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CYP21A2 expression is localized in the developing distal epithelium of the human perinatal lung and is compatible with *in situ* production and intracrine actions of active glucocorticoids

Wafae Bouhaddioui[a,c], Pierre R. Provost[a,b,c], Yves Tremblay[a,b,c,*]

[a] *Reproduction, Mother and Youth Health, Centre de recherche CHU de Québec, Québec, QC, Canada*
[b] *Department of Obstetrics/Gynecology & Reproduction, Faculty of Medicine, Université Laval, Québec, QC, Canada*
[c] *Centre de Recherche en Biologie de la Reproduction (CRBR), Faculté de Médecine, Université Laval, Québec, QC, Canada*

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ABSTRACT

Glucocorticoids play essential roles in lung development. We investigated for expression of *CYP21A2* (21-hydroxylase) as well as for the presence of the corresponding protein and identification of *CYP21A2*-expressing cells in several human developing lungs. Expression of some related genes was also assessed. CYP21A2 and CYP17A1 (P450c17) mRNAs were found in all the 34 lung samples from 17 to 40 weeks' gestation at variable levels. No correlation was found according to sex but a correlation with age was detected for CYP17A1 only. In contrast, CYP11B1 (11β-hydroxylase)- and CYP11B2 (aldosterone synthase)-mRNAs were not detected. Significant levels of the CYP21A2 protein were detected in all the analyzed samples, while only very low signals were detected for CYP17A1 protein. *In situ* hybridization revealed that *CYP21A2* was almost exclusively expressed in the distal epithelium. It was reported that the lung distal epithelium of human fetuses also express 11β-hydroxysteroid dehydrogenase type 2, which catalyzes cortisol inactivation into cortisone. Based on this information, intracrine glucocorticoid actions should take place from CYP21A2 products through the glucocorticoid receptor in the absence of cortisol. In contrast, mineralocorticoid receptor activation did not seem to depend on deoxycorticosterone produced from local activity of CYP21A2 because of the reported circulating amounts of aldosterone.

1. Introduction

Glucocorticoids (GCs) are principally secreted by the adrenal cortex. GCs play important roles in several physiological processes including lung development. The first evidence of GC benefit on lung maturation was established by Liggins in 1968 [1]. Acceleration of lung maturation was observed in preterm lambs after antenatal GC therapy. This important role was confirmed with a glucocorticoid receptor (GR) knockout mouse model, which showed an altered respiratory system leading to newborn death [2]. GCs are known to play a positive role in fibroblast-epithelial cell-cell communication by increasing the secretion of fibroblast factors leading to the surge of surfactant synthesis [3–5]. GCs also have other effects on the developing lung including a decrease in alveolar wall thickness [6]. For several decades, antenatal GCs have been administered to mothers at risk for premature delivery to accelerate fetal lung maturation and prevent respiratory distress syndrome (RDS) of neonates [7,8]. RDS is the first cause of early neonatal death affecting 20% of neonates weighting less than 2500 g and two thirds of babies of less than 1500 g [9].

In addition to GR, mineralocorticoid receptor (MR) is also expressed in the developing lung [10]. In contrast to GR gene disruption, MR gene disruption did not induced any obvious morphological change in the neonatal lung [11]. In addition, MR-KO mice did not die from a respiratory failure, but rather from a failure of sodium reabsorption between day 8 and 13 [12]. While α-ENaC is known to be involved in lung liquid resorption at birth [13] and MR is known for its role in the induction of ENaC in the kidney [14,15], it was demonstrated that, in the fetal lung, ENaC was modulated by glucocorticoids through GR rather than by mineralocorticoids through MR [16,17]. Aldosterone was associated with the control of cell proliferation in the developing lung [17].

In addition to blood stream contribution to GCs levels in the lung, local synthesis of GCs can also be observed. Peripheral GC synthesis is generally associated with type 1 11β-hydroxysteroid dehydrogenase (HSD) (Fig. 1) [4,18]. This enzyme is expressed in

* Corresponding author at: Department of Obstetrics/Gynecology & Reproduction, Reproduction, Mother and Youth Health, Centre de recherche du CHU de Québec, 2705 Laurier Boulevard, Rm T-3-67, Québec, QC G1V 4G2, Canada.
E-mail address: yves.tremblay@crchudequebec.ulaval.ca (Y. Tremblay).

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the rodent developing lung and its knockout has been shown to impair lung maturation [19]. Emerging evidences from the mouse indicate that active GC should also be produced through the expression of "adrenal" enzymes within the developing lung. Since more than a decade, we know that genes encoding for enzymes involved in GC synthesis in adrenals are expressed in some extra-adrenal tissues such as the thymus [20] and skin [21]. We have demonstrated that it is also the case for the fetal mouse lung [22]. All the genes required for corticosterone synthesis are expressed in the fetal mouse lung on gestation day (GD) 15, near the end of the pseudoglandular stage: *Cyp11a1* (P450scc), *Hsd3b1* (3β-HSD), *Cyp21a1* (*P450c21* or 21-hydroxylase), and *Cyp11b1* (*P450c11β* or 11β-hydroxylase) (Fig. 1) [22]. From GD 17.5 to postnatal day 15.0, all these genes, but *Cyp11b1*, are expressed [23]. This is interesting knowing that deoxycorticosterone is an agonist of GR [24–27] and MR [25,26,28]. *Cyp11b2* (*P450c11AS* or aldosterone synthase) and *Cyp17a1* (*P450c17*) are either not expressed or expressed at very low levels in the tested mouse fetal lung samples [22]. *In vitro* activity assays with fetal mouse lung explants showed synthesis of deoxycorticosterone and corticosterone in specific conditions [29], while aldosterone synthesis was never observed.

There is a difference between human and rodent adrenal GC synthesis pathways. This difference relies to the expression of *CYP17A1* which is found in the human adrenal but not in the rodent adrenal (Fig. 1). As a consequence, the active GC synthesized by *CYP11B1* and secreted in the blood by adrenals is cortisol in human and corticosterone in rodents [30]. Whether the "adrenal" pathway of GC synthesis also exists in the human fetal lung remains to be demonstrated. The products of CYP21A2 (21-hydroxylase in the human) can be transformed into a GC or a mineralocorticoid (aldosterone) depending on the expression of *CYP11B1* and *CYP11B2* in the fetal lung. In the present study, we investigated the expression of *CYP21A2*,*CYP11B1*, *CYP11B2*, and *CYP17A1* in order to characterize the capability of the human fetal lung to produce GC/mineralocorticoid through the "adrenal" steroidogenic pathway. In addition, cell specificity of *CYP21A2* gene was determined by *in situ* hybridization.

2. Materials and methods

2.1. Human tissues

Human fetal lung samples from 17 to 24 6/7 weeks of pregnancy (n = 29, 12 females and 17 males) were obtained from women who gave birth prematurely or were undergoing termination of pregnancy from the same obstetrics unit. The mothers completed a consent form before participating in the project (protocol no. 62-05-13). Two exclusion factors were taken into account: mothers' antenatal glucocorticoid treatment and malformations/diseases affecting the developing respiratory system. Tissues were collected on ice and extensively rinsed in PBS (135 mM NaCl, 2.65 mM KCl, 4.22 mM Na_2HPO_4, 1.45 mM KH_2PO_4). Then, tissues were either frozen at −80 °C for RNA or protein extraction, or fixed during 48 h in 4% paraformaldehyde in PBS before paraffin embedding. Human fetal lung RNA samples from 29 to 40 weeks of pregnancy (n = 5, 2 females and 3 males) and human adult adrenal RNA sample (normal woman, 88 years-old) were purchased from BioChain® (Newark, Ca, USA). A fragment of human adult adrenal tissue obtained before 1999, before the establishment of the ethics committee of Université Laval, was used for microsome and total protein analyses. Another adrenal total protein sample (from a 63 year old man) was obtained from BioChain®.

2.2. RNA extraction, reverse transcription and real-time quantitative PCR

Total RNA was extracted using Tri-reagent (Sigma-Aldrich, Saint Louis, Missouri, USA), a mixture of phenol and guanidine

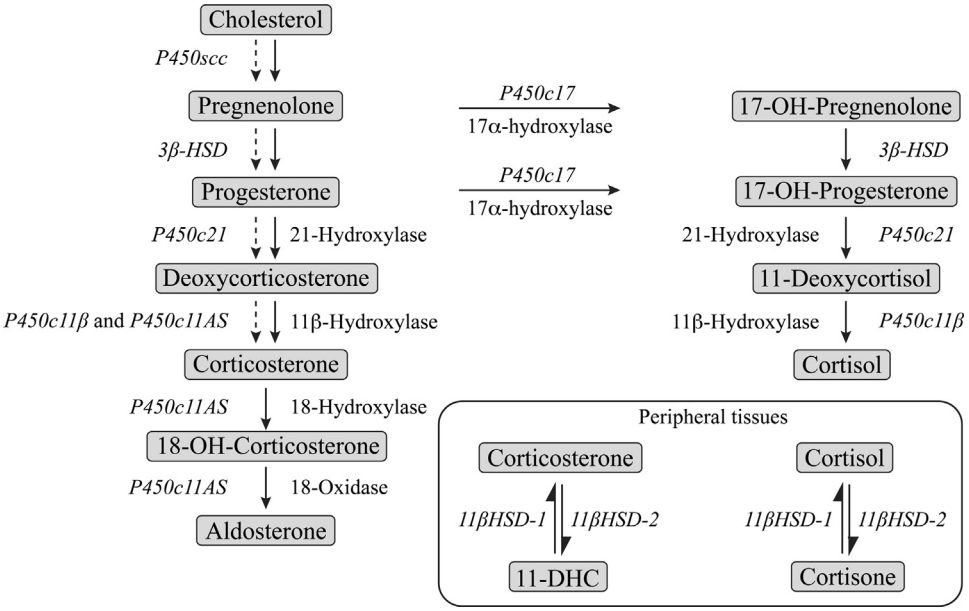

Fig. 1. Adrenal glucocorticoid and mineralocorticoid pathways. Human and rodent mineralocorticoid synthesis pathways (from cholesterol to aldosterone) and human glucocorticoid synthesis pathway (to cortisol through P450c17) are shown with plain arrows. Dotted arrows show the rodent adrenal glucocorticoid pathway (from cholesterol to corticosterone). The "classical" peripheral glucocorticoid metabolic reactions are presented in the box. The protein encoded by *P450c11AS* exerts 11β-hydroxylase, 18-hydroxylase, and 18-oxidase activities. *P450scc*: cytochrome P450 side chain cleavage (*CYP11A1* and *Cyp11a1* in human and mouse adrenals, respectively); *3β-HSD*: 3β-hydroxysteroid dehydrogenase/Δ^5-Δ^4 isomerase (*CYP3B2* and *Cyp3b1* in human and mouse adrenals, respectively, and *Cyp3b1* in mouse lungs); *P450c21*: cytochrome P450 21-hydroxylase (*CYP21A2* and *Cyp21a1* in human and rodent adrenals, respectively); *P450c11β*: cytochrome P450 11β-hydroxylase (*CYP11B1* and *Cyp11b1* in human and rodent adrenals, respectively); *P450c11AS*: cytochrome P450 aldosterone synthase (*CYP11B2* and *Cyp11b2* in human and rodent adrenals, respectively); *P450c17*: cytochrome P450c17 or steroid 17α-hydroxylase/17,20 lyase (*CYP17A1* and *Cyp17a1* in human and rodent adrenals, respectively); *11β-HSD-1* and *11βHSD-2*; type 1 and type 2 11β-hydroxysteroid dehydrogenases, *HSD11B1* and *HSD11B2*.

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