Ribosome Function in Livers of Porphyric Mice

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1. A porphyrinogenic drug, 3,5-diethoxycarbonyl-1,4-dihydrocollidine, caused a decrease in the proportion of single ('run-off') ribosomes, and an increase in the number of polyribosomes, in the livers of treated animals.
2. No change could be detected in the distribution of amino acid incorporation among hepatic polyribosomes.

The porphyrias are a group of inherited diseases associated with overproduction of porphyrins or porphyrin precursors. An unusual biochemical aspect of these diseases is that they are associated with increased activity of an enzyme. A reasonable explanation for this phenomenon is that synthesis of the first enzyme in the haem-biosynthetic pathway (5-aminolaevulinate synthetase) is normally controlled by feedback repression from haem. A defect in any one of the intermediate enzymes of the pathway would then decrease haem formation and result in increased production of 5-aminolaevulinate synthetase (for review, see Elder *et al.*, 1972).

Animal models have been developed by using drugs which induce porphyria. One of these drugs, 3,5-diethoxycarbonyl-1,4-dihydrocollidine, appears to act by inhibiting conversion of protoporphyrin into haem. As with human porphyria, 5-aminolaevulinate synthetase would again be induced as a result of decreased feedback repression from haem (De Matteis *et al.*, 1973). In common with some other porphyrinogenic drugs, 3,5-diethoxycarbonyl-1,4-dihydrocollidine resembles phenobarbital in its ability to increase liver RNA (Nawata & Kato, 1973), protein and lipid (Waterfield, 1967). Thus 3,5-diethoxycarbonyl-1,4-dihydrocollidine seems to have both specific and generalized effects.

Treatment of mice with 3,5-diethoxycarbonyl-1,4-dihydrocollidine results in a 5-fold increase in 5-aminolaevulinate synthetase after 6h, and this increase in activity is prevented by injecting the animals with actinomycin 1h before treatment with 3,5-diethoxycarbonyl-1,4-dihydrocollidine (Norman, 1971). Thus it seems probable that 3,5-diethoxycarbonyl-1,4-dihydrocollidine increases the overall rate of protein synthesis in the liver, while exerting a particularly marked effect on formation of 5-aminolaevulinate synthetase. The experiments described in

the present paper were designed to detect any changes in hepatic ribosome function that might occur 6h after treatment of mice with 3,5-diethoxycarbonyl-1,4-dihydrocollidine.

Experimental

Materials. 3,5-Diethoxycarbonyl-1,4-dihydrocollidine, which was supplied by Eastman Kodak Ltd., Kirkby, U.K., was recrystallized twice from an ethanol–water mixture before use.

The sources of other materials were as described previously (Norman *et al.*, 1973).

Treatment of animals. Male albino mice weighing 25–30g were maintained on an Oxoid 41B diet and water *ad libitum*. 3,5-Diethoxycarbonyl-1,4-dihydrocollidine was ground into a fine powder and suspended in a 0.5% solution of methylcellulose to give a final solution containing 31.3mg/ml. The drug was administered by stomach tube between 8:30 and 9:30 a.m., a dose of 500mg/kg being used. Control animals were treated with 0.5% methylcellulose only, and in each experiment three control mice and three mice treated with the drug were used.

Radioactively labelled polypeptide and RNA precursors were injected intraperitoneally in 0.2ml of a 9g/litre NaCl solution.

[1-¹⁴C]Leucine (62 mCi/mmol; 10 μ Ci/mouse) and [4,5-³H]-leucine (30 Ci/mmol; 100 μ Ci/mouse) were injected 6min before the mice were killed. [6-¹⁴C]Orotic acid (61 mCi/mmol; 10 μ Ci/mouse) and [5-³H]orotic acid (26 Ci/mmol; 100 μ Ci/mouse) were injected 24h before the mice were killed. The preparation and analysis of hepatic polyribosomes and measurement of radioactivity were performed as described previously (Norman *et al.*, 1973).

Results

Size distribution of mouse liver polyribosomes after administration of 3,5-diethoxycarbonyl-1,4-dihydrocollidine. To determine whether or not the drug

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affects the size distribution of mouse liver polyribosomes, rRNA was labelled radioisotopically and the distribution of this RNA was studied 6h after administration of the drug. Treated mice were injected with [^{14}C]orotic acid and control mice were injected with [^3H]orotic acid 24h before the animals were killed (Fig. 1a). In separate experiments (not shown), the radioisotopic labels were reversed and mice treated with 3,5-diethoxycarbonyl-1,4-dihydrocollidine were injected with [^3H]orotic acid, and controls with [^{14}C]orotic acid.

In six experiments, a marked decrease in the proportion of monomeric ribosomes, with a corresponding slight increase in the number of poly-

ribosomes, was found in animals treated with 3,5-diethoxycarbonyl-1,4-dihydrocollidine. Although the drug apparently caused an increase in monomeric ribosomes in one other experiment, we believe this unexpected result was due to a technical error.

Incorporation of amino acid into mouse liver polyribosomes after administration of 3,5-diethoxycarbonyl-1,4-dihydrocollidine. To determine whether the drug produces any change in the pattern of protein-synthetic activity among mouse liver polyribosomes of different sizes, amino acid incorporation into polyribosomes was studied 6h after mice had been treated with the drug. Treated mice were injected with [^{14}C]leucine and control mice were injected with

Fig. 1. Density-gradient analysis showing (a) the distribution of radioactively labelled RNA of ribosomes and (b) the distribution of radioactively labelled nascent polypeptides attached to polyribosomes

(a) Rate zonal centrifugation of a postmitochondrial supernatant fraction was at 7°C for 6h at 30000 rev./min ($\omega^2 t = 20.1 \times 10^{10} \text{ rad}^2/\text{s}$) in an MSE BXIV zonal rotor with 380 ml of a 15–45% (w/w) sucrose gradient and a 250 ml cushion of 50% (w/w) sucrose. Mice treated with 3,5-diethoxycarbonyl-1,4-dihydrocollidine were injected with [^{14}C]orotic acid (●); control mice were injected with [^3H]orotic acid (○). Individual points are presented as a percentage of total d.p.m. in the gradient, which were: ^3H , 578800; ^{14}C , 105300. Abbreviations: nS, native subunits; M, monomers; P, polyribosomes. —, % transmittance at 254 nm. (b) Rate zonal centrifugation of a postmitochondrial supernatant fraction was at 7°C for 3h 20 min at 30000 rev./min ($\omega^2 t = 11.8 \times 10^{10} \text{ rad}^2/\text{s}$) in an MSE BXIV zonal rotor with a 15–45% (w/w) sucrose gradient which was linear with volume. Mice treated with 3,5-diethoxycarbonyl-1,4-dihydrocollidine were injected with [^{14}C]leucine (●); control mice were injected with [^3H]leucine (○). Individual points are presented as a percentage of total d.p.m. in the gradient, which were: ^3H , 67430; ^{14}C , 12440. Abbreviations are as in Fig. 1(a).

[^3H]leucine 6 min before the animals were killed (Fig. 1b). In a separate experiment (not shown), the radioisotopic labels were reversed, and treated mice were injected with [^3H]leucine and controls with [^{14}C]leucine. No consistent change in the distribution of radioactivity between polyribosomes of different sizes could be detected.

Composition of the monomer fraction in livers of mice treated with 3,5-diethoxycarbonyl-1,4-dihydrocollidine. To determine whether the fraction of monomeric ribosomes which decreases after treatment with the drug consists of active monoribosomes or inactive ('unprogrammed') single ribosomes, monomeric ribosomes were recovered by centrifugation of the monomer fraction after separation from other ribosomes by density-gradient centrifugation as in Fig. 1(a). The particles were then centrifuged through a sucrose density gradient containing a lower concentration of Mg^{2+} and an increased concentration of K^+ . The conditions used

have been shown to cause preferential dissociation of inactive single ribosomes (Norman *et al.*, 1973). The RNA of monomeric ribosomes from treated mice had been radioactively labelled by using [^{14}C]orotic acid as precursor, whereas the ribosomes from control mice had been labelled with [^3H]orotic acid (Fig. 2). In a separate experiment (not shown), the radioactive labels were reversed. Both experiments showed that the proportion of unprogrammed single ribosomes is decreased in livers of mice treated with 3,5-diethoxycarbonyl-1,4-dihydrocollidine.

Discussion

The experiments described in this paper show that 3,5-diethoxycarbonyl-1,4-dihydrocollidine induces a decrease in unprogrammed single ribosomes and a corresponding increase in polyribosomes (Figs. 1a and 2). This change is compatible with the postulate that the drug causes an overall increase in protein-synthetic activity. When amino acid incorporation by ribosomes *in vitro* was measured, a slight increase was found 6 h after treatment with the drug, though the increase was not statistically significant (S. Gamulin & M. R. Norman, unpublished work). After 2 days of treatment with 3,5-diethoxycarbonyl-1,4-dihydrocollidine, Gayathri & Padmanaban (1974) found a significant increase in amino acid incorporation by ribosomes of treated mice, though they found decreased incorporation with treatment lasting more than 2 days.

There are at least three different mechanisms which could account for the redistribution of ribosomes that we observe. A closer spacing of ribosomes on mRNA could decrease the proportion of unprogrammed single ribosomes, but should also cause a shift of polyribosomes to larger and faster-sedimenting aggregates. This mechanism is unlikely, since there was no evidence for a change in the profile of polyribosomes (apart from the decreased proportion of monomers). An altered ratio of mRNA to ribosomes resulting either from increased mRNA or a decreased number of ribosomes would also account for the redistribution. Since the increase in activity of 5-aminolaevulinic synthetase after treatment with 3,5-diethoxycarbonyl-1,4-dihydrocollidine is prevented by actinomycin (Norman, 1971), the most likely explanation is that there is a relative increase in the amount of mRNA. Nawata & Kato (1973) also concluded that increased RNA synthesis in rats treated with 3,5-diethoxycarbonyl-1,4-dihydrocollidine was associated primarily with increased synthesis of informational RNA.

The lack of any change in the pattern of amino acid incorporation by polyribosomes (Fig. 1b) would be expected in a situation where the overall rate of protein synthesis is increased. Since the size of a polyribosome

Fig. 2. Density-gradient analysis of monomer fraction subjected to 1 mM- Mg^{2+} and 150 mM- K^+

Rate zonal centrifugation of the monomer fraction was at 7°C for 8 h at 30000 rev./min ($\omega^2 t = 28 \times 10^{10} \text{ rad}^2/\text{s}$) in an MSE BXIV rotor with a 15–45% (w/w) sucrose gradient. Monomers were isolated after 3,5-diethoxycarbonyl-1,4-dihydrocollidine-treated mice had been injected with [^{14}C]orotic acid (●), and control mice had been injected with [^3H]orotic acid (○). Individual points are presented as a percentage of total d.p.m. in the gradient, which were: ^3H , 67860; ^{14}C 8540. Abbreviations: dS, derived subunits; M, monomers. ----, % transmittance at 254 nm.

is related to the length of mRNA (and hence the number of amino acid residues in the protein coded for), it might be expected that a specific increase in the synthesis of one protein (such as 5-aminolaevulinate synthetase) would result in a corresponding increase in amino acid incorporation by polyribosomes of the appropriate size. Since there is no evidence for any such peak of activity (Fig. 1*b*), it must be concluded that any changes are masked by increased synthesis of a number of other proteins.

If our interpretation is correct, the recruitment of unprogrammed single ribosomes to translate newly synthesized mRNA provides further evidence for the flexibility of ribosome activity as conditions in the cell vary. As protein synthesis decreases in the livers of starved mice, the pool of inactive single ribosomes increases (Norman *et al.*, 1973), whereas experiments described in the present paper suggest that a porphyrinogenic drug increases protein synthesis by utilizing ribosomes from the inactive pool of single ribosomes. A similar decrease in monomeric ribosomes of liver has been reported when protein synthesis is increased after partial hepatectomy (Webb

et al., 1966), or in response to the trauma of laparotomy (Liu & Neuhaus, 1968). The availability of a drug that increases ribosome activity could be useful for studies on the control of protein synthesis.

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