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Prostaglandin-Metabolizing Enzymes During Pregnancy: Characterization of NAD^+-Dependent Prostaglandin Dehydrogenase, Carbonyl Reductase, and Cytochrome P450-Dependent Prostaglandin Omega-Hydroxylase

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ABSTRACT: Prostaglandins E_2 and $F_{2\alpha}$ regulate a number of physiological functions in reproductive tissues, and concentrations of these bioactive modulators increase during pregnancy. Corresponding to the increase in circulating levels of prostaglandins during pregnancy is an increase in enzymes that metabolize these agents. Three prostaglandin-metabolizing enzymes induced during pregnancy are NAD^+-dependent 15-hydroxyprostaglandin dehydrogenase (PGDH), NADPH-dependent carbonyl reductase, and cytochrome P450-dependent prostaglandin ω- or 20-hydroxylase. This review discusses the biochemical properties, regulation, and possible functions of these three enzymes.

KEY WORDS: prostaglandin-metabolizing enzymes, pregnancy, NAD^+-dependent prostaglandin dehydrogenase

I. INTRODUCTION

Prostaglandins (PGs) are important regulators of numerous physiological activities in vascular, reproductive, intestinal, and inflammatory tissues (Patrono, 1983; Nies, 1986; Holtzman, 1991). During pregnancy, circulating levels of PGs increase dramatically in a number of species (Venuto and Donker, 1982; Mucha and Losonczy, 1990), and corresponding to the increase in PG levels are changes in the expression of specific enzymes that are involved in PG metabolism. Three enzymes involved in PG metabolism whose activities change during pregnancy are NAD^+-dependent 15-hydroxyprostaglandin dehydrogenase (PGDH), NADPH-dependent carbonyl reductase, and cytochrome P450-dependent ω- or 20-hydroxylase.

This review focuses on the biochemical properties of these PG-metabolizing enzymes, with a major emphasis on PGDH, which has been recognized as a key enzyme in regulating the biological activities of PGs in the circulation. For example, the lung

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enzyme has been reported to metabolize 80 to 90% of an infused PG dose in a single pass through the lung (Piper et al., 1970). PGDH (E.C. 1.1.1.141) catalyzes the reversible oxidation of the 15-hydroxy group of PGs of the E and F series (Figure 1), and recent studies have also demonstrated that the enzyme metabolizes polyunsaturated fatty acids that contain a hydroxy group at their ω-6 position, such as 15-hydroxyeicosatetraenoic acid (15-HETE, Figure 1). There has been renewed interest in the metabolic pathways that are involved in PG metabolism during pregnancy because of recent investigations of the reactions catalyzed by PGDH, carbonyl reductase, and PG ω-hydroxylase. The importance of PGDH in initiating PG inactivation has been recognized for many years, but studies also suggest that the enzyme may work in conjunction with a NADPH-dependent carbonyl reductase (Figure 2) to form a metabolite that regulates ovarian function (Iwata et al., 1990a, b). The PG ω-hydroxylase catalyzes the hydroxylation of PGs, arachidonic acid, and 15-HETE at their terminal methyl carbon atom (Figure 3), and the enzyme has been shown to be induced in the lungs of pregnant rabbits (Powell, 1978). This enzyme may function in the further oxidation of PG metabolites for elimination in the urine. For example, PG ω-hydroxylase metabolizes 13,14-dihydro-15-keto-PGE_2 to 13,14-dihydro-15-keto-20-hydroxy-PGE_2 (Figure 3), which may be further oxidized to a carboxyl derivative and undergo β-oxidation reactions. However, this enzyme may also catalyze the 20-hydroxylation of arachidonic acid to a product that may have potent biological activity (Figure 3). In this review, studies on PGDH, carbonyl reductase, and PG ω-hydroxylase that have helped to characterize their molecular and biochemical properties and their possible role during pregnancy are described.

II. NAD^+-DEPENDENT 15-HYDROXYPROSTAGLANDIN DEHYDROGENASE (PGDH)

A. Purification and Molecular Properties of PGDH

The high specific activities observed for the NAD^+-dependent PGDH activity in human placenta and in the lungs of pregnant rabbits and the ready availability of these tissues have made these organs excellent sources to isolate this enzyme. Improved methods for isolating the purified enzyme in greater yields have permitted the production of antibodies for use in immunochemical studies. Progress in purifying the human placental NAD^+-dependent PGDH was certainly enhanced by the development of affinity chromatography for pyridine nucleotide-dependent enzymes such as Blue-Sepharose or NAD-agarose (Braithwaite and Jarabak, 1975; Mak et al., 1982; Nagai et al., 1987; Jarabak and Watkins, 1988; Krook et al., 1990). Krook et al. (1990) reported that the human placental enzyme could be purified in a relatively short period of time (3 to 4 d) with high recovery by utilizing Pharmacia Mono-Q columns and high-performance liquid chromatography. Elimination of the acid precipitation step was credited for the high yield of the purified human placental enzyme. Porcine and rat kidney PGDH have also been purified by a similar protocol (Kung-Chao and Tai, 1980; Chang et al., 1990; Mak et al., 1990). Bergholte and Okita (1986a) and Bergholte et al. (1987) used hydrophobic resins such as octylamino agarose or lauroyl-Sepharose to obtain highly purified preparations of a lung PGDH from pregnant rabbits, because the lung PGDH did not bind to NAD-affinity columns.

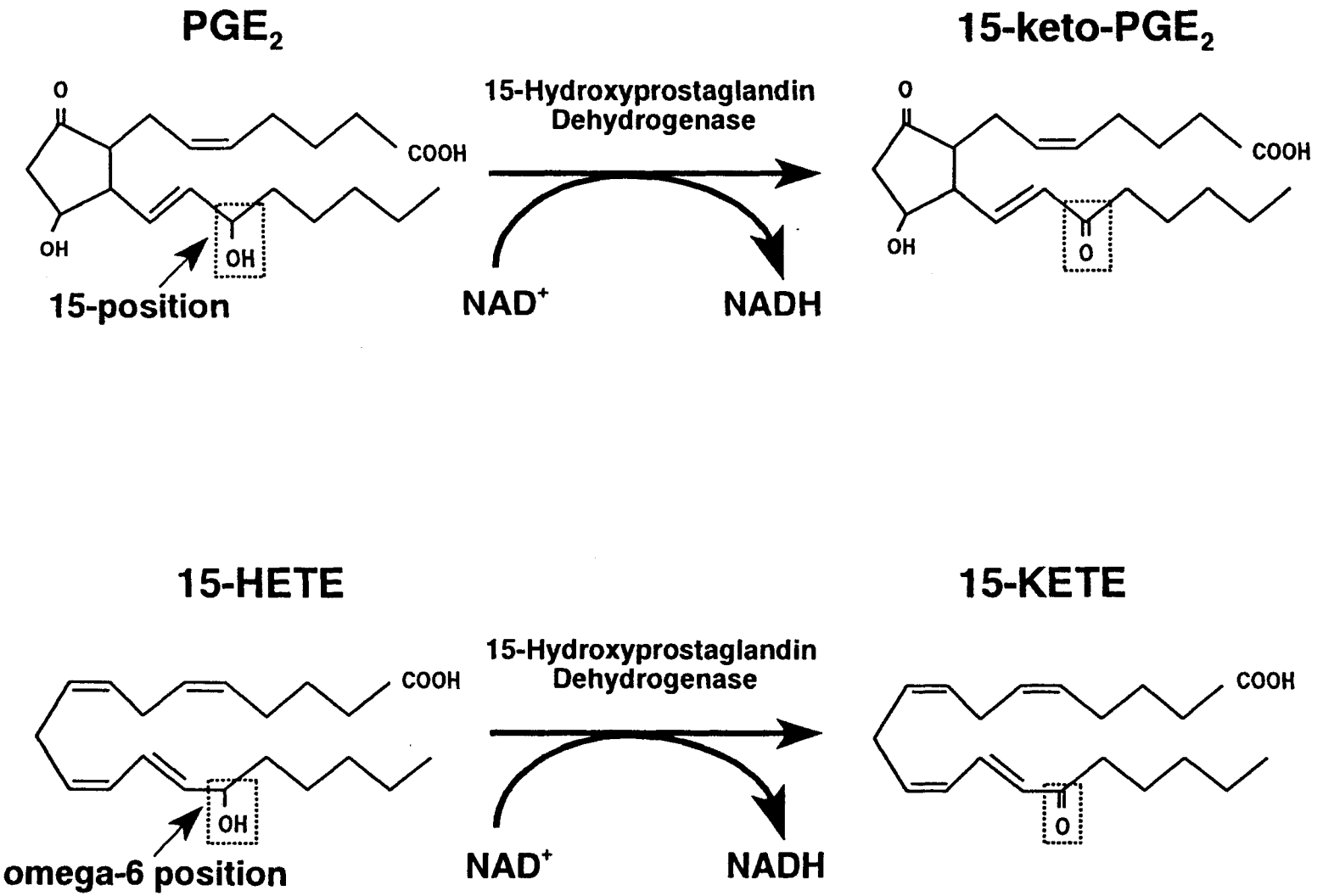

FIGURE 1. Dehydrogenation of PGE_2 and 15-HETE by the NAD^+-dependent PGDH. 15-KETE, 15-keto-eicosatetraenoic acid.

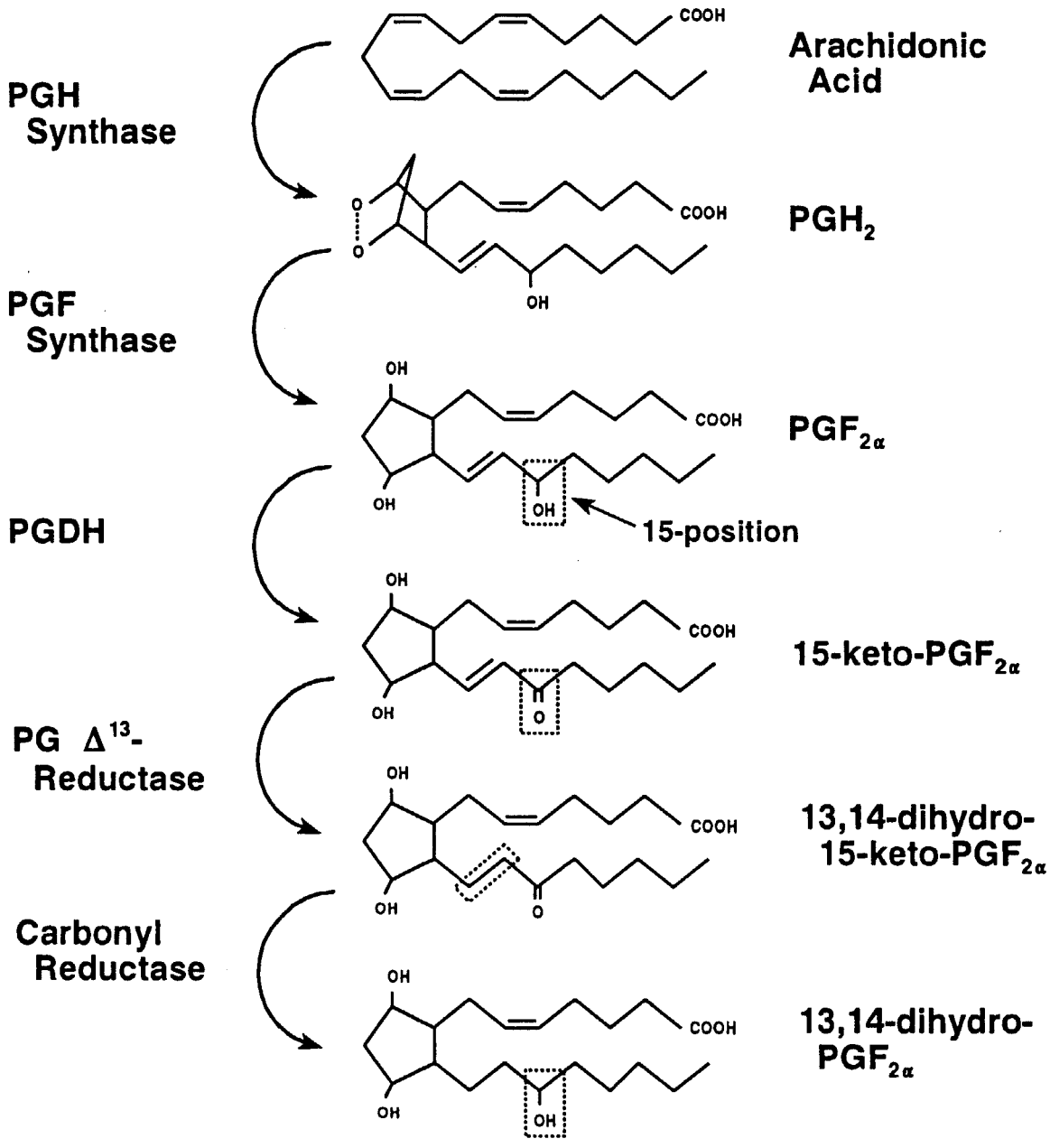

FIGURE 2. Reaction sequence leading to the formation of 13,14-dihydro-$PGF_{2\alpha}$ from arachidonic acid. Reactions are catalyzed by PGH synthase, PGF synthase, PGDH, PG Δ^{13}-reductase, and carbonyl reductase.

The isolation of highly purified PGDH samples improved the accuracy and reproducibility of M_r values because earlier studies reported a range between 20 and 70 kDa, and it was suggested that PGDH may exist in multiple forms (Hansen, 1976; Braithwaite and Jarabak, 1975; Nagai et al., 1987). M_r values between 25.5 and 30 kDa were reported by several laboratories for purified protein preparations from kidney (Chang et al., 1990), placenta (Mak et al., 1982; Tanaka et al., 1986), and lung (Bergholte and Okita, 1986a) by SDS-gel electrophoresis. Western blot analysis of

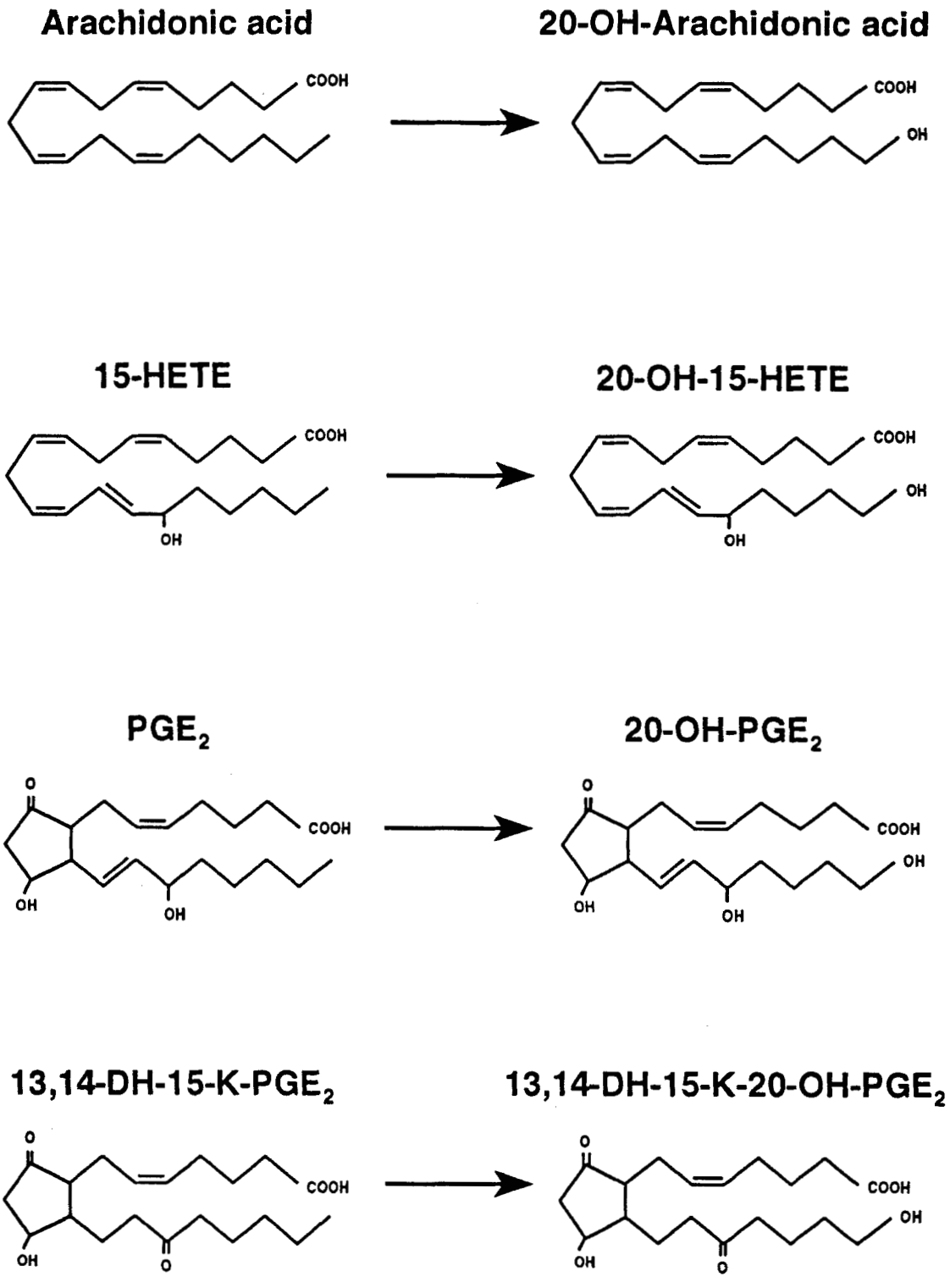

FIGURE 3. Metabolism of arachidonic acid, 15-HETE, PGE_2, and 13,14-dihydro-15-keto-PGE_2 to 20-hydroxy derivatives by the PG ω- or 20-hydroxylase. This reaction is catalyzed by a cytochrome P450, and the enzyme that catalyzes this reaction in lung microsomes of pregnant rabbits was initially called $P\text{-}450_{PG\omega}$. The current designation is CYP4A4. 20-OH, 20-hydroxy; 13,14,DH, 13,14-dihydro; 15-K, 15-keto.

the 105,000 × g supernatant fraction prepared from the lungs and ovaries of rabbits demonstrated immunoreactive proteins at approximately 29 kDa in pregnant rabbits. Tai et al. (1990) reported that the monoclonal antibody to human PGDH recognized a protein band at 28 kDa in Western blots of highly purified and partially purified preparations of human placental PGDH, but a protein band of 56 kDa was also recognized in the cytosolic fraction from placental homogenates. The presence of the higher M_r protein band at 56 kDa in human placenta homogenates may reflect the tighter dimeric association of the human PGDH than the rabbit enzyme and that it may require more extensive SDS treatment or reduction to dissociate it into its monomeric subunits when examining the enzyme in its nonpurified form. Once highly purified, the human placental PGDH was found to contain 266 amino acids (Figure 4) and calculated to have a molecular mass of 28,975 Da (Krook et al., 1990), which was confirmed from the cDNA studies of Ensor et al. (1990). Höhl et al. (1993) reported a molecular mass of 28,740 Da for the human placental PGDH, as determined by matrix-assisted laser desorption/ionization mass spectrometry, and demonstrated that the enzyme did not contain any significant amounts of carbohydrate.

The first definitive report that the NAD^+-dependent PGDH isolated from human placenta was a homodimeric protein with a subunit molecular mass of approximately 28 kDa was published by Mak et al. (1982). This initial report on human PGDH classified the enzyme as a member of the short-chain alcohol dehydrogenases. Human placental PGDH shows approximately 25% homology with a number of distantly related dehydrogenases in prokaryotic and eukaryotic organisms, including alcohol dehydrogenase from *Drosophila melanogaster,* ribitol dehydrogenase from *Klebsiella aerogenes,* glucose dehydrogenase from *Bacillus megaterium,* steroid 7α-dehydrogenases from *Eubacterium,* 3α/20β-hydroxysteroid dehydrogenase from *Streptomyces hydrogenans,* 17β-hydroxysteroid dehydrogenase from human placenta (Krook et al., 1990, 1992), 3β-hydroxysteroid dehydrogenase from *Rhizobium meliloti,* and the NodG protein from *Pseudomonas testosteroni* (Baker, 1994). A second enzyme, which also oxidizes the 15-hydroxyl group of PGs but utilizes $NADP^+$ and is called a carbonyl reductase, also has approximately 20% homology with the NAD^+-dependent PGDH (Wermuth, 1992; Krook et al., 1993a). Four regions of the NAD^+-dependent PGDH (residues 6 to 31, 82 to 92, 130 to 154, and 177 to 187) were identified by Wermuth (1992) as having above-average sequence relatedness with the other short-chain alcohol dehydrogenases and with the carbonyl reductase.

PGDH was found to have a free methionine as its N-terminal residue, and glutamine was found to be the C-terminal amino acid. Ensor et al. (1990) cloned human PGDH from a placental cDNA library and found that the amino acid sequence coded for by the cDNA corresponded to the sequence identified by Krook et al. (1990). These authors observed that the PGDH cDNA coded for a tyrosine at position 217, whereas Krook et al. (1990) reported that they found a microheterogeneity at this position, with either cysteine or tyrosine expressed at this position.

A specific tyrosine residue (at position 151 in human placental PGDH) was found to be conserved in several short-chain, nonmetallo dehydrogenases, suggesting that it may be essential for enzymatic activity (Krook et al., 1990, 1992). Ensor and Tai (1991) reported that substitution of alanine for this tyrosine residue by site-directed mutagenesis abolished enzymatic activity,

1	Met	His	Val	Asn	Gly^{5}	Lys	Val	Ala	Leu	Val^{10}	Thr	Gly	Ala	Ala	Gln^{15}	Gly	Ile	Gly	Arg	Ala^{20}
21	Phe	Ala	Glu	Ala	Leu^{25}	Leu	Leu	Lys	Gly	Ala^{30}	Lys	Val	Ala	Leu	Val^{35}	Asp	Trp	Asn	Leu	Glu^{40}
41	Ala	Gly	Val	Gln	Cys^{45}	Lys	Ala	Ala	Leu	Asp^{50}	Glu	Gln	Phe	Glu	Pro^{55}	Gln	Lys	Thr	Leu	Phe^{60}
61	Ile	Gln	Cys	Asp	Val^{65}	Ala	Asp	Gln	Gln	Gln^{70}	Leu	Arg	Asp	Thr	Phe^{75}	Arg	Lys	Val	Val	Asp^{80}
81	His	Phe	Gly	Arg	Leu^{85}	Asp	Ile	Leu	Val	Asn^{90}	Asn	Ala	Gly	Val	Asn^{95}	Asn	Glu	Lys	Asn	Trp^{100}
101	Glu	Lys	Thr	Leu	Gln^{105}	Ile	Asn	Leu	Val	Ser^{110}	Val	Ile	Ser	Gly	Thr^{115}	Tyr	Leu	Gly	Leu	Asp^{120}
121	Tyr	Met	Ser	Lys	Gln^{125}	Asn	Gly	Gly	Glu	Gly^{130}	Gly	Ile	Ile	Ile	Asn^{135}	Met	Ser	Ser	Leu	Ala^{140}
141	Gly	Leu	Met	Pro	Val^{145}	Ala	Gln	Gln	Pro	Val^{150}	Tyr	Cys	Ala	Ser	Lys^{155}	His	Gly	Ile	Val	Gly^{160}
161	Phe	Thr	Arg	Ser	Ala^{165}	Ala	Leu	Ala	Ala	Asn^{170}	Leu	Met	Asn	Ser	Gly^{175}	Val	Arg	Leu	Asn	Ala^{180}
181	Ile	Cys	Pro	Gly	Phe^{185}	Val	Asn	Thr	Ala	Ile^{190}	Leu	Glu	Ser	Ile	Glu^{195}	Lys	Glu	Glu	Asn	Met^{200}
201	Gly	Gln	Tyr	Ile	Glu^{205}	Tyr	Lys	Asp	HIs	Ile^{210}	Lys	Asp	Met	Ile	Lys^{215}	Tyr	Tyr	Gly	Ile	Leu^{220}
221	Asp	Pro	Pro	Leu	Ile^{225}	Ala	Asn	Gly	Leu	Ile^{230}	Thr	Leu	Ile	Glu	Asp^{235}	Asp	Ala	Leu	Asn	Gly^{240}
241	Ala	Ile	Met	Lys	Ile^{245}	Thr	Thr	Ser	Lys	Gly^{250}	Ile	His	Phe	Gln	Asp^{255}	Tyr	Asp	Thr	Thr	Pro^{260}
261	Phe	Gln	Ala	Lys	Thr^{265}	Gln														

FIGURE 4. Amino acid sequence for the NAD^{+}-dependent PGDH (Krook et al., 1990; Ensor et al., 1990). The underlined amino acids from valine^{32} to glutamate^{40} represent the region identified by Chavan et al. (1993) as the adenine-binding region.

confirming the importance of this amino acid in catalysis.

The NAD^+-binding domain is reported in the N-terminal region of the protein. Chavan et al. (1993) identified a nine-residue segment from valine[32] to glutamate[40] (Val-Ala-Leu-Val-Asp-Trp-Asn-Leu-Glu) that bound the adenine ring of the photoaffinity analog of NAD^+, [α-^{32}P]nicotinamide-2-azidoadenine. Baker (1994) proposed that the aspartate residue at position 36 conferred the NAD^+ specificity for this enzyme, because the charge of this amino acid would reduce affinity for $NADP^+$.

Krook et al. (1993a, b) developed a computer-simulated model for the NAD^+- and $NADP^+$-dependent PGDHs based on the coordinates determined from the crystallographic analysis of the 3α/20β-hydroxysteroid dehydrogenase from *S. hydrogenans*. The models indicate similar homology of the tertiary structure over the first two thirds of the polypeptide chains, with the remaining third demonstrating contrasting tertiary structure. Residues that form the coenzyme and substrate-binding regions were identified from the model and confirmed the importance of the tyrosine residue at position 151 in the catalytic site (Krook et al., 1990) and the region identified by Chavan et al. (1993) for binding of the adenine ring of NAD^+. Wermuth (1992) reported 52 amino acid residues were conserved between the NAD^+- and $NADP^+$-dependent PGDHs, and there were an additional 92 residues that represented conservative substitutions. Wermuth (1992) also reported that unlike the other short-chain alcohol dehydrogenases, which have a strictly conserved glycine near position 130 and a conserved tyrosine-X-X-X-tryptophan doublet around position 150, the $NADP^+$-dependent PGDH (carbonyl reductase) contains a 41 amino acid insertion between the glycine residue and the tyrosine-X-X-X-tryptophan doublet.

B. PGDH Substrate Specificity

It has been well established that PGs of the E, F, and A series containing a 15(S)-hydroxy group are substrates for PGDH and that this enzyme is an important regulator of circulating levels of PGs (Hansen, 1974; Pace-Asciak and Smith, 1983; Casey et al., 1989). The metabolism of PGs has been discussed in the previous reviews of Hansen (1976) and Pace-Asciak and Smith (1983), and the reader is referred to these reviews for a description of studies concerning substrate specificity. It should be noted that not all PGs are substrates for NAD^+-dependent PGDH; for example, PGB_2 is not metabolized and PGD_2 is reported to be metabolized by other enzymes in brain (Watanabe et al., 1983) or in liver and kidney (Pugliese et al., 1985; Hoult et al., 1988; Ohara et al., 1994). Prostacyclin (PGI_2) was found to be metabolized by NAD^+-dependent PGDH (Jarabak et al., 1984), whereas thromboxane (Tx) B_2 was not a substrate (Wu et al., 1990). A distinct NAD^+-dependent TxB_2 dehydrogenase has been described by Wu et al. (1990) in liver, kidney, and the gastrointestinal tract of pigs. It should be noted that changes at the ω-end of PGs reduce the rate at which PGDH metabolizes these compounds, and such analogs have been used as long-acting PG derivatives (Nelson et al., 1982). We have observed that the addition of a hydroxy group at the 19- or 20-carbon atom of PGE_1 increases the K_m two- to ninefold and reduces the k_{cat}/K_M value substantially (Table 1). This may have significance, for it has been reported that primate and ram seminal fluid contain 19- and 20-hydroxylated PGs, respectively (Kelly et al., 1976; Oliw et al., 1986). In the case of primate seminal fluid, 19-hydroxy-PGE_1 and 19-hydroxy-PGE_2 are present in significant amounts (Kelly et al., 1976; Pourian et al., 1995) and may play a role in sperm function

TABLE 1
K_M and k_{cat}/K_M Values Obtained for PG and ω-6 Hydroxy Fatty Acids with the Purified Rabbit Lung NAD-Dependent PGDH

Substrate	K_m[a]	k_{cat}/K_M[b]
PGE_1	30	6.5
19-OH-PGE_1	77[c]	0.2[c]
20-OH-PGE_1	283[c]	0.2[c]
PGE_2	59	3.5
PGE_3	77	2.4
$PGF_{2\alpha}$	133	1.2
PGA_1	38	4.3
PGD_2	741	0.1
15-HETE	16	5.4
12HHTrE	12	7.5

[a] K_M values given in micromolar. Values taken from Bergholte et al. (1987).
[b] Units of k_{cat}/K_M values = $\times 10^{-5}$ *M*/s.
[c] Unpublished results.

and male fertility. It has been proposed that seminal prostaglandins may suppress immune activity in the female reproductive tract and prevent recognition of sperm cells as foreign bodies (Quayle et al., 1989). It is interesting to speculate whether hydroxylation near the ω-terminus will prevent the rapid inactivation of these 19-hydroxy-PGs after they enter the female reproductive tract.

Recent studies have demonstrated that PGDH also catalyzes the dehydrogenation of a number of ω-6 hydroxy fatty acid. 12-Hydroxy-heptadecatrienoic acid (12-HHTrE), a metabolite formed during the synthesis of TxA_2 (Diczfalusy et al., 1977) and various derivatives of 15-HETE also undergo dehydrogenation at the ω-6 hydroxy group (Liu et al., 1985; Bergholte et al., 1987). The common features among 15-HETE, 12-HHTrE, and PGs are the presence of the hydroxy-group six carbon atoms from the ω-carbon atom and the *trans* double bond located seven carbon atoms from the ω-carbon atom. The K_m and k_{cat}/K_m values for these substrates are listed in Table 1. Based on the lower K_m values for 15-HETE and 12-HHTrE than for PGs, the cyclopentane ring of PGs is unnecessary for recognition at the active site of PGDH. Liu et al. (1985) were the first to report that PGDH could metabolize 12-HHTrE and 15-HETE. Agins et al. (1985, 1987) also observed that 12-HHTrE was metabolized to its 12-keto derivative in HL-60 cells by the PGDH. Höhl et al. (1993) also described the ability of purified human placental PGDH to metabolize 12-HHTrE. 12-HHTrE is also oxidized in red blood cells, but a $NADP^+$-dependent enzyme was reported to catalyze this reaction (Hecker and Ullrich, 1988). 13-Hydroxyoctadecadienoic acid (13-HODE) was not observed to be a substrate for purified lung PGDH (Bergholte et al., 1987) even though it also possesses the ω-6 hydroxy group. A reason why 13-HODE was not a substrate for the lung PGDH has not been determined. Earles et al. (1991) characterized a 13-HODE dehydrogenase enzyme in several tissues in the rat and proposed that this enzyme is distinct from PGDH. Whether these compounds are substrates for PGDH *in vivo* has not been studied. The metabolism of 15-HETE may have particular physiological significance because 15-HETE is a major pulmonary product in normal and asthmatic lung tissue (Kumlin et al., 1990; Sigal and Nadel, 1991).

Jarabak (1992) described the interesting observation that certain polyaromatic hydrocarbons may undergo oxidation-reduction reactions with the NAD^+-dependent PGDH in the presence of NADH, producing semiquinone metabolites, superoxide anions, and hydrogen peroxide. 9,10-Phenanthrenequinone and 5,6-chyrsenequinone served as substrates for the enzyme in this reaction (Jarabak, 1992). The addition of superoxide dismutase to the reaction inhibited formation of the quinone metabo-

lites and demonstrated the need for superoxide anions to drive the reaction. The significance of this interaction between PGDH and polycyclic aromatic hydrocarbons *in vivo* has not been studied, and whether it may cause oxidative damage to tissues or interfere with PG metabolism is not known.

C. Inhibitors of PGDH Activity

A large number of drugs and protein-modifying agents have been recognized to inhibit PGDH activity, and these have been reviewed in earlier reports by Hansen (1976) and Pace-Asciak and Smith (1983). Several additional compounds have been found to inhibit PGDH activity since these review articles were published, and they are discussed in this section. Chang et al. (1984) observed that pulmonary PGDH activity decreased by 30 to 39% in male rats exposed to cigarette smoke for 4 to 13 weeks. Cigarette smoke inhibited lung PGDH, but PGDH activity in kidney and intestine was unaffected (Chang et al., 1984). Liu and Tai (1985) found that acrolein, an α,β-unsaturated aldehyde and a component of cigarette smoke and automobile exhaust, is an irreversible inhibitor of PGDH activity in porcine lung. The inhibition by acrolein was proposed to act at the NAD^+-binding site because if NAD^+ was added to the reaction mixture prior to the addition of acrolein, the pyridine nucleotide protected the enzyme from inactivation.

It has been established that PGDH is sensitive to a variety of protein-modifying reagents and that loss of activity is prevented if NAD^+ is coincubated with the enzyme (Krook et al., 1992). *N*-Chlorosuccinimide, *N*-ethylmaleimide, iodoacetamide, and 2,4,6-trinitrobenzene-sulfonic acid were found to inactivate pig kidney PGDH (Mak et al., 1990) in a time-dependent process. These studies indicate that lysine, cysteine, and methionine residues are present in the NAD^+-binding site, located in the N-terminal region of the enzyme. Phenylglyoxal also inhibited the human placental enzyme, but the inactivation was not prevented by inclusion of NAD^+ (Mak et al., 1990). In contrast to the renal enzyme, Krook et al. (1992) reported that human placental PGDH was not inactivated by iodoacetamide and other protein-modifying reagents, but was inhibited by pyridoxal phosphate and tetranitromethane. The rabbit lung enzyme was also inhibited by *N*-ethylmaleimide and *para*-chloromercuriphenylsulfonic acid (Bergholte and Okita, 1986a).

Jarabak (1992) also reported that the polycyclic aromatic hydrocarbon quinones, 1,2-naphthoquinone, 4,5-benzo[*a*]pyrene quinone, and 7,8-benzo[*a*]pyrene quinone were inhibitors of human placental PGDH, causing a time-dependent inactivation at doses of 2 μ*M*. Addition of glutathione to these reactions provided varying degrees of protection of the enzyme from inactivation by these quinones.

Berry et al. (1983) described the inhibition of bovine lung and human placental PGDH activity by analogs of sulfaphasalazine, an anticolitic drug that was shown to inhibit PGDH in a noncompetitive manner. It was determined that homosalazine was the basic structure of a number of compounds that inhibited PGDH activity. A $-CH_2COOH$ group at position 1 and –OH group at position 2 in the salicyl ring of homosalazine were required for inhibition. Based on these initial findings, Berry et al. (1985) synthesized the compound 2-hydroxy-5-(3,5-dimethoxycarbonyl-benzoyl)-benzene acetic acid and demonstrated that it was 1000 times more potent than sulfasalazine in inhibiting PGDH activity.

It was also shown that fatty acids are inhibitors of PGDH activity. Marrazzi and

Matschinsky (1972) published the initial study that fatty acids inhibited PGDH activity. Kung-Chao and Tai (1980) observed that renal PGDH from pigs was inhibited by saturated and unsaturated fatty acids. In this study, oleic acid was found to inhibit PGDH activity with a K_i of 1.25 μ*M*. Linoleic acid, arachidonic acid, and palmitic acid also inhibited PGDH with K_i values of 4 to 6 μ*M*, whereas the value for stearic acid was 40 μ*M*. Since the report by Kung-Chao and Tai (1980), several other investigators have reported that inhibitory factors are present in tissues that have been identified as lipid components. Bergholte and Okita (1986b) isolated a heat-stable factor from lungs of pregnant and nonpregnant rabbits during the purification of PGDH that inhibited the purified lung PGDH. Analysis of this lipid factor revealed that it contained a variety of fatty acids that could inhibit PGDH activity (Bergholte and Okita, 1986b). Nagai et al. (1988) also reported that a small-molecular-weight lipid-like material was present in human milk and placenta that inhibited PGDH activity. Sakuma et al. (1992, 1994) observed that arachidonic acid and 13-hydroperoxyoctadecadienoic acid inhibited renal and gastric PGDH activity, respectively. Whether fatty acids may serve as physiological regulators of PGDH activity has not been determined.

Another reported inhibitor of PGDH activity that may have profound clinical importance is ethanol (Randall et al., 1987; Schenker et al., 1990). The inhibition of PGDH activity by ethanol has been reported in several studies and may contribute to abnormal fetal development in alcohol-related diseases (Randall et al., 1987). Pennington and Taylor (1983) reported that chronic exposure to moderate (17% of calories) or high (35% of calories) doses of ethanol resulted in a decrease in PGDH activity in rat kidney. However, Treissman and Brien (1991) and Treissman et al. (1991) reported that incubating ethanol (10 to 80 m*M*) with the 105,000 × g supernatant fraction of brain homogenates prepared from fetal guinea pigs or sheep did not inhibit PGDH activity. In our laboratory, we observed an inhibition of PGDH activity when ethanol was added to reactions containing the purified enzyme or cytosolic fractions of lung tissue from pregnant rabbits, but we observed no inhibitory effect on rabbit lung PGDH activity when 20% ethanol was administered to pregnant rabbits in their drinking water.

It is interesting to speculate whether these endogenous or exogenous compounds may affect PG levels *in vivo* and if they may affect enzyme function, resulting in abnormal cell function during pregnancy.

D. Distribution of NAD⁺-Dependent PGDH in Tissues and Immunochemical Studies

Änggard et al. (1971) carried out an in-depth study of PGDH activity in the cytosolic fractions (100,000 × g supernatant) of several tissues of pigs and observed that kidney, spleen, and lung were very high in NAD^+-dependent activity. Similar studies were also performed in a number of other animal species (Pace-Asciak and Smith, 1983).

Monoclonal and polyclonal antibodies were made to the human placental PGDH (Tai et al., 1990), and a polyclonal antibody was made to the lung PGDH of pregnant rabbits (Okita et al., 1990). These antibodies have been used to identify PGDH proteins in Western blots and in immunohistochemical studies. The antibody to placental PGDH demonstrated positive staining in human placenta in intermediate trophoblasts and syncytiotrophoblastic cells, but not in cytotrophoblasts or villous stromal tissue

(Cheung et al., 1990). In fetal membranes, PGDH was localized in the trophoblast layer of chorion and in trophoblast cells that invaded decidua, but was not found in amnion or decidual stromal cells. Sangha et al. (1994) observed a substantial decrease in immunodetectable PGDH in trophoblast cells of the chorion in patients with idiopathic preterm labor. The reduction in immunohistochemical staining correlated with the decrease in PGDH activity and PGDH mRNA levels. In lung of pregnant rabbits, positive staining was found in the cytoplasm of epithelial cells of the respiratory bronchiole, but identification of cell types could not be positively made (Okita et al., 1990). In isolated lung cells of pregnant rabbits, Devereux et al. (1987) reported that alveolar type II cells catalyzed formation of 15-keto-$PGF_{2\alpha}$ and 13,14-dihydro-15-keto-$PGF_{2\alpha}$ and that preparations of enriched Clara cells were also capable of metabolizing $PGF_{2\alpha}$; however, due to contamination of Clara cells by alveolar type II cells, no definite conclusion could be made about this cell type. There was no indication that PGDH was located in lung endothelial cells of pregnant rabbits (Okita et al., 1990). It had been speculated that PGDH activity was present in endothelial cells because of the rapidity with which PGs are metabolized and released when perfused through the lung. However, our finding that PGDH protein was not present in endothelial cells was supported by an earlier report that pulmonary endothelial cells did not metabolize PGs (Ryan, 1982).

E. Regulation of PGDH Activity: Whole-Animal Studies

NAD^+-dependent PGDH activity has been observed to be regulated by steroid hormones. Sun and Armour (1974) published one of the earliest reports that described a 20-fold increase in the specific activity of lung NAD^+-dependent PGDH in the 100,000 × g supernatant fraction from pregnant rabbits that were between 28 and 30 d of gestation (the gestational period for a rabbit is 31 or 32 d) as compared to nonpregnant rabbits. A similar observation was made by Edgerton-Vernon and Bedwani (1975), who reported that PGDH activity increased dramatically during the third trimester of pregnancy in rabbit lung but not in kidney or spleen. The increase in activity was not observed in the lungs of pregnant rats (Edgerton-Vernon and Bedwani, 1975). Recently, Nagai et al. (1991) reported a 1.5-fold increase in PGDH specific activity, which peaked at day 21 of the gestational period (the gestational period for rat is 22 d). This 1.5-fold increase in rat lung PGDH activity is minor compared with the 20-fold or greater increase observed in the lungs of pregnant rabbits. Bedwani and Marley (1975) observed that treatment of male rabbits with progesterone (10 mg/kg) or a combination of progesterone (10 mg/kg) and estradiol (5 μg/kg) for 12 or 26 d increased the rate of PGE_2 inactivation in the pulmonary system. Treatment of female rabbits with human chorionic gonadotropin (hCG) also increased PGDH activity in the lung (Bedwani and Marley, 1975).

Okita et al. (1990) demonstrated that the increase in pulmonary PGDH activity corresponded to an increase in the immunoreactive protein that was observed using a polyclonal antibody to lung PGDH purified from pregnant rabbits. This confirmed the results of Bedwani and Marley (1975) that lung PGDH specific activity began to increase in the second trimester and remained elevated until parturition. As shown in Figure 5, Western blot analysis demonstrated intense staining in the 105,000 × g fraction of lungs of 28-d pregnant rabbits, with only minor immunoreactive protein

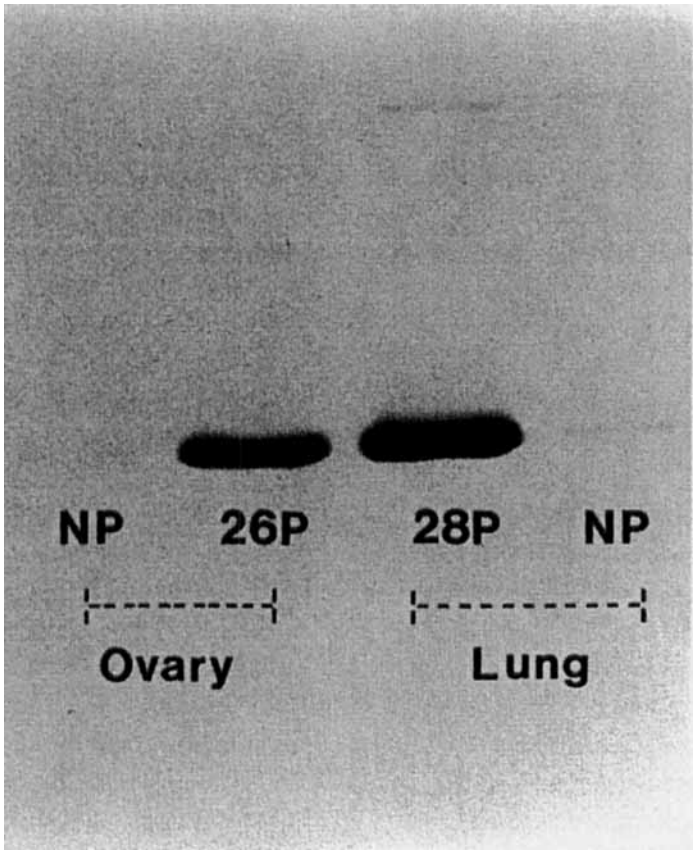

FIGURE 5. Western blot analysis of ovarian and lung 105,000 × g supernatants demonstrating the presence of an immunoreactive protein band corresponding to PGDH in 26- or 28-d pregnant (P) rabbits, but not in the cytosol of nonpregnant (NP) rabbits.

bands in nonpregnant rabbits. This increase in lung PGDH activity was localized in epithelial cells at the level of the respiratory bronchiole in cells that were preliminarily identified as Clara cells (Okita et al., 1990).

The function of the elevated levels of NAD^+-dependent PGDH activity in rabbit lung during pregnancy is not known. The lung is a major site for PG metabolism in many animal species (Piper et al., 1970), causing a rapid inactivation of circulating PGE_2 and $PGF_{2\alpha}$ in a single pass through the lung. The induction of lung PGDH enzymatic activity and protein levels during pregnancy has not been observed in species other than rabbit. It should be noted that placental tissue is a major source for NAD^+-dependent PGDH activity in many species, including humans (Jarabak, 1980; Nagai et al., 1991), and this organ may regulate circulating levels of PGs, which increase dramatically during pregnancy (Venuto and Donker, 1982). However, in the rabbit, placental PGDH activity remains low and does not approach the values obtained in human placenta (Okita et al., 1990). This was confirmed in Western blot studies in which only a faint protein band corresponding to the M_r value of PGDH was observed (Okita et al., 1990). This suggests the lung PGDH may substitute for the placental enzyme in the pregnant rabbit. Corresponding to the

temporal increase in PGDH activity in pregnant rabbits is an increase in circulating levels of 13,14-dihydro-15-keto-PGE_2, which increased between 12- and 15-fold in the late-gestation pregnant vs. nonpregnant rabbits (Mucha and Losonczy, 1990).

Okita et al. (1992) also reported an increase in PGDH activity and immunoreactive protein (Figure 5) in the ovary of pregnant rabbits. The specific activity of ovarian PGDH increased approximately 15-fold in 14- to 18-d pregnant rabbits compared with nonpregnant rabbits. This increase in ovarian PGDH activity occurred primarily in the corpora lutea (CL) and was also observed in ovaries of rabbits treated with pregnant mare's serum gonadotropin (PMSG) and hCG to induce a state of superovulation and pseudopregnancy. Administration of the hCG stimulates PG and progesterone secretion and induces a state of pseudopregnancy in rabbits because it causes changes in the ovary that resemble the initial stages of pregnancy. This state of pseudopregnancy exists for 12 d, at which time the CL regress and serum progesterone levels return to basal levels. Elevated ovarian PGDH activity in pseudo-pregnant rabbits was observed only on day 11 during this time period (Okita et al., 1992). Whether this increase in PGDH activity has a role in CL regression has not been studied. In contrast to the studies of Okita et al. (1990, 1992), Schlegal et al. (1988) reported that PGDH activity did not increase in the CL of pregnant or pseudopregnant rabbits.

Nagai et al. (1991) found that placental PGDH activity also increased dramatically during the course of the gestational period in rats, increasing 50-fold between day 13 and day 22. Keirse et al. (1985) reported that human placental PGDH increased 7.5-fold between 7 to 8 and 15 to 16 weeks of gestation. The activity of PGDH at term was the same as that found in 15- to 16-week placentae. Jarabak et al. (1987) observed that placental PGDH activity is elevated in women who were diagnosed with preeclampsia or eclampsia. The study by Jarabak et al. (1987) reported that placental NAD^+-dependent PGDH activity was 2.7 times greater in preeclamptic patients than in normal subjects, whereas no change was observed in the $NADP^+$-dependent PGDH activity. It was proposed that the increase in NAD^+-dependent PGDH activity may increase PGI_2 and PGE_2 metabolism and may serve to decrease levels of vasodilatory PGs in preeclamptic patients.

Induction of NAD^+-dependent PGDH activity was observed in human endometrial tissue during early and midsecretory phases of the ovarian cycle (Casey et al., 1980). A 3.5-fold increase in PGDH specific activity was reported in the 105,000 × g supernatant fraction of endometrial tissue that was obtained during the secretory phase compared with the proliferative phase. Alam et al. (1976) reported increases in PGDH activity in decidual and myometrial tissue of the uterus in pseudopregnant rats between days 7 and 10 of this period. They also reported that progesterone and estrogen administration to ovariectomized pseudopregnant rats increased PGDH activity in decidual and myometrial tissues when PGE_1 was the substrate. Falkay and Sas (1978) also reported that progesterone may regulate PGDH activity in human placental tissue.

It was reported that estradiol may decrease PGDH activity (Chang and Tai, 1985; Chang, 1987). Chang (1987) reported that administration of 0.5 mg of estradiol per kilogram every 3 or 4 d over a 4-week period to ovariectomized rats decreased PGDH activity by 50% in the kidney. An analysis of the K_m and V_{max} values of the estradiol-treated and control rat kidney fractions indicated the K_m value was unchanged, whereas V_{max} decreased 50%, and it was suggested that estradiol may inhibit PGDH synthesis. Erman et al. (1987) also

reported that renal PGDH activity in rats treated with dexamethasone for 2 weeks was reduced by 57%.

F. Regulation of PGDH Activity: Isolated Cell Studies

Several elegant studies investigating the regulation of PGDH activity in cultured cells have been reported by Tai's group at the University of Kentucky. Xun et al. (1991b) showed that treatment of promyelocytic leukemia (HL-60) cells with phorbol 12-myristate 13-acetate (PMA) or dimethyl sulfoxide (DMSO) induced PGDH activity. The increase in PGDH activity was blocked by both cycloheximide and actinomycin D, indicating *de novo* synthesis of PGDH protein. If the concentrations of PMA used were greater than 10 n*M*, PGDH levels were observed to be increased in PMA-treated human erythroleukemia (HEL) cells, but the newly synthesized enzyme was inactive (Xun et al., 1991a). This inhibition of PGDH activity was presumably caused by enzyme phosphorylation that was also mediated by protein kinase C activity, because the addition of staurosporin prevented the rapid loss of PGDH activity (Xun et al., 1991a). This dual effect of PMA on PGDH activity was shown by Xun et al. (1991b) to be concentration dependent. Concentrations of PMA less than 10 n*M* induced PGDH synthesis, whereas if higher concentrations were used, an inactivation of PGDH also occurred that was mediated by protein phosphorylation. Xun et al. (1991b) determined that a protein kinase C-mediated phosphorylation was responsible for the inactivation of PGDH activity.

Xun et al. (1991a) also reported that PGDH activity in HEL cells may be induced by steroid hormones. Dexamethasone was observed to be the optimal inducer, followed by progesterone and triamcinolone, prednisolone, cortisone, and corticosterone. Estradiol and testosterone were reported to have only minor stimulatory properties. Agins et al. (1985, 1987) also reported that dimethyl formamide increased PGDH activity in HL-60 cells. The human placental PGDH has also been expressed in *E. coli* using the bacterial plasmids pUC-18 and pUC-19, containing the isopropyl-1-thio-β-D-galactopyranoside (IPTG)-inducible Lac 2 promoter (Ensor and Tai, 1992). The specific activity of the PGDH in *E. coli* extracts were found to be severalfold greater than the activity found in human placental fractions.

III. CARBONYL REDUCTASE

A. Introduction

A second enzyme involved in PG metabolism that has been reported to be induced during pregnancy is a $NADP^+$-dependent PGDH that catalyzes the NADPH-dependent reduction of the 9-keto group of PGE_2 to form $PGF_{2\alpha}$ and the reversible reduction of 15-keto groups of PGs. This enzyme was initially referred to as PGDH II, whereas the NAD^+-dependent enzyme was designated PGDH I, but the enzyme is now designated as a carbonyl reductase. This NADPH-dependent enzyme primarily acts as a carbonyl reductase and has been shown to reduce a number of xenobiotic compounds in addition to PGs (Iwata et al., 1989; Krook et al., 1993b). Because several enzymatic activities were described for this protein before it was recognized that a single enzyme catalyzed these reactions, three enzyme numbers (E.C. 1.1.1.141, E.C. 1.1.1.184, and E.C. 1.1.1.189) have been assigned to this protein (Krook et al., 1993b).

B. Ovarian Carbonyl Reductase Activity

Presently, there is renewed interest in the carbonyl reductase as it relates to PG metabolism because of studies demonstrating that it catalyzes the reduction of 13,14-dihydro-15-keto-$PGF_{2\alpha}$ to 13,14-dihydro-$PGF_{2\alpha}$ in ovaries (Figure 2). This enzyme may have a major role in determining the levels of PGE_2, $PGF_{2\alpha}$, 13,14-dihydro-15-keto-$PGF_{2\alpha}$, and 13,14-dihydro-$PGF_{2\alpha}$ in tissues. One of the initial studies to demonstrate the importance of the carbonyl reductase in the metabolism of PGs in reproductive tissues was reported by Aizawa et al. (1980), who observed the conversion of 13,14-dihydro-15-keto-$PGF_{2\alpha}$ to 13,14-dihydro-$PGF_{2\alpha}$ in rat ovaries. Iwata et al. (1990a, 1990b) have suggested that 13,14-dihydro-$PGF_{2\alpha}$ is a novel, biologically active metabolite that may stimulate ovarian steroidogenesis or initiate luteal regression. Kehl and Carlson (1981) have reported that 13,14-dihydro-$PGF_{2\alpha}$ has potent luteolytic activity on rabbit ovaries. However, it should be noted that studies by Powell et al. (1974, 1975) have shown that 13,14-dihydro-$PGF_{2\alpha}$ has two- to fourfold higher dissociation constants than $PGF_{2\alpha}$ for $PGF_{2\alpha}$ receptors in ovine and bovine CL. Further studies are needed to determine the function of the carbonyl reductase in CL and if 13,14-dihydro-$PGF_{2\alpha}$ participates in luteolysis.

Ovarian carbonyl reductase activity was shown to be stimulated in rats treated with PMSG and/or hCG (Inazu et al., 1983). Two distinct proteins were isolated from rat ovary by Iwata et al. (1989) and were designated as carbonyl reductases 1 and 2 (CR1 and CR2). The two proteins were found to have broad substrate specificity, catalyzing the reduction of aromatic aldehydes, ketosteroids, and quinones at rates greater than PGs, but the K_M values for xenobiotics and steroids were tenfold greater than the values for PGs. K_M and k_{cat}/K_M values are given in Table 2 for selected substrates of CR1 and CR2. CR1 and CR2 had nearly identical migration patterns on SDS-PAGE at an M_r of 33 kDa and pI values of 6.0 and 5.9, respectively. Inhibition studies using antibodies directed to CR1 and CR2 demonstrated that these proteins catalyzed the reduction of 4-benzoylpyridines and various PG derivatives extensively, whereas other unidentified reductases also reduced 4-nitroacetophenone, 4-nitrobenzaldehyde, and steroids. Immunoreactive carbonyl reductase protein bands were detected in Western blot studies in the 105,000 × g supernatant fraction of rat adrenal glands, liver, testes, epididymus, seminal vesicles, prostate, and vas deferens.

C. Effect of Pregnancy on Ovarian Carbonyl Reductase Activity

In examining ovarian tissues of rabbits, Iwata et al. (1990b) observed that a carbonyl reductase protein was present in pregnant, but not in nonpregnant rabbits. This observation is of interest because of the increase in ovarian NAD^+-dependent PGDH activity and protein during the pregnancy in rabbits described above (Okita et al., 1992). The interaction between the two PG-metabolizing enzymes and their role in ovarian function has not been examined.

In rats, ovarian carbonyl reductase activity, as measured by the reduction of 13,14-dihydro-15-keto-$PGF_{2\alpha}$ or 4-benzoylpyridine, was found to decrease at the onset of pregnancy in rats, reaching a value of 20 to 30% of the initial activity by day 14 of pregnancy (Iwata et al., 1990b). This decrease in reductase activity was maintained

TABLE 2
K_M and k_{cat}/K_M Values for Various Substrates by the Purified Rat Ovarian NADPH-Carbonyl Reductases

Substrate	K_m[a]	k_{cat}/K_M[b]
Carbonyl Reductase 1		
PGE_1	65	2.5[c]
13,14-Dihydro-15-keto-$PGF_{2\alpha}$	45	2.5
PGE_2	110	0.7
13,14-Dihydro-15-keto-PGE_2	19	13.0
4-Nitroacetophenone	930	6.7
5α-Androstan-17β-ol-3-one	500	1.1
Carbonyl Reductase 2		
PGE_1	27	8.6
13,14-Dihydro-15-keto-$PGF_{2\alpha}$	35	6.6
PGE_2	89	2.9
13,14-Dihydro-15-keto-PGE_2	173	5.3
4-Nitroacetophenone	1430	1.4
5α-Androstan-17β-ol-3-one	820	2.2

[a] K_M values given in micromolar. Values taken from Tables 2 and 3 from Iwata et al. (1989).
[b] Units of k_{cat}/K_M values = $\times 10^{-3}$ *M*/s.
[c] Results calculated from Tables 2 and 3 from Iwata et al. (1989).

for the remainder of the gestational period. One day following parturition, carbonyl reductase activity had returned to levels observed in the ovaries of nonpregnant rats. Western blot studies indicated a decline in immunoreactive protein after day 14 of the gestational period; however, there was no apparent decrease in protein levels during the first 14 d, at which time enzymatic activity declined approximately 70%. This finding suggested to the authors that an unidentified endogenous factor may be responsible for the early decline in carbonyl reductase activity (Iwata et al., 1990b). A rise in immunoreactive protein was observed at parturition that corresponded to the increase in enzymatic activity. Whether a direct relationship exists between changes in carbonyl reductase activity and PG function has not been ascertained.

IV. CYTOCHROME P450-DEPENDENT PG ω-HYDROXYLASE

A. Introduction

A third prostaglandin-metabolizing enzyme that is induced during pregnancy is the cytochrome P450-dependent PG 20-hydroxylase that catalyzes hydroxylation of PGs, arachidonic acid, and other ω-6 hydroxy fatty acid molecules such as 15-HETE at their terminal methyl carbon atoms (Figure 3). The initial report of this enzymatic activity in a pregnant animal was made by Powell and Solomon (1978), who observed that lung and liver microsomes from pregnant New Zealand white rabbits catalyzed the ω-hydroxylation of PGE_2 and $PGF_{2\alpha}$, whereas little activity was found in nonpregnant rabbits. This study showed that PG 20-hydroxylation specific activities were 126- and 20-fold greater in lung and liver microsomes, respectively, of pregnant vs. nonpregnant rabbits (Powell and Solomon, 1978). PG 20-hydroxylase activity was also present in placental and uterine microsomes from rabbits (Powell, 1980). The induction of this activity during pregnancy was found to be unique to rabbits. Further studies by Powell (1978) established that progesterone administration induced this enzymatic activity and that treatment of male rabbits

with this steroid hormone induced this hydroxylation reaction in lung microsomes. The discovery of the lung enzyme system led Williams et al. (1984) and Yamamoto et al. (1984) to purify this PG ω-hydroxylase from lung microsomes of pregnant female or progesterone-treated male rabbits, respectively, and characterized the enzyme as a cytochrome P450. This cytochrome P450-mediated PG ω-hydroxylase activity was also identified in placental microsomes of rabbits (Yamamoto et al., 1986).

B. Regulation of Cytochrome P450-Dependent PG ω-Hydroxylase (CYP4A4)

Matsubara et al. (1987) initially identified the cDNA of this P450 and reported its amino acid sequence, which was designated CYP4A4 by the P450 Nomenclature Committee (Nelson et al., 1993). CYP4A4 was found to share 74% amino acid homology with CYP4A1 (Matsubara et al., 1987), which is found in rat liver and is inducible by agents such as clofibrate or phthalate plasticizers, which are classified as peroxisome proliferators (Okita, 1994). Further studies by Muerhoff et al. (1987) demonstrated that translatable mRNA levels peaked at approximately day 25 of pregnancy and that immunoreactive protein reached a maximum at day 28 of pregnancy. Following the onset of parturition and delivery of the fetuses, the level of immunoreactive P450 and translatable message decreased rapidly, falling to control values 3 to 4 d postpartum (Muerhoff et al., 1987). Palmer et al. (1993) reported isolating the genomic DNA encoding the entire CYP4A4, which was found to contain two transcriptional start sites 37 and 40 nucleotides upstream from the initiation codon, but no TATA consensus sequence was found for the CYP4A4 gene. Palmer et al. (1993) reported that the CYP4A4 mRNA was absent from lung, liver, and kidney of nonpregnant rabbits, but was identified in all three organs in pregnant rabbits. The amount of mRNA was highest in the lung of pregnant rabbits and was lowest in the kidney. No consensus response element for the progesterone or glucocorticoid receptors were found in the gene isolated by Palmer et al. (1993), although these steroid hormones are inducers of the CYP4A4 protein when administered to rabbits.

C. Substrate Specificity of CYP4A4

An interesting question is why CYP4A4 is induced in rabbit lung during pregnancy. The increase in P450 levels and PG ω-hydroxylase activity in pregnant rabbits is also associated with an increase in pulmonary PGDH activity, which is stimulated 20- to 50-fold and was described previously. It was established that PG metabolites excreted in urine have undergone ω-oxidation reactions (Granström and Samuelsson, 1971a, b; Hamberg and Samuelsson, 1971). ω-Oxidation of eicosanoid derivatives consists of a series of reactions at the terminal methyl carbon atom that involve an initial hydroxylation that is subsequently followed by dehydrogenation reactions to form aldehyde and carboxyl groups. In a study by Pace-Asciak and Edwards (1980), urinary metabolites of $PGF_{2\alpha}$ were identified in rats of different age groups to determine whether age affected the metabolite profile. Rats younger than 30 d of age metabolized $PGF_{2\alpha}$ primarily to 13,14-dihydro-15-keto-$PGF_{2\alpha}$, whereas rats older than 30 d excreted products that had also undergone ω-oxidation reactions. It is interesting to speculate

whether the increase in PG ω-hydroxylase activity in the lungs of pregnant rabbits is to convert the inactive 13,14-dihydro-15-keto-PG to a more excretable metabolite and prevent it from accumulating and possibly being converted to the biologically active 13,14-dihydro-15-$PGF_{2\alpha}$ by the carbonyl reductase. Another possibility is that this enzyme will metabolize PGs or other arachidonic acid derivatives to produce biologically active compounds.

Isolated lung perfusion studies have shown that infusion of PGE_1 results in rapid metabolism to 13,14-dihydro-15-keto-20-hydroxy-PGE_1 and 20-hydroxy-PGE_1 in pregnant rabbits, whereas 13,14-dihydro-15-keto-PGE_1 is the major metabolite in nonpregnant rabbits (Leit-hauser et al., 1988). Additional studies on pregnant rabbits demonstrated that circulating levels of PGE_2 rise dramatically during pregnancy, reaching a peak during the third trimester (Venuto and Donker, 1982), and the increase in pulmonary PGDH and PG ω-hydroxylase activities may serve to prevent biologically active PGs from passing from the venous to the arterial side.

It should be noted that the preferred endogenous substrate for CYP4A4 has not been established, and other compounds such as 15-HETE and arachidonic acid have been found to be ω-hydroxylated in addition to PGs (Okita et al., 1987; Nishimoto et al., 1993). The P450 purified by Williams et al. (1984) and Yamamoto et al. (1984) catalyzed the ω-hydroxylation of PGs and long-chain fatty acids, but not that of short-chain fatty acids such as laurate or xenobiotics. It was established that K_m values for arachidonic acid, 15-HETE, PGE_1, and palmitic acid for the CYP4A4 form purified from *E. coli* expression systems were approximately 3 μ*M* for all substrates in a reconstituted P450 system and that the V_{max} value for arachidonic acid (37 pmol/min/pmol) was 4.4 times greater than for PGE_1 (8.4 pmol/min/pmol) (Nishimoto et al., 1993). Therefore, although earlier studies referred to the rabbit lung P450 that was induced during pregnancy as a PG ω-hydroxylase, CYP4A4 is also a fatty acid ω-hydroxylase and catalyzes the conversion of arachidonic acid to 20-HETE. However, Roman et al. (1993) found that if CYP4A4 was expressed in COS cells, the specific activity for PGE_1 20-hydroxylation was approximately five times greater than the activity for arachidonic acid. The differences in activities observed in the two studies probably reflect the two assay systems used to measure CYP4A4-mediated activity. In the study described by Nishimoto et al. (1993), CYP4A4 was purified from *E. coli* membranes and added to an artificial membrane system consisting of dilauroylphosphatidylcholine. NADPH-cytochrome P450 oxidoreductase and cytochrome b_5 were added to the artificial membrane system to reconstitute enzymatic activity (Nishimoto et al., 1993). In the study by Roman et al. (1993), no additional flavoprotein reductase or cytochrome b_5 was added to the microsomes prepared from COS cells containing the expressed CYP4A4. Thus, further studies are needed to characterize the substrate specificity of this P450 form and to determine the preferred endogenous substrate.

Several studies have demonstrated that 20-HETE is an important biological regulator of vascular smooth muscle (Escalante et al., 1990; Ma et al., 1994; Escalante et al., 1994; Zou et al., 1994) and also reduces platelet activation (Hill et al., 1992), which may represent important physiological processes that must be regulated during pregnancy. It has also been proposed that 20-HETE may be acted upon by PGH synthase to form 20-OH-PGs, which regulate important biological activities (Schwartzman et al., 1989; Escalante et al., 1990; Carroll et al., 1991). A number of studies have dem-

onstrated that P450 4A forms are fatty acid ω-hydroxylases. Whether 20-HETE, 13,14-dihydro-15-keto-20-hydroxy-PGs, or 20-hydroxy-PGs are formed *in vivo* must be determined to understand the physiological importance of this P450 or other P450 forms that hydroxylate fatty acids and PGs.

V. SUMMARY

Significant information has been obtained in the last decade recognizing the induction of PGDH, carbonyl reductase, and prostaglandin ω-hydroxylase in different tissues during pregnancy and elucidating the molecular properties of these enzymes. It still is unclear whether these enzymes work in sequence with each other in the lungs, ovaries, or placentas of pregnant animals. Changes in the amount of these enzymes may have dramatic effects on the inactivation of PGs in tissues or the circulation or in the production of biologically active metabolites such as 13,14-dihydro-$PGF_{2\alpha}$. Whether humans undergo similar changes in PGDH expression in the lungs or ovaries has not been determined. Understanding the roles of these enzymes during pregnancy and determining how they affect the levels of arachidonic acid metabolites requires our further attention.

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