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Activation of PPARγ reverses a defect of surfactant synthesis in mice lacking two types of fatty acid binding protein

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ABSTRACT

Lung surfactant is a lipid–protein-film covering the inner alveolar surface. We have previously shown that double knock-out (d-ko) mice lacking both the epidermal-type (E-) and the heart-type (H-) fatty acid binding protein (FABP) exhibit a defect of surfactant synthesis in alveolar type II cells that can be corrected by feeding pioglitazone, a drug that activates peroxisome proliferator-activated receptor gamma (PPARγ). Here, we demonstrate first that healthy surfactant at collapse pressure produces protrusions composed of bilayers but not folds, second that the d-ko effect profoundly perturbs lipid/hydrophobic protein composition, pressure-area isotherm, and structural organisation of the surfactant at nanoscale, parameters that are critical for the normal breathing cycle. In support of these data in vivo measurements of lung function reveal that maximum compliance in d-ko vs. wild-type mice is significantly reduced. Further, we show that the biophysical phenotype can be corrected substantially with pioglitazone. Finally, we show that d-ko alveolar cells up-regulate liver-type (L-) FABP, a member of the FABP family that we have previously shown to interact with PPARγ. Taken together, these data suggest that PPARγ agonists could be a tool to repair surfactant damage caused by dysfunctional alveolar lipid metabolism, and provide in vivo support for L-FABP aided signaling.

1. Introduction

Peroxisome proliferator-activated receptor (PPAR) subtypes α, β and γ are ligand-activated transcription factors that are involved in the regulation of lipid homeostasis [reviews: [1–3]]; they target genes having a PPAR responsive element (PPRE) in their promoters, examples being genes encoding liver-type (L-) and adipocyte-type (A-) fatty acid binding proteins (FABPs). The latter belong to a class of intracellular proteins which, in general, provide soluble binding sites for fatty acids from which they are mobilized in response to metabolic needs [4–6]. For example, fatty acids are transported by FABPs from the cytosol into the nucleus where they become pan-agonists of the PPARs. The specific interaction of the pairs L-FABP/PPARα and L-FABP/PPARγ, A-FABP/PPARγ and epidermal-type (E-) FABP/PPARβ provided in vitro proof for such targeted signaling mechanism [7–9].

Recently we recognized co-expression of PPARβ and γ subtypes with E- and heart-type (H-) FABP in alveolar type II (TII) cells [10]. This cell type is specialized in the formation, storage and secretion of surfactant lipids [11]. Therefore, we assumed an essential role for PPAR subtypes and E- and H-FABP in the regulation of surfactant lipid metabolism. We generated an E/H-FABP double knock-out (d-ko) mouse model and examined its biochemical phenotype in the lung [10]. TII cells from d-ko mice exhibited a significant drop in palmitic acid uptake, oxidation and incorporation into glycerolipids, which included a drop in synthesis of unsaturated phosphatidylcholine and of de novo synthesis of dipalmitoyl phosphatidylcholine (DPPC). A further salient feature was the down-regulation of PPARβ and up-regulation of PPARγ, which prompted us to feed pioglitazone, a PPARγ specific agonist, to the d-ko mice. The biochemical characterization of TII cells isolated from these mice revealed an almost complete biochemical phenocopy of wild-type (wt) TII cells. We concluded that in TII cells E- and H-FABP are involved in fatty acid transport, metabolism and signaling [10].

Abbreviations: PPAR, peroxisome proliferator-activated receptor; PPRE, PPAR response element; L-FABP, liver-type fatty acid binding protein; A-FABP, adipocyte-type FABP; E-FABP, epidermal-type FABP; H-FABP, heart-type FABP; TII, alveolar type II; d-ko, double knock-out; DPPC, dipalmitoyl phosphatidylcholine; wt, wild-type; SP, surfactant protein; LB, Langmuir–Blodgett; FM, fluorescence microscopy; SFM, scanning force microscopy; LPS, lipopolysaccharide

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These previous data lead to two important questions that are the subject of the present paper. First, what are the consequences of altered lipid metabolism in the d-ko TII cells for the structure and function of the surfactant? Here, we address this question with a focus on the biophysical properties of the surfactant.

According to a new concept [12,13], large areas of the surfactant layer are folded below the surface forming lipid protrusions (wrinkles) during expiration while maintaining adherence with the monolayer [14,15]. At inspiration the protrusions in the subphase simply unfold and the monolayer expands without disturbing its continuity. For the formation and stabilization of these protrusions, small proportions of non-bilayer forming phospholipids and the hydrophobic surfactant proteins B and C (SP-B, SP-C) seem to be essential [12,13]. Using this model of a surface-associated reservoir it is conceivable that reduced surfactant in the lung exhibits a biophysical phenotype characterized by a decrease of folded surfactant in the subphase at end-expiration; the number of protrusions decreases. We also hypothesized that these changes would impact lung function in vivo. Moreover, we hypothesized that wt surfactant would be rescued in d-ko mice by administration of pioglitazone to the animals which would open up a perspective to apply this drug in the fields of pulmonology and neonatology.

Second, the present paper addresses the mechanism whereby pioglitazone rescues the biochemical and potentially the biophysical phenotype of E- and H-FABP double deficiency in the lung. A search for candidate FABPs that could compensate for the transport and signaling capacity of E- and H-FABP with respect to interaction between drug, FABP and PPARγ would highlight the in vivo role of the binding proteins in these processes.

2. Materials and methods

2.1. Animals

Generation of homozygous E/H-FABP d-ko mice (of C57BL/6 genetic background) has been described previously [10]. Wt and d-ko mice were fed with normal lab chow (Altromin, Ladbergen, Germany) or lab chow containing pioglitazone (40 mg/kg) for 7 days ad libitum [10]. Feed uptake and increase of body mass did not differ among these groups (data not shown). All animal experiments were approved by the local "Animal Care and Usage Committee" and by the District Governor of Münster (G 63/2001).

2.2. Isolation of lung surfactant

Each mouse lung was lavaged with 150 mM NaCl solution and 1.5 mL of the lavage obtained was centrifuged first at 150 ×*g* to remove cells and then at 42,000 ×*g* for 25 min to sediment surfactant. The resulting pellet was suspended in 300 µl purified water and extracted with butanol (1:66 v/v) to give a hydrophobic extract, which contains all surfactant lipids and the hydrophobic surfactant proteins SP-B and SP-C. This extract was taken to dryness by evaporation at 37 °C in vacuo [16] and is termed surfactant in this work. Phospholipid and protein contents of surfactant were determined according to Rouser et al. [17] and by the fluorescamine assay [18], respectively.

2.3. Film-balance measurements

The analytical Langmuir system with a maximal interfacial area of 927 cm^2 was used (FW2 from Lauda, Lauda-Königshofen, Germany). Surfactant isolated from 1 mouse was dissolved in 3 mL chloroform/methanol (1:1 v/v) and an aliquot of this solution (containing approximately 14 µg phospholipids) was spread at 20 °C onto the water surface of the Langmuir through. The water used was purified with the Milli-Q185 Plus system (Millipore GmbH, Eschborn, Germany). The solvent was allowed to evaporate for 20 min before compression/expansion (9.27 cm^2 min^{-1}) experiments were carried out. During the experiment the surface area was reduced (or reversibly expanded) by a movable mechanical barrier thus compressing the monolayer film. The surface pressure was measured continuously (unit is milliNewton per meter, mN/m) during the area reduction yielding a so-called surface area/surface pressure isotherm, since the measurement was performed at constant temperature (e.g. Figs. 1B, and 2B and B′).

In a further approach a Wilhelmy balance with an operating area of 144 cm^2 (Riegler and Kirstein, Mainz, Germany) was used in combination with a setup enabling FM as described earlier [19]. Prior to spreading onto the aqueous surface in the through, surfactant was mixed with 0.5 mol% phosphatidylcholine labeled with BODIPY® (Avanti Polar Lipids, Alabaster, MS, USA) based on the amount of phospholipids. Images were taken at collapse pressure of the surfactant.

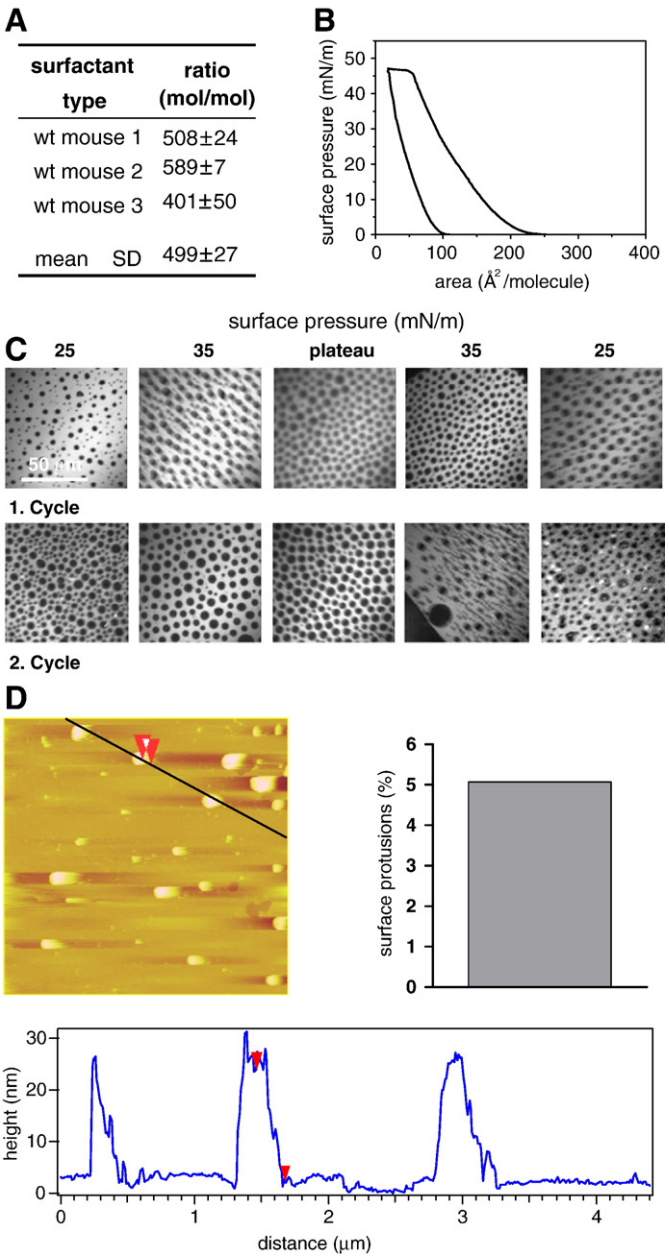

Fig. 1. Analysis of surfactant from wt mouse. A, Phospholipid/hydrophobic protein ratio; phospholipid and hydrophobic protein contents of surfactant were determined by phosphorus and fluorescamine assays, respectively. B, Film-balance measurements; a collapse pressure of around 47 mN/m was applied in an analytical Langmuir system to produce protrusions from surfactant monolayers in this and the following experiments. C, A Wilhelmy balance equipped with a fluorescence microscope provides images, where dark areas represent liquid condensed domains of DPPC and surrounding bright area the liquid expand domain; the latter contains unsaturated and BODIPY-labeled phospholipids together with proteins SP-B and SP-C. D, LB-films of surfactant (prepared with a Wilhelmy balance) were mounted onto mica plates and inspected by SFM. Bright spots in the panels obtained represent protrusions. These were evaluated by a color code to determine height (traces below); by scanning width, the area occupied within the monolayer was assessed in % of total surfactant surface (bar plots to the right). Representative graphs and images shown pertain to mouse 1.

2.4. SFM measurements

Langmuir–Blodgett (LB) films were prepared at 20 °C according to Ross et al. [20]. The Wilhelmy balance used had an operation area of 38.5 cm^2 (Riegler and Kirstein) and, in addition, contained a removable cup at the bottom of the balance for placing the mica plate (SCI-Science Services GmbH, Munich, Germany) into it. In brief, the mica plate was freshly cleaved and a DPPC monolayer transferred onto it rendering the surface hydrophobic. Then, the surfactant layer in the film balance was compressed to a pressure of nearly 50 mN/m to produce protrusions and the hydrophobic mica sheet