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Expression of 11β-Hydroxysteroid Dehydrogenase Type 1 in Alveolar Epithelial Cells in Rats

SATOSHI SUZUKI*,***, HIROYOSHI TSUBOCHI**, ANDREW DARNEL**, TAKASHI SUZUKI**, HIRONOBU SASANO**, ZIGMUNT S. KROZOWSKI*** AND TAKASHI KONDO*

**Department of Thoracic Surgery, Institute of Development, Aging and Cancer, Tohoku University, 4-1 Seiryo-machi, Aoba-ku, Sendai 980-8575 Japan*
***Department of Pathology, School of Medicine, Tohoku University, 2-1 Seiryo-machi, Aoba-ku, Sendai 980-8575 Japan*
****Laboratory of Molecular Hypertension, Baker Medical Research Institute, PO Box 6492, Melbourne 8008, Victoria, Australia*

Abstract. 11β-Hydroxysteroid dehydrogenase type 1 (11β-HSD1) behaves predominantly as an oxoreductase converting the receptor-inactive glucocorticoids to their active forms *in vivo*, while the type 2 isoform (11β-HSD2) possesses only dehydrogenase activity and inactivates cortisol in human or corticosterone in rat. We determined enzyme activity of 11β-HSD in rat lungs from fetus to adult, and examined whether 11β-HSD1 exists in alveolar type II cells, the most important site for the synthesis of pulmonary surfactant in mature lungs, by immunohistochemistry and reverse transcription-polymerase chain reaction (RT-PCR). Enzyme activity of 11β-HSD1 and 2 in lung tissue homogenate were determined as $NADP^+$- and NAD^+-dependent conversion of corticosterone to 11-dehydrocorticosterone, respectively. We found that 11β-HSD1 activity was increased progressively from 21 days gestation to 7 weeks after birth. 11β-HSD2 activity was significantly lower than that of 11β-HSD1 throughout gestation and after birth. Immunoreactivity for 11β-HSD1 was detected in the cytoplasm of the cells in the alveolar region of adult rats. Some of these expressing 11β-HSD1 were considered to be alveolar type II cells, because of their cuboid shape and localization at the corner of the alveoli. RT-PCR demonstrated 11β-HSD1 mRNA in isolated alveolar type II cells. Our results suggest that alveolar type II cells enhance intracellular glucocorticoid availability via 11β-HSD1. 11β-HSD1 in alveolar type II cells is thought of as an autocrine amplifier of glucocorticoid action in the lung.

Key words: Alveolar type II cells, 11β-Hydroxysteroid dehydrogenase type 1 (11β-HSD1), Lung development, Rat
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GLUCOCORTICOIDS, through interaction with glucocorticoid receptors (GR), exert a diverse array of powerful biological effects such as modulation of cellular metabolism, cellular proliferation and differentiation, water and electrolyte homeostasis and anti-inflammatory actions on target tissues. However, their biological actions would be in part determined by 11β-hydroxysteroid dehydrogenase (11β-HSD), which interconverts biologically active glucocorticoids and their receptor inactive 11-oxometabolites. To date, two different isoforms have been cloned and characterized in man and other species including rat [1]. The type 2 isoform (11β-HSD2), requires NAD^+ as a cofactor, possesses only dehydrogenase activity and inactivates cortisol in human or corticosterone in rat, and is believed to confer aldosterone specificity on non-selective mineralocorticoid receptors (MR) [2]. In contrast, the type 1 isoform (11β-HSD1), requires $NADP^+$ as a cofactor, may behave predominantly as an oxoreductase converting the inactive 11-oxometabolites to their active forms *in vivo* [3], while enzymatic studies *in vitro* show both dehydrogenase and reductase activities [4]. Therefore, 11β-HSD1 is thought of as an autocrine amplifier of glucocorticoid action [5].

In the lung, 11β-HSD1 may play an important role

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Correspondence to: Satoshi SUZUKI, M.D., Department of Thoracic Surgery, Institute of Development, Aging and Cancer, Tohoku University, 4-1 Seiryo-machi, Aoba-ku, Sendai 980-8575 Japan

in surfactant-associated phosphatidylcholine synthesis [6, 7]. Previous studies have shown that rat fetal lungs have enzyme activity of 11β-HSD1 and the activity increases towards the end of gestation and after birth [6], although enzyme activity of another isoform of 11β-HSD (11β-HSD2) in normal lung development and maturation has yet to be determined. These observations seem to indicate that alveolar epithelium of mammals is an active glucocorticoid-target site. However, it is still unclear whether 11β-HSD1 exists in alveolar type II cells, the most important site for the synthesis of pulmonary surfactant in mature lungs. In the present study, we sought to obtain a better understanding of local actions of glucocorticoids in the lung. We measured enzyme activity of 11β-HSD1 and 2 separately in rat lungs from fetus to adult. We then examined whether 11β-HSD1 exists in alveolar type II cells by immunohistochemistry and reverse transcription-polymerase chain reaction (RT-PCR).

Materials and Methods

Tissue collection and preparation

Specific pathogen free Sprague-Dawley rats (Funabashi Farm, Sendai, Japan) were used in this study. All animals received humane care in compliance with guidelines from the University Committee on Animal Resources, Tohoku University. Peripheral lung tissue samples at 17 and 21 days in gestation, and 3 days and 2 and 7 weeks after birth were either freshly frozen in liquid nitrogen for enzyme assay or fixed in 4% paraformaldehyde for immunohistochemistry.

Enzyme assay

Frozen lung tissue samples were homogenized in the homogenize buffer (0.25 M sucrose, 140 mM NaCl, 10 mM sodium phosphate buffer, pH 7.4) on ice, then centrifuged at 4°C to obtain supernatant. Protein concentration was determined by the Bradford method [8]. Enzyme activity of 11β-HSD1 and 2 were determined by measuring the conversion of radiolabelled corticosterone (B) to 11-dehydrocorticosterone (A) in the presence and absence of cofactor $NADP^+$ or NAD^+, respectively [9]. 100 μg protein of lung homogenate was incubated in the homogenize buffer containing 2 nM of [^{3}H]-corticosterone (New England Nuclear, Boston, MA, USA) in the presence or absence of 0.5 mM NADPH (Sigma, St. Louis, MO, USA) or NADH (Sigma, St. Louis, MO, USA) at 37°C for 2 hours. Enzyme reaction was then terminated by adding ethylacetate. The organic solvent was dried down and the steroids were dissolved in ethanol containing unlabelled corticosterone (Sigma, St. Louis, MO, USA) and 11-dehydrocorticosterone (Sigma, St. Louis, MO, USA), and separated on plastic silica gel plates (Merck, Darmstadt, Germany) using chloroform/ethanol mixture as a solvent [9]. Areas corresponding to cold carriers were visualized under UV light, cut out into scintillation vials, and counted in a β-counter. Enzyme activity for 11β-HSD1 and 2 was expressed as $NADP^+$- and NAD^+-dependent percent conversion of B to A/100 μg protein/2 hours, respectively. The results were shown in the groups of mean ± SD for three different samples.

Immunohistochemistry

The lung tissues were fixed with 4% paraformaldehyde at 4°C for 18 hours and embedded in paraffin wax, and sectioned at 3 μm thick. Immunohistochemistry was performed employing the streptavidin-biotin amplified method using a Histofine Kit (Nichirei, Tokyo, Japan) as described previously [10]. For 11β-HSD1 immunostaining, a polyclonal anti-rat 11β-HSD1 antibody (RAH113), an immunopurified polyclonal antibody raised in rabbits against the last eight residues of rat 11β-HSD1 [11], was used at a final concentration of 2 μg/ml. In addition, distribution of GR was determined by using a polyclonal antibody for rat GR (M-20) (Santa Cruz Biotechnology, Santa Cruz, CA, USA) at a final concentration of 0.5 μg/ml. The antigen-antibody complex was visualized with 3.3'-diaminobenzidine (DAB) solution (1 mM DAB, 50 mM Tris-HCl buffer (pH 7.6), and 0.006% H_2O_2), and hematoxylin was used for the counterstaining. Rat liver tissue was used as a positive control for 11β-HSD1 and GR immunoreactivity. The specificity of immunohistochemical staining was confirmed by replacing the primary antibodies with pre-immune rabbit serum, and no immunoreactivity was detected in these tissue sections.

RT-PCR

Alveolar type II epithelial cells were isolated from

rats at 7 weeks after birth by the enzymatic tissue digestion using elastase (Worthington Biochemical, Freehold, NJ, USA), and purified by the differential adherence technique in rat Ig-G coated plastic plates [12]. The purity of the freshly isolated cells determined by alkaline phosphatase staining [13] was >90% in this study. Total RNA was extracted from freshly isolated rat alveolar type II epithelial cells and rat liver tissue homogenates with an RNA isolation reagent (RNA-Bee; Tel-Test, Friendswood, TX, USA) according to the manufacturer's protocols. RNA concentration was determined by ultraviolet absorption at 260 nm. 5 μg of total RNA were converted to cDNA by the reverse transcription (RT) reaction using Superscript II reverse transcriptase (GIBCO-BRL, Rockville, MD, USA). The reverse-transcribed cDNA products were then amplified by the polymerase chain reaction (PCR) technique with rat gene-specific primers for 11β-HSD1 (forward: 5'-GGA GCC CAT GTG GTA TTG ACT GCT GCA AGG TCG-3', reverse: 5'-GCT TCC TAC TCT GCA AGC AAG TTT GCT CTG-3', 399 bp) [4]. Polymerase chain reaction (PCR) was performed in a thermocycler (PTC-200 DNA Engine, MJ Research, Watertown, MS, USA) under the following conditions; 4 minutes of denaturation at 94°C, followed by annealing and extension for 35 cycles at 94°C for 1 minute, 55°C for 1 minute, and 72°C for 1 minute with a final extension step at 72°C for 10 min. Amplification products were electrophoresed on a 1.5% agarose gel and visualized by staining with ethidium bromide. PCR products were purified and subjected to direct sequencing (ABI PRISM Big-Dye Terminator Cycle Sequencing Ready Reaction Kit, Perkin Elmer Applied Biosystems, Foster City, CA, USA) to verify amplification of the correct sequences. RNA samples extracted from rat liver tissue homogenate were used as a positive control. Negative control experiments contained water instead of cDNA and no amplified products were observed under these conditions.

Results

11β-HSD1 activity was very low at 17 days gestation and progressively increasing from 21 days gestation to 7 weeks after birth (25.3 ± 4.1% conversion of B to A/100 μg protein/2 hours, n = 3) (Fig. 1). 11β-HSD2 activity was significantly lower than that of 11β-HSD1 throughout gestation and after birth (Fig. 1).

11β-HSD1 immunoreactivity was present in the cytoplasm, and GR was present in the nuclei. At 17 days gestation, immunoreactivity of 11β-HSD1 was not detected in lung epithelial cells or mesenchymal cells. From 21 days gestation to 2 weeks after birth, immunoreactivity of 11β-HSD1 was detectable

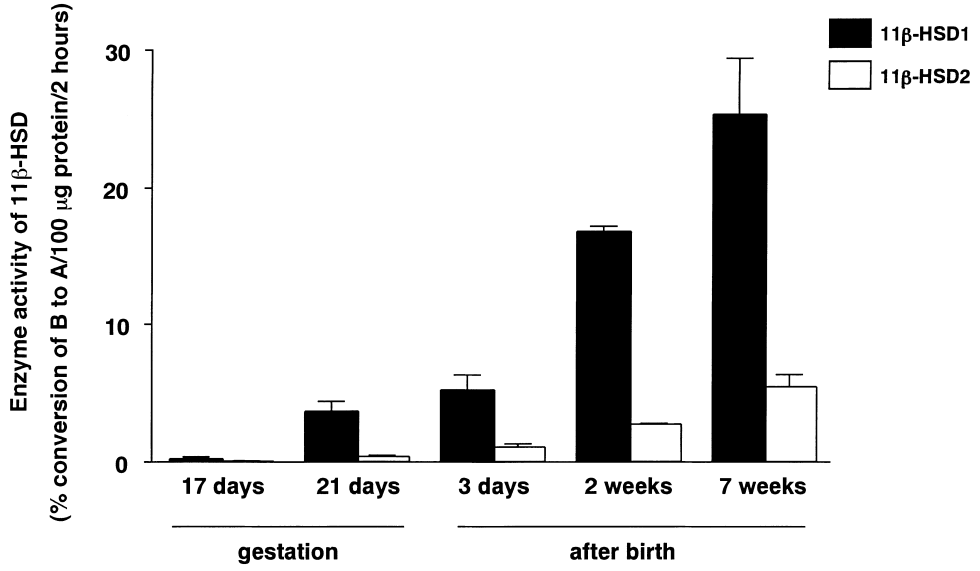

Fig. 1. Enzyme activity of 11β-HSD1 and 2 in rat lungs during development. There was progressive increase in 11β-HSD1 activity from 21 days gestation to 7 weeks after birth. The enzyme activity of 11β-HSD2 was significantly lower than that of 11β-HSD1 throughout gestation and after birth. Data are presented as mean ± SD for 3 different samples.

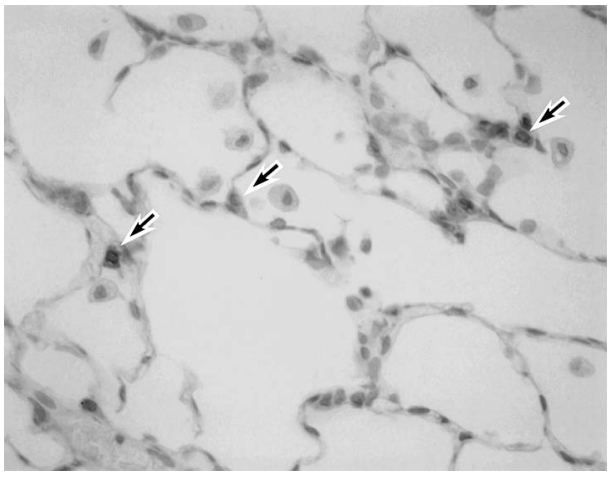

Fig. 2. Immunohistochemistry of 11β-HSD1 in rat lungs. Positive immunoreactivity of 11β-HSD1 was marked in the cytoplasm of the cells in the alveolar wall, but not in alveolar macrophages, in mature rat lungs (7 weeks after birth). Some of those expressing 11β-HSD1 located at the corner of alveoli (arrows). Original magnification X280.

Table 1. Immunohistochemical localization of 11β-HSD1 and GR in developing and mature rat lungs

	11β-HSD1	GR
17 days gestation		
Bronchus (columnar epithelia)	–	+
Mesenchyme	–	+
21 days gestation		
Bronchus (ciliated epithelia)	–	+
Alveolar region	+	+
3 days after birth		
Bronchus (ciliated epithelia)	–	+
Alveolar region	+	+
7 weeks after birth		
Bronchus (ciliated epithelia)	–	+
Alveolar region	++	+

Immunoreactivity was graded as follows: ++, strongly positive; +, weakly positive, and –, negative.

in the alveolar region of developing lung, while bronchial epithelial cells were negative. In mature lung at 7 weeks after birth, immunoreactivity of 11β-HSD1 was observed in the cytoplasm of the cells in the alveolar wall, but not in alveolar macrophages (Fig. 2). Some of these expressing 11β-HSD1 located at the corner of the alveoli (Fig. 2). GR immunoreactivity was detected in all cell types including alveolar epithelial cells at any developmental stage from fetus to adult. The results of immunohistological staining of

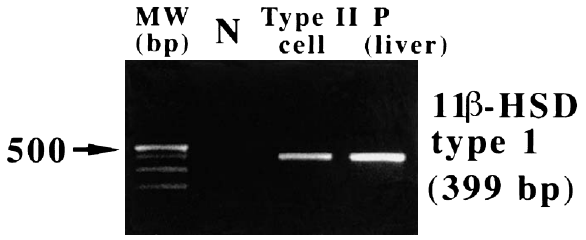

Fig. 3. Reverse transcription-polymerase chain reaction (RT-PCR) analysis of 11β-HSD1 in isolated rat alveolar type II cells. mRNA expression for 11β-HSD1 was detected as a specific single band (399 bp). P; positive control (rat liver), N; negative control (no cDNA substrate). Results were representative of three different samples.

11β-HSD1 and GR in developing and mature rat lungs were summarized in Table 1.

RT-PCR product of 11β-HSD1 mRNA was detected as a specific single band (399 bp) in the preparation of rat alveolar type II epithelial cells as well as rat liver tissue homogenate (Fig. 3).

Discussion

Enzyme activity of 11β-HSD1 was detected in lung tissue homogenates from 21 days in gestation, and progressively increased after birth. This time course is very similar to that reported by Hundertmark *et al*. previously [6]. Importantly, we found that 11β-HSD1 activity was significantly higher than 11β-HSD2 throughout gestation and after birth. This suggests that peripheral rat lung tissue possess reductase activity predominantly, thereby increasing tissue glucocorticoid availability by converting the receptor-inactive glucocorticoids to its active forms.

Does 11β-HSD1 exist in alveolar type II cells, the most important site for the synthesis of pulmonary surfactant in the lungs? Our immunohistochemical examination demonstrated that expression of 11β-HSD1 was restricted to the cells in the alveolar region and was not present in bronchial epithelium or alveolar macrophages, in contrast to ubiquitous distribution of GR. Some of those expressing 11β-HSD1 are thought to be alveolar type II epithelial cells, because of their cuboid shape and localization at the corner of the alveoli. Our RT-PCR data also support the presence of 11β-HSD1 in alveolar type II cells. The cell preparations utilized for RT-PCR in the present study contained small number of other cell types such as ciliated

airway epithelial cells. However, our immunohistochemical examination demonstrated that 11β-HSD1 is not expressed such cells types. Therefore, it is likely that PCR product obtained from the cell preparations indicates the presence of 11β-HSD1 in alveolar type II cells. It is likely that alveolar type II cells expressing 11β-HSD1 is an active glucocorticoid-target site in the lung. In addition to alveolar epithelial cells, 11β-HSD1 may also exist in the pulmonary interstitial fibroblasts [11].

Normal lung development and maturation follows five distinct stages; embryonic, psuedograndular, canalicular, saccular and alveolar, alveolization occures postnatally during weeks 1-3 in the rats [14]. A progressive increase in 11β-HSD1 activity from the late gestation suggests that lung tissue may enhance glucocorticoid availability during final development and maturation by increasing not only circulating glucocorticoid levels, but also local glucocorticoid production. Glucocorticoids play very important role in the modulation of phosphatidylcholine and surfactant protein synthesis in the late gestation fetal lung in alveolar type II cells [15]. Phosphatidylcholine reduces surface tension of the alveolar wall and prevents atelectasis, and may play an important role not only in normal lung development, but also in maintaining normal alveolar structure. Surfactant proteins are known to be essential in the host defense system after birth [16]. In addition, alveolar epithelium plays an important role in absorption of excess alveolar fluid [17]. Alveolar fluid absorption is essential not only in clearing of lung fluid at birth, but also in keeping the alveolar space fluid-free for efficient gas exchange after birth [18]. Although large volumes of Cl^- enriched fluid is secreted from lung epithelium through fetal lung development, alveolar epithelial cells are involved in fluid resorption during late gestation [19]. This dramatic switch to fluid absorbing epithelium is regulated by a range of hormones including glucocorticoids. Expression of alveolar epithelial Na^+ channels and Na^+/K^+-ATPase, which are involved in alveolar fluid absorption [20], are enhanced by glucocorticoids [21–23]. Northern blot analysis showed that the α-subunit of rat epithelial Na^+ channel is first detected at 19 days gestation and progressively increases toward birth, and the β- and γ-subunits appear at the latest stage of the fetal lung development [24]. The existence of 11β-HSD1 in alveolar type II cells suggests that glucocorticoid action could be amplified *in situ* by increasing intracellular glucocorticoid levels via 11β-HSD1. However, it has been reported that 11β-HSD1 knockout mice have no visible abnormality, and their birth weight, postnatal growth and development were reported to be indistinguishable from wild type litter mates [25], indicating that their lungs develop normally. In contrast, maternal carbenoxolone, a potent 11β-HSD inhibitor, decreases the function of placental 11β-HSD and increases fetal glucocorticoid exposure that results in a significant decrease in offspring birth weight [26]. This study suggests that fetal organ development is influenced by extremely high levels of maternal glucocorticoids rather than the steroid that is synthetized in fetus. In addition, fetal and postnatal growth of mice lacking another isoform of 11β-HSD (11β-HSD2) seems to be normal, even though they displays hypertension, cardiomyocyte hypertrophy and significantly enlarged kidney [27]. The exact mechanism of normal lung development may be more complex than the action of glucocorticoids.

Interestingly, 11β-HSD2, which exclusively inactivates glucocorticoids, may also exist in alveolar type II cells of the adult rat [28]. Other cell types including epithelium and endothelium of rat eyes [29] and human endometrial stromal cells [30] were reported to express both 11β-HSD1 and 2. At present, there is no clear explanation for the co-existence of two functionally opposing types of 11β-HSDs in mature alveolar epithelial cells. However, the recent observation demonstrating that 11β-HSD1 and 2 are localized to different regions in placental syncytiotrophoblasts [31] may provide a possible answer.

In summary, we found in the present study that 11β-HSD1 exists in alveolar epithelial cells. Alveolar epithelial cells from late gestation to adult may require higher levels of glucocorticoids than other type of cells in the lung not only in accelerating the surfactant-related lipids and proteins syntheses and establishing an active Na^+ transport system, but also in maintaining their functions in the adult. 11β-HSD1 in alveolar epithelial cells may play a role by amplifying glucocorticoid action *in situ* during normal lung development and maturation.

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