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Neonatal ACE inhibition in rats interferes with lung development

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Summary

The renin–angiotensin system (RAS) is developmentally up-regulated and it is essential for kidney development in several species. Given the fact that the rat lung undergoes postnatal development, the mammalian lung possesses the highest angiotensin-converting enzyme (ACE) levels and ACE activity increases during the first weeks postpartum, we tested the hypothesis that ACE inhibition influences postnatal lung development. Rats were given the ACE inhibitor enalapril (10 mg kg^{-1}) from 0 to 9 days of age and their lungs were examined at day 4 and 9. Lung structure was evaluated by means of light microscopy, and surface tension of bronchoalveolar lavage fluid was measured by means of a Wilhelmy balance. Neonatal ACE inhibition lowered the surface tension of bronchoalveolar lavage fluid and caused widening of respiratory airspaces and thinning of alveolar septa. Our results suggest that early postnatal ACE inhibition in rats interferes with lung development.

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angiotensin-converting enzyme; lung development; renin–angiotensin system; surface tension; Wilhelmy balance

Introduction

The activity and distribution of the renin–angiotensin system (RAS) are developmentally regulated in a tissue-specific manner, and angiotensin II (Ang II) can act as a modulator of growth in a variety of cells and tissues (Gomez, 1990; Touyz & Schiffrin, 2000). The RAS has also been shown to be critically important in development. There is ample evidence for its mandatory role in kidney development (Friberg *et al.*, 1994; Tufro-McReddie *et al.*, 1995; Guron & Friberg, 2000). Different components of the RAS, including angiotensin-converting enzyme (ACE) and both angiotensin type 1 (AT_1) and type 2 (AT_2) receptors, are expressed in lung endothelial and type II epithelial cells, respectively (Morrell *et al.*, 1996; Wang *et al.*, 1999). The mammalian lung possesses the highest ACE levels (Esther *et al.*, 1996) and ACE activity increases during the first weeks postpartum (Wallace *et al.*, 1978). Rats are born with immature lungs and, within the first two postnatal weeks, the alveolization (Scheuermann *et al.*, 1988), together with subcellular reorganization in type II epithelial cells (Young *et al.*, 1991) and changes in surfactant homeostasis (Griese *et al.*, 1999), occurs in rats. The aim of this study was to test the hypothesis that ACE inhibition (ACEI) influences lung development. Hence, rats were given ACEI during 9 days starting at day 0 after birth and their lungs were examined at day 4 and 9. Structural changes in ACEI lung were evaluated at light microscopical level, and surface tension of bronchoalveolar lavage fluid (BALF), a reflector of quantitative and/or qualitative abnormalities of alveolar surfactant, was measured by means of a Wilhelmy balance (Schmekel *et al.*, 1992).

Methods

General procedures

Time-mated, female Wistar rats (B & K Universal AB, Sollentuna, Sweden) were transported to our facility on the 14th day of pregnancy. They were observed carefully for determination of the day of delivery. Both male and female pups were included in the study. Weight matched pups were divided into two groups receiving daily intraperitoneal injections of either enalapril maleate (10 mg kg^{-1}; Sigma-Aldrich Sweden AB) or isotonic saline vehicle in equivalent volumes of 10 ml kg^{-1} from day 0 (within 12 h after birth) to day 9. We chose 10 mg kg^{-1} dose of enalapril maleate, which was demonstrated to inhibit nearly completely ACE activity (Guron *et al.*, 1999). Throughout the study rats had free access to standard rat chow and tap water and were kept in rooms with a controlled temperature of 24°C and a

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12 h dark (6 p.m.–6 a.m.)/light cycle. The regional animal ethics committee in Gothenburg approved all experiments.

Light microscopy and semiquantitative assessment

For microscopical evaluation, seven to nine pups from enalapril maleate and saline treated group were anaesthetized with sodium pentobarbital (60 mg kg^{-1}, intraperitoneally; Apoteksbolaget, Umeå, Sweden) at day 4 and 9 after birth. After thoracotomy, the lungs were excised and immersion-fixed in 4% paraformaldehyde. The upper, middle and lower part of the left lung was cross-dissected into ≃4 mm thick planes and their fixation was continued for 24 h. After fixation planes were dehydrated through an ethanol series and all three planes from each lung were embedded in paraffin en bloc. Keeping the distance of 50 μm in between, three 3-μm-thick serial sections were cut and stained with haematoxylin and eosin. Nine sections from each lung were used for the semiquantitative assessment of lung structural changes. Assessments were made by an investigator blind to treatment group. The presence of wider respiratory airspaces and thinner alveolar septa was scored semiquantitatively with the use of arbitrary scale: 1, mild; 2, moderate; and 3, severe changes.

Measurement of surface tension of bronchoalveolar lavage fluid

For measurements of surface tension of BALF, seven to nine pups from enalapril maleate and saline treated groups were anaesthetized with sodium pentobarbital (60 mg kg^{-1}, i.p.) at day 4 and 9 after birth. After thoracotomy the lungs of each animal were washed gently three times with 37°C saline (30 ml kg^{-1}) and BALF aliquots were pooled, yielding in 2–2·5 ml of lavage fluid. The lavage fluid was centrifuged at 150 *g* for 10 min to remove any cells and frozen at −20°C. Surface tension of BALF was measured with a Wilhelmy balance (Biegler Electronic, Mauerbach, Austria). A teflon trough (115 × 18 mm, depth 17 mm) was filled with 35 ml of saline. The measurements were made at room temperature. A platinum plate, partly immersed in the liquid, is suspended in a balance. The trough contains a movable barrier, which changes the area into which the platinum plate is immersed. The duration of a cyclic area change to 20% of the initial area is 3 min. A baseline recording of the surface tension of saline was first established. The surface tension of saline is 73 mN m^{-1} and is independent of area change. A 200 μl of lavage fluid was added to the trough. A stable recording of surface tension versus area was rapidly obtained, and the maximum (γ_{max}) and minimum (γ_{min}) surface tension of the third cycle were recorded, thus, yielding in total two measurements on each sample of lavage fluid. We measured the surface tension of alveolar surfactant rather than its amount or composition since the surface tension reflects quantitative and/or qualitative abnormalities of alveolar surfactant.

At the end of experiments rats were killed by an overdose of sodium pentobarbital.

Statistical analysis

Values are expressed as mean values ± SD. All data were analysed by ANOVA followed by Fisher's *post hoc* least significant difference (LSD) test, using the computer software SYSTAT (version 5·02; Evanston, IL, USA). $P < 0·05$ was considered statistically significant.

Results

Morphological and semiquantitative observations

Enalapril treatment induced structural alterations in the lungs on postnatal days 4 and 9. Wider respiratory airspaces and thinner alveolar septa were observed in enalapril-treated rats (Figs 1b, d and 2), but it remains open how much of it reflects a real widening of the airspaces and how much is caused by the thinning of the septa.

Surface tension of bronchoalveolar lavage fluid

Enalapril treatment lowered minimum (γ_{min}) surface tension of BALF, as measured with 200 μl quantity (Fig. 3). The maximum (γ_{max}) surface tension of BALF did not differ between groups either on postnatal day 4 (70·3 ± 1·7 versus 68·4 ± 2·6 mN m^{-1}, in control and enalapril treated rats, respectively) or day 9 (69·4 ± 3·2 versus 69·9 ± 2·1 mN m^{-1}, in control and enalapril treated rats, respectively).

Discussion

The main finding of this study was that ACEI decreased surface tension of BALF, reflecting higher amount of alveolar surfactant, in neonatal rats on day 4 and 9. This was paralleled by structural alterations in the lungs. Thus, early postnatal ACE inhibition in rats interferes with lung development.

Surface tension of BALF, as measured in this study, mirrors the functional integrity of alveolar surfactant system. Pulmonary surfactant is a surface-active material composed of phospholipid-rich lipoproteins that reduce surface tension at the alveolar surface, promoting lung expansion during inspiration and preventing lung collapse during expiration (De Paepe *et al.*, 1998). The lower minimum surface tension of BALF in enalapril treated rats suggests that there is more surface-active surfactant in alveolar space, which, in turn, could result in lung structural alterations, characterized by thinner alveolar septa and wider respiratory airspaces, as evident from our study. Pulmonary surfactant is synthesized, stored, secreted, taken up and catabolized by type II pneumocytes (Griese *et al.*, 1999). The lamellar body within type II pneumocyte is the final secretory structure of pulmonary surfactant (Voorhout *et al.*, 1993). In a pilot study, we evaluated the morphology of type II pneumocytes at ultrastructural level and observed no alterations in enalapril-subjected lungs, implying preserved intracellular stores of

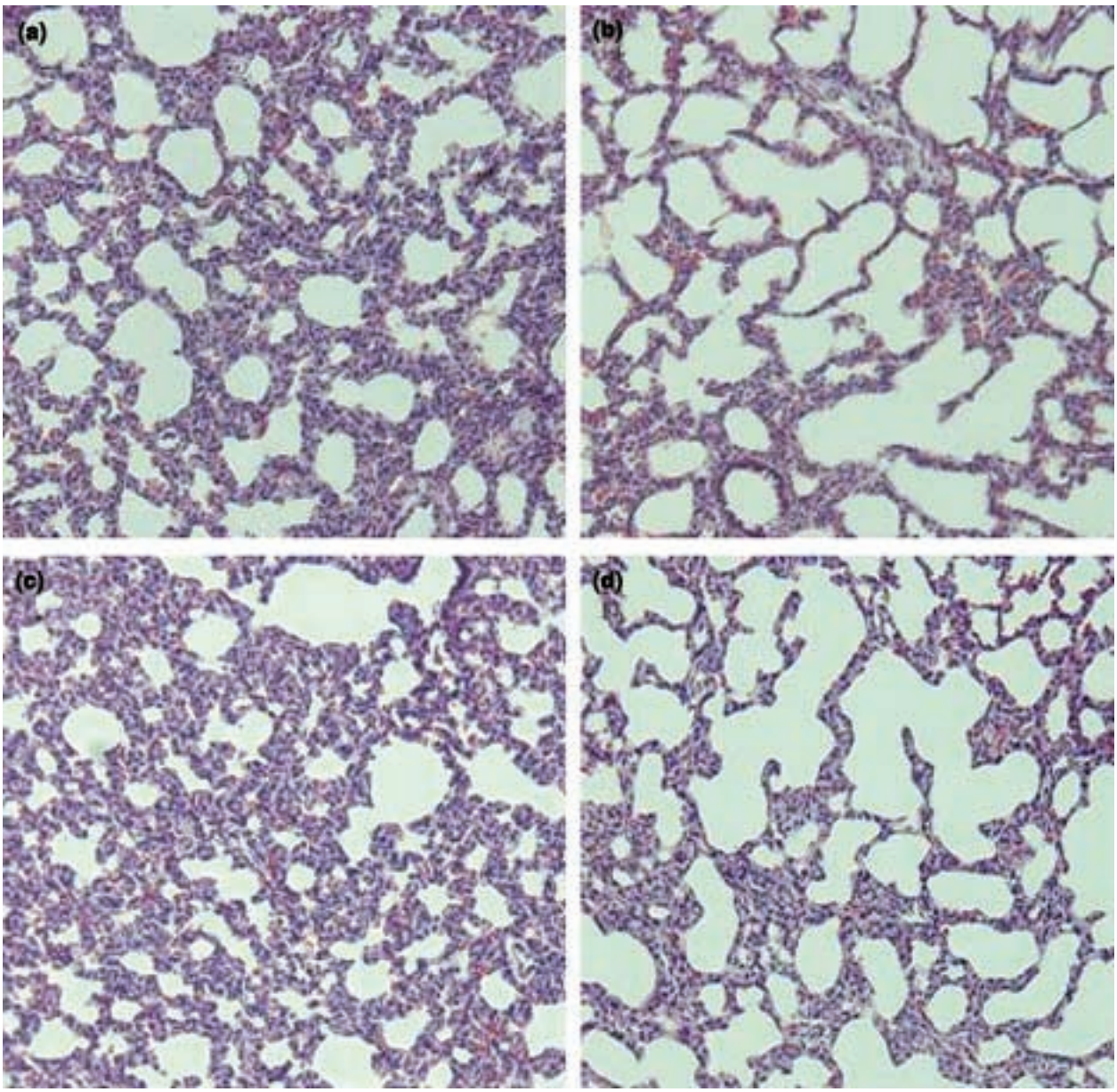

Figure 1 (a)–(d) Lung sections from 4 days-old (a, b) and 9 days-old (c, d) rats treated with saline vehicle (a, c) or enalapril (b, d). Enalapril-treated rat shows thinner alveolar septa and wider respiratory airspaces. Magnification 10×.

surfactant (data not shown). Collectively, it appears that early postnatal ACE inhibition increases the alveolar surfactant pool.

At the present time, we could only speculate about the mechanism of the changes we observed because we have not established whether the alveolar surfactant pool is increased due to enalapril-stimulated secretion or enalapril-inhibited uptake or both. Our finding of the disturbance in alveolar surfactant system in enalapril-treated rats suggests a role for ACE in the regulation of surfactant homeostasis in developing rat lungs. In this context, there is evidence to suggest that ACE inhibition results in increased levels of bradykinin and/or prostaglandins (PGs) (Buttar, 1997). Prostaglandins, in turn, enhance production and secretion of surfactant by type II pneumocytes (Oyarzun & Clements, 1978; Nagai *et al.*, 1995). Therefore, it is possible that the disturbance in surfactant homeostasis following neonatal ACEI is due to increased PGs, such as PGE_2 (Marin-Castano *et al.*, 2002). Indeed, rat PGE_2 receptors are expressed in lung tissue (Nemoto *et al.*, 1997), and PGE_2 is a potent local mediator of cell growth and differentiation in various tissues, and it plays a role in early postnatal lung development (Nagai *et al.*, 1995).

In summary, ACE regulates surfactant homeostasis in immature rat lung and, therefore, may play an important role in lung development.

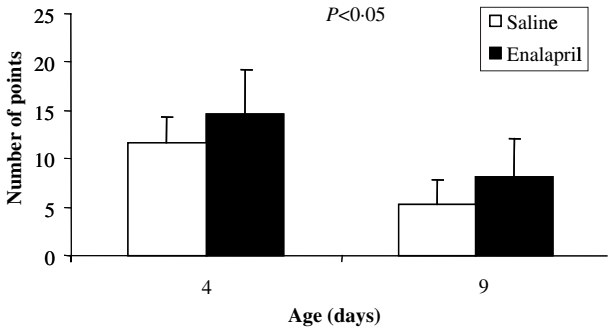

Figure 2 Semiquantitative assessment of lung structural changes, expressed as a number of points, in enalapril and saline vehicle treated neonatal rats at 4 and 9 days of age. The presence of wider respiratory airspaces and thinner alveolar septa was scored semiquantitatively with the use of arbitrary scale: 1, mild; 2, moderate; 3, severe changes. Nine sections per each lung were evaluated. *P < 0·05, indicating a statistically significant overall effect of enalapril versus saline vehicle. Values are mean values ± SD (n = 7–9 per group and time point).

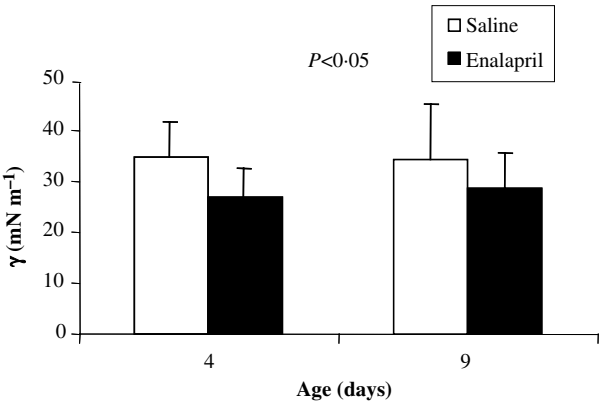

Figure 3 Minimum surface tension of bronchoalveolar lavage fluid in enalapril and saline vehicle treated neonatal rats at 4 and 9 days of age. *P < 0·05, indicating a statistically significant overall effect of enalapril versus saline vehicle. Values are mean values ± SD (n = 7–9 per group and time point).

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