Histochemical distribution of Δ^5 -3 β - and 17 β -hydroxysteroid dehydrogenases in hamster trophoblast

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Summary. The histochemical distribution of Δ^5 -3 β - and 17 β -hydroxysteroid dehydrogenases was demonstrated in hamster trophoblast between Days 8 and 15 of pregnancy. The Δ^5 -3 β -hydroxysteroid dehydrogenase activity in the ectoplacental trophoblast of 8-day embryos was demonstrated by use of Δ^5 -pregnenolone and dehydroepiandrosterone as substrates; between Days 11 and 15, activity was demonstrated in the trophoblastic giant cells of the placenta and in the intra-arterial trophoblast cells when Δ^5 -pregnenolone was the substrate. Between Days 11 and 15, 17 β -hydroxysteroid activity was present in the spongiotrophoblast, labyrinth, placental giant cells and intra-arterial trophoblast cells, as shown by use of testosterone and oestradiol as substrates. Both enzymes were demonstrated in ectopic trophoblast cells, indicating that these activities are autonomous.

Introduction

The Δ^5 -3 β - and 17 β -hydroxysteroid dehydrogenases are known to be essential for the metabolism of Δ^5 -3 β -hydroxysteroids (Δ^5 -pregnenolone, 17-hydroxypregnenolone, dehydroepiandrosterone) and 17 β -hydroxysteroids (testosterone, oestradiol-17 β) during pregnancy. These enzymes have been detected in hamster embryos at 3–5 days *post coitum* (Dickmann & Gupta, 1974) and at the 1- and 2-cell cleavage stages (Niimura & Ishida, 1976). Many authors have described the histochemical distribution of Δ^5 -3 β - and 17 β -hydroxysteroid dehydrogenases in the trophoblast of rats (Deane, Rubin, Dricks, Lobel & Leipsner, 1962; Deane & Rubin, 1965; Botte, Materazzi & Chieffi, 1966; Ferguson & Christie, 1967; Schmidt, Wendler & Gabler, 1970; Legrand & Nguyen Son Hung, 1972; Legrand, 1975), mice (Botte, Tramontana & Chieffi, 1968) and guinea-pigs (Ferguson & Christie, 1967), but the hamster does not appear to have been investigated similarly.

Materials and Methods

Samples of conceptuses were removed from 16 pregnant hamsters (*Mesocricetus auratus*), 2 months old, on Days 8, 11, 13 or 15 *post coitum*. Day 1 of pregnancy was the day on which spermatozoa were found in the vaginal smear. Embryos at 8 days *p.c.* were grafted into the testis of 6 cryptorchid male hamsters as previously described (Legrand & Hernandez-Verdun, 1974) and removed 4 days later.

All samples were frozen in liquid nitrogen and 10 μ m sections were cut in a cryostat at -18°C . The sections were collected on glass cover-slips and placed in 0.1 M-phosphate buffer, pH 7.2, to wash out any blood containing endogenous plasma steroids. The sections were incubated by the procedures of Deane *et al.* (1962), Baillie, Ferguson & Hart (1966) and Botte *et al.* (1966) to demonstrate Δ^5 -3 β - and 17 β -hydroxysteroid dehydrogenases. The incubation medium was of 0.1 M-sodium phosphate buffer, pH 7.2, containing 0.06 mM-NAD/l, 0.08 mM-tetrazolium nitroblue/l, 0.9 mM-nicotinamide/l and the specific substrate (1.7 mM/l). Nicotinamide was added to prevent decomposition of NAD. The four substrates used were Δ^5 -pregneneolone, dehydroepiandrosterone, testosterone and oestradiol-17 β ; they were dissolved in dimethyl-formamide before addition to the incubation medium. Control sections were incubated in the phosphate buffer with NAD, tetrazolium nitroblue, nicotinamide and dimethyl-formamide but without the specific substrate.

After incubation at 37°C for 2 h the sections were fixed in formalin-alcohol and mounted on slides in water-soluble 'Apathy Syrup' for microscopic observation. About 30 sections, from material obtained from different pregnant animals, were assessed and compared for each reaction and each stage of pregnancy.

Results

The activities of the Δ^5 -3 β - and 17 β -hydroxysteroid dehydrogenases were demonstrated by the formation of diformazan granules in the cytoplasm after reduction of the tetrazolium salt (Levy, Deane & Rubin, 1959). Enzyme activities were evaluated in the spongiotrophoblast, the labyrinth, the trophoblastic giant cells and in the intra-arterial trophoblast cells as well as in the intra-testicular grafts. The results are summarized in Table 1. The reactions in all the controls were absolutely negative.

Table 1. Hydroxysteroid dehydrogenase (HSD) activities in tissues of the hamster placenta incubated with different substrates

Tissue	Δ^5 -3 β -HSD		17 β -HSD	
	Δ^5 -Pregnenolone	DHA	Testosterone	Oestradiol-17 β
Ectoplacental trophoblast				
Day 8	++	+	—	—
Trophoblastic giant cells				
Day 11	++	—	++	++
Day 13	++	—	+++	+++
Day 15	++	—	+++	+++
Spongiotrophoblast				
Day 11	—	—	+	+
Day 13	—	—	+	+
Day 15	—	—	+	+
Labyrinth				
Day 11	—	—	+	+
Day 13	—	—	+	+
Day 15	—	—	++	++
Intra-arterial trophoblast				
Day 11	+	—	+++	+++
Day 13	+	—	+++	+++
Day 15	+	—	+++	+++
In testicular grafts				
Intra-arterial trophoblast	+	—	+++	+++
Trophoblastic giant cells	++	—	++	++

—, Absent; +, moderate; ++, intense; +++, very intense.

EXPLANATION OF PLATE

Demonstration of hydroxysteroid dehydrogenases (HSD) in the placental tissues of the hamster.

Fig. 1. 17 β -HSD in trophoblastic giant cells at Day 13 of pregnancy. Substrate, oestradiol-17 β . $\times 330$.

Fig. 2. Δ^5 -3 β -HSD in trophoblastic giant cells at Day 13 of pregnancy. Substrate, Δ^5 -pregnenolone. $\times 330$.

Fig. 3. 17 β -HSD in the placental labyrinth at Day 15 of pregnancy. Substrate, oestradiol-17 β . $\times 330$.

Fig. 4. 17 β -HSD in the spongy zone of the placenta at Day 11 of pregnancy. Substrate, oestradiol-17 β . $\times 330$.

Fig. 5. 17 β -HSD in intra-arterial trophoblast cells of the mesometrial arteries at Day 11 of pregnancy. Substrate, testosterone. $\times 330$.

Fig. 6. 17 β -HSD in intra-arterial trophoblast cells migrating along the testicular arteries. Substrate, oestradiol-17 β . $\times 470$.

PLATE 1

(Facing p. 406)

At Day 8 of pregnancy there were no histochemically demonstrable enzymes in the ectoplacental trophoblast cells when testosterone and oestradiol-17 β were used as substrates. Between Days 11 and 15, 17 β -hydroxysteroid dehydrogenase was present in the various trophoblast tissues of the placenta (Pl. 1, Figs 1, 3 and 4) and was also active in the intra-arterial trophoblast cells which invade the mesometrial arteries of the pregnant uterus (Pl. 1, Fig. 5).

On the 8th day of pregnancy, a moderate Δ^5 -3 β -hydroxysteroid dehydrogenase activity was observed in the ectoplacental cone when dehydroepiandrosterone was used as a substrate. This activity was not present between Days 11 and 15 but when Δ^5 -pregnenolone was used as substrate enzymes were demonstrated in the trophoblastic giant cells (Pl. 1, Fig. 2) and in intra-arterial trophoblast cells.

The distribution pattern of the enzymes in the trophoblastic giant cells (differentiated in the haemorrhagic nodule) and in intra-arterial trophoblast cells (migrating along testicular arteries; Pl. 1, Fig. 6) appeared similar to that observed in the pregnant uterus at the equivalent stage of gestation.

Discussion

It has been shown that Δ^5 -3 β -hydroxysteroid dehydrogenase remains active at Day 8 *post coitum* in some ectoplacental cells when dehydroepiandrosterone and Δ^5 -pregnenolone are used as substrates. Later, 11 to 15 days *post coitum* (i.e. the time of placental morphogenesis: Carpenter, 1975), the placental giant cells and intra-arterial trophoblast cells are able to synthesize progesterone from Δ^5 -pregnenolone but are no longer able to convert dehydroepiandrosterone into androstenedione. This observation indicates a metabolic pathway in hamster trophoblast which differs from that described for the mouse and rat. The trophoblastic giant cells and labyrinth in the rat and mouse, and the intra-arterial trophoblast cells in the rat, show an active metabolism of dehydroepiandrosterone during the second half of pregnancy (Botte *et al.*, 1968; Legrand, 1975; Okker-Reitsma, 1976). In the pregnant hamster, the conversion of dehydroepiandrosterone into androstenedione is effected by the ovaries between Days 11 and 15 because Δ^5 -3 β -hydroxysteroid dehydrogenase remains intensively active when dehydroepiandrosterone is used as the substrate (C. Legrand, unpublished results). The activity of 17 β -hydroxysteroid dehydrogenase appears much later and is present in all tissues derived from trophoblast, indicating that these tissues are able to effect the reversible conversion of testosterone into androstenedione and of oestradiol-17 β into oestrone.

The distribution patterns of Δ^5 -3 β - and 17 β -hydroxysteroid dehydrogenases in the ectopic trophoblast and trophoblast tissues *in utero* of the hamster agrees with previous findings of Dickmann & Dey (1974) and Marcal, Chew, Salomon & Sherman (1975), who suggest that hydroxysteroid dehydrogenase activities in the rat blastocyst and placenta are autonomous and do not depend on maternal hormonal control. This is also the case in the mouse, because Chew & Sherman (1975) have shown that 3 β -hydroxysteroid activity of the trophoblast "can be monitored both *in vitro* and *in vivo*".

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