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REVIEW

Kinins and respiratory tract diseases

A. Trifilieff, A. Da Silva, J-P. Gies

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ABSTRACT: Bradykinin and related kinins are peptidic hormones, formed in tissues and fluids during inflammation. Various functional sites have been proposed as mediators of the biological effects of kinins, including the B_1, B_2 and B_3 receptors. The existence of the B_1 and the B_2 receptor has largely been confirmed, whilst that of the B_3 receptor is controversial and needs further confirmation.

The role of bradykinin in the pathophysiology of asthma is not well understood, but bradykinin was proposed as a putative mediator of asthma, since asthmatic subjects are hyperresponsive to bradykinin, and since immunoreactive kinins are increased in the bronchoalveolar lavage fluids of asthmatic patients. Kinins could provoke bronchoconstriction by acting directly on smooth muscle and/or indirectly by their inflammatory properties. They may also contribute to the symptomatology of allergic and viral rhinitis, since they are the only mediators detected to date that are generated in nasal secretion during experimental and natural rhinovirus colds. Moreover, they can induce relevant symptoms when applied to airway mucosa.

It has also been proposed that coughing during treatment with angiotensin-converting enzyme (ACE) inhibitors is linked to the action of kinins, since ACE is able to degrade kinins, and since the effects of ACE inhibitors are reduced by kinin antagonists. Due to their mitogenic properties, kinins have been proposed to regulate lung carcinoma growth. Their action remains speculative, but some findings are of great interest in order to define their role in these pathologies.

Despite many studies in animals and in humans, the mode of action of kinins in airways is still poorly understood. The recent cloning and sequencing of the complementary deoxyribonucleic acid (cDNA) of a human B_2 receptor, and the availability of selective B_2 antagonists will be useful for defining more precisely the action of kinins on airways functions.

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Laboratoire de Neuroimmuno-pharmacologie-Pulmonaire, INSERM CJF-9105, Université Louis Pasteur-Strasbourg I, Faculté de pharmacie, Illkirch, France.

Correspondence: J-P. Gies
Laboratoire de Neuroimmuno-pharmacologie-Pulmonaire
INSERM CJF-9105
Université Louis Pasteur-Strasbourg I
Faculté de pharmacie
BP 24, 67401 Illkirch Cedex
France

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The history of kinins began in 1909 when ABELOUS and BARDIER [1] observed a transient fall in blood pressure in man after injection of fractions extracted from human urine. It was not until 40 yrs later that WERLE and BEREK [2] attributed the hypotensive effect of urine to the presence of an enzyme, named "kallikrein", which could release a substance from plasma which was capable of contracting smooth muscle. This generated material was named "kallidin". At the same time, ROCHA E SILVA *et al.* [3] coined the name "bradykinin" for a similar smooth muscle spasmogen liberated from plasma by the action of trypsin. Bradykinin was subsequently characterized as a nonapeptide [4] (table 1). The presence of kinins in the tracheobronchial tree [6], and their pharmacological properties, including the ability to increase vascular permeability and secretion of mucus, to cause vasodilatation or oedema, and to contract airway smooth muscle *in vivo* or *in vitro*, would suggest that these peptides are involved in inflammatory responses of the airway and in bronchial hyperresponsiveness.

Kinin receptor subtypes

Two receptor subtypes have been identified on the basis of the kinin receptors literature since 1980, when REGOLI and BARABÉ [7] proposed the classification of B_1 and B_2 kinin receptors based upon the relative bioassay potencies of a series of bradykinin analogues on vascular smooth muscle preparations. Two different patterns of agonist activities have been identified in the rabbit aorta (B_1 tissue), and in the rabbit jugular vein (B_2 tissue). In B_1 tissue, the agonist potencies are: des-Arg^{10}-kallidin > des-Arg^9-bradykinin = kallidin >> bradykinin; whereas in B_2 tissue the agonist potencies are: bradykinin = kallidin >>des-Arg^{10}-kallidin > des-Arg^9-bradykinin [7].

Table 1. – Structure and classification of kinin agonists and antagonists

Name	Structure	Classification
Bradykinin (BK)	Arg-Pro-Pro-Gly-Phe-Ser-Pro-Phe-Arg	Natural agonist
Kallidin (KD)	Lys-Arg-Pro-Pro-Gly-Phe-Ser-Pro-Phe-Arg	Natural agonist
Des-Arg^9-BK	Arg-Pro-Pro-Gly-Phe-Ser-Pro-Phe	Natural B_1 agonist
Des-Arg^{10}-KD	Lys-Arg-Pro-Pro-Gly-Phe-Ser-Pro-Phe	Natural B_1 agonist
Des-Arg^9-[Leu^8]-BK	Arg-Pro-Pro-Gly-Phe-Ser-Pro-Leu	Synthetic B_1 antagonist
[D-Phe^7]-BK	Arg-Pro-Pro-Gly-Phe-Ser-Phe-Phe-Arg	Synthetic B_2 antagonist
D-Arg^0[Hyp^3,D-Phe^7]-BK	Arg-Arg-Pro-Hyp-Gly-Phe-Ser-Phe-Phe-Arg	Synthetic B_2 antagonist
D-Arg^0[Hyp^3,Thi^{5,8},D-Phe^7]-BK	Arg-Arg-Pro-Hyp-Gly-Thi-Ser-Phe-Thi-Arg	Synthetic B_2 antagonist
D-Arg^0[Hyp^3,Leu^{5,8},D-Phe^7]-BK	Arg-Arg-Pro-Hyp-Gly-Leu-Ser-Phe-Leu-Arg	Synthetic B_2 antagonist
D-Arg^0[Hyp^3,Thi^5,D-Tic^7,Oic^8]-BK	Arg-Arg-Pro-Hyp-Gly-Thi-Ser-Tic-Oic-Arg	Synthetic B_2 antagonist
D-Arg^0[Hyp^3,Thi^5,D-Tic^7,Tic^8]-BK	Arg-Arg-Pro-Hyp-Gly-Thi-Ser-Tic-Tic-Arg	Synthetic B_2 antagonist
D-Arg^0[Hyp^3,D-HypE(*trans*-propyl)^7,Oic^8]-BK	Arg-Arg-Pro-Hyp-Gly-Phe-Ser-HypE (*trans*-propyl)Oic-Arg	Synthetic B_2 antagonist

Hyp: trans-4-hydroxyproline; Thi: β-(2-thienyl)-alanine; Tic: (1, 2, 3, 4-tetrahydroisoquinolin-3-yl-carbonyl); Oic: [(3aS, 7aS)-octahydroindol-2-yl-carbonyl]. Underlined residues are of the D-series. Modified from [5] with permission.

B_1 receptors

Bradykinin B_1 receptors are activated by the kinin metabolites, des-Arg-kinins, induced by kininase I. In addition, effects mediated *via* B_1 receptors can be competitively inhibited by the antagonist des-Arg^9-[Leu^8]-bradykinin (table 1). In general, B_1 receptors appear to be distributed predominantly in vascular smooth muscle [7], although non-vascular localization also exists [8]. Interestingly, B_1 responsiveness can be induced under inflammatory conditions or trauma [9, 10]. The mechanism by which this up-regulation of response to B_1 agonist occurs has not been entirely clarified, but may be due to *de novo* synthesis of B_1 receptors during incubation [9, 11], which may be mediated *via* the production of interleukin-1 [12].

B_2 receptors

Most of the actions of kinins are mediated *via* the occupancy of the B_2 receptors. Specific binding of radiolabelled bradykinin to a variety of tissues and cell populations has been demonstrated [13–18]. The affinity constant (K_d) for bradykinin binding has generally been reported to range from picomolar to nanomolar affinities, whilst maximum binding (B_{max}) varies from 10 to 230 fmol per mg of protein or per 10^6 cells. Ligand binding has been shown to correlate with physiological functions in many tissues [19–22]. With the availability of B_2 antagonists [23], a sizeable body of knowledge has been accumulated regarding the properties and regulation of these receptors. The major characteristics of these early B_2 antagonists is the replacement of proline^7 with a D-phenylalanine residue in the bradykinin structure (table 1). This modification has led to a series of compounds which antagonize the action of bradykinin in tissues thought to contain B_2 receptors. However, these analogues have several limitations, since they are partial agonists in various biological systems [24], and display some affinity for B_1 receptors following their degradation by kininase I [25]. Moreover, these antagonists show low affinity for B_2 receptors, at least two log units lower than kinin agonists, and can induce the release of histamine from mast cells [26] by acting on a G_i-like protein [27]. The most potent of the early series of B_2 antagonists is D-Arg^0[Hyp^3,Leu^{5,8},D-Phe^7]-bradykinin (table 1) [28]. Recently, LEMBECK *et al.* [29] have proposed new bradykinin B_2 receptor antagonists, in which unusual amino acids have been inserted. The most potent of these compounds, D-Arg^0[Hyp^3,Thi^5,D-Tic^7,Oic^8]-bradykinin (Hoe 140) (table 1), has been used in many biological assays [30–32]. Hoe 140 does not act as a partial agonist, is selective for B_2 receptors, has a similar affinity for B_2 receptors as bradykinin, and is resistant to enzyme cleavage. Therefore, Hoe 140 is likely to contribute to the investigation of the pathophysiological role of bradykinin. A derived compound, showing a similar binding affinity, has been proposed by FARMER *et al.* [33], D-Arg^0[Hyp^3,Thi^5,D-Tic^7,Tic^8]-bradykinin (NPC 16731) (table 1). These two B_2 antagonists, Hoe 140 and NPC 16731, are conformationally constrained in the backbone of the four C-terminal residues. On the basis of these conformational constraints, KYLE *et al.* [34] have proposed a series of bradykinin analogues, each having the generic primary sequences D-Arg^0-Arg^1-Pro^2-Hyp^3-Gly^4-Phe^5-Ser^6-X^7-Y^8-Arg^9, in which X and Y are chosen to obtain a β-turn. The most potent of these analogues has been obtained by the replacement of X-Y with the dipeptide N-acetyl-(D-4-hydroxyproline*trans*-propyl ether)-(Oic)-N'-methylamide. This compound, named NPC 17731, has the following sequence: D-Arg^0[Hyp^3,D-HypE(*trans*-propyl)^7,Oic^8]-BK (table 1).

Another route to developing more potent bradykinin receptor antagonists, has been to dimerize the early B_2 antagonist D-Arg^0[Hyp^3,D-Phe^7, Leu^8]-BK, in which the seryl^6 residue had been replaced with a cystyl residue to allow the dimerization performed with the thiol-specific homobifunctional cross-linking agent *bis*-maleimidohexane. The compound obtained (CP-0127) is an antagonist, approximately 50–100 times more potent than the parent compound, with pharmacological profiles comparable to those of Hoe 140 and NPC 17731 [35].

The earlier B_2 antagonists [23] have been used extensively *in vivo* and *in vitro*, and a complex picture of B_2 receptors is emerging [36]. Several studies have demonstrated the presence of multiple classes of binding sites in tissues or cells [13, 14, 18, 37], and the different effects of B_2 antagonists in several bioassays imply the multiplicity of bradykinin B_2 receptors [38, 39]. Moreover, the recent cloning of a B_2 bradykinin receptor complementary deoxyribonucleic acid (cDNA), and the tissue distribution of messenger ribonucleic acid (mRNA) encoding this receptor, suggest the possible existence of subtypes of B_2 receptors [40]. More recently, FARMER and co-workers [41, 42] have proposed a new pulmonary receptor subtype, referred to as B_3. But it must be kept in mind that kinins exert complex indirect effects, which might depend on independent receptor mechanisms which are not blocked by antagonists [43], such as the release of histamine from rat mast cells [27, 44], and the release of epinephrine from the adrenal medulla [45].

The cloning of the B_2 bradykinin rat receptor predicted a protein sequence of 366 amino acids having a molecular mass of 41,696 Da. Hydrophobicity analysis reveals seven putative transmembrane domains consistent with the structure observed in the G protein-coupled receptor family. Moreover, the sequence also contains three potential N-linked glycosylation sites in the predicted extracellular domain [40], suggesting that rat bradykinin B_2 receptor is a glycoprotein. Following the cloning of this rat B_2 receptor, HESSE *et al.* [46] have cloned and sequenced the cDNA of a B_2 receptor from a human lung fibroblasts cell line. This cDNA encodes a 364 amino acid protein, which also has the characteristics of a seven transmembrane domain G-protein coupled receptor. The predicted amino acid sequence is 81% identical to the rat receptor, and the three potential sites of glycosylation present in the rat receptor are all conserved in the human one, suggesting high identity between the two species. Analysis of the tissue distribution of the human B_2 receptor suggests that the highest level of receptor is in kidney, lung and uterus.

If cloning can determine the molecular weight and give structural information as to the carbohydrate moieties, it fails, however, to give any information as to the native form (*i.e.* glycosylated form). The production of anti-bradykinin antibodies would be useful to extend the knowledge of the bradykinin receptor. Anti-bradykinin antibodies might be regarded as a model structure for bradykinin receptors and can, therefore, be used as immunogens to produce antibodies which are cross-reactive with the bradykinin receptor. Since all bradykinin antibodies described to date cross-react with des-Arg^9-bradykinin [47], this anti-idiotypic approach cannot be applied specifically to the B_2 receptor. Recently, monoclonal anti-bradykinin antibodies have been described (MBK 1, MBK 2, MBK 3) [48]. MBK 1 appears to be specific for des-Arg^9-bradykinin, and could be correlated with the B_1 receptor system. MBK 3, which does not bind to des-Arg^9-bradykinin, has been correlated with the B_2 receptor system. MBK 2 did not conform to the profiles of any of the known kinin receptor systems. Therefore, MBK 3 was used to produce anti-idiotype antisera (AIA 3). As expected, AIA 3 decreased the binding of [^{3}H]-bradykinin to the guinea-pig ileum receptor. Moreover, AIA 3 acts as an agonist at the B_2 receptor, increasing the production of inositolphosphates on HF 15 fibroblasts and inducing secretion of prostaglandin E_2 in murine SV-T2 cells. This secretion was inhibited by the addition of the B_2 antagonist D-Arg^0[Hyp^3,D-Phe^7]-bradykinin. Therefore, anti-idiotypic antibody AIA 3 interacts specifically with the B_2 kinin receptor at the structural and functional level. Unfortunately, the immunoprint analysis was unsuccessful, probably due to the denaturation of the receptor during gel electrophoresis. The anti-idiotypic approach, therefore, provides a powerful tool to further characterize the kinin receptors.

B_3 receptors

The existence of the pulmonary B_3 receptor is largely controversial. This new receptor has been proposed in view of the weak inhibition produced by B_1 and B_2 antagonists on bradykinin-induced bronchoconstriction *in vivo*, and the failure to antagonize the ability of bradykinin to contract the epithelium-denuded guinea-pig trachea *in vitro*. Binding studies have confirmed that antagonists are unable to displace specific binding in membrane preparations [41]. However, other groups have failed to reproduce these results. *In vivo* experiments do not agree with those of JIN *et al.* [49] and ICHINOSE and BARNES [50], who have shown that bradykinin-induced bronchoconstriction in the guinea-pig is inhibited by the B_2 antagonist D-Arg^0[Hyp^3,Thi^{5,8},D-Phe^7]-bradykinin. By direct binding studies, we have shown that bradykinin binding in the guinea-pig lung is similar to that found in other tissues [14], and shows a typical pattern of B_2 receptors [18]. The failure of the B_2 antagonists used by FARMER and co-workers. [41] to antagonize bradykinin-induced bronchoconstriction may be due to degradation by carboxypeptidase N [25]. In fact, B_2 antagonists have a short half-life in the guinea-pig lung *in vivo* [49]. Moreover, the low affinity of the B_2 antagonists used can explain the weak inhibition observed. Indeed, Hoe 140 [29, 31, 51, 52] and NPC 16731 [33], which have high affinities for kinin receptors, completely inhibit the action of bradykinin in guinea-pig airways *in vivo* or *in vitro*. We have tested the action of NPC 17731 on bradykinin binding in the guinea-pig lung and trachea. This compound is able to completely displace the specific binding, with an affinity comparable to that of bradykinin (Trifilieff A., Da Silva A., Landry Y., Gies J-P., submitted). Based on all of these observations, it would be premature to conclude that bradykinin receptors in airways are different from other bradykinin B_2 receptors. The existence of these putative B_3 receptors needs confirmation, perhaps using molecular cloning techniques.

A comparaison of the relative potencies of Hoe 140, NPC 16731 and NPC 17731 in guinea-pig ileum, trachea and lung (table 2) reveals a similar binding affinity (in the nanomolar range) for the three compounds for B_2 receptors. In *in vitro* functional studies, all compounds

Table 2. — Relative potencies of the most potent B_2 receptor antagonists in different guinea-pig tissues

	Ileum			Trachea			Lung		
	Binding affinity	Functional		Binding affinity	Functional		Binding affinity	Functional	
		Antagonism	AA		Antagonism	AA		Antagonism	AA
Hoe 140	nM [30]	NC [53]	No [53]	nM [52]	NC [52, 53]	No [53] Yes [52]	nM*	-	-
NPC 16731	nM [33]	C [33]	No [33]	nM [33]	C [33]	No [33]	-	-	-
NPC 17731	nM [34]	C [34]	No [34]	nM*	NC*	No*	nM*	-	-

*: parameters from our laboratory. nM: nanomolar; NC: non-competitive; C: competitive; AA: agonist activity. Functional parameters are measured in isolated organs, and represent the antagonism exerted by the compounds used against bradykinin-induced contraction.

inhibited bradykinin-induced contraction in the ileum and trachea. In the trachea, Hoe 140 and NPC 17731 appear to act non-competitively, whereas NPC 16731 exerts a competitive antagonism. Therefore, the use of this latter compound seems to be more appropriate for a study of bradykinin receptors in airways.

Natural bradykinin antagonists

Several natural compounds have been studied for potential bradykinin antagonist activity (review [45]). The most promising natural antagonists have been extracted from the rhizome of *Mandevilla velutina* (Apocynaceae). *Mandevilla velutina* is a native plant of Brazil, and its rhizome is used in folk medicine for the treatment of inflammatory states and for venomous snake bites. A crude extract of its rhizome was found to functionally antagonize bradykinin-induced uterine contraction in the rat. This action was reversible, and selective for bradykinin, since the response to other agonists was unaffected [54]. The extract was also found to be active in antagonizing the contractile response induced by bradykinin and des-Arg^9-bradykinin, mediated by both B_1 and B_2 receptors, in the rabbit vascular bed [55]. Purification of this extract gave five steroid glycoids (MV 8609, MV 8611, MV 8612, MV 9610), which antagonized the bradykinin-induced contraction in rat uterus and guinea-pig ileum [56]. However, only MV 8612 appears to be a competitive antagonist, since the other compounds induce a decrease in the maximal response [54]. Despite the high doses (10–20 μM) employed for these antagonistic actions, these findings strongly suggest that such steroids may constitute a new class of putative non-peptidic bradykinin antagonists.

Bradykinin and asthma

Bronchoconstrictor effect

During the past 30 yrs, evidence that kinins are involved in the pathogenesis of asthma has been accumulating. Inhalation [57, 58], or intravenous injection [59], of bradykinin causes bronchoconstriction in asthmatic subjects, but has little or no effect (cough and retrosternal discomfort) in non-asthmatics. These observations have been reinforced by *in vivo* and *in vitro* studies. Bradykinin does not contract normal human bronchus [60], whereas bronchus from patients with airway obstruction shows an increased sensitivity to bradykinin [61]. These studies have recently been confirmed by FULLER *et al.* [62]. Moreover, circulating plasma kinin is significantly increased in patients with severe bronchial asthma [63]. After allergen challenge in asthmatics, CHRISTIANSEN *et al.* [64] found elevated amounts of tissue kallikrein and kinins in bronchoalveolar lavage fluids. This increase may be due to the slow rate of inhibition of human kallikrein in the airway of asthmatic subjects [65]. Interpreting lung volumes and capacities, NEWBALL *et al.* [59] suggested that the bronchoconstrictor effect induced by intravenously injected bradykinin is localized at the level of the alveolar duct. This localization might lead to sufficient distortion or stimulation of pulmonary receptors to cause the respiratory distress experienced by asthmatics. This hypothesis is in agreement with the autoradiographic localization of bradykinin receptors in human lung: the smooth muscle of large airways is sparsely labelled, whilst greater labelling was observed in the smaller airways [66].

The mode of action of this bronchoconstrictor effect in humans remains to be determined, but a cyclo-oxygenase inhibitor (aspirin) does not affect bronchoconstriction induced by bradykinin, whereas pre-inhalation of ipratropium, a muscarinic antagonist, significantly reduces this bronchoconstrictor response [62]. Moreover, bradykinin applied to mixed isolated human lung cells is unable to release prostaglandins and thromboxanes [67]. Therefore, the product of cyclo-oxygenase does not appear to be involved in the bradykinin-induced bronchoconstriction in asthmatic subjects. Similar conclusions have been drawn for histamine [68]. The non-adrenergic non-cholinergic (NANC) system seems to be involved in the action of kinins in the airways. But the contribution of NANC nerves is controversial regarding the animal model used. It is not involved in the contraction of ferret trachea [69], whereas it has been shown to play a role in the bradykinin-induced contraction of guinea-pig trachea [70]. In asthmatic patients, the

bronchoconstrictor effect of bradykinin is markedly inhibited by cromolyn sodium and nedocromil sodium [71]. Both of these drugs are able to inhibit the activation of sensory nerves in airways [72]. Therefore, part of the bronchoconstrictor response induced by bradykinin may be mediated *via* the NANC system.

Bradykinin and inflammation

Besides this bronchoconstrictor effect, bradykinin could play a role in asthma *via* its pro-inflammatory properties. The circulating level of immunoreactive bradykinin increases three- to fourfold in a clinical model of acute inflammation (oral surgery), or in chronic inflammation (rheumatoid arthritis) [73]. Furthermore, myriad metabolic imbalances and minor traumas activate the cascade of events leading to the formation of kinins, and all components of this system have been reported from inflammatory exudates [74]. Allergic sheep, similar to asthmatic patients, respond to the inhalation of an antigen (*Ascaris suum*) with acute anaphylactic bronchoconstriction, followed 6–8 h later by a late phase of increased airway resistance [75]. In addition, these animals develop nonspecific airway hyperresponsiveness 2 and 24 h following the challenge, and airway inflammation as assessed by the number of oesinophils, neutrophils and macrophages in the bronchoalveolar lavages before and after the challenge by *Ascaris suum*. A B_2 antagonist, D-Arg^0[Hyp^3,D-Phe^7]-bradykinin, had no effect on the acute bronchial response to antigen, but blocked bradykinin-induced bronchoconstriction in a dose-dependent manner. The antigen-induced increase in airway responsiveness and the associated inflammatory responses that occur 2 h after the challenge were also blocked [76]. The same antagonist inhibited the bronchoconstriction, inflammation and mediator generation in the late phase responses (24 h) [77]. Moreover, many inflammatory cells are able to generate kallikreins following stimulation by diverse compounds. Peritonial rat mast cells, stimulated by catecholamines, could liberate an active kininogenase [78], and activation of human basophils by an anti-immunoglobin E (IgE) antibody generates kallikreins [79]. These data suggest a role for kinins in hyperresponsiveness and inflammation in the airways.

Bradykinin is able to increase microvascular leakage of plasma proteins in guinea-pig airways by acting on a B_2 receptor [50]. This effect on plasma leakage may contribute significantly to airway oedema, and may be involved in the development of bronchial hyperresponsiveness. In guinea-pigs, ROGERS *et al.* [80] have demonstrated an interaction between kinin and the platelet activating factor (PAF), which is a potent mediator of asthma [81]. The late phase of this bradykinin-induced leakage is mediated exclusively by PAF [80].

Effect of tracheal epithelium

The actions of kinins on the tracheal epithelium might be important in the understanding of the role of these peptides in asthma. Kinins could possibly generate an epithelium-derived relaxant factor (EpDRF) [82]. The EpDRF, which can be generated by many endogenous substances (acetylcholine, serotonin, histamine) [83], may be different from nitric oxide. It has not yet been identified, but might be an inhibitory prostaglandin [83], and could regulate the bronchoconstrictor action of agonists. Epithelial damage observed in the respiratory tract of asthmatic patients [84, 85] might perturb the liberation of EpDRF and would, therefore, explain the acute bronchoconstriction observed in these patients. On the other hand, *de novo* synthesis of B_1 receptors following tissue damage [9, 10] could be involved in this hypersensitivity to kinins. However, a recent study has shown that the B_1 agonist, des-Arg^9-bradykinin, has no effect on asthmatic subjects [86].

In addition, bradykinin could act on epithelium; elicit a rapid and transient increase in ciliary beat frequency in cultured rabbit tracheal epithelium [87], and increase mucus secretion [88]. In cultured cells of human tracheal mucosa, WIDDICOMBE *et al.* [89] showed that bradykinin could increase short-circuit current. These authors suggested that Na^+ absorption and Cl^- secretion account for at least some of this increase. Both Na^+ absorption and Cl^- secretion across the epithelium can lead to fluid movement into the airway from the lumen [90, 91]. Autoradiographic studies [92], and selective application of bradykinin to submucosal or mucosal surfaces of dog tracheal monolayers [93], have localized bradykinin receptors on the apical and basolateral membranes of epithelial cells. These receptors belong to the B_2 subtype [39, 94]. In human airways, the labelling of [^{3}H]BK was sparse over the luminal surface of the epithelium, but was dense in the lamina propria immediately subjacent to the basal epithelial cells layer [66].

Tachyphylaxis to bradykinin

Repeated inhalation of bradykinin by asthmatic subjects is associated with a loss of bronchoconstrictor response [62, 95]. This tachyphylaxis may be due to the secondary generation of relaxant prostanoids, such as prostaglandin I_2 [96], but the inability of a cyclo-oxygenas inhibitor (flubiprofen) to protect against the impairment of bradykinin responsiveness provides convincing evidence that refractoriness to bradykinin does not result from the release of protective prostaglandins [95]. In dogs, bradykinin-induced bronchoconstriction is largely mediated by the activation of C-fibres [97], which liberate tachykinins (substance P, neurokinin A and B), which are potent bronchoconstrictor agents [98]. Depletion of these neuropeptide stores could explain this tachyphylaxis, as has been proposed in humans for capsaicin [99], which had a similar effect to bradykinin on C-fibres [100].

Bradykinin and upper airways

In recent years, evidence has accumulated to support the hypothesis that kinins may play a role in the

pathogenesis of inflammatory disease in the upper airways. It has been demonstrated that these peptides are generated in nasal secretions during allergic reactions [101, 102]. In addition, kinins are the only mediators detected in nasal secretions during experimental [103], or natural rhinovirus colds [104]. Kinin generation in these inflammatory responses correlates with the onset of symptoms. When adult volunteers, with and without nasal allergy, were challenged by the inhalation of bradykinin, they developed unilateral obstruction of nasal conductance, rhinorrhoea and a persistent sore throat [105, 106]. These symptoms are independent of mast cell or basophil mediator release [105, 107]. Similar studies, conducted by DOYLE *et al.* [108], were unable to entirely confirm the symptoms evoked by bradykinin, but these discrepancies may be a reflection of the different methods of challenge administration, and differences in subjectively reported symptoms.

Autoradiographic examination of human nasal mucosa revealed that bradykinin binds specifically to small muscular arteries, venous sinusoids and submucosal fibres [88]. The identity of the fibres could not be determined, but the authors suggested that they might be nociceptive sensory nerve fibres. Other studies have shown that kinin applied to human nasal mucosa produces an algesic response, which was not blocked by capsaicin desensitization [109]. Therefore, sensory motor nerve fibres do not appear to be involved in the action of kinins on the upper airways. In human nasal mucosa, kinins are also able to increase vascular permeability, as measured by albumin and N-α-tosyl-L-arginine methyl ester (TAME)-esterase activities. This increase cannot be blocked by the competitive B_2 receptor antagonist, D-Arg^0[Hyp^3,D-Phe^7]-bradykinin, suggesting that B_2 receptors are not involved [110]. The possibility of activation of B_1 receptors cannot be excluded. By studying glandular secretion in human nasal fragments in response to kinins, BARANIUK *et al.* [88] reported the ability of B_1 receptor antagonist (des-Arg^9-[Leu^8]-bradykinin) to block this pro-secretory effect. On the other hand, the failure of D-Arg^0[Hyp^3,D-Phe^7]-bradykinin to antagonize the effect of bradykinin on vascular permeability, may be due to its degradation by carboxypeptidase N [25], which has been shown to be one of the major enzymes in kinin degradation in nasal secretions [111]. If a bradykinin-induced increase in glandular secretion, and possibly in vascular permeability, seems to be mediated by the activation of B_1 receptors [88, 110], other effects on human nasal mucosa appear to be mediated by B_2 receptors [109, 112]. The cellular events leading to all of the symptoms observed in nasal mucosa are still not known.

The production of kinins during experimental or natural rhinovirus cold, together with their pharmacological properties, has led to the suggestion that these autacoids may play a role in the pathogenesis of inflammatory disease of the upper airways. In order to establish a definite role for kinins in these disorders, however, it is essential to intervene pharmacologically, in order to alter their activity and to observe the effects of such interventions on the symptoms. Unfortunately, many of the approaches for interfering with the kinin action are not appropriate for use in humans. It is known that the ferret is a suitable model for studying infections with the influenza A virus. Study of this viral infection in this animal showed that it is a convenient model to further evaluate the role of kinins in the pathogenesis of upper respiratory tract infections [113].

Converting enzyme inhibitor induced-cough and kinins

A dry, non-productive cough and, less frequently, exacerbation or development of asthma are well-recognized side-effects of angiotensin-converting enzyme (ACE) inhibitors (review [114]). Although its pathogenesis is unknown, some properties of this class of drugs have been described. ACE inhibitors may generate bronchoactive mediators, such as prostaglandins [115], and may decrease the bronchodilatator effect of vasoactive intestinal peptide (VIP) or beta-agonists by preventing the accumulation of cyclic adenosine monophosphate in smooth muscle [116]. ACE inhibitors may also decrease the breakdown of substance P [117], and bradykinin [7], which are potent bronchoconstrictor agents. Here, we review only the evidence for the kinin-mediated pharmacological activity of ACE inhibitors.

Despite one study, reporting that the antihypertensive action of ACE inhibitors is not a kinin-dependent mechanism [118], convincing evidence suggests that bradykinin plays a major role in the side-effects of ACE inhibitors. HOFFMAN *et al.* [119] demonstrated a stimulation of vascular prostaglandin I_2 synthesis by captopril, lisinopril and bradykinin, which was very strongly suppressed by a competitive B_2 receptor antagonist (D-Arg^0[Hyp^3,Thi^{5,8},D-Phe^7]-bradykinin). Investigating the role of bradykinin in the infarct-limiting effect of the ACE inhibitors, MARTORANA *et al.* [120] showed that the effect of ramiprilat was reversed by the new B_2 receptor antagonist Hoe 140. These and other studies [121, 122] provide evidence for the involvement of kinins in the side-effects of ACE inhibitors. Thus, inhibition of ACE would allow accumulation of bradykinin, provided that this was a rate-limiting step in the metabolic pathway. However, ACE inhibitors cause no [123], or only a minor increase [124, 125] in plasma level of bradykinin. Although this might, in part, be due to the technical problems in bradykinin determination, it might also suggest that ACE inhibitors induce an increase of tissue kinins rather than plasma kinins.

Bradykinin and lung cancer

Lung cancer is the leading cause of cancer death, with approximately 90% of affected patients dying within one year of diagnosis. On the basis of both clinical behaviour and prognosis, lung cancers can be divided into small cell lung cancer (SCLC), and non-small cell lung cancer (NSCLC). Multiple neuropeptides, including vasopressin, galanin, neurotensin, cholecystokinin, gastrin-releasing-peptide, and bradykinin, have been proposed to act as

paracrine or autocrine growth factors for SCLC and NSCLC [126, 127].

The mitogenic action of bradykinin on human lung fibroblasts has been known since the eighties [128, 129], but at this period no possible role in carcinoma growth had been described. A few years later, MAEDA *et al.* [130] reported, for the first time, the presence of bradykinin in human tumour ascites from a patient with gastric cancer. Thereafter, similar observations were reported in various human malignant effusions [131], and in the plasma of advanced cancer patients [132].

The role of peptides in cancer has been more widely studied in SCLC, probably since SCLC produce a greater number of neuropeptides (vasopressin, galanin, gastrin-releasing peptide, and others) [133]. Moreover, SCLC are resisitant to chemotherapy, so that an increased understanding of SCLC growth regulation may identify novel targets for treatment. Bradykinin has been reported to be the most potent peptide in increasing intracellular calcium in a number of SCLC cells lines [134], but other peptides were also observed to produce a response [134, 135]. Because of this heterogeneity, a specific bradykinin antagonist or antibody might not have a great antitumour effect. Future therapeutic strategies must recognize this heterogeneity in neuropeptide response, and the application of a combination of peptide antagonists might be more useful. Alternatively, since all of these peptides utilize the same intracellular signal (*i.e.* an increase in calcium), another approach might be to interfere with this calcium signal. Promisingly, a recent study has shown that this strategy can be utilized successfully using the experimental SCLC in a hamster model [136].

What is the exact role of kinins in the clinical setting and in the pathogenesis of lung cancer? A dual action could be suggested; due to their permeability-enhancing properties, kinins could increase vascular permeability and facilitate a greater supply of nutrients and oxygen to the tumour cells [132]; or, alternatively, kinins could directly stimulate the proliferation of tumour cells [134, 135].

Conclusion

The effects of kinins on airways reviewed in this article are mediated mainly *via* activation of B_2 receptors (table 3 and fig. 1). Concerning the contraction of tracheal smooth muscle, the putative B_3 receptor [41, 42] can be classified as a B_2 receptor [18, 49–52]. The knowledge of kinin receptors requires further investigations, but the recent cloning of a rat [40], and human B_2 receptor [46], and the development of potent antagonists [29–31, 33–35] lead us to expect substantial advances in the understanding of the mechanism of kinin action. Effective hormone antagonists should have several characteristics, the principal being the possession of high affinity for the agonist receptor without any agonist activity. Receptor selectivity is also essential. Another criterion for practical drug development is the long half-life of the antagonist *in vivo*, which generally correlates with a resistance to enzymatic degradation, especially critical for peptides. The new B_2 bradykinin receptor antagonists (Hoe 140, NPC 16731, and NPC 17731) possess all of these qualities, which makes them appropriate for further investigation of the physiological and pathophysiological role of kinins.

With regard to the mode of action of kinins in asthma, the predominant mechanism (contraction of airway smooth muscle, or intervention in the inflammatory process) is still unknown. But the lack of a constrictor effect due to bradykinin in airway smooth muscle of normal subjects [57, 58, 62] suggests that the bronchoconstrictor effect of bradykinin in asthmatic patients is most likely indirect, probably mediated by inflammatory effects. In fact, kinins may play a role in all steps of the inflammation cascade of asthma *e.g.* they can induce a hypersecretion of mucus [88], increase vascular permeability [50, 80], which accounts for the mobilization of inflammatory cells and oedema formation, and can also act directly on inflammatory cells, such as mast cells [27, 44]. These different inflammatory actions could result in the contraction of airway smooth muscle.

Table 3. – Action of kinins in the airways

Site	Action	Receptor	References
Bronchial smooth muscle	Bronchoconstriction	B_2	[57–62, 86]
	Bronchorelaxation	B_2	[43]
Vessels	Vasodilatation	B_1	[7]
	Plasma exudation	B_2	[50, 80]
Epithelium	Secretion of mucus	B_1	[88]
	Generation of EpDRF	B_2	[82, 94]
	Increase in ciliary beat frequency	B_2	[87]
Mast cells	Histamine secretion	Protein G_i-like	[26, 27, 44]
Eosinophils and neutrophils	Proliferation	B_2	[76, 77]
Cholinergic nerves	Activation	B_2	[38, 62]
NANC nerves	Activation (rabbit, guinea-pig, dog)	B_2	[50, 71, 72]

EpDRF: epithelium derived relaxant factor; NANC: non-adrenergic non-cholinergic. Modified from [5] with permission.

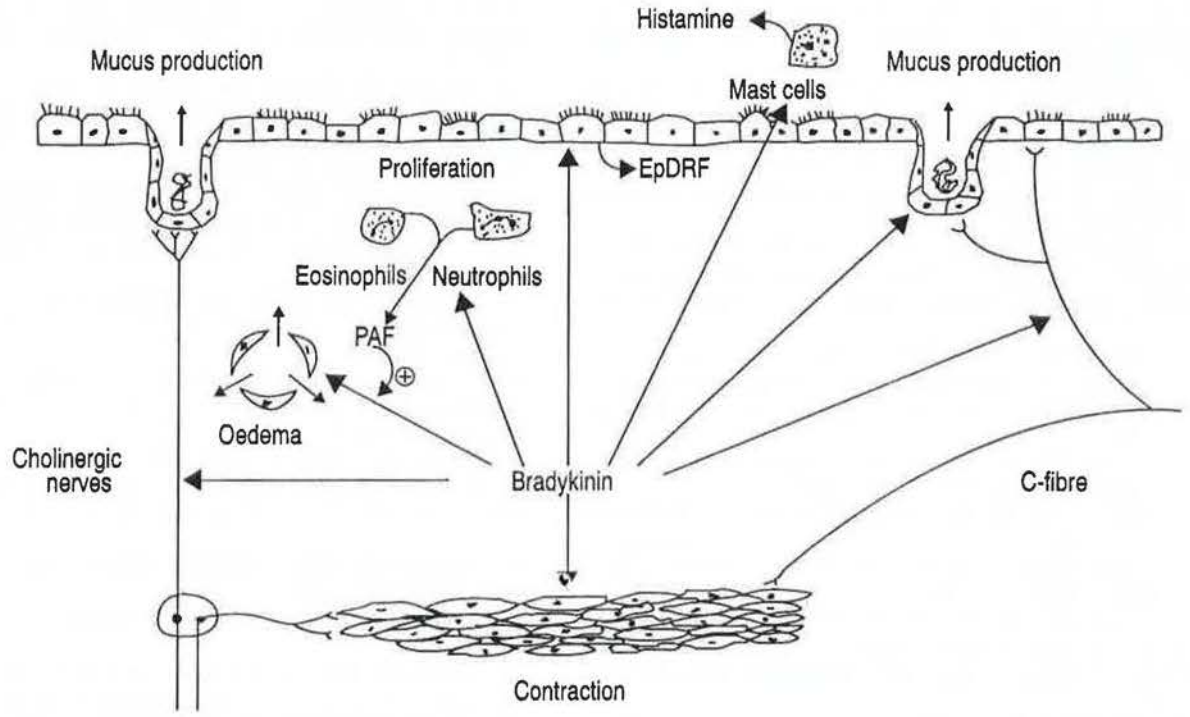

Fig. 1. – Schematic representation of possible levels of action of bradykinin in the airways. Bradykinin may induce the proliferation of neutrophils and eosinophils, the release of histamine from mast cells and the formation of oedema. Bradykinin may also increase mucus secretion by acting directly on submucosal glands or indirectly by the activation of C-fibres and cholinergic nerves. Finally, all of these inflammatory events could be followed by the contraction of airway smooth muscle. PAF: platelet activating factor; EpDRF: epithelium-derived relaxing factor. For references see table 2. Modified from [5] with permission.

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