Inhibition of hepatic ribonuclease activity by chronic administration of phenobarbitone

Acute or chronic administration of phenobarbitone enhances the biotransformation of other drug molecules (Conney, Davison & others, 1960) and increases the incorporation of radiolabelled amino-acids into microsomal protein in *in vitro* and *in vivo* systems (Kato, Jondorf & others, 1966; Kuriyama, Omura & others, 1969; Shuster & Jick, 1966). Although the molecular basis for this phenomenon has not been established it may be due to an enhanced DNA-RNA polymerase activity, since it has been shown that actinomycin D inhibits phenobarbitone-induced enzyme activity (Orrenius, Ericsson & Ernster, 1965). This increase in rate of transcription may be attributed to an inhibition of repressor molecules synthesized by regulator genes (Jacob & Monod, 1961a, b). Alternatively, phenobarbitone may cause enzyme activation by interacting with the endoplasmic reticulum in such a way as to enhance the translation process (Conney, 1967). In addition, a decrease of hepatic ribonuclease (RNase) activity by phenobarbitone may be related in part to enzyme induction, since an inhibition of this enzyme by triamcinolone was associated with an increase in amino-transferase activity (Sarkar, 1969). Therefore, the purpose of this communication is to describe the effect of phenobarbitone on RNase activity.

Male Sprague-Dawley rats, 160 to 200 g, were pretreated with either 100 mg/kg phenobarbitone intraperitoneally, or equivalent volumes of physiologic saline on a daily basis for 2, 6 and 8 injections. Liver, excised from these animals fasted for 24 h after the last injection, was homogenized in 9 volumes of cold 0·24 M sucrose; 50mM tris-HCl, pH 7·6; 12mM $MgCl_2$; and 100mM KCl solution (medium I). Liver microsome and postmicrosomal supernatant fractions were prepared by the method of Zomzely, Roberts & Rapaport (1964). Ribonuclease activity of these fractions was measured (Barondes & Nirenberg, 1962, as modified by Zomzely, Roberts & others, 1968). Approximately 1 mg of microsomal or 0·7 mg of postmicrosomal supernatant protein was used in the incubation system. The protein concentration was measured by the method of Lowry, Rosenbrough & others (1951). Undergraded [^{14}C] polyuridylic acid (Poly U) was collected on Millipore filters (A-AWR-025-00, AAO-8μ), air dried, placed in scintillation vials with 5 ml of a toluene scintillation system composed of 0·5% PBD (phenylbiphenylyloxadiazole, Packard) and 0·01% dimethyl POPOP (1,4-bis-2-(4-methyl-5-phenyloxazoyl) benzene, Packard), and counted in a Packard Tri-Carb scintillation counter.

Six or 8 injections of a 100 mg/kg does of phenobarbitone causes an 80 and 60% reduction of RNase activity in hepatic microsomal and postmicrosomal supernatant fractions respectively, at the 30 min incubation interval (Table 1). There was also no difference in RNase activity of the latter fraction between control animals and rats

Table 1. *The effect of phenobarbitone on hepatic ribonuclease activity*

Number of phenobarbitone injections	Postmicrosomal supernatant			Microsomes		
	5 min	15 min	30 min	5 min	15 min	30 min
0	1250*	970	550	350	280	180
2	1450	1020	550	880	380	230
6	1590	1470	1120	1400	1200	900
8	1590	1470	1200	1400	1200	900

* The recovery of [^{14}C]Poly U in counts/min mg^{-1} protein. Each mean value is based on three independent incubations in duplicate.

receiving two injections of the barbiturate at all designated time intervals. However, at the 5 and 15 min interval, the recovery of [^{14}C]Poly U by microsomes from the latter group was greater than the controls. Therefore it appears that the degree of RNase inhibition is dependent on the number of injections of drug. Additional experiments in our laboratory on *N*-demethylation of aminopyrine (McMahon & Easton, 1962) indicates a positive correlation of enzyme activity and the number of phenobarbitone injections, which confirms Orrenius & Ernster's (1964) findings on *N*-demethylase and NADPH-cytochrome C reductase activity.

Sarkar (1969) has also shown a positive correlation of an increase in aspartate and alanine aminotransferase activity, and a decrease in RNase activity in rats pretreated with triamcinolone. These observations indicate that RNase inhibition may be associated with hepatic enzyme induction.

In vitro addition of phenobarbitone in concentrations up to 1×10^{-3} M to the RNase incubation system of control fractions did not alter the rate of [^{14}C]Poly U degradation. These results indicate that phenobarbitone did not inhibit RNase activity by direct action. It may be possible this inhibition is caused by induced inhibitor formation.

Our experiments suggest that ribonuclease activity inhibited in hepatic microsomal and postmicrosomal supernatant fractions upon chronic phenobarbitone pretreatment was not caused by direct action of the barbiturate and may in part play an essential role in enzyme induction.

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