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DEHYDROGENASE AND OXOREDUCTASE ACTIVITIES OF PORCINE PLACENTAL 11ß-HYDROXYSTEROID DEHYDROGENASE[1]

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Summary

Dehydrogenase (cortisol to cortisone) and oxoreductase (cortisone to cortisol) activities of porcine placental 11ß-hydroxysteroid dehydrogenase (11ß-HSD) were measured in tissue fragment cultures on day 75 of gestation. Dehydrogenase activity was over fivefold greater than oxoreductase activity ($p < .001$). There were positive linear associations ($p < .01$) between net dehydrogenase activity (dehydrogenase minus oxoreductase) and fetal weight, fetal length, and placental weight. These data indicate a predominance of placental 11ß-HSD dehydrogenase activity at this gestational stage that would insure a net conversion of cortisol to cortisone as it traverses the placenta. The data further suggest that 11ß-HSD activities may provide an optimal glucocorticoid environment that is supportive of enhanced fetal and placental growth.

Key Words: 11β-HSD, cortisol, placental metabolism, swine, placental development

Many factors regulate the availability of biologically active cortisol to its target tissues. One such factor is the enzyme 11ß-hydroxysteroid dehydrogenase that reversibly converts cortisol or corticosterone to their biologically inactive 11-keto metabolites cortisone and 11-dehydrocorticosterone. In mineralocorticoid target tissues, 11ß-HSD is responsible for insuring that glucocorticoids do not gain access to mineralocorticoid receptors (1, 2). In glucocorticoid target tissues, dehydrogenase activity of this enzyme may protect the tissues from excessively high concentrations of glucocorticoids or affect the response of these tissues to glucocorticoids (3). Conversely, reductase activity may be important in some tissues for ensuring optimum concentrations of biologically active glucocorticoids (4). It has been demonstrated that there are two predominant isoforms of 11ß-HSD.

[1] Mention of trade names or commercial products in this article is solely for the purpose of providing specific information and does not imply recommendation or endorsement by the U.S. Department of Agriculture.

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11ß-HSD Type I has both dehydrogenase and oxoreductase activities, uses $NADP^+$ and NADPH respectively as coenzymes (5, 6), and has K_m values in the low μM range for both dehydrogenase (7) and reductase activities (6). Under some conditions 11ß-HSD Type I has predominantly oxoreductase activity (7-9). 11ß-HSD Type II is exclusively a NAD^+-dependent dehydrogenase for naturally occurring substrates (10) and has a K_m in the low nM range for cortisol (11).

The placenta expresses both 11ß-HSD isoforms in humans (12-14), baboons (15), sheep (16), and rats (17). Placental 11ß-HSD activity undoubtedly assists in providing an optimal glucocorticoid environment for fetal tissues (18) as glucocorticoids are needed for final development of numerous tissues (19-20) whereas excessive concentrations cause fetal and placental growth retardation and fetal death (21-25). We have measured porcine placental 11ß-HSD dehydrogenase activity under optimum *in vitro* conditions of pH, substrate, and coenzyme concentration (26). Both NAD^+ and $NADP^+$-dependent dehydrogenase activities were present and increased between day 50 and 100 of gestation. Placental oxoreductase activity has not yet been reported for the porcine placenta.

The ultimate influence of 11ß-HSD on accessibility of maternal cortisol to the fetal compartment and on cortisol effects on placental function is dependent upon the balance achieved between 11ß-HSD oxidative and reductive activities. Hence, the current study was conducted to determine both dehydrogenase and oxoreductase activities in porcine placentae under physiological conditions using placental explant culture and endogenous concentrations of coenzymes and substrate in a manner similar to those used by previous investigators (17-18, 27-28). Tissue fragments were used because tissue homogenization causes significant loss of 11ß-HSD oxoreductase activity in other species (27, 28). Associations between fetal and placental size and placental 11ß-HSD activity were also examined.

Methods

Animals. White crossbred gilts were bred naturally to boars of the same breed and were maintained in groups of 10-20 in swine confinement facilities at the Roman L. Hruska U.S. Meat Animal Research Center (MARC; Clay Center, NE) at a temperature of 18-22°C. Gilts received water ad libitum and 1.8-2.3 kg daily of a corn and soybean meal diet. Pregnant gilts were subsequently slaughtered on day 75 of gestation (term = 114 days) in the abattoir at MARC. This gestational age is midway in our previous study that demonstrated the presence of placental 11ß-HSD activity (26). Furthermore, it is at a gestational age when placental size has begun to plateau in white crossbred pigs (26, 29) whereas fetuses are still growing rapidly. Hence at this time, concentrations of placental enzymatic activities that influence fetal development might assume greater importance. Immediately after slaughter the reproductive tract was removed, taken to an adjacent room, and embryos with their placentae removed. Fragments (~ 3 grams) of placentae from 7-8 fetuses from each of three gilts were removed and placed in ice cold sterile Eagle's Minimum Essential Medium with Earle's salts and L-Glutamine (Sigma, St. Louis, MO) to which had been added per liter: 2.2 g $NaHCO_3$, 3 g ß-D glucose, 10 ml vitamins, 10 ml non-essential amino acids, 10 ml glutamine, and 10 ml antibiotic/antimycotic solution (hereafter referred to as MEM; 30). Placental fragments were taken from the antimesometrial area at a point directly opposite to the site of the umbilicus. Fetuses and remaining placental tissue were then removed, weighed, and sex of fetuses noted. All procedures involving use of animals were reviewed and approved by our institution's Animal Care and Use Committee.

Tissue incubations. Working in a laminar flow hood and using sterile instruments, twelve fragments (~ 40 mg each) were removed from each placenta, weighed, and placed into six tubes (two fragments each tube, 80 ± 5 mg per tube) containing 1 ml of sterile MEM. After a preincubation period of 10

min at 37°C, .29 μCi of ^{3}H-cortisol (Amersham, Arlington Heights, IL) in 50 μl of MEM were added to one set of three tubes, and .29 μCi of ^{3}H-cortisone (synthesized from ^{3}-H cortisol; 31) were added to a second set of three tubes for each placenta. Placental samples were then incubated with constant gentle rocking in an atmosphere of 50% N_2, 45% O_2, and 5% CO_2 for 90 min at 37°C. Triplicate incubations with ^{3}H-cortisone and ^{3}H-cortisol in the absence of tissue were conducted to measure nonspecific conversions of substrate to product. Reactions were terminated by setting all tubes simultaneously in a tray of liquid nitrogen to rapidly freeze the incubates. Subsequently, samples were thawed and extracted with ethyl acetate to obtain tritiated steroids. These extracts were subjected to thin layer chromatography using chloroform:methanol (9:1) as the solvent system, and zones corresponding to ^{3}H-cortisol and ^{3}H-cortisone were removed and counted in a liquid scintillation counter. Tissue fragments were then washed twice in distilled water and once in sodium phosphate (.05 M) buffered saline (3 M NaCl) containing .02 M EDTA (PBS) to remove most radioactivity. Tissue fragments were then homogenized in PBS and aliquots removed for DNA analysis (32).

In a preliminary study, placental tissue from one day-75 pregnant gilt was evaluated for product formation with time of incubation. Approximately 80 mg of tissue were incubated for 10, 20, 40, 60, 120, or 240 min (triplicate incubations at each time period and for each substrate). For each time period, triplicate incubates without tissue were also conducted to measure nonspecific conversion of substrate to product. Approximately .25 μCi of either ^{3}H-cortisol or ^{3}H-cortisone were used as substrate. Incubations were conducted as above-noted and DNA measures conducted.

Statistical procedures. All data are expressed as means ± SEM. Data for experimental samples were analyzed by mixed model multifactorial nested analysis of variance using the SAS PROC MIXED procedure (33). Analyses of covariance to relate fetal measures to enzymatic activities were conducted using PROC MIXED. Effects of time on enzymatic activity were analyzed by ANOVA using PROC GLM (34). Data were tested for homogeneity of variance using an F_{max} test, and data were transformed to a log function where necessary to fulfill assumptions of ANOVA. A probability level of $\leq .05$ was considered significant.

Results

In preliminary studies, 11ß-HSD dehydrogenase activity increased steadily between 10 and 120 min ($p < .01$) and then increased at a slower rate between 120 and 240 min (Fig. 1A). 11ß-HSD oxoreductase activity was quite low during the first 60 min, increased dramatically between 60 and 120 min ($p < .01$), and remained constant thereafter (Fig. 1B). Based on these results, subsequent incubations with experimental samples were conducted for 90 min for both dehydrogenase and oxoreductase activity during the apparent time period of initial velocity when the rate of product formation is proportional to the steady-state concentration of enzyme-substrate complex (35).

Twelve male and 10 female fetuses were obtained from the three gilts. There were no differences in fetal weight or length, but placental weights were greater in male compared with female fetuses (Table I).

Neither dehydrogenase nor oxoreductase activity was dependent on the sex of the fetus ($p = .064$); therefore, data were combined across sexes. In the presence of endogenous substrate and coenzymes, dehydrogenase activity was 5.4-fold greater ($p < .001$) than oxoreductase activity (Fig. 2). Consequently, there was a net placental dehydrogenase activity (Fig. 2) that did not differ between sexes ($p = .77$). Significant gilt differences existed in enzymatic activity. Therefore for graphical presentation of associations among fetal measures, activities within each gilt were converted to a percent

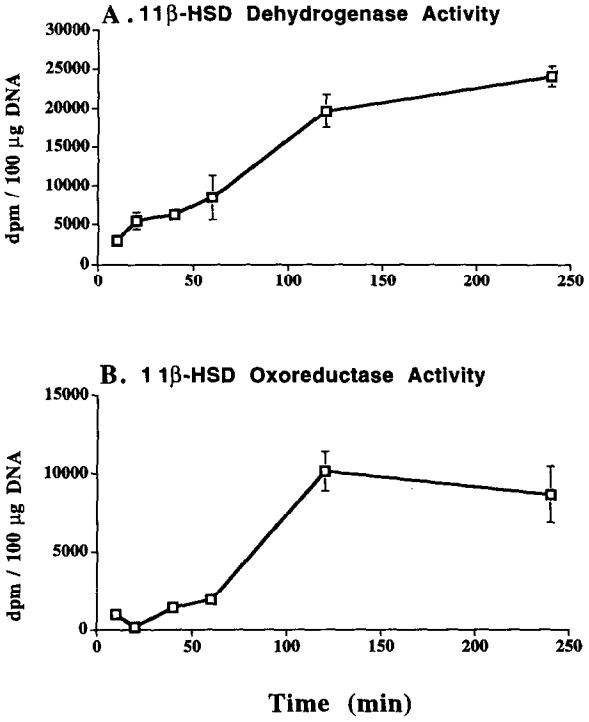

Fig. 1

The relationship between time and 11ß-HSD dehydrogenase (^{3}H-cortisol conversion to ^{3}H-cortisone; A) or 11ß-HSD oxoreductase (^{3}H-cortisone conversion to ^{3}H-cortisol; B) activities in porcine placental fragments. Each datum point represents the mean ± SEM of triplicate determinations using 80 mg of day 75 porcine placental tissue and relying on endogenous concentrations of substrates and coenzymes.

TABLE I

Fetal Measures on Day 75 of Gestation.[a]

Measure	(n)	Females	(n)	Males
Fetal weight (grams)	12	272.7 ± 20.7[b]	10	302.5 ± 12.8[b]
Fetal length (mm)	12	185.9 ± 5.4[b]	10	196.4 ± 4.8[b]
Placental weight (grams)	12	99.7 ± 10.0[b]	10	132.9 ± 9.0[c]

[a] Data represent mean ± SEM.

Means within a measure with different superscripts are different ($p \leq .05$).

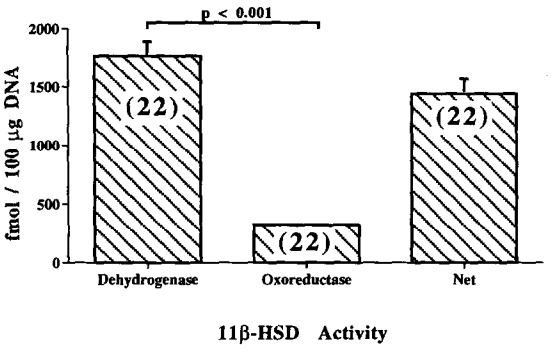

Fig. 2

Placental 11ß-HSD dehydrogenase and oxoreductase activity on day 75 of gestation. Net activity is the difference between dehydrogenase and oxoreductase activity. Data represent the mean + SEM of the number of fetuses shown in parentheses. For oxoreductase activity, the SEM is too small to be visible at the scale used.

of the maximal activity for that gilt. There were no linear relationships among fetal weight ($p = .21$), fetal length ($p = .06$), or placental weight ($p = .37$) and oxoreducatse activity. Fetal weight ($b = .07$ grams/(fmol activity/100 µg DNA); $p = .04$) and fetal length ($b = .02$ mm/(fmol activity/100 µg DNA); $p = .02$), but not placental weight ($b = .04$ grams/(fmol activity/100 µg DNA); $p = .06$), were linearly associated with dehydrogenase activity. When associations with net 11ß-HSD activity (dehydrogenase minus oxoreductase) were examined, linear associations existed for each measure (Fig. 3 A,B,C): fetal weight ($b = .09$ grams/(fmol activity/100 µg DNA); $p < .001$), fetal length ($b = .02$ mm/(fmol activity/100 µg DNA); $p = < .001$), and placental weight ($b = .05$ grams/(fmol activity/100 µg DNA); $p < 0.001$). Statistical evaluations of graphically presented percent activity versus fetal or placental size provide similar interpretations (Fig. 3 A,B,C).

Discussion

This study demonstrates the presence of porcine placental 11ß-HSD dehydrogenase and oxoreductase activity at day 75 of gestation. At this stage and in the presence of endogenous coenzymes and substrate concentrations, dehydrogenase activity is greater than oxoreductase activity. A valid argument could be made for addition of exogenous substrate to achieve saturating substrate concentrations that would obviate potential problems of varying endogenous substrate concentrations (35). However, our *in vitro* system was specifically designed in an attempt to duplicate *in vivo* conditions. Hence, no exogenous coenzymes or saturating substrate concentrations were added. Similar procedures demonstrated close associations between 11ß-HSD Type I and Type II activities and their corresponding mRNA expression (17). The current *in vitro* results correspond with *in vivo* data wherein maternal cortisol accounted for 50% of fetal cortisone at 50 and 100 days of gestation (36). At 100 days when tritiated cortisol was infused into the maternal circulation, fetal tritiated cortisol concentrations were less than 20% of tritiated cortisone concentrations (36). Maternal plasma cortisol exceeded fetal plasma cortisol and fetal umbilical arterial cortisol exceeded umbilical venous cortisol at day 75 of gestation (37). Together, these data provide evidence that under *in vivo* conditions at this gestational stage, dehydrogenase activity predominates, and as maternal and fetal cortisol traverses the placenta net dehydrogenase activity insures much of its conversion to cortisone.

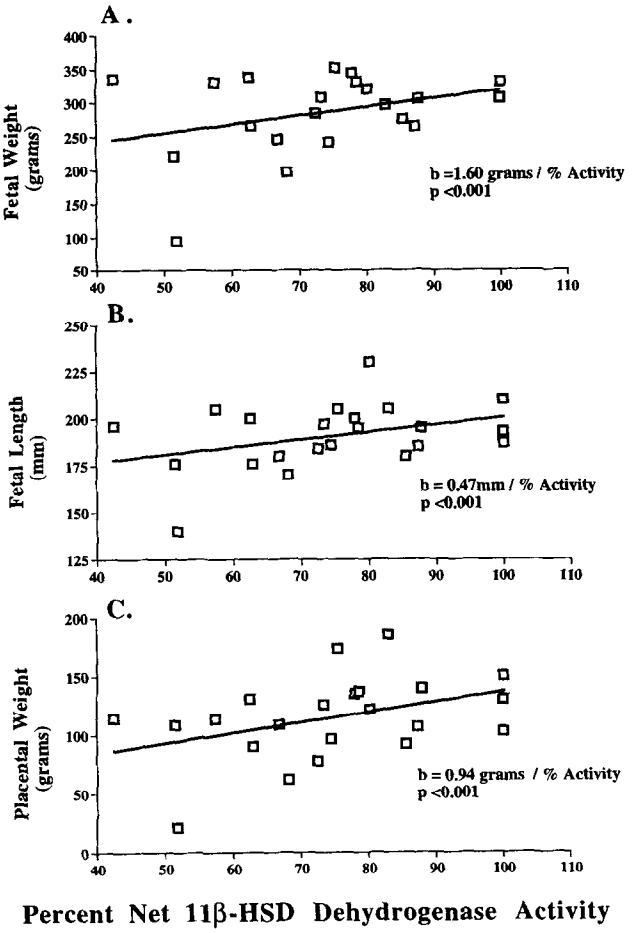

Fig. 3

Associations between fetal weight (A), fetal length (B), or placental weight (C) and placental 11ß-HSD net dehydrogenase activity. For graphical representation, the enzymatic activity for each fetal placenta was expressed as a percent of the maximal placental activity measured within the pregnant pig from which the fetus came. Slopes (b) of linear regressions between size and percent 11ß-HSD activity are indicated.

We were unable previously to show a relationship between placental 11ß-HSD dehydrogenase activity and fetal or placental size using different assay procedures (26). In this new study, there was a positive, linear relationship between 11ß-HSD dehydrogenase activity and fetal and placental size, especially when net activity was considered. Such relationships suggest that an optimal glucocorticoid environment, which is in part provided by 11ß-HSD dehydrogenase activity, is supportive of better fetal and placental growth. Positive associations also were reported between fetal size and placental 11ß-HSD dehydrogenase activity in term rat (23) and human (38) fetuses. Conversely, either no association (38) or a negative association (23) existed between placental weight and placental 11ß-HSD dehydrogenase activity. Discrepancies between human and rat placentae and the current data may reflect: 1) stage of placentae, term in humans and rats versus near mid gestation; 2) type

of placentation, hemochorial versus epitheliochorial; or 3) incubation conditions, homogenates with added cofactors versus tissue fragments without exogenous cofactors.

In this study, one cannot determine the nature of the 11ß-HSD isoforms responsible for the dehydrogenase and oxoreductase activity. We measured $NADP^+$- and NAD^+-dependent 11ß-HSD dehydrogenase activity in day-75 porcine placentae (26). This suggests the presence of both 11ß-HSD Type I and Type II (39, 40), which probably contributed to the measured activities. Indeed, net dehydrogenase activity may in part reflect the lower Km (nM range) for cortisol that is prevalent for 11ß-HSD II when compared with the Km of 11ß-HSD I for cortisone (μM range). In human placental tissue fragments at midterm and late pregnancy (12,18) and in trophoblast cell cultures from term placentae (41), oxidative and reductive activities were present with oxidative predominating. In baboon (mid to late pregnancy; 42), cultured trophoblast cells had greater dehydrogenase than reductase activity. In rat placental fragments, reductase activity was greater at day 16; but on days 19 and 22, dehydrogenase activity was predominant (27). Such distributions of activities were concordant with a strong mRNA expression of 11ß-HSD Type II and weak expression of 11ß-HSD Type I in human term placentae (43). The profile of 11ß-HSD activity measured in intact rat placentae appears to reflect increased mRNA expression of 11ß-HSD Type II in the basal zones (17).

In summary, the current study demonstrates for the first time the presence of 11ß-HSD oxoreductase activity in porcine placentae that is substantially less than the dehydrogenase activity under the *in vitro* conditions used. There were strong positive associations between fetal and placental size and 11ß-HSD net dehydrogenase activity that provide support for the hypothesis that optimal glucocorticoid concentrations are supportive of porcine fetal development.

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