Early Changes in Liver Cytoplasmic RNA of Hydrocortisone-Treated Rats

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SUMMARY

We have investigated certain events occurring in rat liver cytoplasm during the time period between the administration of hydrocortisone and the hormone-induced increase in the level of several hepatic enzymes. At various times after the administration of hydrocortisone to adrenalectomized rats, ^{14}C-orotic acid was injected intraperitoneally. After a 40-min labeling period the rats were killed and liver cytoplasm was examined on sucrose gradients. Two hours post hydrocortisone administration the isotopic labeling of a 45 *S* cytoplasmic particle was markedly increased. Previous data suggest that the 45 *S* particle carries newly synthesized messenger RNA from the nucleus to the cytoplasmic polyribosomes. The stimulation of labeling occurred at a time consistent with the postulate that the excess of labeled RNA in the 45 *S* particle includes messenger RNA coding for the synthesis of the hormone-stimulated enzymes. A simultaneous fall in membrane-free, monomeric ribosomes was noted.

INTRODUCTION

The increased activity of several hepatic enzymes observed following the administration to rats of glucocorticoids (1–3) has been shown to result from induction of new protein synthesis (4–6). In bacteria, enzyme induction is known to be preceded by an accelerated synthesis of RNA (7), and the newly formed RNA has been identified as messenger specific for the protein to be made (8, 9). In rats given cortisone, nuclear RNA synthesis is increased before an effect on enzyme levels can be detected. However, little increase in labeling of cytoplasmic RNA has been described at such early intervals (10–12). Although the intracellular site of synthesis of individual proteins is not known, the bulk are surely made in the cytoplasm. Therefore, we sought evidence for the presence of new messenger RNA in this compartment after hydrocortisone treatment. Recently, there has been demonstrated in mammalian cells a rapidly labeled particle with sedimentation coefficient approximating 45 *S* (13) which appears to bear mRNA[1] from the nucleus to the cytoplasm (14–17). The studies to be presented indicate that hydrocortisone markedly increases the labeling of the cytoplasmic particle. This effect occurs at a time consistent with the postulate that the particle transports newly synthesized mRNA for the induced proteins to their presumed site of synthesis, the cytoplasmic polyribosomes.

METHODS

Male 150-g rats, both normal and adrenalectomized, were purchased from the Charles River Breeding Laboratories, Wilmington, Massachusetts. Adrenalectomized rats were offered 1% saline in their drink-

[1] Abbreviations: TMN, 0.01 M Tris-HCl, 0.001 M $MgCl_2$, 0.01 M NaCl, pH 7.5; mRNA, messenger RNA; rRNA, ribosomal RNA; PMS, postmitochondrial supernatant fraction.

ing water and were studied from 24 to 48 hr after operation. All animals were starved for 18 hr before death. The equivalent of 5 mg of hydrocortisone was given intraperitoneally as the sodium succinate salt in 0.1 ml of buffer, which also contained 0.04 mg sodium phosphate exsiccated, 0.13 mg methylparaben, and 0.01 mg propylparaben (Solucortef Mix-O-Vial, Upjohn) at the times indicated. ^{14}C-Orotic acid, 10 μC per 100 g body weight, was given intraperitoneally 40 min prior to death.

Postmitochondrial supernatant fractions were prepared in TMN and centrifuged through 10–30% sucrose gradients as described previously (14). Ten-drop fractions were precipitated and washed with cold 5% trichloroacetic acid on Millipore filters, and were counted.

RNA was obtained from the 45 *S* and 80 *S* particles by treatment with sodium dodecyl sulfate (18). The particles were collected from the peaks of at least six sucrose gradients in TMN into tubes containing sodium dodecyl sulfate at a final concentration of 1%. The tube contents were made 0.25 M and 67% with respect to NaCl and ethanol, respectively, and held at −10° overnight. The RNA precipitate was recovered by centrifugation, dissolved in 0.02 M sodium phosphate buffer, pH 7, and 0.3% sodium dodecyl sulfate, and centrifuged through linear 5–20% sucrose gradients prepared in 0.02 M potassium phosphate buffer, pH 7, at 2°. Ten-drop fractions were taken, cooled to 0°, and precipitated with cold 12.5% trichloroacetic acid. Precipitates were washed with cold 10% trichloroacetic acid on Millipore filters and were counted.

RESULTS

Effect of Hydrocortisone on 45 S Particles

In Fig. 1 is shown the labeling pattern of postmitochondrial supernatant fractions prepared from adrenalectomized rats killed 40 min after orotic acid administration. In material from untreated animals most of the counts appeared in the soluble RNA close to the top of the gradient, and there

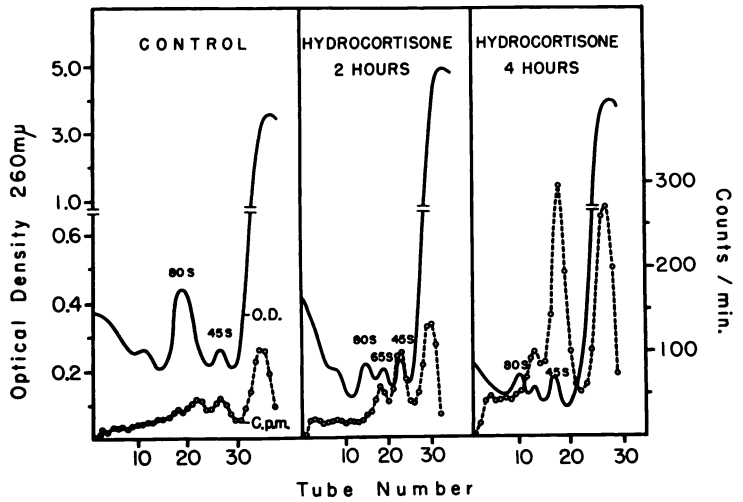

FIG. 1. *Effect of hydrocortisone on isotope distribution in postmitochondrial supernatant fractions from adrenalectomized rats*

One animal was given 5 mg of hydrocortisone intraperitoneally 4 hr prior to death (right-hand panel), the second received the hormone 2 hr prior to death (middle panel), and the third was not treated (control). All animals received ^{14}C-orotic acid 40 min prior to death. A PMS fraction was prepared from the liver of each rat, and these preparations were analyzed on 10–30% sucrose gradients prepared in the buffer used for homogenization. Centrifugation was for 5 hr at 63,600 *g*. Similar patterns were observed in material from at least 8 different animals for each time period shown.

were small radioactive peaks in the 45 *S* and 65 *S* regions. In most experiments the radioactivity in the 65 *S* peak was considerably less than that in the 45 *S* peak. In animals treated with hydrocortisone 2 hr prior to death there was an increase in 45 *S* labeling from a control level of 11% of total counts in the PMS fraction to a stimulated level of 20% of total PMS counts. This increase was maximal 4 hr after hydrocortisone administration (Fig. 1). By 7 hr, labeling in the 45 *S* particles had returned to control levels. The distribution of counts in the liver cytoplasm of hydrocortisone-treated and untreated rats is summarized in Table 1. At 2 hr after the steroid injection there was no detectable increase in total PMS counts. However, variations in isotope incorporation from one control animal to another were of the order of 30%; thus, increases of this magnitude might have been undetected. At 3–5 hours after hydrocortisone the peak 45 *S* labeling was comparable to that in soluble RNA, and there was an increase in total PMS labeling of over 100%, as Table 1 shows.

TABLE 1

Effect of Hydrocortisone on Isotope Distribution in the Postmitochondrial Supernatant Fraction

PMS fractions were analyzed on sucrose gradients, as described in Fig. 1. Counts were calculated from the gradients and pellets, and the counts in the various subfractions were expressed as a percentage of the total PMS counts. The range of counts is shown in brackets, and the number of animals in parentheses.

Time after hydrocortisone (hr)	Total radioactivity (cpm)		Distribution of radioactivity (% of total)			
	PMS	45 *S*	Heavier than 80 *S*[a]	80 *S* and 65 *S*	45 *S*	4 *S*
0	1870 (6) [1480–2480]	206 [84–315]	43	18	11	28
2	1990 (6) [1660–2510]	390 [336–512]	32	17	20	31
3–5	4020 (3) [3780–4190]	1010 [962–1050]	28	13	27	32

[a] Including pellet formed during centrifugation of gradient.

Effect of Hydrocortisone on 80 S Ribosomes

In the experiments of Fig. 1, 260 mμ absorbing material sedimented with peaks, the coefficients of which approximated 80 *S*, 65 *S*, and 45 *S*. These included ribosomal and probably subribosomal particles (13). Ferritin, which can be distinguished by its high 320:260 mμ absorption ratio (19, 20), sedimented with a coefficient of about 65 *S* and was the major contributor to the peak of absorbance in this area. However, a 65 *S* subribosomal particle can also be purified by special procedures from this region (13; Henshaw, unpublished). Polyribosomes were sedimented into the pellet. The 80 *S* material was found to contain 18 *S* and 28 *S* RNA and likely represented single ribosomes, which exist free in the cytoplasm, or which easily separate from membrane during homogenization (21).

In untreated, adrenalectomized animals the amount of 80 *S* material per unit of liver was relatively constant from animal to animal and was about 1½–2 times that of the 45 *S* particles. The administration of 5 mg of hydrocortisone consistently produced a marked decrease in the 80 *S* peak (Fig. 1) to a level equal to or below the 45 *S* peak of ultraviolet-absorbing material. The latter peak served as a convenient standard of reference, since it was constant and, in contrast to the level of isotope in the particles, was unaffected by hydrocortisone given 2 hr before sacrifice, or by adrenalectomy. The relative quantity of 80 *S* particles remained depressed for several hours after hydrocortisone, beginning

to rise at 5 hr and reaching control level at about 7 hr.

It appeared possible that untreated adrenalectomized animals might have high levels of degradative enzymes which might lead to release of particles from membrane, and, therefore, to the higher levels of "free" 80 *S* particles observed. However, it was found in mixing experiments that liver homogenates from untreated animals did not release 80 *S* ribosomes from the hydrocortisone-treated preparations. Nor did the addition of hydrocortisone to a control liver during homogenization effect a reduction in the 80 *S* ribosomes, as might have been anticipated if cortisone were stabilizing lysosomes (22) *in vitro*.

The level of the 80 *S* peak in nonadrenalectomized animals varied considerably from experiment to experiment, ranging from twice the 45 *S* peak to a value equal to it. A decrease in the 80 *S* peak could be produced consistently by stressing the normal animals with sudden noises 2 hr prior to sacrifice. This suggests that the variations in the level of 80 *S* particles noted in normal animals resulted from changes in endogenous adrenocortical secretion. Normal rats responded to hydrocortisone with an increased labeling of the 45 *S* particles comparable to that seen in the adrenalectomized animals.

Effect of Hydrocortisone on RNA from 45 S Particles

Most RNA purified from 45 *S* particles, which were isolated from sucrose gradients, sedimented with a constant of 18 *S* (14). Sucrose gradient profiles of the RNA obtained from the 45 *S* peaks from treated and untreated adrenalectomized rats are shown in Fig. 2. Isotope was virtually confined to the 18 *S* area, with some trailing of counts to the light side. However, recovery of radioactivity from the 45 *S* particles did not exceed 70%, so that as much as a third of its isotope could conceivably be accounted for by species of RNA with a sedimentation pattern different from that seen in Fig. 2. Despite its coincidence in sedimentation with the 18 *S* rRNA, some of the rapidly labeled 18 *S* RNA is probably

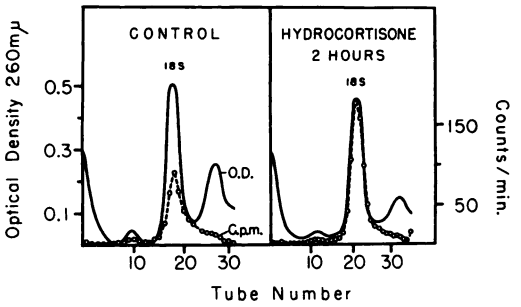

FIG. 2. *Effect of hydrocortisone on the labeling pattern of RNA purified from 45 S particles*

Six identical gradients similar to that shown in the middle panel of Fig. 1 were prepared from an adrenalectomized rat treated with hydrocortisone and ^{14}C-orotic acid 2 hr and 40 min, respectively, prior to death. The 45 *S* particles were isolated from the gradients and were pooled; 45 *S* particles were likewise pooled from gradients similar to the left-hand panel of Fig. 1, from an animal not treated with hydrocortisone. Each preparation of 45 *S* particles was made 1% with respect to sodium dodecyl sulfate and precipitated with NaCl and ethanol, and the RNA was analyzed on 5–20% sucrose gradients prepared in 0.02 *M* potassium phosphate, as described in Methods. Gradients were centrifuged for 18 hr at 63,600 *g* at 2°.

mRNA, as shown previously (14). Material sedimenting between 18 *S* and 4 *S* may represent partially degraded RNA. Hydrocortisone led to a more than twofold increase in isotope incorporation in the 18 *S* RNA of the 45 *S* particle, but the pattern of isotope distribution was unchanged.

DISCUSSION

Recent studies (13–17) strongly suggest the presence of mRNA in 45 *S* particles of liver and HeLa cells. A model for liver polyribosome formation has been proposed according to which newly synthesized mRNA becomes associated with a 45 *S* ribosomal subunit in the nucleus, emerges into the cytoplasm, and is then incorporated into polyribosomes. Evidence from unrelated work (23) indicates that the enzyme induction which follows hydrocortisone administration requires synthesis of new RNA, presumably messenger, and that this is transferred from nucleus to cytoplasm.

The data presented in this communication are consistent with the postulate (14) that such a transfer takes place on 45 *S* particles. Newly synthesized (i.e., isotopically labeled) RNA in the 45 *S* particles rose by 50% at a time before any increase in total cytoplasmic labeling was detectable. It should be emphasized that although a portion of the labeled RNA of the 45 *S* particle is believed to be mRNA (14–17), an unequivocal demonstration that this is true, and that the newly synthesized RNA borne by these particles after hydrocortisone treatment includes the mRNA species specific for the enzymes induced, must await techniques by which the particles or their RNA can be shown to direct the synthesis of specific proteins.

The fall in 80 *S* ribosomes seen after hydrocortisone treatment may also play a role in the steroid-mediated induction of rapidly induced hepatic enzymes. Under the conditions used in these studies, the maximum rate of tryptophan pyrrolase synthesis following hydrocortisone is attained in about 3½ hr (24), or very shortly after the 80 *S* ribosomes reached their nadir and the 45 *S* particles were stimulated. The increased tryptophan pyrrolase synthesis is over at 7 hr, at which time the 80 *S* and 45 *S* particles have returned to control levels. Membrane-bound ribosomes are probably the major site of protein synthesis in the liver, whereas free monomeric ribosomes are much less active in the absence of exogenous messenger (21). In our experiments, hydrocortisone may have stabilized polyribosomes on the membrane, preventing their disruption into nonfunctioning 80 *S* ribosomes. In addition, hydrocortisone may have stimulated the formation of new membrane-bound polyribosomes from the pool of free 80 *S* particles, perhaps by making available more mRNA on 45 *S* particles.

Hydrocortisone probably stimulates the synthesis of both mRNA and rRNA (10–12, 25), and the resultant increase in total cytoplasmic RNA becomes detectable some hours after treatment (10). Evidence has been presented (13–15) that the newly synthesized RNA of the 45 *S* particle includes rRNA as well as mRNA. The increased labeling of this particle after hydrocortisone administration is thus consistent with this interpretation, likely reflecting an increased synthesis of both rRNA and mRNA. Increases in the synthesis of transfer RNA (26) and in the uptake of amino acids (27, 28) have also been noted after hydrocortisone administration. However, no information is as yet available to permit a decision as to which or, indeed, whether one of these several phenomena reflects the primary action of the hormone.

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