **40**, 1633–1643.
- NALINE, E., DEVILLIER, P., DRAPEAU, G., TOTY, L., BAKDACH, H., REGOLI, D. & ADVENIER, C. (1989). Characterization of neurokinin effects and receptor selectivity in human isolated bronchi. *Am. Rev. Respir. Dis.*, **140**, 679–686.
- NIGAM, S., NODES, S., CICHON, G., COREY, E.J. & PACE-ASCIAC, C.R. (1990). Receptor-mediated action of hepoxilin A₃ releases diacylglycerol and arachidonic acid from human neutrophils. *Biochem. Biophys. Res. Commun.*, **171**, 944–948.
- PACE-ASCIAC, C.R., GRANSTROM, E. & SAMUELSSON, B. (1983). Arachidonic acid epoxides: isolation and structure of two hydroxy epoxide intermediates in the formation of 8,11,12- and 10,11,12-trihydroxyeicosatrienoic acids. *J. Biol. Chem.*, **258**, 6835–6840.
- PACE-ASCIAC, C.R. & MARTIN, J.M. (1984). Hepoxilin, a new family of insulin secretagogues formed by intact rat pancreatic islets. *Prostaglandins Leukotrienes Med.*, **16**, 173–180.
- REGOLI, D., DRAPEAU, G., DION, S. & COUTURE, R. (1988). New selective agonists for neurokinin receptors: pharmacological tools for receptor characterization. *Trends Pharmacol. Sci.*, **9**, 290–295.
- UCHIDA, Y., NOMURA, A., OHTSUKA, M., HASEGAWA, S., GOTO, K., KIMURA, S., SUGITA, Y. & UCHIYAMA, Y. (1987). Neurokinin A as a potent bronchoconstrictor. *Am. Rev. Respir. Dis.*, **136**, 718–721.

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