SUPPRESSION OF HEPATIC RIBONUCLEASE DURING PHENOBARBITAL STIMULATION OF DRUG METABOLISM*

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Summary

Hepatic microsomal oxidative demethylase activity of phenobarbital-treated animals 1 or 3 days following 6 daily administrations of phenobarbital (100 mg/kg) was significantly ($p < 0.05$) greater than that of controls. Conversely hepatic microsomal ribonuclease activity of phenobarbital-treated rats was significantly ($p < 0.05$) reduced in rats killed on days 7 and 9 by 99% and 94% respectively. Administration of phenobarbital (50 mg/kg) for 5 days resulted in significant ($p < 0.05$) inhibition (45%) of ribonuclease and increased microsomal oxidative demethylase activity. Phenolpthalein B-glucuronidase activity of hepatic microsomal fractions obtained from phenobarbital-treated animals was not significantly ($p > 0.05$) different from controls. Phenobarbital (2.3 mg/ml) did not inhibit ribonuclease activity following in vitro additions to the assay system. These data suggest the possibility that induction of drug-metabolizing enzymes by phenobarbital may be mediated in part through suppression of enzymes capable of degrading RNA.

Introduction

Increased drug metabolizing enzyme activity following administration of inducing agents such as phenobarbital (Pb) has been associated with increased RNA polymerase activity (Gelboin et al., 1967) and enhanced microsomal amino acid incorporation (Kato et al., 1965). Induction of drug metabolizing enzymes by Pb is prevented by actinomycin D (Orrenius et al., 1965) indicating that Pb induction is mediated through DNA-dependent RNA polymerase activity. Results of this investigation indicate that induction of drug metabolizing enzymes by Pb is associated with reduction of hepatic ribonuclease (RNase) activity.

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Materials and Methods

Chemicals used were analytical reagent grade or equivalent. Highly polymerized yeast RNA was used as obtained from Calbiochem, Los Angeles, California or from Mann Research Laboratories, New York City.

Adult male Sprague-Dawley rats were guillotined following overnight fast. Livers were perfused in situ with ice-cold 0.25M sucrose and were homogenized with a coaxial homogenizer at 1000 RPM for one minute with 5 volumes of ice-cold 0.25M sucrose. Homogenates were centrifuged at 14,000 x g for 15 minutes at 0°C in a Model B-60 I.E.C. preparative ultracentrifuge, and the resulting supernate was centrifuged at 105,000 x g for 2 hrs at 0°C. The 105,000 x g pellet was rinsed with 0.25M sucrose and was assayed for RNase and aminopyrine (Ap) demethylase activity. Protein content of microsomal suspensions was estimated according to the method of Lowry et al. (1951).

Microsomal demethylation of Ap was estimated by assaying for formaldehyde formed as previously described (McMahon and Easton, 1962). The incubation medium (5 ml) for Ap demethylase determinations contained: aminopyrine 10 µM, semicarbazide 45 µM, glucose-6-phosphate 15 µM, NADP 2 µM, $MgCl_2$ 50 µM, nicotinamide 50 µM, 0.8 ml 105,000 x g supernatant (8.8 mg protein), 0.2 ml microsomal suspension (6 mg protein), and 1 ml 0.1M phosphate buffer pH 7.4.

RNase activity was estimated by a modification of a procedure described by Tsukada (1969). The incubation medium (1 ml) for RNase determinations contained: 0.2 ml 0.2M Tris-HCl buffer pH 7.6, 1.0 mg highly polymerized yeast RNA and 10-25 µl of microsomal suspension containing 18-39 mg protein/ml. RNA was added last and the incubation was

carried out in air at 37°C for 20 minutes in a Dubnoff incubator. Following the incubation 1.0 ml 1M HCl in 76% ethanol was added as a precipitant and the mixture was shaken thoroughly. The soluble fraction was separated by centrifugation at 10,000 RPM at 0°C in the #874 rotor of an I.E.C. preparative ultracentrifuge for 15 minutes. The absorbance of 1 ml of the clear supernatant after dilution with 2-5 ml of distilled water was read at 260 mμ vs. distilled water using a Beckman double-beam spectrophotometer. Readings obtained were corrected for absorbances of suitable tissue and RNA blanks.

Phenolpthalein-B-glucuronidase activity was estimated as described by Talalay et al. (1946).

TABLE I

INHIBITION OF HEPATIC RIBONUCLEASE DURING PHENOBARBITAL[a] (100 mg/kg) INDUCTION OF MICROSOMAL AMINOPYRINE DEMETHYLASE

Group	N	RNase[b] (OD 260/mg protein) ± S.E.	Aminopyrine demethylase mμm formaldehyde formed/mg protein/hr
Control	4	0.313 ± 0.056	17.7 ± 1.7
Killed on day 7	3	0.004 ± 0.002[c]	93.4 ± 10.9[c]
Killed on day 9	3	0.018 ± 0.015[c]	79.2 ± 7.6[c]

[a]Two groups of rats 400-550 gm treated with 100 mg/kg Pb for 6 days controls received distilled water.

[b]1.0 ml of incubation medium was diluted with 5 ml HOH and read at 260 mμ vs. distilled water.

[c]Significantly different from controls ($p < 0.05$) but not statistically different from each other.

Results

Table I shows the influence of Pb treatment (100 mg/kg i.p.) for 6 days on Ap demethylase and RNase activity associated with liver microsomal fractions 1 or 3 days following the last administration of Pb. Hepatic microsomal Ap demethylase of rats killed on day 7 was increased approximately five-fold relative to controls. Conversely RNase activity of microsomal fractions from Pb-treated animals was significantly ($p < 0.05$) reduced by 99% of control values. Hepatic microsomal Ap demethylase of rats sacrificed on day 9 was increased four-fold relative to controls while RNase activity was significantly ($p < 0.05$) reduced by 94% of control activity. RNase activities on day 9 appear to increase while corresponding Ap demethylase activities decrease toward control values.

The above experiment was repeated with hepatic microsomal fractions

TABLE II

INHIBITION OF HEPATIC RIBONUCLEASE DURING PHENOBARBITAL[a] (50 mg/kg) INDUCTION OF MICROSOMAL AMINOPYRINE DEMETHYLASE

Group	N	RNase[b] (OD 260/mg protein) ± S.E.	Aminopyrine demethylase mum formaldehyde formed/mg protein/hr
Control	5	0.357 ± 0.039	8.7 ± 3.2
Phenobarbital Treated	4	0.197 ± 0.007[c]	78.6 ± 10.6[c]

[a]One group of rats 375-410 gms was treated with 50 mg/kg phenobarbital for 5 days. Controls received distilled water. All animals were killed on day 6.

[b]1.0 ml of incubation medium was diluted with 2.0 ml distilled water and read at 260 mu vs. distilled water.

[c]Significantly different from controls ($p < 0.05$).

of rats sacrificed one day following administration of 5 daily doses of Pb with the dose reduced to 50 mg/kg i.p. (Table II). Stimulation of Ap demethylase by Pb was associated with a significant $p < 0.05$ reduction of RNase activity relative to controls.

Phenolpthalein-B-glucuronidase activity of microsomal fractions obtained from Pb-treated animals was not significantly different from controls thereby indicating that differential lysosomal contamination was not a factor in these studies. The addition of Pb 2.3 mg to the RNase assay system did not inhibit RNase activity of the microsomal fractions.

Discussion

These data demonstrate suppression of hepatic RNase during Pb induction of microsomal Ap demethylase activity. Reduction of RNase by Pb appears to be dose-related. Induction of Ap demethylation by Pb treatment (50 mg/kg) daily for 5 days is associated with a 45% reduction of RNase activity while 100 mg/kg of Pb daily for 6 days results in a 99% reduction of hepatic RNase.

Enhanced protein synthesis following administration of inducing agents such as Pb or cortisone is reported to result from stimulation of DNA-dependent RNA polymerase activity (Barnabei and Ottolenghi, 1968; Gelboin et al., 1967) and increased microsomal amino acid incorporating activity (Kato et al., 1965). A reciprocal relationship between liver nuclear RNA polymerase and microsomal RNA hydrolysis in cortisone-treated-adrenalectomized rats has been shown by other workers (Barnabei and Ottolenghi, 1968). Furthermore, reduction of hepatic acid and alkaline RNase activity in triamcinolone-treated rats has been correlated with simultaneous increases in aspartate and alanine aminotransferase activities (Sarkar, 1969). Loss of

microsomal amino acid incorporating activity from 3 methylcholanthrene-treated rats has been reported to occur at a lower rate relative to controls (Gelboin, 1964). Collectively these data suggest that stimulated RNA production is associated with suppression of liver RNase.

Whether suppression of RNase activity by Pb is attributable to altered lysosomal or microsomal RNase turnover or increased RNase inhibitor levels (Shortman, 1961) cannot be ascertained from these results. However, the absence of measurable RNase activity in the 105,000 x g liver supernatant from untreated rats and the failure to detect differences in B-glucuronidase suggest that the changes in RNase activities associated with the microsomes in these studies is not due to contamination attributable to disruption of lysosomes. The in vitro addition of Pb (2.3 mg) to incubation media did not inhibit RNase activity, thereby excluding direct inhibition of RNase activity by Pb.

In conclusion these data suggest that one of the resultant effects of the inducing agent Pb is its suppression of ribonuclease activity which in turn may be indirectly responsible for enhanced synthesis of enzyme protein.

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