Developmental changes in the intraplacental distribution of placental lactogen and alkaline phosphatase in the rat

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Summary. The junctional and labyrinth regions of the rat chorioallantoic placenta during the second half of gestation showed different patterns of development with regard to DNA, protein, placental lactogen and alkaline phosphatase content. DNA and protein measurements indicated that growth of the labyrinth region was more rapid and persisted for longer during gestation than did growth in the junctional zone. At midpregnancy the junctional zone was the main source of placental lactogen whereas by late pregnancy both regions contributed considerable amounts. On Day 20 of gestation the labyrinth region contained significantly more placental lactogen than did the junctional zone. Alkaline phosphatase activity was predominant in the labyrinth zone throughout the second half of gestation. The results indicate that the chorioallantoic placenta is composed of two functionally distinct regions.

Introduction

The chorioallantoic placenta of the rat is comprised of two morphologically distinct regions: junctional zone and labyrinth zone (Bridgman, 1948; Jollie, 1964; Davies & Glasser, 1968). The junctional zone is also known as the basal zone and is located between uterine decidual tissue and the labyrinth zone. The labyrinth zone is located deep to the junctional zone and is in direct contact with the developing embryo. Trophoblast giant cells, trophospongiosal cells, and germinal trophoblast cells are the predominant cell types in the junctional zone. The labyrinth region contains two types of syncytial trophoblast cells and possibly a germinal trophoblast cell population (Enders, 1965; Davies & Glasser, 1968; Peel & Bulmer, 1977). Trophoblast giant cells appear in the labyrinth zone during the later stages of gestation (Davies & Glasser, 1968). Endothelial and connective tissue cells are also present in the junctional (Ferguson & Palm, 1976) and labyrinth (Enders, 1965) regions. Although these two regions of the chorioallantoic placenta have a very different cellular make-up, they are generally considered together as a single entity.

Trophoblast giant cells are the putative cellular source of placental lactogens (Hall & Talamantes, 1984; Soares *et al.*, 1985). The content of placental lactogen in the entire chorioallantoic placenta of the rat and mouse increases as gestation progresses (Kelly *et al.*, 1975; Soares & Talamantes, 1982). The contribution of each of the placental zones to the content of placental lactogen has not been reported.

The purpose of this investigation was to determine the intraplacental distribution of placental lactogen during the second half of gestation. Additionally, developmental changes in the intraplacental distribution of alkaline phosphatase were monitored.

Materials and Methods

Animals

All experiments were performed on Holtzman rats obtained from SASCO breeders (Omaha, Nebraska). The animals were housed in an environmentally controlled animal facility with lights on from 06:00 to 20:00 h and allowed

free access to food and water. Female rats were housed with male Holtzman rats. Vaginal smears were taken daily during the cohabitation. Successful matings were confirmed by the presence of a copulatory plug and/or the presence of spermatozoa in the vaginal smear (designated as Day 1 of gestation).

Chorioallantoic placenta dissection and tissue preparation

The procedure for dissecting the chorioallantoic placenta and its separation into junctional and labyrinth zones was similar to that previously described for the mouse (Jenkinson & Owen, 1980). Embryos with their encapsulating decidua tissue (conceptuses) were dissected from the uteri of rats on Days 13, 15, 17, 19 or 21 of gestation. Conceptuses were dissected with the aid of a dissecting microscope ($\times 10$ – 20 magnification). The tissues were collected into and washed with Hank's balanced salt solution without Ca^{2+} and Mg^{2+} (KC Biological, Lenexa, KS, U.S.A.). The overlying decidua tissue and the underlying yolk sac/umbilical insertion were removed as well as possible with fine forceps and iridectomy scissors. The junctional zone was identified by its pale appearance, due to the absence of fetal blood, and separated from the labyrinth zone, a richly vascularized tissue, with fine forceps and 23-gauge needles. The completeness of this dissection procedure has been verified by historical examination. The tissues were immediately frozen on solid CO_2 and stored frozen at -25°C until further processing for DNA, protein, placental lactogen and alkaline phosphatase measurements. The tissues were homogenized in a Brinkman Polytron tissue homogenizer (Brinkman Instruments, Westbury, NY, U.S.A.) for 60 sec at a setting of 6.5 in a Tris-saline buffer (10 mM-Tris, 150 mM-NaCl, 1 mM-phenylmethylsulphonyl fluoride, pH 8.2). Aliquants of the homogenates were precipitated with perchloric acid for DNA determinations, trichloroacetic acid for protein determinations or centrifuged at 4000 g. Supernatants from the centrifugation were used for assessment of placental lactogen and alkaline phosphatase activity.

Prolactin radioreceptor assay

Placental lactogen was measured with a modification of the procedure described by Shiu *et al.* (1973). Briefly, the prolactin receptor source was mammary gland membranes isolated from the lactating rabbit. Ovine prolactin (NIAMDD-oPRL-15) was used for radioiodination and as a reference standard for the radioreceptor assay. Radioiodination was accomplished with the solid-phase reagent 'Iodo-Gen' (Pierce Chemical Company, Rockford, IL, U.S.A.) as described by Markwell & Fox (1978). The radioiodinated hormone was purified by gel filtration on Sephadex G-100 (Pharmacia Fine Chemicals, Piscataway, NJ, U.S.A.). The specific activity of the radioiodinated ovine prolactin ranged from 55 to 95 $\mu\text{Ci}/\mu\text{g}$. The buffer for the radioreceptor assay was 25 mM-Tris-HCl, pH 7.6, containing 10 mM- CaCl_2 and 0.5% bovine serum albumin. The remainder of the procedure was similar to the method developed by Shiu *et al.* (1973). The sensitivity of the assay ranged from 0.1 to 0.2 ng/tube and within- and between-assay coefficients of variation were 7% and 11%, respectively.

Alkaline phosphatase assay

Alkaline phosphatase activity was determined as previously described by Lowry *et al.* (1954). The procedure measures the cleavage of *p*-nitrophenyl phosphate to *p*-nitrophenol in a 1 M-2-amino-2-methyl-1 propanol buffer at pH 10.3. Aliquants of the placental homogenates were appropriately diluted with phosphate-buffered saline. A 50- μl sample was added to tubes placed in an ice bath, followed by the addition of 200 μl 8 mM-disodium *p*-nitrophenyl phosphate, 1 M-2-amino-2-methyl-1-propanol, pH 10.3. The reaction vessels were then incubated for 30 min at 37°C . The reaction was stopped by placing the tubes in an ice bath and adding 750 μl 0.25 N-NaOH. Samples were then read by spectrophotometry at 410 nm. A standard curve of *p*-nitrophenol from 1 to 50 nmol was generated. Results were expressed in nanomoles of *p*-nitrophenol released per mg protein per min or per placenta per min. The within- and between-assay coefficients of variations were 5% and 10%, respectively.

Protein and DNA determinations

Protein and DNA contents of the junctional and labyrinth zones of the chorioallantoic placenta were determined by the procedures of Lowry *et al.* (1951) and Burton (1956) respectively.

Statistical analysis

DNA, protein, placental lactogen and alkaline phosphatase content of the junctional and labyrinth zones were analysed with two-way classification analyses of variance (day of gestation $\times$ placental zone). The source of variation from significant F-ratios was determined with Newman-Keuls multiple comparison test (Keppel, 1973).

Table 1. DNA and protein content of the labyrinth and junctional zones of the rat chorioallantoic placenta during the second half of gestation

	Day of gestation				
	13	15	17	19	21
Junctional zone					
DNA (mg)	0.033 ± 0.002	0.184 ± 0.012 ^a	0.171 ± 0.009	0.185 ± 0.011	0.221 ± 0.015 ^c
Protein (mg)	0.71 ± 0.03	6.61 ± 0.37 ^a	11.30 ± 0.83 ^b	14.33 ± 0.81	16.30 ± 0.65 ^c
Labyrinth zone					
DNA (mg)	0.015 ± 0.001	0.158 ± 0.010 ^a	0.335 ± 0.012 ^{*b}	0.323 ± 0.014 [*]	0.342 ± 0.017 [*]
Protein (mg)	0.29 ± 0.02	3.76 ± 0.30 ^d	11.69 ± 0.47 ^b	19.20 ± 0.81 ^{*c}	28.96 ± 1.49 ^{*c}

All values are mean ± s.e.m. for 12 determinations or placentas from at least 5 different animals.

*Values significantly different from junctional zone values on the same day of gestation, $P < 0.01$.

^a $P < 0.01$ compared with Day 13 value.

^b $P < 0.01$ compared with Day 15 value.

^c $P < 0.01$ compared with Day 19 value.

^d $P < 0.05$ compared with Day 13 value.

^e $P < 0.01$ compared with Day 17 value.

Results

DNA content of the labyrinth zone increased significantly from Day 13 to Day 15 and from Day 15 to Day 17 of gestation (Table 1). DNA content of the junctional zone increased significantly from Day 13 to Day 15 and from Day 19 to Day 21 of gestation (Table 1). DNA content of the labyrinth zone on Days 17, 19 and 21 was significantly greater than that of the junctional zone on Days 17, 19, and 21.

Protein content of the labyrinth zone increased progressively as gestation proceeded (Table 1). Values for each day sampled were significantly greater than for the preceding day sampled. Protein content of the junctional zone demonstrated attenuating increases as gestation proceeded. Protein content of the junctional zone increased significantly from Day 13 to Day 15, Day 15 to Day 17, and Day 17 to Day 21 of gestation. The protein content of the labyrinth zone on Days 19 and 21 of gestation was significantly greater than that of the junctional zone on Days 19 and 21 of gestation.

Placental lactogen content expressed as ng hormone activity per placenta increased significantly in the labyrinth region from Day 13 to Day 17 and from Day 17 to Day 21 of gestation, while that in the junctional zone increased significantly from Day 13 to Day 15 (Table 2). Content expressed as ng hormone activity per placenta was significantly greater in the junctional zone than in the labyrinth zone on Day 15 of gestation, and in the labyrinth zone than in the junctional zone on Day 21. When content was expressed as ng hormone activity per mg total tissue protein, there was a significant increase in the labyrinth region from Day 13 to Day 17 of gestation, but in the junctional zone it decreased significantly from Day 13 to Day 15 and from Day 15 to Day 17 (Table 2). The junctional zone had a significantly greater specific activity (ng hormone activity per mg total tissue protein) of placental lactogen than did the labyrinth region on Day 13 and Day 15 of gestation. Significant differences between the labyrinth and junctional zones were not evident on Days 17, 19 and 21 of gestation.

Alkaline phosphatase activity increased progressively as gestation proceeded in the labyrinth zone but did not change significantly in the junctional zone during gestation (Table 2). Labyrinth zone alkaline phosphatase activity increased significantly from Day 13 to Day 17, Day 17 to Day 19 and decreased significantly from Day 19 to Day 21 of gestation. Alkaline phosphatase activity was significantly greater in the labyrinth zone than in the junctional zone on Days 17, 19 and 21 of gestation.

Table 2. The content of placental lactogen and alkaline phosphatase of the labyrinth and junctional zones of the rat chorioallantoic placenta during the second half of gestation

	Day of gestation				
	13	15	17	19	21
Junctional zone					
Placental lactogen (ng/placenta)	329.7 ± 27.7	1194.1 ± 112.5 ^a	1100.8 ± 92.0	1115.5 ± 79.3	906.4 ± 75.0
(ng/mg protein)	465.3 ± 26.6	174.5 ± 11.9 ^a	97.7 ± 5.53 ^d	80.8 ± 4.6	55.4 ± 3.8
Alkaline phosphatase nmol/placenta/min	0.17 ± 0.01	1.01 ± 0.08	1.28 ± 0.11	0.93 ± 0.05	0.63 ± 0.03
nmol/mg protein/min	0.24 ± 0.01	0.15 ± 0.01	0.11 ± 0.01	0.06 ± 0.004	0.04 ± 0.002
Labyrinth zone					
Placental lactogen (ng/placenta)	3.7 ± 0.3	154.2 ± 10.7 [*]	690.9 ± 65.4 ^a	1016.9 ± 89.4	1487.6 ± 140.9 ^{*b}
(ng/mg protein)	13.0 ± 0.9 [*]	44.2 ± 5.5 [*]	63.2 ± 2.9 ^c	53.1 ± 4.7	51.1 ± 3.6
Alkaline phosphatase nmol/placenta/min	0.07 ± 0.01	1.61 ± 0.10	6.94 ± 0.45 ^{*a}	9.48 ± 0.98 ^{*b}	7.23 ± 0.69 ^{*c}
nmol/mg protein/min	0.23 ± 0.01	0.44 ± 0.02	0.61 ± 0.03 ^{*a}	0.50 ± 0.04 [*]	0.25 ± 0.02 ^b

All values are mean ± s.e.m. for 12 determinations or placentas from at least 5 different animals.

^{*}Values significantly different from junctional zone values on the same day of gestation, $P < 0.01$.

^a $P < 0.01$ compared with Day 13 value.

^b $P < 0.01$ compared with Day 17 value.

^c $P < 0.05$ compared with Day 13 value.

^d $P < 0.01$ compared with Day 15 value.

^e $P < 0.01$ compared with Day 19 value.

When values were expressed as nanomoles per mg total tissue protein per minute alkaline phosphatase activity in the labyrinth zone increased significantly from Day 13 to Day 17 and decreased significantly from Day 17 to Day 21 of gestation but did not change in the junctional zone. Labyrinth zone alkaline phosphatase activity expressed per mg protein was significantly greater than junctional zone activities on Days 15, 17 and 19 of gestation.

Discussion

The results of this study indicate that within the chorioallantoic placenta the junctional and labyrinth zones demonstrate different patterns of development during gestation.

DNA and protein measurements indicated that growth of the labyrinth region was more rapid and persisted for longer during gestation than did growth in the junctional zone. These observations are consistent with previous reports (Winick & Noble, 1966; Butterstein & Leatham, 1974).

The chorioallantoic placenta of the rat begins producing placental lactogens at midpregnancy (Days 10–11 of gestation) and continues to produce them for the remainder of gestation (Robertson *et al.*, 1982; Soares *et al.*, 1985). At least two biochemical variants of placental lactogen are produced; a high molecular weight form, placental lactogen-1, that is predominant at mid-pregnancy and a low molecular weight form, placental lactogen-2, that is predominant during the second half of gestation (Robertson *et al.*, 1982; Soares *et al.*, 1985). A similar pattern of placental lactogen expression has also been found in the mouse (Soares *et al.*, 1982, 1983). In the rat, from Day 13 to the end of gestation, the chorioallantoic placenta produces primarily placental lactogen-2 (Robertson *et al.*, 1982; Soares *et al.*, 1985). During the second half of gestation the content of placental lactogen in the chorioallantoic placenta increases progressively (Kelly *et al.*, 1975). The labyrinth zone is responsible for the continued increase in placental lactogen content during gestation (Table 2). Placental lactogen content in the junctional zone peaks at Day 15 of

gestation and does not change significantly thereafter (Table 2). Changes in the specific activity (hormone activity/tissue protein) of placental lactogen in the labyrinth and junctional zones also reflect an important contribution of the labyrinth zone as gestation progresses. The present observation of an increase in placental lactogen content in the labyrinth zone during the later stages of gestation is in good agreement with immunocytochemical evidence for increased numbers of placental lactogen-2-containing cells in the mouse labyrinth during this same gestational stage (Hall & Talamantes, 1984). The roles of the junctional and labyrinth regions as sources of placental lactogen therefore change as gestation progresses. At mid-pregnancy the junctional zone is the principal source of placental lactogen, whereas by late pregnancy both regions contribute considerable placental lactogen at comparable levels. The contribution of placental lactogen production by the labyrinth and junctional zones may have different impacts on the maternal and fetal environments. The location of placental lactogen-secreting cells in the labyrinth region might be more responsive to signals from the fetus and have greater accessibility and influence on the fetal environment, whereas placental lactogen-secreting cells in the junctional region might be more responsive to signals from the maternal environment and have greater accessibility and influence on the maternal environment.

Alkaline phosphatase is a well established biochemical marker of the chorioallantoic placenta (Padykula, 1959). Our studies indicate that alkaline phosphatase is a particularly good marker for the labyrinth zone of the rat (Table 2). Alkaline phosphatase activity increases during the maturation of the labyrinth zone, whereas junctional zone alkaline phosphatase activity is minimal and without significant changes during the second half of gestation (Table 2). Müller *et al.* (1983) have reported similar differences in the expression of alkaline phosphatase in the junctional and labyrinth zones of the mouse.

Further evidence for a difference in the function of the two components of the chorioallantoic placenta has been demonstrated by others. The oncogene *c-fos* has been shown to be differentially expressed in the junctional and labyrinth zones of the mouse during gestation, whereas the oncogenes *c-fms* and *c-ras* are expressed similarly in both regions (Müller *et al.*, 1983). Additionally, receptors for transferrin and epidermal growth factor are located in greater numbers in the labyrinth zone than in the junctional zone of the mouse (Müller *et al.*, 1983; Adamson & Meek, 1984), whereas pregnancy-specific β_1 -glycoprotein is localized exclusively to the junctional zone of the rat chorioallantoic placenta (MacPherson *et al.*, 1985).

These results indicate that the chorioallantoic placenta is composed of two distinct regions that function independently. I contend that a greater appreciation of placental physiology would be achieved if the labyrinth and junctional zones of the rat or mouse placenta were studied as individual entities.

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