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Minireview: Cellular Redox State Regulates Hydroxysteroid Dehydrogenase Activity and Intracellular Hormone Potency

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Hydroxysteroid dehydrogenases (HSDs) interconvert potent and relatively inactive forms of individual steroid hormones using nicotinamide cofactors NADPH/$NADP^+$ and NADH/NAD^+ [nicotinamide adenine dinucleotide (phosphate), reduced/oxidized forms]. Although reactions with purified enzymes *in vitro* may be driven in either direction depending on the assay conditions, HSD enzymes appear to function in one direction or the other in intact cells. At least for some of these enzymes, however, the apparent unidirectional metabolism actually reflects bidirectional catalysis that reaches a pseudo-equilibrium state with a strong directional preference. This directional preference, in turn, derives from intracellular concentration gradients for the nicotinamide cofactors and the relative affinities of each HSD for these cofactors. Because the concentrations of free cofactor exceed those of steroids by many orders of magnitude, the activities of these enzymes are predominantly driven by cofactor abundance, which is linked to intermediary metabolism. Consequently, the amount of active steroids in cells containing HSDs may be modulated by cofactor abundance and, hence, intracellular redox state. We will review the evidence linking cofactor handling and HSD activity, speculate on additional ways that intracellular metabolism can alter HSD activity and, thus, hormone potency, and discuss fruitful avenues of further investigation. (*Endocrinology* 146: 2531–2538, 2005)

Cellular Redox State and Redox Sensing

Organisms must adapt to environmental stress and fuel supply to maintain cellular homeostasis within certain boundaries. The complex regulation of intermediary metabolism, which includes glycolysis, oxidation of fats and carbohydrates, and the balance between anabolic and catabolic processes, reflects the preeminence of cellular energy balance over specialized functions. Enzyme-catalyzed oxidation and reduction reactions are major components of intermediary metabolism, and although enzymes accelerate the rates of these reactions, the reactions must proceed according to the laws of thermodynamics; the entire system must have a favorable change (lowering) of free energy. Consequently, cells must defend their supplies of oxidizing and reducing equivalents to perform these reactions, and the capacity to execute these enzymatic transformations requires not only the expression of the enzyme itself but the presence of oxidized or reduced cofactors.

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Abbreviations: AKR, Aldo-keto reductase; GFP, green fluorescent protein; G6P, glucose-6-phosphate; G6PDH, glucose-6-phosphate dehydrogenase; H6PDH, hexose-6-phosphate dehydrogenase; HSD, hydroxysteroid dehydrogenase; NMR, nuclear magnetic resonance; 6PG, 6-phosphogluconate; 6PGDH, 6-phosphogluconate dehydrogenase; PPP, pentose phosphate pathway; SDR, short-chain dehydrogenase reductase; TCA, tricarboxylic acid cycle; TIM, triosephosphate isomerase.

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Nicotinamide cofactors and redox state

The relative ratio of oxidized and reduced cofactors varies with the amount and type of metabolic fuels and with oxygen supply. Reducing equivalents are generated by the oxidation of hydrocarbon substrates, and, collectively, the abundance of reducing equivalents determines the redox state of the cell. In the fed state with abundant fuel and oxygen, cells have high reductive capacity, reflected by a high ratio of the reduced and oxidized forms of nicotinamide adenine dinucleotide phosphate (NADPH $\gg$ $NADP^+$) (1). However, specialized cellular enzymatic activities, including the synthesis or degradation of biomolecules, also require nicotinamide cofactors. Consequently, the capacity of cells to perform these specialized functions is inherently linked to intermediary metabolism, redox state, and the supply of proper cofactors. However, little is known about the capacity of eukaryotic cells to monitor redox state for supporting specialized functions.

Cellular mechanisms for sensing and responding to redox state

Although formerly an area of intense study when the pathways of intermediary metabolism was first elucidated, interest in mechanisms controlling the redox state of cells and sensing mechanisms has surfaced again over the last few years. Lower eukaryotes have evolved proteins like Rex in *Streptomyces coelicolor* A3(2) (2) or OxyR (3) and RegB (4), which react to changing redox state of the cells. Most commonly, these proteins contain disulfide bonds, which can be oxidized (-S-S-) or reduced (-SH HS-) conveniently and pro-

portionately to redox state. Additional protein prosthetic groups that undergo redox chemistry and, hence, serve as sensors include iron-sulfur clusters, iron-protoporphyrins (hemes), and flavins (flavin adenine dinucleotide and flavin mononitrate) (5). Redox-sensing prosthetic groups can alter the function of the protein itself, or the redox-sensing protein can alter the function of other proteins. In most cases, electrons from NADPH, the common electron currency of the cytoplasm, reduce the prosthetic groups, and the extent to which reduction occurs depends on the abundance of NADPH. The process may be reversible or irreversible, and the mechanism may involve a two-electron hydride transfer or two discreet one-electron steps.

Cellular Redox State and the Nicotinamide Cofactor Gradients

Maintaining a high NADPH/NADP$^+$ gradient

The principal and best studied pathway for regenerating NADPH from NADP$^+$ is via the pentose phosphate pathway (PPP) (6). The enzymes of this pathway are mainly cytoplasmic and provide most of the reducing equivalents for cellular needs (Fig. 1). The two key enzymes involved in this process, glucose-6-phosphate (G6P) dehydrogenase (G6PDH) and 6-phosphogluconate (6PG) dehydrogenase (6PGDH), sequentially oxidize G6P and use those electrons to reduce NADP$^+$ to NADPH. Because glucose is a major fuel for many

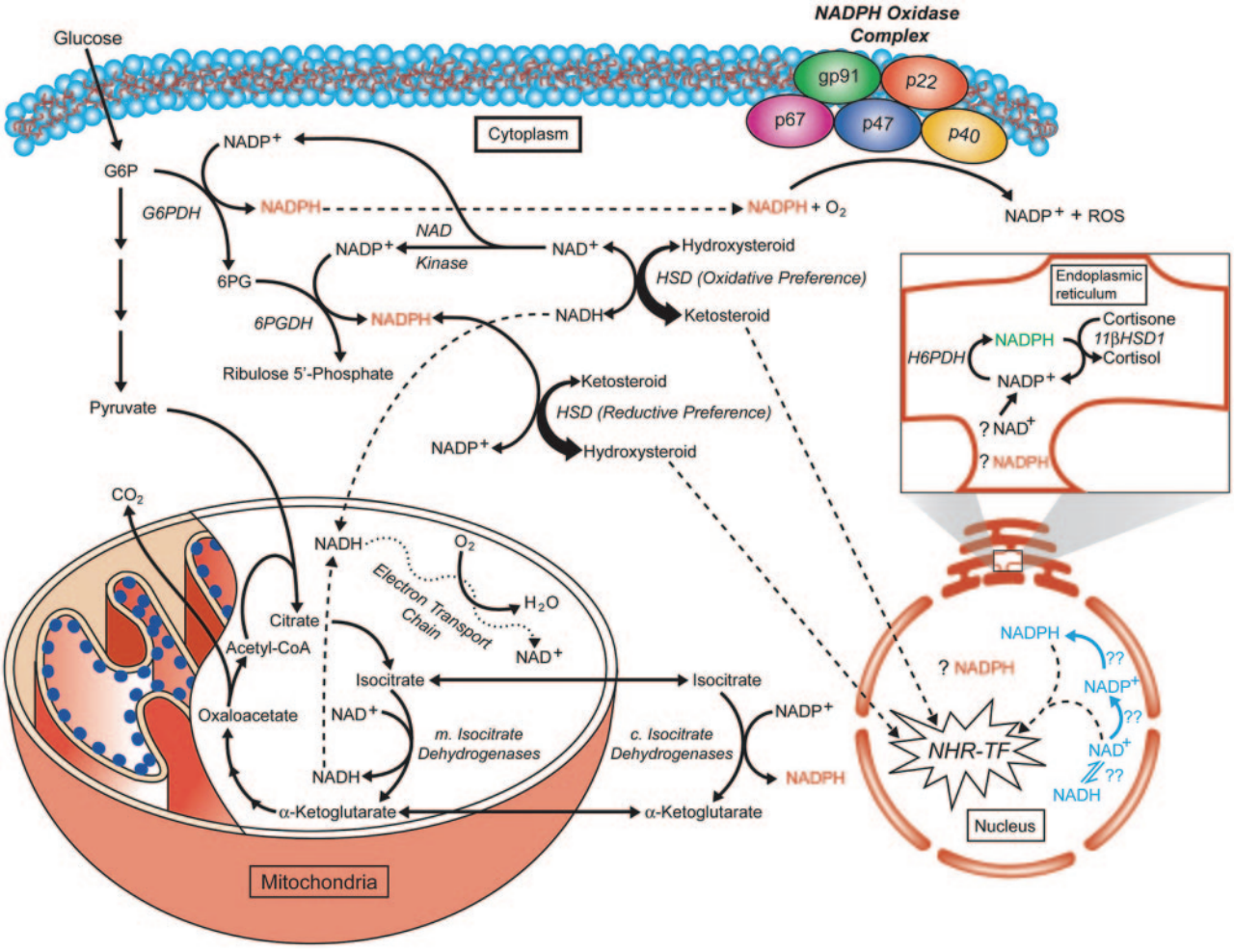

FIG. 1. Pathways of NADH/NADPH regeneration and consumption in a stylized cell. Upon entering the cell, glucose is phosphorylated by hexokinase to G6P, which may enter the PPP. In the first step, G6P is oxidized to 6PG by G6PDH, and 6PG is further oxidized to ribulose 5′-phosphate by 6PGDH. In both reactions, one equivalent of NADP$^+$ is reduced to NADPH (*red*). In glycolysis, G6P is also metabolized to pyruvate, which enters the mitochondria for further oxidation in the TCA. Isocitrate, a TCA intermediate, may be oxidized in the cytosol to α-ketoglutarate by NADP$^+$-specific isocitrate dehydrogenase, which also regenerates NADPH. Cytoplasmic NADPH provides reducing equivalents for biosynthetic and catabolic enzymes, including cytochromes P450 and reductive HSDs (*tapered arrows*). NAD$^+$ is the common electron acceptor for many dehydrogenases, such as fatty acyl dehydrogenases and oxidative HSDs (*tapered arrows*). Activation of nuclear hormone receptors and other transcription factors (NHR-TF) by steroids and by cofactors (*blue*) is shown in the nucleus. In the endoplasmic reticulum lumen, H6PDH supports NADPH regeneration (*green*) and is necessary for the reductase activity of 11β-HSD1, which reduces cortisone and dehydrocorticosterone to cortisol and corticosterone, respectively. Compartmentalization of different cofactor pools in the cytoplasm (*red*), endoplasmic reticulum lumen (*green*), and nucleus (*blue*) is suggested. Shown also is conversion of NAD$^+$ to NADP$^+$ by NAD kinase in the cytosol, whereas this conversion has not been demonstrated in the endoplasmic reticulum lumen and nucleus (marked by ?). Proteins of the NADPH oxidase complex, gp91 and p22, are shown on the plasma membrane, and these subunits recruit p67, p47, and p40. The heteropentameric complex oxidizes NADPH and reduces molecular oxygen, an energetically favorable and mechanistically irreversible reaction that produces reactive oxygen species (ROS) and/or water.

tissues, these two enzymes are the major source of NADPH in most cells for biosynthetic purposes. Because most tissue culture is performed in high-glucose medium, the PPP is also the principal mechanism for regenerating NADPH in cultured cell lines used for various experiments. In addition, G6PDH plus G6P is commonly employed as an NADPH regeneration system for *in vitro* experiments. G6PDH is the first committed step in the PPP, and dysregulation of the enzyme will have an effect on the abundance of NADPH in cells. Glucocorticoids increase G6PDH activity in the rat hepatocytes, and insulin exerts a cooperative effect on the enzyme in the presence of glucocorticoids. Similar regulation by glucocorticoids and insulin are also observed in the adipose tissue, but the comprehensive hormonal, nutritional, and transcriptional regulation of this isoform is not well understood. The 6PGDH enzyme of the PPP also generates NADPH but demonstrates product inhibition by high concentrations of NADPH, and additional regulatory mechanisms for this enzyme are not known.

A second source of cytoplasmic NADPH derives from a reaction of the tricarboxylic acid cycle (TCA, or citric acid cycle). In the TCA, citrate is isomerized to isocitrate by aconitase; then, isocitrate dehydrogenase oxidizes isocitrate to α-ketoglutarate (Fig. 1). All of the reactions of the TCA occur in the mitochondria, and the mitochondrial isoform of isocitrate dehydrogenase reduces NAD^+ to NADH. However, a cytoplasmic form of isocitrate dehydrogenase uses $NADP^+$ as the electron acceptor and therefore provides another mechanism for generating cytoplasmic NADPH (7). Other minor pathways also regenerate NADPH, but flux through these pathways is not a significant source of NADPH.

Although these major routes of NADPH regeneration service the cytoplasm, nicotinamide pools appear to be compartmentalized within cells and may not equilibrate across organelles. In the endoplasmic reticulum, which is primarily an oxidizing environment that favors protein processing and disulfide bond formation, hexose-6-phosphate dehydrogenase (H6PDH; or salivary and microsomal glucose-1-dehydrogenase) provides a local supply of NADPH (8). H6PDH, the microsomal counterpart of the cytoplasmic G6PDH, has much broader substrate specificity than G6PDH and oxidizes other substrates such as glucose-6-sulfate and galactose-6-phosphate to generate reduced cofactor. H6PDH is ubiquitously expressed, except in erythrocytes, and this enzyme is not part of any known biosynthetic pathways. Proteins or enzymes that are localized to the lumen of the endoplasmic reticulum and that require NADPH for its activity are likely to be dependent on the action of H6PDH (Fig. 1). The regulation of H6PDH activity by hormones and nutrient supply has not been studied in detail, and the mechanisms for supplying the intralumenal compartment with nicotinamide cofactors remain unknown.

Maintaining an inverse NADH/NAD^+ gradient

In contrast, cells maintain a concentration gradient that favors the oxidized form of NADH, with NAD^+ concentrations exceeding NADH by nearly 3 orders of magnitude (9). Analogous to the role of NADPH as the common electron currency in cells, NAD^+ is the prototypical electron acceptor for several dehydrogenase reactions involved in glycolysis and fatty acid oxidation. Just as NADPH is constantly regenerated when glucose is abundant, electrons from NADH are constantly removed for use in the mitochondrial electron transport chain that drives oxidative phosphorylation and ATP generation during aerobic metabolism. This insatiable drain of electrons from NADH maintains a high NAD^+ to NADH ratio and assures that cofactor is available for oxidation of fuel substrates.

Despite elaborate systems for maintaining cellular cofactor gradients, disruption of fuel and oxygen supply will lower these gradients. Massive changes may not be compatible with cell survival, but small changes are tolerated given the magnitude of these gradients. However, alterations in cofactor gradients may have consequences on other cellular functions that require large gradients for optimal function.

Nontraditional redox-sensing transcription factors and steroid hormones

The abundance of reduced and oxidized pairs of intermediary metabolism products, such as NADPH/$NADP^+$ and malate/oxaloacetate, reflect the redox state of the cell. Although these molecules are present at high concentrations in cells, it is now recognized that the amounts and activities of other, less abundant molecules and proteins are also influenced by redox state. For example, the activities of transcription factor Sir2 (10) and the transcriptional corepressor carboxyl-terminal binding protein (11) are regulated via their binding NADH/NAD^+; hence, these proteins sense the cellular composition of these cofactors. PAS domain proteins are basic helix-loop-helix transcription factors that are regulated by ligands, light, and other factors (12). The NPAS2-BMAL1 heterodimer, which maintains the circadian rhythm, senses the cellular redox state by binding NADPH/$NADP^+$, which in turn regulates its transcriptional activity (13).

Additional metabolic pathways that require oxidized or reduced cofactor(s) require intermediary metabolism to maintain cellular cofactor concentration gradients necessary for these reactions. Consequently, these pathways are vulnerable to disruption if cellular redox state and, hence, cofactor gradients are not maintained. Steroid hormones are examples of a low-abundance ($\sim$1 nM) compounds that undergo redox reactions. Cytochrome P450 reactions, including the steroid hydroxylation and side-chain cleavage reactions, all require NADPH and molecular oxygen (14), and these reactions irreversibly oxidize the steroid nucleus (15). P450 reactions are rather limited, encompassing the biosynthetic transformations in the adrenal, gonad, and placenta plus the catabolic reactions in the liver and some other tissues. More globally, peripheral steroid metabolism is dominated by the hydroxysteroid dehydrogenases (HSDs). HSD reactions convert hydroxysteroids to ketosteroids and vice versa using nicotinamide cofactors (15). The key positions of metabolism are C-11 for glucocorticoids and mineralocorticoids, C-20 for progestins, C-17 for androgens and estrogens, and C-3 for all 5α-reduced steroids. The two members of a ketosteroid-hydroxysteroid pair often differ dramatically in affinities for their cognate receptors; consequently, HSD reactions are critical to determining steroid bioactivity.

Structural and Functional Characteristics of HSDs

Types of HSDs

HSDs may be grouped by either their structural fold or physiologic function. Structurally, HSDs belong to two groups of enzymes: the short-chain dehydrogenase reductase (SDR) family or the aldo-keto reductase (AKR) family. The SDR enzymes exhibit a β-α-β fold with core of parallel β-strands fanning across the center and α-helices draped on the outside (16). The SDR enzymes contain a Rossman fold and GXXXGXG motif (where G is glycine and X is any other amino acid) characteristic of oxidation/reduction enzymes that bind nicotinamide cofactors, plus the YXXXK (where Y is tyrosine and K is lysine) sequence that participates in catalysis. This structural fold, which was first described for glyceralde 3-phosphate dehydrogenase, is the fold observed in x-ray structures of bacterial 3α,20β-HSD (17), human 17β-HSD1 (18), and guinea pig 11β-HSD1 (19). Sequence analysis indicates that 17β-HSDs1–3, 11β-HSDs1 and 2, and the oxidative 3α-HSDs of the retinol dehydrogenase variety all belong to the SDR superfamily. The SDR-type HSDs are active only as dimers or tetramers, and most are tightly membrane-bound (Fig. 2, *left*).

In contrast, the AKR enzymes are soluble, monomeric proteins that contain the triosephosphate isomerase (TIM) barrel motif (20). The TIM barrel is named after the prototypical structure of TIM, and all AKR enzymes, including rat liver 3α-HSD (AKR1C9) (21), AKR1C2 (22), and AKR1C3 (23) also demonstrate this fold. A TIM barrel also exhibits a continuous β-α-β pattern, but the eight β-strands lie in a tilted circular array like the staves of a barrel, with α-helices stitching the strands together on the outside of the barrel. Analogous tyrosine and lysine residues that participate in catalysis are adjacent in space but distant in linear sequence, and cofactor drapes across the edge of the barrel without a Rossman fold (Fig. 2, *right*).

Functionally, the HSDs can be divided into true dehydrogenases, which oxidize hydroxysteroids to ketosteroids, and reductases, which reduce ketosteroids to hydroxysteroids. This distinction is based on the observed flux of substrates obtained when the cDNA for the enzyme in question is expressed in a cell line with low background steroid metabolism, such as HEK-293 cells. For example, the reductive 17β-HSD1 converts estrone almost completely to estradiol (24), whereas oxidative 17β-HSD2 converts over 95% of estradiol to estrone (25). However, all HSDs employ a common hydride transfer mechanism using nicotinamide cofactors, and all steps of these reactions are reversible. Consequently, some HSDs, when purified and studied *in vitro*, have been shown to catalyze either steroid oxidation or reduction depending on assay conditions. So what factors control HSD directionality in intact cells and explain the discrepancy of results from *in vivo* and *in vitro* experiments?

Regulation of HSD activity by cofactor preference and redox state

Under conditions where enzyme concentrations and pH remain stable, HSD reactions obey second order kinetics. For a reductive HSD:

$$\text{ketosteroid} + \begin{matrix}\text{reduced}\\ \text{cofactor}\end{matrix} + H^+ \leftrightarrow \text{hydroxysteroid} + \begin{matrix}\text{oxidized}\\ \text{cofactor}\end{matrix}$$

$$\text{Rate}_{red} = k_{red} \times [\text{ketosteroid}] \times [\text{reduced cofactor}].$$

Unlike the P450 reactions that are mechanistically irreversible, all steps of the hydride transfer reactions are freely reversible. Therefore, the reactions are also governed by the kinetic expression for the oxidation reaction:

$$\text{Rate}_{ox} = k_{ox} \times [\text{hydroxysteroid}] \times [\text{oxidized cofactor}].$$

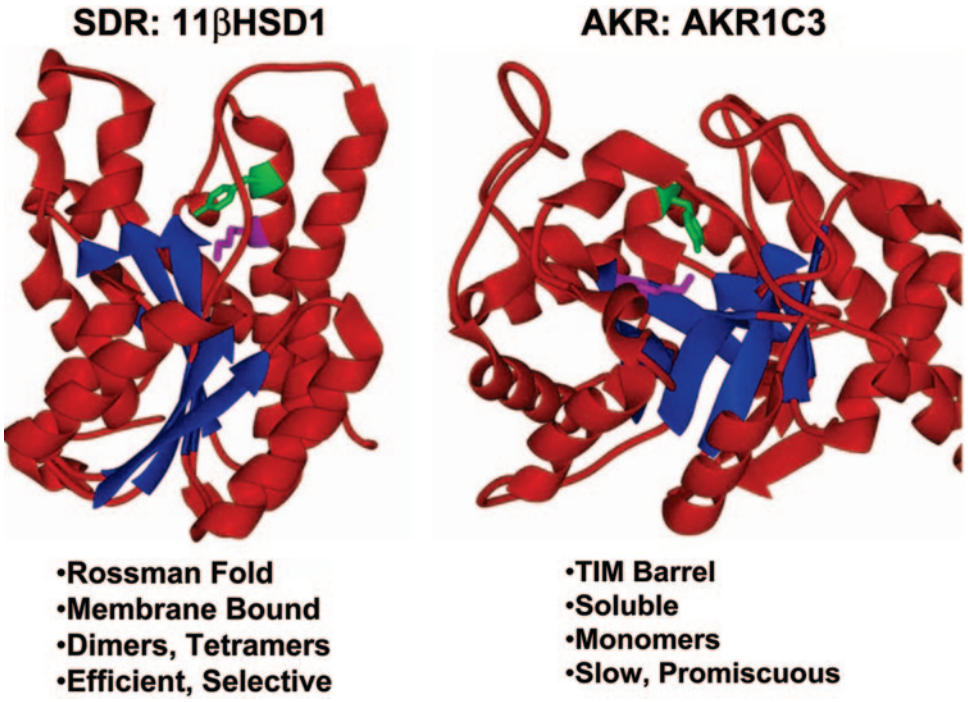

FIG. 2. Two major structural classes of HSD enzymes and the differences in key properties. *Left*, Ribbon diagram of guinea pig 11β-HSD1 (pdb ID 1XU9), an SDR enzyme; *right*, ribbon diagram of human AKR1C3 (17β-HSD5, 3α-HSD2; pdb ID 1XF0), an AKR enzyme. The β-sheet cores are shown as *blue block arrows* in *red structures*. The tyrosine and lysine residues in active site are shown in *green* and *magenta*, respectively (tyrosines 183 and 55 and lysines 187 and 84 in 11β-HSD1 and AKR1C3, respectively).

Theoretically, these reactions should come to an equilibrium state when enough substrate has been depleted that the rate of the reverse reaction rises and $Rate_{red} = Rate_{ox}$. These equilibrium states are observed when purified enzyme is studied *in vitro*, and the equilibrium steroid distribution can be shifted by altering cofactor concentrations and pH (26). Do similar reversible equilibria also occur in intact cells?

The simplistic equations above neglect the fact that the nicotinamide cofactors are used by other cellular processes and are constantly regenerated via intermediary metabolism as described above (Fig. 1). Consequently, cofactor concentrations remain relatively constant during short-term experiments in transfected cells, allowing steroid conversion to proceed nearly to completion. Yet, no matter how long incubations are continued or how much enzyme is expressed, some steroid substrate persists, and metabolism proceeds asymptotically to a final state in which metabolism appears to cease because no net mass flux occurs. Nonetheless, double-isotope experiments demonstrate that, at least in the case of 17β-HSDs types 1–3, steroids are rapidly interconverted at these apparent equilibrium states and that the rates of the forward and reverse reactions are essentially equal (27). Cell culture experiments are not a closed system as are enzyme assays in a test tube that achieve a true equilibrium, but to a first approximation, these HSD reactions achieve a pseudoequilibrium state in intact cells. However, in all cases, the final steroid proportions prominently favor either oxidation or reduction depending on the enzyme.

Structural and Cellular Determinants of Directional Preference

17β-HSDs

A thermodynamic analysis of HSD reactions would conclude that all HSDs should favor reduction of ketosteroids. Based on studies with alcohol dehydrogenases and lactate dehydrogenases, the free energy change for oxidation of NAD(P)H is about −30 kcal/mol, whereas the free energy change for interconversion of a hydroxysteroid and its ketosteroid congener is comparatively small. Therefore, the observed chemistry of reductive HSDs, such as 17β-HSD1 and 3, is not a mystery, but the directional preference of oxidative enzymes, such as 17β-HSD2, requires a mechanism to overcome the uphill thermodynamic barrier to hydroxysteroid oxidation. Enzymes are catalysts, which increase the rate of reactions but cannot by themselves alter the thermodynamics of the process. However, enzymes can functionally alter metabolic processes by altering the kinetics of a reaction.

In HSD-catalyzed reactions, the hydride transfer or chemical step occurs in the ternary enzyme-steroid-cofactor complex. If both 17β-HSD1 and 17β-HSD2 interconvert estrone and estradiol from similar ternary complexes, then how does 17β-HSD1 achieve net steroid reduction, whereas 17β-HSD2 efficiently oxidizes estradiol? The answer cannot be in the handling of steroid but rather in the use of cofactor. Indeed, cytoplasmic free nicotinamide cofactor concentrations (~1 mM) exceed steroid hormone concentrations (~1 nM) by many orders of magnitude, even for the low-abundance members of the cofactor pairs NADH and $NADP^+$. Consequently, rate expressions are dominated not by steroid but by the cofactor term. As explained above, intermediary metabolism normally establishes large cofactor concentration gradients, with [NADPH] ≫ [$NADP^+$] and [NAD^+] ≫ [NADH]. Therefore, if cofactor binding by each HSD isoform is restricted to one pair of cofactors, then mass action will drive steroid flux for either ketosteroid reduction (by NADPH) or hydroxysteroid oxidation (by NAD^+). Although this paradigm may be oversimplified, it does rationalize the observed chemistry. Steroid oxidation by 17β-HSD2 proceeds because the NADH so generated is reoxidized via the electron transport chain and coupled to reduction of oxygen, providing the ultimate thermodynamic drive (Fig. 2).

Both 17β-HSD1 and 17β-HSD2 are SDR enzymes, for which considerable functional and structural information is available. The SDR enzymes bind cofactor within a Rossman fold, and residues within this fold interact with cofactor in the vicinity of the 2′ position, where a phosphate group distinguishes NADP(H) from NAD(H). In the early 1990s, crystal structures and sequence alignments suggested that enzymes such as glutathione reductase, which use NADPH, contain a positively charged arginine that forms a salt bridge with the 2′-phosphate of NADPH, stabilizing the binding of NADP(H). In contrast, oxidative enzymes like dihydrolipoamide dehydrogenase and alcohol dehydrogenase contain a negatively charged residue at the corresponding position, which would repel the 2′-phosphate of NADP(H) but might hydrogen bond to the 2′-hydroxyl group of NAD(H). Site-directed mutagenesis experiments demonstrated that swapping the charged residues of these enzymes reversed the relative affinities of purified enzymes for these cofactor pairs (28, 29).

Analogously, mutation of leucine 36 to aspartate reverses the cofactor preference of 17βHSD1 from NADPH/$NADP^+$ to NAD^+/NADH by introducing a negative charge adjacent to the 2′-phosphate (30) (Fig. 3). This mutation also reverses the directional preference of 17βHSD1 in intact cells, strongly favoring estradiol oxidation yet still catalyzing rapid and bidirectional metabolism that achieves a pseudoequilibrium state (27). Mutation of arginine 80, the corresponding residue in 17β-HSD3, to glutamine causes male pseudohermaphroditism primarily by disrupting NADPH binding, not steroid binding (31, 32). Conversely, mutation aspartate 36 to alanine + lysine 37 to arginine reverses cofactor preference of the oxidative human 3β-HSD1 from NAD(H) to NADP(H) (33). However, it is not known whether these simple rules for cofactor binding, which translate to directional preference in intact cells, are unique to these few HSDs or general. The oxidative 3α-HSD from prostate (34), which belongs to the retinol dehydrogenase family (35), appears to be a hydroxysteroid epimerase (36), ultimately reducing the 3-ketosteroid (*i.e.* 5α-androstane-3,17-dione) to a 3β-hydroxysteroid (*i.e.* epi-androsterone) after prolonged incubations, somehow managing to reverse its directional preference. Finally, the cofactor binding determinants for the AKR isoforms, which lack a Rossman fold, have received limited study (37, 38) (Fig. 3).

11β-HSDs

The two isoforms of the 11β-HSDs, the liver type (11β-HSD1) and the kidney type (11β-HSD2), illustrate many of

the principles of HSD function and cofactor use. The 11β-HSD2 enzyme is mutated in the apparent mineralocorticoid excess syndrome (39), in which cortisol oxidation to cortisone is impaired. All experiments *in vivo* and *in vitro* suggest that 11β-HSD2 has a strong directional preference for oxidizing cortisol to cortisone using NAD^+ as cofactor. The studies of 11β-HSD1, however, have yielded much more complex results.

The directional preference of 11β-HSD1 in transfected cells favors the reduction of cortisone to cortisol, presumably using NADPH as do all other reductive HSDs. Paradoxically, recombinant 11β-HSD1, overexpressed in cultured cells infected with vaccinia virus (40) or in *Escherichia coli* (41), reduces cortisone poorly *in vitro* with NADPH. In contrast, recombinant 11β-HSD1 readily oxidizes cortisol to cortisone *in vitro* using either $NADP^+$ or NAD^+, a result that seemingly violates all of the principles established above. Addition of an NADPH-regenerating system (G6PDH plus G6P) to the *in vitro* assay, however, enables recombinant 11β-HSD1 to reduce cortisone almost completely to cortisol. Therefore, it appears that the reductive activity of 11β-HSD1 *in vivo* requires a particularly high $NADPH/NADP^+$ gradient for maximal activity. This condition is fulfilled in some cells for which 11β-HSD1 activity appears important such as adipocytes, where its cortisone reductase activity may contribute to fat accumulation (42). During lipogenesis, NADPH abundance increased considerably to provide reducing equivalents necessary to elongate fatty acid chains with two-carbon acetate units (43). This rise in the NADPH to $NADP^+$ ratio would activate the reductase activity of 11β-HSD1 (6).

To complicate matters further, 11β-HSD1 resides in the endoplasmic reticulum lumen (42), which normally maintains a highly oxidative redox state (44). Within the intralumenal compartment, the processing of nascent proteins occurs, including the formation of intramolecular disulfide bonds and their rearrangement by protein disulfide isomerase. How, then, can an enzyme that functions as a reductase only when available $NADP^+$ is scrupulously reoxidized to NADPH function in a strongly oxidizing environment? The answer appears to be that this requisite supply of NADPH derives from the intralumenal enzyme H6PDH (Fig. 1). This role of H6PDH has been substantiated by studies of subjects with apparent cortisone reductase deficiency in whom cortisone reduction to cortisol is impaired. These subjects have intronic polymorphisms in the *HSD11B1* gene that reduce expression and, more importantly, mutations in the *H6PDH* gene that impair intralumenal NADPH regeneration (45). Although these results are consistent with a model whereby H6PDH supplies NADPH to 11-βHSD1, the stoichiometry of the two proteins appears to show less H6PDH than expected (46), and other intralumenal HSDs, such as 17β-HSD2, retain an oxidative preference in this environment (25). Therefore, additional and yet unknown properties of H6PDH and 11-βHSD1 appear to cooperate and allow this unique intralumenal reductive preference. Alternatively, excess NADPH from the cytoplasmic pool might also transport, actively or passively, into the endoplasmic reticulum lumen to provide needed reducing equivalents under some conditions.

Significance of bidirectional metabolism and directional preference

Our analysis of the kinetic and metabolic determinants of HSD function illustrates the fundamental principle that HSD-catalyzed reactions are not driven by steroid but by cofactor concentration gradients. Although each HSD has a limited repertoire of keto- and hydroxysteroid substrate pairs that it will metabolize, it is cofactor abundance and relative affinities of the enzymes for cofactors that determine the directional preference of HSDs and, thus, their physiologic roles. Because the chemical mechanisms of these reactions are inherently reversible, the HSDs can only shuttle steroid pairs to a certain pseudoequilibrium distribution that is limited by cofactor gradients.

Two important correlates of this analysis emerge. First, when HSDs are presented with the wrong steroid, which is normally the product of the preferred half-reaction, rapid bidirectional metabolism will force some steroid flux in the

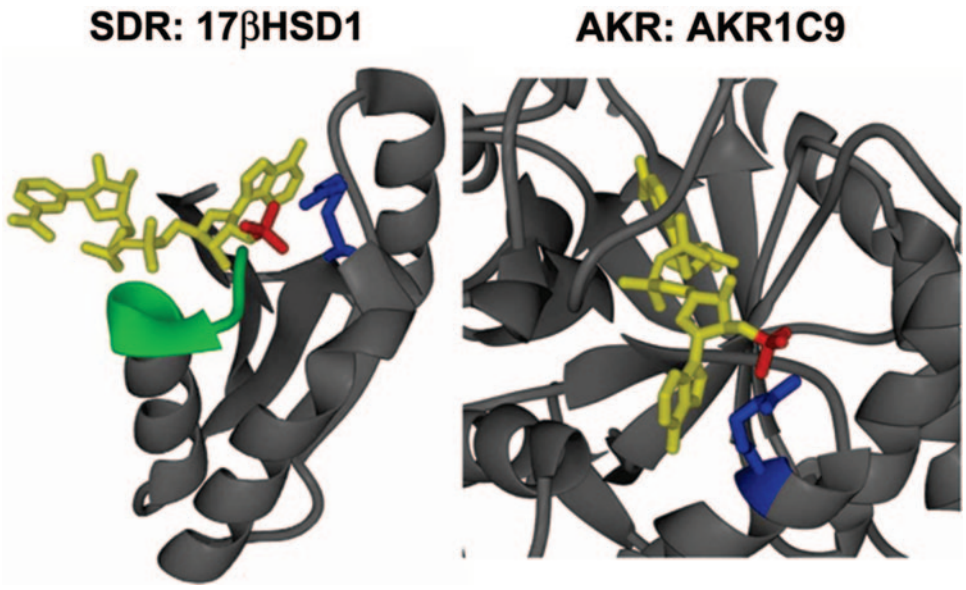

FIG. 3. Cofactor binding regions of human 17β-HSD1, an SDR enzyme (*left*, pdb ID 1QYV), and rat AKR1C9, an AKR enzyme (*right*, pdb ID 1AFS). In both cases, an arginine residue (*blue*) forms a salt bridge with the 2′-phosphate (*red*) of NADPH (*yellow*). Both enzymes also demonstrate preference for ketosteroid reduction in transfected cells and higher affinities for NADP(H) than for NAD(H). The GXXXGXG motif of 17β-HSD1 is shown in *red*.

reverse direction. In model systems, this backwards metabolism by 17β-HSD2 is sufficient to activate reporter gene expression with physiologic concentrations of estrone via its conversion to estradiol (27). Second, because the magnitude of the directional preference is intimately linked to cofactor abundance, conditions of altered intermediary metabolism that significantly deplete intracellular cofactor gradients should shift the pseudoequilibrium of HSDs in cells and tissues. These shifts in intermediary metabolism can occur when the supply, composition, or combustion of fuel is changed, such as in fasting, hypoxia, and reduced blood flow. It is difficult to predict how the results obtained in model systems extrapolate the tissues of an animal; nonetheless, the regulation of steroid hormone potency via intermediary metabolism, mediated by the impact of cofactor abundance on HSD activities, requires further study.

Future Studies

To advance these studies beyond those obtained thus far using model systems, it is critical that sensitive and convenient methods to monitor the intracellular abundance of all four nicotinamide cofactors be developed. Recently, green fluorescent protein (GFP)-based systems have been employed with some success (47). In one assay, the GFP protein was engineered to place two cysteine residues in close approximation with the fluorophore, and the emission spectrum of this fluor shifts upon formation of a disulfide bond between these cysteines (44, 48). Consequently, changes in intracellular NADPH, which shift the balance between oxidized and reduced disulfide, manifest as changes in the emission spectrum and report the redox state of the cell. Using this method, intracellular cofactor abundance can be monitored by fluorescence microscopy in real time under physiologic conditions that alter cofactor abundance. To distinguish among various subcellular pools of cofactors, this method can be extended by targeting the modified GFP protein to different intracellular compartments. Furthermore, it is possible that additional modifications, such as the incorporation of NAD(H) or NADP(H) binding domains, might render these fluors selective for one pair of cofactors.

Additional methods to track cofactor flux involve stable isotope and nuclear magnetic resonance (NMR) techniques. By incorporating stable isotopes into cofactors, flux may be quantitated by mass spectrometry, which is an extremely accurate and sensitive yet destructive technique. NMR methods are theoretically nondestructive, but only in cells and tissues that can be studied in an NMR magnet. Both methods might be adapted to tracking specific subcellular pools by selectively introducing stable isotopes to one compartment or by exploiting differences in relaxation times for cofactors in different compartments. Significant technical hurdles must be overcome for any of these approaches can be exploited experimentally.

Finally, the regulatory mechanisms for sensing, synthesizing, and consuming nicotinamide cofactors in higher eukaryotes have not been adequately defined. For example, NAD^+ is converted to $NADP^+$ by NAD kinase, affording a mechanism for transfer of cofactor mass from the NADH/NAD^+ pool to the NADPH/$NADP^+$ pool. However, it is not known how this flux is regulated and whether different mechanisms exist for pools in different compartments such as the cytoplasm, endoplasmic reticulum lumen, and nucleus, which all have characteristic proteins that respond to cofactor gradients (Fig. 1). Rapid depletion of NADPH by NADPH oxidase during the oxidative burst phase of neutrophil activation has been studied for years; however, it is now clear that other cells posses the NADPH oxidase protein complex. This system can rapidly alter cofactor gradients either in response to stimuli or as a primary process, which will necessarily alter both the potency of steroid hormones in cells containing HSDs and the activity of redox-sensitive transcription factors.

Although it has long been recognized that steroid hormones regulate cellular metabolism, it is only recently that we are beginning to recognize that cellular metabolism can regulate the response to steroid hormones. Our understanding of how cellular redox state translates to altered responses to circulating hormones remains in its infancy. Significant advances in this area await the application of better methods to monitor several cellular processes simultaneously.

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