Placenta 37 (2016) 79–84

Contents lists available at ScienceDirect

Placenta

journal homepage: www.elsevier.com/locate/placenta

Species differences of 11beta-hydroxysteroid dehydrogenase type 2 function in human and rat term placenta determined via LC-MS/MS

Kirsten Heussner [a], Matthias Ruebner [b], Hanna Huebner [b], Wolfgang Rascher [a], Carlos Menendez-Castro [a], Andrea Hartner [a], Fabian B. Fahlbusch [a, *, 1], Manfred Rauh [a, 1]

[a] Department of Pediatrics and Adolescent Medicine, University of Erlangen-Nürnberg, Erlangen, Germany
[b] Department of Gynecology and Obstetrics, University of Erlangen-Nürnberg, Erlangen, Germany

ARTICLE INFO

Article history:
Received 5 August 2015
Received in revised form
16 November 2015
Accepted 20 November 2015

Keywords:
LC-MS/MS
Placenta
Glucocorticoid
Steroids
CRH
11beta-hydroxysteroid dehydrogenase
Microsomes
Rat
Human

ABSTRACT

Introduction: Glucocorticoid-induced fetal programming has been associated with negative metabolic and cardiovascular sequelae in the adult. The placental enzyme 11beta-hydroxysteroid dehydrogenase type 2 (11β-HSD2) shields the fetus from maternal glucocorticoid excess by catalyzing the conversion of these hormones into biologically inactive derivatives. In vivo experiments addressing placental barrier function are mostly conducted in rodents. Therefore we set out to characterize species-specific differences of rat and human placental 11β-HSD2 steroid turnover, introducing Liquid Chromatography Tandem Mass-Spectrometry (LC-MS/MS) as a tool for rat tissue analysis.
Materials and Methods: Using LC-MS/MS we determined corticotropin-releasing hormone (CRH), cortisol (F), cortisone (E), corticosterone (B) and 11-dehydrocorticosterone (A) in human and rat placenta at term and measured the enzymatic 11β-HSD glucocorticoid conversion-rates in placental microsomes of both species. In parallel, further glucocorticoid derivatives and sex steroids were determined in the same placental samples.
Results: In contrast to the human placenta, we did not detect CRH in the rat placenta. While cortisol (F) and cortisone (E) were exclusively present in human term placenta (E/F-ratio >1), rat placenta showed significant levels of corticosterone (B) and 11-dehydrocorticosterone (A), with an A/B-ratio <1. In line with these species-specific findings, human placenta showed a prominent 11β-HSD2 activity, while in rat placenta higher 11β-HSD1 glucocorticoid turnover rates were determined.
Discussion: Placental steroid metabolism of human and rat shows relevant species-specific differences, especially regarding the barrier function of 11β-HSD2 at term. The exclusive expression of CRH in the human placenta further points to relevant differences in the regulation of parturition in rats. Consideration of these findings is warranted when transferring results from rodent placental glucocorticoid metabolism into humans.

1. Introduction

The concept of fetal programming describes the association of a stimulus or insult during a critical phase of in utero organ development with persistent structural/functional changes after birth. In humans, fetal programming can result in low birth weight and the subsequent development of insulin resistance, type 2 diabetes and cardiovascular disease [1]. Evidence from animal studies by us [2–4] and others [5] suggests that manipulation of the fetal environment can account for pathophysiological changes in the adult. Glucocorticoids are highly influential on fetal organ development [6,7]. While this knowledge is clinically applied for the induction of lung maturation [8], overexposure to these hormones during pregnancy reduces birth weight and can be detrimental to fetal development in both animals and humans [9–11].

In mammals, the placental enzyme 11beta-hydroxysteroid dehydrogenase type 2 (11β-HSD2) shields the fetus from maternal glucocorticoid excess [12]. Located in placental compartments that act as interfaces of feto-maternal exchange - the syncytiotrophoblast in human placenta and the labyrinth layer in the rat placenta

* Corresponding author. Department of Pediatrics and Adolescent Medicine, Friedrich-Alexander-University of Erlangen-Nürnberg, Loschgestr. 15, 91054 Erlangen, Germany.
E-mail address: fabian.fahlbusch@uk-erlangen.de (F.B. Fahlbusch).
[1] Authors contributed equally.

http://dx.doi.org/10.1016/j.placenta.2015.11.009
0143-4004/

[13–15] - 11β-HSD2 catalyzes the conversion of active glucocorticoids (cortisol in humans, corticosterone in rats) to their inactive 11-keto metabolites (cortisone and 11-dehydrocorticosterone, respectively) [14,16,17]. As a consequence, only up to 20% of maternal cortisol passes to the human fetus [18,19]. In contrast, the placental enzyme 11β–HSD1 converts inactive glucocorticoids to cortisol/corticosterone [14,20].

In humans, reduced placental 11β-HSD2 mRNA levels are found in pregnancies complicated by intrauterine growth restriction (IUGR) [21]. Rats exposed to dexamethasone (poorly catalyzed by 11β-HSD2) or to 11β-HSD inhibitors during the last third of pregnancy, are of low birth weight and develop hypertension and glucose intolerance in adulthood [22–25]. Furthermore, recent studies in animals and humans suggest a negative influence of glucocorticoid-induced fetal programming on offspring neurodevelopment and behavior [26,27]. Interestingly, prenatal stress was found to epigenetically regulate 11β-HSD2 gene expression in the rat placenta and brain [27].

So far, studies have assessed placental 11β-HSD2 activity indirectly via placental perfusion studies or via measurement of the respective hormone levels at the maternal and fetal side, mostly using immunoassays [16,28]. Recently, we have introduced liquid chromatography tandem mass-spectrometry (LC-MS/MS) as a reliable method for the combined quantification of steroids and corticotropin-releasing hormone (CRH) in human placental tissue [29]. CRH is an important regulator of placental 11β-HSD2 expression in humans [30], where the peptide hormone regulates the timing of birth via its interaction with progesterone [31]. Moreover CRH indirectly drives maturation of fetal organ systems via induction of adrenocorticotropic hormone (ACTH) release and subsequent fetal glucocorticoid production. This feedback loop is regulated by the transplacental transport of biologically active glucocorticoids, which is limited by 11β-HSD2 [32].

While only a few mass-spectrometric studies (GC-MS/MS) exist on the analysis of tissue (neuro-)steroids in the rat brain [33–35], the LC-MS/MS method has not been used in rat placental tissue, so far. Moreover, as indirect measurements of 11β-HSD2 substrate- and product-glucocorticoids in the placenta are only surrogates for its enzymatic activity, we set out to determine its direct enzymatic turnover in vitro via microsome preparation. Because the rodent glucocorticoid-excess model is frequently used to address questions of human fetal programming, we were interested in the placental 11β-HSD2 activity of the different species.

Typical assays of enzyme activity are difficult, since they commonly utilize radiolabeled steroid substrates [36,37] followed by separation techniques such as thin layer chromatography to remove unreacted substrate and liquid scintillation counting. Such approaches are time-consuming and impractical for high-throughput screening. Moreover handling and disposal of radioactive material require special licensing for their handling [38,39]. To address this problem, we have developed a sensitive LC-MS/MS method for assessing enzyme activity and show its utility to quantify enzyme activity for a variety of steroid substrates. The aim of our study is the introduction of the LC-MS/MS method as a novel, sensitive technique for the comparison of human and rat 11β-HSD-activities in placental tissue at term, offering the possibility of direct and indirect determination of glucocorticoid turnover rates along with the ability to study multiple steroids simultaneously.

2. Materials and Methods

2.1. Animals and surgical procedures

All procedures performed on animals were in line with the *Guide for the Care and Use of Laboratory Animals* published by the US National Institutes of Health (NIH Publication No. 85-23, revised 1996). The *EU Directive 2010/63/EU* for animal experiments was followed. The experimental protocol was approved by the appropriate Institutional and Governmental Review Boards (Regierung von Mittelfranken, AZ #54-2531.31-12/06). Placental tissue was obtained from healthy rats at E21.5, as previously described in detail [40,41]. Briefly, time-mated pregnant Wistar rats were ordered form Charles River (Sulzfeld, Germany). Animals were housed under standard conditions with free access to standard chow (no. 1320; Altromin, Lage, Germany) and tap water. On day E21.5 of pregnancy, all dams were anesthetized by isoflurane inhalation and underwent Cesarean section. Rat brain hemispheres were obtained from adult rats, as a reference for CRH after sacrifice.

2.2. Sample collection and preparation

Rat placenta and brain were collected as described above. All collected tissues and tissue samples were immediately snap-frozen in liquid nitrogen and stored at −80 °C until further usage. Healthy human placental tissue at term was obtained and processed immediately after birth, as previously described [42]. In short, after removal of decidua and fetal membranes placental tissue (~8 g per sample) was collected from three different sites with regard to their proximity to the umbilical cord to minimize sampling site influences. Samples were snap-frozen in liquid-nitrogen and stored at −80 °C until further use. For the LC-MS/MS measurement of tissue steroids along with CRH we used total rat placenta ($n = 6$, range 157–269 mg), total brain hemispheres ($n = 4$, range 299–532 mg) and fractions from each of the three samples from 5 human placentas (range 84–445 mg). For the measurement of 11β-HSD enzymatic activity, total rat placentas and a ~2.5 g fraction from each of the three samples from 5 human placentas was used.

Steroid and CRH measurements were conducted in rat and human placentae at term (range of gestational age 37.5–40.3 weeks, mean maternal age 35.3 years, all cesarean sections, 3 from male, 2 from female newborns). These time-points were chosen for a better comparison of our findings between species and the literature [13,14,43]. LC-MS/MS measurements were carried out using rat total placenta ($n = 6$), rat brain hemispheres ($n = 4$) and samples of human placenta ($n = 5$). Per 0.5 g of tissue, 1.02 ml of ethanol containing 20 μl/ml proteinase-inhibitor cocktail (cOMPLETE, Roche Diagnostics Deutschland GmbH, Penzberg, Germany) were added. Tissues were homogenized at 4 °C using a Precellys Ceramic Kit on a Precellys® 24 tissue grinder equipped with a Cryolys-module for liquid-nitrogen cooling (Peqlab, Erlangen, Germany). Conditions were 2 × 30 s at 224 × g for rat tissue and 6 × 30 s at 224 × g for human tissue with an inter-cycle pause of 40 s. Subsequently, samples were ultra-sonificated on ice (UW2070, Bandelin Electronic, Berlin, Germany) (settings: cycle 5, power 50%, 40 s). The homogenized samples were transferred into Eppendorf LoBind-tubes (Fisher Scientific GmbH, Schwerte, Germany). After centrifugation for 10 min (23,000 × g, 4 °C), supernatant was used for further analysis.

2.3. Microsomal preparation

Microsomes were prepared from total placentae of Wistar rats ($n = 6$) and humans ($n = 5$, with 3 samples from different sites of the placenta, which makes a total of 15 samples). All subsequent steps were performed at 0–4 °C. Tissues were homogenized in 0.01 M sodium phosphate buffer (pH 7.4) containing 0.25 M sucrose (Precellys® 24-Dual homogenizer, Peqlab, Erlangen, Germany). Homogenates were ultrasonificated, followed by filtration through shredder spin columns (QIAshredder homogenizer, QIAgen GmbH, Hilden, Germany) at 21,000 × g for 2 min. For further extraction,