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Differential expression of 11β-hydroxysteroid dehydrogenase types 1 and 2 mRNA and glucocorticoid receptor protein during mouse embryonic development

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Abstract

Accumulating evidence suggests that the actions of glucocorticoids in target tissues are critically determined by the expression of not only the glucocorticoid receptor (GR) but also the glucocorticoid-metabolizing enzymes, known as 11β-hydroxysteroid dehydrogenase types 1 and 2 (11β-HSD1 and 11β-HSD2). To gain insight into the role of glucocorticoids in fetal development, the expression patterns of the two distinct 11β-HSD isozymes and GR were studied in the mouse embryo from embryonic day 12.5 (E12.5, term = E19) to postnatal day 0.5 (P0.5) by in situ hybridization and immunohistochemistry, respectively. 11β-HSD1 mRNA was detected in the heart as early as E12.5 and maintained thereafter. In the lung and liver, 11β-HSD1 mRNA was first detected between E14.5 and E16.5, increased to high levels towards term and maintained after birth. Relatively low levels of 11β-HSD1 mRNA were also detected in the kidney, adrenal glands and gastrointestinal tract at E18.5. However, the mRNA for 11β-HSD1 was undetectable in all other embryonic tissues including the brain. In contrast, kidney was the only organ that expressed appreciable levels of 11β-HSD2 mRNA during embryonic life. The level of 11β-HSD2 mRNA in the kidney increased dramatically in the newborn, which coincided with expression of 11β-HSD2 mRNA in the whisker follicle, tooth and salivary gland. Distinct from the profiles of 11β-HSD1 and 11β-HSD2 mRNA, GR protein was detectable in all tissues at all ages studied except for the thymus, salivary gland, and bone. Taken together, the present study demonstrates that tissue- and developmentally-stage specific expression of 11β-HSD1 and 11β-HSD2 as well as GR occurs in the developing mouse embryo, thus highlighting the importance of these two enzymes and GR in regulating glucocorticoid-mediated maturational events in specific tissues during murine embryonic development.

Keywords: Embryo; Glucocorticoid receptor; Pregnancy; Gene regulation; Developmental biology

1. Introduction

During embryonic life, glucocorticoids play a critical role in processes leading to differentiation and maturation of fetal tissues [1]. Specifically, in the fetal liver, glucocorticoids promote the maturation of enzymes responsible for glycogen deposition shortly before birth [1,2]. In the perinatal period glucocorticoids help promote structural and biochemical maturation of the developing lung [3]. In utero, administration of exogenous corticosterone in corticotrophin releasing factor (CRH)-deficient mice can prevent the abnormal lung histology usually seen in the genotype [4]. The critical role of corticosterone during murine embryonic development is further demonstrated by the findings from two recent studies with reduced [5] or absent [6] GR gene expression. In both studies, severe abnormalities in the

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structure and function of major organs were evident in the offspring. Conversely, exposure of the developing fetus to high levels of glucocorticoids results in intrauterine growth restriction (IUGR) [7–9], and potentially leads to the subsequent development of disease in adult life [10]. Thus, the correct level of fetal glucocorticoid exposure appears to be vital for normal embryonic development.

Glucocorticoid actions are mediated through their specific glucocorticoid receptors (GR). Upon binding the ligand, GR translocate to the nucleus, where they interact with specific DNA elements and/or other transcription factors to induce or repress target genes [11]. In adult mammals, GR are known to be expressed ubiquitously [12]. In target tissues, the actions of glucocortiocoids are critically regulated by the intracellular enzyme, 11β-hydroxysteroid dehydrogenase (11β-HSD), which catalyzes the interconversion of active glucocorticoids (cortisol in humans, corticosterone in rodents) and their inactive metabolites (cortisone and 11-dehydrocorticosterone). To date, two distinct isoforms of 11β-HSD have been cloned and characterized [13,14]. In vivo, 11β-HSD1 is thought to function as a reductase [15,16], increasing active glucocorticoid levels in target tissues. By contrast, 11β-HSD2 inactivates glucocorticoids [17,18]. In view of the deleterious effects of both excess [7–9], and absent [6], glucocorticoid on fetal life, the control of glucocorticoid action by GR, 11β-HSD1 and 11β-HSD2 is likely to play a crucial role in the developing embryo. Moreover, given the widespread use of the mouse as an animal model in gene targeting experiments, a thorough understanding of the precise localization of both GR and 11β-HSD1 and 11β-HSD2 in the mouse embryo during development is invaluable. Although there is evidence for functional GR in murine embryonic tissues [6], and a previous study has localized only 11β-HSD2 mRNA in the mouse embryo [19], the precise localization of 11β-HSD1 and GR in the developing mouse embryo is unknown. Therefore, the objectives of the present study were to determine and contrast the cellular localization of 11β-HSD1 and 11β-HSD2 mRNA by in situ hybridization in the developing mouse embryo from embryonic day 12.5 (E12.5) to E18.5 as well as in the newborn mouse at postnatal day 0.5 (P0.5). In parallel, we also utilized immunohistochemistry to examine the distribution of GR protein over the same gestational period, in order to gain insight into the intricate relationship between 11β-HSD enzymes and GR in the control of glucocorticoid action in the developing mouse embryo.

2. Materials and methods

2.1. Animals and materials

Pregnant BALB/c mice from day 5 of gestation were housed under standard conditions and provided with food and water ad libitum. Animals were killed by cervical dislocation as approved by the Animal Care Committee of the University of Western Ontario. Embryos were collected at E12.5, E14.5, E16.5, E18.5 and P0.5. A total of 4–5 mice per gestational age and 4–6 embryos per mouse were studied/analyzed. Unless stated otherwise, restriction enzymes and other molecular biological reagents were obtained from Gibco BRL (Burlington, Ontario) or Pharmacia Canada Inc. (Baie D'Urfe, Quebec). All solvents used were Omni-Solve grade from BDH Inc. (Toronto, Ontario). All other chemicals were purchased from Sigma–Aldrich Canada Limited (Oakville, Ontario). Slides and other histology supplies were from Fisher Scientific Ltd. (Unionville, Ontario). Oligonucleotides were synthesized using a Pharmacia Gene Assembler and purified using NAP-50 columns (Pharmacia) according to the manufacturer's instructions.

2.2. Immunohistochemistry

Embryos were fixed by immersion with 4% paraformaldehyde in 70 mM phosphate buffer, pH 7.0, at 4 °C for 24 h. They were then embedded in paraffin, and 5 μm sections were prepared by standard methods and mounted onto Superfrost slides. After deparaffinization and rehydration, tissue sections were incubated sequentially in 1% hydrogen peroxide for 10 min to quench endogenous peroxidase activity and then in 10% normal swine serum for 30 min. Tissue sections were incubated in rabbit anti-GR antiserum (1:300; Santa Cruz Biotech Inc., CA) at 4 °C overnight. Sections were immunostained using an avidin–biotin–peroxidase method (LSAB plus-kit; DAKO Corporation, CA), with 3,3-diaminobenzidene as the chromagen. Slides were counterstained with methyl green (DAKO Corporation, CA) and mounted with permount.

2.3. In situ hybridization

Antisense and sense mouse 11β-HSD1 and 11β-HSD2 riboprobes were labeled with [^{35}S]UTP (Du Pont Canada Inc.; Markham, Ontario) by in vitro transcription from the 900 and 580 bp mouse 11β-HSD1 and 11β-HSD2 cDNA, respectively, in pBluescript KS+ [20] using commercially available reagents (Promega Riboprobe Gemini II system). While 11β-HSD1 riboprobes of 150–200 bp were obtained by limited alkaline hydrolysis in 0.2 M bicarbonate buffer, pH 10.2 at 60 °C for 51 min, 11β-HSD2 riboprobes were not subjected to alkaline lysis due to their short length.

In situ hybridization was performed as previously described [20]. Briefly, tissue sections were treated sequentially in PBS containing 0.2% (v/v) Triton-X100 at room temperature for 1 h; 0.2 units proteinase-K/ml in 100 mM Tris–HCl (pH 8.0), and 50 mM EDTA at 37 °C for 30 min; and 0.1 M triethanolamine containing 25 mM acetic anhydride at room temperature for 10 min. They were then dehydrated by increasing (70–100%) ethanol concentrations and air-dried. Sections were prehybridized in a hybridization buffer containing of 50% (v/v) formamide, 0.3 M NaCl, 20 mM Tris–HCl, pH 8.0, 1 mM EDTA, 1× Denhardt's

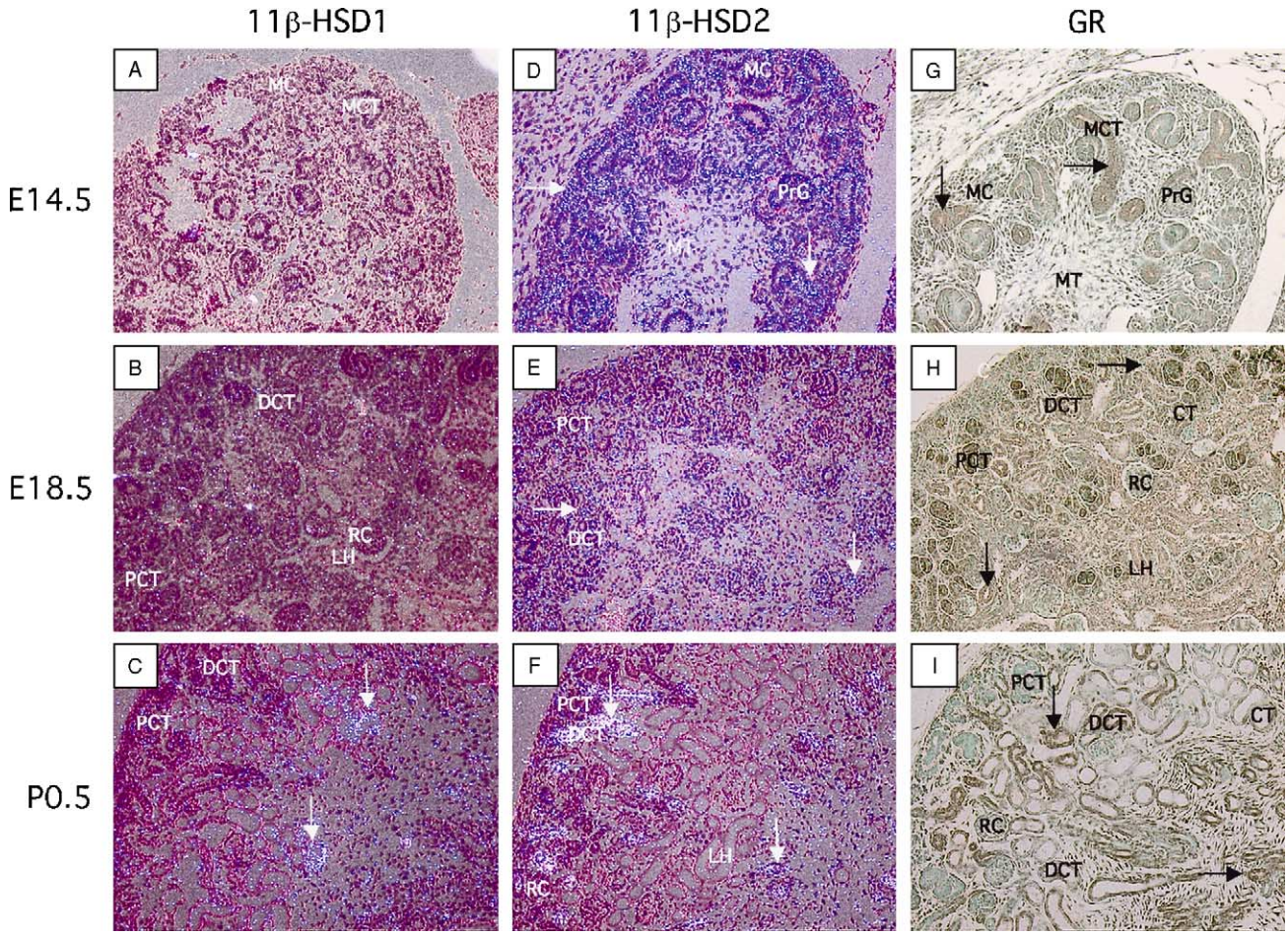

Fig. 1. Localization of 11β-HSD1 and 11β-HSD2 mRNA and GR immunoreactivity in the developing mouse kidney at E14.5, E18.5 and in the newborn. Parts (A–F) are dark field photomicrographs of in situ hybridization of mouse kidney showing localization of mRNA (white dots) for 11β-HSD1 and 11β-HSD2; parts (G–I) are bright field photomicrographs of immunohistochemistry to show localization of GR protein (brown staining). At both E14.5 (A) and E18.5 (B), 11β-HSD1 mRNA is undetectable. In the newborn (C), 11β-HSD1 mRNA is expressed primarily in the collecting duct. At E14.5 (D), 11β-HSD2 mRNA is highly and uniformly expressed in the developing kidney. At E18.5 (E), 11β-HSD2 mRNA begins to be expressed in a cell-specific manner, detected principally in distal convoluted tubules and collecting ducts. In the newborn (F), the expression of 11β-HSD2 mRNA is confined to distal convoluted tubules and collecting ducts. GR immunoreactivity is localized in distal convoluted tubules at both E12.5 (G) and E16.5 (H) and in the newborn (I), indicating that unlike 11β-HSD1 and 11β-HSD2 mRNA, GR immunoreactivity is unchanged with gestation. G, glomerulus; PrG, primitive glomerulus; MCT, metanephronic collecting tubule; MT, mesenchymal tissue; PCT, proximal convoluted tubule; DCT, distal convoluted tubule; LH, loop of Henle; CT, collecting ducts. Arrows indicate positive signals for in situ hybridization and immunohistochemistry. Original magnification 20×.

solution, 500 μg/ml yeast transfer RNA, 100 μg/ml salmon sperm DNA (Loftstrand Labs, MD, USA), 0.1% (w/v) SDS, and 100 mM DTT in a humidified chamber at 45 °C for 2 h. Sections were then hybridized with the same hybridization buffer, except this also included 10% (w/v) dextran sulphate, at 55 °C overnight. The solution containing the riboprobes was removed and sections were incubated for an additional 10 min in pre-hybridization buffer at 55 °C, followed by incubation in 40 μg RNase-A/ml 10 mM Tris–HCl, pH 8.0, 0.5 M NaCl, and 1 mM EDTA, at 37 °C for 30 min. Slides were then taken through the following series of washes: three washes at 10 min each in 2× SSC (1× = 0.15 m NaCl and 0.015 M sodium citrate) at room temperature, four 15 min washes in 2× SSC at 55 °C, and two washes in 0.1× SSC at 55 °C for 10 min each. Sections were then dehydrated in ascending ethanols (70–100%), air dried, and exposed to XAR5 film (Kodak) overnight to determine the intensity of the signal. They were then coated with NTB3 photoemulsion (Kodak) and exposed at 4 °C in light-tight boxes for 1–2 weeks. Slides were developed in D19 developer (Kodak), fixed in Kodafix (Kodak), stained with Harris's hematoxylin and eosin, dehydrated, and mounted with permount.

3. Results

3.1. Localization of 11β-HSD1 mRNA

During late embryonic development, 11β-HSD1 mRNA was undetectable in most of the embryonic tissues except for the heart, lung and liver where it was detected at low levels (Table 1). This pattern of 11β-HSD1 mRNA persisted in the

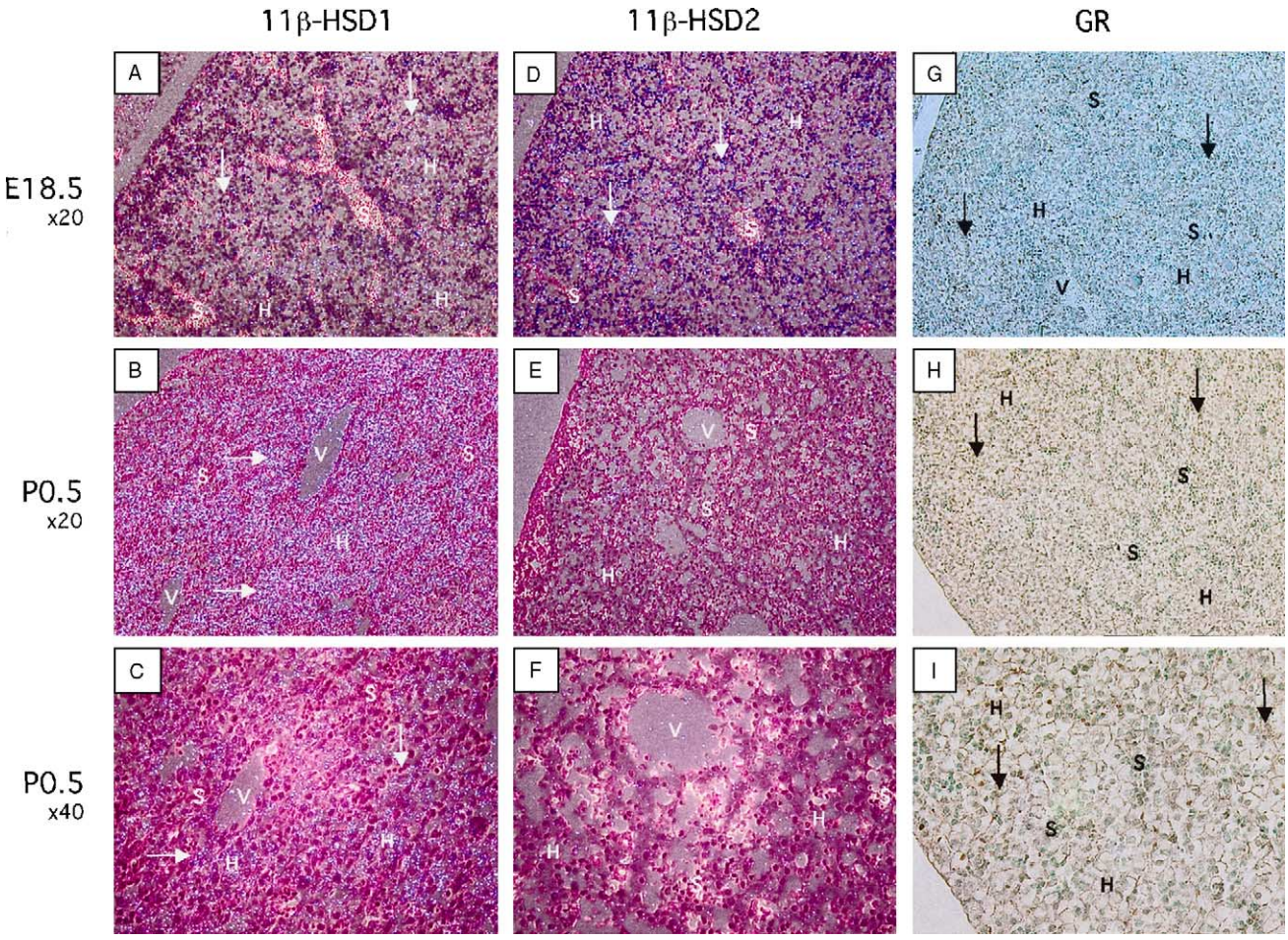

Fig. 2. Localization of 11β-HSD1 mRNA and 11β-HSD2 mRNA and GR immunoreactivity in the developing mouse liver at E18.5 and at P0.5. Parts (A–F) are dark field photomicrographs of in situ hybridization of mouse liver to show localization of mRNA (white dots) for 11β-HSD1 and 11β-HSD2; parts (G–I) are bright field photomicrographs of immunohistochemistry to show localization of GR protein (brown staining). At E18.5 (A), 11β-HSD1 mRNA is expressed at moderate levels, but increased dramatically in the newborn (B and C). In contrast, abundant 11β-HSD2 mRNA is detected in the developing liver at E18.5 (D), its expression decreased to undetectable levels in the newborn (E and F). GR immunoreactivity is clearly detectable in the liver at E18.5 (G) and increased in the newborn (H and I). H, hepatocytes; S, sinusoids; V, hepatic venules. Arrows indicate positive signals for in situ hybridization and immunohistochemistry. Original magnification 20× or 40×, as indicated.

newborn, but with increased level of expression in all three tissues. While evenly distributed in all cellular structures in the heart and liver (Fig. 2), the mRNA for 11β-HSD1 in the lung was detected only in the alveolar epithelium but not in the airway epithelium (Fig. 3). Both the kidney and adrenal glands expressed low levels of 11β-HSD1 mRNA at E18.5 and P0.5 (Table 1; Fig. 1). No specific hybridization signal was detected when the tissue sections were incubated with 11β-HSD1 sense riboprobe (Fig. 5).

3.2. Localization of 11β-HSD2 mRNA

11β-HSD2 mRNA was absent or barely detectable in all the embryonic tissues at all gestational ages studied except for the kidney where variable levels of 11β-HSD2 mRNA were detected (Table 2). In the kidney, 11β-HSD2 mRNA was detectable at E12.5, its level increased at E14.5, but decreased thereafter and remained low for the rest of gestation. The level of 11β-HSD2 mRNA increased dramatically in the newborn kidney. It is noteworthy that although 11β-HSD2 mRNA was uniformly distributed throughout the nephron during embryonic life, its expression in the newborn became cell-specific (distal convoluted tubules and collecting ducts (Fig. 1)). In the developing tooth, whisker follicle and salivary gland, 11β-HSD2 mRNA was expressed at low levels at E18.5, but increased dramatically at P0.5. Low levels of 11β-HSD2 mRNA were also detected in the hypothalamus at P0.5 (Table 2). No specific hybridization signal was detected when the tissue sections were incubated with 11β-HSD2 sense riboprobe (Fig. 5).

3.3. Localization of GR protein

By comparison, GR immunoreactivity was widely detected in the developing mouse embryo except in the cerebellum, salivary gland, thymus and bone at all the ages

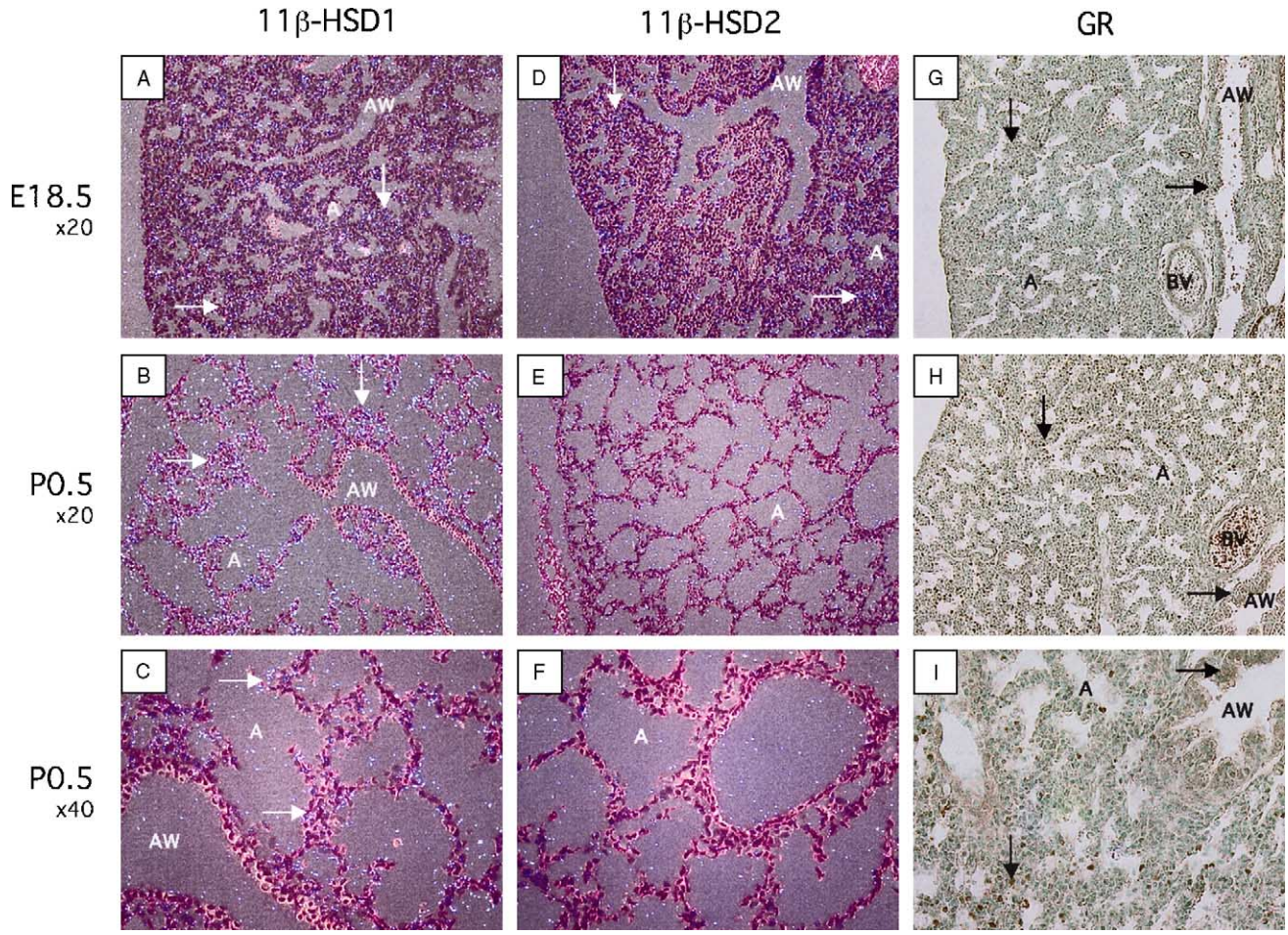

Fig. 3. Localization of 11β-HSD1 mRNA and 11β-HSD2 mRNA and GR immunoreactivity in the developing mouse lung at E18.5 and at P0.5. Parts (A–F) are dark field photomicrographs of in situ hybridization of mouse lung to show localization of mRNA (white dots) for 11β-HSD1 and 11β-HSD2; parts (G–I) are bright field photomicrographs of immunohistochemistry to show localization of GR protein (brown staining). At E18.5 (A), 11β-HSD1 mRNA is barely detectable, but its levels increased dramatically in the newborn (B and C). It is noteworthy that 11β-HSD1 mRNA is detected primarily in the alveoli epithelium and is absent from the airway epithelium. Although low levels of 11β-HSD2 mRNA are detected in the developing lung at E18.5 (D), its expression decreased to undetectable levels in the newborn (E and F). By contrast, constant and high levels of GR immunoreactivity are detected in the lung at E18.5 (G) and in the newborn (H and I). In contrast to the cell-specific expression of 11β-HSD1 mRNA, GR immunoreactivity is detected in both airway and alveoli epithelium. A, alveoli; AW, airway; BV, blood vessel. Original magnification 20× or 40×, as indicated.

studied. GR protein was also absent from developing tooth and whisker follicle until after birth when moderate levels of GR protein were expressed. Consistently high levels of GR protein were detected in the choroid plexus, heart (Fig. 4), kidney, adrenal gland, pancreas, diaphram, lung and liver at al ages studied (Table 3). Immunohistochemical staining was abolished after absorption of antiserum with 10-fold excess concentration of GR (data not shown).

4. Discussion

Tissue growth and differentiation during embryonic life is a complex process regulated by multiple signals including growth factors, transcription regulators and hormones. Glucocorticoids, produced by the fetal adrenals [21] or reaching the embryo via the placenta [22], act as powerful morphogenetic factors influencing a wide range of functions in the fetus. However, both the absence [6] and excess [7–9] of glucocorticoids are deleterious to normal fetal development. As such, the intricate control of glucocorticoid action within fetal tissues by GR, and intracellular glucocorticoid metabolism via actions of 11β-HSD1 and 11β-HSD2, is likely to play a crucial role in prenatal development. Although there is evidence for functional GR in murine fetal tissues [6], and a previous study has localized 11β-HSD2 mRNA in the mouse embryo [19], little is known about the precise localization of either 11β-HSD1 or GR in the developing mouse embryo. The present study demonstrates, for the first time, the relative distribution of mRNAs for 11β-HSD1 and 11β-HSD2 in the mouse embryo, highlighting the potential importance of the two enzymes (if confirmed at protein and activity levels) as regulators of glucocorticoid action during embryonic

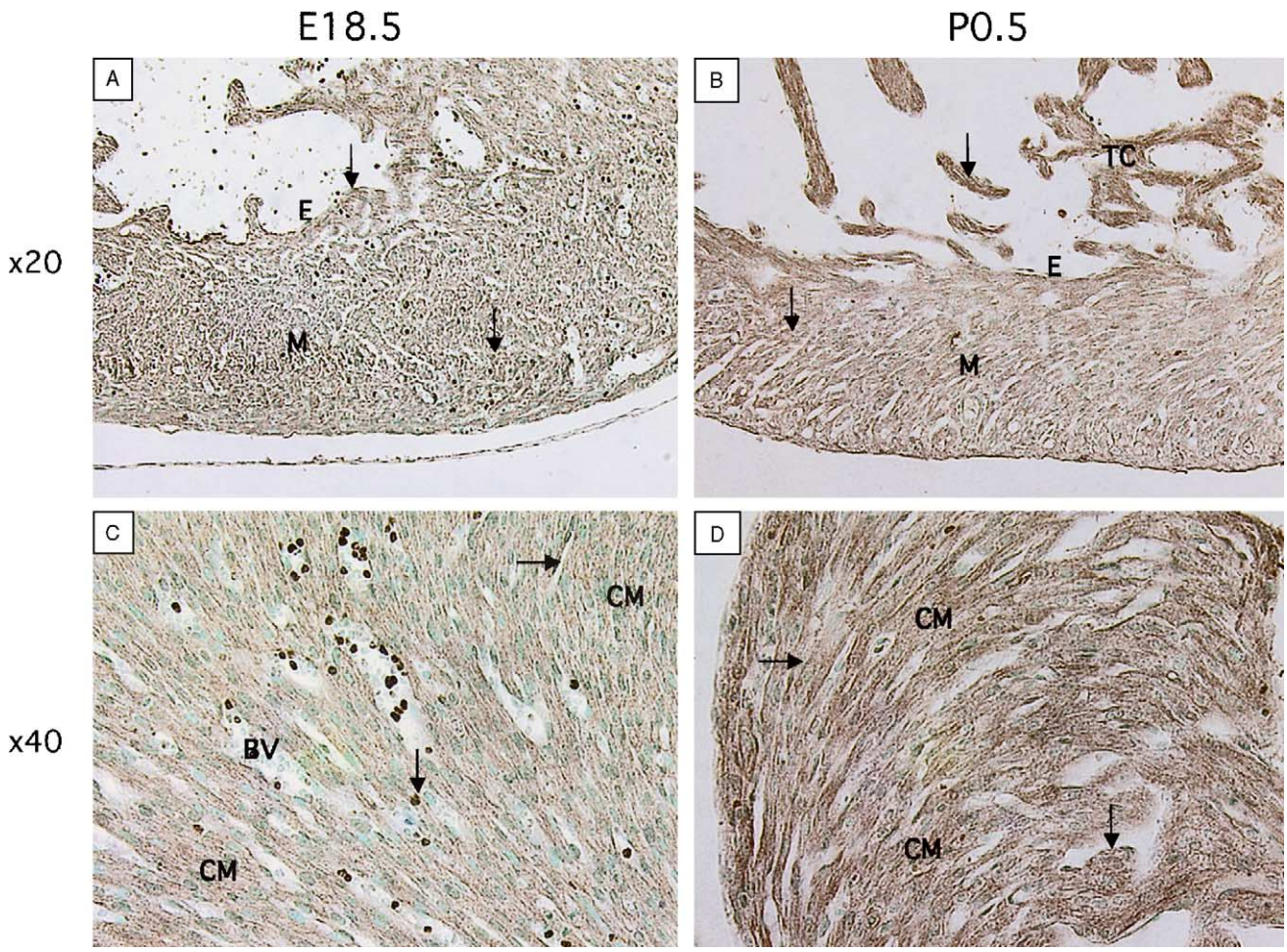

Fig. 4. Localization of GR immunoreactivity in the developing mouse heart at E18.5 and P0.5. Parts (A–D) are bright field photomicrographs of immunohistochemistry to show localization of GR protein (brown staining). At both E18.5 (A and B) and P0.5 (C and D), GR immunoreactivity is distributed uniformly throughout the heart. E, endocardium; M, myocardium; BV, blood vessel; CM, cardiac myocyte; TC, tubeculae carnea. Original magnification 20× in A and B, and 40× in C and D.

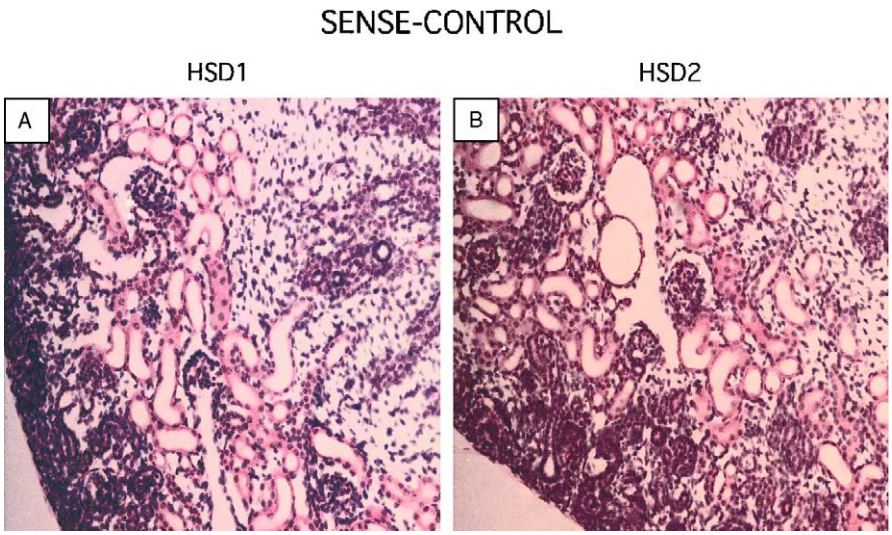

Fig. 5. Dark filled photomicrographs of in situ hybridization of newborn (P0.5) mouse kidney to show sense control for 11β-HSD1 mRNA (A) and 11β-HSD2 mRNA (B). Original magnification 20×.

Table 1
Expression of 11β-HSD1 mRNA in the mouse embryo at E12.5, E14.5, E16.5 and E18.5, and in the newborn (P0.5)

Structure/age	E12.5	E14.5	E16.5	E18.5	P0.5
Brain-pituitary	−	−	−	−	−
Brain-choroid plexus	−	−	−	−	−
Brain-cerebellum	−	−	−	−	−
Brain-hypothalamus	−	−	−	−	−
Developing tooth	−	−	−	−	−
Whisker follicle	−	−	−	−	−
Salivary gland	−	−	−	−	−
Thymus	−	−	−	−	−
Heart	+/−	+/−	+/−	+/−	+/−
Lung	−	+/−	+/−	+	++
Liver	−	−	+/−	+/−	++
Diaphragm	ND	−	−	−	−
Kidney	ND	−	−	−	+
Adrenal	ND	−	−	+/−	+/−
Pancreas	ND	−	−	−	−
Colon/gastrointestinal tract	−	−	−	+/−	+/−
Bone	−	−	−	−	+/−

− or + signs indicate differences in signal intensities observed by optical microscopy and reflect levels of 11β-HSD1 expression. The number of + signs does not represent a strict linear measure of mRNA levels. −, absent; +/−, barely detectable; +, weak expression; ++, moderate expression; ND, not determined.

Table 3
Expression of GR protein in the mouse embryo at E12.5, E14.5, E16.5 and E18.5, and in the newborn (P0.5)

Structure/age	E12.5	E14.5	E16.5	E18.5	P0.5
Brain-pituitary	ND	ND	+	+	+
Brain-choroid plexus	ND	+++	+++	+++	+++
Brain-cerebellum	−	−	−	−	−
Brain-hypothalamus	+/−	+/−	+/−	+/−	+/−
Developing tooth	ND	ND	ND	−	++
Whisker follicle	NA	NA	−	−	++
Salivary gland	−	−	−	−	−
Thymus	−	−	−	−	−
Heart	+++	+++	+++	+++	+++
Lung	+/−	+	++	++	++
Liver	−	+	+	++	++
Diaphragm	ND	++	++	++	++
Kidney	ND	++	++	+++	++
Adrenal	ND	++	++	++	++
Pancreas (islets)	ND	+	++	++	++
Colon/gastrointestinal tract	ND	+	+	+	+
Bone	−	−	−	−	−

− or + signs indicate differences in signal intensities observed by optical microscopy and reflect levels of GR expression. The number of + signs does not represent a strict linear measure of protein levels. −, absent; +/−, barely detectable; +, weak expression; ++, moderate expression; +++, high expression; ND, not determined.

development. Moreover, although tissue-specific changes in 11β-HSD1 and 11β-HSD2 expression occur throughout the latter half of gestation, in the newborn, both enzymes have established localization patterns similar to those described in adult mice [23].

During embryonic life, glucocorticoids play a critical role in processes leading to differentiation and maturation of fetal tissues, particularly the liver and lung [1]. Consistent with this, the expression of GR and 11β-HSD1 in the mouse liver and lung increased substantially between E18.5 and P0.5, which would ensure that the maximal effect of glucocorticoids takes place in these two vital organs immediately prior to birth. This view is also supported by the demonstration of very low levels of 11β-HSD2 mRNA in these two organs during embryonic development and at birth. Since glucocorticoids promote the maturation of organ systems crucial for the successful transition to extrauterine life [24], the observation that fetal plasma glucocorticoid levels decrease just prior to birth [25] has, until now, been intriguing. However, the present findings provide evidence for the precise and distinct regulation of glucocorticoid actions specifically within these crucial target tissues, such that a few days before birth, GR and 11β-HSD1 expression both increase simultaneously, to ensure maximal glucocorticoid-induced maturation of lung and liver.

In the kidney, isozyme-specific changes occur over the gestational period studied. The factors responsible for the cell- and developmental stage-specific changes in 11β-HSD2 expression in the developing mouse kidney are presently unknown. Although it is conceivable that high levels of 11β-HSD2 may be required to confer aldosterone-specificity on renal MR [26,27], a previous study failed to detect MR in the mouse embryo until E18.5 [19]. Thus, it is more likely that in the developing mouse embryo, unlike the adult where 11β-HSD2 is known to restrict the access of glucocorticoid to MR, 11β-HSD2 may function to regulate glucocorticoid access to GR.

Table 2
Expression of 11β-HSD2 mRNA in the mouse embryo at E12.5, E14.5, E16.5 and E18.5, and in the newborn (P0.5)

Structure/age	E12.5	E14.5	E16.5	E18.5	P0.5
Brain-pituitary	−	−	−	−	−
Brain-choroid plexus	−	−	−	−	−
Brain-cerebellum	−	−	−	−	−
Brain-hypothalamus	−	−	−	−	+
Developing tooth	ND	ND	ND	+/−	++
Whisker follicle	ND	−	−	+/−	++
Salivary gland	ND	−	−	+/−	+
Thymus	−	−	−	−	−
Heart	+	−	−	−	−
Lung	−	−	+/−	+/−	−
Liver	+/−	+/−	+/−	+/−	−
Diaphragm	ND	−	−	−	−
Kidney	+	+++	++	+	+++
Adrenal	ND	−	−	−	−
Pancreas	−	−	−	−	−
Colon/gastrointestinal tract	ND	−	−	−	−
Bone	−	−	−	−	−

− or + signs indicate differences in signal intensities observed by optical microscopy and reflect levels of 11β-HSD2 expression. The number of (+) signs does not represent a strict linear measure of mRNA levels. −, absent; +/−, barely detectable; +, weak expression; ++, moderate expression; +++, high expression; ND, not determined.

The adrenal and diaphragm all expressed consistently high levels of GR throughout the gestational period studied, suggesting that glucocorticoids are vital for normal development of these tissues. Moreover, the fact that neither 11β-HSD1 nor 11β-HSD2 is co-localized with GR in these tissues at any gestational age studied, suggests that glucocorticoid access to their receptors may not be strictly regulated, and is thus consistent with a basal 'housekeeping' role for glucocorticoids in these mouse embryonic tissues. In the pancreas, GR expression increased at E16.5, the major differentiation period for this organ in the mouse fetus [28], and is then localized to the islets of Langerhan thereafter, consistent with a role for glucocorticoids in influencing β-cell function in the adult mouse pancreas [29,30]. Although 11β-HSD1 activity and mRNA have been localized to the pancreatic islets of the adult ob/ob mouse [30], in the present study, no 11β-HSD1 mRNA was detected in the pancreas at any gestational age studied or in the newborn. This suggested that the expression of 11β-HSD1 in the mouse pancreas is a postnatal event beyond P0.5 studied.

GR and 11β-HSD2 were co-localized in the whisker follicles and teeth of the newborn mouse. Although 11β-HSD2 mRNA has previously been localized to these structures in mouse embryos [19], mineralocorticoid receptors (MR) were absent from these tissues, an intriguing observation given that 11β-HSD2 is usually co-localized with MR. In this study, 11β-HSD2 has been co-localized with GR in several mouse embryonic tissues, suggesting that 11β-HSD2 may serve to inactivate glucocorticoids and restrict glucocorticoid access to GR during mouse embryonic development.

It is noteworthy that GR is absent from the thymus and bone, a surprising observation in view of the fact that in the adult rodent, both are considered classical glucocorticoid-target tissues. Receptor binding studies have demonstrated the presence of GR in adult mouse thymus [31,32] and, furthermore, defects in thymus function are evident in mice lacking GR [33]. However, controversies exist regarding the role of GR in thymocyte differentiation. Studies using $GR^{-/-}$ mice clearly demonstrated that GR signaling was not required for thymocyte differentiation and development [34,35], while those using a transgenic mouse model showed a partial blockade of thymocyte differentiation with impaired GR function [36]. Our present findings provide additional/critical evidence for the notion that GR is not required for thymocyte differentiation. Likewise, glucocorticoids play an important role in bone cell development and in the maintenance of normal bone structure [37]. Furthermore, mice with targeted GR gene disruption exhibit bone marrow disfunctions [33], suggesting that glucocorticoids play a vital role in both fetal bone and thymus development. Although 11β-HSD1 activity has been reported in both adult and fetal human bone [38] and 11β-HSD2 mRNA has been detected in rat osteoblastic cells [39], neither isoform was expressed in the skeleton of the embryonic mice utilized in the current study. It is likely therefore that GR as well as 11β-HSD1 and/or 11β-HSD2 may be switched on in the mouse bone and thymus postnatally.

In conclusion, the current study highlights complex, tissue- and developmental stage-specific synchronization of expression of 11β-HSD1 and 11β-HSD2 with GR in the developing mouse embryo, which serves to regulate glucocorticoid activity in major target tissues such as the lung and liver. In addition, evidence presented in this paper also suggests that in the mouse fetus, unlike the adult where 11β-HSD2 is known to restrict the access of glucocorticoid to MR, 11β-HSD2 may function to regulate glucocorticoid access to GR.

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