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LETTERS TO THE EDITOR

Inhibition by phenobarbitone of oestrogen-stimulated increases in uterine enzymes

Some barbiturates, carcinogens and insecticides enhance the activities of drug-metabolizing enzymes in hepatic microsomes (Conney, 1967). Pretreatment of immature rats with phenobarbitone modifies the responses of uterine tissue to oestrogenic hormones. Levin, Welch & Conney (1967) found phenobarbitone to inhibit the increases in uterine wet weights induced by oestradiol as well as the incorporation of [2-^{14}C]glycine into uterine protein. Subsequently these workers (Levin, Welch & Conney, 1968) demonstrated that phenobarbitone, and several unrelated inducers of liver microsomal enzymes, blocked not only the uterotrophic action of oestradiol and oestrone but also decreased the amount of tritiated oestrogen in the uterus after an injection of the labelled hormone.

We interpreted the rapid increases in the activity of uterine phosphofructokinase induced by oestradiol-17β to represent enzyme synthesis *de novo* (Singhal & Valadares, 1967; Singhal, Valadares & Ling, 1967a), but whether drug-induced stimulation of hepatic microsomal enzyme activities could modify the effects of oestradiol-17β on uterine enzyme biosynthesis remained unknown. We now report the ability of phenobarbitone to alter the oestrogen-induced increases in several glycolytic and hexosemonophosphate shunt enzymes in the uterus of the ovariectomized rat.

Female Wistar rats, 180–200 g were ovariectomized, and 2 weeks later were divided into three groups: control rats injected with physiological saline; animals treated intramuscularly with oestradiol-17β (0·1 μg/100 g); and rats treated by intraperitoneal injections of phenobarbitone (3·7 mg/100 g) twice daily for 3 days before giving the oestrogen. Animals were killed 16 h after injection of the hormone. Uteri were rapidly excised, carefully trimmed of extraneous tissue and homogenates and supernatant fluids were prepared (Singhal & others, 1967a, b). The activities of uterine hexokinase (Valadares, Singhal & Parulekar, 1968), aldolase (Warburg & Christian, 1943), as well as glucose 6-phosphate and 6-phosphogluconate dehydrogenases (Glock & McLean, 1953) were assayed in the supernatant. Uterine pyruvate kinase activity was also measured in the supernatant fluid (Weber, Stamm & Fisher, 1965). All enzyme activities were assayed under strictly linear kinetic conditions and calculated as μmol of substrate metabolized per g of tissue per h at 37° $\times$ the weight of the uterus.

Table 1 summarizes the effects of phenobarbitone pretreatment on the oestradiol-induced alterations in the activities of uterine hexokinase, aldolase, pyruvate kinase and the two NADP-specific dehydrogenases of the hexose monophosphate shunt pathway. Sixteen h after a single injection of oestradiol-17β, uterine hexokinase increased to 229%, aldolase to 331% and pyruvate kinase to 370% of the values of control animals. Likewise, the activities of glucose 6-phosphate and 6-phosphogluconate dehydrogenases were raised respectively, to 268 and 264% of control values in uteri of oestrogenized rats. Chronic treatment with phenobarbitone inhibited completely (98%) the increases in uterine hexokinase activity induced by oestradiol-17β. Phenobarbitone also reduced markedly the oestrogen-stimulated increases in uterine aldolase (138%) and pyruvate kinase (179%). Additionally, the steroid-induced rise in the activities of the two NADP-dependent dehydrogenases was inhibited significantly

Table 1. *Influence of phenobarbitone administration on oestradiol-stimulated increases in several uterine enzymes*

Treatment	Hexokinase	Aldolase	Pyruvate kinase	Glucose 6-phosphate dehydrogenase	6-Phospho-gluconate dehydrogenase
Control	4·8±0·2 (100)	10·2±0·3 (100)	108±4·0 (100)	9·6±0·2 (100)	2·48±0·02 (100)
Oestradiol-17β ..	11·0±0·3 (229)*	33·8±0·3 (331)*	399·5±13·3 (370)*	25·7±0·4 (268)*	6·6±0·3 (264)*
Oestradiol-17β + phenobarbitone	4·7±0·1 (98)†	14·1±0·7 (138)*†	193±10·1 (179)*†	16·0±0·4 (167)*†	3·8±0·2 (152)*†

Each value represents the mean ± s.e. based on 3 assays of enzyme activity in uteri pooled from 2–3 rats. Rats were treated with phenobarbitone (3·7 mg/100 g), twice daily for 3 days. Oestradiol-17β (0·1 μg/100 g) was administered intramuscularly 16 h before death. Enzyme activities are expressed as μmol of substrate metabolized per g of tissue per h at 37° × weight of the uterus. The results are also given in percentages (in parentheses) taking the control values as 100%.

* Statistically significant difference as compared to the values of control rats ($P = <0·05$).

† Statistically significant difference as compared to the values of oestradiol-injected rats without pretreatment with phenobarbitone ($P = <0·05$).

by phenobarbitone and in these animals, uterine glucose 6-phosphate dehydrogenase increased to only 167% and 6-phosphogluconate dehydrogenase to 152% of the values of saline-treated controls. Chronic phenobarbitone treatment did not influence the basal levels of uterine phosphofructokinase although this barbiturate produced an almost complete blockade of the enzyme response elicited by a 2·5 μg/100 g dose of oestradiol-17β (Singhal & others, 1967b).

It has been suggested that phenobarbitone accelerates the metabolism of oestradiol in the body by inducing the synthesis of drug-metabolizing enzymes (Conney, 1967). The observed inhibition by phenobarbitone of the oestradiol-stimulated increases in the activities of several uterine carbohydrate-metabolizing enzymes may be the result of a decrease in the amount of oestradiol-17β available to the uterus.

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