ROLE OF ESTROGENS IN PHENOBARBITAL INDUCTION OF LIVER MICROSOMAL ENZYMES

P. V. Sergeev, N. N. Vedernikova, A. I. Maiskii, N. V. Shapoval, and V. A. Volov

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The action of estradiol dipropionate (250 μg/kg) and phenobarbital (80 mg/kg), both separately and in combination (a single injection of estradiol dipropionate after administration of phenobarbital for 4 days), was studied on ovariectomized rats. Administration of phenobarbital or estradiol dipropionate was shown to increase the incorporation of phenylalanine-H^3 in a cell-free protein-synthesizing system from liver microsomes of ovariectomized rats by 86 and 53% respectively. Combined administration of the estrogen and barbiturate was not followed by summation of that effect. Correlation was found between the increase in the rate of incorporation of phenylalanine-H^3 in vitro and the increase in the concentration of cytochrome P_{450} in vivo in the microsomes of the liver cells after both separate and combined administration of the preparations. The role of phenobarbital as an activator of estradiol metabolism in the liver cells is discussed.

KEY WORDS: estrogens; phenobarbital; protein synthesis; microsomal cytochromes of the liver; ovariectomized rats.

The role of the sex hormones in the induction of enzyme systems of liver cells by barbiturates has so far received little study. It is claimed that the action of these substances involves common mechanisms linked with the regulation of protein synthesis [2].

This paper describes a comparative analysis of the state of the cell-free protein-synthesizing system of the liver of ovariectomized rats and the content of cytochromes b_5 and P_{450} (components of the NADP·H_2-dependent electron transport chain, induced by barbiturates) following the separate and combined administration of phenobarbital and estradiol dipropionate.

EXPERIMENTAL METHOD

Noninbred female albino rats weighing 150-200 g, ovariectomized 3-4 weeks before the experiment, were used. Phenobarbital (sodium salt, Merck, West Germany), dissolved in water, was injected intraperitoneally in a dose of 80 mg/kg daily for 4 days. This time was chosen on the basis of the observations of Welch et al. [10], who showed that administration of phenobarbital for this period is followed by a maximal increase in hydroxylation of estrogens, with the formation of more strongly polar metabolites. An oily solution of estradiol dipropionate in a dose of 250 μg/kg was injected 24 h after the last injection of the barbiturate, and the animals were decapitated 15 h later. The rats were starved for 48 h before sacrifice. The rate of protein synthesis was determined from the incorporation of phenylalanine-H^3 in a cell-free protein-synthesizing system by Hoagland's method with slight modifications [4]. The content of cytochromes b_5 and P_{450} was determined on the DSF-1M differential spectrophotometer by the method of Omura and Sato [8]. Statistical analysis of the experimental results was carried out by Student's method.

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TABLE 1. Effect of Phenobarbital and Estradiol Dipropionate on Weight of the Uterus, Incorporation of Phenylalanine-H^3, and Cytochrome Content in Microsomes of Ovariectomized Rats (M ± m)

Substance	Wt. of uterus (% of control)	Incorporation of phenylalanine-H^3 (% of control)	Cytochrome b_5		Cytochrome P_{450}	
			nmoles/mg protein	%	nmoles/mg protein	%
Estradiol dipropionate	182,3±9,4*	153,4±12,3*	0,418±0,031	108,4	0,758±0,054*	140,4
Phenobarbital	97,4±6,4	186,2±17,9*	0,620±0,045*	160,6	1,163±0,083*	215,3
Estradiol dipropionate + phenobarbital	137,8±8,5*	208,9±13,1*	0,666±0,68*	172,5	1,201±0,042*	222,4
Control	100,0±4,8	100,0±6,2	0,386±0,015	100,0	0,540±0,028	100,0

* $P < 0.05$ (relative to control).

EXPERIMENTAL RESULTS

A single injection of estradiol dipropionate into ovariectomized rats caused a marked increase in weight of the uterus. Administration of phenobarbital for 4 days had no effect on the weight of the uterus but reduced the effect of estradiol dipropionate, when both substances were given, on the average by 44%.

This phenomenon is due to induction by barbiturates of xenobiotic-metabolizing enzyme systems in the liver, the physiological substrates of which are the steroid hormones [6]. It was shown previously that administration of barbiturates leads to a decrease in the quantity of biologically active steroid and to an increase in the number of its metabolites. In particular, the negative uterotropic effect of barbiturates as a result of increased estrogen metabolism has been demonstrated by several workers [3, 6, 10].

Experimental data indicating that estrogens [1] and barbiturates [5] can stimulate RNA and protein synthesis in the liver of animals and that the latter activate predominantly the biosynthesis of specific proteins which are components of NADP·H_2-dependent electron transport chain [2] have recently been obtained. The qualitative characteristics of proteins inducible by estrogen administration have received inadequate study.

As Table 1 shows, incorporation of phenylalanine-H^3 in the cell-free microsomal protein-synthesizing system from the liver of the ovariectomized rats increased by 53% on the average compared with the control after injection of estradiol dipropionate. Phenobarbital also stimulated protein synthesis in the microsomes of the rats' liver. Induction of protein synthesis thus took place at a time when the estrogen level was lowered by castration. Meanwhile it has been shown that the activity of microsomal enzymes which metabolize therapeutic compounds and poisons is controlled by the circulating blood sex hormone level [9]. In Marshall's opinion [7] a steroid hormone deficiency in the body leads principally to a disturbance of the conformation of the liver microsomal enzymes.

Another important aspect of interaction between xenobiotics and hormones is the possibility of competition, which has been found in model experiments in vitro between the substrate (hexobarbital) and the various steroids when incubated together with liver microsomes. Competition of this sort may also take place in vivo. In that case no increase in the induction of protein synthesis would be expected following the combined administration of phenobarbital and estradiol.

This hypothesis is confirmed by the results of the present experiments. Estradiol dipropionate and phenobarbital, when injected separately, stimulated the incorporation of phenylalanine-H^3 by the liver microsomes of the ovariectomized rats. However, combined administration of the substances did not lead to summation of the effects and the incorporation of the labeled amino acid into protein corresponded to the level observed when phenobarbital only had been given.

A similar picture was found during investigation of the content of cytochromes b_5 and P_{450}, components of the NADP·H_2-dependent electron transport chain. The data in Table 1 show that administration of phenobarbital caused the cytochrome b_5 and P_{450} levels to rise on the average by 60 and 115%, whereas injection of estradiol dipropionate led to increases of 8 and 40% respectively compared with the control ovariectomized rats. When estradiol dipropionate was injected after the preliminary injection of pheno-

barbital, the concentrations of cytochromes b_5 and P_{450} corresponded to their levels following administration of phenobarbital alone. This observation correlates with the results of the analysis of phenylalanine-H^3 incorporation in vitro.

Combined administration of estradiol and phenobarbital thus led to an increase in the quantity of metabolites of the hormone and to a corresponding decrease in the number of its biologically active molecules [3]. It is principally the latter that have the property of stimulating protein synthesis in the liver and this fact can be regarded as one condition determining the decrease in the estradiol effect in the presence of phenobarbital.

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