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SHORT COMMUNICATIONS

Changes in rat serum ribonuclease during phenobarbital treatment

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PARTIAL hepatectomy and acute stress cause a decrease in the activity of serum ribonuclease closely related to the alteration of alkaline ribonuclease and RNase cellular inhibitor levels in the liver.[1] After partial hepatectomy, a decrease of ribonuclease activity was noticed in the early stages of liver regeneration, as reported by Arora and De-Lamirande,[2] the absence or reduction of ribonuclease activity being considered by these and other authors[3] as the main stabilizing factor of the ribosomes, that characterizes the regenerating period.

In a previous paper we have reported the production of a constant and early significant reduction in the activity of liver microsomal alkaline RNase after the administration of phenobarbital to adult male rats.[4] The lower RNase activity found has been considered as an important factor for the enhancement of protein synthesis produced by phenobarbital, since its inducing action mainly results from a stabilization of cellular RNA, as it has been recently demonstrated by Cohen and Ruddon.[5] In the present paper, the author reports a study on the free and total RNase activities in blood sera from rats at different periods after phenobarbital administration.

Male Wistar rats, 3–3·5 months old were starved for 24 hr period, receiving water *ad lib.* before they were bled. Control and phenobarbital-treated rats were kept under the same experimental conditions and control animals included within each experiment. Phenobarbital-treated animals were given 80 mg/kg body wt., daily, in aqueous solution (16 mg/ml) intragastrically, for a maximum of 7 days. The rats were bled at different times afterwards, between 10 hr after the first administration and 24 hr after the last one.

An orbital bleeding technique was used, employing the use of small siliconized polyethylene tubing (1·19 mm i.d., 1·70 o.d.) for collecting blood directly from the ophtalmic venous plexus in unanesthetized rats.[6] Individual non hemolyzed blood samples ± 5 ml were collected from a single puncture in less than 1 min per rat.

Clear blood serum was separated and recentrifugated at 3000 r.p.m. for 10 min after complete blood coagulation at room temperature for 30 min.

For the determination of RNase activity 20 μl serum were incubated for 20 min at 37° in a thermostable shaking bath with 0·25 ml of 0·25 M Tris–HCl buffer pH 7·45, 0·1 ml of 20 mM EDTA and 0·25 ml of 1·2% RNA solution, in a total volume of 0·700 ml containing NaCl in a final concentration 0·075 M.

The purified RNA used as substrate was prepared from a commercial RNA (yeast RNA B.D.H.) treated by the method of Kirby[7] and further dialysed against 0·01 M EDTA as described by Shortman.[8] Immediately after the incubation the sample tubes were transferred to an ice bath and precipitated with 0·25 ml of cold 0·75% uranyl–acetate in 25% perchloric acid. The assays were run in duplicate as well as blank tests. After precipitation (30 min) the soluble fractions were separated by centrifugation at 0° for 30 min at 12,500 *g*. 0·2 ml of the clear supernatants were diluted with 5 ml of distilled water in a test tube and absorbances determined at 260 nm. RNase activities were expressed in units per ml serum; one unit corresponds to an increase in absolute absorption value of 1000, within the range of linearity.[9]

Total RNase was determined by the same method but after releasing the latent RNase, bound to RNase inhibitor, by destruction of the inhibitor with 4×10^{-4} M *p*-chloromercuribenzoic acid (PCMB), included in the incubation medium, and pH adjusted to 7·45 as for the free RNase test.

Sulphydryl reagents easily inactivate RNase inhibitor, allowing the determination of latent ribonuclease activity and *p*-chloromercuribenzoic acid has been considered the most useful sulphydryl inhibitor for this purpose.[10]

The optimal pH and sulphydryl reagent concentration were previously determined, with the incubation system used.

Pools of six individual sera were used for the determination, in order to reduce the inconvenience of individual variation in RNase activity that we have observed in preliminary experiments. Percentual values are refered to the controls included in the same experiment.

Quantitative determination of protein was made by the Lowry method,[11] with LAB-TROL (DADE) as standard.

The pH optimum for rat serum ribonuclease was found to be 7·4–7·5. These results are in agreement with those found by Rabinovitch and Dohi,[12] using 0·05 M phosphate and 0·01 M veronal buffers and by Umeda *et al.*[13] for the purified rat serum Ribonuclease, in Tris-HCl buffer.

As it is shown in Fig. 1, there is a rise in the RNase activity released by PCMB, the maximum activation occurring at a concentration of 4×10^{-4} M. The same result was obtained both with sera from normal and from phenobarbital-treated rats.

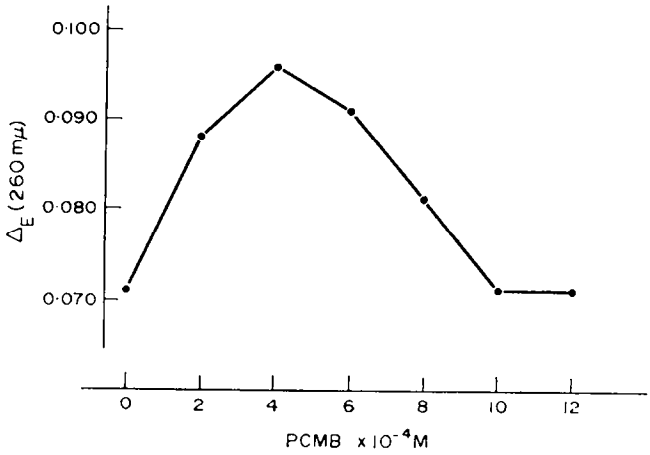

FIG. 1. The effect of PCMB on rat serum ribonuclease activity. Assay procedure is described in the text.

When the animals were treated with phenobarbital as described above, serum RNase was reduced to 86% ± 8 (S.D.) of the normal value 10 hr after the initiation of the treatment reaching the lower values 24 hr after the first phenobarbital administration, 72% ± 9 (S.D.) (Fig. 2).

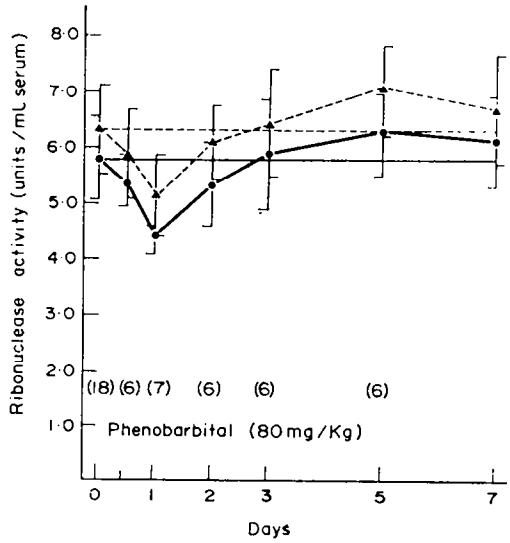

FIG. 2. Course of free and total RNase activities in rat serum as a function of time during phenobarbital treatment (mean values ±S.D.). ▲---▲ total RNase, ●——● free RNase. Figures in brackets represent the number of experiments for each point.

In the 6 subsequent days, during chronic phenobarbital treatment, 80 mg/kg daily, RNase activity rose again gradually reaching values higher than the normal at the 5th and 7th day, respectively 116% ± 9 (S.D.) and 111% ± 6 (S.D.).

Total serum RNase, determined in the presence of PCMB, followed similar percentual variations, presenting maximal reduction at 24 hr, 74% ± 10 (s.d.), of the normal total serum RNase, and then a progressive enhancement of the activity. Figure 2 represents the values found for free and total RNase activities, in units/min/ml serum.

The latent RNase, as determined by the difference between total and free RNase activities, did not show important modifications, the mean absolute values varying in the range 0·52–0·77 units per min per ml serum, along the whole period studied. At any time, the free RNase represented the most important part of total serum RNase (Table 1) showing that phenobarbital treatment does not significantly affect the situation present in normal rat sera, where the RNase inhibitor is quite negligible.[14]

We did not observe any significant difference in the total serum protein, under the action of the *in vivo* phenobarbital chronic treatment (Table 1).

TABLE 1

Time	0	10 hr	24 hr	2 D	3 D	5 D	7 D
RNase free/total	0·92	0·92	0·85	0·87	0·91	0·89	0·91
Protein	6·77	6·89	6·40	6·80	6·68	6·77	6·85
g.p. 100 ml	±0·25	±0·70	±0·30	±0·19	±0·22	±0·42	±0·27
	(18)	(6)	(7)	(6)	(6)	(6)	(6)

Free/total RNase activities ratios, and total serum protein, in control and phenobarbital-treated rats, as described in the text: Figures in brackets represent the number of experiments.

It has been shown, in this investigation that rat serum RNase activity is decreased 24 hr after phenobarbital administration and that under chronic treatment by this drug it rises subsequently reaching values higher than the normal at the 5th and 7th day.

The reduction of serum RNase observed can be compared with the modification produced in liver microsomal RNase during the induction of liver microsomal enzymes by phenobarbital.[4] However, the subsequent rise of serum RNase activity is no more understandable as the direct reflexion of the alteration of the hepatic microsomal RNase specific activities, that remain at a stable low level during the phenobarbital steady-state. Nevertheless, as it is known, the phenobarbital administration produces an important liver hypertrophy[15] mainly due to the proliferation of the endoplasmic reticulum.[16] Total microsomal phospholipids[17] and proteins are significantly enhanced, reaching near 130 per cent of the normal after 4 consecutive days of phenobarbital treatment,[4] and total RNase activity per liver approaches the normal values at this period. Moreover the blood flow presents an important increase from the 3rd to 4th day after the beginning of barbiturate treatment, as it has been recently demonstrated by Ohnhaus *et al.*[18] These facts could explain the enhancement in RNase activity observed, as it is known that many plasma enzymes essentially arise in liver[19] and that liver endoplasmic reticulum exports proteins to the circulation.[20,21] The problem of the origin of serum RNase is nowadays lacking clear elucidation, though some evidences have been presented, correlating it, particularly in pathological conditions, with different organs and tissues.[22]

From the presented data we can conclude that there are analogies with the situations studied by Tsukada, despite this author has limitated the investigation to short periods, maximum 24 hr, and that our results, although not providing direct evidences, corroborate the hypothesis of an hepatic origin for serum RNase, suggested by the same author.[1] However the variations of RNase activity that we have observed under phenobarbital treatment are not dependent on an increase in the level of ribonuclease inhibitor in the serum, detectable by PCMB inactivation, proving that the decrease of activity produced by phenobarbital is of a different nature from that one observed after partial hepatectomy and acute stress.

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Phosphodiesterase inhibition by papaverine and structurally related compounds*

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CYCLIC 3′,5′-nucleotide phosphodiesterase(s) (PD) catalyze the hydrolysis of cyclic 3′,5′-adenosine monophosphate (cyclic AMP) and other cyclic 3′,5′-nucleotides to their respective 5′-nucleoside monophosphates. Following its discovery by Rall and Sutherland,[1] numerous agents have been found which inhibit the PD-promoted hydrolysis of cyclic AMP.[2] Papaverine, initially observed by, Pöch *et al.*[3] to be a potent inhibitor of rat coronary vessel PD, has been reported to exert a similar effect upon this enzyme activity from several different tissues in a number of species.[4–7] Recently structure–activity relationship studies in regard to modifiers of this enzyme activity have appeared in the literature.[8,9] This report describes the investigation of several compounds structurally related to papaverine as inhibitors of a partially purified PD from beef heart. Analysis of the data proved useful in identifying those structural features of papaverine-like compounds which apparently are necessary for inhibition.

Cyclic nucleotide PD was partially purified from beef heart by the procedure of Butcher and Sutherland.[10] The activity of the PD preparation was approximately 4·8 μmoles of cyclic AMP hydrolyzed/mg of protein/hr with saturating amounts of cyclic AMP (5×10^{-4} M) as substrate. Initial velocity measurements were conducted by determining the rate of ^{3}H-5′-AMP generation from ^{3}H-cyclic AMP according to a procedure described previously.[11] All analyses were carried out in duplicate for 15

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