RESEARCH

Diethylstilbestrol inhibits human and rat 11 β -hydroxysteroid dehydrogenase 2

Yiyan Wang*, Yaoyao Dong*, Yinghui Fang, Yao Lv, Qiqi Zhu, Xiaoheng Li, Qingquan Lian and Ren-Shan Ge

Department of Anesthesiology, The Second Affiliated Hospital and Yuying Children's Hospital of Wenzhou Medical University, Wenzhou, Zhejiang, China

Correspondence should be addressed to R-S Ge: r_ge@yahoo.com

*(Y Wang and Y Dong contributed equally to this work)

Abstract

Glucocorticoid hormone might cause intrauterine growth restriction. The glucocorticoid-metabolizing enzyme 11 β -hydroxysteroid dehydrogenase 2 (HSD11B2) in the placenta eliminates excess levels of glucocorticoids during pregnancy. The aim of the current study was to define the effects of diethylstilbestrol (DES) on HSD11B2 activity in the mammalian placentas and identify its mode of action. Rat and human placental microsomal HSD11B2 were incubated with different concentrations of DES, and IC₅₀ values were determined. The mode of action was analyzed by incubation of DES together with substrates, glucocorticoid and NAD⁺. DES suppressed rat and human HSD11B2 with IC₅₀ values of 5.33 and 12.62 μ M, respectively. DES was a competitive inhibitor of rat and human HSD11B2 when steroid substrates were added, while it was an uncompetitive inhibitor when cofactor NAD⁺ was exposed. Oral administration of DES (0.5 mg/kg) to the rat delayed the cortisol metabolism in adult female Sprague–Dawley rats, as indicated by the increases in cortisol's elimination half-life, maximum concentration and area under the curve. In conclusion, DES is a potent HSD11B2 inhibitor, possibly contributing to the intrauterine growth restriction.

Key Words

- ▶ diethylstilbestrol
- ▶ 11 β -hydroxysteroid dehydrogenase 2
- ▶ glucocorticoid
- ▶ intrauterine growth restriction

Endocrine Connections
(2019) **8**, 1061–1069

Introduction

Diethylstilbestrol (DES) (Fig. 1) is a potent synthetic estrogen (1), as its relative binding affinity to rat estrogen receptors being three-fold higher than that of the natural ligand 17 β -estradiol (2). DES was once prescribed by physicians for pregnant women to treat the loss of pregnancy (3). However, *in utero* exposure to pharmacological doses of DES was demonstrated to be associated with adverse effects on the reproductive tract of both male and female offspring (4). DES has also been demonstrated to cause placental alterations such as a reduction of the labyrinthine region and accumulation of trophoblast giant cells in mid- and late-gestation in mice (5). Animal studies showed that *in utero* exposure to DES induced an intrauterine growth restriction of pups in the placentas (6). However, the mechanism has not been fully elucidated.

Glucocorticoid hormone is an inducing factor of intrauterine growth restriction. Maternal glucocorticoid (cortisol in the human and corticosterone, CORT, in the rat) levels are much higher than those in the circulation of the fetus. Although the maternal glucocorticoid can easily penetrate the placenta to enter the fetal circulation, there is a mechanism to achieve this gap of glucocorticoid level between the mother and the fetus. This mechanism has been found to be achieved by the glucocorticoid metabolizing enzyme 11 β -hydroxysteroid dehydrogenase 2 (HSD11B2) in the placenta (7). HSD11B2 is an NAD⁺ oxidase, catalyzing biologically active cortisol or CORT into inert cortisone or 11-dehydrocorticosterone (11DHC). Thus, even there is a huge fluctuation of maternal cortisol or CORT levels, hardly any of them can get into the fetal circulation. Thus, HSD11B2 is a critical

Figure 1
Chemical structure of cortisol and diethylstilbestrol.

enzyme that protects the fetus from overexposure to the maternal cortisol and CORT, which potentially interferes with fetal development and causes cardiovascular, metabolic and neuropsychiatric disorders in the postnatal period (8, 9, 10). A prospectively controlled multicenter study demonstrated that HSD11B2 expression was associated with the intrauterine growth restriction in human beings (11). Furthermore, inhibition of HSD11B2 activity by a potent inhibitor carbenoxolone in cultured trophoblast cells stimulated secretion of the human chorionic gonadotropin and caused a rapid differentiation of cytotrophoblasts into syncytial trophoblasts (12). Therefore, HSD11B2 might influence the placental function (12). Indeed, fetal and placental weights were reduced in the *Hsd11b2* gene knockout mice (13). It is likely that intrauterine growth restriction of fetal development after knockout of the HSD11B2 enzyme was mediated in part by altered transport of nutrients across the placenta into the developing fetus (13). In the present study, we investigated the effects and the mode of action of DES on HSD11B2 activity in both rat and human placenta.

Materials and methods

Chemical and animals

[1,2,6,7- ^3H]corticosterone (^3H -CORT) and [1,2,6,7- ^3H]cortisol (^3H -cortisol) were from Dupont-New England Nuclear (Boston, MA, USA). ^3H -11-dehydrocorticosterone

(^3H -11DHC) and ^3H -cortisone were prepared from labeled ^3H -corticosterone or ^3H -cortisol as previously described (14). Cold corticosterone, 11-dehydrocorticosterone (11DHC), cortisol, pirfenidone and cortisone were purchased from Steraloids (Newport, RI, USA). Trifluoroacetic acid (purity >98.0%) was obtained from Sigma-Aldrich Company. Female Sprague–Dawley rats (250–300 g) were purchased from Shanghai Laboratory Animal Center (Shanghai, China). The animal protocol was approved by the Wenzhou Medical University's Animal Care and Use Committee. Full-term human placentas (Table 1) were obtained from the Second Affiliated Hospital of Wenzhou Medical University under the approval of The Ethics Committee of the hospital and consent of the subjects.

Preparation of microsomal protein

Four female dams (gestational day 18) were killed by CO_2 asphyxiation, and one placenta from each rat was randomly collected (Table 2). Four full-term human placentas were obtained (Table 1). Both rat and human placental microsomal fractions were prepared as previously described (15). In brief, samples were homogenized in 0.01 mM phosphate-buffered saline (PBS) containing 0.25 M sucrose. Nuclei and large cell debris were removed by centrifugation at 750 g for 30 min. The post-nuclear supernatants were transferred to new tubes and centrifuged at 10,000 g for 30 min. Then, the supernatants were transferred to ultracentrifugation tubes and centrifuged twice at 105,000 g and the resultant microsomal particulates were resuspended in PBS by homogenization. Protein concentrations were measured using the Bio-Rad Dye Reagent Concentrate (Bio-Rad, cat. no. 500-0006) and adjusted to 20 mg/mL for storage. Microsomal suspensions were used for the measurement of HSD11B2 activity.

HSD11B2 assay

Corticosterone or cortisol was used as the enzyme-substrate to measure HSD11B2 activity as described (16). HSD11B2 activity was assayed in an enzyme reaction mixture containing 25 nM CORT (for rats) or cortisol (for humans) spiked with 60,000 dpm of their respective (radiolabeled) ^3H -steroids, 0.2 mM NAD^+ , 0.1 mM DTT and microsomes and the reaction mixture was incubated at 37°C in a shaking water bath (75 rpm) for 15 min. DES was dissolved in dimethyl sulfoxate (as the control, CON) with a final concentration of 0.4%, at which dimethyl sulfoxate did not inhibit HSD11B2 activity as described (16).

Table 1 Relevant information about the human placentas used.

Parameter	ID number			
	01570740	01569309	01589146	01588002
Maternal age (years)	27	24	31	25
Weight (kg)	60	59	67.5	56
Gestational age (weeks)	39 ⁺²	40	40 ⁺¹	39 ⁺²
Gravidity	1	1	2	1
Parity	1	1	2	1
Birth weight (g)	2820	3280	3280	3210
Blood pressure	133/88	125/74	115/63	112/56
Baby's sex	Female	Male	Female	Female

The HSD11B2 reaction was linear within this time frame. The reaction was stopped by adding 2 mL ice-cold ether into reaction tubes. The tubes were vortexed vigorously for 1 min and the steroids were extracted with ether. Then, the ether layer was transferred into a glass tube and was dried up under nitrogen. The steroids were plotted onto a thin layer plate and separated chromatographically in chloroform and methanol (90:10, v/v). The plate was dried up and the steroid radioactivity was read using a scanning radiometer (System AR2000, Bioscan Inc., Washington, DC, USA) as previously described (15). The percentage conversion of CORT to 11DHC or cortisol to cortisone was calculated by dividing the radioactive counts identified as 11keto-steroids by the total counts.

Determination of the half maximum inhibitory concentration (IC₅₀) and mode of action

IC₅₀ value was determined by adding 25 nM of CORT or cortisol and 0.2 mM NAD⁺ with various concentrations (0.1–100 μM) of DES to the 250 μL reaction buffer (0.1 mM PBS) containing 2–10 μg rat or human microsomal protein as previously described (17). The mode of action was measured by adding various concentrations (0.1–1 μM) of either CORT or cortisol to the assay tubes.

Animals and treatment for *in vivo* inhibition of HSD11B2

Cortisol is not the endogenous glucocorticoid in rats (18). Therefore, administration of exogenous cortisol to

rats can be used to monitor its metabolism as an index of *in vivo* HSD11B2 activity (19). Twelve female rats, weighing 300 ± 20 g, were divided into two groups that were orally administered with either DES 0.5 mg/kg or the vehicle (dimethyl sulfonate versus water = 1:9 as control; v/v) once. Thirty minutes later, rats received intraperitoneal injections of cortisol at 20 mg/kg body weight based on the previous study (20). A preliminary study found that at least 0.5 mg/kg DES is required to alter the cortisol pharmacokinetics. Animals were then anesthetized by the administration of 10% chloral hydrate. Blood samples (800 μL) were collected directly from the tail vein into glass tubes (Sigma) at 5, 10, 20, 40, 60, 90, 120, 150, 180 and 240 min. Blood samples were centrifuged at 2000 g for 10 min to obtain sera, which were then stored at –20°C until analysis. Because the kidney expresses high HSD11B2 levels (21, 22), six rats in each group were killed 40 min after DES administration to obtain the kidneys for processing in order to measure HSD11B2 activity *ex vivo*. CORT at 25 nM spiked with 60,000 dpm tracer were added to and mixed with sliced kidney samples. Reactions were stopped 10 min later by adding 3 mL ice-cold ether. The tubes were vortexed vigorously. The steroids were extracted and separated and detected as described earlier.

Serum steroid extraction

Serum samples were prepared by the liquid–liquid extraction technique. A 300 μL aliquot of the serum sample was placed in a 10 mL glass test-tube, on which 20 μL of pirfenidone (internal sample, IS, 100 μg/mL) was spiked and 150 μL of sodium hydroxide solution (0.01 mol/L) was added. 3 mL of extraction solvent (ethyl acetate) was added and the mixture was vortexed for 2 min. The organic layer was separated after centrifugation at 4000 g for 15 min. The organic layer was transferred into a new glass tube and dried up under a nitrogen gas and then steroids extracted were dissolved in 200 μL of the mobile

Table 2 Relevant information about the rat placentas.

Parameter	ID number			
	Rat 1	Rat 2	Rat 3	Rat 4
Dam weight	377	391	365	375
Placental weight (g)	1.01	0.82	0.95	0.85
Pup	12	14	13	12

phase. An aliquot (20 μ L) of the final supernatant was injected into an HPLC-MS/MS system for analysis as below.

UPLC-MS/MS conditions

Liquid chromatography for detection of cortisol was performed on an Acquity Ultra Performance Liquid Chromatography (UPLC) unit (Waters Corp.) with an Acquity BEH C18 column (2.1 mm $\times$ 50 mm, 1.7 μ m particle size) and inline 0.2 μ m stainless steel frit filter (Waters Corp.) as previously described (23). A gradient program was employed with the mobile phase combining solvent A (0.1% formic acid in water) and solvent B (acetonitrile) as follows: 5–5% B (0–0.5 min), 5–95% B (0.5–1.0 min), 95–95% B (1.0–2.0 min), 95–5% B (2.0–2.1 min). A subsequent re-equilibration time (0.9 min) was performed before next injection. The flow rate was 0.40 mL/min and the injection volume was 6 μ L. The column and sample temperature were maintained at 40°C and 4°C, respectively. An XEVO TQD triple quadrupole mass spectrometer equipped with an electrospray ionization (ESI) source (Waters Corp.) was used for mass spectrometric detection. The detection was operated in a multiple reaction monitoring modes under unit mass resolution in the mass analyzers. The multiple reaction monitoring mode transitions were m/z 358.3 $\rightarrow$ 340.3 and m/z 237.2 $\rightarrow$ 194.3 for cortisol and IS, respectively. The Masslynx 4.1 software (Waters Corp.) was used for data acquisition and instrument control.

Calculation of cortisol pharmacokinetics

Serum cortisol concentration versus time for each rat was analyzed by DAS (Drug and statistics) software (Version 2.0, Shanghai University of Traditional Chinese Medicine, China). The parameters of pharmacokinetics calculated were followed: elimination half-life ($t_{1/2}$), the peak plasma concentration of a drug after administration (C_{max}), time to reach C_{max} (T_{max}), the integral under the concentration–time curve (AUC) and the volume of plasma cleared of the drug per unit time (CL_z/F).

Statistics

Each experiment of the enzyme assay was repeated four times. Data were subjected to nonlinear regression analysis by GraphPad (version 6, GraphPad Software Inc., San Diego, CA, USA) for IC_{50} . Lineweaver–Burk plot was used in the analysis of the mode of action. Data were

subjected to analysis by one-way ANOVA followed by *ad hoc* Tukey multiple comparisons, testing significant differences between groups. Serum cortisol concentration versus time data of each rat was analyzed by DAS software. All data are expressed as means $\pm$ s.e.m. Differences were regarded as significant at $P < 0.05$.

Results

DES inhibits rat placental HSD11B2 activity

The effect of DES on rat placental HSD11B2 activity was examined at 100 μ M, at which concentration it inhibited the enzyme over 50% (Fig. 2A). Analysis of concentration-dependent inhibition by DES estimated an apparent IC_{50} value of 5.33 ± 0.08 μ M (Fig. 3A).

DES inhibits human placental HSD11B2 activity

The effect of DES on human placental HSD11B2 activity was examined at 100 μ M. At this concentration, DES inhibited enzyme activity over 50% (Fig. 2B).

Figure 2

Diethylstilbestrol (DES) inhibits rat and human placental 11 β -hydroxysteroid dehydrogenase 2 (HSD11B2) activities. HSD11B2 activity was measured in the presence of 100 μ M DES as described in Materials and methods. Panel A, Rat HSD11B2; Panel B, Human HSD11B2. Mean $\pm$ s.e.m. ($n = 4$). *** designates significant difference when compared to control (CON, DMSO) at $P < 0.001$.

Figure 3

DES concentration-dependently inhibits rat and human placental 11 β -hydroxysteroid dehydrogenase 2 (HSD11B2) activities. Panel A, Rat HSD11B2; Panel B, Human HSD11B2. Mean $\pm$ S.E.M. ($n = 4$).

Based on a concentration-dependent inhibition by DES, its apparent IC₅₀ value was 12.65 $\pm$ 0.08 μ M (Fig. 3B).

Mode of action of DES

The mode of action of DES-mediated inhibition of HSD11B2 activity was determined by enzyme kinetic analysis. Using rat placental microsomes, the Lineweaver–Burk plot showed that DES was a competitive inhibitor of HSD11B2 when CORT was used as the substrate (Fig. 4A) and an uncompetitive inhibitor in the presence of the cofactor NAD⁺ (Fig. 4B). DES exhibited a similar inhibitory mode for human placental HSD11B2 (Fig. 5A and B).

DES *in vivo* inhibits rat HSD11B2 activity

Serum cortisol concentrations were measured after DES (0.5 mg/kg) was gavaged to adult female Sprague–Dawley rats 30 min before the administration of cortisol as the probe. Concentration–time course analysis showed a significant increase of bioavailability of DES (Fig. 6). Further analysis showed that there was a delay in cortisol elimination from blood because the elimination half-life ($t_{1/2}$), the maximum concentration (C_{max}) and area under

Figure 4

Lineweaver–Burk plot of rat placental 11 β -hydroxysteroid dehydrogenase 2 (HSD11B2) in the presence of diethylstilbestrol (DES). Panel A, 1/V versus 1/(CORT); Panel B, 1/V versus 1/(NAD⁺) V = velocity (pmol/mg protein $\times$ min). Mean $\pm$ S.E.M., $n = 4$.

the curve (AUC) that reflects the level of glucocorticoid were significantly increased after DES administration (Fig. 7). This indicates that DES blocks cortisol metabolism. Furthermore, analysis of HSD11B2 activity *ex vivo* showed that renal HSD11B2 activity was significantly decreased after DES treatment (Fig. 8).

Discussion

DES is a potent estrogen and it has been associated with disruption of the endocrine axis in several animal species (24, 25, 26, 27, 28). The mechanism associated with DES-related intrauterine growth restriction is still unclear. In the present study, we performed experiments to demonstrate that DES is a powerful HSD11B2 enzyme inhibitor *in vitro* and *in vivo*. The IC₅₀ values of DES on rat and human HSD11B2 activities were calculated to be 5.33 and 12.62 μ M, respectively.

In the placenta, HSD11B2 is known to protect the fetus from high circulating levels of glucocorticoids in the mother (10, 29, 30) by catalyzing cortisol into inert cortisone (31, 32). Indeed, mouse models with a null mutation of the *Hsd11b2* gene showed placental

Figure 5

Lineweaver-Burk plot of human placental 11 β -hydroxysteroid dehydrogenase 2 (HSD11B2) in the presence of diethylstilbestrol (DES). Panel A, 1/V versus 1/(cortisol); Panel B, 1/V versus 1/(NAD⁺) V = velocity (pmol/mg protein $\times$ min). Mean $\pm$ S.E.M., $n = 4$.

anomalies and dysfunction that resulted in intrauterine fetal growth retardation (33). Thus, our observations imply that DES-related suppression of HSD11B2 activity during pregnancy may be a factor in disruption of glucocorticoid-mediated activity that causes adverse consequences on fetal development. The indirect effect via the direct

Figure 6

Effect of co-administration with diethylstilbestrol (DES) and cortisol on serum cortisol concentrations. Close circle: control (dimethyl sulfonate versus water = 1:9, v/v); open circle: DES administration. Mean $\pm$ S.E.M., $n = 6$.

inhibition of HSD11B2 by endocrine disruptors often goes unnoticed. Although some endocrine disruptors are inhibitors of HSD11B1 and HSD11B2 (34), there is very limited information on placental HSD11B2 inhibitors. One HSD11B2 inhibitor is the metabolite of methoxychlor, 2-bis(p-hydroxyphenyl)-1,1,1-trichloroethane (HPTE) (35). In the present study, we presented another placental HSD11B2 inhibitor, DES. Here, we demonstrated that DES directly inhibited both human and rat placental HSD11B2 enzyme activities at the micromolar concentration range. These effects were not exerted via gene expression and translation for HSD11B2. Other studies also showed that DES at pM can inhibit human β -chorionic gonadotropin (β -hCG) secretion by the human trophoblast cell lines BeWo and HTR-8 (36), although there are no studies to report its effects on HSD11B2 expression in these cell lines or human and rat placentas.

Moreover, HSD11B2 is thought to be the gate-keeper for the mineralocorticoid receptor in several target tissues, including the kidney and colon (37). Usually, mineralocorticoid receptors are without selectivity between aldosterone (a natural mineralocorticoid) and cortisol, a natural glucocorticoid (38). Because serum concentration of cortisol is approximately 100-fold greater than that of serum aldosterone, without HSD11B2, mineralocorticoid receptors are always bound by cortisol, thus causing hypertension and hypokalemia (38). HSD11B2 is a high-affinity enzyme and effectively eliminates cortisol through converting it into cortisone, which has the negligible binding affinity to the mineralocorticoid receptor (39, 40). This interpretation is supported by observations from null *Hsd11b2* mice (41) or the results of experiments of pharmacological suppression by carbenoxolone (42), showing that mineralocorticoid receptors in the kidneys are occupied by cortisol, causing apparent mineralocorticoid excess. The pathophysiology of hypertension and hypokalemia is associated in part with mineralocorticoid excess (43, 44). Thus, it appears that the HSD11B2 is essential for maintaining physiological levels of aldosterone. The present results demonstrated further that DES is a more potent inhibitor of HSD11B2 activity *ex vivo* compared to activity *in vitro*. It is likely that DES suppression of HSD11B2 activity in the kidneys may have a role in the incidence of mineralocorticoid excess that is associated with hypokalemia and hypertension.

There is evidence within the literature, indicating that DES treatment may impair the development of the testis and male reproductive tract. In this regard, HSD11B2 has been localized to fetal as well as the adult mammalian (human, rat and pig) Leydig cells (22, 45, 46).

Figure 7

Effect of co-administration with diethylstilbestrol (DES) and cortisol on pharmacokinetic parameters of the cortisol rat model. C_{max} = the peak plasma concentration; $t_{1/2}$ = elimination half-life; T_{max} = time to reach C_{max} ; CLz/F = clearance; AUC = area under the curve (the integral of the concentration-time curve after a single dose). Mean $\pm$ S.E.M., $n = 6$. *, ** and *** designate significant differences of DES when compared to control (CON, dimethyl sulfonate versus water = 1:9, v/v) at $P < 0.05$, 0.01 and 0.001, respectively.

It was reported previously that cortisol bound directly to the glucocorticoid receptor in Leydig cells and thereby caused an inhibition of androgen biosynthesis (46, 47, 48). Therefore, HSD11B2 activity may play an important role in glucocorticoid inactivation in Leydig cells in order to prevent glucocorticoid-mediated suppression of testosterone production.

The present study showed that enzymatic cortisol turnover rates after a single dose of DES and implied possible implications for intrauterine growth restriction. There is a limitation that there is no comparison of long-term low versus high DES dose effects and that the effects of DES *in vivo* were examined in non-pregnant only. Other limitations for human and rodent placentas at term are that in human placental HSD11B2 activity rises during gestation, while in rodents HSD11B2 activity declines gradually (49). Future studies are required for investigating these consequences.

Overall, data from the present study demonstrated that the synthetic estrogen DES is a potent inhibitor of HSD11B2 activity. Structurally, DES is similar to cortisol or CORT, indicating that it may competitively bind at the

steroid-binding site on the enzyme. Indeed, the modes of action of DES implied a competitive inhibition of the enzyme for a steroid substrate. On the other hand, DES acted as an uncompetitive inhibitor in the presence of the cofactor NAD^+ . These findings imply that DES can act on the enzyme-substrate complex.

Figure 8

Effect of co-administration with diethylstilbestrol (DES) and cortisol on the conversion of cortisol to cortisone in the rat kidney. Mean $\pm$ S.E.M., $n = 6$. *** designates significant difference of DES when compared to control (CON, dimethyl sulfonate versus water = 1:9, v/v) at $P < 0.001$.

In conclusion, we have shown that DES is an inhibitor of both rat and human placental HSD11B2. Thus, inhibition of HSD11B2 activity by DES may be a factor in the pathophysiology of intrauterine growth restriction of fetuses associated with endocrine anomalies.

Declaration of interest

The authors declare that there is no conflict of interest that could be perceived as prejudicing the impartiality of the research reported.

Funding

The present study was supported in part by National Natural Science Foundation of China with grant number 81730042 and 81601266, Health & Family Planning Commission of Zhejiang Province with grant numbers 11CX29, 2017KY483 and 2015RCB021.

Statement

Full-term human placentas were obtained from the Second Affiliated Hospital of Wenzhou Medical University under the approval of The Ethics Committee of the hospital. Consent has been obtained from each patient or subject after full explanation of the purpose and nature of all procedures used.

References

- Smith OW. Diethylstilbestrol in the prevention and treatment of complications of pregnancy. *American Journal of Obstetrics and Gynecology* 1948 **181** 1570–1571. ([https://doi.org/10.1016/s0002-9378\(99\)70409-6](https://doi.org/10.1016/s0002-9378(99)70409-6))
- Okulicz WC & Johnson LD. The relative binding affinity of diethylstilbestrol to uterine nuclear estrogen receptor: effect of serum and serum albumin. *Proceedings of the Society for Experimental Biology and Medicine* 1987 **185** 478–483. (<https://doi.org/10.3181/00379727-185-42573>)
- Bamigboye AA & Morris J. Oestrogen supplementation, mainly diethylstilbestrol, for preventing miscarriages and other adverse pregnancy outcomes. *Cochrane Database of Systematic Reviews* 2003 **3** CD004353. (<https://doi.org/10.1002/14651858.CD004353>)
- Marselos M & Tomatis L. Diethylstilboestrol: I, pharmacology, toxicology and carcinogenicity in humans. *European Journal of Cancer* 1992 **28A** 1182–1189. ([https://doi.org/10.1016/0959-8049\(92\)90482-H](https://doi.org/10.1016/0959-8049(92)90482-H))
- Scott JN & Adejokun F. Placental changes due to administration of diethylstilbestrol (DES). *Virchows Archiv: B, Cell Pathology Including Molecular Pathology* 1980 **34** 261–267. (<https://doi.org/10.1007/BF02892423>)
- Graffmann N, Ring S, Kawala MA, Wruck W, Ncube A, Trompeter HI & Adjaye J. Modeling nonalcoholic fatty liver disease with human pluripotent stem cell-derived immature hepatocyte-like cells reveals activation of PLIN2 and confirms regulatory functions of peroxisome proliferator-activated receptor alpha. *Stem Cells and Development* 2016 **25** 1119–1133. (<https://doi.org/10.1089/scd.2015.0383>)
- Krozowski Z, Albiston AL, Obeyesekere VR, Andrews RK & Smith RE. The human 11 beta-hydroxysteroid dehydrogenase type II enzyme: comparisons with other species and localization to the distal nephron. *Journal of Steroid Biochemistry and Molecular Biology* 1995 **55** 457–464. ([https://doi.org/10.1016/0960-0760\(95\)00194-8](https://doi.org/10.1016/0960-0760(95)00194-8))
- Seckl JR. Prenatal glucocorticoids and long-term programming. *European Journal of Endocrinology* 2004 **151** (Supplement 3) U49–U62. (<https://doi.org/10.1530/eje.0.151u049>)
- Doyle LW, Faber B, Callanan C & Morley R. Blood pressure in late adolescence and very low birth weight. *Pediatrics* 2003 **111** 252–257. (<https://doi.org/10.1542/peds.111.2.252>)
- Seckl JR & Holmes MC. Mechanisms of disease: glucocorticoids, their placental metabolism and fetal ‘programming’ of adult pathophysiology. *Nature Clinical Practice: Endocrinology and Metabolism* 2007 **3** 479–488. (<https://doi.org/10.1038/ncpendmet0515>)
- Holmes MC, Abrahamsen CT, French KL, Paterson JM, Mullins JJ & Seckl JR. The mother or the fetus? 11beta-hydroxysteroid dehydrogenase type 2 null mice provide evidence for direct fetal programming of behavior by endogenous glucocorticoids. *Journal of Neuroscience* 2006 **26** 3840–3844. (<https://doi.org/10.1523/JNEUROSCI.4464-05.2006>)
- Nacharaju VL, Divald A, McCalla CO, Yang L & Muneyyirci-Delale O. 11beta-Hydroxysteroid dehydrogenase inhibitor carbenoxolone stimulates chorionic gonadotropin secretion from human term cytotrophoblast cells differentiated in vitro. *American Journal of Reproductive Immunology* 2004 **52** 133–138. (<https://doi.org/10.1111/j.1600-0897.2004.00195.x>)
- Wyrwoll CS, Seckl JR & Holmes MC. Altered placental function of 11beta-hydroxysteroid dehydrogenase 2 knockout mice. *Endocrinology* 2009 **150** 1287–1293. (<https://doi.org/10.1210/en.2008-1100>)
- Lakshmi V & Monder C. Extraction of 11 beta-hydroxysteroid dehydrogenase from rat liver microsomes by detergents. *Journal of Steroid Biochemistry* 1985 **22** 331–340. ([https://doi.org/10.1016/0022-4731\(85\)90435-2](https://doi.org/10.1016/0022-4731(85)90435-2))
- Ge RS, Gao HB, Nacharaju VL, Gunsalus GL & Hardy MP. Identification of a kinetically distinct activity of 11beta-hydroxysteroid dehydrogenase in rat Leydig cells. *Endocrinology* 1997 **138** 2435–2442. (<https://doi.org/10.1210/endo.138.6.5165>)
- Li X, Mao B, Dong Y, Li Y, Zhan M, Bai Y, Lian Q, Ge RS & Ye L. Effects of ziram on rat and human 11beta-hydroxysteroid dehydrogenase isoforms. *Chemical Research in Toxicology* 2016 **29** 398–405. (<https://doi.org/10.1021/acs.chemrestox.5b00527>)
- Zhao B, Lian QQ, Chu Y, Hardy DO, Li XK & Ge RS. The inhibition of human and rat 11beta-hydroxysteroid dehydrogenase 2 by perfluoroalkylated substances. *Journal of Steroid Biochemistry and Molecular Biology* 2011 **125** 143–147. (<https://doi.org/10.1016/j.jsmb.2010.12.017>)
- Lin D, Sun W, Wang Z, Chen LG, Chen XL, Wang SH, Li WS, Ge RS & Hu GX. The effect of glycyrrhetic acid on pharmacokinetics of cortisone and its metabolite cortisol in rats. *Journal of Biomedicine and Biotechnology* 2012 **2012** 856324. (<https://doi.org/10.1155/2012/856324>)
- Libby AE, Bales E, Orlicky DJ & McManaman JL. Perilipin-2 deletion impairs hepatic lipid accumulation by interfering with sterol regulatory element-binding protein (SREBP) activation and altering the hepatic lipidome. *Journal of Biological Chemistry* 2016 **291** 24231–24246. (<https://doi.org/10.1074/jbc.M116.759795>)
- Chen L, Guo L, Wang J, Ni L, Chen D, Hu G, Li W, Sun W & Ge RS. The effects of glycyrrhetic acid on the pharmacokinetics of cortisol in rats. *Latin American Journal of Pharmacy* 2012 **31** 1182–1186.
- Agarwal AK, Mune T, Monder C & White PC. NAD(+)-dependent isoform of 11 beta-hydroxysteroid dehydrogenase. Cloning and characterization of cDNA from sheep kidney. *Journal of Biological Chemistry* 1994 **269** 25959–25962.
- Albiston AL, Obeyesekere VR, Smith RE & Krozowski ZS. Cloning and tissue distribution of the human 11 beta-hydroxysteroid dehydrogenase type 2 enzyme. *Molecular and Cellular Endocrinology* 1994 **105** R11–R17. ([https://doi.org/10.1016/0303-7207\(94\)90176-7](https://doi.org/10.1016/0303-7207(94)90176-7))
- Wu C, Cao S, Hong T, Dong Y, Li C, Wang Q, Sun J & Ge RS. Taxifolin inhibits rat and human 11beta-hydroxysteroid dehydrogenase. *Fitoterapia* 2017 **121** 112–117. (<https://doi.org/10.1016/j.fitote.2017.07.004>)
- Asbhy J. Dose levels of 0.01–0.2 microg/kg/day diethylstilbestrol are not suitable for use as a positive control in endocrine toxicity

- studies. *Regulatory Toxicology and Pharmacology* 1999 **29** 235–237. (<https://doi.org/10.1006/rtp.1999.1310>)
- 25 Atanassova N, McKinnell C, Fisher J & Sharpe RM. Neonatal treatment of rats with diethylstilboestrol (DES) induces stromal-epithelial abnormalities of the vas deferens and cauda epididymis in adulthood following delayed basal cell development. *Reproduction* 2005 **129** 589–601. (<https://doi.org/10.1530/rep.1.00546>)
 - 26 Atanassova N, McKinnell C, Turner KJ, Walker M, Fisher JS, Morley M, Millar MR, Groome NP & Sharpe RM. Comparative effects of neonatal exposure of male rats to potent and weak (environmental) estrogens on spermatogenesis at puberty and the relationship to adult testis size and fertility: evidence for stimulatory effects of low estrogen levels. *Endocrinology* 2000 **141** 3898–3907. (<https://doi.org/10.1210/endo.141.10.7723>)
 - 27 Goyal HO, Braden TD, Mansour M, Williams CS, Kamaleldin A & Srivastava KK. Diethylstilbestrol-treated adult rats with altered epididymal sperm numbers and sperm motility parameters, but without alterations in sperm production and sperm morphology. *Biology of Reproduction* 2001 **64** 927–934. (<https://doi.org/10.1095/biolreprod64.3.927>)
 - 28 Kaufman RH, Adam E, Hatch EE, Noller K, Herbst AL, Palmer JR & Hoover RN. Continued follow-up of pregnancy outcomes in diethylstilbestrol-exposed offspring. *Obstetrics and Gynecology* 2000 **96** 483–489. (<https://doi.org/10.1097/00006250-200010000-00001>)
 - 29 Kajantie E, Dunkel L, Turpeinen U, Stenman UH, Wood PJ, Nuutila M & Andersson S. Placental 11 beta-hydroxysteroid dehydrogenase-2 and fetal cortisol/cortisone shuttle in small preterm infants. *Journal of Clinical Endocrinology and Metabolism* 2003 **88** 493–500. (<https://doi.org/10.1210/jc.2002-021378>)
 - 30 Benediktsson R, Calder AA, Edwards CR & Seckl JR. Placental 11 beta-hydroxysteroid dehydrogenase: a key regulator of fetal glucocorticoid exposure. *Clinical Endocrinology* 1997 **46** 161–166. (<https://doi.org/10.1046/j.1365-2265.1997.1230939.x>)
 - 31 Seckl JR, Benediktsson R, Lindsay RS & Brown RW. Placental 11 beta-hydroxysteroid dehydrogenase and the programming of hypertension. *Journal of Steroid Biochemistry and Molecular Biology* 1995 **55** 447–455. ([https://doi.org/10.1016/0960-0760\(95\)00193-X](https://doi.org/10.1016/0960-0760(95)00193-X))
 - 32 Hill M, Paskova A, Kanceva R, Velikova M, Kubatova J, Kancheva L, Adamcova K, Mikesova M, Zizka Z, Koucky M, *et al.* Steroid profiling in pregnancy: a focus on the human fetus. *Journal of Steroid Biochemistry and Molecular Biology* 2014 **139** 201–222. (<https://doi.org/10.1016/j.jsbmb.2013.03.008>)
 - 33 Ma X, Lian QQ, Dong Q & Ge RS. Environmental inhibitors of 11beta-hydroxysteroid dehydrogenase type 2. *Toxicology* 2011 **285** 83–89. (<https://doi.org/10.1016/j.tox.2011.04.007>)
 - 34 Vitku J, Starka L, Bicikova M, Hill M, Heracek J, Sosvorova L & Hampl R. Endocrine disruptors and other inhibitors of 11beta-hydroxysteroid dehydrogenase 1 and 2: tissue-specific consequences of enzyme inhibition. *Journal of Steroid Biochemistry and Molecular Biology* 2016 **155** 207–216. (<https://doi.org/10.1016/j.jsbmb.2014.07.007>)
 - 35 Guo J, Deng H, Li H, Zhu Q, Zhao B, Chen B, Chu Y & Ge RS. Effects of methoxychlor and its metabolite 2,2-bis(p-hydroxyphenyl)-1,1,1-trichloroethane on 11beta-hydroxysteroid dehydrogenase activities in vitro. *Toxicology Letters* 2013 **218** 18–23. (<https://doi.org/10.1016/j.toxlet.2013.01.002>)
 - 36 Bechi N, Sorda G, Spagnoletti A, Bhattacharjee J, Vieira Ferro EA, de Freitas Barbosa B, Frosini M, Valoti M, Sgaragli G, Paulesu L, *et al.* Toxicity assessment on trophoblast cells for some environment polluting chemicals and 17beta-estradiol. *Toxicology in Vitro* 2013 **27** 995–1000. (<https://doi.org/10.1016/j.tiv.2013.01.013>)
 - 37 White PC, Mune T, Rogerson FM, Kayes KM & Agarwal AK. 11beta-Hydroxysteroid dehydrogenase and its role in the syndrome of apparent mineralocorticoid excess. *Pediatric Research* 1997 **41** 25–29. (<https://doi.org/10.1203/00006450-199704001-00158>)
 - 38 Arriza JL, Weinberger C, Cerelli G, Glaser TM, Handelin BL, Housman DE & Evans RM. Cloning of human mineralocorticoid receptor complementary DNA: structural and functional kinship with the glucocorticoid receptor. *Science* 1987 **237** 268–275. (<https://doi.org/10.1126/science.3037703>)
 - 39 Agarwal AK, Mune T, Monder C & White PC. Cloning of cDNA encoding an NAD(+) dependent isoform of 11 beta-hydroxysteroid dehydrogenase in sheep kidney. *Endocrine Research* 1995 **21** 389–397. (<https://doi.org/10.3109/07435809509030455>)
 - 40 Krozowski Z, McGuire JA, Stein-Oakley AN, Dowling J, Smith RE & Andrews RK. Immunohistochemical localization of the 11 beta-hydroxysteroid dehydrogenase type II enzyme in human kidney and placenta. *Journal of Clinical Endocrinology and Metabolism* 1995 **80** 2203–2209. (<https://doi.org/10.1210/jcem.80.7.7608280>)
 - 41 Mune T, Rogerson FM, Nikkila H, Agarwal AK & White PC. Human hypertension caused by mutations in the kidney isozyme of 11 beta-hydroxysteroid dehydrogenase. *Nature Genetics* 1995 **10** 394–399. (<https://doi.org/10.1038/ng0895-394>)
 - 42 van Uum SH, Hermus AR, Smits P, Thien T & Lenders JW. The role of 11 beta-hydroxysteroid dehydrogenase in the pathogenesis of hypertension. *Cardiovascular Research* 1998 **38** 16–24. ([https://doi.org/10.1016/s0008-6363\(97\)00299-x](https://doi.org/10.1016/s0008-6363(97)00299-x))
 - 43 Edwards CR, Stewart PM, Burt D, Brett L, McIntyre MA, Sutanto WS, de Kloet ER & Monder C. Localisation of 11β-hydroxysteroid dehydrogenase – tissue specific protector of the mineralocorticoid receptor. *Lancet* 1988 **2** 986–989. ([https://doi.org/10.1016/s0140-6736\(88\)90742-8](https://doi.org/10.1016/s0140-6736(88)90742-8))
 - 44 Funder JW, Pearce PT, Smith R & Smith AI. Mineralocorticoid action: target tissue specificity is enzyme, not receptor, mediated. *Science* 1988 **242** 583–585. (<https://doi.org/10.1126/science.2845584>)
 - 45 Honda Y, Ohno S & Nakajin S. Leydig cells from neonatal pig testis abundantly express 11beta-hydroxysteroid dehydrogenase (11beta-HSD) type 2 and effectively inactivate cortisol to cortisone. *Journal of Steroid Biochemistry and Molecular Biology* 2008 **108** 91–101. (<https://doi.org/10.1016/j.jsbmb.2007.07.003>)
 - 46 Ge RS, Dong Q, Niu EM, Sottas CM, Hardy DO, Catterall JF, Latif SA, Morris DJ & Hardy MP. 11beta-Hydroxysteroid dehydrogenase 2 in rat Leydig cells: its role in blunting glucocorticoid action at physiological levels of substrate. *Endocrinology* 2005 **146** 2657–2664. (<https://doi.org/10.1210/en.2005-0046>)
 - 47 Hales DB & Payne AH. Glucocorticoid-mediated repression of P450_{sc} mRNA and de novo synthesis in cultured Leydig cells. *Endocrinology* 1989 **124** 2099–2104. (<https://doi.org/10.1210/endo-124-5-2099>)
 - 48 Hardy MP, Sottas CM, Ge R, McKittrick CR, Tamashiro KL, McEwen BS, Haider SG, Markham CM, Blanchard RJ, Blanchard DC, *et al.* Trends of reproductive hormones in male rats during psychosocial stress: role of glucocorticoid metabolism in behavioral dominance. *Biology of Reproduction* 2002 **67** 1750–1755. (<https://doi.org/10.1095/biolreprod.102.006312>)
 - 49 Brown RW, Diaz R, Robson AC, Kotelevtsev YV, Mullins JJ, Kaufman MH & Seckl JR. The ontogeny of 11 beta-hydroxysteroid dehydrogenase type 2 and mineralocorticoid receptor gene expression reveal intricate control of glucocorticoid action in development. *Endocrinology* 1996 **137** 794–797. (<https://doi.org/10.1210/endo.137.2.8593833>)

Received in final form 12 June 2019

Accepted 26 June 2019

Accepted Preprint published online 26 June 2019