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Pulmonary apelin levels and effects in rats with hypoxic pulmonary hypertension

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KEYWORDS
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Summary
Background: The peptide apelin is localised in the vascular endothelium and highly expressed in pulmonary tissue. The aim of this study was to investigate whether apelin could be a potential lung-derived plasma marker for pulmonary hypertension, and study the effect of apelin in pulmonary arteries.
Methods: Apelin protein levels were measured in the lung, right ventricle, and plasma from normoxic and chronic hypoxic rats with pulmonary hypertension. Isolated intrapulmonary arteries were mounted in microvascular myographs and the effect of apelin investigated. Finally, the distribution of apelin receptors in pulmonary tissue was visualised by immunohistochemistry.
Results: Total pulmonary apelin content was not changed by hypoxia. Right ventricular apelin concentrations and content were lower than in the lung, but increased substantially in hypoxia in correlation with right ventricular pressure. Plasma apelin did not reflect pulmonary or right ventricular apelin levels. In pulmonary arteries from normoxic rats, apelin inhibited vasoconstriction to endothelin-1 and angiotensin-II. However, in arteries from hypoxic rats, apelin failed to inhibit contraction to angiotensin-II and endothelin-1. No difference in immunoreaction for apelin receptors was found in lung sections and arteries from normoxic versus chronic hypoxic rats.
Conclusions: Apelin changes in the right ventricle seem more specific for pulmonary hypertension than do changes in pulmonary tissue, which does not speak in favour of apelin as a lung-derived marker for this disease. During normoxic conditions, apelin has a modulating effect on vasoconstriction which is lost in chronic hypoxia. This may reflect alterations in the signal transduction downstream of the apelin receptor.

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Introduction

Pulmonary hypertension is a severe disease with a median survival of 2.8 years in the idiopathic form, if left untreated.[1] The late occurrence and non-specific character of symptoms and clinical signs can delay diagnosis, which is unfortunate because survival depends on disease stage at the onset of treatment.[2] Plasma biomarkers to help in the diagnosis of pulmonary hypertension are desirable, and various substances involved in the pathophysiology of pulmonary hypertension have been proposed, e.g. endothelin-1, serotonin, and nitric oxide (NO). However, measurements of endothelin-1 are complicated by intrapersonal variability,[3] a short half life in plasma and pulmonary clearance.[4] A few studies on plasma concentrations of serotonin have reported conflicting results of elevated[5] and normal[6] concentrations in patients with severe pulmonary hypertension. NO is difficult to measure in the blood, and the second messenger cGMP is also regulated by the natriuretic peptides.[4] Uric acid levels are higher in patients with pulmonary hypertension,[7] but are influenced by e.g. diet and kidney function. Consequently, these substances are not yet established as markers for pulmonary hypertension. Currently, the best validated marker is brain natriuretic peptide (BNP), which is secreted in response to ventricular overload.[8] However, since the origin of BNP is the heart, it is unlikely that BNP reflect early stages of pulmonary hypertension, when right ventricular strain has not yet occurred. Furthermore, BNP may also be elevated in case of left ventricular dysfunction.[9]

Recently, a newly discovered peptide, apelin, was proposed as a marker of heart failure and pulmonary hypertension.[10] Apelin exists in lengths of e.g. 12, 13 and 36 aminoacids and is present in vascular endothelial cells, and the expression is particularly high in pulmonary tissue.[11] The peptide has a potent positive inotropic effect[12] and in systemic vascular tissue, apelin exerts vasodilatation and inhibits angiotensin-II mediated vasoconstriction through NO-dependent mechanisms.[13,14] These effects seem to be more pronounced for apelin peptides of 12 and 13 aminoacids.[15] Furthermore, the effects of the apelin system are altered in cardiovascular disease.[16,17] We hypothesised that apelin could be a lung-derived plasma marker for pulmonary hypertension and that it may have vasodilatory effects in pulmonary vessels.

In accordance, the present study addressed how apelin concentrations in the lung are affected by hypoxia-induced pulmonary hypertension in rats, and whether pulmonary apelin content is reflected in plasma. Furthermore, apelin was measured in the right ventricle which is also affected in chronic hypoxic rats. Finally, we investigated the effect of apelin and the apelin receptor distribution in pulmonary arteries from normoxic and chronic hypoxic rats.

Methods

Animals

9 Weeks old male Wistar rats were kept for 2 weeks in normoxia or hypobaric hypoxia (550 mBar corresponding to 10% of O_2) to induce pulmonary hypertension. To evaluate whether apelin levels correlated to pressure load rather than hypoxia alone, a group of rats with intermediate right ventricular pressures were created by treating 8 hypoxic rats with the specific pulmonary vasodilator sildenafil, 25 mg/kg/day (Pfizer, Kent, UK), in the drinking water.[18] The animals were kept on a 12:12-h light–dark cycle with free access to water and chow and renewal of the cages twice a week.

All experiments were performed with approval from the Danish Institutional Animal Care and Use Committee.

Measurements of right ventricular systolic pressure and collection of tissue

Rats used for measurements of apelin levels were anaesthetized by inhalation of isoflurane (4.5%, oxygen flow 2 l/min, FORENE®) (Abbott Scandinavia AB, Sweden) followed by injection of dormicum (midazolam 5 mg/ml) (F. Hoffmann-La Roche AG, Basel, Switzerland) and hypnorm (fentanyl citrate 0.315 mg/ml, fluanisone 10 mg/ml) (VetaPharma Ltd, Leeds, UK) 1.8 mg/kg. Right ventricular systolic pressures were measured by insertion through the right jugular vein of a Millar catheter (model SPR-407 2F, Micro-Tip® Catheter) (Millar Instruments Inc, Houston, TX, USA) connected to a QUAD Bridge, Powerlab 4/20 (AD Instruments, UK) and Chart 5.5 software (AD Instruments, UK). Rats were killed by exsanguination and lungs and heart were removed en bloc and kept in cold physiological salt solution (PSS) (NaCl 119 mM, KCl 4.7 mM, $MgSO_4$ ($7H_2O$) 1.17 mM, $NaHCO_3$ 25 mM, KH_2PO_4 1.18 mM, EDTA 0.026 mM, glucose 5.5 mM). Wet weight of lungs, heart, right and left heart ventricle plus septum was measured and the organs were snap frozen in liquid nitrogen. Arterial blood was collected in tubes containing EDTA (1 mg/ml sample) and aprotinin (0.6 TIU/ml) (Sigma–Aldrich, St. Louis, U.S.A.) and centrifuged at 1600 g at 4 °C for 15 min.

Apelin measurements

Lung and right ventricular tissues were squashed in liquid nitrogen, homogenised in lysis buffer (5 ml/g of tissue) with protease inhibitor cocktail for mammalian cell and tissue extracts (Sigma–Aldrich, St. Louis, U.S.A) 100 ul/1 ml, and 10 mM tris base (Sigma–Aldrich, St. Louis, U.S.A), pH 7.4 and centrifuged at 1600 g at 4 °C for 15 min. The supernatant was collected and total protein was measured with Bio-Rad *DC* Protein Assay (Bio-RAD Laboratories, Hercules, California, USA). Lung and right ventricular supernatant was diluted 1:75 and 1:30, respectively. Plasma was diluted 1:10 and the fluids were used in an Apelin-12 kit (Phoenix peptides, Burlingame, California, USA), following the manufacturer's instructions. The kit detects apelin-12, -13 and -36 with 100% cross reactivity, and has an inter-assay variation of $<$14%. All samples were analysed in duplicate. The coefficient of variation was calculated based on the duplicate analyses. All samples from one type of tissue were analysed in the same assay, to avoid inter-assay variations. In each assay, a standard curve was included. Also, the detection of apelin was controlled with a positive control of diluted apelin peptide. Control experiments for

non-specific colour reactions were performed. Absorbance was read at 450 nm on an ELISA plate reader (ELx808 Ultraplate Reader, Biotek Instruments, Inc., UK).

Functional studies

In rats used for functional studies, pulmonary hypertension was confirmed by the presence of right ventricular hypertrophy to avoid influence of anaesthetics.

3rd or 4th order branches of the pulmonary artery were dissected and mounted in microvascular myographs for isometric tension recordings. The organ bath containing PSS was bubbled with 5% of CO_2 in atmospheric air, heated to 37 °C and the arteries were stretched to a level of tension corresponding to a transmural pressure of 3.9 kPa.[18]

Contractile function was tested by depolarisation of the cells with PSS containing 123.7 mM potassium (KPSS). Arteries that responded with an increase in transmural pressure of less than 2.7 kPa were discarded. Endothelial cell function was evaluated by contracting the arteries with the thromboxane analogue, U46619 (30 nM) (Sigma–Aldrich, St. Louis, U.S.A) followed by acetylcholine (ACh) (10 μM) (Sigma–Aldrich, St. Louis, U.S.A).

To evaluate direct effects of apelin-13 (Sigma–Aldrich, St. Louis, U.S.A) on pulmonary arteries from normoxic and hypoxic rats, the arteries were precontracted with U46619 (30 nM) and increasing concentrations of apelin-13 (1 nM–3 μM) were added. Because of development of tachyphylaxis in arteries from normoxic rats, a single concentration of apelin-13 (3 μM) was added in one line of experiments.

To investigate the effect of apelin-13 on contraction to the pulmonary vasoconstrictors endothelin-1 (Sigma–Aldrich, St. Louis, U.S.A) and the thromboxane analogue U46619, half the vessels from each animal were randomized to incubation with apelin (100 nM) for 20 min before performing concentration-response curves for the compounds. As a positive control of findings in systemic vascular tissue, similar experiments were performed with angiotensin-II (Sigma–Aldrich, St. Louis, U.S.A).

Immunohistochemistry

Paraffin embedded sections of the lungs from normoxic and hypoxic rats were obtained as previously described.[18] Sections were pretreated with heat-induced epitopic retrieval and incubated with anti-apelin receptor antibody (Phoenix peptides, Burlingame, California, USA). The sections were rinsed and the signal amplified with biotinylated antibodies from the DAKO LSAB/HPR kit (DAKO™). Negative controls for each section were obtained by omission of the primary antibody. The strength of positive immunoreactions in vascular endothelial cells, vascular smooth muscle cells, and alveoli were scored by a blinded observer.

Data analysis

Results were expressed as means ± SEM. Differences in results between groups were analysed by Student's *t*-test. Correlation between apelin levels and manifestations of pulmonary hypertension was analysed by linear regression only including animals where all measurements had been obtained. The fold change in total apelin was calculated by the following formula: $(C_{sample}/C_{meanN)} \times (OW/BW_{animal})/(OW/BW_{mean})$, where C_{sample} is the concentration in each sample, C_{meanN} is the mean concentration of the samples from normoxic animals, OW/BW_{animal} is the organ to body weight ratio in each animal and OW/BW_{meanN} is the organ to body weight ratio in normoxic animals. Responses to apelin were expressed as percentage of U46619-induced tension and responses to vasoconstrictors as percent of tension induced by KPSS after 3 min (mean ± SEM). Differences between concentration-response curves were evaluated by two-way analysis of variance (ANOVA). *p*-Values below 0.05 were considered statistical significant.

Results

Manifestations of pulmonary hypertension

Hypoxic rats had increased lung weight, increased right ventricular pressures, and right ventricular hypertrophy. Treatment with sildenafil only reduced the increase in right ventricular pressure in the chronic hypoxic rats (Table 1).

Apelin assay

Based on the duplicate results from samples, the intra-assay coefficient of variation was 10.0 ± 1.16%. The functional detection limit was 0.047 ± 0.003 ng/ml with an upper range limit of 0.243 ± 0.03 ng/ml. The kit was able to detect apelin peptide diluted in assay buffer.

Pulmonary apelin content

Total protein concentration pr mg of tissue was not significantly different in the three groups of animals (data not shown).

Pulmonary apelin concentrations were 1.30 ± 0.07 ng/mg protein ($n = 6$) in normoxic rats, and reduced to 1.10 ± 0.06 ng/mg protein ($n = 7$) in hypoxic rats ($p < 0.05$). In sildenafil treated rats, concentrations were 1.00 ± 0.12 ng/mg

Table 1 Animal data.

	N	*H*	*H* + *S*
RVSP (mmHg)	26.5 ± 0.6	55.7 ± 1.9*	44.7 ± 2.8*†
BW (g)	327.2 ± 8.3	281.9 ± 2.7*	288.7 ± 8.7*
LW (g)	1.33 ± 0.04	1.65 ± 0.04*	1.84 ± 0.15*
LW/BW (%)	0.41 ± 0.02	0.59 ± 0.01*	0.64 ± 0.05*
RV (g)	0.12 ± 0.011	0.22 ± 0.011*	0.19 ± 0.009*
RV/LV + S (%)	17.8 ± 1.9	36.7 ± 1.6*	31.4 ± 2.0*
RV/BW (%)	0.037 ± 0.004	0.078 ± 0.004	0.067 ± 0.003
LV + S/BW	0.21 ± 0.004	0.21 ± 0.005	0.22 ± 0.008

*$p < 0.05$ compared to normoxic rats.
†$p < 0.05$ compared to hypoxic rats.
Data are means ± SEM. n (N) = 6, n (H) = 7 and n ($H + S$) = 6. N = normoxic rats, H = hypoxic rats, $H + S$ = hypoxic sildenafil treated rats, RVSP = right ventricular systolic pressure, BW = body weight, LW = lung weight, RV = right ventricle and LV + S = left ventricle + septum.

protein ($n = 6$) which was not different from concentrations in hypoxic rats ($p = 0.32$) (Fig. 1a). The total pulmonary apelin content corrected for lung and body weight was calculated to investigate whether this would be reflected in plasma. This was not changed by hypoxia ($p = 0.1$, $n = 7$) or sildenafil treatment (Fig. 1b). There was no correlation between pulmonary apelin concentrations or content and right ventricular pressures.

Right ventricular apelin content

Right ventricular apelin concentrations were smaller than in the lungs. Thus, in normoxic animals the concentrations were 0.15 ± 0.04 ng/mg protein ($n = 6$). In hypoxic animals concentrations were 0.59 ± 0.03 ng/mg protein ($n = 7$) ($p < 0.05$ versus normoxic rats). In sildenafil treated hypoxic rats lung apelin concentrations were 0.65 ± 0.05 ng/mg protein ($n = 6$), which was not different from apelin concentrations in hypoxic, vehicle treated rats ($p = 0.33$) (Fig. 2a). When adjusted for right ventricular and body weight, the total amount of apelin increased substantially in chronic hypoxic rats (Fig. 2b). There was a strong correlation of apelin concentrations and content to right ventricular systolic pressure (Fig. 2c–d).

Plasma apelin content

Plasma apelin concentrations were not changed by hypoxia alone ($p = 0.12$), but were increased by exposure to the combination of hypoxia and treatment with sildenafil (Fig. 3a).

Plasma apelin did not correlate to total pulmonary apelin, and only weakly to right ventricular apelin content (Fig. 3b). There was no correlation between plasma apelin levels and right ventricular systolic pressure.

Functional studies

Arteries had a diameter of 494 ± 26 μm. Acetylcholine (10 μM) induced relaxations of $69 \pm 3\%$ ($n = 12$) and $69 \pm 6\%$ ($n = 7$) in arteries from normoxic and hypoxic animals, respectively.

In pulmonary arteries from normoxic rats, cumulatively added concentrations of apelin-13 did not elicit any response. A single concentration of apelin-13 (3 μM) induced transient relaxations of $17 \pm 4\%$ ($p < 0.05$, $n = 5$) (Fig. 4a,b). In pulmonary arteries from hypoxic rats, a similar relaxation was induced when a concentration of apelin-13 (3 μM) was reached when performing concentration-response curves (Fig. 4c,d).

In arteries from normoxic rats, incubation with apelin-13 (100 nM) had no effect on U46619 contraction (Fig. 5a), but attenuated the response to endothelin-1 and angiotensin-II (Fig. 5b,c). In the arteries from hypoxic rats, there was no effect of incubation with apelin-13 on vasoconstriction induced by either U46619 (Fig. 5d), endothelin-1 (Fig. 5e), or angiotensin-II (Fig. 5f).

Apelin receptor distribution

Immunoreaction for apelin receptors was present in the vascular endothelium and to a lesser extent in smooth muscle cells of pulmonary arteries with internal diameters of 400–600 μm corresponding in size to those used for functional experiments (Fig. 6a,b). Also, immunoreaction for apelin receptors was present in the respiratory epithelium. No immunoreaction was detected in control sections when the primary antibody was omitted (Fig. 6c). When scoring the strength of the expression, no difference in the distribution or density of the immunoreaction for apelin receptors in the endothelium (1.6 ± 0.4 versus 2.1 ± 0.4, $p = 0.3$), smooth muscle cells (0.54 ± 0.15 versus 0.54 ± 0.12, $p = 0.9$), and alveoli was detected between pulmonary tissue from normoxic and hypoxic rats.

Discussion

This study is the first to address pulmonary apelin protein levels and the effect of apelin in pulmonary arteries in a rat model of chronic hypoxic pulmonary hypertension. The main findings are that while apelin protein concentration in

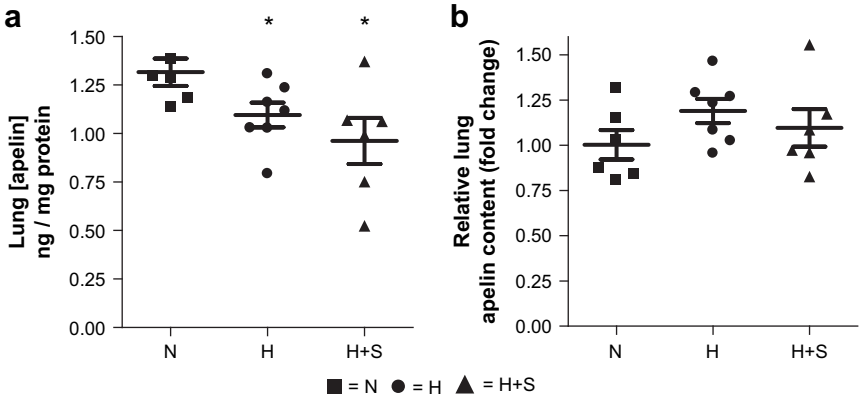

Figure 1 Pulmonary apelin content. (a) Apelin concentration pr mg tissue. (b) Apelin content adjusted for lung and body weight expressed as fold change of content in normoxic animals (normalised to 1). Data are shown as mean ± SEM. n (N) = 6, n (H) = 7 and n ($H + S$) = 6. *$p < 0.05$ compared to normoxic rats. N = normoxic rats, H = hypoxic rats and $H + S$ = hypoxic sildenafil treated rats.

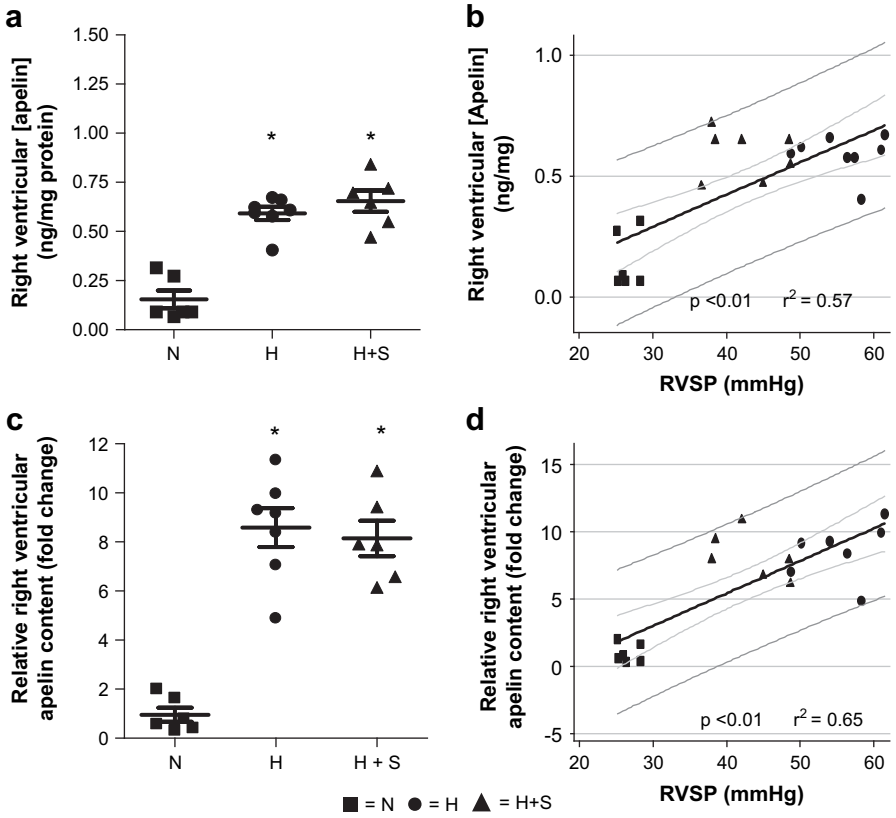

Figure 2 Right ventricular apelin content. (a) Apelin concentration pr mg tissue. (b) Apelin content adjusted for lung and body weight expressed as fold change of content in normoxic animals (normalised to 1). (c) And (d) correlation of concentrations and content to right ventricular systolic pressure. Data are shown as mean ± SEM. n (N) = 6, n (H) = 7 and n ($H + S$) = 6. N = normoxic rats, H = hypoxic rats, $H + S$ = hypoxic sildenafil treated rats and RVSP = right ventricular systolic pressure.

lung tissue decreased, the total pulmonary apelin content remained stable because of a corresponding increase in pulmonary tissue mass. In the right ventricle, a substantial increase in apelin content that correlated strongly to right ventricular pressure was found, suggesting that apelin levels in the heart are more specifically related to hemodynamic changes. In pulmonary arteries from normoxic rats, apelin counteracted vasoconstriction in response to endothelin-1. In arteries from hypoxic rats, this effect was abolished suggesting that the effect of the peptide is altered in chronic hypoxic rats. However, no difference in the expression of apelin receptors was detected. Therefore, alterations in the intracellular signalling pathways activated by apelin may account for the altered response in pulmonary arteries from chronic hypoxic rats.

Pulmonary apelin

Previous studies have suggested that hypoxia alters apelin in the lungs. Thus, in mice an increase in total pulmonary apelin mRNA was reported to take place after one week of hypoxia,[19] and in another study in mice, approximately a doubling of apelin mRNA and an increase of apelin protein of about 25% was found after 5 h of hypoxia.[20] However, some endothelial proteins are regulated in opposite directions by acute and chronic hypoxia,[21] and the species may influence the response of the apelin pathway to hypoxia. Thus, in the present study chronic hypoxia (two weeks) caused an increase of lung weight with a decreased amount of apelin pr mg of tissue. Two weeks of hypoxia has been shown to cause inflammation, collagen deposition in alveolar septae and vessel walls, decreased staining for endothelial cells,[22] and increased muscularization of small pulmonary arteries,[18] resulting in increased lung weight. The unaltered total apelin amount and lowered apelin protein concentrations pr mg of tissue could, therefore, reflect a decrease of the relative mass of apelin containing endothelial cells.

Apelin in the heart

In the heart, heart failure and hypoxia have been shown to regulate apelin levels. In a murine study, short-term hypoxia upregulated left ventricular apelin mRNA,[19] and in models of ischemic heart failure total heart apelin protein was upregulated 6 weeks after induction of myocardial infarction.[23] Because of apelins well-demonstrated positive inotropic effect,[12,23] the early upregulation is thought to be a compensatory mechanism.[23] However, in rats with decompensated heart failure, levels were downregulated,[24] suggesting that apelin levels in the heart are regulated differently in short- and long-term heart failure. In agreement with the previous studies,[19,23] the rats in the present study had right ventricular hypertrophy, and both concentration and total content of

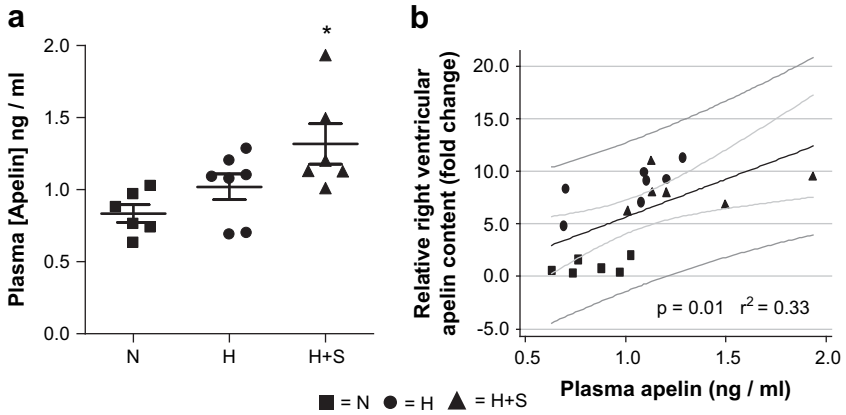

Figure 3 Plasma apelin content. (a) Plasma apelin concentrations. (b) Correlations of plasma apelin and adjusted right ventricular apelin levels. n (N) = 6, n (H) = 7 and n ($H+S$) = 6. N = normoxic rats, H = hypoxic rats and $H+S$ = hypoxic sildenafil treated rats. *$p < 0.05$ compared to normoxic animals.

apelin was markedly increased in the right ventricle. The correlation to the right ventricular pressure suggests that the changes in apelin levels are induced by pressure load of the right ventricle.

Plasma apelin

Plasma apelin concentrations have been shown to be decreased in patients with pulmonary hypertension, severe chronic obstructive lung disease (COPD), and left heart failure.[10,25,26] However, it is unclear whether plasma apelin reflects apelin levels in the organs involved in the diseases. As apelin is highly expressed in pulmonary tissue,[11] it was speculated that pulmonary apelin could be a major source of plasmatic apelin.[10] However, apelin is also present in adipose tissue and has been found in systemic vessels, where the mRNA expression was also upregulated by hypoxia or heart failure in animal models or cell cultures.[19,27] Since COPD, pulmonary hypertension and heart failure all result in some degree of tissue hypoxia, apelin from these sources could also contribute to disease-related alterations in plasmatic apelin levels. Indeed, in the present study, plasma apelin did not reflect pulmonary concentrations or content and correlated only weakly to apelin levels in the right ventricle. These findings suggest that apelin from other sources may play a role for plasma apelin levels.

Interestingly, we found that while plasma concentrations were not changed by exposure to hypoxia per se, the combination of hypoxia and sildenafil increased the concentration significantly. Previous studies have shown that NO increases apelin receptor mRNA in rat brain,[28] but so far it is unknown whether the NO—cGMP pathway may also influence apelin formation.

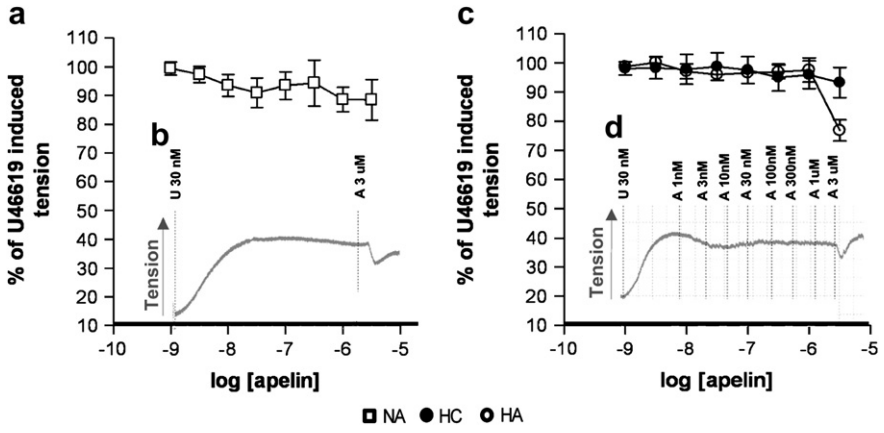

Figure 4 Direct effect of apelin. (a) Effect of cumulatively added concentrations of apelin-13 in pulmonary arteries from normoxic rats. (b) Example of original trace showing the effect of one single concentrations of apelin added to a pulmonary artery from normoxic animal without prior exposure to the peptide. (c) Effect of cumulatively added concentrations of apelin-13 in pulmonary arteries from hypoxic rats. (d) Example of original trace from concentration-response curve for apelin in pulmonary artery from hypoxic animal. The effect of apelin is expressed as percentage of contraction to U46619 (30 nM). *$p < 0.05$ by two-way ANOVA compared to normoxic vessels. n = 3—7. NA = apelin treated pulmonary arteries normoxic animals, HA = apelin treated pulmonary arteries from hypoxic animals, HC = vehicle treated pulmonary arteries from hypoxic animals, U = U46619 and A = apelin.

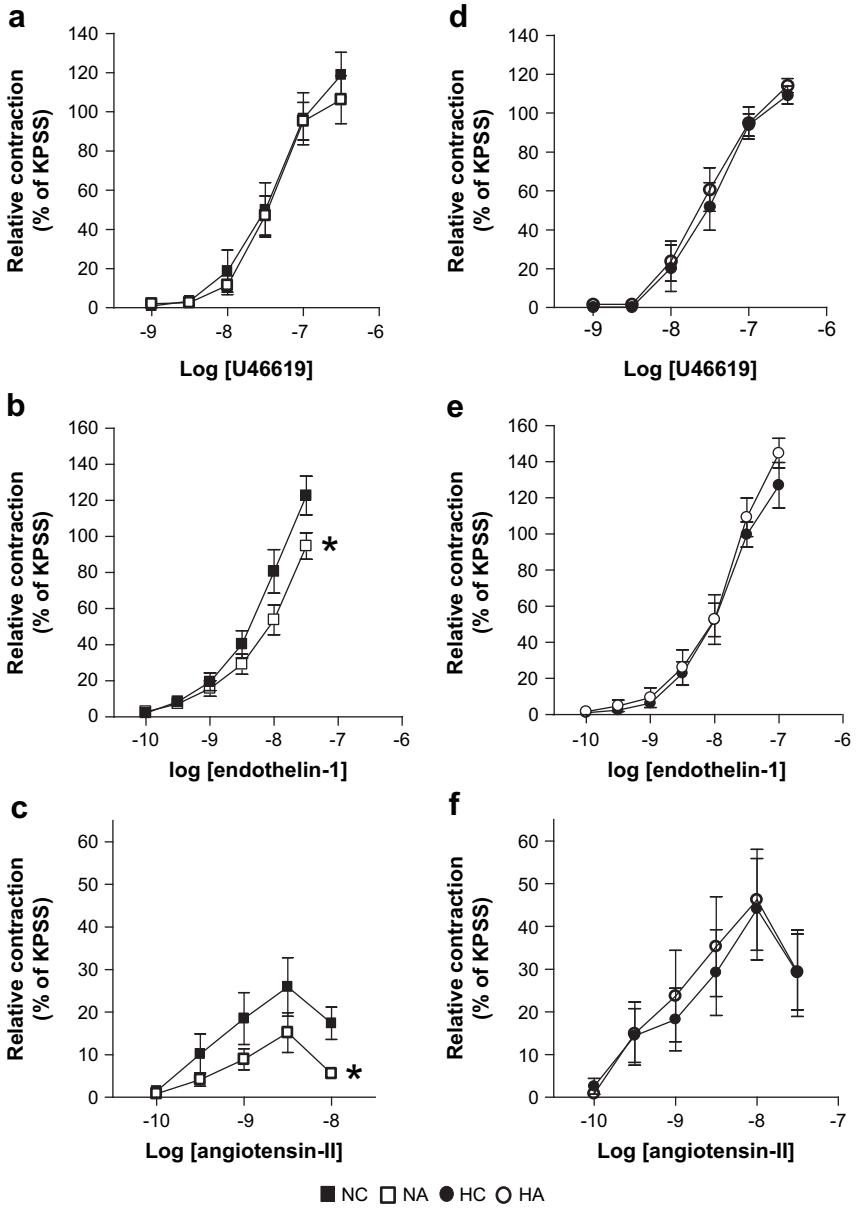

Figure 5 Effects of incubation with apelin and hypoxia on responses to vasoconstrictors in pulmonary arteries from normoxic and hypoxic rats. (a—c) Effect of apelin-13 on contraction to (a) U46619, (b) endothelin-1 and (c) angiotensin-II in arteries from normoxic rats. (d—f) Effect of apelin-13 on contraction to (d) U46619, (e) endothelin-1 and (f) angiotensin-II in arteries from hypoxic rats. Data are shown as mean ± SEM of tension in percent of the maximal contraction to KPSS 124 mM. *$p < 0.05$ by two-way ANOVA compared to normoxic control vessels. KPSS = physiological salt solution with potassium, 124 mM. NC = pulmonary arteries from normoxic animals, vehicle treated; NA = pulmonary arteries from normoxic animals, apelin treated; HC = pulmonary arteries from hypoxic animals, vehicle treated and HA = pulmonary arteries from hypoxic animals, apelin treated. $N = 5—10$.

Functional effects of apelin in pulmonary arteries and distribution of receptors

Apelin receptors have been proven to be localised in vascular endothelial cells, vascular smooth muscle cells, and cardiomyocytes in humans as well as in bronchial epithelial cells in rats.[29] It is believed that apelin receptor activation in smooth muscle cells and cardiomyocytes results in contraction, whereas in endothelial cells it causes NO release leading to vasorelaxation.[30] Apelin receptor protein quantified by western blotting has been shown to be upregulated in pulmonary tissue in mice after one week of hypoxia.[19]

Whether the relaxation in response to 3 μM of apelin-13 is of physiological significance is uncertain, but due to the close proximity of the peptide and receptors, the local concentration around the receptors could be significantly higher than measured in plasma. However, compared to other substances, such as sildenafil,[18] apelin does not seem to be a potent pulmonary vasodilator.

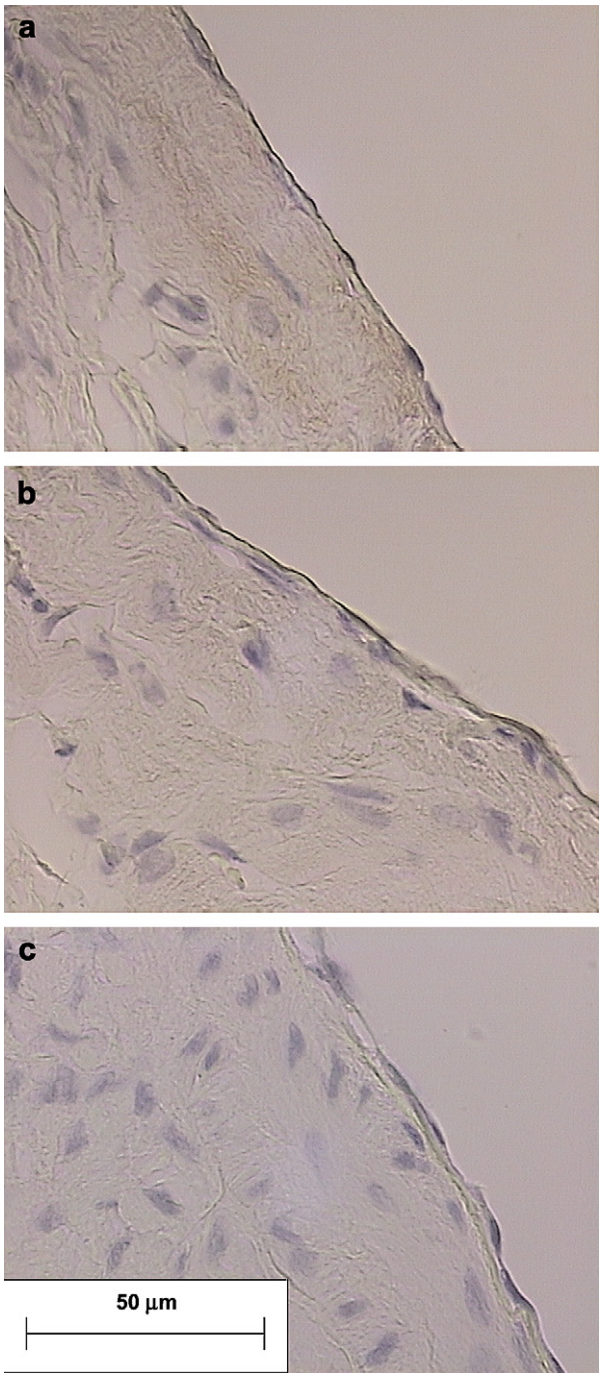

Figure 6 Receptor distribution and density. (a—b) Lung section with pulmonary artery stained with anti-apelin receptor antibody from normoxic (a) and hypoxic rat (b). (c) Negative control.

The fact that 100 nM of apelin counteracted contraction to endothelin-1 and angiotensin-II, but not to U46619 in arteries from normoxic animals suggests that apelin does not have a general inhibitory effect on vasoconstriction.

The absence of the inhibitory effect of apelin on endothelin-1 and angiotensin-II contraction in pulmonary arteries from chronic hypoxic rats was probably not caused by a generally impaired endothelial NO production, since relaxation induced by acetylcholine was similar in arteries from normoxic and hypoxic animals. With respect to apelin receptors, localisation in the endothelium, smooth muscle cells and epithelium concurs with previous findings.[29] However, immunoreaction for the apelin receptor did not suggest differences in receptor density or localisation. These findings suggest that the changed apelin response rather can be ascribed to alterations in the signalling pathways downstream of the apelin receptor.

Conclusions

Pulmonary apelin content was unchanged by hypoxia and did not correlate to plasma apelin levels, which does not speak in favour of apelin as a lung-derived plasma marker of pulmonary hypertension. Furthermore, apelin only modulated vasoconstrictor tone in arteries from normoxic rats, an effect which was lost in hypoxia. On the other hand, apelin levels in the right ventricle were closely related to right ventricular pressure. This is indeed an attractive quality for a biomarker of pulmonary hypertension, but right ventricular levels were only weakly reflected in plasma, suggesting that additional tissue sources contribute to plasma apelin concentrations.

Conflict of interest statement

Authors have no conflicts of interests.

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