STEROIDOGENIC CELL LINES: POTENTIAL USE AS BIOANALYTICAL TOOLS TO SCREEN CHEMICAL INTERFERENCES WITH STEROID HORMONE SYNTHESIS

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Introduction

Various environmental contaminants have the potential to disrupt sex hormone function in exposed organisms, which may result in adverse effects on reproduction, sexual differentiation, growth and development. Current research has focused mainly on potential interactions with sex hormone receptors such as the estrogen (ER) receptor. Certain chemicals have been shown to be agonists (or antagonists) for the ER, and although receptor affinities are usually much lower than endogenous ligands such as 17β-estradiol, exposures to these xenoestrogens can be relatively high. Bioanalytical screening methods are currently being developed to identify compounds in complex mixtures that activate (or block) the ER. Complex mixtures can thus be rank-ordered based on their biological potency as ER agonists or antagonists in a way analogous to the toxic equivalency concept used for dioxin-like aryl hydrocarbon receptor (AhR) ligands (1), although this process is complicated by the relatively lower persistencies of ER ligands compared with AhR ligands.

Despite the emphasis on xeno(anti)estrogens, many other mechanisms of potential interference with hormonal functions exist, including non-receptor-mediated effects such as those on enzymes involved in sex hormone synthesis. Several classes of environmental contaminants have been shown to inhibit or induce steroidogenic enzymes (2,3). Developing bioanalytical methods to identify and quantify chemicals or complex mixtures based on their effects on expression and catalytic activity of these enzymes, however, is not straightforward. Below we discuss some of the possibilities for interference with the biosynthetic pathway of steroid hormones, with particular emphasis on the synthesis of estrogens. In addition, several human cell lines are discussed with respect to their usefulness as screening tools to identify and quantify effects of xenobiotics on steroidogenesis, with particular emphasis on the H295R adrenocortical carcinoma cell line.

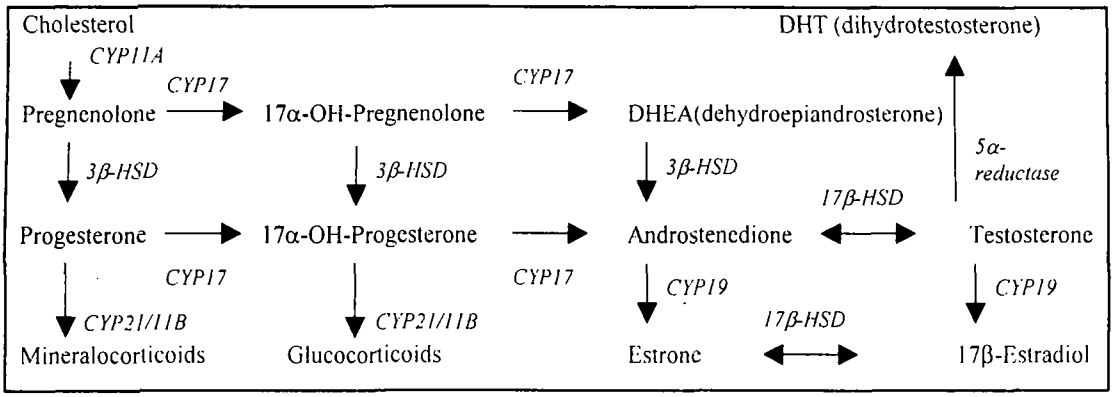

Fig. 1. Enzymes involved in biosynthesis of mineralo- and glucocorticoids, androgens and estrogens.

Enzymes involved in steroid synthesis

Steroidogenic enzymes are responsible for the biosynthesis of various steroid hormones including glucocorticoids, mineralocorticoids, progestins and sex hormones (Fig. 1), and consist of various specific cytochrome P-450 enzymes (CYPs), and several hydroxysteroid dehydrogenases (HSDs) and reductases (4). *De novo* synthesis of 17β-estradiol starts with the conversion of cholesterol to pregnenolone by CYP11A (cholesterol side-chain cleavage). In the subsequent steps 3β-HSD, CYP17 (17α-hydroxylase and 17,20 lyase activity), 17β-HSD and CYP19 (aromatase) are involved. Dependent on the tissue and the types of enzymes expressed cell types may produced predominantly androgens, estrogens, mineralo- or glucocorticoids. CYP19 is of particular interest as it is the rate-limiting catalyst in the formation of estrogens, not only in cells engaged in *de novo* synthesis of estrogens, but also in tissues such as the brain and adipose, which utilize circulating levels of androstenedione or testosterone as precursors. Aromatase is expressed in various tissues and plays an important role in sexual differentiation, development and behavior, particularly in the brain (5), but is also involved in diseases such as estrogen-dependent tumors (6).

Suitable cell systems to screen interferences with steroidogenesis

Our laboratory has used several human cell lines to investigate the effects of xenobiotics on steroidogenic enzymes, including MCF-7 breast tumor, JEG-3 and JAR placental choriocarcinoma, H295R adrenocortical carcinoma cells, and more recently, several human ovarian and rat Leydig cell lines, and primary human cell cultures. Each cell system has its advantages and disadvantages (7). We have given a preference to cell systems that have the capability of *de novo* synthesis of the hormones of interest and stably express the enzymes required. The H295R cell line has proven particularly useful as a screening tool because it stably expresses a wide range of steroidogenic enzymes, including all the enzymes required to produce mineralocorticoids, glucocorticoids, androgens and estrogens (8,9).

Qualitative use of the H295R bioassay

The H295R cell line can be used in several ways to assess the potential for compounds and complex mixtures to interfere with steroidogenesis. Among interactions with steroidogenic enzymes various mechanisms play a role, including direct reversible or irreversible catalytic inhibition, and up- or down regulation of enzyme expression. An initial screening approach could

involve the exposure of H295R cells to various chemicals or complex mixtures in the presence of a stimulating concentration of a steroid hormone precursor early in the steroidogenic pathway (Fig. 1), such as pregnenolone or the cholesterol analogue 22R-hydroxycholesterol (cholesterol itself is not bioavailable to CYP11A when added exogenously as it partitions into the cell membrane). The relative amounts of the various steroids produced over time by the H295R cells can be determined using HPLC or radioimmunoassay. Changes in the formation of the various steroids may reflect an effect on one or more steroidogenic enzymes, thus helping to narrow down the target enzymes affected by a given test compounds or mixture. Subsequently, catalytic assays using selective substrates may identify the individual enzymes affected. Enzyme kinetic studies may follow to determine whether induction or inhibition of an enzyme has occurred, and in the case of inhibition see whether it is of a competitive or non-competitive nature. In the case of induction, mRNA measurements may determine whether the observed increase in enzyme activity is the result of increased gene transcription.

The approach above uses the H295R bioassay in a qualitative way. It can be used to determine the ability of a complex mixture to inhibit or induce one or more steroidogenic enzymes. This will firstly provide information on a specific biological activity of the mixture; in other words, whether a mixture has the ability to increase or decrease estrogen, androgen or other steroid hormone levels in a particular cell system, and thus potentially in an intact organism. It may also direct the investigator toward uncovering the identity of certain classes of 'likely candidate' contaminants known to interfere with certain steroidogenic enzymes. These compounds include known inhibitors of CYP17 and 19 such as imidazole-type fungicides (2).

Quantitative use of the H295R bioassay

The quantitative use of H295R cells to compare the effects of environmental mixtures to that of known inhibitors/inducers of steroidogenic enzymes is complex, as there are various mechanisms of induction and inhibition. Induction of steroidogenic enzymes is highly tissue- and cell-type specific and is controlled by different promoters and second messenger pathways, which in turn provides various targets for interaction with xenobiotics. Inhibition of steroidogenic enzymes may occur by mechanisms such as substrate competition, or mechanism-based inactivation and other forms of non-competitive inhibition. Although inhibition by xenobiotics is likely to be less cell-type and organism dependent, it may be differentially influenced by cell-type and organism-specific biokinetics.

To complicate matters the resultant hormone products have varying physiological functions dependent on the tissue of formation and the stage of development of the organism (10). For example, estrogens are involved in determining sex-dependent behavior in the brain, whereas peripherally they control growth of bone, lipid metabolism and distribution, and the reproductive cycle in tissues such as the ovaries and uterus. In some tissues aromatase plays a crucial role by forming the required estrogens locally using circulating levels of androgens, in others circulating estrogens are required which originate mainly from the ovaries. Thus effects of chemicals on aromatase activity may result in altered estrogen synthesis, but the ultimate tissue and organism responses will be harder to predict.

Furthermore, although interferences with aromatase activity will mainly alter estrogen synthesis, effects on various other key enzymes in the steroidogenic pathway may result in a more complex pattern of altered hormone synthesis (Fig. 1). A potent and selective inhibitor of CYP11A would be expected to result in decreased overall steroid hormone synthesis, whereas inhibition of CYP17

(17,20-lyase activity) may result in decreased androgen synthesis and secondary to that decreased estrogen synthesis, but possibly also increased synthesis of mineralo- and glucocorticoids (in the appropriate tissues) due to the accumulation of precursor steroids.

To systematically rank-order the inhibition or induction potencies of various complex mixtures or individual environmental contaminants, comparisons need to be made with the potencies of well established reference compounds, such as known pharmacological inhibitors/inducers of the various enzymes under study. Highly selective and potent inhibitors are available for aromatase, such as 4-hydroxyandrostenedione or vorozole (11). However, inhibitors of CYP11A (aminoglutethimide) and 11B1 (ketoconazole) are considerably less selective and potent. Prototype inducers of steroidogenic enzymes, which may be used as reference compounds, are harder to find due to the high cell-type specificity of this response, and unless the mechanism of induction of the xenobiotic in a particular cell system is understood comparisons are not very useful.

Conclusions and future directions

Bioassay systems such as the H295R cell line are expected to be useful for the qualitative screening of the potential for complex environmental mixtures and individual contaminants to interfere with specific steroidogenic enzymes. Inhibitory effects may be compared to highly selective prototype enzyme inhibitors to obtain a rank-order, whereas inductive effects will require further mechanistic studies before interpretation is possible. However, given the complexities in the steroid synthesis pathways and the numerous biological activities of the resultant steroid hormones, together with the unknown biokinetic properties of the complex mixtures, we do not see steroidogenesis bioassays, at present, being used effectively to quantify complex mixtures based on their ability to interfere with steroid hormone synthesis.

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