EFFECT OF VARIOUS INHIBITORS ON NUCLEAR PROTEIN SYNTHESIS IN RAT LIVER

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INTRODUCTION

During the past decade it has become clear that in cells of higher organisms most protein synthesis is mediated by polysomes occurring free in the cytoplasm or attached to the endoplasmic reticulum. There is ample evidence, however, that synthesis of proteins may also occur at other loci within the cell; for example, in mitochondria (1–3), in chloroplasts (4–6), and in the nucleus (7–11).

Protein synthesis in both mitochondria and chloroplasts differs from that occurring elsewhere in the cytoplasm, resembling, rather, that of bacterial systems. Thus, ribosomes from mitochondrial and chloroplasts, like those of bacteria, are smaller than those from microsomal systems (3, 5), and the response of mitochondrial and chloroplast protein-synthesizing systems to inhibitors of protein synthesis is similar to that of bacterial, rather than of microsomal, systems (2, 4, 6).

Nuclear protein synthesis has generally been found to resemble the predominant type of cytoplasmic protein synthesis (7, 8, 10). Several workers, however, have described a chromatin-catalyzed incorporation of amino acids into polypeptide chains which differs from both bacterial and microsomal protein-synthesizing systems in many respects, including its response to several antibiotics (12, 13).

To learn more about the role of the cell nucleus in protein synthesis and to define more closely the

nature of this synthesis, we have investigated the effect of various inhibitors of protein and RNA metabolism on the incorporation of labeled amino acids into protein by the cell nucleus in vivo.

MATERIALS AND METHODS

Male Holtzman albino rats weighing 270–370 g were used; animals were fasted for 23–25 hr before administration of the radioisotope. Inhibitors were injected interperitoneally: actinomycin D, as a solution in 33% propylene glycol–0.10 M NaCl; chloramphenicol, as a solution in 25% propylene glycol–0.11 M NaCl; and all other inhibitors, as neutral solutions in 0.15 M NaCl. At various times after administration of the inhibitors, the animals were anesthetized with ether and injected with 0.16 ml per 100 g body weight of a solution containing 407 μc (0.081 μmoles) of L-leucine-4,5-^{3}H per milliliter and unlabeled amino acids and inorganic salts in the same concentrations as in previous studies (11).

Animals that had received actinomycin D and the corresponding controls were given the isotope solution via the femoral vein; in all other cases the isotope was injected into the hepatic portal vein. 2 min after injection of isotope, livers were rapidly frozen *in situ*, excised, lyophilized, and separated into cytoplasmic and nuclear fractions in nonaqueous solvents (11). The specific activities of the total nuclear and cytoplasmic proteins were determined essentially as described earlier (11).

RESULTS AND DISCUSSION

The effect of actinomycin D, an inhibitor of DNA-dependent RNA synthesis, of thioacetamide, a drug which has been shown to interfere with the metabolism of ribosomal precursor RNA (14), and of several widely used inhibitors of protein synthesis on the ability of rat liver nuclei and cytoplasm to incorporate labeled amino acid into protein has been studied. The results are presented in Table I.

For those treatments in which repeat determinations were run, the absolute level of incorporation was found to vary greatly from one trial to the next. This is not unexpected since the absolute level of isotope incorporation attained after 2 min, the time employed in the present experiments, is still very low, whereas the rate of incorporation is near its maximum value (11). We attach no significance, therefore, to moderate differences in the absolute level of incorporation between two treatments; for example, between the various thioacetamide treatments and the corresponding control. Much more significant is the ratio of incorporation in the cytoplasm to incorporation in the nucleus, a value which, as might be expected, is subject to much less variation than the absolute levels of incorporation. A change in this ratio as a result of a treatment with an inhibitor would indicate that the inhibitor had affected nuclear and cytoplasmic protein synthesis to different degrees.

The following assumptions are implicit in our interpretation of the above data: (*a*) no exchange or preferential loss of protein occurs during isolation of the nuclear and cytoplasmic fractions; (*b*) the concentration of labeled amino acid is the same in the nucleus and the cytoplasm during the labeling period; (*c*) movement of significant amounts of protein between the nucleus and cytoplasm does not occur during the labeling period. Evidence for the validity of these assumptions has been presented in a previous paper (11).

Except for puromycin, none of the inhibitors tested showed a significant difference in its effect on nuclear and cytoplasmic protein synthesis. The difference in the ratio of cytoplasmic incorporation to nuclear incorporation between the 25 mg/kg–20 m puromycin treatment and the appropriate control is significant at the 2% level by the *t*-test. It should be noted that in puromycin-treated animals the specific activity of the total nuclear protein is higher than that of the cytoplasmic protein, whereas in the control animals exactly the reverse is true. Because of its ability to reverse the relative level of cytoplasmic to nuclear incorporation, puromycin promises to be a useful tool in investigating nuclear protein synthesis.

When nuclear proteins were separated into a fraction soluble in 0.15 M NaCl and a residual fraction, the specific activity of both fractions was found to decrease proportionally upon puromycin treatment. This observation is compatible with the hypothesis that puromycin exerts its differential effect by a greater over-all inhibition of cytoplasmic as compared with nuclear protein synthesis rather than by a selective inhibition of the synthesis of certain proteins. Further data will be required to decide this question, however.

Taken as a whole, the data support the view that the synthesis of proteins in the nucleus proceeds by a mechanism which is similar, if not identical, to the mechanism predominating in the cytoplasm. The differential effect of puromycin at the two sites does not necessarily indicate a

TABLE I

Effect of Inhibitors on Nuclear and Cytoplasmic Protein Synthesis

Treatment	Disintegrations/min per mg protein		
	Cytoplasm	Nucleus	Cytoplasm/Nucleus
Control	7010	4170	1.68
Actinomycin D 2 mg/kg–4 hr	8090	5000	1.62
Actinomycin D 2 mg/kg–16 hr	1530	1160	1.32
Control	12070	9160	1.32
Chloramphenicol 30 mg/kg–5 min	19370	12870	1.50
Chloramphenicol 30 mg/kg–70 min	28200	19560	1.44
Control	10100	7930	1.28 ± 0.22
Streptomycin 50 mg/kg–5 min	18600	14400	1.29
Streptomycin 50 mg/kg–70 min	15000	10000	1.50
Cycloheximide 0.25 mg/kg–5 min	2150	1490	1.44
Cycloheximide 1.00 mg/kg–5 min	651	461	1.41
Puromycin 25 mg/kg–5 min	2750	3160	0.87
Puromycin 25 mg/kg–20 min	1900	2470	0.77 ± 0.10
Puromycin 75 mg/kg–20 min	511	681	0.75
Thioacetamide 50 mg/kg–50 min	10300	9840	1.05
Thioacetamide 50 mg/kg–23 hr	13500	9860	1.37
Thioacetamide 8 × 50 mg/kg*	16600	11700	1.42

Experimental design is described under Materials and Methods. Most values were obtained from a single determination; where more than one determination was made, the values presented are the averages. The figures beneath each inhibitor indicate the dosage and time between injection of the inhibitor and injection of the labeled amino acid.

* Animals were injected with 50 mg/kg of thioacetamide every day for 8 days; labeled amino acid was given 1 day after the final injection of drug.

difference in the protein-synthesizing systems, but might result from some unrelated factor such as a differential rate of uptake of puromycin by the nucleus and the cytoplasm.

The failure of chloramphenicol to inhibit nuclear protein synthesis and the pronounced inhibition caused by even low concentrations of cycloheximide are in marked contrast to the results obtained with mitochondria and chloroplasts. Chloramphenicol has been shown to inhibit protein synthesis in mitochondria, chloroplasts, and bacteria, but not in microsomal systems (2, 4, 6), whereas cycloheximide is known to inhibit protein formation in microsomal systems but not in mitochondria or bacteria and probably not in chloroplasts (2, 6). Thus, the

protein-synthesizing system in the cell nucleus differs from that found in chloroplasts or in mitochondria.

Chromatin-catalyzed amino acid incorporation is inhibited by chloramphenicol (12). The finding that nuclear protein synthesis in vivo is not depressed by this antibiotic indicates that the chromatin system, if not an artifact, plays a quantitatively minor role in nuclear protein synthesis in vivo.

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