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CYP17- and CYP11B-dependent steroid hydroxylases as drug development targets

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Abstract

Steroid hormone biosynthesis is catalyzed by the action of a series of cytochrome P450 enzymes as well as reductases. Defects in steroid hydroxylating P450s are the cause of several severe defects such as the adrenogenital syndrome (AGS), corticosterone methyl oxidase (CMO) I or II deficiencies, or pseudohermaphroditism. In contrast, overproduction of steroid hormones can be involved in breast or prostate cancer, in hypertension, and heart fibrosis. Besides inhibiting the action of the steroid hormones on the level of steroid hormone receptors by using antihormones, which often is connected with severe side effects, more recently the steroid hydroxylases themselves turned out to be promising new targets for drug development. Since the 3-dimensional structures of steroid hydroxylases are not yet available, computer models of the corresponding CYPs may help to develop new inhibitors of these enzymes. During the past years, the necessary test systems have been developed and new compounds have been synthesized, which displayed selective and specific inhibition of CYP17, CYP11B2, and CYP11B1. With some of these potential new drugs, clinical trials are under way. It can be expected that in the near future some of these compounds will contribute to our arsenal of new and selective drugs.

Keywords: CYP11B1; CYP11B2; CYP17; Hypertension; Heart fibrosis; Prostate cancer

Abbreviations: ACE, angiotensin-converting enzyme; ADME, absorption, distribution, metabolism, and excretion; AdR, adrenodoxin reductase; Adx, adrenodoxin; AGS (CAH), Adrenogenital syndrome (congenital adrenal hyperplasia); CMO, corticosterone methyl oxidase; EPHESUS, Eplerenone Post-Acute Myocardial Infarction Heart Failure Efficacy and Survival Study; DHEA, dehydroepiandrosterone; DOC, 11-deoxycorticosterone; FH, familial hyperaldosteronism; IC_{50}, concentration of inhibitor required to give 50% inhibition; RALES, Randomized Aldosterone Evaluation Study; RSS, 11-deoxycortisol; T, testosterone.

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1. Introduction

Steroid hormone research began in a broader sense with the crystallization of sex steroid hormones in the years 1929–1935, of the glucocorticoids in 1935–1938, and finally of aldosterone in 1953. All of these hormones possess the basic parent cyclopentanophenanthrene ring structure provided by cholesterol, which is modified by an array of enzymes expressed at various levels, in numerous tissues, throughout the body.

The enzymes involved in steroid hormone metabolism can be divided into 2 broad groups, the cytochromes P450 and the reductases, each of which exhibits important, biochemically distinct properties (Miller, 1988; Bureik et al., 2002a; Lisurek & Bernhardt, 2004). P450 enzymes comprise a large family, the CYP family of highly conserved proteins that incorporate molecular oxygen into lipophilic substrates with the provision of reducing equivalents from the cofactor nicotinamide adenine dinucleotide (phosphate) (NAD(P)H). Cytochrome P450 proteins in humans are enzymes that are used to synthesize cholesterol, steroids, and other important endogenous substrates such as prostacyclins and thromboxane A2, and to degrade xenobiotics and drugs. They catalyze many types of reactions, but the one that is most important is hydroxylation. These enzymes are classified as mixed function oxidases or monooxygenases, because they incorporate 1 atom of molecular oxygen into the substrate and 1 atom into water. These reactions are essentially irreversible, not easily product inhibited, and are so poised in the steroidogenic pathway that they determine the formation of each of the 5 major classes of steroid hormones: progestagens, mineralocorticoids, glucocorticoids, androgens, and estrogens.

To activate oxygen in the substrate binding pocket of P450s, electrons must be transferred from NAD(P)H to the P450, and this requires the participation of additional proteins acting as redox partners during the reaction. A protein complex forms transiently between the P450 and the redox partner allowing the effective transfer of electrons. There are 2 redox protein systems in mammals, 1 for the P450 enzymes anchored in the mitochondrial membrane and 1 for P450s located in the endoplasmic reticulum (microsomal compartment). The mitochondrial electron transfer chain consists of 2 components, a FAD containing flavoprotein, adrenodoxin reductase (AdR), and an iron–sulfur protein of the [2Fe-2S] ferredoxin type, adrenodoxin (Adx) (Lambeth et al., 1982).

Microsomal P450s are supported by a single redox partner protein, the highly conserved FAD and FMN containing flavoprotein NADPH-cytochrome P450 reductase (Black & Coon, 1987; Porter, 1991).

Thus, the subcellular location (Tamoaki, 1973), and corresponding electron transfer, or redox system, also defines a subclassification of mitochondrial or microsomal cytochrome P450s involved in steroid synthesis, collectively known as the steroid hydroxylases. Within the mitochondrial class of steroid hydroxylases of most species, there are 3 functionally distinct P450 enzymes. The first one, the cholesterol side-chain cleavage P450 (CYP11A, also known as $P450_{scc}$), utilizes cholesterol for the formation of pregnenolone, which is the universal precursor for all subsequent steroids (Fig. 1). A second enzyme, cytochrome P450 11β-hydroxylase (CYP11B1, also known as $P450_{11\beta}$ or P450c11), catalyzes the last steps in cortisol and corticosterone biosynthesis. In addition, CYP11B1 catalyzes the subsequent conversion of corticosterone to aldosterone in some species such as bovine and porcine, and therefore this enzyme is critical in mineralocorticoid metabolism in these animals. In humans, baboons, rats, mice, and guinea pigs, however, a third mitochondrial cytochrome P450, aldosterone synthase (CYP11B2, also known as $P450_{aldo}$), is encoded by another gene (CYP11B2), which has evolved by duplication of CYP11B1 to specifically catalyze aldosterone synthesis (Fig. 1) (Bureik et al., 2002a).

The enzymes comprising the microsomal steroid hydroxylase group include 3 P450s involved in steroid hormone biosynthetic steps subsequent to CYP11A1 leading to both corticoid and sex steroid hormone synthesis.

CYP17 (17α-hydroxylase/17,20-lyase, also known as P45017α or P450C17) catalyzes 17-hydroxylation of pregnenolone and progesterone and 17,20-lyase reaction of the corresponding 17-hydroxylated products. Progesterone and 17-hydroxyprogesterone are substrates for 21-hydroxylase cytochrome P450 (CYP21, also known as P450C21), which catalyzes the formation of 11-deoxycorticosterone (DOC) and 11-deoxycortisol (RSS), intermediates