Histochemical demonstration of prostaglandin dehydrogenases in the uterine and chorionic epithelia of the pregnant pig

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Summary. The NAD-dependent 15-hydroxyprostaglandin dehydrogenase (PGDH) was active in the uterine and chorionic epithelia of the pig at 30, 60 and 90 days of pregnancy when PGs of the A, E or F series were used as the substrate. The activity of NADP-dependent PGDH in the same epithelia appeared only when PGB-1 or PGB-2 was used as the substrate.

Introduction

Since the biochemical detection of 15-hydroxyprostaglandin dehydrogenase (PGDH) activity in guinea-pig lungs and the isolation of this enzyme from pig lungs (Änggård & Samuelsson, 1964, 1966), this enzyme has been demonstrated in various other kinds of animal tissues, and its biochemical nature clarified (see Hansen, 1976). The characteristics and activity of PGDH in the human placenta have been studied by several investigators (Jarabak, 1972; Schlegel, Demers, Hildebrandt-Stark, Behrman & Greep, 1974; Thaler-Dao, Saintot, Baudin, Descomps & Crastes de Paulet, 1974; Braithwaite & Jarabak, 1975; Schlegel & Greep, 1975; Jarabak & Braithwaite, 1976; Keirse, Hicks & Turnbull, 1976; Keirse, Hanssens, Hicks & Turnbull, 1976; Westbrook, Lin & Jarabak, 1977a, b). Because the human placenta contains a lot of PGDH (Jarabak, 1972; Schlegel *et al.*, 1974), Jarabak (1972) considered that it might be a major site of prostaglandin metabolism during pregnancy. The present study was an investigation of PGDH activities in the placenta of the pig at different stages of pregnancy.

Materials and Methods

One Large Yorkshire sow, weighing about 170 kg, and two Landrace sows, both weighing about 150 kg, were mated as required. The animals were slaughtered at 30, 60 and 90 days after service. The pieces of placenta for histological observations were fixed in 10% neutral formalin or Bouin's solution, embedded in paraffin wax, sectioned at 7 µm, and stained with haematoxylin and eosin. Tissue for the demonstration of PGDH was quickly frozen in liquid nitrogen, sectioned at 15 µm on a cryostat, and incubated at 37°C for 2 h. The composition of the incubation medium was a slight modification of that used for kidney incubations by Nissen & Andersen (1968, 1969): 0.3 mM substrate (Ono Pharmaceutical Co.), 0.75 mM cofactor (Sigma Co.), 0.075 mM nitro-blue tetrazolium (Sigma Co.), and 0.1 M-sodium phosphate buffer, pH 8.0, in a total volume of 10 ml. The substrates used were prostaglandin (PG) A-1, A-2, B-1, B-2, E-1, E-2, F-1α and F-2α, each of which was dissolved in 0.5 ml ethanol solution and added to the incubation medium. The cofactors were NAD and NADP. Substrate-free but ethanol- and cofactor-containing solution was used for the control incubations.

The activity of NADH₂- and NADPH₂-dehydrogenases was detected by the method of Barka & Anderson (1965) and of alcohol dehydrogenase by the method of Nachlas, Walker & Seligman (1958) to determine the absence of 'nothing dehydrogenase'. The tissues were all incubated at the same time.

After incubation, the sections were fixed in 10% formalin solution and mounted on glass slides with glycerine jelly. The intensity of the enzyme activity was judged by the amount of deposited diformazan granules and estimated on a 5-point scale: strong (++++), moderate (+++), weak (++), slight (+) and absent (–).

Results

PGDH

When NAD was the cofactor, diformazan granules were uniformly deposited in the cytoplasm of the uterine and chorionic epithelia when sections were incubated in medium containing PGs of the A, E or F series (Pl. 1, Figs 1, 2 and 3), but were not present in the sections incubated in medium containing PGB-1 or PGB-2, or in sections incubated in a substrate-free medium. The intensities of the enzyme activity during pregnancy are shown in Table 1.

Table 1. The activities of PGDH with different substrates in the uterine and chorionic epithelia of the pig placenta

Substrate	Cofactor	Days after service					
		30		60		90	
		Uterine	Chorionic	Uterine	Chorionic	Uterine	Chorionic
PGA-1	NAD	++	++	++	+++	++	++++
PGA-2	NAD	++	++	++	+++	++	++++
PGB-1	NAD	–	–	–	–	–	–
PGB-2	NAD	–	–	–	–	–	–
PGE-1	NAD	++	+	++	++	+++	+++
PGE-2	NAD	++	+	++	++	++++	+++
PGF-1 α	NAD	++	+	+++	+++	++++	++++
PGF-2 α	NAD	++	+	+++	+++	++++	++++
PGA-1	NADP	–	–	–	–	–	–
PGA-2	NADP	–	–	–	–	–	–
PGB-1	NADP	+	+	++	++	++	++
PGB-2	NADP	++	++	++	++	+++	+++
PGE-1	NADP	–	–	–	–	–	–
PGE-2	NADP	–	–	–	–	–	–
PGF-1 α	NADP	–	–	–	–	–	–
PGF-2 α	NADP	–	–	–	–	–	–

++++, strong; +++, moderate; ++, weak; +, slight; –, absent.

When NADP was the cofactor diformazan granules were found only in the sections incubated with PGB-1 or B-2 and were spread uniformly throughout the cytoplasm of cells in the uterine and chorionic epithelia (Pl. 1, Fig. 4). There was very little difference in enzyme activity at the 3 stages of pregnancy (Table 1).

Other dehydrogenases

Moderate to strong activities of NADH₂- and NADPH₂-dehydrogenases were seen in the uterine and chorionic epithelial cell cytoplasm (Pl. 1, Figs 5 and 6), but no alcohol dehydrogenase activity was found in either tissue at any period.

Discussion

Nissen & Andersen (1968, 1969), who were the first to demonstrate histochemically the presence of PGDH in the rat kidney, used PGs of the E series as the substrate and reported that the enzyme was NAD-dependent and that it was specific. Lee & Levine (1975) advocated that PGDH should be

Histochemical demonstration of various dehydrogenases in the uterine (UE) and chorionic (CE) epithelia of the pig placenta. $\times 250$.

Fig. 1. PGDH with PGA-2 as substrate at 30 days of pregnancy.

Fig. 2. PGDH with PGE-1 as substrate at 90 days of pregnancy.

Fig. 3. PGDH with PGF-2 α as substrate at 60 days of pregnancy.

Fig. 4. PGDH with PGB-1 as substrate at 90 days of pregnancy.

Fig. 5. NADH₂-dehydrogenase at 60 days of pregnancy.

Fig. 6. NADPH₂-dehydrogenase at 30 days of pregnancy.

(Facing p. 150)

divided into two types according to their cofactor dependency, i.e. type I (NAD-dependent) and type II (NADP-dependent). The PGDH of type I has been extracted from various kinds of mammalian tissues (Hansen, 1976), while that of type II has been found only in monkey brains, human erythrocytes, pig kidneys (see Hansen, 1976) and human placentae (Westbrook *et al.*, 1977a, b). The human placenta does, however, contain both types of PGDH, the NAD-dependent PGDH oxidizing PGs of the A, E and F series (Jarabak, 1972; Schlegel *et al.*, 1974; Thaler-Dao *et al.*, 1974; Braithwaite *et al.*, 1975; Schlegel & Greep, 1975; Jarabak & Braithwaite, 1976) but hardly at all those of the B series (Westbrook *et al.*, 1977a, b) while the NADP-dependent PGDH oxidizes PGA-1 and PGB-1 but not PGEs or PGFs (Westbrook *et al.*, 1977a, b). The present results indicate that the pig placenta also contains two types of PGDH which have a substrate specificity similar to that of the enzymes in the human placenta. The trend to an increased activity of the PG dehydrogenases in later pregnancy needs to be confirmed with more animals and related to the hormonal changes which occur during pregnancy (see Dziuk, 1977).

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