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Bradykinin in asthma: modulation of airway inflammation and remodelling

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ABSTRACT

Bradykinin, a pro-inflammatory molecule, and its related peptides have been studied for their effects on acute reactions in upper and lower airways, where they can be synthesised and metabolized after exposure to different stimuli including allergens and viral infection. Bradykinin B₁ and B₂ receptors are constitutively expressed in the airways on several residential and/or immune cells. Their expression can also be induced by inflammatory mediators, usually associated with eosinophil and neutrophil recruitment, such as IL-4, IL-13, TNF- α , IL-6 and IL-8, via intracellular MAPK and NF- κ B signalling. In turn, the latter up-regulate both bradykinin receptors. Bradykinin activates epithelial/endothelial and immune cells, neurons and mesenchymal cells (such as fibroblasts, myofibroblasts and smooth muscle cells), which are implicated in the development of airway chronic inflammation, responsiveness and remodelling (a major feature of severe asthma). This review highlights the role of bradykinin and its receptors in respect to chronic inflammatory response involving eosinophils/neutrophils and to vascular/matrix-related airway remodelling in asthmatic airways. This scenario is especially important for understanding the mechanisms involved in the pathogenesis of eosinophilic and/or neutrophilic asthma and hence their therapeutic approach.

KEY WORDS: bradykinin, bradykinin receptors, asthma, severe asthma, airway inflammation, airway remodelling.

1. Introduction

Bradykinin is a key regulator of vascular tone, acute inflammation, pain and cancer (Kaplan et al., 2002; Leeb-Lundberg et al., 2005; Kashuba et al., 2013). In asthma, bradykinin induces acute inflammatory responses in the airways, including plasma protein extravasation and smooth muscle contraction leading in turn to airway obstruction (Abraham et al., 2006). Acute anaphylactic response in allergic asthma is characterised by mast cells-derived mediators of inflammation and kinins formation with subsequent involvement of tachykinins (Geppetti et al., 1995). Furthermore, bradykinin is an indirect stimulus of bronchial hyperresponsiveness (Van Schoor et al., 2000) and a negative modulator of excessive bronchoconstriction, via the release of epithelium-derived bronchoprotective factors in asthma (Figini et al., 1996; Ricciardolo et al., 1996).

The bradykinin pathway is also involved in several processes related to asthma pathophysiology, such as the recruitment of eosinophils (Gurusamy et al., 2016) and neutrophils (Sodhi et al., 2017), the synthesis of “nitrosative stress” products (MacPherson et al., 2001; Fitzpatrick et al., 2009; Ricciardolo et al. 2006; Ricciardolo et al., 2012) and vascular/matrix-related remodelling (Ricciardolo et al., 2013). This review is focused on recent discoveries regarding the capability of bradykinin to amplify inflammatory responses and to influence cellular/molecular pathways involved in airway remodelling in asthma.

2. Synthesis and physiological effects of bradykinin

Bradykinin is produced by enzymatic cleavage of high and low molecular weight kininogens located, respectively in plasma and tissues. Specifically, plasma kallikrein cleaves high molecular weight kininogens while tissue kallikrein acts on low molecular weight kininogens. Both enzymes produce two low-molecular-weight vasoactive peptides: the nonapeptide bradykinin (arg-pro-gly-phe-ser-pro-phe-arg), and the 10-amino-acid peptide lysyl-bradykinin or kallidin (excellent reviews exist on kinins formation (Kaplan et al., 2002; Kaplan and Ghebrehiwet, 2010)).

Bradykinin and kallidin are collectively referred to as kinins.

Kinins participate in inflammatory processes by their ability to activate structural and inflammatory cells. Bradykinin has been shown to induce vasodilatation (Yamawaki et al., 1994) and vascular permeability (Ricciardolo et al., 1994a); it mediates the recruitment of inflammatory cells (Sato et al., 2000; Perron et al. 1999; Duchene et al. 2007), the production of nitric oxide (Ricciardolo et al., 2000) and the release of prostanoids (Li et al., 1998), tachykinins (Geppetti et al., 1995) and cytokines/chemokines (Koyama et al., 2000). In addition, bradykinin induces spasm of airway smooth muscle (Figini et al., 1996) with subsequent increase of airway resistance (Ricciardolo et al., 1994b). As illustrated in Fig. 1, bradykinin can also provoke stimulation of sensory neurons resulting in chronic hyperalgesia (Schuelert et al., 2015), alter the ion secretion by epithelial cells (Cozens et al., 1994) and modulate cell migration and tissue injury (Abraham et al., 2006) (Fig. 1). Bradykinin and kallidin display their biological effects upon binding and activation of their specific receptors B₁ and B₂. Bradykinin B₁ receptor usually binds [des-Arg⁹]-bradykinin and [lys-des-Arg⁹]-bradykinin, the latter is considered more selective (Leeb-Lundberg, 2005). Bradykinin B₁ receptor is constitutively expressed in inflammatory, but not in residential, cells and it is regularly induced as a result of inflammation (Christiansen et al., 2002) in the presence of cytokines such as interleukin (IL)-1 and tumor necrosis factor (TNF)- α (Gurusamy et al., 2016). Bradykinin B₂ receptors show high affinity for kallidin and bradykinin and are instead constitutively expressed on most cell types including those in the airways (Leeb-Lundberg, 2005). Both bradykinin receptors are G protein-coupled receptors. Their stimulation, via the activation of G α q/11 family of heterotrimeric G proteins, induces a number of intracellular events related to inflammation (Leeb-Lundberg, 2005; Ricciardolo et al., 2000, 1998).

3. Kinin metabolism

Metabolic degradation of kinins is a major mechanism for the regulation of their actions and virtually all tissues and biological fluids contain enzymes that are capable of degrading these

peptides. Such enzymes include kininase I, also known as plasma carboxypeptidase N, and kininase II (Kaplan et al., 2002), the most important kinin-degrading enzymes in the endothelium of pulmonary vessels, which is identical to angiotensin-converting enzyme. Inhibition of this enzyme, with compounds such as captopril, results in a marked blockade of the metabolism of bradykinin. This enzyme has a higher affinity for bradykinin than for angiotensin I. Although the kininase II-mediated metabolism may predominate in plasma and lungs, other enzymes such as aminopeptidase M and P, and neutral endopeptidase, also called enkephalinase, are involved in the inactivation of bradykinin as well (Kaplan et al., 2002).

4. Role of bradykinin in airway inflammation

The activation of the kinin-kallikrein system plays a fundamental role in sustaining an acute inflammatory response by means of the synthesis of kinins. Their action on tissues is associated with vasodilation (“rubor and calor”), increased vascular permeability (“tumor”), pain (“dolor”) and smooth muscle contraction (“function laesa”) which are defined as “cardinal signs” by Aulus Cornelius Celsus and therefore implicated in the pathogenesis of acute inflammation. During the onset and progression of inflammation the kinin-kallikrein system activation liberates bradykinin, whose half-life is very short (30 s) due to metabolism by kininases. However, this is long enough to trigger intracellular signalling, initiated via the activation of bradykinin B₂ receptors (Colman, 2006). Bradykinin, hence, initiates an acute inflammatory response and promotes the synthesis of other mediators, including prostaglandins, nitric oxide and tachykinins, modifying acute inflammatory response (Sainz et al., 2007) (Fig. 1).

Kinins are well known as mediators of bronchial hyperresponsiveness in asthma (Roisman et al., 1996). Different stimuli, including allergen exposure and viral infection are able to increase airway responsiveness associated with bradykinin, either in animal models (Ellis et al, 2004; Folkerts et al., 2000; Ricciardolo et al., 1994b) or in human asthmatics (Ricciardolo et al., 2001; Ricciardolo et al., 2012a).

In asthmatic airways, kinins are able to provoke inflammatory oedema, mucus production, cough and smooth muscle contraction (cardinal signs of acute asthma) through the direct activation of bradykinin B₂ receptor, neural cholinergic pathway, capsaicin-sensitive type C sensory nerve fibres (effect referred as 'neurogenic inflammation') (Geppetti et al., 1995) and the release of various inflammatory mediators acting on endothelial, epithelial and smooth muscle cells.

Some of these actions (direct activation of bradykinin B₂ receptor on smooth muscle and stimulation of capsaicin-sensitive type C sensory fibres and vagal nerves) are excitatory by provoking acute bronchoconstriction (Geppetti et al., 1995; Ricciardolo et al., 1994b) while, on the other hand, others (activation of epithelial cells through release of PGE₂ and nitric oxide produced by constitutive nitric oxide synthase) are protective in terms of reducing bronchoconstriction itself (Figini et al., 1996; Ricciardolo et al., 1994c, 1996).

Another mechanism of action of bradykinin in the airways concerns its involvement in cough regulation. Inhaled bradykinin is able to induce cough: this effect is augmented by ACE inhibitors and seems to be dependent on capsaicin-sensitive non-myelinated bronchopulmonary C-fibers (Hewitt et al., 2016). Furthermore, bradykinin is able to upregulate the TRPV1 receptor sensitivity on C fibres (Lee et al 2010) provoking the release of tachykinins (substance P and neurokinin A) (Geppetti et al., 1995) and, in addition, to induce the production of eicosanoids and prostaglandin in the airways (Lavorini et al., 2014; Hewitt et al., 2016) enhancing cough sensitivity (Lee et al 2010). Interestingly, a substantial increase of TRPV1-immunoreactive nerve profiles was found in the bronchial tissue of patients with chronic cough (Lee et al 2010).

The expression of bradykinin B₂ receptor is regulated by bradykinin itself as reported in *in vitro* studies on epithelial cells (Ricciardolo et al., 2012b) and lung/bronchial fibroblasts (Sabatini et al., 2012, 2013). Bradykinin B₂ receptor expression is constitutively enhanced in bronchial fibroblasts from asthmatics compared to normal bronchial fibroblasts (Sabatini et al., 2013) and bradykinin itself increases the expression of bradykinin B₂ receptor, particularly in asthmatic bronchial

fibroblasts (Sabatini et al., 2013), showing a different cellular phenotype in relation to asthma (Th2-driven) inflammatory milieu.

Bradykinin-induced bradykinin B₂ receptor expression in human lung fibroblasts is suppressed by corticosteroid treatment (budesonide) (Sabatini et al., 2012), suggesting a potential role for transcription factors in the modulation of bradykinin B₂ receptor expression. Bradykinin B₂ receptor expression is increased in the bronchial mucosa of mild allergic asthmatics after allergen compared to diluent challenge and, in addition, in bronchial fibroblasts from asthmatics after exposure to the Th2 cytokines IL-4 and IL-13 compared to the untreated fibroblasts (Ricciardolo et al., 2016). The nuclear factor- κ B (NF- κ B) p65 (Ser 276) inhibitor suppressed bradykinin B₂ receptor up-regulation induced by bradykinin and IL-4/IL-13 on bronchial fibroblasts *in vitro*, indicating that bradykinin B₂ receptors protein expression is modulated by NF- κ B pathway (Ricciardolo et al., 2016).

In human epithelial cells, the bradykinin B₂ receptor mediates inflammatory signalling through activation of NF- κ B and an increase in cyclooxygenase-2 expression (Chen et al., 2004). Moreover, the activation of MAPK/NF- κ B and their related signal pathways stimulates the production of cytokines (Hayashi et al., 2000). Of interest, the release of neutrophil-related cytokines like IL-6 and IL-8 could amplify the MAPK/NF- κ B signalling inducing airway kinin receptor upregulation as well (Bryborn et al., 2004; Newton et al., 2002; Zhang et al., 2007a). Elevated levels of bradykinin (Christiansen et al., 1992), IL-8 and human tissue kallikrein activation are reported in bronchoalveolar lavage fluids from allergic subjects and asthmatic patients with rhinovirus infection (Christiansen et al., 2008), which underlines the relationship between bradykinin and IL-8 and their possible role in virus-induced asthma exacerbation.

Less information is available on the role of bradykinin B₁ receptor in asthma. The bradykinin B₁ receptor antagonist R954 inhibited monocyte chemotactic protein-1 and interferon-gamma release, abolished TNF- α and IL-1 β in bronchoalveolar lavage fluid in a mouse model of acute lung injury caused by lipopolysaccharide inhalation (Campanholle et al., 2010). Also an inhibitory effect of

blocking bradykinin B₁ receptor was observed in terms of eosinophil activation, proliferation and migration in a murine model of asthma (Vasquez-Pinto et al., 2010). The presence of bradykinin B₁ and B₂ receptors was confirmed on neutrophils and eosinophils from asthmatic and non-asthmatic subjects. A significantly greater protein expression of bradykinin B₁ and B₂ receptors was observed on eosinophils from asthmatic compared to non-asthmatic subjects (Bertram et al., 2007) (Bertram et al., 2009). A recent study showed the inhibitory effect of the bradykinin B₁ receptor antagonist on BAL cell recruitment (eosinophils, neutrophils, macrophages and lymphocytes) after allergen challenge in a mouse model demonstrating the involvement of bradykinin B₁ in the processes regulating inflammatory cell migration towards the airway lumen in asthmatic mice (Gurusamy et al., 2016). Administration of a bradykinin B₂ receptor antagonist given before, during and 4 hours after antigen challenge significantly inhibited the late bronchial response, inflammatory mediators and the number of recruited neutrophils in an ovine model of asthma, suggesting that bradykinin B₂ receptor selective antagonists may be potential drugs to reduce inflammatory cell infiltration in the late asthmatic response (Abraham et al., 2006).

A new role for bradykinin in modifying the cellular inflammatory response in the airways has recently been depicted. Bradykinin stimulates the release of the neutrophilic chemotactic agent IL-8, via both bradykinin B₂ and B₁ receptors stimulation, in epithelial bronchial cells (BEAS2B). Furthermore, in BEAS2B cells co-cultured with human neutrophils, bradykinin increased myeloperoxidase release and 3-nitrotyrosine production suggesting that bradykinin induces “nitrosative stress”, potentially mediated by IL-8 (Ricciardolo et al., 2012). “Nitrosative stress” is a pathogenic pathway involved in severe asthma (Fitzpatrick et al. 2009; Ricciardolo et al. 2006) and on the basis of the results obtained in our *in vitro* study using epithelial cells co-cultured with neutrophils we may speculate that bradykinin-induced “nitrosative stress” might contribute to chronic airway inflammation in neutrophilic-dependent (non-type 2) asthma.

Bradykinin is also a mediator of hereditary and ACE-Inhibitor-induced angioedema (Riha et al 2017). Bradykinin-induced angioedema results from overproduction or reduced degradation of

bradykinin (Misra et al., 2016). This rise of bradykinin concentration causes an increase in vascular permeability, vasodilation and oedema formation (Hsu et al., 2012). Icatibant, a bradykinin receptor competitive antagonist (Guo et al., 2017), has been tested in several clinical trials (Baş et al., 2015; Farkas et al., 2017; Sinert et al., 2017) reporting significant efficacy as a new therapeutic option in hereditary and ACE-Inhibitor-induced angioedema.

5. Bradykinin in asthma

Asthma is a chronic airway disease showing variable bronchial motor tone and airflow limitation (GINA Report 2017). Bronchial hyperresponsiveness, chronic inflammation and remodelling (structural alterations of airway wall) are major features of asthma and they are modulated, at least in part, by the epithelium layer (Ojiaku et al., 2017; Nair et al., 2017; Lambrecht and Hammad, 2012; Ricciardolo et al., 2003; Davies, 2009).

Different stimuli (allergen exposure, viral infection, cold air, various pollutants such as ozone and acidic molecules) may lead to acute airway inflammation, which triggers the production of pro-inflammatory mediators like TNF- α , interleukins and, mostly, kinins (Christiansen et al., 1992; Folkerts et al., 2000; Yoshihara et al., 1996; Ricciardolo et al., 1999). These pro-inflammatory mediators can result in activation of intracellular signal pathways that induce upregulation of bradykinin receptors in the airways (Sabatini et al., 2013; Ricciardolo et al., 2016; Zhang et al., 2007). In allergic mild asthma the expression of bradykinin B₂ receptors is higher compared to control subjects (Ricciardolo et al., 2016). Subsequently, the kinin receptor upregulation and the epithelium dysfunction result in excessive bronchial hyperresponsiveness (Ricciardolo et al., 2001; Ricciardolo et al., 2003; Ricciardolo et al., 2016), which is a feature of uncontrolled asthma. Kinins are detected in bronchoalveolar lavage fluid from stable asthmatics (Christiansen et al., 1992). Furthermore, in atopic asthmatic patients, allergen exposure is associated with increased kinin concentrations in the epithelial lining fluid (Christiansen et al., 1992) and enhanced bronchial hyperresponsiveness to bradykinin (Ricciardolo et al., 2001), suggesting that anaphylactic response

is able to amplify kinin formation and to upregulate kinin receptors. Both bradykinin receptor subtypes seem to contribute to allergen-induced bronchial hyperresponsiveness in rats (Huang et al., 1999). However, patients with asthma exhibit airway hyperresponsiveness to aerosolized bradykinin (bradykinin B₂ receptor agonist), but not to des-Arg⁹-bradykinin (bradykinin B₁ receptor agonist) indicating that, in the human model, bradykinin B₁ receptor is not involved in acute bronchoconstriction. In healthy humans inhalation of bradykinin has little or no effect, but in asthmatics it produces significant bronchoconstriction associated with the perception of cardinal asthma symptoms (“chest tightness, cough, wheezing and dyspnoea”) resembling a natural asthma attack (Abraham et al., 2006).

Bradykinin is a weak constrictor in isolated human bronchi with epithelial integrity, while *in vivo* it is a potent bronchoconstrictor in asthmatic patients. This effect is due to the capability of bradykinin to activate cholinergic nerves (Reynolds et al., 1999), since bradykinin can stimulate airway parasympathetic ganglion neurons independently of sensory nerve activation, therefore providing an alternative mechanism for regulating airway parasympathetic tone (Kajekar and Myers, 2000).

On the other hand, epithelium-derived bronchoprotective factors such as nitric oxide are released by bradykinin (Figini et al., 1996; Ricciardolo et al., 2000) negatively modulating enhanced bronchial hyperresponsiveness in mild asthma (Ricciardolo et al., 1996, 2001) (Fig. 1). A further study also showed the impairment of the bronchoprotective nitric oxide on the bradykinin-induced airway hyperresponsiveness in moderate-to-severe asthma suggesting the loss of this protective mechanism in difficult-to-control patients (Ricciardolo et al., 1997). In addition, clinical studies confirmed the role of corticosteroids in decreasing bronchial hyperresponsiveness to bradykinin and the bradykinin receptor expression in eosinophils/neutrophils from asthmatics in association with the improvement of respiratory symptoms (Bertram et al., 2007, 2009, Reynolds et al., 2002) indicating that the bradykinin-induced effects on the asthmatic airways seem to be corticosteroid sensitive. In line with the latter studies, dexamethasone, a well-known anti-inflammatory corticosteroid drug, inhibited the kinin receptor upregulation and bronchial hyperresponsiveness to kinins in murine

airways *in vitro* and the cigarette smoke-induced bronchial hyperresponsiveness *in vivo* (Zhang et al., 2004) (Lei et al., 2008) confirming a steroid sensitive modulation of kinin-induced airway responses. In a nice review, Zhang et al. pointed out that exposure to allergen, cigarette smoke or viral infection stimulated MAPK/NF- κ B pathway promoting the gene upregulation of bradykinin receptors and subsequently increased bronchial responsiveness. These environmental risk factors result in the synthesis of pro-inflammatory mediators, such as TNF- α and ILs, that activate intracellular MAPK and NF- κ B (Zhang et al., 2013).

Incubation of murine airway segments for 24 h in medium significantly increased bradykinin B₁ receptors and cyclooxygenase-2 expression which were significantly decreased by dexamethasone. (Xu and Cardell, 2014) Interestingly, incubation with nicotine significantly suppressed the cyclooxygenase-2 mediated and epithelium-dependent relaxations to bradykinin in organ culture (not reversed by dexamethasone) and, additionally, nicotine long-term exposure enhanced murine airway contractile responses to kinins suggesting that the nicotine-potentiating contractile effects in the airways might explain the mechanism of corticosteroid resistance in smoking asthma (Xu et al., 2010; Xu and Cardell, 2014).

Tabet et al. demonstrated a relationship between bradykinin-induced relaxation by means of hyperpolarization in human bronchi and the synthesis of the bronchorelaxing/hyperpolarizing factors epoxyeicosatrienoic acids produced by the endogenous CYP450 epoxygenase indicating a role for these arachidonic acid metabolites in hyperpolarization, via the large conductance Ca²⁺-sensitive K⁺ channel, of airway smooth muscle cells induced by bradykinin (Tabet et al., 2013).

Finally, we would like to point out that a main risk factor for asthma inception and/or worsening is chronic rhinitis (Brozek et al., 2017). Bradykinin- or allergen-induced nasal blockage and plasma extravasation are mediated by bradykinin B₂ receptors in the human nasal airway (Shirasaki et al., 2009; Thornton et al., 2013) and in animal models (Ricciardolo et al., 1994a; Valenti et al., 2008). Moreover, in allergic rhinitis, the expression of bradykinin B₁ receptor mRNA was significantly higher than in healthy subjects. Nasal allergen challenge increased bradykinin B₁ receptor mRNA

expression in subjects with allergic rhinitis (Christiansen et al., 2002) indicating that both kinin receptors are involved in vascular responses of chronic rhinitis.

6. B₁ and B₂ bradykinin receptor antagonists

In normal human airways there is minimal expression of constitutive bradykinin B₁ receptors in residential cells; this implies that the bronchoconstrictor response is mediated by bradykinin B₂ receptors (Abraham et al 2006; Ricciardolo et al., 2013).

Activation of bradykinin B₂ receptor is able to induce bronchoconstriction, vascular permeability, mucus secretion and cholinergic/sensory nerve stimulation. To counteract these effects several studies developed peptide and non-peptide receptor antagonists as potential therapeutic agents (Abraham et al 2006).

The class of peptide B₂ receptor antagonist is subdivided in three generation: the first is represented by NPC349 and later NPC567 and NPC17731: these are selective B₂ receptor antagonist but with a reduced biological activity because of their susceptibility to enzymatic cleavage; the second generation of peptide antagonists is more potent and selective than the previous generation and it is resistant to peptidases: major representative is HOE 140 (Icatibant); the third generation is represented by B-9430, a longer acting peptide antagonist with a dual interaction with both receptors (Abraham et al 2006).

Non-peptide antagonists were extensively studied: the first one WIN64338 had low affinity for human bradykinin B₂ receptor, then FR173657 and FR184280 seem to be more effective and lastly MEN16132 that is a selective B₂ receptor antagonist with good efficacy in inhibiting the bradykinin-induced bronchoconstriction in guinea pigs airways (Abraham et al 2006).

Peptide antagonists, including Icatibant, were widely studied both in animal models and in humans showing a potential effect in blocking bradykinin-induced bronchoconstriction (Leeb-Lundberg 2005). More than 20 years ago a double blinded, randomized, placebo-controlled phase II study has been performed in order to investigate the efficacy of the B₂ bradykinin receptor antagonist Icatibant on

moderate to severe asthmatics (Akbariy et al., 1996). Four weeks treatment of nebulized Icatibant (t.i.d. 900 µg or 3000 mcg) led to a dose-dependent improvement in pulmonary function tests (PFTs) but no clinically relevant improvement in global symptom score was noted compared to placebo. During the study it has not been shown an acute bronchodilator effect but a gradually (week by week) increase of functional parameters until the end of active treatment suggesting a role as anti-inflammatory drug. No other trials with Icatibant have been performed and currently it would be interesting to test its effect in selected population of asthmatics on the basis of phenotypes.

Non-peptide receptor antagonists, such as MEN16132 (Valenti et al 2008), seems to be more effective than peptide antagonists in cell cultures and in guinea pigs airways, but there are still too little data available on their efficacy in humans.

Activation and upregulation of bradykinin B₁ receptor in the airways of asthmatics is supported by the fact that its expression could be induced by several proinflammatory cytokines. Studies on B₁ receptor antagonists, R-715 (El-Kady et al., 2016) and BI113823 (Gurusamy et al., 2016), were conducted mainly on allergic airway inflammation. The former demonstrated a decrease in bronchial leukocyte infiltration, albumin, IL-1 β and serum OVA-specific IgE level and suppressed both bronchial cyclooxygenase-2 and inducible nitric oxide synthase expression (El-Kady et al., 2016). The latter reduced inflammatory cell infiltration, cytokine production and reduced mucus expression (Gurusamy et al., 2016). Also these B₁ receptor antagonists, as well as non-peptide B₂ receptor antagonists, were tested only in animal models.

7. Bradykinin and matrix-related airway remodelling

In asthma, the inflammatory milieu generates a state of abnormal activation of epithelial cells with release of growth factors, particularly TGF-β (Brightling et al., 2012; Ojiaku et al., 2017).

Consequently, the latter stimulates mesenchymal cell proliferation and promotes differentiation of fibroblasts into myofibroblasts which express alpha-smooth muscle actin and secrete increased

amount of extracellular matrix components, such as type I collagen (Al-Muhsen et al., 2011; Ojiaku et al., 2017). This cascade, known as the “epithelial mesenchymal trophic unit”, probably drives the remodelling of the asthmatic airways (Davies et al., 2003). TGF- β increases intracellular calcium mobilization in human airway smooth muscle cells, therefore enhancing airway smooth muscle responsiveness to bradykinin (Kim et al., 2005). Moreover, TGF- β modulates the responsiveness to bradykinin by increasing mRNA and protein expression of bradykinin B₂ receptor in human airway smooth muscle cells (Kim et al., 2005). In an *in vitro* model of embryonic kidney cells, bradykinin B₂ receptor-mediated G α q/11 activation was involved in cell proliferation via the mitogen-activated protein kinases (MAPK) activation, particularly p38 and extracellular-regulated kinase (ERK)1/2, and/or cross-talk with EGF-receptor (Zimmerman et al., 2011). Investigations of intracellular molecular signal mechanisms showed that activation of MAPK/NF- κ B signal pathways regulates cellular events like differentiation, proliferation, apoptosis and production of pro-inflammatory mediators as well as expression of GPCR in airways (Damera et al., 2012; Gerthoffer and Singer, 2003). In asthmatic patients, there are significant levels of phosphorylated ERK1/2 and p38 MAPK that correlate to the severity of airway disease (Liu et al., 2008; Vallese et al., 2015). Bradykinin induced the differentiation of normal human lung fibroblasts into myofibroblasts (α -smooth muscle actin [α -SMA]⁺ cells) via bradykinin B₂ receptor activation (Vancheri et al., 2005). We showed that bradykinin at low concentration (in the range of picomolar) induced cell proliferation in human bronchial fibroblasts from asthmatics and healthy controls, whereas at higher concentration (10⁻⁹-10⁻⁶M) bradykinin induced myofibroblast differentiation and blocks proliferation (Sabatini et al., 2013). In addition, the bradykinin-induced proliferation in asthmatic/normal fibroblast was dependent on metalloproteinase cleavage of the EGFR ligand precursors and EGFR transactivation, upon the bradykinin B₂ receptor-ligand interaction, through activation of the G-protein cascade. The subsequent phosphorylation of ERK1/2 was involved in the regulation of cell proliferation in response (Sabatini et al., 2013) (Fig. 2). On the other hand, the p38-MAPK pathway mediated bradykinin-induced human bronchial fibroblast differentiation and polymerization of α -SMA⁺ fibres

(Sabatini et al., 2013). These experimental observations, showing that different concentrations of bradykinin trigger various cellular responses, suggest a temporal sequence of fibroblast driven airway remodelling, able to produce a progressive reduction in the airway lumen diameter in chronic asthma. Bradykinin triggered a concentration-dependent fibroblast gel contraction in conjunction with α -SMA overexpression, a phenomenon inhibited in α -SMA-siRNA-treated cells (Petcchia et al., 2010). In addition, bradykinin increased F-actin⁺ stress fibres polymerization and intracellular calcium levels, with phosphorylation of the myosin light-chain. All these data indicate that bradykinin-induced differentiation of fibroblasts into myofibroblasts and their contraction is mediated via bradykinin B₂ receptor activation involving Ca²⁺-calmodulin phosphorylation of the myosin light-chain dependent pathway (Petcchia et al., 2010) (Fig.2).

8. Bradykinin and vascular airway remodelling

Angiogenesis, a process of formation of new capillaries, from existing microvessels involves several steps beginning with localised degradation of the basement membrane of the existing vessels. Endothelial cells detach from the basement membrane and migrate into the perivascular space where they proliferate. The new endothelial cells then form tube-like structures that eventually join and form new capillaries. Different promoting or inhibiting factors regulate this process. VEGF stimulates endothelial cell migration and proliferation and is widely expressed in highly vascularised organs such as lungs. Angiogenin is implicated as a mitogen for vascular endothelial cells and induces vascular endothelial cell proliferation, migration and tubule formation. The kinin-kallikrein system is also involved in angiogenesis; in particular high molecular weight kininogens, the residue derived from high molecular weight kininogens hydrolysis to form bradykinin, is an angiogenesis inhibitor able to block endothelial cell proliferation and migration. Moreover, bradykinin promotes angiogenesis by upregulation of bFGF or VEGF, by regulation of vascular permeability, by stimulation of cell proliferation through bradykinin B₂ receptor and by endothelial-nitric oxide synthase activation in endothelial cells (Colman, 2006). Angiogenesis is

recognised as a fundamental event in the development of airway remodelling that leads to irreversible obstruction (Postma and Timens, 2006). Proteins involved in angiogenesis, like VEGF and angiogenin are increased in asthma, and are associated with vascular remodelling (Hashimoto et al., 2005; Hoshino et al., 2001). We demonstrated that the VEGF-A⁺ cell counts in the bronchial mucosa (*lamina propria*) of old and young asthmatics were higher than in age matched non-smoking controls, and that endothelial CD31⁺, angiogenin⁺, bradykinin B₂ receptor⁺ and bradykinin B₁ receptor⁺ cell numbers in the *lamina propria* were higher in old asthmatics than in age matched non-smoking controls, smoking controls and COPD patients (Ricciardolo et al., 2013). Furthermore, angiogenin⁺ cells were significantly increased in patients with severe asthma compared with patients with mild asthma, in conjunction with a higher number of CD4⁺ cells, neutrophils and eosinophils. All of these data suggest that angiogenin-related vascular remodelling is a feature of severe asthma and asthma in the elderly (Ricciardolo et al., 2013). In asthmatic patients, the number of bradykinin B₂ receptor⁺ cells is positively related to the number of bradykinin B₁ receptor⁺, angiogenin⁺ and CD31⁺ cells, indicating a functional interplay between the kinin system and neoangiogenesis in asthma (Ricciardolo et al., 2013). In addition, only the numbers of angiogenin⁺ cells were negatively related to forced expiratory volume 1s which confirms a role for angiogenin in developing progressive airflow limitation and fixed airway obstruction in chronic asthma. Interestingly, the number of neutrophils in the *lamina propria* of asthmatic bronchial biopsies was related to the number of CD31⁺ cells and B₂ receptor⁺ cells (Fig. 3) suggesting that neutrophilic asthma is associated with bradykinin B₂ receptor expression and increased vascularization in asthmatic airways.

Confocal microscopy showed that CD31⁺ cells (marker of endothelial cells) of capillary vessels in the *lamina propria* of asthmatic patients also co-express bradykinin B₂ receptors, demonstrating a role for bradykinin B₂ receptors in airway microvessel function (Ricciardolo et al., 2013). In addition, the large majority of cells in the *lamina propria* of asthmatics were fibroblasts (5B5⁺) co-expressing bradykinin B₂ receptors (72%), VEGF-A (87%) and angiogenin (80.5%). In an *in vitro*

study, bradykinin induces a significant increase of VEGF and angiogenin release in human asthmatic bronchial fibroblasts, and in particular angiogenin release was relatively higher in comparison with human bronchial fibroblast from COPD patients (Ricciardolo et al., 2013). These data infer the involvement of bradykinin receptors in modulating bronchial vascular remodelling in asthma, via fibroblasts activation and subsequent release of vascular growth factors (Fig. 1).

9. Conclusion

Kinins and their receptors are detected in the respiratory tract and could be highly produced and expressed under specific pathophysiological conditions in asthma. For a long time, bradykinin has been considered a mediator of acute responses in asthma (mainly as a mediator of neurogenic inflammation and airway responsiveness), but most recent evidence sheds light on additional long-term effects of bradykinin in mesenchymal and endothelial cells activating downstream cascades (including release of growth factors and stimulation of MAPK/EGFR) involved in the chronicity and severity of asthma such as matrix- and vascular-related airway remodelling.

Bradykinin and their receptors are highly involved in allergen-induced responses and it seems that bradykinin B₂ receptor expression and bradykinin-related activities are specifically modulated by Th2 responses (due to IL-4/IL-13) in asthmatic fibroblasts pointing to a role in the remodelling process related to allergic severe asthma. On the other hand, also smoke- and virus-induced asthma showed up-regulation of bradykinin receptors. The latter seems to be associated with neutrophil-dependent inflammatory process with a relative steroid resistance (“neutrophilic asthma”).

Future studies are warranted in order to focus on the role of bradykinin and related receptors in specific asthma phenotypes (type 2 or non-type 2) (Wenzel et al., 2012) where the bradykinin pathway should be involved as molecular targets (personalized therapy). Actually, therapeutic strategies might be directed against the synthesis of kinins or the expression of bradykinin receptors

in order to modulate one of the most important clinically relevant features of asthma, namely airway remodelling.

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Legend to the figures

Fig. 1: Effects of bradykinin on asthmatic airways.

In airway epithelial cells bradykinin stimulates the release of bronchorelaxing factors PGE₂ and nitric oxide, the mucus secretion from human submucosal glands and the ion transport. Bradykinin is a potent inducer of airway microvascular leakage and vascular permeability and causes prolonged leakage at all airway levels. Moreover it has direct effects on the recruitment and activation of inflammatory cells. Upon stimulation of bradykinin receptors, fibroblasts activation provokes the release of vascular growth factors (VEGF and angiogenin) able to modulate bronchial vascular remodelling in asthma. In airway smooth muscle bradykinin induces bronchoconstriction via direct stimulation of its receptors and via indirect neural activation (cholinergic nerves and C-fibre sensory nerves); nevertheless, epithelium-derived PGE₂ and nitric oxide reduce excessive bronchoconstriction in asthma.

Fig. 2: Bradykinin-induced intracellular pathways in human bronchial fibroblasts.

Bradykinin B₂ receptor coordinates human bronchial fibroblast proliferation and differentiation. These effects on bronchial fibroblast are due to activation of bradykinin B₂ receptor and to subsequent stimulation of different MAPK/ERK pathways with the involvement of the metalloproteinase/EGF receptor signalling cascade.

Low concentration of bradykinin is able to induce fibroblast proliferation via phosphorylation of p38 and ERK1/2. The latter is phosphorylated through metalloprotease-mediated cleavage of the EGF receptor ligand precursor. Higher concentration of bradykinin provokes the differentiation from fibroblast to myofibroblast via p38 pathway only. Myofibroblast differentiation is characterized by α -SMA polymerization in F-actin⁺ stress fibres and increased intracellular calcium levels in conjunction with phosphorylation of myosin light-chain. The organization in cytoskeletal

filaments of α -SMA and F-actin⁺ stress fibres reflects the plasticity of activated myofibroblasts promoting contractile behaviour.

Fig. 3: Correlations between the number of neutrophils and CD31⁺ cells or bradykinin B₂ receptor⁺ cells in the *lamina propria* of asthmatic bronchial biopsies.

Panel A: correlation between number of neutrophils and CD31⁺ cells in the *lamina propria* of bronchial biopsies in asthmatics (cell/mm²) $r_s=0,47$, $P=0,02$

Panel B: correlation between number of neutrophils and B₂ receptor⁺ cells in the *lamina propria* of bronchial biopsies in asthmatics (cell/mm²) $r_s=0,44$, $P=0,007$

These data are derived from: Ricciardolo et al. Expression of vascular remodelling markers in relation to bradykinin receptors in asthma and COPD. *Thorax*. 2013 Sep;68(9):803-11.

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