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Short communication

Evidence for expression and function of angiotensin II receptor type 1 in pulmonary epithelial cells

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ABSTRACT

There is a growing evidence that the peptide hormone angiotensin II (ANGII) can act as an auto-/paracrine mediator to regulate epithelial ion transport processes. The present study focused on the impact of ANGII on transepithelial ion transport in pulmonary epithelia. Transcripts for the ANGII receptor type 1 (ATR_1) were detected in lungs of *Xenopus laevis* and H441 cells (human pulmonary epithelial cell line). Native *Xenopus* lung preparations were used for Ussing chamber recordings and apically applied ANGII (10 μM) induced a significant increase of short-circuit current (I_{SC}: 8 ± 2%, n = 13). Pre-incubation with losartan (LOS), an antagonist of ATR_1 prevented the effect of ANGII on I_{SC}.

Transcripts for ATR_1 in *Xenopus* lungs and H441 cells were detected and an increase of I_{SC} was observed by ANGII in native *Xenopus* lung epithelia. This indicates that ANGII is a potential auto-/paracrine mediator for ion transport regulation in pulmonary epithelia.

1. Introduction

Angiotensin II (ANGII) is a peptide hormone and part of the renin–angiotensin–aldosterone-system (RAS) that controls fluid homeostasis and blood pressure (de Gasparo et al., 2000). Within the RAS, activation of ANGII is controlled by complex interactions: Renin, that is released from the juxtaglomerular apparatus of the kidney, converts angiotensinogen, that is released by the liver, to form angiotensin I (ANGI). As ANGI circulates in the blood, it flows through the lungs where angiotensin-converting enzyme (ACE), converts ANGI to ANGII. ANGII binding to its receptors (angiotensin II receptor type 1: ATR_1) induces the release of aldosterone from the adrenal cortex and causes vasoconstriction in the arterial system (de Gasparo et al., 2000).

Apart from being a part of the canonical RAS, there is accumulating evidence that ANGII can be produced locally by lung epithelia and alveolar macrophages indicating a role of ANGII as an auto-/paracrine mediator (Paul et al., 2006). The function of this local RAS (lRAS) is involved in different lung diseases (Kaparianos and Argyropoulou, 2011) although its function for normal lung physiology is unknown.

The main functions of the pulmonary epithelium are to facilitate gas exchange and to protect the host against pathogens from the environment. Both functions rely on a certain amount of fluid at the surface of the epithelium (Fronius et al., 2012). The amount of this fluid needs to be balanced within certain limits – to much fluid in the distal area of the lung will impair gas exchange, whereas too little fluid in the airways affects the viscosity of the airway surface layer and impairs host defense (Fronius et al., 2012). Both situations are associated with/or caused by dysfunctional ion transport processes and highlight the relevance of transepithelial pulmonary ion transport.

Considering the evidence that ANGII and its precursors can be produced by pulmonary epithelia (Uhal et al., 2007) and by alveolar macrophages (Paul et al., 2006) and that ion transport is a crucial role of the pulmonary epithelium for lung fluid homeostasis, the present study questioned whether ANGII might affect transepithelial ion transport and if ATR are expressed in lung epithelia, since they are crucial for the detection of ANGII and the initiation of cellular responses.

2. Material and methods

2.1. Animals and tissue preparation

All procedures were approved by the Regional Board Giessen as the responsible authority for animal welfare and are in agreement

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Table 1
Primer sequences against angiotensin II receptor transcripts.

Species	Gene	Primer sequence (5′ → 3′)	Amplicon size (bp)	NCBI reference sequence	Sequence identity (%)
Human	agtr1	for: gatgattgtcccaaagctgg rev: aggtgactttggctacaagc	400	NM_000685.4	100[a]
Xenopus	agtr1-a	for: catgaagatgaagacagtggccagca rev1: agctaccattgcagtgcgcca	268	NM_001089663.1	99[a]
		rev2: ctagctaccattgcagtgcgcc	271		100[a]

bp: base pairs; for: forward; rev: reverse.
[a] Identity of the sequenced amplicon (Eurofins MWG Operon) compared with the reference sequences (NCBI: National Center for Biotechnology Information).

with the German Law of Animal Welfare. Lungs of adult female South African clawed frogs (*Xenopus laevis*) were used for this study. The animals were purchased from H. Kaehler (Hamburg, Germany), or *Xenopus* express (Le Bourg, France) kept in tap water and fed once a week with commercial fish-food. Preceding removal of the lungs, all animals were cooled down to make them manageable and killed by decapitation. Immediately after decapitation a cannula was introduced into the vertebral canal to destroy the spinal cord. The removed lungs were then either used for RNA isolation, or for transepithelial measurements using modified Ussing chambers.

2.2. Reverse transcription-PCR

Xenopus lungs: Isolated *Xenopus* lungs were frozen and homogenized in liquid nitrogen. Total RNA was extracted with RNeasy® Midi Kit (Qiagen, Hilden, Germany) according to the manufacturer's protocol including a DNase digestion step. RNA was reversed transcribed with Oligo-dT primers and the ImProm-II Reverse Transcription Kit (Promega, Mannheim, Germany). Negative controls were performed under the same conditions but in the absence of reverse transcriptase. Specific primers were designed from the published sequence of *Xenopus* ATR_1 (Table 1).

H441 cells: Cells were grown on Snapwell filters (Corning, Amsterdam, The Netherlands) in RPMI medium 1640 L-glutamine (PAA, Cölbe, Germany) as described before (Althaus et al., 2011).

For PCR experiments filters with confluent grown monolayers (7–10 days after seeding to Snapwell filters) were frozen and stored at −80 °C. For RNA isolation cells were scrapped from the filters and lysed/homogenized with a syringe. RNA was extracted using an RNeasy Mini Kit (Quiagen) and applying the provided protocol. Specific primers (Table 1) were used to perform the PCR reaction.

Amplicons from the PCR reactions were separated on a 1.5% agarose gel and visualized by Midori Green. All obtained bands (*Xenopus* lungs and H441 cells) were cut out and used for sequencing (Eurofins MWG Operon, Ebersberg, Germany).

2.3. Electrophysiological recordings

Functional recordings of transepithelial ion transport (short-circuit current: I_{SC}) were performed in Ussing chambers as described before (Richter et al., 2014). Briefly, freshly isolated lungs were dissected to flat sheets, fixed by a Lucite ring containing pins and mounted into custom-made Ussing chambers. Both compartments of the chamber were bathed with Ringer's solution containing (in mM): 100 NaCl, 3 KCl, 1 $CaCl_2$, 1 $MgCl_2$, 10 glucose and 5 HEPES (pH 7.4, adjusted with trizma base). The effective aperture of the used chambers was 0.5 cm^2, although all measured I_{SC} values were normalized to an area of 1 cm^2. All experiments were performed at room temperature.

2.4. Solutions and chemicals

All chemical used for the preparation of the Ringer's solution were obtained from Sigma (Seelze, Germany). The following substances were used in this study: amiloride (Sigma) to inhibit epithelial Na^+ channels, angiotensin II (human, Sigma) as an ATR agonist and losartan potassium (Sigma) as ATR antagonist. All compounds were dissolved as stock solutions and diluted in Ringer's solution for application.

2.5. Statistical analyses

Values are given as means ± standard error of the mean. The number of experiments is indicated with "*n*". Paired *Student*'s *t*-test was used to estimate the significance between means (before and after drug application). Significantly different means are indicated by asterisk and a *P*-value ≤0.05 was considered as statistically significant.

3. Results and discussion

3.1. Angiotensin II receptor transcripts are expressed in Xenopus lungs and H441 cells

To investigate whether or not ATR are expressed in *Xenopus* lungs, RT-PCR experiments were performed. The primers to verify the putative expression of ATR_1 in *Xenopus* lung were designed against the published sequence of the *Xenopus* angiotensin II receptor type 1 (NCBI reference sequence: NM_001089663.1). For the detection of *Xenopus* ATR_1 ($xATR_1$) one forward and two reverse primers were used. These primer pairs detected specific products of expected sizes with reverse primer 1 (268 bp, Fig. 1A and Table 1) as well as with reverse primer 2 (271 bp, Table 1). RT-PCR reactions were performed on RNA extracts derived from lung tissues of three different animals. Two different isoform of ATR_1 receptors were identified in *Xenopus*, ATR_{1a} (Ji et al., 1993) and ATR_{1b} (Bergsma et al., 1993). Sequencing and alignment of the amplicons with the reference sequences confirmed the identity of $xATR_{1a}$ (NCBI reference sequence: NM_001089663.1; Table 1) by 99 (reverse primer 1) and 100% (reverse primer 2) that has been cloned from *Xenopus* myocardium and whose expression was confirmed in the lung (Ji et al., 1993). Alignment with the $xATR_{1b}$ sequence (NCBI reference sequence: NM_001085781.1) revealed 94% sequence identity (for both primer pairs). These results indicate that the $xATR_{1a}$ subtype is expressed in *Xenopus* lung rather than the $xATR_{1b}$ subtype. Amplicons derived by the primers against the human ATR_1 were sequenced and BLAST with the reference sequence of the humane ATR_1 (NCBI reference sequence: NM_000685.4; Fig. 1B) revealed 100% alignment. This confirms the expression of ATR_1 in native lungs of *X. laevis* (Fig. 1A) as well as in isolated cultured human pulmonary epithelial cells. These findings are in agreement with prior reports indicating the existence of lRAS in pulmonary epithelia (Paul et al., 2006; Kaparianos and Argyropoulou, 2011) and the expression of ATR in human and rat alveolar epithelial cells (Wang et al., 1999).