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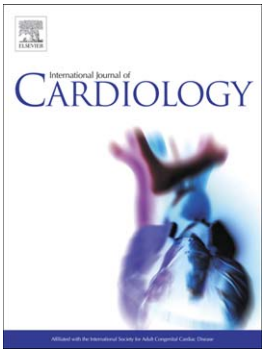

Brain natriuretic peptide: Much more than a biomarker

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Brain natriuretic peptide: much more than a biomarker

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Abstract

Brain natriuretic peptide (BNP) modulates several biological processes by activating the natriuretic peptide receptor A (NPR-A). Atria and ventricles secrete BNP. BNP increases natriuresis, diuresis and vasodilatation, thus resulting in a decreased cardiac workload.

BNP and NT-proBNP, which is the biologically inactive N-terminal portion of its pro-hormone, are fast and sensitive biomarkers for diagnosing heart failure. The plasma concentrations of both BNP and NT-proBNP also correlate with left ventricular function in patients with acute exacerbation of COPD, even without history of heart failure. Several studies have been conducted *in vitro* and *in vivo*, both in animals and in humans, in order to assess the potential role of the NPR-A activation as a novel therapeutic approach for treating obstructive pulmonary disorders. Unfortunately, these studies have yielded conflicting results.

Nevertheless, further recent specific studies, performed in *ex vivo* models of asthma and COPD, have confirmed the bronchorelaxant effect of BNP and its protective role against bronchial hyperresponsiveness in human airways. These studies have also clarified the intimate mechanism of action of BNP, represented by an autocrine loop elicited by the activation of NPR-A, localized on bronchial epithelium, and the relaxant response of the surrounding ASM, that does not expresses NPR-A.

Therefore, this review explores the teleological activities and paradoxical effects of BNP with regard of chronic obstructive respiratory disorders, and

provides an excursus on the main scientific findings that explain why BNP should be considered much more than a biomarker.

Keywords

BNP, NPR-A, asthma, COPD, human airway smooth muscle

1. Background

Natriuretic peptide (NP) hormones are small cardiovascular-derived peptides characterized by a 17 aminoacid ring and include atrial natriuretic peptide (ANP), brain natriuretic peptide (BNP) and C-type natriuretic peptide (CNP) [1]. NPs are encoded by different genes, synthesized as prepropeptides and stored as high molecular mass propeptides (proANP, proBNP and proCNP). The cleavage of propeptide results in the formation of the daughter ANP, BNP and CNP, which are characterized by lower molecular mass [1].

NPs modulate several biological effects by interacting with specific natriuretic peptide receptors (NPRs) including NPR-A, NPR-B and NPR-C, a family of homologous single-transmembrane, glycosylated receptors [2, 3]. The stimulation of NPR-A and NPR-B activates an intracellular particulate domain with guanylate cyclase (GC) activity that promotes the synthesis of cyclic guanosine monophosphate (cGMP) [4]. NPR-C does not modulate cGMP levels but inhibits adenylyl cyclase (AC) and activates phospholipase C (PLC), and removes NPs from the circulation. In fact NPR-C serves as a “clearance” receptor leading to internalization and lysosomal degradation of NPs [5, 6].

ANP and BNP are the biological ligands of NPR-A, whereas CNP preferentially binds to NPR-B. ANP and BNP both have a relatively high affinity for their respective receptor sub-types, although ANP is about 10 fold more potent than BNP [1, 7]. NPR-A is expressed in the cardiovascular system (cardiac atria and ventricles, aorta and peripheral vasculature), kidney, skin, platelets, and sympathetic fibers [1]. In both animals and man NPR-A has also been widely identified on a variety of pulmonary cells such as endothelial and smooth muscle cells of pulmonary blood vessels, type II

alveolar cells, and epithelial and airway smooth muscle cells in bronchi and bronchioles [8-12], whereas NPR-B is mainly expressed in veins as compared with arteries [7].

2. Cardiovascular and renal actions of NPs

Under physiological conditions ANP is secreted from cardiac atria, BNP from both atria and ventricles, whereas CNP is mainly released primarily from nervous tissue and vascular endothelium [1]. However, in a number of cardiovascular disorders and conditions associated with elevated blood pressure or volume overload, increased gene expression of ANP may be detected in the left ventricle [13] in association with rapid and constant enhancement of BNP transcripts [14, 15].

ANP and BNP have similar pharmacological profiles since they act on the same NP receptor and can induce natriuresis, vasodilatation and inhibition of aldosterone synthesis. Furthermore, these NPs have anti-mitogenic effects on endothelial and vascular smooth muscle cells [16]. In the central nervous system both ANP and BNP induce thirst suppression, inhibition of the release of antidiuretic and adrenocorticotropic hormones, as well as a reduction of sympathetic tone. Altogether these effects contribute to the hypotensive properties of NPs [16].

The action of CNP is different compared with that of ANP and BNP since it acts as an autocrine/paracrine mediator in blood vessels, through the modulation of vascular tone and cell growth [1, 16]. Thus, CNP is less effective at inducing diuresis and natriuresis compared with the other NPs but

is more effective at modulating the autonomic control of vascular tone [17, 18].

These pharmacological properties of NPs underpin why they have been implicated in the pathogenesis of congestive heart failure, as proposed by Woodard and Rosado [19]. In fact ANP and BNP increase both natriuresis and diuresis and induce local vasodilatation in response to cardiac failure, whereas CNP modulates the cardiac remodeling and inhibits the proliferation of vascular smooth muscle cells (VSMCs) [19]. Taken together, these actions of NPs lead to a reduction in blood pressure and circulatory volume, resulting in a decreased cardiac workload [19].

3. BNP as a biomarker

There is a large body of evidence that the levels of BNP and the biologically inactive N-terminal portion of its pro-hormone, NT-proBNP, correlate well with the severity of heart failure [20]. Since they function as an indicator of increased ventricular mass and a surrogate marker for heart failure, NT-proBNP and BNP are regarded as biomarkers, namely biological parameters that are objectively measured and evaluated as indicators of normal biological processes, pathogenic processes or pharmacological responses to a therapeutic intervention [21].

NT-proBNP has a longer plasma half-life and exists at considerably higher concentrations compared with BNP [22]. It is significantly more stable at room temperate and current laboratory assays are highly sensitive and specific.

Furthermore, all commercially available NT-proBNP assays utilize the same set of antibodies, which greatly simplifies intra-laboratory comparisons.

When used in conjunction with other clinical information, BNP and NT-proBNP levels are useful in establishing or ruling out the diagnosis of heart failure in patients with acute dyspnea [23]. For both BNP and NT-proBNP to exclude acute heart failure in symptomatic patients, very low values are necessary [24]. For BNP, the value is approximately 20 to 30 pg/mL, while for NT-proBNP, a cut point that has a negative predictive value of 98% to 99% is 300 pg/mL. Values above these levels, whether or not they are below the rule in cut point, may be associated with heart failure. However, the International Collaborative Of NT-proBNP (ICON) study [25] suggested that, for the exclusion of acute heart failure, a general age-independent cut-point of 300 pg/ml should be used, whereas for diagnosis of heart failure, age-dependent cut-points are more useful: namely NT-proBNP >450 pg/ml for patients <50 years; >900 pg/ml for patients in between 50 and 75 years; and NT-proBNP >1,800 for patients >75 years.

Since the use of BNP and NT-proBNP for the diagnosis of heart failure has dramatically impacted the standard of care in this pathological condition, all major societies recommend the use of these biomarkers for the diagnosis of heart failure in their clinical practice guidelines [20, 26, 27]. BNP and NT-proBNP concentrations typically fall with therapies proven to improve mortality in heart failure with decreased left ventricular ejection fraction [28-30]. BNP-guided therapies decrease mortality and reduce cardiovascular events, although do not decrease overall hospitalizations.

Adding routine BNP testing in patients with a history of asthma or chronic obstructive pulmonary disease (COPD) increases the detection of newly diagnosed or previously unrecognized chronic heart failure by approximately 20% [31]. In any case, BNP levels are elevated in patients with pulmonary diseases, at least in those with concomitant right ventricular (RV) dysfunction and pulmonary arterial hypertension [32], although BNP levels are significantly lower in right heart failure due to COPD compared with right heart failure due to left ventricular systolic heart failure [33].

Elevated BNP concentrations identify significant pulmonary hypertension with a sensitivity of 0.85 and specificity of 0.88 and predicted mortality [34]. It has been shown that plasma BNP levels may be elevated in patients with COPD and correlate not only with pulmonary arterial pressure but also with forced vital capacity (FVC), forced expiratory volume in 1 s (FEV_1) and partial arterial oxygen pressure [35]. However, there is contrasting documentation indicating that plasma BNP levels are also elevated in patients with stable COPD without pulmonary hypertension or cor pulmonale [36]. In these patients, there is no significant correlation between plasma BNP level and pulmonary function or hypoxia, but there is a significant correlation between plasma BNP level and % ejection fraction and pulmonary artery systolic pressure. Intriguingly, they are also increased in patients with COPD with normal right ventricular function after exercise [37].

Several studies have highlighted the importance of the BNP dosage in detecting left ventricular dysfunction in patients with acute exacerbation of COPD (AECOPD), even without history of heart failure [38-40], although echocardiographic examinations are able to document cardiac systolic and

diastolic dysfunction in only a small number of patients during the AECOPD [41]. Whatever the case may be, the period until initial AECOPD in subjects with high plasma BNP level seems to be significantly shorter [36]. Furthermore, in patients with AECOPD, BNP levels independently predict the need for intensive care [42], and elevated levels of NT-proBNP are strong predictors of early mortality among patients admitted to hospital with acute exacerbations of COPD independently of other known prognostic indicators [43]. There may be a link between an elevated level of BNP or NT-proBNP and increased cardiovascular mortality in AECOPD, although the data currently available are not conclusive [44].

4. NPs and airway smooth muscle cell

Several studies have revealed that airway smooth muscle (ASM) cells obtained from subjects with asthma display mechanical and phenotypical differences from that of ASM obtained from non-asthmatic subjects. ASM cells obtained from subjects with asthma showed a marked increase in force generation, capacity of shortening, degree of shortening and sensitivity to agonists [45, 46]. Moreover, hypertrophy of ASM in patients with severe asthma has been associated with a 5-fold greater positivity for markers of proliferating than ASM obtained from healthy subjects [47]. However, other studies failed to document mechanical differences between ASM derived from asthmatic and non-asthmatic donors [48, 49]. An increased amount of expression of contractile cytoskeletal proteins that characterizes the contractile phenotype have also been described [50], as have phenotypic differences in the sensitivity to proliferative and apoptotic stimuli for ASM [51].

Studies on the expression of components of the contractile cytoskeletal have also been observed in ASM obtained by endobronchial biopsy from subjects with asthma, demonstrated as an increased mRNA expression of myocitic markers, including myosin light chain kinase and total smooth muscle myosin heavy chain, when compared to ASM obtained from non-asthmatic donors [52, 53]. The increased expression of specific smooth muscle markers has been linked to increased bronchial smooth muscle mass and ASM cell functional differences observed in asthmatic patients [54]. Most of the information concerning the effects of BNP on ASM cell proliferation has been obtained from *in vitro* models. For example, BNP inhibited angiotensin II-induced smooth muscle cell proliferation, likely mediated by a decreased calcium influx, reduced ROS production and Akt signal transduction [55].

5. Expression of NPRs in human bronchi

RT- and qRT-PCR both documented that human bronchial tissue expresses significant levels of NPR-A transcripts, whereas the gene expression of NPR-B and NPR-C were scarce or even not detectable [8]. As evidenced by immunohistochemistry, NPR-A was localized at the level of bronchial epithelium and inflammatory cells of lamina propria, whereas NPR-A was barely detected in ASM and absent on the surface of goblet cells [8]. NPR-A transcripts were also detected on BEAS-2B cells, an immortalized human bronchial epithelial cell line that has been widely used to study the effect of BNP on human bronchi and ASM [8, 56, 57].

Neither the passive sensitization of human bronchi, an *ex vivo* model that closely mimics important characteristics of BHR in asthmatic patients [8, 56, 58-60], did not modify the NPR-A gene expression [8], nor the stimulation with methacholine or histamine altered the NPR-A levels [8]. NPR-A transcripts were increased after BNP treatment in both non-sensitized and passively sensitized bronchi, whereas the selective antagonism of M_2 muscarinic receptors by methoctramine prevented the receptor over-expression, suggesting that antagonizing M_2 muscarinic receptors may act as a negative feedback loop in the NPR-A transcriptional pathway [8].

6. NPR-A activation and airways: past and recent knowledge

NPR-A is expressed in the airways of several animal species [9-11] and administration of exogenous ANP induced relaxation of ASM of rats, guinea pigs and cows *in vitro* [61-63]. Unfortunately, only a few studies have investigated the ability of ANP to induce relaxation of human ASM *in vitro*, and the limited data available in the literature have proposed conflicting conclusions, with some studies documenting weak relaxation [64] and others showing no significant relaxant effect [65]. In any case, ANP seemed to have a protective effect on propranolol-induced bronchoconstriction in allergic guinea pigs *in vivo* [66]. ANP reduce airway resistance in normal subjects [67]. Further studies have demonstrated that ANP may induce bronchodilation and prevent bronchial hyperresponsiveness (BHR) in asthmatic patients when given intravenously or by inhalation, and have also demonstrated the ability of ANP to modify the bronchoconstriction in response to inhaled histamine or nebulized water in man [68-74].

Studies carried out in laboratory animals have also demonstrated that BNP may relax tracheal smooth muscle *in vitro* and prevent ovalbumin-induced bronchoconstriction and microvascular leakage *in vivo* [75, 76]. Nevertheless, until some years ago specific research on the ability of BNP to relax human ASM were still lacking in the literature. However, it has been documented that the administration of nesiritide, a human recombinant BNP, induces bronchodilation in patients with asthma and that PL-3994, a novel NPR-A agonist resistant to neutral endopeptidase, relaxes the tone of human precision-cut lung slices (PCLS) pre-contracted with carbachol [77, 78]. Combined, these evidences (Table 1) would suggest that the activation of NPR-A might modulate bronchial tone in patients suffering from chronic obstructive pulmonary disorders.

7. Influence of BNP on the contractile tone of human ASM: current knowledge

BNP was ineffective at relaxing medium isolated human bronchi pre-contracted with carbachol, and produced a weak relaxant response of passively sensitized airways pre-contracted with histamine (≃60% vs. maximal relaxation induced by papaverine) [58]. This modest effectiveness of BNP may be correlated with the absence of NPR-A at the level of ASM. Moreover, the modest effect of BNP on isolated human bronchi [58] is in contrast with the ability of nesiritide to produce considerable bronchodilation in patients with asthma [78]. This discrepancy may be explained by the fact that BNP probably does not act as a direct bronchodilator on ASM [56]. It has also

been suggested that the ability of BNP to improve lung function *in vivo* may be related to other effects, such as the reduction of airway microvascular leakage and plasma exudation into the airway [79, 80].

The bronchodilator effect of BNP has also been investigated in human small airways by using PCLS preparations and it has been compared with the effect elicited by PL-3994 [77]. In contrast to what has been observed in human isolated human bronchi [58], BNP was able to induce a relaxant response of bronchioles pre-contracted with carbachol (≃50% vs. maximal relaxation induced by salbutamol) [77]. Furthermore, although PL-3994 induced a potent and concentration-dependent relaxation of PCLS preparations, its effectiveness was modest (≃30% vs. maximal relaxation induced by salbutamol) and was less effective when compared with BNP [77].

Further studies have investigated why BNP appears to be more effective as a bronchoprotective agent than as a direct bronchodilator. Thus, recent studies have demonstrated that BNP shifts rightward the contraction-response curve induced by histamine in passively sensitized bronchi (potency reduced by ≃1 logarithms) and inhibits the contractile tone induced by carbachol in non-sensitized airway (maximal effect reduced by ≃70%), compared with BNP-untreated bronchi [8]. Intriguingly, in these experimental settings [8] the removal of epithelial cells from the bronchial lumen completely abolished the relaxant effects of BNP, suggesting that epithelium integrity is crucial for the modulatory role of BNP on the tone of human ASM.

Although *in vitro* studies carried out by using human isolated airways are useful to investigate the effect of drugs on airway tone, this experimental approach has certain limitations. For example, one cannot rule out the potential influence of cell types other than ASM in regulating bronchial tone.

Indeed, in an attempt to identify the downstream mechanisms regarding the BNP-mediated relaxation of human ASM, the functional evidence obtained from *in vitro* experiments with isolated human bronchi have been supplemented by other types of *in vitro* laboratory studies using BEAS-2B cells and cultured human ASM cells [56]. Whilst BNP was unable to modulate *per se* the contractile response of asthmatic ASM cells, incubation with the supernatant from BEAS-2B cells pre-treated with BNP was effective in inhibiting the contractile response to histamine, reducing the contractile potency more than 1.5 logarithms [56]. Furthermore, the indirect relaxant effect of BNP in asthmatic ASM cells was comparable with that elicited by salbutamol [56].

The pharmacological characterization of BNP in modulating the human ASM contractility is reported in Table 2.

The relative activation of myosin light chain kinase (MLCK) and myosin light chain phosphatase (MLCP), a trimeric enzyme with a catalytic subunit called myosin phosphatase target subunit 1 (MYPT1) regulates ASM tone. The phorphorylation of MYPT1 makes MLCP inactive, resulting in a sustained myogenic tone [81]. Recently, it has been demonstrated that ASM cells, collected from asthmatic donors and incubated with the medium of BEAS-2B cells pre-treated with BNP, over-expressed the gene and protein levels of unphosphorylated and active form of MYPT1, whereas the phosphorylated

and inactive pMYPT1^{Thr696} form was inhibited [56]. These findings provide an important piece of information concerning the functional antagonistic effect of BNP to protect against stimulation of ASM cells with histamine [8].

Since asthmatic ASM cells may directly alter their microenvironment by producing extracellular matrix proteins, pro-inflammatory mediators and adhesion receptors [82], further studies have been performed in human asthmatic ASM to elucidate the molecular and intracellular gene pathways involved in response to BNP challenge. Interestingly, although BNP indirectly modulated the activation of myosin light chain (MLC) by inhibiting the MLC phosphorylation (MLC-P) and the consequent interaction with actin, recent results have demonstrated that the BNP-induced relaxation of ASM did not depend upon immediate changes of expression of alpha-smooth muscle actin isoform (α-SMA). In fact, both immunofluorescence and western blotting analysis documented an analogous distribution and expression of α-SMA in asthmatic ASM cells incubated with the supernatant of BEAS-2B cells treated with BNP, compared with untreated cells [57]. On the other hand, the supernatant of BNP-treated BEAS-2B cells induced a rapid down-regulation of both calcium homeostasis-associated and epidermal growth factor receptor (EGFR) gene levels in asthmatic ASM cells [57].

8. The mechanism of action of BNP in human bronchi: from teleological activity to paradoxical effect

The prevalent localization of NPR-A at the level of respiratory epithelium, and the absence of this receptor on ASM, suggests that the bronchoprotective role

elicited by BNP in both medium and small airways is indirect and potentially mediated by some autocrine mechanism. In addition, the results concerning the influence of BNP on the contractile tone of human ASM suggest that this NP may have a noteworthy teleological effect at the level of human respiratory system. Nevertheless, the specific mechanisms of action and the intimate pathways modulated by BNP via the activation of epithelial NPR-A have been only recently elucidated.

As in the case of epithelium removal, the selective antagonism of M_2 muscarinic receptor by methoctramine and the selective inhibition of inducible nitric oxide synthase (iNOS) by aminoguanidine completely abolished the bronchoprotective effect of BNP in human isolated bronchi and asthmatic ASM cells [8, 56]. BNP indirectly enhanced the gene transcripts and the protein expression of iNOS in epithelium-intact bronchi and asthmatic ASM cells, but not in epithelium-denuded airways and human bronchial epithelial cells [8, 56]. In addition, inhibiting the vesicular release of endogenous acetylcholine from bronchial epithelial cells by quinine, an organic cation transporters (OCT) inhibitor [83, 84], also reduced the bronchoprotective effect of BNP in a concentration dependent manner [8].

These evidences indicated that the integrity of bronchial epithelial cells, the activation of M_2 muscarinic receptor and the activity of iNOS synthase are necessary conditions to allow the BNP-mediated effect on lung function.

Further experiments demonstrated that BNP enhances the acetylcholine release from both epithelium-intact bronchi and BEAS-2B cells, but not from epithelium-denuded airways [8]. Therefore, we supposed that the acetylcholine itself, released from the respiratory non-neural cholinergic

system, might play a crucial role in the pathway that links the activation of epithelial NPR-A by BNP and the ASM relaxant response.

This hypothesis has been confirmed by results obtained from experiments carried out by stimulating isolated bronchi and asthmatic ASM cells with very low concentrations of exogenous acetylcholine, in order to mimic the endogenous release of acetylcholine from non-neural cells [83, 84]. Paradoxically, we have evidenced that acetylcholine administered at pico-nanomolar concentrations induced a modest but significant relaxation of both human isolated bronchi (≃30% vs. maximal relaxation induced by papaverine) and asthmatic ASM cells (≃20% vs. maximal relaxation induced by papaverine) pre-contracted by histamine whereas, as expected, at higher concentrations acetylcholine induced contractile response [83, 84]. Moreover, very low concentrations of acetylcholine enhanced the NO levels in both epithelium-intact and epithelium-denuded bronchi, an effect that was abolished by methoctramine and aminoguanidine [8]. The direct exposure of asthmatic ASM cells and BEAS-2B cells to BNP did not modulate the NO levels [8, 56], whereas the supernatant of BEAS-2B cells treated with BNP significantly increased the NO levels of asthmatic ASM cells, an effect that, also in this case, was abolished by methoctramine and aminoguanidine [56].

9. BNP: clinical considerations

BNP and NT-proBNP are fast and sensitive biomarkers for diagnosing heart failure. In patients with COPD, the plasma concentrations of both BNP and NT-proBNP increase proportionally to the severity of right ventricular diastolic

dysfunction. For this reason we strongly suggest the addition of routine BNP testing in patients with a history of COPD increases the detection of newly diagnosed or previously unrecognized heart failure. Furthermore, BNP testing could represent advance in the management of patients with AECOPD because potentially it allows treatment monitoring. Interestingly, BNP levels can fall not only when patients are treated with diuretics, inotropes, and vasopressors. We documented that β_2-agonists are able to induce a rapid reduction in BNP levels in patients admitted to emergency department for AECOPD [85]. It is not easy to explain why β_2-agonists decrease BNP levels. The most plausible hypothesis is that they are able to influence the pulmonary hemodynamics. Alternatively, we can suggest that β_2-agonists are able to cause an attenuation of air trapping, leading to a reduction of intrathoracic pressure, including pressure on the whole heart, and, consequently, to an improvement of right ventricular overload and left ventricular diastolic dysfunction.

The big issue is that, in our opinion, BNP is not only a biomarker. The BNP Consensus Panel 2004 already highlighted that the rapidly evolving spectrum of therapeutic benefit and the emerging realm of additional therapeutic potential positions BNP as an increasingly important treatment option in the management of a growing number of cardiovascular conditions [86]. Our findings, which support a teleological role for elevated BNP concentrations, at least in patients with COPD in whom BNP might be part of a response aimed at mitigating the effects of the disease [87], suggest that this concept can also be applied to patients with COPD, in which BNP might be part of a response aimed at mitigating the effects of the disease.

In order to test this hypothesis it would be of interest to evaluate the effect of BNP administration in patients with COPD. Nesiritide is the recombinant form of human BNP and has been tested in patients with acute heart failure and it is approved for the treatment of acute decompensated congestive heart failure although its use is not associated with a change in mortality or re-hospitalizations. To date nesiritide has never been tested in patients with COPD in whom it may have, based on the above-mentioned assumptions, a beneficial effect on airway responsiveness.

10. Conclusions

The findings of recent studies carried out by our research group [8, 56-58] have permitted to clarify the pathway leading to the bronchorelaxant effect induced by BNP (Figure 1).

BNP binds to NPR-A expressed at the level of airway epithelium with consequent vesicular release of very low concentrations of acetylcholine from bronchial epithelial cells, such as ciliated cells and neuroendocrine cells [8, 83, 84]. Although there is less acetylcholine released from the airway epithelium compared with that from neurons [83, 84], it seems to be sufficient to activate prevalently postsynaptic M_2 muscarinic receptors localized on the surface of surrounding ASM cells. The stimulation of M_2 muscarinic receptor modulates the gene and protein expression of iNOS, that increases the NO levels in ASM and activates the NO/cGMP signaling. The NO/cGMP signaling is a proved pathway involved in the relaxation of ASM, which results in bronchodilation [88]. In fact the NO-mediated relaxation of ASM is controlled

by the activation of a soluble pool of GC that enhances the cGMP levels [56]. Thus, bronchial epithelium regulates the BNP-induced relaxant activity by an autocrine loop inducing the activation of the NO/cGMP pathway at the level of ASM. The NO/cGMP signaling stimulates specific protein kinases that, in turn, activate a number of targets such as MYPT1 [56]. MYPT1 fine tunes the MLCP activity with consequents ASM relaxation. In addition, the NO/cGMP pathway activated by BNP may prevent the BHR through a rapid modulation of calcium homeostasis and EGFR signaling in ASM, leading to the inhibition of MLCK activity [57, 89].

Concluding, the integrity of airway epithelium and its cooperation with ASM is crucial for the bronchorelaxant activity of BNP, suggesting for a teleological influence of this NP against the BHR and airway obstruction in asthma and COPD.

11. Expert opinion

BNP may represent an alternative therapeutic option for the treatment of chronic obstructive pulmonary disorders. The pharmacological rationale for administering BNP in asthmatic patients has been proved from bench to bedside [8, 56, 57, 78]. Furthermore, it has been suggested that BNP may also modulate the bronchial tone in COPD [8], since this NP prevented the cholinergic tone in human isolated airways.

The bronchorelaxant activity of BNP is mediated by the interaction with the NPR-A localized at the level of bronchial epithelial cells. Therefore, the administration of BNP via inhalation is required to deliver this NP topically on

the airway epithelium. The inhalant administration of BNP should reduce the risk of hypotension, the main potential cardiovascular adverse event related with the systemic activation of NPR-A [56]. Epithelium integrity is an essential condition for the effectiveness of BNP but, unfortunately, it has been widely documented that asthmatic patients can present epithelium abnormalities and destruction at all levels of the airways [90, 91]. In addition, also COPD is associated with bronchial epithelial changes [92] that may potentially affect the BNP activity. These peculiarities suggest that BNP may have a role as a bronchodilator agent prevalently in stable and controlled patients, and that should be administered in combination with further bronchodilators in order to optimize the therapeutic approach [93].

The density of vagal innervation is greatest in proximal airways and diminishes peripherally, being almost insignificant or absent at the level of bronchioles, at the least in human airways [94, 95]. BNP modulates the human bronchial tone independently from the interaction with the parasympathetic system, thus it may have a relevant influence as a bronchodilator agent at the level of small airways. Nevertheless, the relatively high molecular weight of BNP (3.7 kDa) [96], compared with that of the bronchodilators that are currently used in the clinical practice (overall <0.4 kDa) [97], might imply technological difficulties in developing specific devices that are able to deliver this NP up to small airways.

In any case, BNP has the noteworthy characteristic to stimulate a physiologic autocrine loop at the level of bronchial wall, leading to bronchorelaxation and protection against BHR, representing a potential emerging drug for chronic obstructive pulmonary disorders [98]. Moreover, we cannot exclude potential

synergistic interaction of BNP when administered by inhalation in combination with low doses of further bronchodilators characterized by different mechanisms of action, such as long-acting β_2 agonists (LABAs) and long-acting muscarinic receptor antagonists (LAMAs). Finally, this combination approach may be of benefit in the treatment of chronic obstructive airway disorders by optimizing bronchodilation and preventing potential adverse events [60, 93, 99-103].

Conflict of interest

The authors report no relationships that could be construed as a conflict of interest.

References

[1] S.P. D'Souza, M. Davis, G.F. Baxter, Autocrine and paracrine actions of natriuretic peptides in the heart, Pharmacol. Ther. 101 (2004) 113-129.

[2] T. Omland, T.A. Hagve, Natriuretic peptides: physiologic and analytic considerations, Heart Fail. Clin. 5 (2009) 471-487.

[3] L.R. Potter, A.R. Yoder, D.R. Flora, L.K. Antos, D.M. Dickey, Natriuretic peptides: their structures, receptors, physiologic functions and therapeutic applications, Handb. Exp. Pharmacol. 191 (2009) 341-366.

[4] L.R. Potter, S. Abbey-Hosch, D.M. Dickey, Natriuretic peptides, their receptors, and cyclic guanosine monophosphate-dependent signaling functions, Endocr. Rev. 27 (2006) 47-72.

[5] K.N. Pandey, Biology of natriuretic peptides and their receptors, Peptides 26 (2005) 901-932.

[6] M.B. Anand-Srivastava, P.D. Sehl, D.G. Lowe, Cytoplasmic domain of natriuretic peptide receptor-C inhibits adenylyl cyclase. Involvement of a pertussis toxin-sensitive G protein, J. Biol. Chem. 271 (1996) 19324-19329.

[7] C.Y. Lee, J.C. Burnett Jr, Natriuretic peptides and therapeutic applications, Heart Fail. Rev. 12 (2007) 131-142.

[8] M.G. Matera, L. Calzetta, D. Passeri, F. Facciolo, E.A. Rendina, C. Page, et al., Epithelium integrity is crucial for the relaxant activity of brain natriuretic peptide in human isolated bronchi, Br. J. Pharmacol. 163 (2011) 1740-1754.

[9] C. Bianchi, J. Gutkowska, G. Thibault, R. Garcia, J. Genest, M. Cantin, Radioautographic localization of 125I-atrial natriuretic factor (ANF) in rat tissues, Histochemistry 82 (1985) 441-452.

[10] Y. Ishii, T. Watanabe, M. Watanabe, S. Hasegawa, Y. Uchiyama, Effects of atrial natriuretic peptide on type II alveolar epithelial cells of the rat lung, Autoradiographic and morphometric studies, J. Anat. 166 (1989) 85-95.

[11] S. Kawaguchi, K. Uchida, T. Ito, M. Kozuka, M. Shimonaka, T. Mizuno, et al., Immunohistochemical localization of atrial natriuretic peptide receptor in bovine kidney and lung, J. Histochem. Cytochem. 37 (1989) 1739-1742.

[12] G. Hellermann, X. Kong, J. Gunnarsdóttir, H. San Juan, R. Singam, S. Behera, et al., Mechanism of bronchoprotective effects of a novel natriuretic hormone peptide, J. Allergy Clin. Immunol. 113 (2004) 79-85.

[13] H. Ruskoaho, Atrial natriuretic peptide: synthesis, release, and metabolism, Pharmacol. Rev. 44 (1992) 479-602.

[14] A.J. de Bold, B.G. Bruneau, M.L. Kuroski de Bold, Mechanical and neuroendocrine regulation of the endocrine heart, Cardiovasc. Res. 31 (1996) 7-18.

[15] H. Tokola, N. Hautala, M. Marttila, J. Magga, S. Pikkarainen, R. Kerkelä, et al., Mechanical load-induced alterations in B-type natriuretic peptide gene expression, Can. J. Physiol. Pharmacol. 79 (2001) 646-653.

[16] A. Ahluwalia, R.J. MacAllister, A.J. Hobbs, Vascular actions of natriuretic peptides. Cyclic GMP-dependent and -independent mechanisms, Basic Res. Cardiol. 99 (2004) 83-89.

[17] T. Sudoh, N. Minamino, K. Kangawa, H. Matsuo, C-type natriuretic peptide (CNP): a new member of natriuretic peptide family identified in porcine brain, Biochem. Biophys. Res. Commun. 168 (1990) 863-870.

[18] A.L. Clavell, A.J. Stingo, C.M. Wei, D.M. Heublein, J.C. Burnett Jr., C-type natriuretic peptide: a selective cardiovascular peptide, Am. J. Physiol. 264 (1993) R290-295.
[19] G.E. Woodard, J.A. Rosado, Natriuretic peptides in vascular physiology and pathology, Int. Rev. Cell. Mol. Biol. 268 (2008) 59-93.
[20] P. Bettencourt, NT-proBNP and BNP: biomarkers for heart failure management, Eur. J. Heart Fail. 6 (2004) 359-363.
[21] M. Cazzola, G. Novelli, Biomarkers in COPD, Pulm. Pharmacol. Ther. 23 (2010) 493-500.
[22] B. Smit, A.M. Spoelstra-de Man, A.R. Girbes, M.C. de Waard, NT-proBNP in cardiopulmonary resuscitated patients treated with mild therapeutic hypothermia is not independently associated with mortality: a retrospective observational study, BMC. Anesthesiol. 15 (2015) 48.
[23] S.P. Wright, R.N. Doughty, A. Pearl, G.D. Gamble, G.A. Whalley, H.J. Walsh, et al., Plasma amino-terminal pro-brain natriuretic peptide and accuracy of heart-failure diagnosis in primary care: a randomized, controlled trial, J. Am. Coll. Cardiol. 42 (2003) 1793-1800.
[24] A.A. Mohammed, J.L. Januzzi Jr., Natriuretic peptides in the diagnosis and management of acute heart failure, Heart Fail. Clin. 5 (2009) 489-500.
[25] J.L. Januzzi, R. van Kimmenade, J. Lainchbury, A. Bayes-Genis, J. Ordonez-Llanos, M. Santalo-Bel, et al., NT-proBNP testing for diagnosis and short-term prognosis in acute destabilized heart failure: an international pooled analysis of 1256 patients: the International Collaborative of NT-proBNP Study, Eur. Heart. J. 27 (2006) 330-337.
[26] S.A. Hunt, W.T. Abraham, M.H. Chin, A.M. Feldman, G.S. Francis, T.G. Ganiats, et al., 2009 Focused update incorporated into the ACC/AHA 2005 Guidelines for the Diagnosis and Management of Heart Failure in Adults A Report of the American College of Cardiology Foundation/American Heart Association Task Force on Practice Guidelines Developed in Collaboration With the International Society for Heart and Lung Transplantation, J. Am. Coll. Cardiol. 53 (2009) e1-e90.
[27] J. Lindenfeld, N.M. Albert, J.P. Boehmer, S.P. Collins, J.A. Ezekowitz, M.M. Givertz, et al., HFSA 2010 Comprehensive Heart Failure Practice Guideline, J. Card. Fail. 16 (2010) e1-194.
[28] P. Porapakkham, P. Porapakkham, H. Zimmet, B. Billah, H. Krum, B-type natriuretic peptide-guided heart failure therapy: A meta-analysis, Arch. Intern. Med. 170 (2010) 507-514.
[29] J.L. Januzzi Jr., S.U. Rehman, A.A. Mohammed, A. Bhardwaj, L. Barajas, J. Barajas, et al., Use of amino-terminal pro-B-type natriuretic peptide to guide outpatient therapy of patients with chronic left ventricular systolic dysfunction, J. Am. Coll. Cardiol. 58 (2011) 1881-1889.
[30] H.K. Gaggin, Q.A. Truong, S.U. Rehman, A.A. Mohammed, A. Bhardwaj, K.A. Parks, et al., Characterization and prediction of natriuretic peptide "nonresponse" during heart failure management: results from the ProBNP Outpatient Tailored Chronic Heart Failure (PROTECT) and the NT-proBNP-Assisted Treatment to Lessen Serial Cardiac Readmissions and Death (BATTLESCARRED) study, Congest. Heart Fail. 19 (2013) 135-142.
[31] P.A. McCullough, J.E. Hollander, R.M. Nowak, A.B. Storrow, P. Duc, T. Omland, et al., Uncovering heart failure in patients with a history of

pulmonary disease: rationale for the early use of B-type natriuretic peptide in the emergency department, Acad. Emerg. Med. 10 (2003) 198-204.
[32] L.B. Yap, D. Mukerjee, P.M. Timms, H. Ashrafian, J.G. Coghlan, Natriuretic peptides, respiratory disease, and the right heart, Chest 126 (2004) 1330-1336.
[33] N. Flessas, I. Alexanian, J. Parissis, D. Kremastinos, J. Lekakis, G. Filippatos, Plasma activity of B-type natriuretic peptide in patients with biventricular heart failure versus those with right heart failure due to chronic obstructive pulmonary disease, J. Cardiovasc. Med. (Hagerstown) 15 (2014) 476-480.
[34] H.H. Leuchte, R.A. Baumgartner, M.E. Nounou, M. Vogeser, C. Neurohr, M. Trautnitz, et al., Brain natriuretic peptide is a prognostic parameter in chronic lung disease, Am. J. Respir. Crit. Care Med. 173 (2006) 744-750.
[35] E. Bozkanat, E. Tozkoparan, O. Baysan, O. Deniz, F. Ciftci, M. Yokusoglu, The significance of elevated brain natriuretic peptide levels in chronic obstructive pulmonary disease, J. Int. Med. Res. 33 (2005) 537-544.
[36] Y. Inoue, T. Kawayama, T. Iwanaga, H. Aizawa, High plasma brain natriuretic peptide levels in stable COPD without pulmonary hypertension or cor pulmonale, Intern. Med. 48 (2009) 503-512.
[37] G. Gemici, R. Erdim, A. Celiker, S. Tokay, T. Ones, S. Inanir, et al., B-type natriuretic peptide levels in patients with COPD and normal right ventricular function, Adv. Ther. 25 (2008) 674-680.
[38] F. Abroug, L. Ouanes-Besbes, N. Nciri, N. Sellami, F. Addad, K.B. Hamda, et al., Association of left-heart dysfunction with severe exacerbation of chronic obstructive pulmonary disease: diagnostic performance of cardiac biomarkers, Am. J. Respir. Crit. Care Med. 174 (2006) 990-996.
[39] G.H. Abusaid, A. Barbagelata, E. Tuero, A. Mahmood, G. Sharma, Diastolic dysfunction and COPD exacerbation, Postgrad. Med. 121 (2009) 76-81.
[40] K. Gariani, A. Delabays, T.V. Perneger, T. Agoritsas, Use of brain natriuretic peptide to detect previously unknown left ventricular dysfunction in patients with acute exacerbation of chronic obstructive pulmonary disease, Swiss Med. Wkly. 141 (2011) 13298-13300.
[41] K. Nishimura, T. Nishimura, K. Onishi, T. Oga, Y. Hasegawa, P.W. Jones, Changes in plasma levels of B-type natriuretic peptide with acute exacerbations of chronic obstructive pulmonary disease, Int. J. Chron. Obstruct. Pulmon. Dis. 9 (2014) 155-162.
[42] D. Stolz, T. Breidthardt, M. Christ-Crain, R. Bingisser, D. Miedinger, J. Leuppi, et al., Use of B-type natriuretic peptide in the risk stratification of acute exacerbations of COPD, Chest 133 (2008) 1088-1094.
[43] C.L. Chang, S.C. Robinson, G.D. Mills, G.D. Sullivan, N.C. Karalus, J.D. McLachlan, et al., Biochemical markers of cardiac dysfunction predict mortality in acute exacerbations of COPD, Thorax 66 (2011) 764-768.
[44] A. Buchan, R. Bennett, A. Coad, S. Barnes, R. Russell, AR. Manuel, The role of cardiac biomarkers for predicting left ventricular dysfunction and cardiovascular mortality in acute exacerbations of COPD, Open Heart 2 (2015) e000052.

[45] R.J. Thomson, A.M. Bramley, R.R. Schellenberg, Airway muscle stereology: implications for increased shortening in asthma, Am. J. Respir. Crit. Care Med. 154 (1996) 749-757.
[46] T.R. Bai, Abnormalities in airway smooth muscle in fatal asthma, Am. Rev. Respir. Dis. 141 (1990) 552-557.
[47] M. Hassan M, T. Jo, P.A. Risse, B. Tolloczko, C. Lemière, R. Olivenstein, et al., Airway smooth muscle remodeling is a dynamic process in severe long-standing asthma, J. Allergy Clin. Immunol. 125 (2010) 1037-1045.e3.
[48] S.D. Whicker, C.L. Armour, J.L. Black, Responsiveness of bronchial smooth muscle from asthmatic patients to relaxant and contractile agonists, Pulm. Pharmacol. 1 (1988) 25-31.
[49] J. Cerrina, M. Le Roy Ladurie, C. Labat, B. Raffestin, A. Bayol, C. Brink, Comparison of human bronchial muscle responses to histamine in vivo with histamine and isoproterenol agonists in vitro, Am. Rev. Respir. Dis. 134 (1986) 57-61.
[50] A. Orlandi, H.P. Ehrlich, P. Ropraz, L.G. Spagnoli, G. Gabbiani, Rat aortic smooth muscle cells isolated from different layers and at different times after endothelial denudation show distinct biological features in vitro, Arterioscler. Thromb. 14 (1994) 982-989.
[51] A. Orlandi, A. Francesconi, Cocchia D, A. Corsini, L.G. Spagnoli, Phenotypic heterogeneity influences apoptotic susceptibility to retinoic acid and cis-platinum of rat arterial smooth muscle cells in vitro: Implications for the evolution of experimental intimal thickening, Arterioscler. Thromb. Vasc. Biol. 21 (2001) 1118-1123.
[52] J.K. Bentley, M.B. Hershenson, Airway smooth muscle growth in asthma: proliferation, hypertrophy, and migration, Proc. Am. Thorac. Soc. 5 (2008) 89-96.
[53] X. Ma, Z. Cheng, H. Kong, Y. Wang Y, H. Unruh, N.L. Stephens, et al. Changes in biophysical and biochemical properties of single bronchial smooth muscle cells from asthmatic subjects, Am. J. Physiol. Lung Cell. Mol. Physiol. 283 (2002) L1181-1189.
[54] P.J. Plant, M.L. North, A. Ward, M. Ward, N. Khanna, J. Correa, et al., Hypertrophic airway smooth muscle mass correlates with increased airway responsiveness in a murine model of asthma, Am. J. Respir. Cell. Mol. Biol. 46 (2012) 532-540.
[55] J.H. Hsu, S.F. Liou, S.N. Yang, B.N. Wu, Z.K. Dai, I.J. Chen, et al., B-type natriuretic peptide inhibits angiotensin II-induced proliferation and migration of pulmonary arterial smooth muscle cells, Pediatr. Pulmonol. 49 (2014) 734-744.
[56] L. Calzetta, D. Passeri, V. Kanabar, P. Rogliani, C. Page, M. Cazzola, et al., Brain natriuretic peptide protects against hyperresponsiveness of human asthmatic airway smooth muscle via an epithelial cell-dependent mechanism, Am. J. Respir. Cell. Mol. Biol. 50 (2014) 493-501.
[57] A. Orlandi, L. Calzetta, E. Doldo, C. Tarquini, M.G. Matera, D. Passeri, Brain natriuretic peptide modulates calcium homeostasis and epidermal growth factor receptor gene signalling in asthmatic airways smooth muscle cells, Pulm. Pharmacol. Ther. 31 (2015) 51-54.
[58] M.G. Matera, L. Calzetta, V. Parascandolo, G. Curradi, P. Rogliani, M. Cazzola, Relaxant effect of brain natriuretic peptide in nonsensitized and

passively sensitized isolated human bronchi, Pulm. Pharmacol. Ther. 22 (2009) 478-482.
[59] L. Calzetta, D. Spina, M. Cazzola, C.P. Page, F. Facciolo, E.A. Rendina, et al., Pharmacological characterization of adenosine receptors on isolated human bronchi, Am. J. Respir. Cell. Mol. Biol. 45 (2012) 1222-1231.
[60] L. Calzetta, C.P. Page, D. Spina, M. Cazzola, P. Rogliani, F. Facciolo, et al., Effect of the mixed phosphodiesterase 3/4 inhibitor RPL554 on human isolated bronchial smooth muscle tone, J. Pharmacol. Exp. Ther. 346 (2013) 414-423.
[61] L.B. Fernandes, J.M. Preuss, R.G. Goldie, Epithelial modulation of the relaxant activity of atriopeptides in rat and guinea-pig tracheal smooth muscle, Eur. J. Pharmacol. 212 (1992) 187-194.
[62] M. O'Donnell, R. Garippa, A.F. Welton, Relaxant activity of atriopeptins in isolated guinea pig airway and vascular smooth muscle, Peptides 6 (1985) 597-601.
[63] K. Ishii K, F. Murad, ANP relaxes bovine tracheal smooth muscle and increases cGMP, Am. J. Physiol. 256 (1989) C495-C500.
[64] R.M. Angus, J.E. Nally, R. McCall, L.C. Young, J.C. McGrath, N.C. Thomson, Modulation of the effect of atrial natriuretic peptide in human and bovine bronchi by phosphoramidon, Clin. Sci. (Lond.) 86 (1994) 291-295.
[65] M.L. Candenas, E. Naline, L. Puybasset, P. Devillier, C. Advenier, Effect of atrial natriuretic peptide and on atriopeptins on the human isolated bronchus. Comparison with the reactivity of the guinea-pig isolated trachea, Pulm. Pharmacol. 4 (1991) 120-125.
[66] M. Mizuguchi, S. Myo, M. Fujimura, Bronchoprotective effects of atrial natriuretic peptide against propranolol-induced bronchoconstriction after allergic reaction in guinea pigs, Clin. Exp. Allergy 30 (2000) 439-444.
[67] G. Hulks, A.G. Jardine, J.M. Connell, N.C. Thomson, Effect of atrial natriuretic factor on bronchomotor tone in the normal human airway, Clin. Sci. (Lond.) 79 (1990) 51-55.
[68] R.M. Angus, MJ McCallum, N.C. Thomson, Effect of inhaled atrial natriuretic peptide on methacholine induced bronchoconstriction in asthma, Clin. Exp. Allergy 24 (1994) 784-788.
[69] R.M. Angus, MJ McCallum, G. Hulks, N.C. Thomson, Bronchodilator, cardiovascular, and cyclic guanylyl monophosphate response to high-dose infused atrial natriuretic peptide in asthma, Am. Rev. Respir. Dis. 147 (1993) 1122-1125.
[70] R.M. Angus, E.A. Millar, G.W. Chalmers, N.C. Thomson, Effect of inhaled atrial natriuretic peptide and a neutral endopeptidase inhibitor on histamine-induced bronchoconstriction, Am. J. Respir. Crit. Care Med. 151 (1995) 2003-2005.
[71] G. Hulks, A. Jardine, J.M. Connell, N.C. Thomson, Bronchodilator effect of atrial natriuretic peptide in asthma, B.M.J. 299 (1989) 1081-1082.
[72] P. Chanez P, C. Mann, J. Bousquet, P.E. Chabrier, P. Godard, P. Braquet, et al., Atrial natriuretic factor (ANF) is a potent bronchodilator in asthma, J. Allergy Clin. Immunol. 86 (1990) 321-324.
[73] G. Hulks, A.G. Jardine, J.M. Connell, N.C. Thomson, Influence of elevated plasma levels of atrial natriuretic factor on bronchial reactivity in asthma, Am Rev Respir Dis 143 (1991) 778-782.

[74] L.G. McAlpine, G. Hulks, N.C. Thomson, Effect of atrial natriuretic peptide given by intravenous infusion on bronchoconstriction induced by ultrasonically nebulized distilled water (fog), Am. Rev. Respir. Dis. 146 (1992) 912-915.
[75] H. Ohbayashi, H. Suito, K. Takagi, Compared effects of natriuretic peptides on ovalbumin-induced asthmatic model, Eur. J. Pharmacol. 346 (1998) 55-64.
[76] K. Takagi, N. Araki, Relaxant effects of brain natriuretic peptide on guinea-pig tracheal smooth muscle, Clin. Exp. Pharmacol. Physiol. 20 (1993) 239-243.
[77] J.D. Edelson, M. Makhlina, K.R. Silvester, S.S. Vengurlekar, X. Chen, J. Zhang, et al., In vitro and in vivo pharmacological profile of PL-3994, a novel cyclic peptide (Hept-cyclo(Cys-His-Phe-d-Ala-Gly-Arg-d-Nle-Asp-Arg-Ile-Ser-Cys)-Tyr-[Arg mimetic]-NH(2)) natriuretic peptide receptor-A agonist that is resistant to neutral endopeptidase and acts as a bronchodilator, Pulm. Pharmacol. Ther. 26 (2013) 229-238.
[78] M.J. Akerman, M. Yaegashi, Z. Khiangte, A.T. Murugan, O. Abe, J.D. Marmur, Bronchodilator effect of infused B-type natriuretic peptide in asthma, Chest 130 (2006) 66-72.
[79] C.G. Persson, Role of plasma exudation in asthmatic airways, Lancet 2 (1986) 1126-1129.
[80] C. Passino, A.M. Sironi, B. Favilli, B. Favilli, R. Poletti, C. Prontera, et al., Right heart overload contributes to cardiac natriuretic hormone elevation in patients with heart failure, Int. J. Cardiol. 104 (2005) 39-45.
[81] D. Schaafsma, I.S. Bos, A.B. Zuidhof, J. Zaagsma, H. Meurs, Inhalation of the Rho-kinase inhibitor Y-27632 reverses allergen-induced airway hyperresponsiveness after the early and late asthmatic reaction, Respir. Res. 7 (2006) 121.
[82] D.B. Wright, T. Trian, S. Siddiqui, C.D. Pascoe, O.O. Ojo, J.R. Johnson, et al., Functional phenotype of airway myocytes from asthmatic airways, Pulm. Pharmacol. Ther. 26 (2013) 95-104.
[83] I. Wessler, C.J. Kirkpatrick, Acetylcholine beyond neurons: the non-neuronal cholinergic system in humans, Br. J. Pharmacol. 154 (2008) 1558-1571.
[84] W. Kummer, KS Lips, U. Pfeil, The epithelial cholinergic system of the airways, Histochem. Cell. Biol. 130 (2008) 219-234.
[85] A. Segreti, E. Fiori, L. Calzetta, M. Sabatini, V. Segreti, P. Rogliani, et al., The effect of indacaterol during an acute exacerbation of COPD, Pulm. Pharmacol. Ther. 26 (2013) 630-634.
[86] M.A. Silver, A. Maisel, C.W. Yancy, P.A. McCullough, J.C. Burnett Jr., G.S. Francis, et al., BNP Consensus Panel 2004: A clinical approach for the diagnostic, prognostic, screening, treatment monitoring, and therapeutic roles of natriuretic peptides in cardiovascular diseases, Congest. Heart Fail. 10 (2004) 1-30.
[87] M. Cazzola, M.G. Matera, B-type Natriuretic Peptide – Not Only a Biomarker, Eur. Respir. Dis. 7 (2011) 80-83.
[88] F. Mullershausen, A. Lange, E. Mergia, A. Friebe, D. Koesling, Desensitization of NO/cGMP signaling in smooth muscle: blood vessels versus airways, Mol. Pharmacol. 69 (2006) 1969-1974.

[89] J.J. Khatri, K.M. Joyce, F.V. Brozovich, S.A. Fisher, Role of myosin phosphatase isoforms in cGMP-mediated smooth muscle relaxation, J. Biol. Chem. 276 (2001) 37250-37257.
[90] L.A. Laitinen, M. Heino, A. Laitinen, T. Kava, T. Haahtela, Damage of the airway epithelium and bronchial reactivity in patients with asthma, Am. Rev. Respir. Dis. 131 (1985) 599-606.
[91] S.T. Holgate, The airway epithelium is central to the pathogenesis of asthma, Allergol. Int. 57 (2008) 1-10.
[92] T.S. Lapperre, J.K. Sont, A. van Schadewijk, M.M. Gosman, D.S. Postma, I.M. Bajema, et al., Smoking cessation and bronchial epithelial remodelling in COPD: a cross-sectional study, Respir. Res. 8 (2007) 85.
[93] L. Calzetta, M.G. Matera, M. Cazzola, Pharmacological interaction between LABAs and LAMAs in the airways: optimizing synergy, Eur. J. Pharmacol. 761 (2015) 168-173.
[94] M.G. Belvisi, Overview of the innervation of the lung, Curr. Opin. Pharmacol. 2 (2002) 211-215.
[95] B.J. Canning, A. Fischer, Neural regulation of airway smooth muscle tone, Respir. Physiol. 125 (2002) 113-127.
[96] H. Shimizu H, K. Masuta, K. Aono, H. Asada, K. Sasakura, M. Tamaki, et al., Molecular forms of human brain natriuretic peptide in plasma, Clin. Chim. Acta 316 (2002) 129-135.
[97] PubChem, Open Chemistry Database, Available at http://pubchem.ncbi.nlm.nih.gov, Last accessed 21 April 2016.
[98] M.G. Matera, L. Calzetta, A. Segreti, M. Cazzola, Emerging drugs for chronic obstructive pulmonary disease, Expert Opin Emerg Drugs 17 (2012) 61-è82.
[99] L. Calzetta, M. Cazzola, C.P. Page, P. Rogliani, F. Facciolo, M.G. Matera, Pharmacological characterization of the interaction between the dual phosphodiesterase (PDE) 3/4 inhibitor RPL554 and glycopyrronium on human isolated bronchi and small airways, Pulm. Pharmacol. Ther. 32 (2015) 15-23.
[100] M. Cazzola, L. Calzetta, C.P. Page, P. Rogliani, F. Facciolo, A. Gavaldà, et al., Pharmacological characterization of the interaction between aclidinium bromide and formoterol fumarate on human isolated bronchi, Eur. J. Pharmacol. 745 (2014) 135-143.
[101] M. Cazzola, L. Calzetta, A. Segreti, F. Facciolo, P. Rogliani, M.G. Matera, Translational study searching for synergy between glycopyrronium and indacaterol, COPD 12 (2015) 175-181.
[102] M. Cazzola, L. Calzetta, J. Ora, E Puxeddu, P. Rogliani, M.G. Matera, Searching for the synergistic effect between aclidinium and formoterol: From bench to bedside, Respir. Med. 109 (2015) 1305-11.
103. P. Rogliani, L. Calzetta, E.A. Rendina, D. Massullo, M. Dauri, B. Rinaldi, et al., The influence of propofol, remifentanil and lidocaine on the tone of human bronchial smooth muscle, Pulm. Pharmacol. Ther. 26 (2013) 325-331.

Figure legend

Figure 1. Autocrine loop and mechanism of action of BNP inducing human airways relaxation. BNP activates NPR-A expressed at the level of airway epithelium, with consequent vesicular release of very low concentrations of acetylcholine. Picomolar concentrations of acetylcholine activate prevalently postsynaptic M_2 muscarinic receptors localized on surrounding ASM cells. The M_2 muscarinic receptor stimulation modulates the iNOS activity, which in turn increases the NO levels and activates the intracellular NO/cGMP signaling. This pathway stimulates specific protein kinases that activate MYPT1 and MLCP, with consequent increase of MLC and ASM relaxation. The NO/cGMP pathway activated by BNP also prevents ASM contractility through a rapid modulation of calcium homeostasis, leading to the inhibition of MLCK activity and reduction of MLC-P. ACh: acetylcholine; ASM: airway smooth muscle; BNP: brain natriuretic peptide; Ca^{++}: calcium; cGMP: cyclic guanosine monophosphate; iNOS: inducible nitric oxide synthase; MLC: myosin light chain; MLC-P: myosin light chain phosphorylation; MLCK: myosin light chain kinase; MLCP: myosin light chain phosphatase; MYPT1: myosin phosphatase target subunit 1; NO: nitric oxide; NPR-A: natriuretic peptide receptor A; pMYPT1: inactive (phosphorylated) myosin phosphatase target subunit 1.

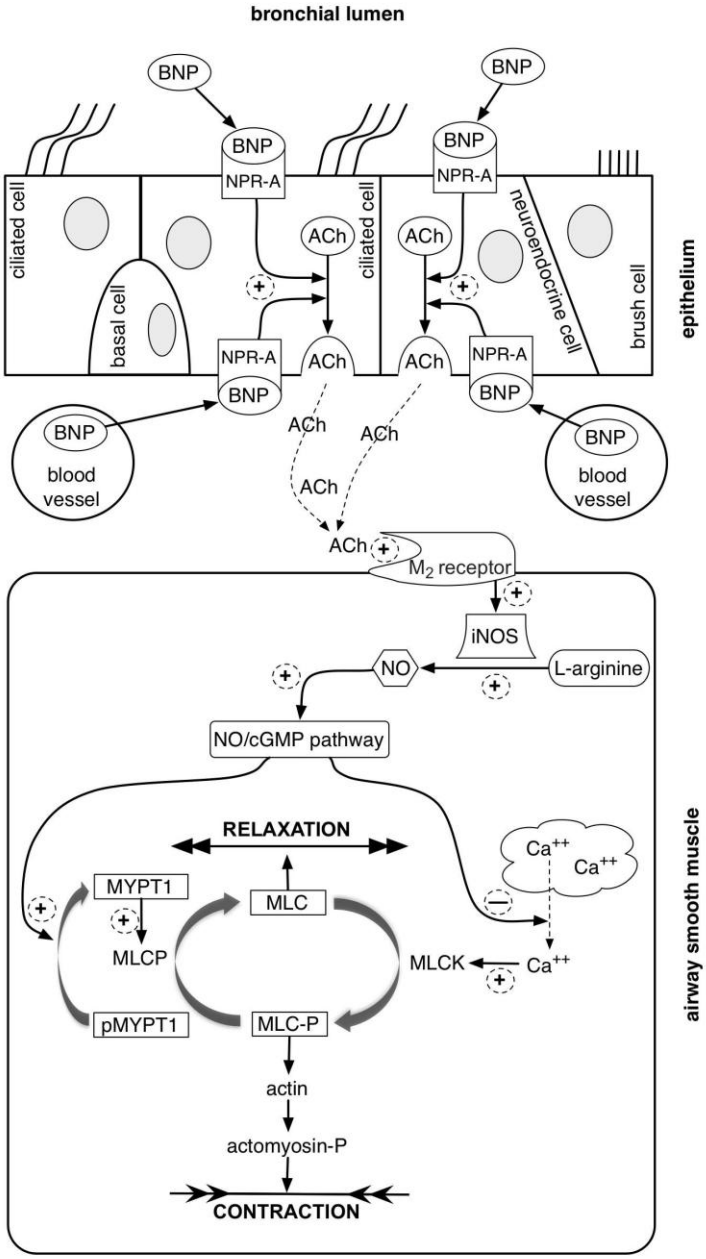

Fig. 1

Tables

Table 1. Influence of NPR-A activation on airways.

Study	NPR-A agonist	Specie	Experimental setting	Functional effect
Fernandes *et al.*, 1992 [61]	ANP	Guinea pig	*In vitro*	Relaxation of tracheal smooth muscle
Candenas *et al.*, 1991 [65]	ANP	Guinea pig	*In vitro*	Relaxation of tracheal smooth muscle
O'Donnell *et al.,* 1985 [62]	ANP	Rat	*In vitro*	Relaxation of tracheal smooth muscle
Ishii *et al.,* 1989 [63]	ANP	Bovine	*In vitro*	Relaxation of tracheal smooth muscle
Angus *et al.*,1994 [64]	ANP	Bovine	*In vitro*	Weak relaxation of bronchial smooth muscle
Mizuguchi *et al.*, 2000 [66]	ANP	Guinea pig	*In vivo*	Protection against propranolol-induced bronchoconstriction after allergic reaction
Candenas *et al.*, 1991 [65]	ANP	Human	*In vitro*	No relaxation of bronchial smooth muscle
Angus *et al.*, 1994 [64]	ANP	Human	*In vitro*	Weak relaxation of bronchial smooth muscle
Hulks *et al.*, 1989 [71]	ANP	Human	*In vivo*	Bronchodilator response in asthmatic patients
Chanez *et al.*, 1990 [72]	ANP	Human	*In vivo*	Bronchodilator response in asthmatic patients
Hulks *et al.*, 1990 [67]	ANP	Human	*In vivo*	Reduction of airway resistance in normal subjects
Hulks *et al.*, 1991 [73]	ANP	Human	*In vivo*	Reduction of bronchial reactivity to inhaled histamine in asthmatic patients
McAlpine *et al.*, 1992 [74]	ANP	Human	*In vivo*	Reduction of bronchial reactivity to ultrasonically nebulized distilled water in asthmatic patients
Angus *et al.*, 1993 [69]	ANP	Human	*In vivo*	Bronchodilator response in asthmatic patients
Angus *et al.*, 1995 [70]	ANP	Human	*In vivo*	Reduction of bronchial reactivity to inhaled histamine
Takagi *et al.*, 1993 [76]	BNP	Guinea pig	*In vitro*	Relaxation of tracheal smooth muscle
Ohbayashi *et al.*, 1998 [75]	BNP	Guinea pig	*In vivo*	Prevention of ovalbumin-induced bronchoconstriction and microvascular leakage
Akerman *et al.*, 2006 [78]	Nesiritide (human recombinant BNP)	Human	*In vivo*	Bronchodilation in patients with asthma
Edelson *et al.*, 2012 [77]	PL-3994 (NPR-A agonist)	Guinea pig	*In vivo*	Reduction in pulmonary inflation pressure
Edelson *et al.*, 2012 [77]	PL-3994 (NPR-A agonist)	Human	*In vitro*	Relaxation of bronchial smooth muscle

Table 2. Pharmacological characterization of BNP on the human airways contractility.

			***Ex vivo* COPD models (cholinergic stimulus)**		***In vitro* / *ex vivo* asthma models (histaminergic stimulus)**	
Study	**Specimen**	**Experimental setting**	**Delta E_{max} (% cholinergic tone)**	**Delta potency (pEC_{50})**	**Delta E_{max} (% histaminergic tone)**	**Delta potency (pEC_{50})**
Matera *et al.*, 2009 [58]	Medium bronchi	Effect on pre-contracted airways	<-30%	NC	-61%	NC
Matera *et al.*, 2011 [8]	Medium bronchi	Effect on CRC to contractile agonists	-71%	-1.5	-33%	-0.9
Edelson *et al.*, 2012 [77]	Bronchioles	Effect on pre-contracted airways	-50%	NC	NA	NA
Calzetta *et al.*, 2014 [56]	ASM cells	Effect on CRC to contractile agonists	NA	NA	NC	-1.6
Calzetta *et al.*, 2014 [56]	ASM cells	Effect on pre-contracted ASM cells	NA	NA	-50%	NC

ASM: airway smooth muscle

CRC: concentration response curve

E_{max}: maximal effect

NA: data not available

NC: data not calculable

pEC_{50}: negative logarithm of concentrations inducing 50% E_{max}

Highlights

- BNP and NT-proBNP are fast and sensitive biomarkers for diagnosing heart failure
- BNP testing in COPD patients increases the detection of chronic heart failure by about 20%
- BNP and NT-proBNP levels may be elevated in patients with COPD without a history of heart failure
- *Ex vivo* studies documented a BNP bronchorelaxant and bronchoprotective effect in human airways
- BNP induces a physiologic autocrine loop at the level of bronchial wall and is a potential drug for COPD