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RIBOSOMAL AND MICROSOMAL RNASE ACTIVITIES IN RAT LIVER AFTER METHYLCHOLANTHRENE ADMINISTRATION*

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Abstract—The administration of a single dose of 3-methylcholanthrene to adult male Wistar rats causes a fleeting reduction in microsomal RNase but an important and lasting reduction in ribosomal RNase. Aniline hydroxylase activity, cytochrome P-450 and liver weight rise more markedly during the first 24 hr whilst microsomal and ribosomal RNases are at their lowest level. Microsomes from 3-MC treated rats show an enhanced protein synthesizing capacity at 10 hr, concomitantly with diminished RNase. Ribosomes are better able to incorporate L-[^{14}C]-phenylalanine *in vitro* 10 and 24 hr after the treatment, they have reduced RNase activities and are stabilized as revealed by polysomes autodegradation tests. This suggests the possibility of an inverse relationship between protein synthesizing capacities and RNase activities. It is concluded that there is a polysomes stabilization in rat liver, following MC treatment that may be, at least in part, explained by the reduction in RNase activities, now described.

IN A PREVIOUS investigation Lechner and Pousada[1] have demonstrated that during the stimulation of oxidative drug metabolism by phenobarbital and other inducing agents of the same group there is a constant and early significant reduction in liver microsomal alkaline RNase that precedes the rise of cytochrome P450, related enzymes activities and liver weight. In that paper the authors have concluded that microsomal alkaline RNase may play a role in the mechanism of stimulation of oxidative drug metabolism by inducers of the phenobarbital group. This same hypothesis has been advanced by other authors based on results obtained with phenobarbital treated rats in different conditions.[2–4]

It is known that the hepatic microsomal enzyme induction phenomena are mainly the result of an enhancement in the synthesis of new molecules of drug metabolizing enzymes,[5,6] the phenobarbital affecting the liver protein synthesis in a general way particularly at microsomal level.[7,8] 3-Methylcholanthrene is the prototype of inducers of the polycyclic hydrocarbon group, characterized by affecting in a more specific way the synthesis of certain drug metabolizing enzymes. Gelboin, from investigations on protein synthesizing capacities[9,10] of rat liver after MC administration, has concluded that at least part of the increased incorporation of L-[^{14}C]-phenylalanine in microsomes is not dependent on alterations in the endoplasmic reticulum (as it is the case with phenobarbital)[11] but in the ribosome itself.

The presence of an alkaline RNase in rat liver ribosomes has been reported by Arora and De Lamirande.[12] From the changes observed in rat liver during the

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regeneration period, after partial hepatectomy, these authors have postulated that ribosomal RNase is an important control factor in protein synthesis on which the stability of active polysomes depends to a large extent.

In the present investigation variations of alkaline RNase activities in microsomes and in isolated ribosomes from rat liver, were studied at different times after 3 MC-administration. Simultaneously, amino acid incorporation and ribosomes autodegradation tests have been performed in parallel with determinations of cytochrome P450 and aniline hydroxylase.

The purpose of this investigation was to bring additional information on the importance of ribonucleases in the control mechanism of liver protein synthesis in general[13] and in the inductive response in particular.

MATERIAL AND METHODS

Male Wistar rats 3–3½ months old were used and deprived of food for 24 hr before sacrifice but provided with water *ad lib*. Groups of 4 rats were used for each sample.

The treated rats were injected intraperitoneally with a single dose of 3-methylcholanthrene (50 mg/kg body wt) in corn oil solution (10 mg/ml). For each time of treatment a control was run in parallel, the animals being injected with corn oil.

At different times after the administration of the drug, the rats were kileld by decapitation, bled and the livers removed, weighed, pooled and homogenized in 6·5 vol. of 0·25 M sucrose solution. All operations were carried out at low temperature and sample containers kept in ice baths.

Liver microsomes were prepared as previously described.[1] The washed microsomes were then resuspended in 2 vol. of 0·15 M KCl, 25 mM Tris-buffer (pH 7·5), and adjusted to a protein concentration of 10 mg/ml for L-[^{14}C]-phenylalanine incorporation tests and 6·67 mg for determinations of aniline hydroxylase and cytochrome P450.

The ribosomes were isolated essentially by the method of Tashiro and Siekevitz,[14] although slightly modified.[15] Tissue samples were homogenized in 2·5 vol. of 0·25 M sucrose solution, centrifuged for 10 min at 7000 *g*, the supernatant collected and re-centrifuged at 12,500 *g* for 10 min. The post-mitochondrial supernatant was then spun down by centrifugation at 105,000 *g* for 1 hr.

The microsomal pellet was suspended in 25 ml of 50 mM Tris-buffer (pH 7·5), containing 12·5 mM $MgCl_2$ and 10 mM KCl. Sodium deoxycholate was added to a final concentration of 0·5 per cent, and ribosomes collected by centrifugation at 105,000 *g* for 90 min. The resuspended ribosomal pellet was passed through 2 M sucrose layer in 50 mM Tris-buffer (pH 7·5) containing 12·5 mM $MgCl_2$ and 10 mM KCl by centrifugation at 105,000 *g* for 16 hr, in order to separate the ribosomes from the less dense membranes eventually present as impurities.[16] The purified ribosomes were finally resuspended in 5 ml of 0·05 M Tris-buffer (pH 7·5) 10 mM KCl and 12·5 mM $MgCl_2$.

RNase activities were determined as described before,[1] in microsomal suspensions adjusted to a protein concentration of 6·67 mg/ml and in ribosomal suspensions with 2 mg protein per ml. Results are expressed in units per min per milligram microsomal or ribosomal protein.

The liver ribosomes autodegradation tests were performed by the method described

by Arora and De Lamirande,[12] at the optimal pH for ribosomal RNase that was found to be 7·8. The results were expressed in terms of optical density at 260 nm per milligram ribosomal protein.

The L-[^{14}C]-phenylalanine incorporation tests were performed in an incubation medium containing in a total volume of 0·300 ml; 5,74 μmoles potassium phosphate (pH 7·4), 0·54 μmoles ATP, 0·57 μmoles GTP, 4·50 μmoles magnesium acetate, 13 μmoles phosphocreatine, 50 μg creatine phosphokinase (EC 2.7.3.2), 19·80 μmoles GSH, pH adjusted to 7·4; 0·04 μmoles of uniformly labelled L-[^{14}C]-phenylalanine (specific activity 10 mc/mM), normal rat liver cytoplasmic soluble fraction (supernatant of 105,000 *g* freshly prepared) corresponding to 0·48 mg soluble protein. The incubations were run in duplicate at 37° for 20 min, in the presence of either microsomes or ribosomes (obtained as prescribed by Korner[17]) in a quantity corresponding to 0·60 and 0·30 mg microsomal or ribosomal protein respectively. 100 μl of these incubation mixtures were removed at zero time and at 20 min and uniformly spread over a small filter paper disc 2·5 cm dia. (Schleicher and Shüll 598). Incorporated L-[^{14}C]-phenylalanine was fixed in cold 10% TCA solution and unincorporated labelled amino acid washed out by the method of Mans and Novelli.[18] The incorporated radioactivity was measured in a Packard model 3320, Tri-carb liquid scintillation spectrometer. Phenylalanine incorporation activity in these cell free systems was expressed in cpm per milligram microsomal or ribosomal protein.

Proteins were estimated by the method of Lowry *et al.*[19] Aniline hydroxylase activities and P450 were measured in microsomal fractions as described elsewhere and expressed in nmoles *p*-aminophenol formed per min and in nmoles, per milligram microsomal protein, respectively.[1]

RESULTS

After the administration of a single dose of 3-methylcholanthrene, aniline hydroxylase activity and cytochrome P450 increased, attaining 127 and 110 per cent respectively after 10 hr, and 168 and 125 per cent of the normal values after 24 hr.

Microsomal RNase activity was decreased at 10 hr (75 per cent of the normal values) rising subsequently to 87 per cent of the normal values, 24 hr after MC administration. Ribosomal RNase showed a pronounced reduction, both at 10 and 24 hr, falling to near 50 per cent of the normal activity. At 48 and 72 hr after drug administration there was a progressive rise in RNase activities, as shown in Fig. 1. Autodegradation tests have shown that liver ribosomes from MC treated animals, prepared 10 and 24 hr after drug administration are more stable than the normal (Fig. 2). Protein synthesizing capacity of the studied subcellular fractions was affected by MC treatment, revealing that whole microsomes from induced animals are more able to incorporate L-[^{14}C]-phenylalanine *in vitro* (168 per cent of the normal), 10 hr after the drug administration. After 24 hr amino acid incorporation capacity was decreased, approaching normal values.

Concomitantly ribosomal fractions of the treated animals showed an enhanced amino acid incorporation at 10 and 24 hr, representing 165 and 156 per cent of control ribosomes activity respectively (Fig. 3).

All the results were calculated in percentages of the control values included in each experiment.[1]

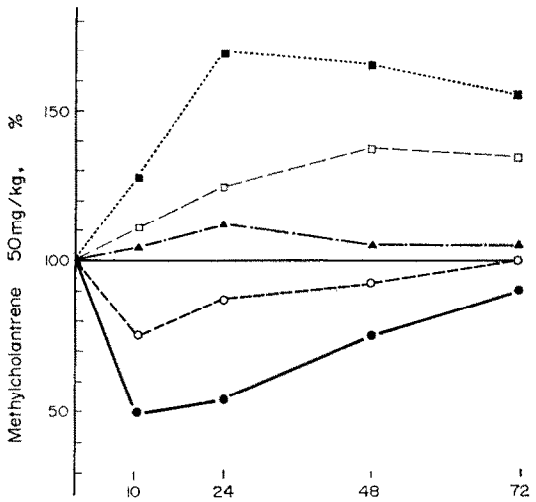

FIG. 1. Plot of the mean percentual values obtained in methylcholanthrene treated rats ●——● Rib. RNase; ○- - -○ Mic. RNase; ▲——▲ Liver weight; □——□ Cytochrome P 450; ■ · · · ■ Aniline hydroxylase.

Control values were: P 450, 1·16 ± 0·12 nmoles (n = 8); Mic. RNase 0·192 ± 0·034 (n = 8) units; Rib. RNase, 0·378 ± 0·057 (n = 7) units; Aniline hydroxylase, 0·217 ± 0·011 (n = 6) nmoles *p*-hydroxyaniline, all expressed per min per milligram microsomal protein; liver weight, 2·66 ± 0·09 (n = 8) per 100 g body wt.

Each point represents the mean of four experiments.

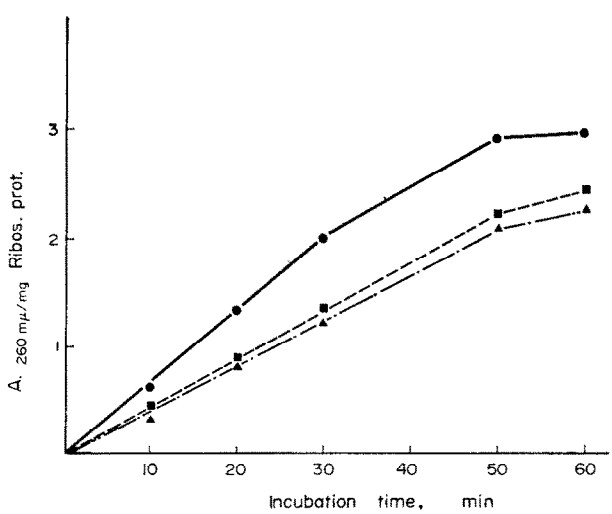

FIG. 2. Rate of ribosomes autodegradation. Control, ●——●, 3-MC treated animals: 10 hr, ■- - -■; 24 hr, ▲- · -▲.

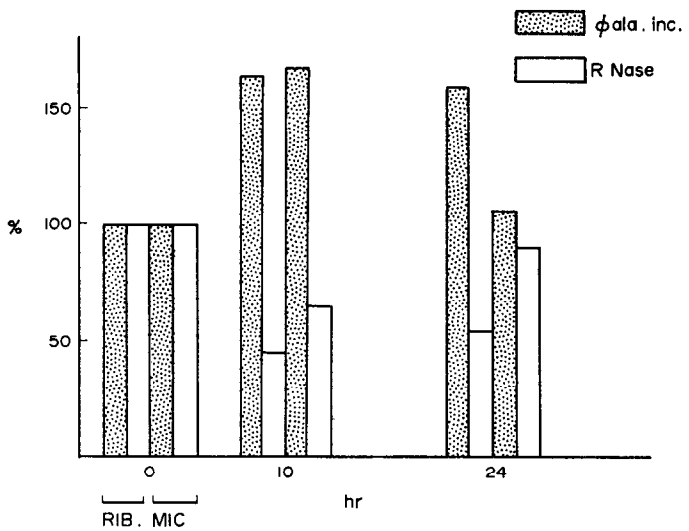

FIG. 3. Effect of *MC on L-[^{14}C]-phenylalanine incorporation and on RNase activities in rat liver microsomes and ribosomes.
Specific activities found for controls: 67·5 and 614 cpm per milligram microsomal and ribosomal protein, respectively. Experimental details as in Materials and Methods.

DISCUSSION

The molecular mechanism of the enhancement in the capacity of drug metabolizing activities, produced at the hepatic microsomal level, by MC, is not completely understood. It is believed that the greater enzymatic protein synthesis, on which the enhancement of drug metabolizing capacities depends, is the consequence of the accumulation of certain RNA species in the cell.[20] Several authors[21,22] have searched for a possible activation of the DNA-dependent RNA polymerase, that would explain the increase in the nuclear RNA content, produced in the liver cell by MC. However, the results already published do not entirely account for the situation, and are not always in agreement with each other.

Bresnick *et al.*[21] failed to detect an increase of the orotic acid incorporation, after MC pretreatment, but Gelboin *et al.* found that 3 hr after drug administration there was a stimulation of DNA-dependent RNA polymerase in rat liver as determined by *in vitro* [^{3}H]-CTP incorporation tests.[22]

Loeb and Gelboin[23] and Hishizawa[24] have also found an increase in orotic acid incorporation into RNA, after MC treatment. These investigators suggested that the higher specific activity of the RNA found, indicates an MC-induced increase in the rate of RNA synthesis. Nevertheless, as has been recently stated by Gelboin, this conclusion must be viewed with caution, since the intracellular and extranuclear pool sizes of precursors of RNA, as well as the rate of degradation of RNA, may markedly affect the specific activity of RNA.[25]

In this connection, Hopkinson and Argyris,[26] studying the rate of incorporation of [^{3}H]-orotic acid into free cellular uridine nucleotides and nuclear RNA, 12, 24 and

72 hr after MC injection, have found only a modest increase in the rate of RNA synthesis (considering the changes in RNA precursor pool sizes), not sufficient to account for the RNA accumulation induced by MC in the liver.

The results found in the present investigation show that 3 MC causes a fleeting reduction in microsomal RNase that attains minimal values 10 hr after administration of the drug but an important reduction in ribosomal RNase persisted at 10, 24 and 48 hr. Aniline hydroxylase activity, cytochrome P450 and liver weight rose more markedly during the first 24 hr when microsomal and ribosomal RNases attained lower values. Concomitantly, microsomes from 3 MC rats have shown an enhanced protein synthesizing capacity at 10 hr, when RNase activity was reduced, but both microsomal amino acid incorporation capacities and RNase activity approach normal values at 24 hr.

Liver ribosomes are better able to incorporate phenylalanine *in vitro*, 10 and 24 hr after the treatment, have reduced RNase activities and are stabilized as revealed by polysomes autodegradation tests.

These results suggest that there is an important decrease in the rate of RNA degradation in liver cell, associated with 3 MC induction, particularly concerned with polysomes, that appears to be related to the enhancement in amino acid incorporation capacities of both ribosomes and microsomes.

We may therefore consider the hypothesis, also supported by the results of Hopkinson and Argyris that the decrease in RNA degradation may be an important step to the inductive response to MC, in the hepatic cell. This RNA stabilization may be, at least in part, the consequence of the alterations in microsomal and ribosomal RNase activities described in the present paper.

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