Enzyme Systems that Metabolise Drugs and Other Xenobiotics.
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6 Prostaglandin Synthases

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Introduction

Prostaglandin H synthase (PGHS; EC 1.14.99.1) is a haem-containing protein with two enzymic activities: a cyclooxygenase and a peroxidase. The bifunctional enzyme catalyses key reactions in the biosynthesis of prostanoids (prostaglandins and related compounds). Since these products are important mediators of several physiologic and pathophysiologic processes, there is a great deal of interest in PGHS and its modulation. Moreover, a large and diverse number of chemicals including carcinogens have been found to be oxidised by PGHS-peroxidase or by peroxyl radicals generated during prostanoid biosynthesis. This chapter addresses two major aspects: one is the involvement of the enzyme in the metabolism and bioactivation of xenobiotics, the other relates to compounds affecting the activity and/or expression of PGHS or more precisely its two known isoforms (PGHS-1 and PGHS-2). In addition to compounds which inhibit PGHS-cyclooxygenase activity, there is emerging evidence that various xenobiotics, by inducing predominantly PGHS-2, modulate the synthesis of biologically active prostanoids.

PGHS genes and isozymes

There are two structurally related isoforms of PGHS encoded by separate genes. The two PGHS isozymes called PGHS-1 and PGHS-2 or COX-1 and COX-2 are homodimeric, haem-containing, glycosylated membrane-bound proteins (Smith and DeWitt 1996; Smith *et al*. 1996). PGHS-1, first purified in 1976 (Miyamoto *et al*. 1976) and cloned in 1988 (Merlie *et al*. 1988; DeWitt and Smith 1988, 1990; Yokoyama *et al*. 1988), is considered as the constitutive enzyme and synthesises prostaglandins involved in 'housekeeping' functions. PGHS-2, identified in 1991 by several laboratories, is inducible by various stimuli including proinflammatory cytokines and growth factors implying a role for PGHS-2 in inflammation and control of cell growth (for reviews see DuBois *et al*. 1998; Hershman 1996, 1999).

The PGHS-isozymes differ considerably with respect to their expression and

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biology, but have a very similar structure and similar kinetic properties (Garavito and DeWitt 1999; Smith and DeWitt 1996). Both isoforms convert free arachidonic acid to PGH_2; the release of free fatty acid substrate from membrane lipids is predominantly catalysed by phospholipases A_2 (Dennis 1994). PGH_2 is then metabolised by distinct synthases to prostaglandins (e.g. PGE_2, PGD_2, $PGF_{2\alpha}$), prostacyclins (PGI_2) or to thromboxanes (TxA_2). Being key enzymes in the synthesis of these biologically active prostanoids (Figure 6.1) make PGH-synthases a prime target for an important class of pharmaceutical compounds, the non-steroidal antiinflammatory drugs (see below).

PGHS-1 is located on human chromosome 9 (Funk *et al.* 1991). The genomic DNA sequence of human and murine PGHS-1 of approximately 22 kb contain eleven exons and ten introns (Yokoyama and Tanabe 1989; Kraemer *et al.* 1992). The PGHS-1 gene

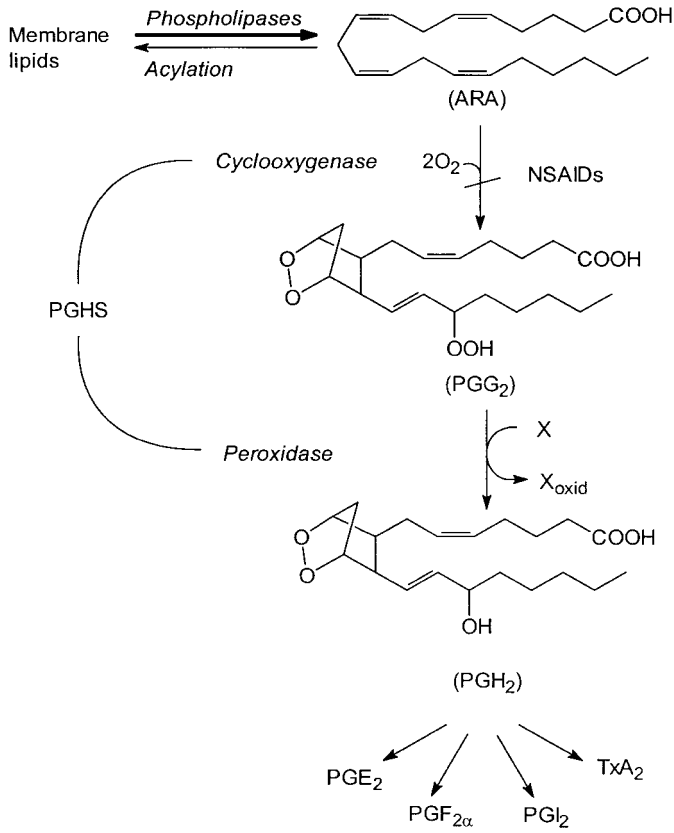

Figure 6.1 Arachidonic acid metabolism by PGHS. PGHS expresses two enzymic activities: its cylooxygenase catalyses bisoxygenation of free fatty acid substrate, and converts arachidonate to PGG_2. The peroxidase reduces this intermediate to PGH_2, a reaction which requires the presence of reducing cosubstrates (X). Depending upon additional enzymes present in a given cell, the PGH_2 product is converted to prostaglandins, prostacyclins or thromboxanes, biologically active compounds collectively termed prostanoids (see text for more details).

has no TATA box (Kraemer *et al*. 1992), consistent with its constitutive expression as a housekeeping gene in most tissues and cells. Two Sp1 elements have been identified in the PGHS-1 promoter region responsible for the basal PGHS-1 gene transcription (Xu *et al*. 1997). PGHS-1 codes for a protein of approximately 70-72 kDa; the mature processed forms of various species all have 576 amino acids and highly conserved functional domains (Smith and DeWitt 1996). The active enzyme contains haem as a prosthetic group. It exists as a head-to-tail homodimer and is attached to lipid bilayers by means of a unique membrane binding domain which was first recognised by analysis of the X-ray crystal structure of ovine PGHS-1 (Picot *et al*. 1994).

The human PGHS-2 gene was mapped to chromosome 1 (Jones *et al*. 1993). PGHS-2 was first identified on the basis of its structural similarity to PGHS-1 (Kujubu *et al*. 1991; Xie *et al*. 1991). Molecular cloning revealed that the PGHS-2 gene contains ten exons and nine introns, and is considerably smaller (approximately 8 kb) than the PGHS-1 gene (Fletcher *et al*. 1992; Xie *et al*. 1991; Kosaka *et al*. 1994; Tazawa *et al*. 1994; Appleby *et al*. 1994). PGHS-2 transcribes an mRNA of about 4.4 kb in length, which is larger than the 2.8 kb transcript for PGHS-1. The PGHS-2 mRNA contains several RNA instability sequences (AUUUA), and is more rapidly degraded than PGHS-1 mRNA (Evett *et al*. 1993; Ristimaki *et al*. 1994). Cytoplasmic proteins of 90 to 35 kDa have been identified which interact with these instability sequences, and are important in the post-transcriptional control of PGHS-2 (Dixon *et al*. 2000). PGHS-2 obtained from chick, mouse, rat or human code for proteins with 587 amino acids in the mature forms. PGHS-2 often migrates as two bands on SDS-PAGE with apparent molecular masses of approximately 74 and 72 kDA, due to different degrees of glycosylation (Smith and DeWitt 1996). Immunoreactive bands of lower molecular weight (approximately 60 kD) have been observed and seem to correspond to degradation products (Kargman *et al*. 1996).

The subcellular localisation and compartmentation of the isozymes appear to be similar. Both PGHS-1 and PGHS-2 are predominantly associated with the endoplasmic reticulum; they are also found on the nuclear envelope (Spencer *et al*. 1998). Cross-species comparisons of PGHS isozymes show a greater than 85% amino acid identity for human, ovine and rodent homologues, while the PGHS-1 or PGHS-2 sequences from the same organism share about 60% identity (Smith and DeWitt 1996; Feng *et al*. 1993). Yet functional domains and key amino acid residues involved in catalysis are noticeably conserved among the isozymes, and the three-dimensional X-ray crystal structures of PGHS-1 and PGHS-2 are practically superimposable (Picot *et al*. 1994; Kurumbail *et al*. 1996; Luong *et al*. 1996). These and biochemical studies, e.g. on enzyme forms with specific mutations in key amino acid residues, have immensely contributed to our present understanding of the inner workings and catalytic functions of PGHS (reviewed by Marnett and Maddipatti 1991; Smith and DeWitt 1996; Garavito and DeWitt 1999).

Enzyme function in the metabolism and bioactivation of xenobiotics

Despite some differences in inhibitor selectivity and substrate preferences as well as pronounced differences in the expression pattern, PGHS-1 and PGHS-2 are essentially identical in structure and catalytic function. Unless otherwise specified, we refer to

them as PGHS in this section, since both isozymes possess the same two catalytic activities. Upon cyclooxygenase catalysed bisoxygenation of arachidonic acid to PGG_2, the endoperoxide-hydroperoxide is reduced to the corresponding alcohol (PGH_2) by the *peroxidase* activity. As depicted in Figure 6.1, this occurs at the expense of an oxidisable cofactor/co-substrate (X). Since the first report on this reaction termed 'co-oxygenation' or 'co-oxidation' (Marnett *et al*. 1975), a large number and variety of chemicals were found to be oxidised by PGHS. In many cases this involves formation of reactive intermediates and/or products with mutagenic activity.

Prior to discussing examples of PGHS-mediated xenobiotic oxidations and bioactivation mechanisms the following points are of interest with respect to the relation of cyclooxygenase to peroxidase:

- Prostanoid biosynthesis is efficiently inhibited at the level of the cyclooxygenase by non-steroidal antiinflammatory drugs (NSAIDs). Well-known examples are aspirin, indomethacin, ibuprofen, diclofenac and many others. These NSAIDs inhibit the cyclooxygenase, but they do not affect the peroxidase activity. Aspirin is the only NSAID that covalently binds to PGHS; it exerts subtly different effects on the isozymes: Acetylation of PGHS-1 blocks the cyclooxygenase (not the peroxidase) activity whereas acetylation of PGHS-2 converts it to a form that still oxygenates arachidonic acid, but at C-15 instead of C-11 (Lecomte *et al*. 1994; O'Neill *et al*. 1994).
- The substrate fatty acid requirements of the cyclooxygenase are very specific, but the peroxidase activity can use a broad range of substrates other than PGG_2 (see below). Thus, PGHS can oxidise chemicals in the presence of organic hydroperoxides or H_2O_2 instead of arachidonate (ARA). Whereas cyclooxygenase inhibition prevents ARA-dependent co-oxidation of xenobiotics *in vitro*, NSAIDs will not necessarily block their PGHS-mediated metabolism in cells which generate lipid peroxides e.g. HPETES (monohydroperoxy polyunsaturated fatty acids) or hydrogen peroxide (Marnett and Maddipatti 1991; Degen 1993a).
- Hydroperoxides have an essential role to play in the oxygenation of the free fatty acid substrate, arachidonic acid, and are considered to be obligatory initiators of the cyclooxygenase reaction (Lu *et al*. 1999). Reducing co-substrates are important for enzyme activity, since they delay considerably the self-inactivation of PGHS. Thus, with purified PGHS, the number of turnovers can be as high as 1300 and as low as 10 depending on the concentration of reductants and other factors (Markey *et al*. 1987; Marshall *et al*. 1987).

MECHANISMS OF XENOBIOTIC OXIDATION BY PGHS

PGHS-dependent bioactivation of xenobiotics can occur by different mechanisms. The peroxidase can directly oxidise chemicals which act as reducing co-substrates for the enzyme. Another mechanism involves secondary oxidant species formed by peroxidase-generated cosubstrate radicals and molecular oxygen. Moreover, peroxyl radicals generated as intermediates during PG-biosynthesis can act as potent oxidant species. Thus, a number of diverse reactions are involved in the oxidation of

Table 6.1 Examples: prostaglandin H synthase and xenobiotic oxidations

Reaction type	Chemical	References
Dehydrogenation	Benzidine	Zenser *et al.* (1983a)
	Diethylstilboestrol	Degen *et al.* (1982)
	Phenidone	Marnett *et al.* (1982)
Demethylation	Aminopyrine	Lasker *et al.* (1981)
	N-Methylaniline	Sivarajah *et al.* (1982)
C-Hydroxylation	Phenylbutazone	Siedlik and Marnett (1984); Hughes *et al.* (1988)
	Oestradiol	Freyberger and Degen (1989a)
N-Oxidation	IQ*	Morrison *et al.* (1993); Wolz *et al.* (1995)
	N-Acetylbenzidine	Zenser *et al.* (1999)
Sulfoxidation	Sulindac sulphide	Egan *et al.* (1980)
Dioxygenation	Diphenylisobenzofuran	Marnett *et al.* (1979a)
Epoxidation	B[*a*]P-7,8-diol	Marnett *et al.* (1979b); Sivarajah *et al.* (1979)
	Aflatoxin B_1	Battista and Marnett (1985)

* 2-amino-3-methylimidazo[4,5-*f*]quinoline; benzo[a]pyrene-7,8-dihydrodiol
O- and *S*-Dealkylations *not* observed so far, neither dealkylation of nitrosamines.

xenobiotics by PGHS (see Table 6.1; and reviews by Eling *et al.* 1990; Marnett 1990; Smith *et al.* 1991; Zenser and Davis 1990).

PGHS are unique among other peroxidase species because they are able to biosynthesise the hydroperoxide substrate for the peroxidase activity. On the other hand, PGHS-peroxidase resembles a classical peroxidase, with its haem moiety being converted to a higher oxidation state analogous to compound I of other peroxidases (Marnett and Maddipati 1991). Subsequent reduction of enzyme to its resting state is coupled to oxidation of electron donors (reducing co-substrates) or direct oxygen

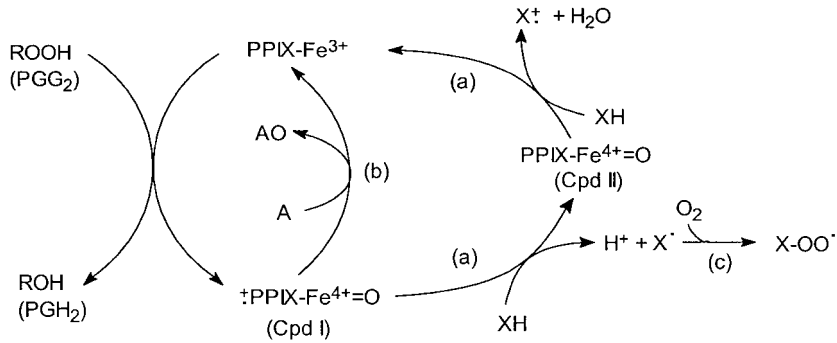

Figure 6.2 Peroxidase catalytic cycle and postulated mechanisms of xenobiotic co-oxidation. The two-electron reduction of hydroperoxide(PGG_2 or ROOH) by PGHS yields compound I. It must be reduced to regenerate the resting enzyme, a task accomplished by an as yet unknown endogenous reducing cofactor and/or by xenobiotics which undergo cooxidation. (see text for more details). The two major mechanism which govern cooxidation involve (a) single electron transfers to the oxidised PGHS intermediates, or (b) direct oxygen transfer from the oxygenated haem to a suitable acceptor compound. Certain radical intermediates may also bind oxygen (c) and thus give rise to secondary oxidant species. Moreover, peroxyl radicals are generated during PG-biosynthesis and act as oxidising agents (not depicted here).

transfer to a suitable acceptor compound (see Figure 6.2). Native PGHS with ferric protoporphyrin IX in the Fe(III) state (Fe^{3+} – PPIX) is oxidised to compound I upon reaction with hydroperoxide (PGG_2 or ROOH). Compound I contains a hypervalent iron-oxo form ($Fe^{4+} = O$ and porphyrin oxidised to a cation radical), and is transformed by one-electron reduction to another intermediate, compound II (in which iron is Fe^{4+} and the porphyrin fully covalent). Compound II is reduced at the expense of another electron donor molecule (XH) to resting enzyme. For more details on spectral intermediates and the catalytic cycle see also recent papers (Koshkin and Dunford 1999; Wu *et al*. 1999).

Chemicals known to act as **reducing co-substrates** for PGHS-peroxidase include several naturally occurring compounds, but also various drugs and carcinogens, e.g. benzidine, 2-aminofluorene, some nitrofurans, paracetamol (acetaminophen), diethylstilboestrol, and the benzene metabolite hydroquinone. By serving as electron donors, compounds are oxidised to free radicals and/or other reactive intermediates (see Figures 6.3–6.8). Thus, direct oxidation of xenobiotics by PGHS-peroxidase represents a potentially important mechanisms of bioactivation.

The common trait with these chemicals is that during conversion of PGG_2 (or reduction of other hydroperoxides) to the corresponding alcohol, the peroxide oxygen is reduced to water rather than being incorporated into the xenobiotic compounds. The overall reaction can be summarised as follows:

$$ROOH + 2\ XH \xrightarrow{\text{Peroxidase}} ROH + 2X' + H_2O \qquad (6.1)$$

With regard to the stoichiometry this means that for each molecule of hydroperoxide that is reduced (a two-electron process), two molecules of xenobiotic (X) will be oxidised (by a one-electron process) to electron-deficient derivatives. Yet, with compounds capable of donating another electron (e.g. adrenalin, diethylstilboestrol) the ratio for molecule co-substrate oxidised/hydroperoxide reduced is close to 1 in incubations with purified PGHS enzyme (Freyberger and Degen 1989b; Marnett and Eling 1983). In cells, however, where endogenous cosubstrates compete for cooxidation, the stoichiometry differs (Degen 1993b).

In some cases, PGHS-peroxidase can also catalyse peroxide reduction by direct transfer of the peroxide oxygen to an acceptor molecule (A). This is evident when ^{18}O-labelled peroxide is used in the reaction.

$$R^{18}O^{18}OH + A \xrightarrow{\text{Peroxidase}} RO^{18}H + AO^{18} \qquad (6.2)$$

Such a peroxygenase reaction (depicted in Figure 6.2, reaction (b) has been reported for sulindac and methyl phenyl sulphide which are oxidised to sulphoxides, with a stoichiometry of one molecule xenobiotic being oxidised per molecule of hydroperoxide reduced (Egan *et al*. 1980, 1981). Recently, a peroxygenase-type reaction was also reported by Zenser and collegues with *N*-acetylbenzidine (see below).

By looking in more detail into the PGHS-dependent oxidation of some chemicals, it becomes apparent that quite a range of metabolites and reactive intermediates is generated by various mechanisms. By donating one electron, *reducing cosubstrates* are initially oxidised to either oxygen-, nitrogen-, or carbon-centred radicals.

The first intermediates in the oxidation of **phenols** are phenoxyl radicals. Examples are diethylstilboestrol (DES) and paracetamol (acetaminophen). These cosubstrates can undergo another one-electron oxidation and thus form two-electron oxidation products, i.e. a quinone and quinone-imine, respectively (see Figures 6.3 and 6.4). Metabolism of DES by PGHS (Degen *et al*. 1982) resembles its oxidation by horseradish peroxidase (HRP), but, this cannot be generalised to all substrates (Table 6.2). DES quinone tautomerises to the stable metabolite Z,Z-dienoestrol; but, reactive DES-intermediates (Figure 3) also bind covalently to proteins (Degen *et al*. 1986; Degen 1990). PGHS-peroxidation results in bioactivation of DES and of steroid oestrogens (Freyberger and Degen 1989a), and this is thought to play a role in their genotoxic effects (see below).

The one-electron oxidation product *N*-acetyl-*p*-benzo-semiquinone imine (Figure 6.4) may be further oxidised to *N*-acetyl-*p*-benzoquinone imine (NAPQI) or dimerise; it can also undergo disproportionation, forming paracetamol and NAPQI. An

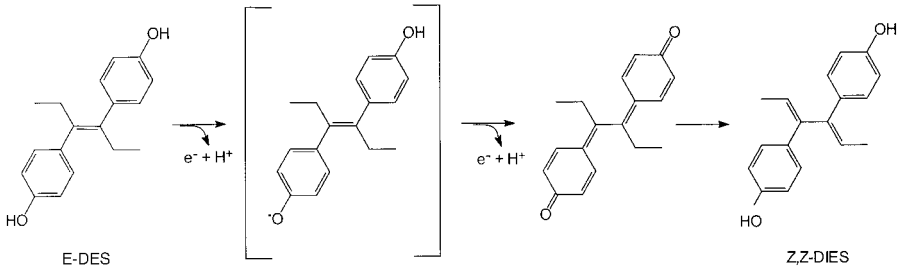

Figure 6.3 Peroxidative metabolism of diethylstilboestrol (E-DES) generates reactive intermediates and the stable product dienestrol (Z,Z-DIES).

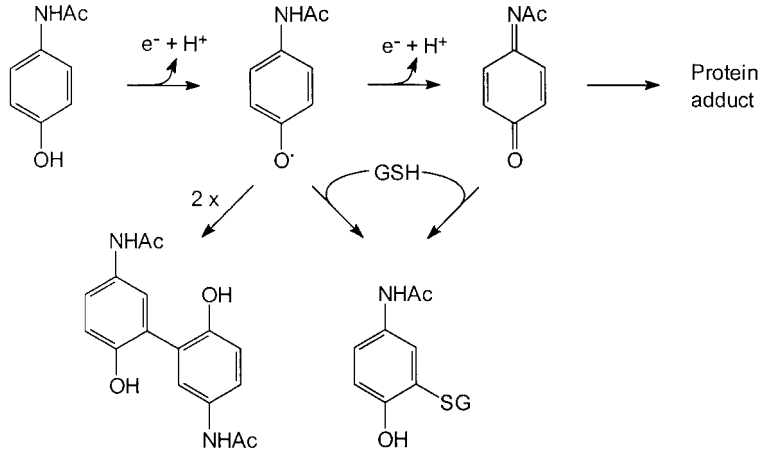

Figure 6.4 PGHS-catalysed oxidation of paracetamol.

interruption after the first step is evidenced by a formation of dimers and other oligomers (Moldeus *et al.* 1985). The latter was decreased by glutathione (GSH) with subsequent formation of the glutathione conjugate in incubations with PGHS or HRP. HRP was proposed to catalyse the one-electron oxidation of this substrate whereas PGHS can catalyse both one- and two-electron oxidations of paracetamol (Potter and Hinson 1987a,b). In contrast, cytochrome P450 metabolism occurs by two-electron oxidation; it did not produce oligomers but NAPQI, and in the presence of GSH resulted only in the formation of the conjugate, 3-(glutathione-*S*-yl)-paracetamol (Potter and Hinson 1987b). NAPQI is known to bind to proteins and held responsible for the observed toxicity of high doses of analgesics like paracetamol and phenacetin in liver and kidney.

Aromatic amines are another important class of compounds that are oxidised by PGHS. Reported products of benzidine (Figure 6.5) are the free radical cation, the two-electron oxidised benzidinediimine, a charge-transfer complex between imine and parent amine, a dimer (azo-bendizine) and oligomers (Zenser *et al.* 1983a). An *N*-hydroxy product was not found with benzidine, and not detected in similarly detailed studies of peroxidatic metabolism of 2-aminofluorene (Figure 6.5) (reviewed by Eling *et al.* 1990; Zenser and Davis 1990). On the other hand, formation of an *N*-

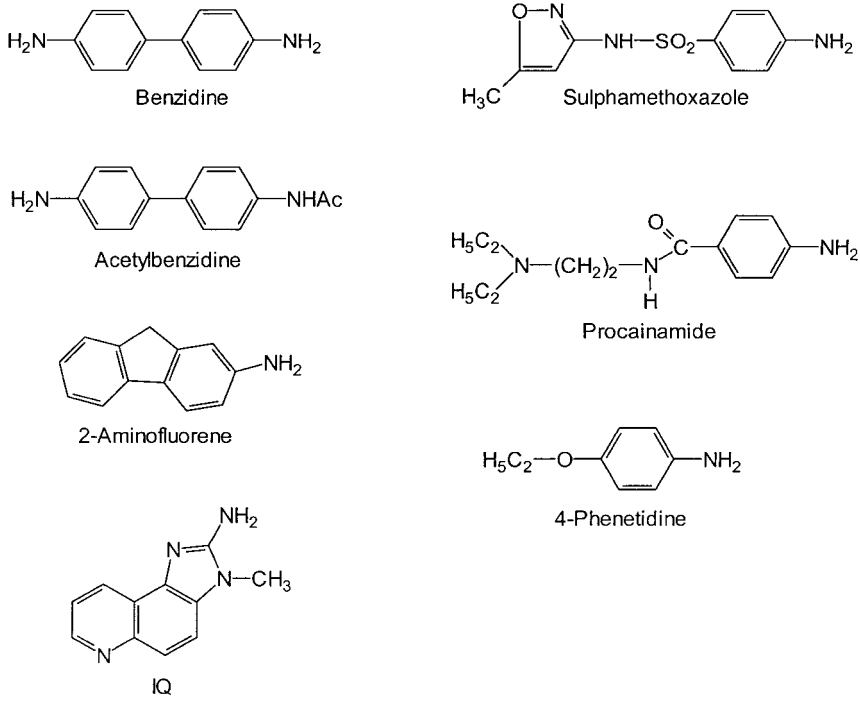

Figure 6.5 Aromatic amines (carcinogens and drugs) metabolised by PGHS. IQ = 2-amino-3-methylimidazo[4,5-*f*]quinoline.

hydroxylamine by PGHS was recently demonstrated with *N*-acetylbenzidine (ABZ; Zenser *et al*. 1999). The PGHS-metabolite *N*′-hydroxy-*N*-acetylbenzidine (N′-HA) is only observed under reducing conditions; it is converted to 4′-nitro-4-aminobiphenyl by PGHS. The results of mechanistic studies suggest a peroxygenase–type reaction rather than one-electron transfer reactions in the stepwise oxidation of ABZ (Figure 6.5) to *N*-oxidation products (Zenser *et al*. 1999). In support of this view, the PGHS metabolite N′-HA was not detected in incubations with HRP (Lakshmi *et al*. 2000). Oxidation of ABZ by PGHS generates DNA adducts (Lakshmi *et al*. 1998). The enzyme is thought to catalyse the bioactivation of this benzidine metabolite in the carcinogen's target tissue, the bladder epithelium which contains rather high PGHS-activity (see below).

The carcinogenic arylamine 2-aminofluorene (Figure 6.5) is metabolised by PGHS probably via free radicals to adduct-forming intermediates, some polymeric species, and to azo-*bis*-fluorene (Boyd and Eling 1984; Krauss and Eling 1985). Formation of nitrofluorene is indicative of its *N*-oxygenation. In addition, PGHS activates 2-AF and other arylamines to mutagens (for a review see Eling *et al*. 1990). Another interesting example is the food-borne heterocyclic amine 2-amino-3-methyl-imidazo[4,5-f]quinoline (IQ; Figure 6.5). Metabolism of IQ by PGHS yields azo-IQ and nitro-IQ as stable metabolites (Wolz *et al*. 1995). PGHS-catalysed oxidation of IQ and related compounds results in their bioactivation to mutagenic species (Wild and Degen 1987; Petry *et al*. 1989). PGHS-mediated metabolism of IQ also yields DNA-adducts (see below).

There are also various arylamines among drugs. Examples of compounds shown to be *N*-oxygenated by PGHS on their primary amine function are sulphamethoxazole and procainamide (Figure 6.5). The metabolites formed are the hydroxylamine and nitro-derivatives. Since PGHS-mediated formation of drug-modified proteins can occur in phagocytic and antigen-presenting cells, this pathway is considered to be of toxicological importance for adverse reactions to these xenobiotics (Cribb et al, 1990; Goebel *et al*. 1999).

Many studies provide evidence for a one-electron oxidation of aromatic amines by PGHS peroxidase to *N*-centred radicals. The fate of such radicals can differ. For instance, PGHS-dependent oxidation of *p*-phenetidine (Figure 6.5) resulted in the formation of several intensely coloured products due to radical coupling (Moldeus *et al*. 1985). In incubations with radiolabelled compound, also binding to protein and DNA, was observed which may be due to radicals and/or quinoid metabolites formed upon dimerisation of the intermediates.

The tertiary amine aminopyrine (AP) is comparable in its basicity to aromatic amines. AP is oxidised by PGHS to a rather stable radical cation detectable by ESR and UV/VIS spectroscopy (Eling *et al*. 1985; Lasker *et al*. 1981). The radical cation can disproportionate to an iminium cation and its parent compound AP (Figure 6.6). The iminium cation (a two-electron oxidation product) is hydrolysed to the demethylated amine and formaldehyde. Also a number of secondary and tertiary *N*-methylated aromatic amines are *N*-demethylated by PGHS (Sivarajah *et al*. 1982). The proposed mechanism of *N*-demethylation is in accord with the inability of PGHS to catalyse *O*- and *S*-dealkylations, reactions which occur by C(α)-hydroxylation rather than heteroatom oxidation.

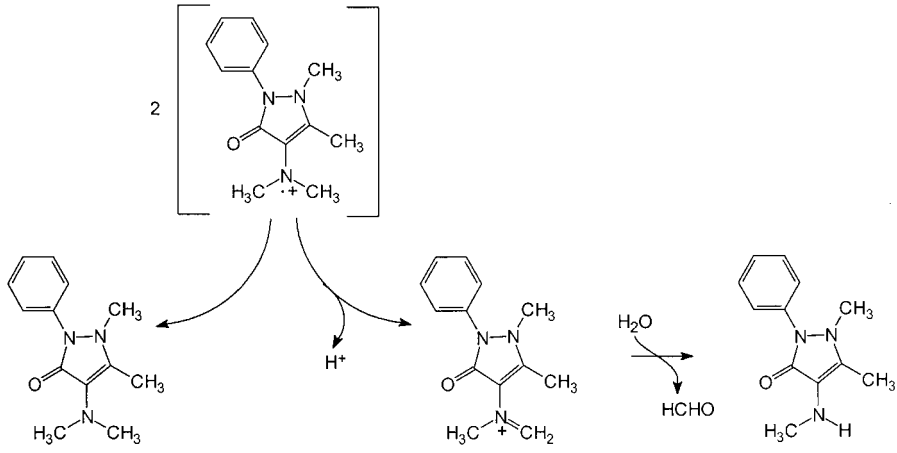

Figure 6.6 PGHS-catalysed oxidation of aminopyrine.

The next examples illustrate reactions of **carbon oxidation** by PGHS. In the case of phenylbutazone (Figure 6.7) and 13-*cis*-retinoic acid, one-electron oxidation by PGHS-peroxidase initially generates the C-centred radicals (Hughes *et al.* 1988; Samokyszyn and Marnett 1987). One molecule of oxygen is added thereby producing peroxyl radicals that undergo secondary reactions. Peroxyl radicals are potent oxidants capable of hydrogen abstraction or epoxidation. Accordingly, 4-hydroperoxy- and 4-hydroxyphenylbutazone as well as 4-hydroxyretinoic acid were isolated as metabolites (Hughes *et al.* 1988; Samokyszyn and Marnett 1987). Moreover, the retinoic acid peroxyl radical adds to the 5,6-double bond of another retinoic acid molecule to form the 5,6-epoxy product (Marnett 1990). Phenylbutazone and retinoic acid exemplify a mechanism of PGHS-mediated drug metabolism depicted in Figure 6.2 as reaction (c). It is based on the PGHS-peroxidase catalysed one-electron oxidation of certain chemicals to C-centred radicals that couple with oxygen to form peroxyl radicals. These secondary oxidants can bioactivate other compounds, e.g. benzo[a]pyrene-7,8-diol (Reed *et al.* 1984). This offers also an explanation for oxidation reactions usually not observed with other peroxidases and a more efficient oxidation of chemicals by PGHS than expected for a stoichiometry of 1:1 or 1:2 for hydroperoxide reduction to xenobiotic oxidation.

Another, although similar, mechanism is probably involved in the PGHS-mediated

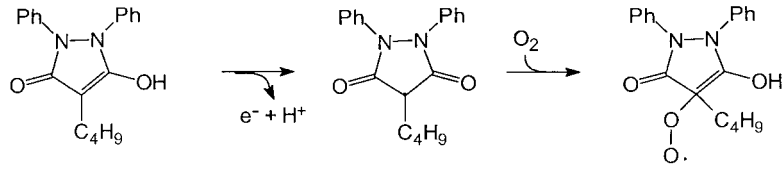

Figure 6.7 PGHS-catalysed oxidation of phenylbutazone.

aryl hydroxylation found with steroid oestrogens (Degen *et al.* 1987). One-electron oxidation of oestradiol (Figure 6.8) produces a phenoxyl radical; its resonance form, a C-centred radical, can trap oxygen thereby producing a peroxyl radical which abstracts hydrogen to form an *ortho*-hydroperoxide. The hydroperoxide is reduced (non-enzymically or by PGHS) to the corresponding alcohol which tautomerises to the catechol product, a process driven by re-establishment of aromaticity. Catechol oestrogens are also cooxidised by PGHS-peroxidase to reactive intermediates, i.e. semiquinone and *ortho*-quinone metabolites that bind to proteins (Freyberger and Degen 1989a).

In essence, chemicals that are reducing co-substrates of PGHS-peroxidase are oxidised by two major mechanisms which involve either single electron transfers to the oxidised enzyme intermediate or direct oxygen transfer from the oxygenated haem to a suitable acceptor compound (Figure 6.2, reactions (a) and (b)). Yet oxygen incorporated into some xenobiotics is not peroxide-derived, but, apparently comes from molecular oxygen (Figure 6.2, reaction (c)). Stepwise one-electron oxidation of chemicals and the formation of xenobiotic peroxyl radicals are illustrated above.

Additional mechanisms exist, since certain compounds are oxidised which are **no** or very poor reducing co-substrates of PGHS-peroxidase. One mechanism of PGHS-dependent bioactivation is indirectly linked to the cyclooxygenase activity of the

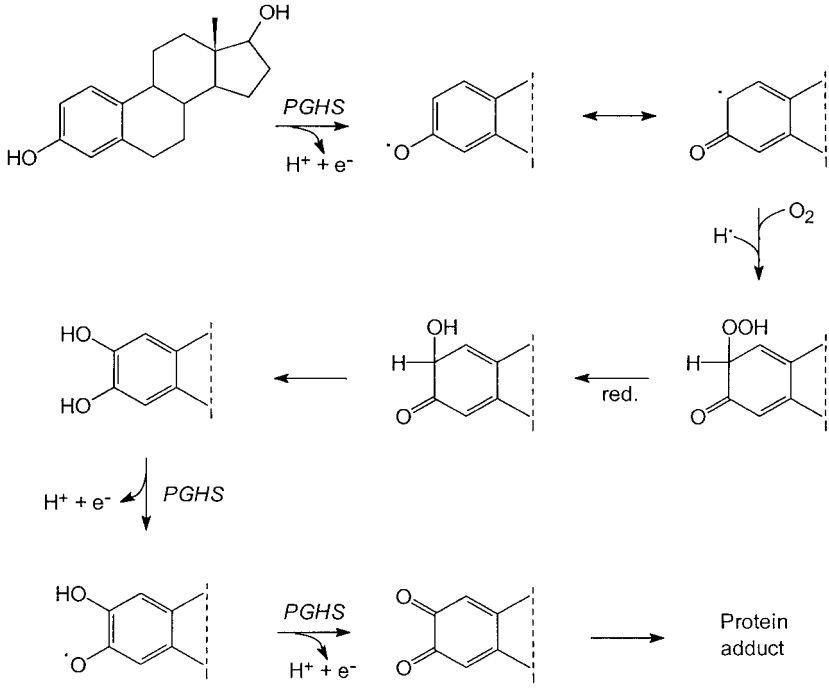

Figure 6.8 PGHS-catalysed oxidation of oestradiol and catecholoestrogens.

enzyme. During conversion of arachidonate to PGG_2, fatty acid peroxyl radicals are formed as intermediates. Similar lipid peroxyl radicals are formed during metabolism of unsaturated fatty acids by lipoxygenases. Peroxyl radicals are potent oxidising agents and rather selective in their reactions (Marnett 1990). They are capable of bioactivating some procarcinogens or promutagens by epoxidation (references in Table 6.1). For example, the carcinogenic mycotoxin aflatoxin B_1, the polycyclic hydrocarbon benzo[a|pyrene (BP), and its metabolite BP-7,8-dihydrodiol (BP-7, 8-diol) are epoxidised by PGHS; but, the stoichiometry is only 0.1:1 for xenobiotic oxidation to hydroperoxide (PGG_2) reduction (Marnett and Eling 1983). Although these reactions are rather sensitive to antioxidant inhibition, peroxyl-radical-dependent epoxidation of BP-7,8-diol has been demonstrated in mouse skin. This was detected by means of a stereochemical approach that can distinguish between peroxyl-radical-dependent and monooxygenase-mediated epoxidation of the (+)-enantiomer of BP-7,8-diol. The findings suggested that peroxyl radicals are important contributors to BP-7,8-diol oxidation in the skin of normal mice, whereas cytochrome P-450 has primacy in β-naphthoflavone-pretreated animals (Marnett 1990; Pruess-Schwartz *et al.* 1989). These and other studies (see below) indicate that PGHS can serve as a complementary/alternative metabolic activation enzyme to the cytochrome P-450 enzymes. Although it is unlikely that PGHS plays a major role in systemic drug metabolism, it is thought to be an important determinant of chemical toxicity in extrahepatic tissues (Eling *et al.* 1990).

As described here and in previous reviews on this topic (Eling *et al.* 1990; Eling and Curtis 1992; Marnett 1990; Smith *et al.* 1991; Zenser and Davis 1990) in many cases oxidation of xenobiotics by PGHS results in the formation of reactive intermediates and/or generates products with mutagenic activity. Ram seminal vesicles microsomes (RSVM) lack monooxygenase activity, but contain the highest PGHS activity of all tissues, and are a good source of enzyme for experimental studies. For example, in bacterial mutagenicity assays, arachidonate supplemented RSVM can serve as bioactivating system instead of NADPH-fortified liver microsomes (or 'S-9 mix'). RSVM thus have a function similar to liver preparations used to study cytochrome P450-dependent drug oxidations.

Whereas ram seminal vesicles express only PGHS-1, many tissues contain both PGHS-1 and PGHS-2. Moreover, PGHS-2 expression is inducible by several endogenous factors and certain xenobiotics. The role of PGHS isozymes with regard to xenobiotic oxidation is the same. This is exemplified by studies with purified PGHS-2 and transiently PGHS-2 cDNA transfected COS cells; bioactivation of 2-aminofluorene was demonstrated by formation of DNA adducts and its inhibition by aspirin and indomethacin (Liu *et al.* 1995) in accord with previous data for 2-AF oxidation by PGHS-1 (RSVM; see above). In studies with the heterocyclic amine IQ (Figure 6.5) and lysates from cells expressing PGHS-1 or PGHS-2 both catalysed IQ-DNA adduct formation (Liu and Levy 1998). As seen with CYP1A2-dependent oxidation, PGHS-isozyme-dependent IQ adduct formation was enhanced by *N*-acetyltransferases. This observation points towards a basic similarity in IQ-activation by the two oxidising enzymes. Similarities seen in studies on the mutagenic activation of IQ by either PGHS or cytochrome P-450 support this view (Wild and Degen 1987), and apparently the same IQ-DNA adducts are formed (Wolz *et al.* 2000).

XENOBIOTIC OXIDATION BY PGHS: BIOLOGICAL IMPLICATIONS

PGHS-dependent oxidation of xenobiotics involves multiple mechanisms and can generate a range of reactive intermediates (free radical species, peroxyl radicals, quinones, quinoneimines, epoxides and others). Unless they are detoxified, such metabolites bind to macromolecular nucleophiles and damage critical cellular components (DNA, certain proteins). Thus, and because of its tissue distribution (see below), PGHS is considered as bioactivating enzyme for xenobiotics, in particular in tissues with low cytochrome P-450 activity. The following studies are indicative of such a role for PGHS in various tissue-specific pathologies of certain carcinogens and drugs.

Zenser and colleagues demonstrated PGHS-mediated bioactivation of acetylbenzidine (AZB) and formation of a DNA adduct; *N*′-(3′-monophospho′-deoxyguanosin-8-yl)-*N*-acetylbenzidine (Lakshmi *et al*. 1998). It is thought to play an important role in the initiation of benzidine-induced bladder cancer in humans, since this adduct has been detected in exfoliated bladder cells of benzidine-exposed workers who excrete AZB in their urine (Rothman *et al*. 1996). It was also detected by postlabelling analysis in *N*-acetylating and PGHS-competent porcine urothelial cell cultures upon exposure to benzidine or ABZ (Degen *et al*. 1998b). This observation supports a role for PGHS in the local bioactivation of this carcinogen.

IQ is a multisite carcinogen in animals and along with related food mutagens considered as risk factor for colon cancer in humans. Bioactivation of IQ by PGHS generates products mutagenic in acetyltransferase-proficient *S. typhimurium* strains, and both IQ and nitro-IQ are genotoxic in ovine seminal vesicle (OSV) cells which express PGHS and *N*-acetyltransferase (NAT), but lack cytochrome P-450 activity (Degen *et al*. 1998a). The IQ-derived DNA-adducts in bacterial and mammalian cells were indistinguishable from those observed with P450/NAT-activation (Wolz *et al*. 2000). Therefore, it is difficult to assess the contribution of PGHS-activation *in vivo*, e.g. in target sites for IQ carcinogenicity such as colon. For another carcinogenic amine, 2-aminofluorene (2-AF), peroxidase-catalysed formation of two extra adducts was described which differed from N-(deoxyguanosin-8-yl)-2-AF formed by the reaction of N-hydroxy-2-AF with DNA (Krauss and Eling 1985). These uncharacterised adducts were detected in the kidney and bladder epithelia of dogs fed radiolabelled 2-AF (Krauss *et al*. 1989); their biological importance for amine-induced canine bladder cancer is unknown.

Not only DNA adduct formation but also covalent binding to certain proteins may be a critical reaction for toxicity of xenobiotics. This appears to be the case for paracetamol and related analgesics (see above). Moreover, peroxidase activation of DES yields reactive intermediates which bind preferentially to tubulin and other spindle proteins (Degen 1990), a reaction which could explain its genotoxic (aneuploidogenic) effects. Induction of micronuclei by DES in PGHS-competent cells was inhibited by indomethacin along with a decrease in DES oxidation (Foth *et al*. 1992). Since PGHS is also present in target cells for neoplastic transformation by synthetic and by steroid oestrogens, cooxidation seems to play a role in their adverse effects (Degen *et al*. 1983; Schnitzler *et al*. 1994).

PGHS is also implicated in the toxicitys of sulphonamides and procainamide (see

above). PGHS-mediated bioactivation can occur in phagocytic and antigen-presenting cells, and yields drug-modified proteins that are involved in idiosyncratic adverse reactions to these xenobiotics (Cribb *et al*. 1990; Goebel *et al*. 1999). PGHS is further implicated in the teratogenicity of hydantoin and oxazolidenedione anticonvulsants, i.e. phenytoin and dimethadione (Kubow 1992; Wells *et al*. 1989a,b), and teratogenic effects of such structurally diverse chemicals as benzo[a]pyrene (Winn and Wells 1997), isotretinoin (Kubow 1992) and thalidomide (Arlen and Wells 1996). These and additional studies with these compounds (Figure 6.9) revealed PGHS-catalysed formation of reactive intermediates and free radical-mediated oxidative damage to cellular macromolecules as well as evidence for protection against the chemical-induced teratogenicity by aspirin and other PGHS-inhibitors (Kubow and Wells 1989; Parman *et al*. 1998, 1999; Winn and Wells 1997).

As pointed out already, PGHS is primarily thought to catalyse xenobiotic metabolism in extrahepatic tissues and cells with low cytochrome P450 activity. The *in vivo* contribution of PGHS is not readily apparent when peroxidase and peroxyl radical-mediated bioactivation of chemicals finally result in the same products as those generated by cytochrome P450-dependent oxidation. This similarity is seen for DNA adducts of IQ and of acetylbenzidine formed by either activating system (see above). Thus, studies often rely on specific enzyme inhibitors; an example is 3-methylindole (3-MI), a fermentation product of tryptophan, known to cause lung toxicity in several species. 3-MI is oxidised by both cytochrome P450 enzymes or PGHS to reactive intermediates that yield glutathione conjugates and covalent protein binding; the modulation of these was inhibited in Clara cells and alveolar macrophages by the cytochrome P450 inhibitor 1-aminotriazole by 94% and by 24% respectively (Thornton-Manning *et al*. 1993). Bray and colleagues used aspirin and indomethacin to investigate the role of PGHS in the 3-MI-induced pneumotoxicity in goats, and observed a clear protective effect when these compounds were administered prior to 3-MI (Acton *et al*. 1992). The above examples indicate that PGHS participates in the

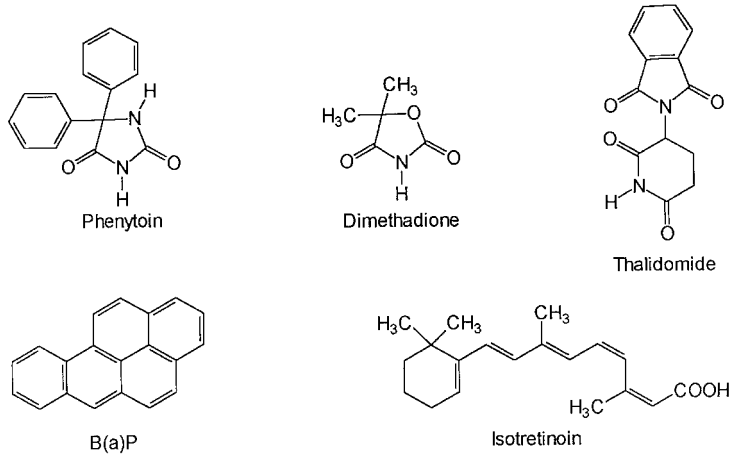

Figure 6.9 Some teratogenic compounds which are oxidised by PGHS. B(a)P, Benzo[a]pyrene.

bioactivation of xenobiotics. PGHS has been also suggested to mediate detoxification reactions, e.g. of styrene (Eling *et al*. 1990; Eling and Curtis 1992).

In summary, PGHS catalyses the bioactivation of a large and diverse number of chemicals, and there is now ample evidence that such reactions contribute to the toxicity of xenobiotics. Another aspect is that of xenobiotics affecting PGHS-activity. Drugs which inhibit cyclooxygenase and agents which induce PGHS, predominantly PGHS-2, by modulating the synthesis of biologically active prostanoids, may contribute to tissue-specific pathologies (see below).

Substrate specificity

PGHS is bifunctional, containing both cyclooxygenase and peroxidase activities. In a strict biochemical sense the term *substrate* refers to fatty acids and hydroperoxides, whereas xenobiotics metabolised by the enzyme are considered as *co-substrates* (or cofactors). Thus, with respect to drug metabolism, the terminology for PGHS-isozymes and, for example, cytochrome P450 enzymes differs.

Whereas PGHS-peroxidases can use various peroxides and a broad range of xenobiotics as co-substrates, the substrate requirements of the cyclooxygenases are very specific. Best substrates for the cyclooxygenase activities of PGHS-1 and PGHS-2 are arachidonate and dihomo-γ-linolenate, i.e. 20-carbon ω-6 polyunsaturated fatty acids. The apparent K_m values with arachidonic acid are about $\sim$5 μM for both isozymes (Meade *et al*. 1993a; Barnett *et al*. 1994; Laneuville *et al*. 1994, 1995). Kinetic studies with purified PGHS-1 show a sigmoidal dependence on arachidonate concentration, whereas PGHS-2 displays simple saturable behaviour (Swinney *et al*. 1997). In addition, PGHS/COX-2 seems to accept a wider range of fatty acids than PGHS/COX-1; in general, 18-carbon polyunsaturated fatty acids are more efficiently oxygenated by PGHS-2 than by PGHS-1. Other ω-3 polyunsaturated fatty acids from fish oil, e.g. docosahexaenoic acid (22:6, w-3) and eicosapentanoic acid (20:5, w-3), are competitive inhibitors of both isozymes without being a substrate (for a review see Smith and DeWitt 1996). Moreover, there are differences in peroxide requirements, with hydroperoxide concentrations needed for activating the cyclooxygenase of PGHS-2 being lower than those needed to activate PGHS-1, which may play a role in their differential cellular control (Capdevila *et al*. 1995; Kulmacz and Wang 1995; Kulmacz 1998). Despite biochemical differences in peroxide requirements and fatty acid substrate specificities, PGHS-isoenzymes are virtually the same in the context of catalytic mechanisms.

Studies with ovine PGHS-1 showed that its peroxidase preferentially reduces fatty acid hydroperoxides, including those generated by lipoxygenases (e.g. 15-HPETE, 12-HPETE). The enzyme is less active with hydrogen peroxide, and almost inactive with tertiary hydroperoxides (Marnett and Maddipatti 1991). The substrate specificity of PGHS-2 peroxidase has not been examined in great detail, but, in various assays, it exhibits an activity that is quantitatively similar to that of PGHS-1 peroxidase (Fletcher et al 1992; Meade et al 1993b; Barnett et al 1994; Laneuville et al 1994).

Since the peroxidase activity of PGHS can function independently of the cyclooxygenase activity, there are convenient assays for measuring the enzyme activity. A typical spectral peroxidase assay employs H_2O_2 and the phenolic compound guaiacol

as oxidising and reducing co-substrates, respectively. Marnett's laboratory has evaluated a large number of reducing co-substrates by quantitating their ability to support the reduction of the stable primary hydroperoxide PPHP (5-phenyl-4-pentenyl hydroperoxide) by catalytic amounts of the enzyme (Markey *et al*. 1987). In this HPLC-based assay, the yield of 5-phenyl-4-pentenyl-alcohol indicates PPHP-reduction and reflects the efficiency of a given compound to act as reducing co-substrate for PGHS-peroxidase. The identity of the endogenous reducing co-substrate is not known, and it may vary between cells; candidates for this role are adrenaline, lipoic acid, ascorbate and uric acid (Markey *et al*. 1987). Among the best reductants for PGHS-peroxidase are aromatic diamines (benzidine, tetramethylphenylenediamine) and polyhydroxylated chemicals (diethylstilboestrol, hydroquinone). These compounds are also known to be readily oxidised by other mammalian peroxidases and by horseradish peroxidase (HRP). Although PGHS catalyses a number of oxidations similar to those of HRP, there are also differences between the two peroxidases (Marnett and Maddipati 1991). Examples of chemicals that are good substrates for PGHS but not for HRP and vice versa are shown in Table 6.2.

Although metabolism of DES and some structural analogues by PGHS shows many similarities to HRP-mediated oxidation, there are notable differences in the extent to which some compounds are oxidised, for instance hexestrol (Degen and McLachlan 1985; Degen *et al*. 1986). Moreover, there are differences in the metabolites produced by either HRP or PGHS. The carcinogen 2-naphthylamine (2-NA) is co-oxidised by PGHS to ring-oxygenated products (metabolites atypical for a peroxidase-type reaction) that are clearly different from polymeric non-oxygenated metabolites of 2-NA that are generated with HRP. The formation of the ring-oxygenated metabolites probably occurred by peroxyl radical-mediated attack or direct transfer of peroxide oxygen from PGHS to 2-NA (Curtis *et al*. 1995). The heterocyclic arylamine IQ

Table 6.2 Substrate specificity of peroxidases

Compound	PGHS	HRP	References
Methylphenylsulphide	+	–	Egan *et al*. (1981)
Sulindac sulphide	+	–	Egan *et al*. (1981)
ANFT[a]	+	(–/+)[d]	Lakshmi *et al*. (1992)
HMN[b]	+	–	Zenser *et al*. (1983b)
Lipoic acid	+	–	Markey *et al*. (1987)
Indole acetic acid	+	–	Markey *et al*. (1987)
Amitrol	+	–	Krauss and Eling (1987)
Paracetamol	+	+	Potter and Hinson (1987a,b)
Diethylstilboestrol	+	+	Degen *et al*. (1982)
2-Aminofluorene	+	+	Boyd and Eling (1984)
2-Naphthylamine	+	(+)[d]	Curtis *et al*. (1995)
Hexestrol	+	(–/+)[d]	Degen *et al*. (1986)
IQ[c]	+	(–/+)[d]	Wild and Degen (1987)
Aniline	–	+	Markey *et al*. (1987)
Iodide	–	+	Markey *et al*. (1987)

[a] ANFT: 2-amino-4-(5-nitro-2-furyl)thiazole.
[b] HMN: 3-hydroxymethyl-1-{[3-(5-nitro-2-furyl)allylidene]amino}hydantoin.
[c] IQ: 2-amino-3-methylimidazo[4,5-f]quinoline.
[d] Difference in the type or extent (-/+) of metabolism (see text).

(2-amino-3-methylimidazo[4,5-f]quinoline) is another interesting example: PGHS-mediated oxidation resulted in the formation of mutagenic metabolites whereas such activation was not seen with HRP (Wild and Degen 1987). In conclusion, HRP is not always a suitable model enzyme for PGHS-mediated oxidations of xenobiotics.

Tissue distribution

PGHS is widely distributed in animal and human tissues, but is not present in all cells of a given tissue (Smith 1985). Enzyme activity is particularly abundant in the accessory sex glands, the kidney, the lung, gastrointestinal and urogenitalial tract, and in the vasculature. Early studies on prostanoid biosynthesis in animal tissues (Christ and VanDorp 1972) showed the wide distribution of PGHS in many tissues of various species, but could not discriminate between the expression of isozymes. This is now achievable with suitable techniques (Northern Blotting, RT-PCR (Reverse Transcription - Polymerase Chain Reaction), Western-Blotting, Immunohistochemistry) for PGHS-1 and PGHS-2 mRNAs or proteins.

PGHS-1 is constitutively expressed in almost all tissues. Consistent with its role for the maintenance of physiological functions of the stomach, mediating normal platelet function, and regulating blood flow, high quantities of PGHS-1 are present in epithelial cells of the crypts (Cohn *et al*. 1997), platelets and endothelial cells (Habib *et al*. 1993). High levels of PGHS-1 were found in the gastrointestinal tract (Kargman *et al*. 1996), the kidney medulla (Harris *et al*. 1994) and the interstitium (Smith and DeWitt 1995; Seibert *et al*. 1994). A high constitutive expression of PGHS-1 was found in foetal organs (heart, kidney, lung, brain), and also in the decidual lining of the uterus (Gibb and Sun 1996; Sawdy *et al*. 1997).

PGHS-2 is normally not detectable by immunohistochemistry in most tissues. A basal expression has been observed in the hippocampus, the cingulate cortex of the brain, in trachea and renal cortex (Yamagata *et al*. 1993; Walenga *et al*. 1996; Harris *et al*. 1994). Sheep placenta is a rich source of PGHS-2 (Johnson *et al*. 1995), and PGHS-2 expression has been reported in human amnion epithelial cells and chorion laeve trophoblasts (Gibb and Sun 1996). The expression of PGHS isoenzymes was also examined in maternal uterine tissues of pregnant sheep (Gibb *et al*. 2000). PGHS-1 or PGHS-2 mRNA were not detectable in the myometrium; in endometrial epithelium only PGHS-2 mRNA was expressed, consistent with PGHS-2 protein localisation. The increased expression of PGHS-2 rather than PGHS-1 is thought to be important for the onset of parturition.

The mRNA expression pattern of PGHS-1 and PGHS-2 in human tissue samples was investigated using the sensitive RT-PCR technique. High mRNA levels of PGHS-1 and PGHS-2 were detected in the human prostate and in human lung. The mRNA levels of both isoforms were low in testis, pancreas, kidney, thymus and liver (O'Neill and Ford-Hutchinson 1993). A similar mRNA expression pattern was found in animals; the highest PGHS-1 and particularly PGHS-2 expression was observed in lung, low PGHS-1 and PGHS-2 mRNA levels in thymus, and no PGHS-2 mRNA expression was detected in the liver of rats (Vogel C, Boerboom AM, Degen GH and Abel J, unpublished observations) and mice (Vogel *et al*. 1997). In human liver parenchymal cells, PGHS-2 was detected at higher levels than PGHS-1 (Koga *et al*. 1999). However,

since the tissue samples were from patients with hepatocellular carcinoma, this finding is not necessarily indicative of a species difference.

Liver may serve as an example of organ heterogeneity. Hepatocytes contain less than a third of liver cyclooxygenase activity (Mion *et al*. 1995). The majority of hepatic PGHS-1 is found in non-parenchymal cells, i.e. sinusoidal endothelial cells, Kupffer cells, and Ito cells; PGHS-2 is inducible by various stimuli (Gallois et al 1998; Suzuki-Yamamoto *et al*. 1999; Zhang *et al*. 1995).

Species distribution

Numerous reports indicate the ability of many mammalian and non-mammalian species to synthesise prostaglandins in various tissues. Surprisingly, there are few studies on comparative aspects of PG-biosynthesis in animal and human tissues. By far the highest PGHS activity of all species is found in the seminal vesicles of rams, with a ten- to twenty-fold lower activity in those of bulls (Christ and Van Dorp 1972). Much lower prostaglandin secretion by vesicular glands of other species was reported (Silvia et al 1994). This is reflected at the protein level, with up to 15% of the total microsomal protein being PGHS-1 in ovine seminal vesicles, but only 1.5% in the bovine tissue, and much less PGHS ($< 0.05\%$ of microsomal protein) in other species including humans (Fischer 1989). With regard to the kidney (where PGHS is highest in inner medulla and papilla) the rabbit tissue showed higher PG-synthesis activity than those of hamster, cow, sheep or pig (Christ and Van Dorp 1972).

Despite these quantitative differences in PGHS-activity between species, on the whole the pattern of PGHS isozyme mRNA expression in human tissues (O'Neill and Ford-Hutchinson 1993) appears to be similar to that reported in several animal species, at least when tissue homogenates are analysed. PGHS-isozyme expression was studied in gastrointestinal (GI-) tract tissues of rat, dog, squirrel monkey, rhesus monkey and human by Western Blotting and immunhistochemical analysis (Kargman *et al*. 1996). It confirmed the presence of PGHS-1 in all GI-tract regions (stomach to caecum) of these species, with higher PGHS-1 protein levels in humans and monkeys than in rats and dogs. In contrast, expression of PGHS-2 protein was absent in most GI-tissues examined; only small amounts were detected, e.g. in rat caecum (Kargman *et al*. 1996).

In studies on the renal localisation of PGHS-isozymes with rats, dogs, monkeys and human kidney specimen Khan *et al*. (1998b) observed a similar regional distribution of PGHS-1 across the species investigated, but, significant interspecies differences in PGHS-2 localisation were noted; a similar distribution between dogs and rats, and between monkeys and humans, respectively, but differences between primate kidneys in comparison to those of dogs and rats. Moreover, PGHS-2 expression was markedly increased in kidneys of volume-depleted rats and dogs, but not in monkeys (Khan *et al*. 1998). In the authors' opinion, interspecies differences in the presence and distribution of PGHS-isozymes may further help to explain the difference in species susceptibility to NSAID-related renal toxicity.

Epidermal expression of PHGS-isozymes was evaluated by immunohistochemistry performed on human and mouse skin biopsy sections, and by Western Blotting of protein extracts from cultures of human keratinocytes (Leong *et al*. 1996). These data

and those of others suggest that in human epidermis and in keratinocyte cultures, expression of PGHS-2 occurs as part of the normal keratinocyte differentiation whereas in murine skin, PGHS-2 is not expressed, but inducible (Scholz *et al*. 1995; Müller-Decker *et al*. 1998a,b, 1999). In light of the above observations, further studies on isoenzyme expression and regulation in different species are indicated.

Influence of age and sex

AGE-DEPENDENT EXPRESSION

PGHS-1 expression and activity are considerably higher in large seminal vesicles (>8 g wet weight) of mature uncastrated sheep than in small organs (<3 g wet weight) from young animals. In addition to PGHS-1 levels being related to overall size and functional status of the gland, seasonal fluctuations in enzyme activity were observed (Koburg 1991). Thus, it was suggested that an increase in PGHS-1 tissue levels is due to elevated production of androgens during puberty. The notion of a developmental regulation of PGHS-1 is further supported by studies demonstrating the stimulating effect of the sex steroid testosterone on PGHS-1 expression and the development of seminal vesicles in young rams (Silvia *et al*. 1994).

PGHS-1 expression was also found to increase during the first four weeks after birth in the developing ovine vasculature (Brannon *et al*. 1994). In lungs from foetal lambs to newborn and four-month-old lambs the PGHS-1 and PGHS-2 protein levels increased four- to fivefold (Brannon *et al*. 1998). An intense immunostaining of PGHS-1 was found in endothelial and airway epithelial cells whereas PGHS-2 was not detected. PGHS-2 immunohistochemistry has been performed on human lung tissues from autopsies of foetuses, preterm infants, and term infants; PGHS-2 staining occurred exclusively in the type II pneumocytes, and in ciliated cells (Lassus *et al*. 2000). Consistent with animal studies, the most intense bronchial staining of PGHS-2 was found in foetuses and the least intense in term infants. These findings illustrate the cell-specific expression of PGHS-1 and PGHS-2 in the lung and are indicative of the developmental role of both isoforms in the perinatal lung. A developmental regulation of PGHS-2 has been reported in rat brain (Yamagata *et al*. 1993). Using *in situ* hybridization, PGHS-2 mRNA was detected in regions of the hippocampus on day 1 (postnatal), with increasing levels on day 5, and reaching adult levels on day 16 after birth. Postnatal development of PGHS-2 has been studied also in rat kidney. The isozyme was found in a subset of thick ascending limb of Henle (TAL) cells; positive immunostaining PGHS-2 was observed in a small number of these cells in early postnatal life, increasing from day 5 to day 15, and decreasing thereafter to reach adult levels (Vio *et al*. 1999). During maximal expression, about 20% of TAL cells expressed PGHS-2, but only 2% in the early postnatal period and in adult animals. The transient induction of PGHS-2 appeared to correspond to a de-repression of PGHS-2 expression secondary to low levels of glucocorticoids (Vio *et al*. 1999).

An age-dependent change in expression has been shown for PGHS-2, although not in the context of early developmental stages as described above. The analysis of PGHS-1 and PGHS-2 revealed a higher PGHS-2 protein and mRNA expression in peritoneal macrophages derived from old mice compared with those from young

mice, whereas levels of PGHS-1 protein were not different (Hayek *et al*. 1997). Moreover, older rats exhibit significantly higher PGHS-2 expression (mRNA and protein) in the kidney than young animals (Chung *et al*. 1999). It has been proposed that the increase of PGHS-2 during lifetime is due to increased formation of reactive oxidant species (ROS) which can activate the redox-sensitive transcription factor NF-kB (Nuclear Factor—KappaB) (Kim *et al*. 2000). The age-associated increase in PGHS-2 and PGE_2 production has been sugggested to be involved in the dysregulation of immune functions in aging animals (Hayek *et al*. 1997).

SEX-SPECIFIC DIFFERENCES

A sex-dependent divergence of the PGHS-1- or PGHS-2-mediated cooxidation of xenobiotics is not documented for any species. Aside from findings which imply a regulatory role for sex hormones in PGHS isozyme expression of certain reproductive organs (see below) there are no obvious sex specific differences for PGHS.

Men are known to have a higher risk of developing cardiovascular diseases than premenopausal women. Regarding the vasculoprotective action of oestrogens, it is of interest that oestradiol has been reported to stimulate prostacyclin production in human vascular endothelial cells (Mikkola *et al*. 1995). An *in vivo* study (Stanzcyk *et al*. 1995) found an increase in the urinary prostacyclin metabolite 6-keto-$PGF_{1\alpha}$ and a decrease of urinary thromboxane in postmenopausal women after infusion of oestradiol. On the other hand, oestradiol treatment caused a decrease in PGHS-2 mRNA levels in *vena cava* of ovariectomised rats; oestradiol was also found to inhibit the PGHS-2 increase seen under inflammatory conditions induced by lipopolysaccharide (LPS) (Diel *et al*. 1996). The significance of these observations is not immediately clear, since various other factors are discussed with respect to cardiovascular diseases.

Regulation of PGHS by endogenous factors (cytokines/hormones)

In general, PGHS-1 failed to respond to cytokines or other stimuli. Under some circumstances, however, PGHS-1 can be induced by certain stimuli which trigger differentiation and/or developmental processes. For instance, experimentally induced differentiation of osteoblasts and monocytes was associated with an increased expression of PGHS-1 (Oshima *et al*. 1991; Smith *et al*. 1993). Treatment of bone marrow-derived mast cells with stem cell factor induced mast cell differentiation and PGHS-1 mRNA accumulation (Samet *et al*. 1995); also stimulation with recombinant c-*kit* ligand (which binds to KIT, a mast/stem cell growth factor receptor) alone or in combination with IL-10 (Interleukin-10) led to increased expression of PGHS-1 transcript (Murakami *et al*. 1995). Treatment of monocytic cells (U937 and THP-1) by TPA resulted in increased levels of PGHS-1 mRNA and protein (Hoff *et al*. 1993; Smith *et al*. 1993). This increase of PGHS-1 expression was associated with enhanced differentiation into a macrophage-like phenotype. A hormonal regulation of PGHS-1 expression has been demonstrated in ovine seminal vesicles (Silvia *et al*. 1994). The castration of young male sheep (wethers) led to decreased levels of PGHS-1 in the involuted accessory sex gland. Administration of testosterone restored the PGHS-1

levels to those seen in age-matched rams and normal seminal vesicle development. The results suggest that PGHS-1 is positively regulated by androgens during the pubertal development of the seminal vesicles (Silvia *et al*. 1994).

The regulation of the PGHS-2 gene by endogenous factors like cytokines and growth factors is documented in numerous studies, and the molecular mechanisms of transcriptional control by various stimuli are an area of active reserach. DNA binding sites for NF-κB, CAAT/enhancer binding protein (C/EBP), ATF/cyclic-AMP response element (CRE), and E-box sequences have been identified in the 5′-flanking region of the PGHS-2 genes of different species (Fletcher *et al*. 1992; Kosaka *et al*. 1994; Inoue *et al*. 1994; Newton *et al*. 1997; Yamamoto *et al*. 1995). They mediate the rapid modulation of PGHS-2 expression upon stimulation of cells with e.g. lipopolysaccharide, hormones, and cytokines (Hwang *et al*. 1997; Inoue *et al*. 1995; Jones *et al*. 1993; Morris and Richards 1996; Reddy *et al*. 2000).

Particularly pro-inflammatory cytokines like interleukin (IL)-1β, IL-1α, tumour necrosis factor (TNF)-α, and interferon (INF)-γ, but also growth factors like epidermal growth factor (EGF), platelet-derived growth factor (PDGF), fibroblast growth factor (FGF), and transforming growth factor (TGF)-α can induce PGHS-2 expression in many cell types (reviewed by Herschman 1996,1999; Smith and De Witt 1996). On the other hand, the anti-inflammatory cytokines IL-10, IL-4, and IL-13 exert an inhibitory effect on PGHS-2 expression (Mertz *et al*. 1994, 1996; Dworski and Sheller 1997; Onoe *et al*. 1996). The effects of the immunosuppressive cytokine TGF-β on PGHS-2 expression are cell-specific; TGF-β suppresses the LPS-induced PGHS-2 expression in macrophages (Reddy *et al*. 1994), but TGF-β stimulates PGHS-2 expression and production of prostaglandins in tetradecanoyl phorbol acetate (TPA-) and IL-1β treated fibroblasts (Gilbert *et al*. 1994a) or epithelial cells (Gilbert *et al*. 1994b). The immunomodulatory suppressive effects of cytokines may be mediated by an altered PGHS-2 expression.

Hormonal regulation of PGHS-2 by gonadotropins was demonstrated by Sirois and Richards (1992) who thereby contributed to the discovery of the inducible isoform. PGHS-2 was induced in granulosa cells of rat preovulatory follicles upon treatment with gonadotropic hormones (Sirois *et al*. 1993; Sirois and Richards 1993). Promoter analysis revealed that the E-box and the C/EBP binding site are involved in the induction of the PGHS-2 gene (Morris and Richards 1996; Liu *et al*. 1999). These and other observations are indicative for an important role of prostaglandins in processes of female reproduction, e.g. ovulation, implantation and parturation (Chakraborty *et al*. 1996; Charpigny *et al*. 1997; Mitchell *et al*. 1995).

Adrenal steroids are important regulators of PGHS-2 expression. The effect of endogenous glucocorticoids is demonstrated by elevated prostanoid levels in adrenalectomised animals; treatment with glucocorticoids completely suppressed the increase of prostanoids (Masferrer *et al*. 1992). Glucocorticoids selectively suppressed PGHS-2, but not PGHS-1, mRNA and protein accumulation (Masferrer *et al*. 1994). Synthetic glucocorticoids such as dexamethasone also block the induction of PGHS-2 mRNA and protein usually seen after stimulation with inducers like serum, IL-1β, PDGF, TPA, and forskolin (O'Banion *et al*. 1992; DeWitt and Meade 1993; Rzymkiewicz *et al*. 1994; Kujubu and Herschman 1992). The strong inhibitory effects of anti-inflammatory glucocorticoids on PGHS-2 expression are mediated via transcriptional

and post-transcriptional processes (DeWitt and Meade 1993; Evett *et al*. 1993; Newton *et al*. 1998). The down-regulation of PGHS-2 by glucocorticoids is not mediated via nGRE enhancer elements which are absent in the promoter of the PGHS-2 genes. Interestingly, in contrast to most other cell types, glucocorticoids induce the expression of PGHS-2 in amnion cells (Zakar *et al*. 1995), thus giving another example for a tissue-specific regulation.

There is evidence for a critical role of PGHS-2 in bone resorption and osteoclast formation. Parathyroid hormone (PTH) is a potent activator of osteoclastic bone resorption and increases PGHS-2 transcription in osteoblasts (Tetradis *et al*. 1997). Osteoclast formation stimulated by PTH or by 1,25-dihydroxyvitamin D_3 (1,25-D) is reduced in cell cultures deficient in PGHS-2, and is reversed by exogenous PGE_2. Furthermore, specific inhibitors for PGHS-2 can repress osteoclast formation in wild type cell cultures indicating that PGHS-2 and PGE_2 are crucial factors for the PTH and 1,25-D-stimulated bone resorption (Okada *et al*. 2000).

The action of 17β-oestradiol on the arachidonate metabolism is cell-specific. Treatment of human peripheral monocytes (Miyagi *et al*. 1993) or cultured endometrial stromal cells (Bulun *et al*. 1999) with oestradiol stimulated their PGE_2 production, whereas in cultured bovine coronary endothelial cells the exposure to oestradiol reduced the production of thromboxane B_2 and 6-keto $PGF_{1\alpha}$ (Stewart *et al*. 1999). Since the mRNA expression of PGHS-1 or PGHS-2 was not altered, oestradiol appeared to act at a post-transcriptional level. A similar tissue-specific effect, yet accompanied by changes in PGHS-2, was noted in other studies. Treatment of ovariectomised rats with oestradiol caused a decrease in PGHS-2 mRNA levels in *vena cava*, but an increased expression of PGHS-2 in the uterus of these animals (Diel *et al*. 1996). These and other reports on changes of PGHS isozyme expression in ovine and baboon endometrium during the menstrual cycle point to oestradiol and progesterone as regulators of prostaglandin production (Charpigny *et al*. 1997; Kim *et al*. 1999). The mechanisms responsible for PGHS regulation by these steroid hormones are unknown.

The importance of PGHS-2 in various diseases, like colon carcinoma, neurological disorders, kidney diseases, and inflammation has been extensively reviewed (recent overviews by DuBois, 2000; Halliday *et al*. 2000; Prescott and Fitzpatrick, 2000; Vane *et al*. 1998; Williams *et al*. 1999).

Modulation of PGHS expression by prior exposure to xenobiotics

Similar to endogenous stimuli, xenobiotics which modulate PGHS expression are predominantly affecting the PGHS-2, but not the PGHS-1 isoform. An exception is the tumour promoter tetradecanoyl phorbol acetate (TPA), in some studies TPA treatment was found to induce also, to some extent, PGHS-1 mRNA expression in specific cell types (Hamasaki *et al*. 1993; Kitzler *et al*. 1995). The mechanism of PGHS-1 induction by TPA is unclear. Most studies, however, demonstrate a pronounced induction of PGHS-2 by TPA in the absence of notable effects on PGHS-1 transcription. This differential response to inducers led to the discovery of the PGHS-2 isoform, first described as a so-called 'immediate-early' gene in Swiss 3T3 cells (Lim *et al*. 1987). Numerous studies followed, showing that TPA induces PGHS-2 expression in a wide

variety of cell types of different species, including chicken, mouse, rat, and human (Table 6.3).

A range of cells including fibroblasts, epithelial, smooth muscle cells as well as keratinocytes can respond to TPA treatment *in vitro* by increased expression of PGHS-2. Induction of PGHS-2 has been demonstrated also *in vivo* in mouse skin; acute

Table 6.3 Xenobiotics and induction of PGHS-2 expression: Studies with various cell types and in tissues

Cell type and tissue	Xenobiotic	References
Murine fibroblast 3T3 cells	TPA	Kujubu *et al.* (1991)
Murine embryo fibroblasts		Gilbert *et al.* (1994a)
Human monocytes		Hoff *et al.* (1993)
Chick embryo fibroblasts		Xie *et al.* (1991)
Rat aortic smooth muscle cells		Rimarachin *et al.* (1994)
Human vascular endothelial cells		Jones *et al.* (1993)
Rat bronchial epithelial cells		Hamasaki Y *et al.* (1993)
Rat intestinal epithelial cells (RIE-1 and IEC-6)		Du Bois *et al.* (1994); Gilbert *et al.* (1994b)
Human breast cancer cells (MCF-7 and MDA-MB 231)	TPA	Liu and Rose (1996)
Murine keratinocytes (MSCP5) mouse epidermis		Scholz *et al.* (1995)
Murine peritoneal macrophages	Procainamide	Goebel *et al.* (1999)
Immortalised mouse liver cells (ML-457)	WY-14,643, monoethylhexyl phthalate, clofibrate, ciprofibrate ethyl ester	Ledwith *et al.* (1997)
Human mammary epithelial cells (184B5) and human colon carcinoma cells (CaCo-2)	WY-14,643, fatty acids	Meade *et al.* (1999)
Rabbit corneal epithelium	WY-14,643	Bonazzi *et al.* (2000)
Canine kidney cells (MDCK)	TCDD	Kraemer *et al.* (1996)
Human umbilical vascular endothelial cells		Liu *et al.* (1997)
Murine hepatoma cells (Hepa1c1c7)		Puga *et al.* (1997)
Murine peritoneal macrophages		Vogel *et al.* (1997)
C57BL/6 mice lung and spleen	TCDD	Vogel *et al.* (1998)
Primary rat hepatocytes		Vogel *et al.* (2000)
Murine fibroblasts (C3H/M2)		Wölfle *et al.* (2000)
Rat thymocytes and murine thymic lymphoma cells (WEHI 7.1)	Down-regulation by TCDD	Olnes *et al.* (1996)
Oral epithelial cells	Benzo[a]pyrene	Kelley *et al.* (1997)
Human airway epithelial cells	Residual oil fly ash	Samet *et al.* (1996)
Rat alveolar macrophages	Silica	Chen *et al.* (1997)

Notes: TPA: Tetradecanoyl phorbolacetate; TCDD, 2,3,7,8-tetrachlorodibenzo-p-dioxin; WY-14,643, [4-chloro-6-(2,3-xylidino)-2-pyrimidyl-thio] acetic acid.

inflammation and epidermal hyperplasia evoked by wounding or by TPA treatment resulted in a transient induction of PGHS-2 expression while PGHS-1 remained unchanged (Scholz *et al*. 1995). Futher studies by the same group imply an important role of PGHS-2 in both regenerative hyperplasia and during multistage carcinogenesis in the mouse model (Müller-Decker *et al*. 1995, 1998a,b).

The molecular mechanisms involved in the TPA-mediated induction of the PGHS-2 gene have been studied by several groups (Xie and Herschman 1995, 1996; Inoue *et al*. 1995; Pilbeam *et al*. 1997; Wadleigh and Herschman 1999). These papers and reviews (Herschman 1999; Vogel 2000) also cover studies on factors and elements important in the transcriptional control of this gene. It may suffice to point out that the exogenous chemical TPA—by mimicking endogenous factors and using established signal transduction pathways—can interfere with the regulation of PGHS-2 expression.

More recently, also non-TPA-type compounds, including several rodent liver and skin tumour promoters were shown to modulate PGHS-2 expression (Table 6.3). Specifically, the peroxisome proliferators, WY-14,643, monoethylhexyl phthalate, clofibrate, ciprofibrate ethyl ester, and eicosatetraynoic acid elevated PGHS-2 mRNA and protein levels *in vitro* (Ledwith *et al*. 1997). In contrast to TPA, these peroxisome proliferators caused little or no increase in PGE_2 levels, and inhibited the serum-induced synthesis of PGE_2 in immortalised mouse liver cells (ML-457). These compounds act cell specifically on PGHS-2 and prostaglandin metabolism via both positive and inhibitory mechanisms. Ledwith *et al*. (1997) also reported induction of PGHS-2 by thapsigargin, okadaic acid and calcium ionophore, but not by phenobarbital or dehydroepiandrosterone sulphate.

An induction of PGHS-2 by WY-14,643 with the ability to activate the peroxisome proliferator-activated receptor (PPAR) was also shown in rabbit corneal epithelial cells (Bonazzi *et al*. 2000). The PGHS-2 induction was independent of prostaglandin synthesis inhibition (by NSAIDs) and not associated with a parallel increase in PGE_2 accumulation. Meade *et al*. (1999) identified a peroxisome proliferator response element in the human PGHS-2 promoter which seems to be responsible for the enhanced PGHS-2 expression seen after treatment of colon and mammary epithelial cells with various peroxisome proliferators including WY-14,643, and several fatty acids.

Evidence for PGHS-2 modulation has been reported for 2,3,7,8-tetrachlorodibenzo-*p*-dioxin (TCDD) and for other chemicals. Treatment of murine macrophage cultures with procainamide or the potent inducer LPS/INF-γ resulted in clearly increased expression of PGHS-2 and enhanced production of PGE_2; with both stimuli the effect was more pronouced in macrophages from C57BL/6 mice than in those from A/J mice (Goebel *et al*. 1999). The treatment of peritoneal macrophages from two mice strains (C57BL/6 and DBA/2) with TCDD resulted in a strain-dependent induction of PGHS-2 mRNA whereas PGHS-1 levels remained unchanged (Vogel *et al*. 1997). Kraemer *et al*. (1996) found that TCDD led to a marked increase of PGHS-2 in MDCK cells. An induction of PGHS-2 by TCDD was also shown in primary human epithelial cells (Liu *et al*. 1997) and in a murine hepatoma cell line (Puga *et al*. 1997). Similar TCDD effects on PGHS-2 as shown in these *in vitro* studies are also documented *in vivo*. A dose-dependent induction of PGHS-2 was observed in lung and spleen of TCDD-

sensitive C57BL/6 mice, but no or weak induction in the organs of insensitive DBA/2 mice (Vogel *et al*. 1997, 1998). The results indicated an involvement of the Ah-receptor (AhR) in the TCDD-mediated induction of PGHS-2. However, the molecular mechanism by which this modulation occurs was still unclear. Transfection studies with reporter plasmids containing XRE (Xenobiotic Responsive Elements) elements of the PGHS-2 promoter were inconsistent regarding the functional activity of these XRE elements. In transfected Hepa 1c1c7 cells, the AhR failed to activate the transcription of the PGHS-2 gene (Kraemer *et al*. 1996), whereas in thymocytes the XRE element appears to be necessary for down-regulation of PGHS-2 (Olnes *et al*. 1996).

Recently, the mechanism of the PGHS-2 activation by TCDD has been studied in more detail. Promoter analysis revealed that the *cis*-acting element C/EBP is involved, rather than the AhR-binding XRE motif identified in the 5'-flanking region of the human, rat and mouse PGHS-2 gene (Fletcher *et al*. 1992; Sirois *et al*. 1993; Hla and Neilson 1992). There is evidence from *in vitro* and *in vivo* studies that the AhR-associated c-Src tyrosine kinase (Enan and Matsumura 1996) takes part in the activation of PGHS-2. The upregulation of PGHS-2 by TCDD was blocked by c-Src inhibitors herbimycin and geldanamycin, and in c-Src deficient mice, PGHS-2 failed to respond to TCDD treatment (Vogel *et al*. 1998, 2000).

Thus, the regulation of PGHS-2 by TCDD differs from the classical AhR/Arnt-pathways described for the induction of several xenobiotic-metabolising enzymes, e.g. cytochromes P450s (Nebert *et al*. 2000; Chapter 2). The exposure of oral epithelial cells to the carcinogen benzo[*a*]pyrene (B[a]P) was found to induce PGHS-2 (Table 6.3); the non-carcinogen benzo[e]pyrene (B[e]P) had no effect on PGHS-2 expression (Kelley *et al*. 1997). Since B[a]P, not B[e]P, is known as an AhR agonist, a similar mechanism as described above for TCDD can be invoked for the induction of PGHS-2 by B[a]P.

Another class of xenobiotic agents such as mineral dust and silica have been shown to interfere with the arachidonic acid cascade (Table 6.3). Treatment of rat alveolar macrophages with silica led to transcriptional activation of the PGHS-2 gene; the effect has been attributed to silica-induced NF-κB activation (Chen *et al*. 1997). Exposure of human airway epithelial cells to the particulate air pollutant residual oil fly ash (ROFA) resulted in an increased PGHS-2 expression and $PGE_2/PGF_{2\alpha}$ production (Samet *et al*. 1996, 1999). ROFA contains significant quantities of transition metals which can generate reactive oxygen species, and thus induce PGHS-2 via the NF-κB signal transduction pathway. Ozone can cause neutrophilic airways inflammation. After inhalation of ozone, increased levels of PGE_2 and thromboxane B_2 were found in bronchoalveolar lavage fluid (Hazucha *et al*. 1996). Although a direct effect on the PGHS-2 expression was not described, it is likely to occur since in other studies the inducible effect of reactive oxygen species and oxidative stress on PGHS-2 expression has been demonstrated and considered to contribute to inflammatory reactions (Feng *et al*. 1995). These findings suggest that induction of PGHS-2 may be a crucial factor in mediating adverse health effects (e.g. lung inflammation and pulmonary injury) of particles and other air pollutants.

Collectively, there is evidence that, in addition to endogenous factors, several exogenous agents are capable of modulating PGHS-2 expression. Table 6.3 lists xenobiotics found to increase PGHS-2 expression. Related to chemical toxicity, this is

important, since induction of PGHS-2 could result: (a) in an inappropriate synthesis of prostanoids, and (b) an increased peroxidative metabolism of xenobiotics.

NSAIDs and other PGHS inhibitors

PGHS as a key enzyme in the synthesis of biologically active prostanoids (Figure 6.1) is a prime target for an important class of pharmaceutical compounds, the non-steroidal antiinflammatory drugs (NSAIDs). Well-known examples are aspirin, indomethacin, ibuprofen, diclofenac, piroxicam, sulindac, and many others. Whereas many NSAIDs inhibit PG-synthesis by both isozymes to a similar extent, new drugs have been developed which are much more potent inhibitors of PGHS-2 than of PGHS-1. When the existence of PGHS-2 was discovered, pharmaceutical research focused on designing such drugs, because the PGHS-2-dependent PG-synthesis is thought to be predominantly responsible for inflammatory processes. Although NSAIDs-specific for PGHS-2 with good efficacy in a low dose-range offer advantages with respect to unwanted side effects, like ulcerogenicity and nephrotoxicity, other undesirable effects cannot be eliminated so far. A detailed discussion on the selectivity of the compounds and the mechanisms by which NSAIDs inhibit the cyclooxygenase isozymes are beyond the scope of this chapter. Interested readers are referred to recent reviews that also cover the role of prostanoids in physiology and in certain diseases (DuBois *et al*. 1998; Hawkey 1999; Vane *et al*. 1998; Taketo 1998a; Wolfe *et al*. 1999).

Of particular interest is the role of PGHS and its inhibitors in cancer. PGHS-2 expression is markedly increased in several tumours, e.g. human colorectal cancer and human invasive transitional cell carcinoma of the bladder, and PGHS-inhibitors show antitumour activity in animal models of these forms of cancer (Mohammed *et al*. 1999; Williams *et al*. 1999; Oshima *et al*. 1996). Moreover, epidemiological studies have shown that chronic administration of PGHS-inhibiting drugs can effectively suppress the development of several tumours (Giovannucci *et al*. 1995; Thun 1996; Taketo 1998b; Williams *et al*. 1999). The underlying mechanisms are not fully understood yet.

The potency of NSAIDs to suppress the synthesis of prostanoids is based on their ability to bind to the active domain of the PGHS protein and thereby inhibiting PGHS cyclooxygenase activity (Bhattacharyya *et al*. 1996; Garavito and DeWitt 1999). More recently, it was described that NSAIDs could act through an additional mechanism to inhibit PG-biosynthesis; aspirin and sulindac markedly suppressed the mRNA expression of cytosolic phospholipase A_2 ($cPLA_2$) in NIH3T3 cells (Yuan *et al*. 2000), an acyl esterase which catalyses the release of arachidonic acid from cellular phospholipids (Figure 6.1). The authors proposed that this pathway contributes, at least in part, to the cancer chemopreventive effects of NSAIDs.

Synthetic glucocorticoids such as dexamethasone inhibit prostanoid synthesis by down-regulating the expression of PGHS-2, an effect found in most tissues except amnion. The modulation of PGHS-2 expression by glucocorticoids has already been discussed in more detail (see above).

In addition to drugs, natural plant compounds such as certain stilbenes and flavonoids can also affect arachidonate metabolism by PGHS. Resveratrol (*trans*-

3,4′,5-trihydroxystilbene), a phytoalexin found in grapes and other food products, exerted chemopreventive activity in assays of multi-stage carcinogenesis (Jang *et al*. 1997). Resveratrol was found to inhibit the cyclooxygenase and hydroperoxidase activity of PGHS (Johnson and Maddipati 1998). In addition, resveratrol affects arachidonic acid release, and can suppress the PGHS-2 induction mediated by LPS and TPA (Martinez and Moreno 2000; Subbaramaiah *et al*. 1999). Similar to resveratrol, various flavonoid derivatives such as oroxylin A, wogonin, skullcapflavone II, tectorigenin and iristectorigenin A show inhibitory activities on PGHS and on lipoxygenases (You *et al*. 1999). Silymarin is used clinically for the treatment of inflammatory liver diseases (Flora *et al*. 1998). Like resveratrol, silymarin possesses cancer-preventive effects in different *in vivo* and *in vitro* carcinogenesis models (Anderson *et al*. 1998; Lahiri-Chatterjee *et al*. 1999). Silymarin acts specifically on PGHS-2 with no change in constitutive PGHS-1 protein levels (Zhao *et al*. 1999). One proposed mechanism of the PGHS-2-specific inhibition by flavonoids is inhibition of activation nuclear factor-kappa B (Chen *et al*. 2000).

The above examples illustrate that a variety of naturally occurring xenobiotics can affect PGHS activity and expression. In contrast to agents which induce PGHS-2 expression, natural plant ingredients appear to inhibit PG-biosynthesis by various mechanisms. On the other hand, also man-made chemicals, e.g. *o*-phenylphenol and its metabolite, have been found to inhibit PGHS activity (Freyberger and Degen 1998).

Genetics and pharmacogenetics

The reason for the existence the two PGHS-isozymes is as yet unknown. PGHS-1 and PGHS-2 knockout animals were constructed by gene targeting techniques to study physiological function and pathophysiological states. Langenbach *et al*. (1995) have shown that mice genetically deficient in PGHS-1 exhibit normal life spans and had no impairment of health. These findings were surprising in view of the constitutive expression and physiological importance of PGHS-1, since severe gastric bleeding and renal dysfunction are seen upon chronic treatment of normal animals with NSAIDs, an experimental model equivalent to gene-deletion studies. The PGHS-1 null mice produced only 1% of the normal PG levels. They failed to produce viable offspring attributable to a disturbed parturition in female animals (Langenbach *et al*. 1995). On the other hand, only about 60% of the pups lacking PGHS-2 expression survived to weaning. The PGHS-2 knockout mice had serious renal developmental deficiencies, peritonitis, and 25% of the animals began to die after 3 weeks of age. About 75% of surviving pups lived to one year of age (Morham *et al*. 1995). Another PGHS-2-deficient mouse showing similar characteristics has been described by Dinchuk *et al*. (1995). In accordance with the results from the PGHS-2 gene-deletion studies are recent findings that a selective inhibitor for PGHS-2 (SC58236, administered during pregnancy until weaning) impairs glomerulogenesis and development of the renal cortex in both mice and rats (Komhoff *et al*. 2000).

The above findings reveal that prostaglandins derived via the individual PGHS isoforms have distinct as well as common functions. It appears that deficiency of PGHS-2 has more profound effects than deficiency of PGHS-1. Recent data from PGHS-deficient mice indicate that both PGHS-1 as well as PGHS-2 are mediating

inflammatory responses, and that both isoforms have significant roles in carcinogenesis (Langenbach *et al*. 1999a,b). Thus, the terms for PGHS isoforms as 'housekeeping' and/or 'response' genes may not be entirely precise.

There are no known polymorphisms for PGHS-1. Only two reports are documenting a genetic polymorphism for the PGHS-2 gene. Molecular cloning of the human PGHS-2 gene and digestion of genomic DNA of 78 individuals with Hind III revealed that a polymorphism is present in about 5% of the population (Jones *et al*. 1993). By sequence analysis of genomic DNA, a silent mutation in exon 3 of the human PGHS-2 was detected (Spirio *et al*. 1998). Phenotypic changes like altered enzyme activity or gene inducibility of PGHS-2 as a consequence of the identified polymorphisms have not been reported so far.

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