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COMMENTARY

XENOBIOTIC CARBONYL REDUCTION AND PHYSIOLOGICAL STEROID OXIDOREDUCTION

THE PLURIPOTENCY OF SEVERAL HYDROXYSTEROID DEHYDROGENASES

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The metabolism of xenobiotics and steroid hormones has been well studied with regard to their oxidation catalyzed by the cytochrome P450 monooxygenase system, but less attention has been paid to their reductive metabolism, the common denominator of which is a carbonyl group (—C═O⟷—CHOH) at the respective substrate molecule. Many enzymes that catalyze the reduction of carbonyl compounds of endogenous and/or exogenous origin to their corresponding alcohols, and simultaneously exhibit specificity for the oxidoreduction of certain hydroxy or keto groups at the steroid nucleus, have already been characterized. These enzymes have been classified either as being hydroxysteroid dehydrogenases having an additional substrate specificity towards non-steroidal xenobiotics or, conversely, the enzymes have been considered as carbonyl reducing enzymes possessing additional steroid-oxidoreducing properties.

Enzyme activities in mediating both the oxidoreduction of steroids as well as the reduction of carbonyl compounds have been reported for many tissues and a large variety of animals, and identical enzymes have been classified under several numbers of the enzyme nomenclature system. In addition, the exceptionally low substrate specificity has precluded the development of a logical nomenclature based on unique substrate specificities.

In contrast to the cytochrome P450 superfamily [1], an establishment of a nomenclature for the carbonyl reducing enzymes/hydroxysteroid dehydrogenases, which is based on protein structure and data of gene composition, including an assignment of the early trivial names, is hampered in two ways. First, only a limited amount of structural data on respective DNA sequences of the pertinent genes, as well as of the encoded proteins, is available today, and, second, the enzymes involved in carbonyl reduction obviously belong to more than one protein superfamily.

This commentary, therefore, tries to combine the current knowledge on functional properties of carbonyl reducing enzymes/hydroxysteroid dehydrogenases with today's available structural data in order to stimulate investigations directed towards their catalytic and mechanistic functions. Such studies should be useful in understanding the physiological roles of these versatile oxidoreductases as well as in providing a means for specific modifications in terms of a therapeutic benefit.

Most of the functional and structural overlaps between carbonyl reducing enzymes and hydroxysteroid dehydrogenases concern aldehyde reductase (EC 1.1.1.2), aldose reductase (EC 1.1.1.21) and carbonyl reductase (EC 1.1.1.184) (previously referred to as aldo-keto reductases due to common functional properties) together with several hydroxysteroid dehydrogenases including 3α-HSD† (EC 1.1.1.50), 3β-HSD (EC 1.1.1.51), 17β-HSD (EC 1.1.1.62), 3α,20β-HSD (EC 1.1.1.53), 20α-HSD (EC 1.1.1.149) and 11β-HSD (EC 1.1.1.146). In addition, enzymes like prostaglandin 9-ketoreductase (EC 1.1.1.189), indanol dehydrogenase (EC 1.1.1.112) and DDH (EC 1.3.1.20) were also shown to be functionally and/or structurally related to these enzymes.

Based on structural data, the above enzymes have been generally classified into two protein superfamilies: (1) the aldo-keto reductases [2], and (2) the short-chain dehydrogenases [3]. Accordingly, the main characteristics of these two protein superfamilies, as far as they concern the pluripotency

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† Abbreviations: 3α-HSD, 3α-hydroxysteroid dehydrogenase; 3β-HSD, 3β-hydroxysteroid dehydrogenase; 11β-HSD, 11β-hydroxysteroid dehydrogenase; 17β-HSD, 17β-hydroxysteroid dehydrogenase; 20α-HSD, 20α-hydroxysteroid dehydrogenase; 3α,20β-HSD, 3α,20β-hydroxysteroid dehydrogenase; and DDH, dihydrodiol dehydrogenase.

of hydroxysteroid dehydrogenases towards non-steroidal xenobiotics, will be considered in this article.

CARBONYL REDUCTION

Carbonyl reduction is a significant step in the phase I biotransformation of a great variety of aromatic, alicyclic and aliphatic xenobiotic carbonyl compounds (for review cf. Ref. 4), including pharmacologically relevant substances such as fenofibrate, oxisuran, ethacrynic acid [4], chloralhydrate [5], acetohexamide [6] and befunolol [7, 8]. However, this metabolic pathway has diverse consequences regarding the pharmacological and toxicological behavior of a parent drug containing a carbonyl group, such that it is impossible to generally and unequivocally ascribe activating or inactivating properties to this reaction.

From the pharmacologist's point of view, carbonyl reduction has been shown to be of significance in various inactivation processes of drugs bearing a carbonyl group, such as warfarin [9, 10] and haloperidol [8, 11]. On the other hand, the respective carbinols formed may retain their therapeutic potency, thus prolonging the pharmacodynamic effect of the parent drug, or, in some instances, a compound gains activation through carbonyl reduction, as is the case for the alcohols of Adriamycin® [12], naloxone [13], pentoxifylline [14], prodrug precursors of the β-adrenergic blockers propranolol and alprenol [15], metyrapone [16] and chloral hydrate [5]. Also, from the toxicologist's point of view, carbonyl reduction plays an important role in the toxification of daunorubicin and Adriamycin (cardiotoxicity) [17], whereas numerous reports also corroborate the concept of carbonyl reducing enzymes being involved in the detoxification processes for quinones [18], aflatoxin B_1 [19, 20], aldophosphamide [21], tripeptidyl aldehydes [22], and chlordecone [23].

The difference in tissue and intracellular distribution suggests that several enzymes may be involved in the enzymatic reduction of carbonyl compounds and that the intracellular multiplicity of the enzymes may have some relation to their physiological function. At physiological pH values, reduction of biological aldehydes and ketones to alcohols can be accomplished by alcohol dehydrogenases (EC 1.1.1.1) [24], which are universally distributed in animal tissues.

However, carbonyl reduction of biologically and pharmacologically active xenobiotic carbonyl compounds to the corresponding alcohols is generally mediated by cytosolic NADPH-dependent members of the aldo-keto reductase superfamily, which are widely distributed in humans and animals [25]. Although it has been suggested that aldo-keto reductases are involved in biogenic amine metabolism, aldehyde and ketone detoxification [4, 26], ascorbic acid synthesis [27] and tetrahydrobiopterin biosynthesis [28], it is not yet possible to ascribe unequivocally a specific physiological role to these enzymes. Interestingly, aldo-keto reductases have been shown in several tissues to be affiliated with hydroxysteroid dehydrogenase activities.

Aldehyde reductase

One member of this family of enzymes is aldehyde reductase (EC 1.1.1.2), previously named alcohol:$NADP^+$-oxidoreductase, high K_m aldehyde reductase, mevaldate reductase, daunorubicin-pH 8.5 reductase, hexonate dehydrogenase, lactaldehyde reductase, glucuronate reductase and ALR 1 [4, 25, 26, 29]. The enzyme has been found in every species investigated, and its substrate specificity, kinetic mechanism and tissue distribution have been studied [26, 30, 31]. In addition, enzymes with the characteristics of aldehyde reductase have also been observed in all non-mammalian animal classes, e.g. amphibia, fish, insects, as well as in yeast (cf. Ref. 32 and references therein).

Some aldehyde reductases utilize NADH as a cosubstrate, but they can be distinguished from alcohol dehydrogenase by the fact that they are not inhibited by pyrazole and are susceptible to inhibitors such as barbiturates [4].

Frequently, aldehyde reductase has been shown in several tissues to be affiliated with hydroxysteroid dehydrogenase activities. Pietruszko and Chen [33] first reported a rat liver aldehyde reductase to be a 3α-HSD, which catalyzes the reversible oxidoreduction of 3α-hydroxysteroids with A/B *cis* configuration, and postulated that the steroid dehydrogenase activity may be connected with its physiological function. Subsequently, Sawada *et al.* [34] purified an aldehyde reductase from guinea pig liver, which catalyzed the oxidoreduction of 17β-hydroxysteroids and 17-ketosteroids. They concluded that this enzyme may play important roles in the metabolism of exogenous aldehydes and ketones as well as of endogenous steroids.

Aldehyde reductase expressing specificity for the synthetic steroids isocortisol and 11-deoxyisocorticosterone in human brain has been speculated to modulate hormonal function [35, 36]. Although these steroids undergo reduction of the aldol side chain, the human brain aldehyde reductase can be considered as a "pluripotent" hydroxysteroid dehydrogenase since it allows access to its active site despite the relatively large structure of the substrates.

Indications for coincident properties of aldehyde reductase and 3α-HSD or 17β-HSD have been reported subsequently for enzymes in rat [5, 37], rabbit [38], and human [23, 36] liver, as well as in chicken kidney [39]. It is conceivable, therefore, that aldehyde reductase plays some role in endogenous steroid metabolism.

Aldose reductase

A second member of the aldo-keto reductase family is aldose reductase (EC 1.1.1.21), previously named alditol:$NADP^+$-oxidoreductase, low K_m aldehyde reductase and ALR 2 [29]. In addition to catalyzing the reduction of a wide range of xenobiotic and endogenous aldehydes and ketones [40], aldose reductase has received special attention recently due to its possible role in the reduction of glucose during diabetic hyperglycemia and its implication in the pathogenesis of various diabetic complications such as neuropathy [41], retinopathy [42] and cataracts [43]. Although the exact mechanisms by which

aldose reductase initiates these complications are not fully understood, in ocular cataracts the osmotic stress caused by the intracellular accumulation of sorbitol is thought to play a primary role in the development of lens opacity.

Aldose reductase has been purified to homogeneity from many tissues (cf. Ref. 44 and references therein), and the amino acid sequence has been determined. Because the aldose reductase protein is blocked at the NH_2 terminus, most of the reported amino acid sequences are deduced from cloned cDNA sequences and reveal a high degree of identity (> 84%) among the reported protein sequences. On the other hand, deduced sequences of aldehyde reductase from human liver and placenta define proteins that are 325 amino acids long and have 52% identity with human placental aldose reductase [2]. Southern blot analysis of human [2] and rat [45] genomic DNA indicates the existence of multiple aldose reductase genes.

The ability of aldose reductase to express hydroxysteroid dehydrogenase activities has obviously received less attention, although there exist two reports on aldose reductase catalyzing the C-21 reduction of isocorticosteroids [36, 46]. Interestingly, molecular cloning of bovine testicular 20α-HSD, an enzyme that plays an essential role in the regulative conversion of progesterone to the biologically less active 20α-hydroxyprogesterone, revealed its complete sequence identity with bovine lens aldose reductase [47].

A recent crystallographic analysis of human placental aldose reductase has pointed out that its active site pocket is large in size and has an extremely hydrophobic nature, which is consistent with the observation that aromatic compounds (such as isocorticosteroids) are the best substrates [48].

Carbonyl reductase

Carbonyl reductase (EC 1.1.1.184), also known as NADPH:secondary-alcohol oxidoreductase, prostaglandin 9-ketoreductase, daunorubicin-pH 6.0 reductase and ALR 3 [29], has long been considered to belong to the aldo-keto reductase superfamily based on its functional properties. Recent structural investigations, however, revealed no significant homologies to the aldo-keto reductases but, in contrast, indicated a relationship to the short-chain dehydrogenase superfamily [2, 49]. The enzyme is ubiquitous in nature, catalyzing the reduction of a large number of biologically and pharmacologically active carbonyl compounds to their corresponding alcohols [4, 25]. The role of carbonyl reductase in humans is at present unknown. The metabolism of activated carbonyl groups, including quinones, suggests a possible role in the detoxification of common cellular metabolites or other natural or xenobiotic compounds. Wermuth *et al.* [18] reported that carbonyl reductase accounted for 50–70% of the NADPH-quinone-reducing activity in human liver and could provide the enzymatic basis for detoxification of quinones in humans [18, 50].

Carbonyl reductase activities have in many cases been affiliated with HSD activities. Wermuth [51] purified an NADPH-dependent carbonyl reductase that was able to reduce a number of biologically and pharmacologically active carbonyl compounds including quinones. The enzyme, surprisingly, exhibited 9-ketoreductase activity towards prostaglandins and 3-ketoreductase activity towards steroids. Chang and Tai [52] isolated a prostaglandin 9-ketoreductase/15-hydroxyprostaglandin dehydrogenase from swine kidney capable of catalyzing steroid oxidoreduction and xenobiotic carbonyl reduction.

Several laboratories have reported on the properties of purified carbonyl reductases, to coincidentally catalyze prostaglandin 9-keto and/or 15-keto reduction as well as expressing 3α-hydroxysteroid dehydrogenase activity in rat ovary [53], testis and vas deferens [54], rabbit liver [7, 10] and kidney [8], human testis [55] as well as in pig and human kidney [56]. For example, Schieber *et al.* [56] have purified a prostaglandin 9-ketoreductase from pig and human kidney. Although the enzymes were shown to catalyze the interconversion of prostaglandin E_2 to prostaglandin $F_{2\alpha}$ and the oxidation of prostaglandins E_2, $F_{2\alpha}$ and D_2 to their corresponding biologically inactive 15-keto metabolites, the catalytic properties measured for prostaglandin 9-ketoreductase suggest that its *in vivo* function is unlikely to be the catalysis of oxidation/reduction at the prostaglandin 9-position. Rather, the enzyme reduces various carbonyl compounds with high efficiency. Sequence homology of tryptic peptides, as well as catalytic and immunological properties, suggest that this enzyme is carbonyl reductase. Moreover, several steroids were also substrates with low specificity. Imamura *et al.* [8] have purified carbonyl reductase from rabbit kidney and shown it to exhibit prostaglandin 9-ketoreductase as well as 3α-hydroxysteroid dehydrogenase activities. Whether this is one and the same enzyme and whether its main physiological function is hydroxysteroid dehydrogenase, prostaglandin 9-ketoreductase or carbonyl reductase remains to be elucidated.

In general, to assess an enzyme's physiological role, structural data, i.e. the three-dimensional modelling of its active site by X-ray crystallographic studies, as well as functional group information by site-directed mutagenesis, are required to elucidate the catalytic mechanism and to image the optimal substrate molecule in the catalytic site. Although carbonyl reductase has been described as having a monomeric structure, a dimeric form [57] identical with that of 17β-HSD has been reported in chicken liver. Hara and coworkers [58, 59] purified a pyrazole-sensitive, tetrameric carbonyl reductase from pig lung, which was composed of identical subunits of 24 kDa. They suspected this enzyme of playing a role in regulating the physiological action of androgenic steroids, because of its high hydroxysteroid dehydrogenase activity towards 5α-androstan-17β-ol-3-one, the active mediator of the androgenic testosterone.

Considering the above data, it is conceivable that the aldo-keto reductases and hydroxysteroid dehydrogenases share overlapping substrate specificities and that respective enzymes may have an evolutionary relationship. Moreover, it is noteworthy to state that several aldo-keto reductases exhibit

higher affinities for physiological steroids than for non-steroidal, xenobiotic carbonyl compounds, from which it might be concluded that the respective enzymes, in fact, are hydroxysteroid dehydrogenases.

Microsomal carbonyl reducing enzymes

One of the features characteristic of the aldo-keto reductases is their subcellular localization in the cytosolic fraction of the cell [4]. However, carbonyl reduction of non-steroidal xenobiotics also takes place in the microsomal fraction, and various reports indicate that some of the enzymes involved are related to microsomal hydroxysteroid dehydrogenases [60–63]. Moreover, based on substrate specificity and kinetic data, these membrane-associated enzymes were considered to actually be 3α- or 17β-HSDs that are physiologically involved in steroid metabolism, but also must have a role as drug-metabolizing enzymes in the detoxification of biologically active carbonyl compounds to more hydrophilic and less active alcohols [64–67].

Indanol dehydrogenase

In addition, enzymes obviously not belonging to the aldo-keto reductases or short-chain dehydrogenases have also been affiliated with steroid dehydrogenases, which is true, for example, for indanol dehydrogenase (EC 1.1.1.112). Several isoforms of indanol dehydrogenase have been purified from rabbit [68] and monkey [69] liver and were shown to catalyze not only xenobiotic carbonyl reduction and benzene dihydrodiol oxidation, but also to exhibit 3α- and/or 17β- (rabbit) and 3(20)α- (monkey) hydroxysteroid dehydrogenase activities, which led to the suggestion that indanol dehydrogenase acts as a hydroxysteroid dehydrogenase or a dihydrodiol dehydrogenase *in vivo*. It is questionable whether indanol dehydrogenases are, in fact, pluripotent hydroxysteroid dehydrogenases which, in addition to their specificity towards physiological steroid substrates, are able to catalyze the oxidation or reduction of xenobiotics.

Dihydrodiol dehydrogenase

DDH (*trans*-1,2-dihydrobenzene-1,2-diol: $NADP^+$-oxidoreductase) catalyzes the $NADP^+$-dependent oxidation of *trans*-dihydrodiols of aromatic hydrocarbons to their corresponding catechols and is thus of importance in the detoxification of mutagenic polycyclic aromatic hydrocarbons [70–72]. The carcinogenic activation of polycyclic aromatic hydrocarbons often proceeds through dihydrodiol precursors to diol-epoxide ultimate carcinogens. By catalyzing the oxidation of these proximate carcinogenic dihydrodiols, DDH reduces their carcinogenic and mutagenic potential. On the other hand, DDH may be involved in the pathogenesis of naphthalene cataract in the rabbit by producing the catechol metabolite 1,2-dihydroxynaphthalene from *trans*-1,2-dihydroxy-1,2-dihydronaphthalene (naphthalene dihydrodiol), which is readily auto-oxidized to cytotoxic 1,2-naphthoquinone and H_2O_2 [73].

DDH exists in multiple forms in most mammalian tissues, and the multiple forms of the enzyme have been reported to be identical to 3α-HSD [74–82], 17β-HSD [68, 75, 78, 83–86], 3α(17β)-HSD [87], 20α-HSD [77] and/or aldehyde reductase [71, 75, 77, 80, 84, 88], depending on the tissue source. For example, in Swiss–Webster mouse liver there appear to be four forms of DDH, three of which are associated with 17β-HSD [83]. In the liver of male ddY mice, a major and a minor form of DDH are identical to 17β-HSD and aldehyde reductase, respectively [84]. In rabbit liver, at least eight isoforms of DDH exist, which were distinguished on the basis of substrate and inhibitor specificity as carbonyl reductase, aldehyde reductase as well as 3α-HSD and 17β-HSD [78].

Although benzene dihydrodiol is a common xenobiotic substrate of all these enzymes, the physiological substrates of several DDH isoforms are often hydroxysteroids, leading to the conclusion that the respective DDHs not only function as steroid-metabolizing enzymes but, in fact, are hydroxysteroid dehydrogenases. Interestingly, several hydroxysteroid dehydrogenases have been purified and characterized, which, in addition to their specificity for steroidal substrates, also can function as dihydrodiol dehydrogenase. For example, 3α-HSD purified from mouse [89], rat [76, 79, 90, 91], rabbit [78], hamster [87] and human liver [81], as well as mouse kidney [80], 17β-HSD purified from mouse [84], rabbit [78], hamster [87] and guinea pig liver [86] and 3(17)α-HSD from mouse kidney [80] were shown to oxidize benzene dihydrodiol (cf. "hydroxysteroid dehydrogenases" in this article).

At this point some clarification of enzyme nomenclature may be required. The term DDH may be directly inferred from the enzyme's functional properties, namely detoxification of dihydrodiols by oxidation, which is probably a special case of alcohol oxidation to a ketone followed by a ketol-enol rearrangement of the oxoalcohol to the aromatic catechol [71]. The aromatic character of the reaction product renders the reaction irreversible, which is not the case with other substrates, such as steroids or xenobiotic aldehydes and ketones. In this case, these enzymes can act as dehydrogenases or reductases depending on the cosubstrate. Accordingly, based on the physicochemical and functional properties, the multitude of DDHs can be classified as either aldo-keto reductases or hydroxysteroid dehydrogenases. Currently, DDH is classified as belonging to subgroup 1.3 in IUB nomenclature (C–C dehydrogenases), which is objected to by Klein *et al.* [78], who suggested that a reclassification of these enzymes to subgroup 1.1 in IUB (C–O dehydrogenases) would be more appropriate. In this context it is noteworthy to state that occasionally 3α-HSD is termed DDH, although values of androsterone oxidation surpass those of benzene dihydrodiol oxidation [92].

HYDROXYSTEROID DEHYDROGENASES OF THE ALDO-KETO REDUCTASE SUPERFAMILY

Hydroxysteroid dehydrogenases are pyridine nucleotide-dependent oxidoreductases, which mediate the interconversion of secondary alcohols to ketones on the steroid nucleus and side chain in a positional and stereoselective manner. The enzymes

are believed to play pivotal roles in the regulation of steroid hormone action [93, 94], displaying either ordered or random Bi-Bi sequential kinetic mechanisms. However, recent reports give evidence that several hydroxysteroid dehydrogenases exhibit other activities besides steroid oxidoreduction, among which xenobiotic carbonyl reduction is the most prominent.

3α-Hydroxysteroid dehydrogenase

3α-HSD was first identified by its activity in converting dihydrocorticosterone to 3α-tetrahydrocorticosterone [95] and was subsequently shown to convert the potent androgen 5α-dihydrotestosterone into the weak androgen 3α-androstanediol [96, 97], thus terminating androgen action. The most thoroughly characterized 3α-HSD is the homogenous enzyme from rat liver cytosol [76, 90], and immunotitration data suggest that it represents 1–3% of the cytosolic protein in this tissue [98].

3α-HSD displays dual pyridine nucleotide specificity for NAD(H) and NADP(H) and accounts for the major DDH and ketone reductase activities present in rat liver cytosol [76]. Initial-velocity and product-inhibition studies are consistent with a sequential ordered Bi-Bi mechanism in which pyridine nucleotide binds first and leaves last [99]. The enzyme reduces a series of biologically important 3-oxo steroids of the androstane (C_{19}), pregnane (C_{21}), and cholane (C_{24}) series, in which the A/B ring fusion may be *cis* or *trans* [5, 100, 101].

In addition to its role in the metabolism of steroid hormones, 3α-HSD serves additional functions in rat liver in terms of bile acid synthesis and transport of bile acids from the sinusoidal to the canalicular pole of the rat hepatocyte [101–104]. For example, rat liver 3α-HSD stereospecifically reduces the bile acid precursors 7α-hydroxy-5β-cholestane-3-one and 7α,12α-dihydroxy-5β-cholestane-3-one to bile acids [105], whereas 3α-HSD from human liver catalyzes the reduction of 3,7-disubstituted 3-keto bile acids but is inactive towards the bile acid precursors [101]. From this example it becomes apparent that similar hydroxysteroid dehydrogenases may already differ in terms of their specificity towards physiological steroid substrates, a fact which, of course, is more pronounced in the case of non-steroidal xenobiotic carbonyl substrates.

After the appearance of several papers reporting on the 3α-HSD properties of aldo-keto reductases or DDHs, genuine 3α-HSDs were described as having the ability of xenobiotic carbonyl reduction and/or dihydrodiol oxidation, thus mimicking the action of aldo-keto reductases or DDH. It, therefore, seems possible that identical enzymes have been classified under several numbers of the enzyme nomenclature!

3α-HSD from rat liver cytosol was purified by Penning and coworkers [76] and, based on physicochemical properties and substrate specificities, assumed to be the same enzyme as DDH described by Vogel *et al.* [71]. The enzyme was shown to be a monomer of M_r 33,000 and to react at comparable rates with both NAD(H) and NADP(H). Examination of the substrate specificity of the purified enzyme for *trans*-dihydrodiol metabolites of polycyclic aromatic hydrocarbons indicates that the enzyme catalyzes the NAD(P)-dependent oxidation of *trans*-dihydrodiols of benzene, naphthalene, phenantrene, chrysene, 5-methylchrysene, and benzo[*a*]pyrene under physiological conditions thus suppressing the formation of *anti*-diol epoxides, which are known to be ultimate carcinogens [70, 71, 90]. However, since 3α-hydroxysteroids were by far the most efficient substrates, this enzyme should be considered to be 3α-HSD, and numerous reports indicate that 3α-HSD and DDH are, in fact, identical enzymes [71, 76, 79, 90, 98, 106]. On the other hand, dissociation of 3α-HSD activity from DDH activity was shown to be species dependent [74].

In addition to catalyzing hydroxysteroid oxidoreduction and benzene dihydrodiol oxidation, 3α-HSD also promotes the NAD(P)H-dependent reductions of various quinones (e.g. 9,10-phenanthrenequinone, 1,4-benzoquinone), as well as that of various aromatic aldehydes and ketones, which suggests another role of 3α-HSD in the detoxification of xenobiotic carbonyl compounds. Interestingly, rat liver 3α-HSD has been demonstrated to catalyze the reduction of camphoroquinone, a natural monoterpene, non-aromatic quinone [107].

The purified enzyme is effectively inhibited by non-steroidal, anti-inflammatory drugs [108] in the rank order of their pharmacological potency and at concentrations relevant to therapeutic doses, suggesting that 3α-HSD may be an additional target for these drugs. In addition, 3α-HSD binds arachidonic acid and prostaglandins with affinity in the low micromolar range at its active site [76, 108], which led to the suggestion that 3α-HSD may also act as a hydroxyprostaglandin dehydrogenase. Indeed, Penning and Sharp [109] provided evidence that homogeneously purified 3α-HSD from rat liver displays 9-, 11-, and 15-hydroxyprostaglandin dehydrogenase activity and that this enzyme may represent a major 9-hydroxyprostaglandin dehydrogenase in this tissue. The ability to function as a prostaglandin dehydrogenase adds another series of substrates to 3α-HSD, which has already been ascribed multiple functions.

However, whereas this enzyme belongs to the aldo-keto reductase superfamily, two other prostaglandin dehydrogenases have been described as belonging to the short-chain dehydrogenases: one, a dimeric form with NAD^+-specificity, and the other an $NADP^+$-dependent, monomeric form that is identical to carbonyl reductase [110, 111] (see below).

Rat hepatic 3α-HSD was also found to act as a chloral hydrate reductase [5, 37, 112], underlining its pharmacological importance in the metabolism of this sedative hypnotic. In addition to a variety of other xenobiotic carbonyl compounds, endogenous biogenic aldehydes derived from serotonin, tryptamine, tyramine, octopamine and norepinephrine were preferred substrates for this enzyme, providing evidence that 3α-HSD also functions as carbonyl reductase for these endogenous carbonyl compounds [5]. Among the substrates tested, C_{24}-3-keto steroids were the best substrates for the rat hepatic enzyme [5].

Several isoforms of 3α-HSD associated with indanol dehydrogenase were found in male rabbit liver cytosol. The monomeric enzymes (M_r 30,000–37,000) showed high carbonyl reductase activity but low dihydrodiol oxidation, which is mainly catalyzed by 17β-HSD in this tissue [68]. All these 3α-HSDs could reduce xenobiotic carbonyl compounds such as pyridine-4-aldehyde, 4-nitrobenzaldehyde, DL-glyceraldehyde, 4-benzoylpyridine and 4-nitroacetophenone, which led the authors to the conclusion that they may be important as drug-metabolizing enzymes.

Interestingly, Imamura *et al.* [7] have purified a befunolol reducing enzyme from rabbit liver which, in addition to other pharmacologically relevant substances such as levobunolol, acetohexamide and daunorubicin, catalyzes the oxidoreduction of prostaglandin and 3α-hydroxysteroids. Moreover, since K_m values of this enzyme were lower for steroids and prostaglandins than those for xenobiotic carbonyl compounds, the authors assumed that this enzyme, in fact, may function as an oxidoreductase for endogenous steroids and prostaglandins. Therefore, befunolol reductase of rabbit liver may be 3α-HSD, but its real relation to 3α-HSD cannot be proven unless its amino acid sequence composition is determined.

Also, carbonyl reductase from rabbit kidney catalyzes 3α-hydroxysteroid oxidoreduction with lower K_m values than xenobiotic carbonyl reduction and might be 3α-HSD [8]. If this were true, 3α-HSD from rabbit kidney mediates carbonyl reduction of a variety of drugs including acetohexamide, metyrapone, haloperidol, trifluperidol, moperone, loxoprofen, befunolol, levobunolol and daunorubicin [8].

Klein *et al.* [78] purified eight isoforms of DDH from the same tissue, some of which share properties of 3α-HSD and/or aldo-keto reductases and, in the authors' opinion, should be classified as such (see under "dihydrodiol dehydrogenase" in this article).

Monomeric 3α-HSD from mouse liver cytosol (M_r 34,000), catalyzing the reversible oxidoreduction of the 3α-hydroxy group of C_{19}-, C_{21}- and C_{24}-steroids, showed a strict specificity for NADP(H) that is clearly different from the dual cosubstrate specificity [NAD(H) and NADP(H)] of the enzyme from rat tissues [74, 76, 112], but it exhibited the same non-specificity towards xenobiotic carbonyl compounds. However, the K_m values for xenobiotics were much higher than those for steroids [89], underlining that this enzyme is 3α-HSD with the additional ability for xenobiotic carbonyl compound reduction, and not vice versa, a carbonyl reductase with HSD properties.

Hamster liver was shown to contain two different 3α-HSDs [87, 113] which were described previously as DDHs [75]. The two monomeric enzymes, although having similar molecular masses of 38,000, differed from each other in pI values, cosubstrate dependence, heat stability and substrate specificity. The NADP(H)-linked enzyme catalyzed the oxidoreduction of various 3α-hydroxysteroids, whereas the NAD(H)-linked enzyme was active also on the 17β-hydroxy group of testosterone and androstanes. The NADP(H)-dependent 3α-HSD of hamster liver has been reported to cross-react immunologically with mouse liver 3α-HSD, but not with hamster liver NAD(H)-dependent 3α-HSD. The two proteins also gave different fragmentation patterns by gel electrophoresis after digestion with protease. Thus, hamster liver contains a 3α(17β)-HSD structurally and functionally distinct from 3α-HSD.

Since the 3α- and 17β-HSD activities were depressed to similar degrees by various inhibitors, the NAD(H)-dependent enzyme may catalyze the dehydrogenation on the two different positions of the steroid molecule at the same catalytic site. Consequently, the hydroxy group at both the A and D rings of the steroid molecule may be able to bind to the active center of the enzyme. This points to the fact that the active site of certain hydroxysteroid dehydrogenases allows access of steroid substrates with dual orientation of the steroid molecule as well as with tolerable variations in their molecular structure as regards stereochemical and functional group configuration of the steroid nucleus. However, both enzymes were active on a variety of aromatic aldehydes and ketones, underlining their importance as a means in the detoxification of xenobiotic carbonyl compounds [75].

Purified human liver 3α-HSD [100] shows extensive similarities in amino acid sequence, immunological reactivity, and enzymatic properties to human chlordecone reductase [114], which was described previously as a 36- kDa monomeric oxidoreductase that is capable of reducing the organochlorine pesticide chlordecone to its alcohol metabolite. There is strong evidence that human 3α-HSD and chlordecone reductase are, in fact, identical proteins [23]. Hence, 3α-HSD catalyzes chlordecone carbonyl reduction, which leads to increased biliary excretion of the pesticide and concomitant reduction of its neurotoxicity, since bile is the major excretory route [115]. Interestingly, human liver has been reported to contain several DDHs, two of which were found to be 3α-HSDs [77, 81] based on functional properties. In addition to dihydrodiol oxidation, both enzymes effectively catalyzed reduction of xenobiotic carbonyl compounds, which emphasizes the role of 3α-HSD in drug metabolism.

Rat liver 3α-HSD has been crystallized for X-ray studies, and its cDNA has been cloned and sequenced [91, 116, 117]. The deduced aminoacid sequence shows 69% identity with bovine lung prostaglandin F synthase [91, 118], thus proving its affiliation to the aldo-keto reductase superfamily [2]. The fact that prostaglandin F synthase is capable of metabolizing some of the same xenobiotics as the rat hepatic 3α-HSD, but inefficiently reduces dihydrotestosterone [119], together with the competitive inhibition of 3α-HSD by indomethacin and its capacity to oxidize hydroxyprostaglandins [76, 109] confirms the close homology between these two proteins.

Microsomal 3α-hydroxysteroid dehydrogenase

Aside from the cytosolic forms, 3α-HSDs are also localized in the microsomal fractions of human [120] and rat liver [121–124] as well as rat kidney [125], among which several exhibit reductase activity towards xenobiotic aldehydes and ketones [64–67].

Rat liver microsomal 3α-HSD is a monomeric 32,000 kDa enzyme, which was found to be active exclusively with NADPH as cosubstrate and which effectively reduces xenobiotic carbonyl compounds such as 2,3-butanedione, 4-nitrobenzaldehyde, acetophenone, pyridine-4-aldehyde and 4-nitroacetophenone. However, 3-keto reduction with 5α-dihydrotestosterone and 5β-dihydrotestosterone gave the lowest K_m values. Testosterone treatment led to significant rises of the enzyme activity, indicating that the enzyme is inducible by androgens [67].

Guinea pig liver microsomes contain two immunologically distinct hydroxysteroid dehydrogenases [65, 66]. The major form is a tetrameric glycoprotein of single subunits of M_r 32,000, which was shown to reduce 5α- and 5β-dihydrotestosterone irreversibly. The minor form is a monomeric protein with M_r 34,000, which not only reduces 17-oxosteroids but also oxidizes 17-hydroxysteroids. The major form may be 3α-HSD (although only described as 3-ketosteroid reductase) and the minor form should be 17β-HSD. Nevertheless, both enzymes were capable of reducing a variety of xenobiotic carbonyl compounds including, for example, 2-(2-amino-5-bromobenzoyl)pyridine (a metabolite of bromazepam), metyrapone, cyclohexanone, menadione, α-naphthoquinone and phenanthraquinone.

Thus, in liver microsomes, at least in rats and guinea pigs, hydroxysteroid dehydrogenases are involved in xenobiotic carbonyl reduction and play a role as drug-metabolizing enzymes in the detoxification of biologically active carbonyl compounds to more hydrophilic and less active alcohols. As no structural data on these membrane-associated hydroxysteroid dehydrogenases are available today, an assignment to any protein superfamily cannot be accomplished.

20α-Hydroxysteroid dehydrogenase

20α-HSD catalyzes the interconversion of 20-oxo pregnanes into 20α-hydroxy derivatives and is distributed in various mammalian and non-mammalian species [47, 126–129]. In general, these are monomeric proteins with M_r values of 35,000–40,000, which are specific for 20α-hydroxysteroid substrates, although some were described to exhibit additional 3α-HSD activity for androstanes and pregnanes [69, 77, 129].

3(20)α-HSD associated with indanol dehydrogenase (cf. "indanol dehydrogenase") from monkey liver [69] catalyzes the reversible conversion of 3α- or 20α-hydroxy groups of several bile acids and 5β-pregnanes to the corresponding 3- or 20-ketosteroids. The k_{cat} values for dehydrogenation of steroids were low, but k_{cat}/K_m values for 3-ketosteroid reduction were comparable to or exceeded those for 1-indanol and xenobiotic carbonyl substrates. Since k_{cat}/K_m values for 3α-hydroxysteroid dehydrogenation were much in favor for a distinct 3α-HSD in the same tissue [130], which may be responsible for the metabolism of various types of steroid hormones and bile acids, this enzyme may act as a 3(20)α-HSD in the metabolism of specific steroids such as progesterone and some bile acids. Nevertheless, high carbonyl reductase activity towards xenobiotic aldehydes and ketones has been observed [69, 77], indicating that 20α-HSD plays an important role in the reductive metabolism of xenobiotic carbonyl compounds.

A dimeric 20α-HSD devoid of 3α-HISD activity has been purified and characterized from the protozoan *Tetrahymena pyriformis* [126]. The enzyme is capable of reducing several non-steroidal carbonyl compounds, such as pyridine-4-aldehyde, pyridine-3-aldehyde and acenaphthenequinone, as well as oxidizing *trans*-benzene dihydrodiol. Although *T. pyriformis* 20α-HSD was inhibited by aldose reductase inhibitors, it resembles aldehyde reductase and dihydrodiol dehydrogenase rather than aldose reductase with respect to substrate specificities. N-terminal amino acid sequence shows (slight) similarity to human placental aldose reductase [2], human liver aldehyde reductase [2], rat liver 3α-HSD [91, 116] and bovine lung prostaglandin F synthase [118], supporting the idea that 20α-HSD from *T. pyriformis* may also belong to the aldo-keto reductase superfamily.

Recently, bovine testicular [47] and rabbit ovarian [127] 20α-HSD have been cloned. Based on amino acid sequence similarities to human chlordecone reductase [114], bovine lung prostaglandin F synthase [118], human aldose reductase [2], human aldehyde reductase [2], and frog lens crystallin [131], this mammalian 20α-HSD can be placed into the aldo-keto reductase superfamily [47, 127]. While oxidoreductase activity for non-steroidal substrates has not been investigated with rabbit ovarian 20α-HSD [127], the bovine testicular enzyme was demonstrated to exhibit reductase activities towards xenobiotic carbonyl compounds [47].

Interestingly, chlordecone reductase [114] has 82% identity with rabbit ovarian 20α-HSD cDNA [127], whereas the organochlorine pesticide chlordecone has ovarian toxicity. Complete identity was reported between bovine testicular 20α-HSD and bovine aldose reductase [47]. This leads to the intriguing suggestion that there may exist several homologies between hydroxysteroid dehydrogenases and aldo-keto reductases near or at the 100% level which have not been recognized yet due to tissue and species variations.

HYDROXYSTEROID DEHYDROGENASES OF THE SHORT-CHAIN DEHYDROGENASE SUPERFAMILY

Procaryotic 3α-hydroxysteroid dehydrogenase

Procaryotic 3α-HSD was first isolated from *Pseudomonas testosteroni* [132], but has been also found in *Clostridium perfringens* [133], *Eubakterium lentum* [134] and *Pseudomonas putida* [133]. 3α-HSD from *P. testosteroni* is inducible by testosterone, and its molecular weight has been reported to be both 100,000 [132] and 47,000 [135] as determined by gel filtration. This discrepancy was explained by the property of the enzyme to exhibit reversible, concentration-dependent monomer–dimer transitions [136]. Analysis by SDS–PAGE revealed that the enzyme consists of two types of subunits of approximately 25,000 [137].

Recently, a novel inducible isoform of 3α-HSD with M_r 28,000 was described in *P. testosteroni* which, in addition to the reversible oxidoreduction

of several 3α-hydroxysteroids with different A/B ring fusions and B ring conformations, is capable of reducing the xenobiotic carbonyl compounds metyrapone, 4-nitrobenzaldehyde and 4-nitroacetophenone [138–140]. No dihydrodiol dehydrogenase activity that distinguishes *P. testosteroni* 3α-HSD from rat liver 3α-HSD could be detected [90]. The N-terminal amino acid sequence shows striking homologies to members of the short-chain dehydrogenase family such that this procaryotic enzyme can be grouped into this family. It thus corresponds to *P. testosteroni* 3β-HSD, which is also classified as a short-chain dehydrogenase [141] and is capable of reducing the carbonyl substrate 4-nitrobenzaldehyde, although no immunological cross-reaction between the two enzymes has been found [140].

11β-Hydroxysteroid dehydrogenase

11β-HSD is a 34 kDa microsomal enzyme responsible for the interconversion of active 11-hydroxyglucocorticoids (cortisol and corticosterone) to inactive 11-oxo forms (cortisone and dehydrocorticosterone) [142]. By this action 11β-HSD protects the Type I mineralocorticoid receptor against active glucocorticoids, the levels of which are 100- to 1000-fold higher than the mineralocorticoids, and thus ensures mineralocorticoid specificity of the non-selective Type I receptor, which has equal affinities to aldosterone and cortisol [143, 144]. Diminished 11β-HSD activity has been associated with the clinical syndrome of apparent mineralocorticoid excess, which is characterized by sodium retention, potassium wasting and hypertension, without measurable increases in aldosterone [145].

11β-HSD activity is also found in glucocorticoid target tissues, notably the liver [146, 147], where it may regulate steroid exposure to the glucocorticoid Type II receptor. It has long been controversially discussed whether 11β-dehydrogenation and 11-oxoreduction are catalyzed by a single bidirectional enzyme or if the 11β-HSD system comprises two kinetically distinct enzymes [148, 149]. Recently, homogenously purified 11β-HSD from mouse liver was shown to contain both 11-dehydrogenation as well as 11-oxoreducing activities of glucocorticoid metabolism, thus disproving the two-enzyme theory [150]. Because the activities of 11β-HSD are much higher in the liver than in the kidney, the liver enzyme was suggested to have other functions in addition to the metabolism of glucocorticoids [151].

Surprisingly, mouse liver 11β-HISD was demonstrated to be capable of catalyzing the reductive metabolism of xenobiotic carbonyl compounds, such as metyrapone, *p*-nitroacetophenone and *p*-nitrobenzaldehyde [152]. Enzyme kinetic studies suggest that both glucocorticoids and xenobiotic carbonyl substances bind to the same catalytically active site of 11β-HSD [152]. Hence, corresponding to other carbonyl reducing enzymes, microsomal 11β-HSD of liver may be considered to play a role in the phase I biotransformation of pharmacologically relevant carbonyl substances as well as protecting organisms against toxic carbonyl compounds by converting them to less lipophilic and more soluble and conjugatable metabolites [152].

The cDNAs of rat liver [153] and human testis [154] 11β-HSD have been cloned, and a comparison of the deduced amino acid sequence to that of other known proteins revealed that 11β-HSD is a member of the short-chain dehydrogenase superfamily [3].

A second, NADH-specific high-affinity isoform of 11β-HSD seems to exist in rat distal tubule and in placenta, which apparently has no capabilities for xenobiotic carbonyl reduction [151, 155]. Several laboratories have been unable to isolate the gene for the kidney 11β-HSD isoform using the liver 11β-HSD cDNA as a probe, which may indicate that the high-affinity kidney form may be encoded by a different gene.

The enzyme 11β-HSD is another example of the versatility of hydroxysteroid dehydrogenases to permit substrates of both steroidal and non-steroidal structures access to the catalytic site. Although steroid oxidoreduction by 11β-HSD is restricted to carbon 11 of the steroid nucleus, thus exhibiting specificity towards glucocorticoids, several other steroids of the androstane and pregnane class were shown to be potent and competitive inhibitors of this enzyme [156]. Hence, on the one hand, a variety of steroids binds to the catalytically active site of the enzyme, but improper orientation of the functional group moiety prevents catalytic activity. On the other hand, certain xenobiotic aldehydes and ketones fit into the substrate binding pocket of 11β-HSD, such that the active site affords hydrogen transfer between the functional groups of the substrate and cosubstrate.

Most carbonyl compounds are lipid soluble and are expected to distribute in membranes rich in lipids. Hence, microsomal hydroxysteroid dehydrogenases may play a more significant role in the reductive metabolism than the cytoplasmic reductases. For a variety of pharmacologically and toxicologically relevant substances, including acetohexamide [157] and haloperidol [158], enzymatic conversion via carbonyl reduction within the microsomal fraction is known, but the lack of exact data prevents an exact assignment to a specific enzyme unless the respective activity can be proved with homogenous enzyme preparations.

17β-Hydroxysteroid dehydrogenase

17β-HSD (EC 1.1.1.62 and EC 1.1.1.64) catalyzes the interconversion of estradiol and estrone as well as testosterone and androstanedione in a variety of tissues with NAD(H) or NADP(H) as cosubstrates [159–161]. The enzyme thus is crucial to the regulation of intracellular levels of biologically active steroid hormones, since both androgens and estrogens bind to their pertinent receptors with higher affinity as 17β-hydroxy steroids than as 17-keto steroids. There is considerable evidence that 17β-reduction and -oxidation of androgens and estrogens are catalyzed by different isozymes with distinct substrate specificities and intracellular localizations. At least hepatic 17β-HSD is present in microsomal and cytosolic fractions [159, 160]. Steroids are not the only substrates for hepatic 17β-HSD, and it has been proposed that the majority of the hepatic benzene dihydrodiol dehydrogenase activity is catalyzed by an $NADP^+$-dependent 17β-HSD [86]. It therefore appears that the hepatic 17β-

HSD is involved in the metabolism of foreign compounds as well as of physiological steroid hormones *in vivo*.

Xenobiotic carbonyl reducing activity of 17β-HSD was first reported by Sawada and coworkers [34], who isolated two NADP(H)-dependent isoforms from guinea pig liver. They showed that both enzymes were capable of reducing a variety of xenobiotic aromatic aldehydes and ketones at the same catalytic site, which is also responsible for 17β-hydroxysteroid oxidoreduction, and proposed that 17β-HSD may play an important role in the metabolism of exogenous carbonyl compounds. In a later study, the same authors purified eight DDHs from the same source [85], seven of which were demonstrated to be 17β-HSD isoforms, whereas one form turned out to be aldehyde reductase. The other seven forms oxidized alicyclic alcohols and benzene dihydrodiol and reduced xenobiotic carbonyl compounds, although 17β-hydroxysteroids showed higher affinities. The identification of most guinea pig DDHs with multiple forms of 17β-HSD isozymes differs from reports that rat liver DDH is identified with 3α-HSD [74, 76], but is in agreement with the observation that mouse liver DDH is 17β-HSD [83, 84]. Nevertheless, mouse liver 17β-HSD also catalyzes carbonyl compound reduction and is even suggested to be identical to indanol dehydrogenase and carbonyl reductase [84]. Multiple forms of 17β-HSD associated with xenobiotic carbonyl reducing activities have been reported from rabbit liver [68, 78]. A dimeric 17β-HSD with dual cosubstrate specificity and carbonyl reductase activity has been described in chicken liver [57], whereas a microsomal form of 17β-HSD that catalyzes a variety of xenobiotic carbonyl compounds has been shown in guinea pig liver [66].

Recently, a human placental 17β-HSD cDNA has been cloned [162], and preliminary X-ray diffraction data of a crystallized active enzyme form have been reported [163]. 17β-HSD belongs to the short-chain dehydrogenase superfamily [3], and one enzyme form (17β-HSD type 2) was demonstrated to possess a pronounced 20α-HSD activity towards progestins [162].

3α,20β-Hydroxysteroid dehydrogenase

3α,20β-HSD, also known as 20β-HSD or as cortisone reductase, catalyzes the interconversion of 20β-hydroxysteroids to their respective 20-keto derivatives as well as the 3α- and 3β-oxidoreduction of a variety of androstanes [164–167], and has thus far been regarded as a polyfunctional enzyme [167]. An NAD(H)-linked 3α,20β-HSD was first purified from *Streptomyces hydrogenans* [168] and subsequently shown to be highly inducible by steroids [169]. Based on the primary structure, the enzyme was classified as a member of the short-chain dehydrogenase superfamily [170]. The enzyme is active as a tetramer [171] and was the first hydroxysteroid dehydrogenase for which the X-ray crystallographic structure was reported [172].

Reductase activity towards non-steroidal substrates was demonstrated by Gibb and Jeffery [173], who showed that the crystalline preparation of *S. hydrogenans* 3α,20β-HSD reduced adamantanone.

A mammalian, NADP(H)-dependent 3α,20β-HSD from neonatal pig testis has been purified [166] and crystallized [174]. The enzyme also exhibits high reductase activities towards non-steroidal carbonyl compounds, which were even higher than the values for steroid oxidoreduction [175]. cDNA cloning and amino acid prediction revealed 85% homology to human carbonyl reductase [175], documenting that 3α,20β-HSD and carbonyl reductase bear both functional as well as structural resemblances. Based on respective sequence homologies, the mammalian 3α,20β-HSD may also be assigned to the short-chain dehydrogenase family [174, 175].

An enzyme–cosubstrate–inhibitor complex of *S. hydrogenans* 3α,20β-HSD with carbenoxolone and NADH was crystallized recently [176]. The preliminary X-ray analysis indicates that the three-dimensional structure is significantly different from the 3α,20β-HSD–NADH (enzyme–cosubstrate) complex [172]. These studies revealed that the steroid-binding pocket of 3α,20β-HSD is a large hydrophobic cleft near the surface and adjacent to the cosubstrate-binding site, in which the carbenoxolone molecule can be accommodated with either its C-3 or C-30 end oriented towards the cosubstrate-binding site [176]. This feature may explain the capability of 3α,20β-HSD to be active on both ends of a steroid substrate, namely as 3α- as well as 20β-oxidoreductase.

Interestingly, an exocyclic-keto reductase activity for progesterone and *S*-warfarin has been described in hepatic microsomes from adult male rats [63]. Although the respective enzyme has not been purified, the functional data obtained suggest that one and the same enzyme catalyzes the reduction of progesterone to its 20β-hydroxy derivative and *S*-warfarin to its 11*S*-hydroxy product. Considering that this enzyme is a microsomal 3α,20β-HSD with putative warfarin reducing activities, this might be another example of the versatility of 3α,20β-HSD to accept steroidal and non-steroidal compounds as substrates.

STRUCTURAL CONSIDERATIONS

The short-chain dehydrogenase superfamily

P. testosteroni 3α-HSD [138–140] and 3β-HSD [141], mammalian 11β-HSD [150, 153, 154], 17β-HSD [162, 163], 20β-HSD [174, 175], *S. hydrogenans* 3α,20β-HSD [170] as well as carbonyl reductase [2, 49] belong to the protein family of the short-chain dehydrogenases [3], which also includes 3β-hydroxy-5-ene-steroid dehydrogenase (EC 1.1.1.145) [177], sepiapterin reductase (EC 1.1.1.153) [178] and dihydropteridine reductase (EC 1.6.99.7) [179]. At least thirty different proteins are known to belong to this family, which forms a diverse group of enzymes, acting on many classes of substrates, e.g. sugars, steroids, alcohols, aromatic aldehydes and ketones, quinones, and prostaglandins. They are found in procaryotes as well as in lower and higher eucaryotes, including humans, which suggests that they originated from a common ancestor at least 1.8 billion years ago. Alignment of the different enzymes reveals large homologous parts with

clustered similarities, indicating regions of special functional and/or structural importance [3].

Although many of the proteins have not yet been characterized enzymatically, there also seem to be similarities in cosubstrate stereospecificity and reaction mechanism in that they transfer the pro-S hydrogen of the pyridine nucleotide and exhibit an ordered Bi-Bi kinetic mechanism by which the cosubstrate binds first and leaves last [6, 165, 180]. Several of the above-described pluripotent hydroxysteroid dehydrogenases show preferential cosubstrate specificity for either NAD(H) or NADP(H) or, in some cases, exhibit dual cosubstrate specificity towards both pyridine nucleotides, a fact that can be explained on the basis of some structural considerations.

A highly conserved region near the N-terminus is part of the coenzyme binding site [3, 181] and is predicted to consist of a $\beta\alpha\beta$ unit, the Rossman fold [182, 183], which is characteristic for dinucleotide binding regions and which has been demonstrated in the crystal structure of 3α,20β-HSD [172]. In addition, three highly conserved glycine residues close to the N-terminus were described in the region predicted to be involved in coenzyme binding [172, 181].

There is also a conserved negatively charged residue (aspartate) at the C-terminus of the second β-strand which forms a hydrogen bond to the 2'-hydroxyl group of the adenine ribose of the NAD^+ [3, 172, 184–186]. This residue is important in discriminating between the cosubstrates NAD(H) and NADP(H), since repulsion between the 2'-phosphate group on the adenosine and the conserved aspartate prevents binding of NADP(H) but permits binding of NAD(H) [184, 187]. Thus, the presence of a negatively charged amino acid in this position appears to explain the preference for NADH over NADPH in enzymes that otherwise are very similar in amino acid sequence. Accordingly, 17β-HSD [57, 161] and 11β-HSD [150, 152] isoforms can use either NADPH or NADH as cofactors, which might be explained by the fact that they contain an alanine and threonine, respectively, in the position where aspartic acid is found in other short-chain dehydrogenases that exhibit NAD(H) specificity [188]. Moreover, both enzymes also contain a nearby arginine residue that could bind the negatively charged phosphate group in the 2'-position of NADP(H) [188].

The C-terminal part of the short-chain dehydrogenases is considered to contain the substrate binding site [3]. This part exhibits many variations in terms of primary structure, which might explain the broad substrate specificities for some short-chain hydroxysteroid dehydrogenases. From the three-dimensional structures of 3α,20β-HSD [172] and dihydropteridine reductase [180] the topologies of the active site with coenzyme bound are known. A well-conserved pentapeptide, beginning at residue 155 in human 17β-HSD [189], together with adjacent conserved residues, is likely to have a general role in catalysis. Chemical modification and site-directed mutagenesis studies identified tyrosine-152 and lysine-156 to be essential for enzyme activity in 3α,20β-HSD, 15-OH-prostaglandin dehydrogenase [190, 191] and *Drosophila* alcohol dehydrogenase [190, 192] as well as their homologues in 11β-HSD [193]. The strong conservation of these two amino acids in enzymes that act on steroids, prostaglandins, sugars and xenobiotic carbonyl compounds suggests that they have an important functional role in substrate binding and catalysis. Nevertheless, in some instances the active site conformation obviously allows access and catalysis of substrates bearing limited variations in terms of their molecular structure.

An important potential for understanding how substrates bind to steroid dehydrogenases has come from the determination of the crystal structure of 3α,20β-HSD by Ghosh *et al.* [172], who successfully modeled cortisone into a putative binding site in the tertiary structure of 3α,20β-HSD of *S. hydrogenans* [176].

The aldo-keto reductase superfamily

As already described above, several hydroxysteroid dehydrogenases have been cloned and sequenced, including rat liver [153] and human testis [154] 11β-HSD, human placental 17β-HSD [194] as well as mammalian 3α,20β-HSD [175] and 3α,20β-HSD from *S. hydrogenans* [170], which all have been shown to belong to the short-chain dehydrogenases, a phylogenetically ancient gene superfamily that includes many enzymes of bacteria and invertebrates as well as mammals [3]. However, rat liver 3α-HSD bears no significant sequence homology with these proteins, indicating that the mammalian liver 3α-HSD does not belong to this group [91, 195]. Rather, comparison of the homology between 3α-HSD and entries in the GenBank and EMBL data banks identifies significant homologies with bovine lung prostaglandin F synthase [118], human placental aldose reductase [2], human liver aldehyde reductase [2], human liver chlordecone reductase [114] and frog lens crystallin [131], suggesting that 3α-HSD belongs to the aldo-keto reductase superfamily [91, 116, 117]. The homology to structural proteins like lens crystallin is worth mentioning since these proteins are known to bind NADPH and to display lactate dehydrogenase [196] as well as low prostaglandin H_2 9,11-endoperoxide reductase activity [197].

As is the case with the short-chain dehydrogenases, the high degree of identity among the members of the aldo-keto reductase superfamily suggests that they have been highly conserved during evolution. In addition, whereas 70% homology has been found between rat liver 3α-HSD and human liver chlordecone reductase [116], even complete identity has been proposed if both enzymes are derived from human liver [23], thus emphasizing species variations.

Alignment of the N-terminal sequence of 20α-HSD from *T. pyriformis* with those of other hydroxysteroid dehydrogenases showed a considerable degree of similarity to bovine testicular 20α-HSD, rat liver 3α-HSD [126], as well as human placental aldose reductase [2], human liver aldehyde reductase [2], and bovine lung prostaglandin F synthase [118], suggesting that this procaryotic 20α-HSD does also belong to the aldo-keto reductase superfamily.

The large number of highly conserved residues makes it difficult to identify residues that might be important in terms of catalytic function. Analysis of the sequences of 3α-HSD and related enzymes revealed a pair of conserved closely spaced glycine residues (Gly-20 and Gly-22) followed 28 residues later by a conserved acidic residue (Asp-50). This region is predicted to be in a βαβ conformation and could, in principle, constitute part of the nucleotide cosubstrate-binding site [116]. Analysis of the deduced amino acid sequence and isolation of the active site peptides by Penning and coworkers [91, 195] are in agreement with this observation and suggest that the pyridine nucleotide binding site may reside at the NH_2-terminus, while the steroid-binding site may reside at the COOH-terminus of rat liver 3α-HSD.

The kinetic mechanism of 3α-HSD predicts an ordered Bi-Bi mechanism in which the cosubstrate binds first and leaves last [99]. These findings imply that during catalysis the pyridine nucleotide binds first to the NH_2-terminus, the steroid substrate binds to the COOH terminus, and the two structural domains are brought together to form the central complex so that dehydrogenation can proceed.

At this point, some common principles between the short-chain dehydrogenases and the aldo-keto reductases should be discussed. In analyzing the primary structures of the SCAD, Jörnvall and coworkers [110] described a motif Tyr-X-X-X-Lys, which is completely conserved. This consensus sequence is also found in 3α-HSD at amino acid residue 205–209 as Tyr-Cys-Lys-Ser-Lys, and is also present in the aldose reductase and rho-crystallin, but not in prostaglandin F synthase [91]. On the other hand, there do not exist any sequence homologies between the aldo-keto reductases and the short-chain dehydrogenases. Hence, the two superfamilies are obviously not related and must have evolved separately. However, they share some common similarities with respect to catalytic function and structural organization. Both families comprise oxidoreductases that can use either NADH and/or NADPH as cosubstrates, among which pluripotent hydroxysteroid dehydrogenases stand out in terms of their additional ability to act as dihydrodiol dehydrogenase, quinone reductase and/or xenobiotic carbonyl reductase. Moreover, the alternating stretches of β-sheets and α-helices with the proposed N-terminal cosubstrate binding site (including conserved glycine residues), together with the Tyr-X-X-X-Lys motif near the putative substrate binding COOH terminus in members of both superfamilies, suggest a common requirement for specific conformational features to afford catalytic activity.

Two interpretations seem feasible: either (1) two different lines branched off from a common ancestor resulting in substantially divergent protein superfamilies, or (2) according to compelling necessities in terms of catalytic function, both superfamilies arose from a different ancestor, thus implying an example of convergent evolution. Nevertheless, the constitutivity and overall abundance emphasize the biological importance of these pluripotent hydroxysteroid dehydrogenases from both protein superfamilies.

FUNCTIONAL CONSIDERATIONS

Dual specificity at one catalytic site

Dual activity at the active site of a single enzyme is a common feature of steroidogenic oxidoreductases. Kinetic studies and affinity-labeling experiments on bacterial and mammalian hydroxysteroid dehydrogenases have shown that some of these enzymes exhibit dual substrate specificity, a single protein catalyzing oxidoreduction at two distinct positions on the steroid molecule. 3β,17β-HSD activity of *P. testosteroni* [198], 3α,20β-HSD activity of *S. hydrogenans* [165, 199, 200], 3α,3β,20β-HSD from pig testis [166], 3(17)α-HSD from mouse kidney [80], 3α(17β)-HSD of hamster and male rabbit liver [68, 75, 87], 3β,20α-HSD from fetal calf [201] and fetal lamb [202] erythrocytes, 3α,20α-HSD from human liver [77] and 17β,20α-HSD activities of human placenta [162, 203] have been reported to be mediated by single enzymes.

Hydroxysteroid dehydrogenases which, on the one hand, show dual steroid substrate specificity for 3α- and 17β-hydroxysteroids and, on the other hand, additionally exhibit specificity towards xenobiotic carbonyl compounds were described in hamster [75, 87, 113] and rabbit liver [68, 78] as well as in the case of 3(17)α-HSD activity in mouse kidney [80]. Since indanol dehydrogenase showed low K_m or high k_{cat}/K_m values for some 3- and 20-ketosteroids, comparable to the values of rat liver 3α-HSD and rat ovary 20α-HSD [69], the enzyme, which has been recognized as a drug-metabolizing enzyme, may act also as a bifunctional 3(20)α-HSD in monkey liver. The bifunctional activity of the enzyme may be important for the conversion of 5β-pregnandione to 5β-pregnane-3α,20α-diol, a major metabolite of progesterone and pregnenolone. Moreover, activities towards even more than two functional groups of the steroid molecule have been reported for 3α,3β,17β,20α-HSD from rabbit liver, which could, therefore, be considered as a polyfunctional hydroxysteroid dehydrogenase [204].

An important advance in understanding the catalytic mechanism of dual activity at the same active site might come from investigations of tertiary structures of enzyme–cosubstrate–substrate/inhibitor complexes. Ghosh *et al.* [176] have crystallized an enzyme–NADH–carbenoxolone complex of *S. hydrogenans* 3α,20β-HSD and provided evidence that the carbenoxolone molecule can be accommodated in the large steroid-binding pocket with either its C-3 or its C-30 end oriented towards the cosubstrate binding site, which might explain the ability of this enzyme to act as a 3α- as well as a 20β-steroid oxidoreductase.

Multiple specificity towards non-steroidal substrates

As described above, several hydroxysteroid dehydrogenases of the two protein superfamilies, the aldo-keto reductases and the short-chain dehydrogenases, exhibit versatility in that they perform catalytic activity towards physiological steroid substrates as well as towards non-steroidal carbonyl-containing xenobiotics. Structural and kinetic data revealed that catalysis proceeds at a common catalytic site. Dual specificity towards

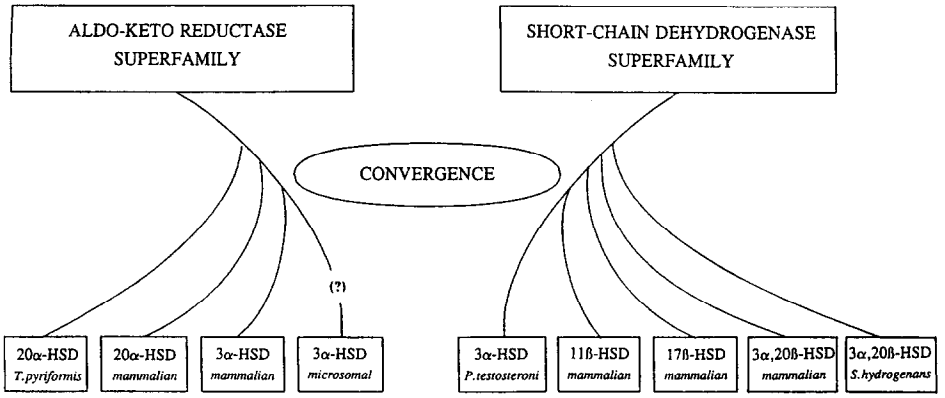

Fig. 1. Pluripotent hydroxysteroid dehydrogenases involved in xenobiotic carbonyl reduction.

different functional groups at the steroid nucleus already emphasizes the complex steroid-binding characteristics and may be consistent with the view that the active site is larger than a steroid molecule [48, 173, 176].

This leads to the intriguing concept that several hydroxysteroid dehydrogenases may have broader substrate specificity than originally anticipated. Possibly, certain xenobiotic aldehydes and ketones either have the same orientation within the active site comparable to the physiological steroid substrate of a given enzyme, or the xenobiotic molecule is small enough to perform flexible orientation within the active site, such that hydrogen transfer between the functional groups of the substrate and cosubstrate can proceed. However, in these cases the large size conformation of the substrate binding pocket provides pluripotency towards the respective enzymes.

To assess the biological importance of the pluripotency of these particular hydroxysteroid dehydrogenases several questions have to be answered: (1) What is the specific physiological function of these enzymes? (2) Do they have more than one specific function? (3) From what kind of ancestral precursor protein have these hydroxysteroid dehydrogenases evolved? (4) What role did these ancestral molecules fulfill in ancient organisms? (5) Were they specific towards yet unknown substrates with perhaps steroid-like structure? (6) What is the reason for their evolutionary conservation? At this point a philosophical approach should be indicated.

As already stated above, the constitutivity and overall abundance of these pluripotent hydroxysteroid dehydrogenases emphasize their biological significance, which is supported by the fact that the coincident ability of enzymes for hydroxysteroid dehydrogenation and xenobiotic carbonyl reduction has evidently been invented twice and subsequently been conserved during evolution in two different protein superfamilies, corresponding to the principle of convergent evolution (Fig. 1). It is possible that early ancestors of today's pluripotent oxidoreductases originally functioned in the catabolism of a variety of carbonyl compounds as a means of using even complex polycyclic hydrocarbons as a source of carbon and energy. Evidence for such considerations is provided by the fact that certain procaryotes, i.e. *P. testosteroni*, can grow on steroids or polycyclic hydrocarbons as its sole carbon and energy source [132, 205]. Moreover, *P. testosteroni* is inducible by various steroids [132] and, upon induction, expresses a 3α-HSD isoform which exhibits versatile substrate specificities towards steroids and non-steroidal carbonyl compounds [138–140].

Two other examples for the occurrence of pluripotent hydroxysteroid dehydrogenases in lower organisms are 3α,20β-HSD from *S. hydrogenans*, which mediates ketone-reduction of adamantanone [173] and 20α-HSD from the protozoan *T. pyriformis*, which reduces several non-steroidal carbonyl compounds and oxidizes *trans*-benzene dihydrodiol [126].

In the course of time and due to evolutionary pressure, some hydroxysteroid dehydrogenases may have acquired absolute specificity towards a given steroid molecule. On the other hand, the enduring presence of xenobiotic carbonyls may have led to the persistence of the multifunctional potency of other hydroxysteroid dehydrogenases, which today may serve several functions. One of these functions, as postulated by numerous authors, is that these hydroxysteroid dehydrogenases play a role as drug-metabolizing enzymes in the detoxification of biologically active carbonyl compounds to more hydrophilic, conjugatable and less active alcohols. However, besides multicellular organisms like mammals, procaryotes and lower eucaryotes also have to deal with injurious and toxic molecules, which suggests that the respective enzymes may already have served as detoxifying tools in ancient organisms.

Another idea is that pluripotent hydroxysteroid dehydrogenases have evolved from ancestral proteins that have been developed as a device in the regulation of hydrophobic signal molecules in unicellular organisms. In higher organisms, the hormone system serves as a complex intercellular communication system and is dependent on intracellular hormone

receptor specificity and the existence of hydroxysteroid dehydrogenases which regulate the intracellular active hormone level. This resembles, in many aspects, the situation in *Rhizobium*/plant signal transduction, where hydrophobic plant flavonoids as well as different bacterial gene products act as signal molecules in the interspecies communication. Sequence analysis of these gene products, NodG protein from *Rhizobium meliloti* [206] and FixR protein from *Bradyrhizobium japonicum* [207] (bacteria that form nitrogen-fixing nodules in the roots of alfalfa and soybeans, respectively), revealed an unexpected relationship to the pluripotent hydroxysteroid dehydrogenases of the short-chain dehydrogenase superfamily [3, 189]. In addition, some flavonoids have steroid-like actions in mammals as well as anti-cancer activity [208–212].

Sequence homologies of procaryotic enzymes, such as *S. hydrogenans* 3α,20β-HSD and *P. testosteroni* 3α-HSD, to 11β-HSD and 17β-HSD of mammalian tissues [138, 140, 213] support the idea that the original function of several hydroxysteroid dehydrogenases could have been the metabolic regulation of hydrophobic signal molecules in unicellular organisms [213], although the hydrophobic signal molecules are unknown.

Nevertheless, the broad substrate specificity of these hydroxysteroid dehydrogenases for non-steroidal compounds suggests that, at an earlier stage of evolution, they were incorporated into bacteria as a means of detoxifying xenobiotic chemicals. This property may have been conserved during evolution, such that these enzymes "today" play an additional role in drug metabolism in mammalian tissues. However, if ancestors of the aldo-keto reductases or short-chain dehydrogenases indeed had more specific functions in unicellular organisms, it would be interesting to speculate by which evolutionary strategy they have acquired their diverse substrate spectrum of today (or pluripotency in case of the hydroxysteroid dehydrogenases). In this context the principle of "gene sharing" may be of prominent significance.

Gene sharing as an evolutionary strategy was first described for duck lactate dehydrogenase and duck lens crystallin, and means that a functional gene acquires and maintains a second function without duplication and without loss of the primary function [196, 214, 215]. This process thus differs from the use of a single gene to generate more than one protein with different functions by alternative RNA splicing, DNA rearrangement, or posttranslational modifications. Such genes are under two or more different constraints, which might significantly slow the evolutionary process for parts of these genes while elsewhere accelerated modifications take place. Gene sharing may occur widely in nature and may, perhaps initiated by the acquisition of new gene promoter elements, precede gene duplication, specialization and subsequent divergence.

Gene sharing adds to the compactness of the genome, which is of considerable priority in procaryotic genomes and which could have been the primary cause for the development and multiplicity of the aldo-keto reductase and short-chain dehydrogenase protein superfamilies. It is possible that an ancestral gene, encoding for a protein with a specific function (for example, metabolic regulation of signal molecules), may have acquired additional roles (for example, catabolism of polycyclic carbonyl compounds as a source of carbon and energy), which during the course of evolution and due to adaptive modifications and selections have resulted in the establishment of a protein superfamily, whose members, on the one hand, are evolutionarily related, but, on the other hand, act on diverse substrates such as steroids, sugars, prostaglandins, quinones and a variety of aromatic aldehydes and ketones. Hence, whereas the aldo-keto reductases and short-chain dehydrogenases obviously have evolved from a different ancestor and show a classic example of convergent evolution, the pluripotent specificity of some of their members, i.e. hydroxysteroid dehydrogenases, may illustrate gene sharing as an alternative evolutionary approach for attaining diversity towards a broad substrate spectrum.

PROSPECTIVE VIEW—CONCLUSIONS

In view of the role that these pluripotent hydroxysteroid dehydrogenases have in regulating the actions of their physiological steroid substrates (and thus contribute to the maintenance of normal cell physiology), together with their widespread capability for the metabolism of non-steroidal carbonyl compounds, insights into mechanistic characteristics of enzyme function as well as predictions of catalytic activity and substrate interactions seem useful and have an important application in providing means for specific modifications in terms of a therapeutic benefit. Information and rational explanations for their broad substrate specificity and non-specific nature towards xenobiotic carbonyl compounds also seem desirable in view of the increasing roles being proposed for several multifunctional hydroxysteroid dehydrogenases in drug metabolism and carbonyl compound detoxification.

First of all, intensive genomic and cDNA analysis will be required to determine the origin and precise relationship of these proteins, which eventually will contribute to the elucidation of their ancient as well as current physiological functions. To define the topography of the active center and to identify the substrate-binding domains, functional residues can be predicted by multiple sequence comparisons and be confirmed by affinity labeling, thus proving the multifunctional nature of the various pluripotent hydroxysteroid dehydrogenases. Site-directed mutagenesis combined with the overexpression of the wild-type and the mutant cDNA in cultured cells in the presence of physiological steroid substrates or non-steroidal xenobiotic carbonyl compounds will then be necessary to assess the importance of these residues.

More direct data concerning the functional significance of conserved residues and the interaction of substrates with the catalytic site may come from the determination of the crystal structure of the enzyme together with the modeling of potential substrates or competitive inhibitors along with

cosubstrates into the putative binding pocket of the tertiary structure of the respective enzyme. Subsequent analysis of enzyme–substrate–cosubstrate complexes is likely to yield new information on the molecular interactions and how hydride transfer occurs.

In conclusion, the pluripotent hydroxysteroid dehydrogenases of both superfamilies, the aldo-keto reductases and the short-chain dehydrogenases, may have a more significant and hitherto not fully appreciated role in general cellular metabolism. Detailed knowledge on structural and functional properties of these pluripotent enzymes possibly provides a molecular understanding of the catalytic mechanism and may allow the rational design of specific effectors that might be used in pharmacologic therapy.

REFERENCES

1. Nelson DR, Kamataki T, Waxman DJ, Guengerich FP, Estabrook RW, Feyereisen R, Gonzalez FJ, Coon MJ, Gunsalus IC, Gotoh O, Okuda K and Nebert DW, The P450 superfamily: Update on new sequences, gene mapping, accession numbers, early trivial names of enzymes, and nomenclature. *DNA Cell Biol* **12**: 1–51, 1993.
2. Bohren KM, Bullock B, Wermuth B and Gabbay KH, The aldo-keto reductase superfamily. cDNAs and deduced amino acid sequences of human aldehyde and aldose reductases. *J Biol Chem* **264**: 9547–9551, 1989.
3. Persson B, Krook M and Jörnvall H, Characteristics of short-chain alcohol dehydrogenases and related enzymes. *Eur J Biochem* **200**: 537–543, 1991.
4. Felsted RL and Bachur NR, Mammalian carbonyl reductases. *Drug Metab Rev* **11**: 1–60, 1980.
5. Ikeda M, Ezaki M, Kokeguchi S and Ohmori S, Studies on NADPH-dependent chloral hydrate reducing enzymes in rat liver cytosol. *Biochem Pharmacol* **30**: 1931–1939, 1981.
6. Higuchi T, Imamura Y and Otagiri M, Kinetic studies on the reduction of acetohexamide catalyzed by carbonyl reductase from rabbit kidney. *Biochim Biophys Acta* **1158**: 23–28, 1993.
7. Imamura Y, Nozaki Y, Higuchi T and Otagiri M, Reactivity for prostaglandins and inhibition by nonsteroidal anti-inflammatory drugs of rabbit liver befunolol reductase. *Res Commun Chem Pathol Pharmacol* **71**: 49–57, 1991.
8. Imamura Y, Higuchi T, Nozaki Y, Sugino E, Hibino S and Otagiri M, Purification and properties of carbonyl reductase from rabbit kidney. *Arch Biochem Biophys* **300**: 570–576, 1993.
9. Moreland TA and Hewick DS, Studies on a ketone reductase in human and rat liver and kidney soluble fraction using warfarin as a substrate. *Biochem Pharmacol* **24**: 1953–1957, 1975.
10. Hermans JJR and Thijssen HHW, Stereoselective acetonyl side chain reduction of warfarin and analogs. Partial characterization of two cytosolic carbonyl reductases. *Drug Metab Dispos* **20**: 268–274, 1992.
11. Chang WH, Reduced haloperidol: A factor in determining the therapeutic benefit of haloperidol treatment? *Psychopharmacology (Berlin)* **106**: 289–296, 1992.
12. Loveless H, Arena E, Felsted RL and Bachur NR, Comparative mammalian metabolism of adriamycin and daunorubicin. *Cancer Res* **38**: 593–596, 1978.
13. Roerig S, Fujimoto JM, Wang RIH, Pollock SH and Lange D, Preliminary characterization of enzymes for reduction of naloxone and naltrexone in rabbit and chicken liver. *Drug Metab Dispos* **4**: 53–58, 1976.
14. Ward A and Clissold SP, Pentoxifylline. A review of its pharmacodynamic and pharmacokinetic properties, and its therapeutic efficacy. *Drugs* **34**: 50–97, 1987.
15. Bodor N and Prokai L, Site- and stereospecific ocular drug delivery by sequential enzymatic bioactivation. *Pharm Res* **7**: 723–725, 1990.
16. Maser E and Legrum W, Alteration of the inhibitory effect of metyrapone by reduction to metyrapol during the metabolism of methacetin *in vivo* in mice. *Naunyn Schmiedebergs Arch Pharmacol* **331**: 283–289, 1985.
17. Cusack BJ, Tesnohlidek DA, Loseke VL, Vestal RE, Brenner DE and Olson RD, Effect of phenytoin on the pharmacokinetics of doxorubicin and doxorubicinol in the rabbit. *Cancer Chemother Pharmacol* **22**: 294–298, 1988.
18. Wermuth B, Platt KL, Seidel A and Oesch F, Carbonyl reductase provides the enzymatic basis of quinone detoxication in man. *Biochem Pharmacol* **35**: 1277–1282, 1986.
19. Hayes JD, Judah DJ and Neal GE, Resistance to aflatoxin B_1 is associated with the expression of a novel aldo-keto reductase which has catalytic activity towards a cytotoxic aldehyde-containing metabolite of the toxin. *Cancer Res* **53**: 3887–3894, 1993.
20. Judah DJ, Hayes JD, Yang J-C, Lian L-Y, Roberts GCK, Farmer PB, Lamb JH and Neal GE, A novel aldehyde reductase with activity towards a metabolite of aflatoxin B_1 is expressed in rat liver during carcinogenesis and following the administration of an anti-oxidant. *Biochem J* **292**: 13–18, 1993.
21. Parekh HK and Sladek NE, NADPH-dependent enzyme-catalyzed reduction of aldophosphamide, the pivotal metabolite of cyclophosphamide. *Biochem Pharmacol* **46**: 1043–1052, 1993.
22. Inoue S, Sharma RC, Schimke RT and Simoni RD, Cellular detoxification of tripeptidyl aldehydes by an aldo-keto reductase. *J Biol Chem* **268**: 5894–5898, 1993.
23. Binstock JM, Iyer RB, Hamby CV, Fried VA, Schwartz IS, Weinstein BI and Southren AL, Human hepatic 3α-hydroxysteroid dehydrogenase: Possible identity with human hepatic chlordecone reductase. *Biochem Biophys Res Commun* **187**: 760–766, 1992.
24. Pietruszko R, Crawford K and Lester D, Comparison of substrate specificity of alcohol dehydrogenases from human liver, horse liver, and yeast towards saturated and 2-enoic alcohols and aldehydes. *Arch Biochem Biophys* **159**: 50–60, 1973.
25. Wermuth B, Aldo-keto reductases. In: *Enzymology of Carbonyl Metabolism: Aldehyde Dehydrogenase, Aldo/Keto Reductase, and Alcohol Dehydrogenase* (Eds. Flynn TG and Weiner H), pp. 209–230. Alan R. Liss, New York, 1985.
26. Flynn TG, Aldehyde reductases: Monomeric NADPH-dependent oxidoreductases with multifunctional potential. *Biochem Pharmacol* **31**: 2705–2712, 1982.
27. Mano Y, Suzudi K, Yamada K and Shinazono N, Enzymic studies on TPN L-hexonate dehydrogenase from rat liver. *J Biochem (Tokyo)* **49**: 618–634, 1961.
28. Park YS, Heizmann CW, Wermuth B, Levine RA, Steinerstauch P, Guzman J and Blau N, Human carbonyl and aldose reductases: New catalytic functions in tetrahydrobiopterin biosynthesis. *Biochem Biophys Res Commun* **175**: 738–744, 1991.
29. Flynn TG and Green NC, The aldo-keto reductases: An overview. In: *Enzymology and Molecular Biology of Carbonyl Metabolism 4* (Eds. Weiner H, Crabb DW and Flynn TG), pp. 251–257. Plenum Press, New York, 1993.
30. Daly AK and Mantle J, Purification and charac-

terization of the multiforms of aldehyde reductase in ox kidney. *Biochem J* **205**: 373–380, 1982.
31. Nakayama T, Hara A, Yashiro K and Sawada H, Reductases for carbonyl compounds in human liver. *Biochem Pharmacol* **34**: 107–117, 1985.
32. Von Wartburg JP and Wermuth B, Aldehyde reductase. In: *Enzymatic Basis of Detoxication* (Ed. Jakoby WB), pp. 249–260. Academic Press, New York, 1980.
33. Pietruszko R and Chen FF, Aldehyde reductase from rat liver is a 3α-hydroxysteroid dehydrogenase. *Biochem Pharmacol* **25**: 2721–2725, 1976.
34. Sawada H, Hara A, Hayashibara M and Nakayama T, Guinea pig liver aromatic aldehyde-ketone reductases identical with 17β-hydroxysteroid dehydrogenase isozymes. *J Biochem (Tokyo)* **86**: 883–892, 1979.
35. Hoffmann PL, Wermuth B and von Wartburg JP, Human brain aldehyde reductases: Relationship to succinic semialdehyde reductase and aldose reductase. *J Neurochem* **35**: 354–366, 1980.
36. Wermuth B and Monder C, Aldose and aldehyde reductase exhibit isocorticosteroid reductase activity. *Eur J Biochem* **131**: 423–426, 1983.
37. Ikeda M, Hattori H and Ohmori S, Properties of NADPH-dependent carbonyl reductases in rat liver cytosol. *Biochem Pharmacol* **33**: 3957–3961, 1984.
38. Sawada H, Hara A, Nakayama T and Kato F, Reductases for aromatic aldehydes and ketones from rabbit liver. Purification and characterization. *J Biochem (Tokyo)* **87**: 1153–1165, 1980.
39. Hara A, Deyashiki Y, Nakayama T and Sawada H, Isolation and characterization of multiforms of aldehyde reductase in chicken kidney. *Eur J Biochem* **133**: 207–214, 1983.
40. Iwata N, Inazu N and Satoh T, The purification and properties of aldose reductase from rat ovary. *Arch Biochem Biophys* **282**: 70–77, 1990.
41. Greene D, The pathogenesis and prevention of diabetic neuropathy and nephropathy. *Metabolism* **37**: 25–29, 1988.
42. Stribling D, Armstrong FM, Hardman M, Perkins CM and Smith JC, Aldose reductase in the etiology of diabetic complications: 4. Retinopathy. *J Diabetic Complications* **4**: 102–107, 1990.
43. Bekhor I, Shi S, Carper D, Nishimura C and Unakar NJ, Relative abundance of aldose reductase mRNA in rat lens undergoing development of osmotic cataracts. *Curr Eye Res* **8**: 1299–1308, 1989.
44. Garcia-Perez A and Burg MB, Renal medullary organic osmolytes. *Physiol Rev* **71**: 1081–1115, 1991.
45. Nishimura C, Graham C, Hohman TC, Nagata M, Robison WG Jr and Carper D, Characterization of mRNA and genes for aldose reductase in rat. *Biochem Biophys Res Commun* **153**: 1051–1059, 1988.
46. Wermuth B, Bürgisser H, Bohren K and von Wartburg JP, Purification and characterization of human-brain aldose reductase. *Eur J Biochem* **127**: 279–284, 1982.
47. Warren JC, Murdock GL, Ma Y, Goodman SR and Zimmer WE, Molecular cloning of testicular 20α-hydroxysteroid dehydrogenase: Identity with aldose reductase. *Biochemistry* **32**: 1401–1406, 1993.
48. Wilson DK, Bohren KM, Gabbay KH and Quiocho FA, An unlikely sugar substrate site in the 1.65 Å structure of the human aldose reductase holoenzyme implicated in diabetic complications. *Science* **257**: 81–84, 1992.
49. Wermuth B, Bohren KM, Heinemann G, von Wartburg JP and Gabbay KH, Human carbonyl reductase. Nucleotide sequence analysis of a cDNA and amino acid sequence of the encoded protein. *J Biol Chem* **263**: 16185–16188, 1988.
50. Jarabak J and Harvey RG, Studies on three reductases which have polycyclic aromatic hydrocarbon quinones as substrates. *Arch Biochem Biophys* **303**: 394–401, 1993.
51. Wermuth B, Purification and properties of an NADPH-dependent carbonyl reductase from human brain. *J Biol Chem* **256**: 1206–1213, 1981.
52. Chang DG-B and Tai H-H, Prostaglandin 9-keto-reductase/type II 15-hydroxyprostaglandin dehydrogenase is not a prostaglandin specific enzyme. *Biochem Biophys Res Commun* **101**: 898–904, 1981.
53. Iwata N, Inazu N and Satoh T, The purification and properties of NADPH-dependent carbonyl reductases from rat ovary. *J Biochem (Tokyo)* **105**: 556–564, 1989.
54. Iwata N, Inazu N, Takeo S and Satoh T, Carbonyl reductases from rat testis and vas deferens. Purification, properties and localization. *Eur J Biochem* **193**: 75–81, 1990.
55. Inazu N, Ruepp B, Wirth H and Wermuth B, Carbonyl reductase from human testis: Purification and comparison with carbonyl reductase from human brain and rat testis. *Biochim Biophys Acta* **1116**: 50–56, 1992.
56. Schieber A, Frank RW and Ghisla S, Purification and properties of prostaglandin 9-ketoreductase from pig and human kidney. Identity with human carbonyl reductase. *Eur J Biochem* **206**: 491–502, 1992.
57. Nishinaka T, Kinoshita Y, Terada N, Terada T, Mizoguchi T and Nishihara T, Characterization of multiple forms of carbonyl reductase from chicken liver. *Enzyme* **46**: 221–228, 1992.
58. Oritani H, Deyashiki Y, Nakayama T, Hara A, Sawada H, Matsuura K, Bunai Y and Ohya I, Purification and characterization of pig lung carbonyl reductase. *Arch Biochem Biophys* **292**: 539–547, 1992.
59. Nakanishi M, Deyashiki Y, Nakayama T, Sato K and Hara A, Cloning and sequence analysis of a cDNA encoding tetrameric carbonyl reductase of pig lung. *Biochem Biophys Res Commun* **194**: 1311–1316, 1993.
60. Maser E, Gebel T and Netter KJ, Carbonyl reduction of metyrapone in human liver. *Biochem Pharmacol* **42** (Suppl): S93–S98, 1991.
61. Oppermann UC, Maser E, Mangoura SA and Netter KJ, Heterogeneity of carbonyl reduction in subcellular fractions and different organs in rodents. *Biochem Pharmacol* **42** (Suppl): S189–S195, 1991.
62. Gebel T and Maser E, Characterization of carbonyl reducing activity in continuous cell lines of human and rodent origin. *Biochem Pharmacol* **44**: 2005–2012, 1992.
63. Apanovitch D, Kitareewan S and Walz FGJ, Exocyclic-keto reductase activities for progesterone and *S*-warfarin in hepatic microsomes from adult male rats. *Biochem Biophys Res Commun* **184**: 338–346, 1992.
64. Sawada H, Hayashibara M, Hara A and Nakayama T, A possible functional relationship between microsomal aromatic aldehyde-ketone reductase and hexose-6-phosphate dehydrogenase. *J Biochem (Tokyo)* **87**: 985–988, 1980.
65. Sawada H, Hara A, Nakayama T, Usui S and Hayashibara M, Comparative studies on distribution and properties of carbonyl reductase in mammalian tissues. In: *Enzymology of Carbonyl Metabolism: Aldehyde Dehydrogenase and Aldo/Keto Reductase* (Eds. Wermuth B and Weiner H), pp. 275–289. Alan R. Liss, New York, 1982.
66. Usui S, Hara A, Nakayama T and Sawada H, Purification and characterization of two forms of microsomal carbonyl reductase in guinea pig liver. *Biochem J* **223**: 679–705, 1984.
67. Hara A, Usui S, Hayashibara M, Horiuchi T, Nakayama T and Sawada H, Microsomal carbonyl reductase in rat liver. Sex difference, hormonal

regulation, and characterization. In: *Enzymology and Molecular Biology of Carbonyl Metabolism: Aldehyde Dehydrogenase, Aldo-Keto Reductase, and Alcohol Dehydrogenase* (Eds. Weiner H and Flynn TG), pp. 401–414. Alan R. Liss, New York, 1987.

68. Hara A, Kariya K, Nakamura M, Nakayama T and Sawada H, Isolation of multiple forms of indanol dehydrogenase associated with 17β-hydroxysteroid dehydrogenase activity from male rabbit liver. *Arch Biochem Biophys* **249**: 225–236, 1986.
69. Hara A, Nakagawa M, Taniguchi H and Sawada H, 3(20)α-Hydroxysteroid dehydrogenase activity of monkey liver indanol dehydrogenase. *J Biochem (Tokyo)* **106**: 900–903, 1989.
70. Glatt HR, Vogel K, Bentley P and Oesch F, Reduction of benzo(*a*)pyrene mutagenicity by dihydrodiol dehydrogenase. *Nature* **277**: 319–320, 1979.
71. Vogel K, Bentley P, Platt KL and Oesch F, Rat liver cytoplasmic dihydrodiol dehydrogenase. Purification to apparent homogeneity and properties. *J Biol Chem* **255**: 9621–9635, 1980.
72. Penning TM, Dihydrodiol dehydrogenase and its role in polycyclic aromatic hydrocarbon metabolism. *Chem Biol Interact* **89**: 1–34, 1993.
73. van Heyningen R and Pirie E, The metabolism of naphthalene and its toxic effect on the eye. *Biochem J* **102**: 842–852, 1967.
74. Wörner W and Oesch F, Identity of dihydrodiol dehydrogenase and 3α-hydroxysteroid dehydrogenase in rat but not in rabbit liver cytosol. *FEBS Lett* **170**: 263–267, 1984.
75. Sawada H, Hara A, Nakagawa M, Tsukada F, Ohmura M and Matsuura K, Separation and properties of multiple forms of dihydrodiol dehydrogenase from hamster liver. *Int J Biochem* **21**: 367–375, 1989.
76. Penning TM, Mukharji I, Barrows S and Talalay P, Purification and properties of a 3α-hydroxysteroid dehydrogenase of rat liver cytosol and its inhibition by anti-inflammatory drugs. *Biochem J* **222**: 601–611, 1984.
77. Hara A, Taniguchi H, Nakayama T and Sawada H, Purification and properties of multiple forms of dihydrodiol dehydrogenase from human liver. *J Biochem (Tokyo)* **108**: 250–254, 1990.
78. Klein J, Thomas H, Post K, Worner W and Oesch F, Dihydrodiol dehydrogenase activities of rabbit liver are associated with hydroxysteroid dehydrogenases and aldo-keto reductases. *Eur J Biochem* **205**: 1155–1162, 1992.
79. Klein J, Post K, Seidel A, Frank H, Oesch F and Platt KL, Quinone reduction and redox cycling catalysed by purified rat liver dihydrodiol/3α-hydroxysteroid dehydrogenase. *Biochem Pharmacol* **44**: 341–349, 1992.
80. Nakagawa M, Tsukada F, Nakayama T, Matsuura K, Hara A and Sawada H, Identification of two dihydrodiol dehydrogenases associated with 3(17)α-hydroxysteroid dehydrogenase activity in mouse kidney. *J Biochem (Tokyo)* **106**: 633–638, 1989.
81. Deyashiki Y, Taniguchi H, Amano T, Nakayama T, Hara A and Sawada H, Structural and functional comparison of two human liver dihydrodiol dehydrogenases associated with 3α-hydroxysteroid dehydrogenase activity. *Biochem J* **282**: 741–746, 1992.
82. Buller AL, Sharp RB and Penning TM, Characterization of dihydrodiol dehydrogenase in rat H-4IIe hepatoma cells. *Cancer Res* **49**: 6976–6980, 1989.
83. Bolcsak LE and Nerland DE, Purification of mouse liver benzene dihydrodiol dehydrogenases. *J Biol Chem* **258**: 7252–7255, 1983.
84. Sawada H, Hara A, Nakayama T, Nakagawa M, Inoue Y, Hasebe K and Zhang Y-P, Mouse liver dihydrodiol dehydrogenases. Identity of the predominant and a minor form with 17β-hydroxysteroid dehydrogenase and aldehyde reductase. *Biochem Pharmacol* **37**: 453–458, 1988.
85. Hara A, Hasebe K, Hayashibara M, Matsuura K, Nakayama T and Sawada H, Dihydrodiol dehydrogenase in guinea pig liver. *Biochem Pharmacol* **35**: 4005–4012, 1986.
86. Hara A, Hayashibara M, Nakayama T, Hasebe K, Usui S and Sawada H, Guinea-pig liver testosterone 17β-dehydrogenase (NADP) and aldehyde reductase exhibit benzene dihydrodiol dehydrogenase activity. *Biochem J* **225**: 177–181, 1985.
87. Ohmura M, Hara A, Nakagawa M and Sawada H, Demonstration of 3α(17β)-hydroxysteroid dehydrogenase distinct from 3α-hydroxysteroid dehydrogenase in hamster liver. *Biochem J* **266**: 583–589, 1990.
88. Smirnov AN, Estrophilic 3α,3β,17β,20α-hydroxysteroid dehydrogenase from rabbit liver—II. Mechanisms of enzyme-steroid interaction. *J Steroid Biochem* **36**: 617–629, 1990.
89. Hara A, Inoue Y, Nakagawa M, Naganeo F and Sawada H, Purification and characterization of $NADP^+$-dependent 3α-hydroxysteroid dehydrogenase from mouse liver cytosol. *J Biochem (Tokyo)* **103**: 1027–1034, 1988.
90. Smithgall TE, Harvey RG and Penning TM, Regio- and stereospecificity of homogenous 3α-hydroxysteroid-dihydrodiol dehydrogenase for *trans*-dihydrodiol metabolites of polycyclic aromatic hydrocarbons. *J Biol Chem* **261**: 6184–6191, 1986.
91. Pawlowski JE, Huizinga M and Penning TM, Cloning and sequencing of the cDNA for rat liver 3α-hydroxysteroid/dihydrodiol dehydrogenase. *J Biol Chem* **266**: 8820–8825, 1991.
92. Hou YT, Xia W, Pawlowski JE and Penning TM, Rat dihydrodiol dehydrogenase: Complexity of gene structure and tissue-specific and sexually dimorphic gene expression. *Cancer Res* **54**: 247–255, 1994.
93. Taurog JD, Moore RJ and Wilson JD, Partial characterization of the cytosol 3α-hydroxysteroid: $NAD(P)^+$ oxidoreductase of rat ventral prostate. *Biochemistry* **14**: 810–817, 1975.
94. Funder JW, Pearce PT, Myles K and Roy LP, Apparent mineralocorticoid excess, pseudohypoaldosteronism, and urinary electrolyte excretion: Toward a redefinition of mineralocorticoid action. *FASEB J* **4**: 3234–3238, 1990.
95. Tomkins GM, A mammalian 3α-hydroxysteroid dehydrogenase. *J Biol Chem* **218**: 437–447, 1956.
96. Dorfman RI and Dorfman AS, The assay of subcutaneously injected androgens in castrated rat. *Acta Endocrinol (Copenh)* **42**: 245–253, 1963.
97. Liao S, Liang T, Fang S, Castanedo E and Shao TC, Steroid structure and androgenic activity. Specificities involved in the receptor binding and nuclear retention of various androgens. *J Biol Chem* **248**: 6154–6162, 1973.
98. Smithgall TE and Penning TM, Electrophoretic and immunochemical characterization of 3α-hydroxysteroid/dihydrodiol dehydrogenase of rat tissues. *Biochem J* **254**: 715–721, 1988.
99. Askonas LJ, Ricigliano JW and Penning TM, The kinetic mechanism catalysed by homogeneous rat liver 3α-hydroxysteroid dehydrogenase. Evidence for binary and ternary dead-end complexes containing non-steroidal anti-inflammatory drugs. *Biochem J* **278**: 835–841, 1991.
100. Iyer RB, Binstock JM, Schwartz IS, Gordon GG, Weinstein BI and Southren AL, Purification and properties of human hepatic 3α-hydroxysteroid dehydrogenase. *J Steroid Biochem Mol Biol* **43**: 343–349, 1992.
101. Kudo K, Amuro Y, Hada T and Higashino K,

Purification and properties of 3α-hydroxysteroid dehydrogenase as a 3-keto bile acid reductase from human liver cytosol. *Biochim Biophys Acta* **1046**: 12–18, 1990.
102. Stolz A, Takikawa H, Sugiyama Y, Kuhlenkamp J and Kaplowitz N, 3α-Hydroxysteroid dehydrogenase activity of the Y'-bile acid binders in rat liver cytosol. Identification, kinetics, and physiologic significance. *J Clin Invest* **79**: 427–434, 1987.
103. Takikawa H, Stolz A, Sugiyama Y, Yoshida H, Yamanaka M and Kaplowitz N, Relationship between the newly identified bile acid binder and bile acid oxidoreductases in human liver. *J Biol Chem* **265**: 2132–2136, 1990.
104. Takikawa H, Fernandez Checa JC, Kuhlenkamp J, Stolz A, Ookhtens M and Kaplowitz N, Effect of indomethacin on the uptake, metabolism and excretion of 3-oxocholic acid: Studies in isolated hepatocytes and perfused rat liver. *Biochim Biophys Acta* **1084**: 247–250, 1991.
105. Takikawa H, Stolz A, Kuroki S and Kaplowitz N, Oxidation and reduction of bile acid precursors by rat hepatic 3α-hydroxysteroid dehydrogenase and inhibition by bile acids and indomethacin. *Biochim Biophys Acta* **1043**: 153–156, 1990.
106. Smithgall TE, Harvey RG and Penning TM, Oxidation of the *trans*-3,4-dihydrodiol metabolites of the potent carcinogen 7,12-dimethylbenz(*a*)anthracene and other benz(*a*)anthracene derivatives by 3α-hydroxysteroid-dihydrodiol dehydrogenase: Effects of methyl substitution on velocity and stereochemical course of *trans*-dihydrodiol oxidation. *Cancer Res* **48**: 1227–1232, 1988.
107. Boutin JA, Camphoroquinone reduction: Another reaction catalyzed by rat liver cytosol 3α-hydroxysteroid dehydrogenase. *Biochim Biophys Acta* **870**: 463–472, 1986.
108. Penning TM and Talalay P, Inhibition of a major NAD(P)-linked oxidoreductase from rat liver cytosol by steroidal and nonsteroidal anti-inflammatory agents and by prostaglandins. *Proc Natl Acad Sci USA* **80**: 4504–4508, 1983.
109. Penning TM and Sharp RB, Prostaglandin dehydrogenase activity of purified rat liver 3α-hydroxysteroid dehydrogenase. *Biochem Biophys Res Commun* **148**: 646–652, 1987.
110. Krook M, Marekov L and Jörnvall H, Purification and structural characterization of placental $NAD^{(+)}$-linked 15-hydroxyprostaglandin dehydrogenase. The primary structure reveals the enzyme to belong to the short-chain alcohol dehydrogenase family. *Biochemistry* **29**: 738–743, 1990.
111. Krook M, Ghosh D, Duax W and Jörnvall H, Three-dimensional model of $NAD^{(+)}$-dependent 15-hydroxyprostaglandin dehydrogenase and relationships to the $NADP^{(+)}$-dependent enzyme (carbonyl reductase). *FEBS Lett* **322**: 139–142, 1993.
112. Ikeda M, Hattori H, Ikeda N, Hayakawa S and Ohmori S, Purification and characterization of the multiple forms of 3α-hydroxysteroid dehydrogenase in rat liver cytosol. *Hoppe Seyler's Z Physiol Chem* **365**: 377–391, 1984.
113. Sawada H, Hara A, Ohmura M, Nakayama T and Deyashiki Y, Kinetic and stereochemical characterization of hamster liver 3α-hydroxysteroid dehydrogenase and 3α(17β)-hydroxysteroid dehydrogenase. *J Biochem (Tokyo)* **109**: 770–775, 1991.
114. Winters CJ, Molowa DT and Guzelian PS, Isolation and characterization of cloned cDNAs encoding human liver chlordecone reductase. *Biochemistry* **29**: 1080–1087, 1990.
115. Molowa DT, Shayne AG and Guzelian PS, Purification and characterization of chlordecone reductase from human liver. *J Biol Chem* **261**: 12624–12627, 1986.
116. Cheng KC, White PC and Qin KN, Molecular cloning and expression of rat liver 3α-hydroxysteroid dehydrogenase. *Mol Endocrinol* **5**: 823–828, 1991.
117. Stolz A, Rahimi-Kiani M, Ameis D, Chan E, Ronk M and Shively JE, Molecular structure of rat hepatic 3α-hydroxysteroid dehydrogenase. A member of the oxidoreductase gene family. *J Biol Chem* **266**: 15253–15257, 1991.
118. Watanabe K, Fujii Y, Nakayama K, Ohkubo H, Kuramitsu S, Kagamiyama H, Nakanishi S and Hayaishi O, Structural similarity of bovine lung prostaglandin F synthase to lens epsilon-crystallin of the European common frog. *Proc Natl Acad Sci USA* **85**: 11–15, 1988.
119. Watanabe K, Yoshida R, Shimizu T and Hayaishi O, Enzymatic formation of prostaglandin F from prostaglandin H and D. *J Biol Chem* **260**: 7035–7041, 1985.
120. Madden S, Back DJ and Orme ML, Metabolism of the contraceptive steroid desogestrel by human liver *in vitro*. *J Steroid Biochem* **35**: 281–288, 1990.
121. Björkhem I, Danielsson H and Wikvall K, Reduction of C_{19}-, C_{21}-, C_{24}-, and C_{27}-3-oxosteroids by rat-liver microsomes. *Eur J Biochem* **36**: 8–15, 1973.
122. Golf SW and Graef V, Isolation of a 3-equatorial-hydroxysteroid dehydrogenase from rat liver microsomes. *FEBS Lett* **64**: 315–318, 1976.
123. Golf SW, Graef V and Nowotny E, Solubilisierung und Anreicherung einer 3α-Hydroxysteroid-Dehydrogenase aus Rattenleber-Mikrosomen. *Hoppe Seyler's Z Physiol Chem* **357**: 35–40, 1976.
124. Golf SW and Graef V, Isolation of a NAD:3β(α)-hydroxy-5β-androstane dehydrogenase from rat liver microsomes. *J Steroid Biochem* **10**: 201–205, 1979.
125. Verhoeven G and DeMoor P, Androgenic control of the microsomal 3α-hydroxysteroid oxidoreductases in rat kidney. *J Steroid Biochem* **8**: 113–119, 1977.
126. Inazu A, Sato K, Nakayama T, Deyashiki Y, Hara A and Nozawa Y, Purification and characterization of a novel dimeric 20α-hydroxysteroid dehydrogenase from *Tetrahymena pyriformis*. *Biochem J* **297**: 195–200, 1994.
127. Lacy WR, Washenick KJ, Cook RG and Dunbar BS, Molecular cloning and expression of an abundant rabbit ovarian protein with 20α-hydroxysteroid dehydrogenase activity. *Mol Endocrinol* **7**: 58–66, 1993.
128. Nakajin S, Fujii S, Ohno S and Shinoda M, [Purification and some properties of 3 alpha-hydroxysteroid dehydrogenase from pig adrenal cytosol]. *Yakugaku Zasshi* **111**: 775–782, 1991.
129. Nakajin S, Fujii S, Ohno S and Shinoda M, 3-Alpha-hydroxysteroid dehydrogenase activity catalyzed by purified pig adrenal 20 alpha-hydroxysteroid dehydrogenase. *J Steroid Biochem Mol Biol* **41**: 179–184, 1992.
130. Nakagawa M, Harada T, Hara A, Nakayama T and Sawada H, Purification and properties of multiple forms of dihydrodiol dehydrogenase from monkey liver. *Chem Pharm Bull (Tokyo)* **37**: 2852–2854, 1989.
131. Tomarev SI, Zinovieva RD, Dolgievich SM, Luchin SV, Krayev AS, Skryabin KG and Gause GG, A novel type of crystallin in the frog eye lens. *FEBS Lett* **171**: 297–302, 1984.
132. Marcus PI and Talalay P, Induction and purification of α- and β-hydroxysteroid dehydrogenases. *J Biol Chem* **218**: 661–674, 1956.
133. Uwajima T, Takayama K and Terada O, Production, purification and crystallization of 3α-hydroxysteroid dehydrogenase of *Pseudomonas putida*. *Agric Biol Chem* **42**: 1577–1583, 1978.
134. MacDonald IA, Jellett JF, Mahony DE and

Holdeman LV, Bile salt 3α- and 12α-hydroxysteroid dehydrogenase from *Eubakterium lentum* and related organisms. *Appl Environ Microbiol* **37**: 992–1000, 1979.
135. Squire PG, Delin S and Porath J, Physical and chemical characterization of hydroxysteroid dehydrogenases from *Pseudomonas testosteroni*. *Biochim Biophys Acta* **89**: 409–421, 1964.
136. Skålhegg BA, On the 3α-hydroxysteroid dehydrogenase from *Pseudomonas testosteroni*. Purification and properties. *Eur J Biochem* **46**: 117–125, 1974.
137. Shikita M and Talalay P, Preparation of highly purified 3α- and 3β-hydroxysteroid dehydrogenase from *Pseudomonas* sp. *Anal Biochem* **95**: 286–292, 1979.
138. Oppermann UC, Maser E, Hermans JJ, Koolman J and Netter KJ, Homologies between enzymes involved in steroid and xenobiotic carbonyl reduction in vertebrates, invertebrates and procaryonts. *J Steroid Biochem Mol Biol* **43**: 665–675, 1992.
139. Maser E, Oppermann UCT, Bannenberg G and Netter KJ, Functional and immunological relationships between metyrapone reductase from mouse liver microsomes and 3α-hydroxysteroid dehydrogenase from *Pseudomonas testosteroni*. *FEBS Lett* **297**: 196–200, 1992.
140. Oppermann CT, Netter KJ and Maser E, Carbonyl reduction by 3 alpha-HSD from *Comamonas testosteroni*—New properties and its relationship to the SCAD family. *Adv Exp Med Biol* **328**: 379–390, 1993.
141. Yin S-J, Vagelopoulos N, Lundquist G and Jörnvall H, *Pseudomonas* 3β-hydroxysteroid dehydrogenase. Primary structure and relationships to other steroid dehydrogenases. *Eur J Biochem* **197**: 359–365, 1991.
142. Monder C and Shackleton CHL, 11β-Hydroxysteroid dehydrogenase: Fact or fancy? *Steroids* **44**: 383–417, 1984.
143. Funder JW, Pearce PT, Smith R and Smith AI, Mineralocorticoid action: Target specificity is enzyme, not receptor mediated. *Science* **242**: 583–585, 1988.
144. Edwards CR, Stewart PM, Burt D, Brett L, McIntyre MA, Sutanto WS, de Kloet ER and Monder C, Localisation of 11 beta-hydroxysteroid dehydrogenase—Tissue specific protector of the mineralocorticoid receptor. *Lancet* **2**: 986–989, 1988.
145. Stewart PM, Corrie JE, Shackleton CH and Edwards CR, Syndrome of apparent mineralocorticoid excess. A defect in the cortisol–cortisone shuttle. *J Clin Invest* **82**: 340–349, 1988.
146. Bush IE, Hunter SA and Meigs RA, Metabolism of 11-oxygenated steroids. *Biochem J* **107**: 239–257, 1968.
147. Keorner DR, Assay and substrate specificity of liver 11β-hydroxysteroid dehydrogenase. *Biochim Biophys Acta* **179**: 377–382, 1969.
148. Lakshmi V and Monder C, Evidence for independent 11-oxidase and 11-reductase activities for 11β-hydroxysteroid dehydrogenase: Enzyme latency, phase transitions and lipid requirement. *Endocrinology* **116**: 552–560, 1985.
149. Monder C and Lakshmi V, Evidence for kinetically distinct forms of corticosteroid 11β-dehydrogenase in rat liver microsomes. *J Steroid Biochem* **32**: 77–83, 1989.
150. Maser E and Bannenberg G, The purification of 11β-hydroxysteroid dehydrogenase from mouse liver microsomes. *J Steroid Biochem Mol Biol* **48**: 257–263, 1994.
151. Maser E, Friebertshäuser J and Mangoura SA, Ontogenic pattern of carbonyl reductase activity of 11β-hydroxysteroid dehydrogenase in mouse liver and kidney. *Xenobiotica* **24**: 109–117, 1994.
152. Maser E and Bannenberg G, 11β-Hydroxysteroid dehydrogenase mediates reductive metabolism of xenobiotic carbonyl compounds. *Biochem Pharmacol* **47**: 1805–1812, 1994.
153. Agarwal AK, Monder C, Eckstein B and White PC, Cloning and expression of rat cDNA encoding corticosteroid 11β-dehydrogenase. *J Biol Chem* **264**: 18939–18943, 1989.
154. Tannin GM, Agarwal AK, Monder C, New MI and White PC, The human gene for 11β-hydroxysteroid dehydrogenase. Structure, tissue distribution, and chromosomal localization. *J Biol Chem* **266**: 16653–16658, 1991.
155. Brown RW, Chapman KE, Edwards CRW and Seckl JR, Human placental 11β-hydroxysteroid dehydrogenase: Evidence for and partial purification of a distinct NAD-dependent isoform. *Endocrinology* **132**: 2614–2621, 1993.
156. Maser E, The purification and properties of a novel carbonyl reducing enzyme from mouse liver microsomes. *Adv Exp Med Biol* **328**: 339–350, 1993.
157. Imamura Y, Iwamoto K, Yanachi Y, Higuchi T and Otagiri M, Postnatal development, sex-related difference and hormonal regulation of acetohexamide reductase activities in rat liver kidney. *J Pharmacol Exp Ther* **264**: 166–171, 1993.
158. Inaba T and Kovacs J, Haloperidol reductase in human and guinea pig livers. *Drug Metab Dispos* **17**: 330–333, 1989.
159. Blomquist CH, Kotts CE and Hakanson EY, Microsomal 17β-hydroxysteroid dehydrogenase of guinea pig liver: Detergent solubilization and a comparison of kinetic and stability properties of bound and solubilized forms. *J Steroid Biochem* **8**: 193–198, 1977.
160. Milewich L, Garcia RL and Gerrity LW, 17β-Hydroxysteroid oxidoreductase: A ubiquitous enzyme. Interconversion of estrone and estradiol-17β in BALB/c mouse tissues. *Metabolism* **34**: 938–944, 1985.
161. Murray M and Horsfield BP, 17β-Hydroxysteroid oxidoreductase activity: Age-dependent profile in rat liver and kinetic properties of the hepatic microsomal enzyme in relation to cytochrome P450-dependent steroid hydroxylation. *J Steroid Biochem Mol Biol* **36**: 569–574, 1990.
162. Wu L, Einstein M, Geissler WM, Chan HK, Elliston KO and Andersson S, Expression cloning and characterization of human 17β-hydroxysteroid dehydrogenase type 2, a microsomal enzyme possessing 20α-hydroxysteroid dehydrogenase activity. *J Biol Chem* **268**: 12964–12969, 1993.
163. Zhu DW, Lee X, Breton R, Ghosh D, Pangborn W, Duax WL and Lin SX, Crystallization and preliminary X-ray diffraction analysis of the complex of human placental 17β-hydroxysteroid dehydrogenase with NADP. *J Mol Biol* **234**: 242–244, 1993.
164. Gibb W and Jeffery J, Studies on the site specificity of a hydroxysteroid dehydrogenase. *Biochem J* **125**: 14P, 1971.
165. Gibb W and Jeffery J, 3-Hydroxysteroid dehydrogenase activities of cortisone reductase. *Biochem J* **135**: 881–888, 1973.
166. Ohno S, Nakajin S and Shinoda M, 20 Beta-hydroxysteroid dehydrogenase of neonatal pig testis: 3 Alpha/beta-hydroxysteroid dehydrogenase activities catalyzed by highly purified enzyme. *J Steroid Biochem Mol Biol* **38**: 787–794, 1991.
167. Ohno S, Nakajin S and Shinoda M, Ontogeny of testicular steroid dehydrogenase enzymes in pig (3 alpha/beta-, 20 alpha- and 20 beta-): Evidence for two forms of 3 alpha/beta-hydroxysteroid dehydrogenase. *J Steroid Biochem Mol Biol* **42**: 17–21, 1992.
168. Hübener HJ, Sahrholz FG, Schmidt-Thomé J, Nesemann G and Junk R, 20β-Hydroxy-Steroid-

Dehydrogenase, ein neues kristallines Enzym. *Biochim Biophys Acta* **35**: 270–272, 1959.
169. Schmidt-Thome J, Nesemann G, Hübener HJ and Alester I, 20β-Hydroxysteroiddehydrogenase III. Substratspezifität der Oxydoreduktion und Induktion. *Biochem Z* **336**: 322–328, 1962.
170. Marekov L, Krook M and Jörnvall H, Prokaryotic 20β-hydroxysteroid dehydrogenase is an enzyme of the "short-chain, non-metalloenzyme" alcohol dehydrogenase type. *FEBS Lett* **266**: 51–54, 1990.
171. Pasta P, Carrea G, Longhi R and Antonini E, Renaturation and urea-induced denaturation of 20β-hydroxysteroid dehydrogenase studied in solution and in the immobilized state. *Biochim Biophys Acta* **616**: 143–152, 1980.
172. Ghosh D, Weeks CM, Grochulski P, Duax WL, Erman M, Rimsay RL and Orr JC, Three-dimensional structure of holo 3α,20β-hydroxysteroid dehydrogenase: A member of a short-chain dehydrogenase family. *Proc Natl Acad Sci USA* **88**: 10064–10068, 1991.
173. Gibb W and Jeffery J, Reduction of the non-steroid adamantanone by crystalline preparations of cortisone reductase. *Biochem J* **126**: 443, 1972.
174. Ghosh D, Erman M, Pangborn W, Duax WL, Nakajin S, Ohno S and Shinoda M, Crystallization and preliminary X-ray diffraction studies of a mammalian steroid dehydrogenase. *J Steroid Biochem Mol Biol* **46**: 103–104, 1993.
175. Tanaka M, Ohno S, Adachi S, Nakajin S, Shinoda M and Nagahama Y, Pig testicular 20β-hydroxysteroid dehydrogenase exhibits carbonyl reductase-like structure and activity. cDNA cloning of pig testicular 20β-hydroxysteroid dehydrogenase. *J Biol Chem* **267**: 13451–13455, 1992.
176. Ghosh D, Erman M, Pangborn W, Duax WL and Baker ME, Inhibition of *Streptomyces hydrogenans* 3 alpha,20 beta-hydroxysteroid dehydrogenase by licorice-derived compounds and crystallization of an enzyme–cofactor–inhibitor complex. *J Steroid Biochem Mol Biol* **42**: 849–853, 1992.
177. Luu-The V, Lachance Y, Labrie C, Leblanc G, Thomas JL, Strickler RC and Labrie F, Full length cDNA structure and deduced amino acid sequence of human 3β-hydroxy-5-ene steroid dehydrogenase. *Mol Endocrinol* **3**: 1310–1312, 1989.
178. Ichinose H, Katoh S, Sueoka T, Titani K, Fujita K and Nagatsu T, Cloning and sequencing of cDNA encoding human sepiapterin reductase—an enzyme involved in tetrahydrobiopterin biosynthesis. *Biochem Biophys Res Commun* **179**: 183–189, 1991.
179. Lockyer J, Cook RG, Milstien S, Kaufman S, Woo SLC and Ledley FD, Structure and expression of human dihydropteridine reductase. *Proc Natl Acad Sci USA* **84**: 3329–3333, 1987.
180. Varughese KI, Skinner MM, Whiteley JM, Matthews DA and Xuong NH, Crystal structure of rat liver dihydropteridine reductase. *Proc Natl Acad Sci USA* **89**: 6080–6084, 1992.
181. Jörnvall H, von Bahr-Lindström H, Jany KD, Ulmer W and Fröschle M, Extended superfamily of short alcohol-polyol-sugar dehydrogenases: Structural similarities between glucose and ribitol dehydrogenases. *FEBS Lett* **165**: 190–196, 1984.
182. Rossmann MG, Moras D and Olson KW, Chemical and biological evolution of a nucleotide-binding protein. *Nature* **250**: 194–199, 1974.
183. Rossmann MG, Liljas A, Bränden CI and Banaszak LJ, Evolutionary and structural relationships among dehydrogenases. In: *The Enzymes* (Ed. Boyer PD), 3rd Edn, Vol. 11, pp. 61–102. Academic Press, New York, 1975.
184. Wierenga RK, De Maeyer MCH and Hol WGJ, Interaction of pyrophosphate moieties with α-helices in dinucleotide binding proteins. *Biochemistry* **24**: 1346–1357, 1985.
185. Chen ZL, Lee WR and Chang SH, Role of aspartic acid 38 in the cofactor specificity of *Drosophila* alcohol dehydrogenase. *Eur J Biochem* **202**: 263–267, 1991.
186. Grimshaw CE, Matthews DA, Varughese KI, Skinner M, Xuong NH, Bray T, Hoch J and Whiteley JM, Characterization and nucleotide binding properties of a mutant dihydropteridine reductase containing an aspartate 37-isoleucine replacement. *J Biol Chem* **267**: 15334–15339, 1992.
187. Scrutton NS, Berry A and Perham RN, Redesign of the coenzyme specificity of a dehydrogenase by protein engineering. *Nature* **343**: 38–43, 1990.
188. Baker ME, Sequence analysis of steroid- and prostaglandin-metabolizing enzymes: Application to understanding catalysis. *Steroids* **59**: 248–258, 1994.
189. Baker ME, Human placental 17β-hydroxysteroid dehydrogenase is homologous to NodG protein of *Rhizobium meliloti*. *Mol Endocrinol* **3**: 881–884, 1989.
190. Krook M, Prozorovski V, Atrian S, Gonzalez-Duarte R and Jörnvall H, Short-chain dehydrogenases. Proteolysis and chemical modification of prokaryotic 3α/20β-hydroxysteroid, insect alcohol and human 15-hydroxyprostaglandin dehydrogenase. *Eur J Biochem* **209**: 233–239, 1992.
191. Ensor CM and Tai HH, Site-directed mutagenesis of the conserved tyrosine 151 of human placental NAD-dependent 15-hydroxyprostaglandin dehydrogenase yields a catalytically inactive enzyme. *Biochem Biophys Res Commun* **176**: 840–845, 1991.
192. Chen Z, Jiang JC, Lin GZ, Lee WR, Baker ME and Chang SH, Site-specific mutagenesis of *Drosophila* alcohol dehydrogenase: Evidence for involvement of tyrosine-152 and lysine-156 in catalysis. *Biochemistry* **32**: 3342–3346, 1993.
193. Obeid J and White PC, Tyr-179 and Lys-183 are essential for enzymatic activity of 11β-hydroxysteroid dehydrogenase. *Biochem Biophys Res Commun* **188**: 222–227, 1992.
194. Peltoketo H, Isomaa V, Mäentausta O and Vihko R, Complete amino acid sequence of human placental 17β-hydroxysteroid dehydrogenase deduced from cDNA. *FEBS Lett* **239**: 73–77, 1988.
195. Penning TM, Abrams WR and Pawlowski JE, Affinity labeling of 3α-hydroxysteroid dehydrogenase with 3α-bromoacetoxyandrosterone and 11α-bromoacetoxyprogesterone. Isolation and sequence of active site peptides containing reactive cysteines; Sequence confirmation using nucleotide sequence from a cDNA clone. *J Biol Chem* **266**: 8826–8834, 1991.
196. Wistow GJ, Mulders WM and DeJong WW, The enzyme lactate dehydrogenase as a structural protein in avian and crocodilian lenses. *Nature* **326**: 622–624, 1987.
197. Fujii Y, Watanabe K, Hayashi H, Urade Y, Kuramitsu S, Kagamiyama H and Hayaishi O, Purification and characterization of rho-crystallin from Japanese common bullfrog lens. *J Biol Chem* **265**: 9914–9923, 1990.
198. Minard P, Legoy MD and Thomas D, 3β,17β-Hydroxysteroid dehydrogenase of *Pseudomonas testosteroni*. *FEBS Lett* **188**: 85–90, 1985.
199. Sweet F and Samant BR, Bifunctional enzyme activity at the same active site: Study of 3α and 20β activity by affinity alkylation of 3α,20β-hydroxysteroid dehydrogenase with 17-(bromoacetoxy)steroids. *Biochemistry* **19**: 978–986, 1980.
200. Strickler RC, Covey DF and Tobias B, Study of 3α,20β-hydroxysteroid dehydrogenase with an enzyme-generated affinity alkylator: Dual enzyme

activity at a single active site. *Biochemistry* **19**: 4950–4954, 1980.
201. Sharaf MA and Sweet F, Dual activity at an enzyme active site: 3β,20α-Hydroxysteroid oxidoreductase from fetal blood. *Biochemistry* **21**: 4615–4620, 1982.
202. Chen Q, Nancarrow CD and Sweet F, Isolation of 3β,20α-hydroxysteroid oxidoreductase from sheep fetal blood. *Steroids* **49**: 447–496, 1987.
203. Strickler RC, Tobias B and Covey DF, Human placental 17β-estradiol dehydrogenase and 20α-hydroxysteroid dehydrogenase. *J Biol Chem* **256**: 316–321, 1981.
204. Smirnov AN, Estrophilic 3α,3β,17β,20α-hydroxysteroid dehydrogenase from rabbit liver—I. Isolation and purification. *J Steroid Biochem* **36**: 609–616, 1990.
205. Garciá Valdés E, Cozar E, Rotger R, Lalucat J and Ursing J, New naphthalene-degrading marine *Pseudomonas* strains. *Appl Environ Microbiol* **54**: 2478–2485, 1988.
206. Debellé F and Sharma SB, Nucleotide sequence of *Rhizobium meliloti* RCR2011 genes involved in host specificity of nodulation. *Nucleic Acids Res* **14**: 7453–7472, 1986.
207. Thöny B, Fischer HM, Anthamatten D, Bruderer T and Hennecke H, The symbiotic nitrogen fixation regulatory operon (*fixRnifA*) of *Bradyrhizobium japonicum* is expressed aerobically and is subject to a novel, *nifA*-independent type of activation. *Nucleic Acids Res* **15**: 8479–8499, 1987.
208. Martin PM, Horwitz KB, Ryan DS and McGuire WL, Phytoestrogen interaction with estrogen receptors in human breast cancer cells. *Endocrinology* **103**: 1860–1867, 1978.
209. Adlercreutz H, Does fiber-rich food containing animal lignan precursors protect against both colon and breast cancer? An extension of the "fiber hypothesis." *Gastroenterology* **86**: 761–764, 1984.
210. Markaverich BM, Roberts RR, Alejandro MA, Johnson GA, Middleitch BS and Clark JH, Bioflavonoid interaction with rat uterine type II binding sites and cell growth inhibition. *J Steroid Biochem* **30**: 71–78, 1988.
211. Pelissero C, Bennetau B, Babin P, Le Menn F and Dunogues J, The estrogenic activity of certain phytoestrogens in the siberian sturgeon. *Acipenser baeri*. *J Steroid Biochem Mol Biol* **38**: 293–299, 1991.
212. Baker ME, Genealogy of regulation of human sex and adrenal function, prostaglandin action, snapdragon and petunia flower colors, antibiotics, and nitrogen fixation: Functional diversity from two ancestral dehydrogenases. *Steroids* **56**: 354–360, 1991.
213. Baker ME, Evolution of enzymatic regulation of prostaglandin action: Novel connections to regulation of human sex and adrenal function, antibiotic synthesis and nitrogen fixation. *Prostaglandins* **42**: 391–410, 1991.
214. Wistow G and Piatigorsky J, Recruitment of enzymes as lens structural proteins. *Science* **236**: 1554–1556, 1987.
215. Piatigorsky J and Wistow GJ, Enzyme/crystallins: Gene sharing as an evolutionary strategy. *Cell* **57**: 197–199, 1989.