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INTERACTION BY 2-HYDROXYESTROGENS WITH ENZYMES OF DRUG METABOLISM

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SUMMARY

Inhibition by estrogens of the demethylation of mestranol, catalyzed by rat liver microsomes was studied. Whereas estradiol, ethynyl-estradiol, and 16α-hydroxyestrone showed a non-competitive type of inhibition, the 2-oxygenated estrogens, 2-hydroxyestradiol and 2-methoxyestradiol, showed un-competitive inhibition. The strongest inhibitor was 2-hydroxyestradiol ($K_i = 3{\cdot}3 \times 10^{-5}$ M). Peroxidation of rat liver microsomal lipids *in vitro* was inhibited by all estrogens tested, but highest inhibitory potency was shown by 2-hydroxyestradiol ($I_{50} = 10^{-6}$ M).

INTRODUCTION

During the last few years it has become apparent that 2-hydroxylated estrogens are quantitatively important metabolic products of both natural [1–4] and synthetic [5–11] estrogens. This finding greatly stimulated research of possible biological effects of the catechol estrogens.

Knuppen and his associates [6, 12–14] demonstrated that 2-hydroxyestradiol, 2-hydroxyestrone, and 2-hydroxyethynylestradiol are effective in inhibiting methylation of catecholamines by the catechol-O-methyl transferase. They suggested that hypertension in pregnancy may be partly due to interference of catechol estrogens with that particular enzyme.

Recently there has been speculation that 2-hydroxylated estrogens may play a role in mediating hypothalamic response to estrogens: microinjections of 2-hydroxyestradiol into amygdala of minipigs produced a decline of plasma LH [15], and sc injections of 2-hydroxyestrone raised the LH levels of rats [16]. Furthermore, hypothalamic rat tissue has been reported to effect 2-hydroxylation of estrogens *in vitro* [17].

The present paper describes some further effects of 2-hydroxyestrogens. It is shown that 2-hydroxyestradiol, even in low concentration, interacts with liver microsomal enzymes of drug metabolism *in vitro*.

EXPERIMENTAL

Materials. 2-Hydroxyestradiol was donated by Schering AG, Berlin. Estradiol, ethynylestradiol, and 16α-hydroxyestrone were obtained from Merck, Darmstadt. 2-Methoxyestradiol was purchased from Steraloids, Pawling, N.Y.

Microsomal incubations. Rat liver microsomes were prepared according to the usual standard procedure [18, 19].

Demethylation of mestranol by rat liver microsomes in presence of NADPH-regenerating system was tested as previously described using a sensitive radiometric assay based on determination of polar ^{3}H-formaldehyde and H^{3}HO formed from the substrate [methoxy-^{3}H]mestranol [20].

Peroxidation of rat liver microsomal lipids in presence of NADPH-regenerating system, ADP and Fe^{2+} was tested by titration with thiobarbituric acid of the malondialdehyde formed according to a modification [21] of the method of Smuckler *et al.* [22].

RESULTS

To examine effects of estrogens on liver microsomal drug metabolism, demethylation of mestranol as a typical cytochrome P-450 dependent reaction [23] was chosen because of the availability of a sensitive radiometric asssay. Most spectrophotometric methods for reactions of drug metabolism *in vitro* are not sufficiently sensitive to permit measurements in presence of large amounts of inhibitors. Demethylation of mestranol was also studied since the product of demethylation, ethynylestradiol, is further hydroxylated by liver microsomes, mostly at C-2 [7, 8], so that inhibition by metabolites of mestranol may occur if metabolites accumulate. It has been shown that mestranol must be demethylated in order to become an estrogen active at target sex organs like the uterus [24, 20].

Table 1 shows the apparent inhibition constants (K_i) of several estrogens for the microsomal demethylation of mestranol. These constants have been calculated from Lineweaver–Burk plots [20]. Differences are found in the type of inhibition exhibited by 2-oxygenated and non-2-oxygenated estrogens. Whereas estradiol and 16α-hydroxyestrone inhibit mestranol demethylation non-competitively as has been reported for inhibition by ethynylestradiol [20],

Table 1. Inhibition by estrogens of enzymes involved in microsomal drug metabolism

Inhibitor	Demethylation of mestranol K_i	type of inhibition	Peroxidation of microsomal lipids I_{50}
Estradiol	1×10^{-4} M	non-competitive	2×10^{-5} M
16α-Hydroxyestrone	$2{\cdot}2 \times 10^{-4}$ M	non-competitive	$1{\cdot}3 \times 10^{-5}$ M
2-Hydroxyestradiol	$3{\cdot}3 \times 10^{-5}$ M	un-competitive	1×10^{-6} M
2-Methoxyestradiol	$7{\cdot}9 \times 10^{-5}$ M	un-competitive	3×10^{-5} M
Ethynylestradiol	$5{\cdot}4 \times 10^{-4}$ M*	non-competitive*	3×10^{-5} M

* see ref. [20].

2-hydroxyestradiol and 2-methoxyestradiol show uncompetitive inhibition (for nomenclature of the formal types of enzyme inhibition, see Dixon and Webb [25]). This difference in the inhibition by estradiol and its 2-hydroxy derivative is demonstrated in Fig. 1.

Among the estrogens tested, the 2-oxygenated compounds, especially 2-hydroxyestradiol, are the most effective in inhibiting demethylation of mestranol.

Table 1 also shows inhibition of microsomal lipid peroxidation by estrogens. In this special case it is not possible to give the (apparent) inhibition constant (K_i) since it would require measurements under different concentrations of substrate. Substrates of lipid peroxidation, however, are the microsomal lipid membranes, and it is not possible to prepare microsomes which differ only in their lipid content. For that reason Dixon plots [25] have been employed for calculation of the so-called I_{50} (concentration of inhibitor effecting 50% inhibition of the enzyme reaction). All estrogens are inhibitors of microsomal lipid peroxidation, but by far the greatest inhibitory effect is observed again with 2-hydroxy-estradiol ($I_{50} = 1$ μM).

DISCUSSION

The present results show that in addition to possible biological effects of 2-hydroxy-estrogens, these compounds appear to interact with the drug metabolizing enzyme system. This is indicated by the inhibition of demethylation of mestranol in the rat liver microsomal system and by inhibition of lipid peroxidation. Two-hydroxyestradiol was the most effective inhibitor among the estrogens examined. It should be claimed that the inhibitory potency of 2-hydroxyestradiol on lipid peroxidation is about one order of magnitude greater than its inhibition of the catechol-O-methyl transferase ($K_i = 1{\cdot}8 \times 10^{-5}$ M; [13]).

Free 2-hydroxyestrogens are physiological constituents of the phenolic steroids in human plasma and in late pregnancy levels of 120–360 ng 2-hydroxyestrone/100 ml plasma, i.e. about 10^{-8} mol/l [26], or even higher levels [27] have been measured. Because 2-hydroxyestrogens are formed in the endoplasmic reticulum of the liver, it is conceivable that at this particular place concentrations may arise which could result in a physiological inhibition of lipid peroxidation. Lipid peroxidation is believed to be involved in the physiological process of ageing of membranes. Furthermore, current publications suggest an implication of lipid peroxidation of hepatic cellular membranes in the mediation of toxicity of halogenated hydrocarbons, especially carbon tetrachloride [28, 29]. Preliminary experiments have shown that the toxicity of carbon tetrachloride in rodents is diminished by administering high doses of ethynylestradiol [30].

The uncompetitive mode of inhibition, differing from that of the other estrogenic compounds, suggests a unique mechanism of interaction with the microsomal enzymes which only takes place when the position ortho to the phenolic group is occupied by an oxygen function. Further studies on the mechanism of inhibition of lipid peroxidation demonstrated that a reactive oxygen species, most probably the superoxide radical (O_2^-) formed by the liver microsomal NADPH-cytochrome c reductase, which initiates lipid peroxidation, is trapped by catechol compounds which in turn are oxidized [31].

The opinion that catechol estrogens should be physiologically inert degradation products of hormonally active estrogens, therefore, deserves revision because evidence is growing that 2-hydroxyestrogens may exert unique biological effects on their own.

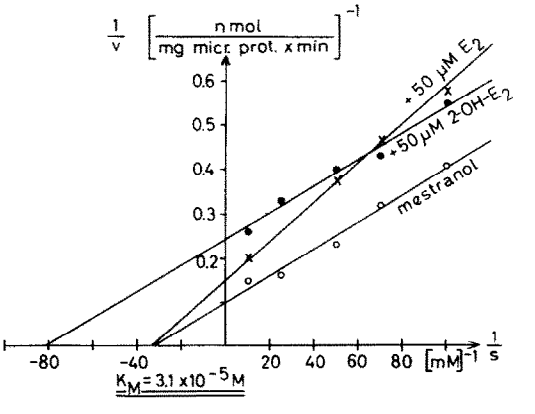

Fig. 1. Inhibition of microsomal demethylation of mestranol by estradiol and 2-hydroxyestradiol. O——O demethylation of mestranol without inhibitor; ●——● demethylation of mestranol in presence of 50 μM 2-hydroxyestradiol; ×——× demethylation of mestranol in presence of 50 μM estradiol. Lineweaver–Burk plots are shown.

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