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Hydrocortisone Induction of Rat-Liver Leucyl-Transfer RNA and Its Synthetases

(hormones/steroid/aminoacyl-tRNA synthetases/isoaccepting tRNAs)

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ABSTRACT **Within 3 hr after the intraperitoneal administration of hydrocortisone to female rats, a new leucine-accepting tRNA and a new leucyl-tRNA synthetase activity appear in the liver cytosol. The new isoaccepting tRNA can be acylated only with the synthetase derived from livers of hormone-treated animals. Both components are transient; by 12 hr after hydrocortisone administration, the isoaccepting tRNA and its synthetase disappear from livers of treated animals.**

The detailed biochemical mechanisms that mediate the effects of corticosteroids on target organs remain largely undefined. The widespread changes in hepatic metabolism that follow cortisone administration imply a marked alteration in the enzymatic constitution of liver cells. It appears reasonable to assume that changes of such magnitude could best be achieved by regulating the synthesis of specific groups of proteins. Indeed, it is well established that the levels of specific enzymes are increased after the injection of glucocorticoids, and that these hormonally induced changes are due to *de novo* synthesis of proteins (1).

Some years ago, we reported (2, 3) that the hydrocortisone-stimulated increase in hepatic levels of tryptophan oxygenase (EC 1.13.1.12) and tyrosine amino transferase (EC 2.6.1.5) required *de novo* synthesis of RNA as well as proteins. These observations, which have been confirmed in a large number of studies in whole animals and in cell cultures, suggest that hormonally-induced mRNA synthesis may account in part for alterations in hepatic enzyme levels. More recently, we have found that the expression of hormone action may be reflected in an alteration of other cellular components involved directly in protein synthesis, such as tRNA and aminoacyl-tRNA synthetases. This report describes hydrocortisone-induced changes that occur in leucyl-tRNA, as well as in leucyl-tRNA synthetase activity, of rat liver.

MATERIALS AND METHODS

Sprague–Dawley female rats (about 200 g) were injected intraperitoneally with hydrocortisone (50 mg/kg) or with 0.9% NaCl, and were killed 3 hr or 12 hr later. The 100,000 × *g* supernatant (S100) of the liver homogenates was used as the source for both the activating enzymes and the tRNA. The tRNA was obtained by phenol extraction of the S100, followed by precipitation from the aqueous phase with 2 volumes of ethanol–2% potassium acetate; tRNA was extracted from the precipitate with 1 M NaCl. After centrifugation, the supernatant fluid was adjusted to pH 9 with NH_4OH and incubated for 10 min at 37°; the tRNA was precipitated as before. The synthetases were prepared by passage of a portion of the S100 fraction through DEAE-cellulose to remove endogenous RNA. The aminoacid acceptor activity of each tRNA was determined in a reaction mixture containing the following components in a final volume of 100 μl: 100 mM Tris·HCl buffer (pH 7.4), 10 mM $MgCl_2$, 2 mM ATP, 300 pmol of rat-liver tRNA, 300 pmol of [^{3}H]leucine (1 μCi/nmol) or [^{14}C]leucine (0.3 μCi/nmol) as indicated, and 0.3 mg of the rat-liver synthetase preparation. Incubation was at 37° for 20 min. The reaction was terminated by the addition of trichloroacetic acid to a final concentration of 5%; the precipitate was collected on nitrocellulose membranes and the radioactivity was counted in Liquiflor (New England Nuclear Co., Boston, Mass.) in a Nuclear-Chicago scintillation counter. The amino-acid acceptor activity for all tRNA preparations was 25–50 pmol of leucine per nmol of tRNA. For reverse-phase chromatography, tRNA was first charged with labeled leucine in a 4-ml reaction mixture, with the incubation components adjusted accordingly as described above. After 20 min of incubation, the RNA was reisolated and loaded onto a Freon 214 column (4) (2.5 cm × 100 cm), at a flow rate of 1 ml/min, and eluted with a linear gradient of 0.25–0.65 M NaCl (1000 ml of each) at 20°. 10-ml Fractions were collected and precipitated with trichloroacetic acid; their radioactivity was determined as described above. Over 80% of the radioactivity applied to the column was recovered. Labeled leucine was obtained from New England Nuclear Corp.

RESULTS AND DISCUSSION

In order to compare isoaccepting tRNAs in control and hydrocortisone-treated animals, a double isotope labeling method was used. The S100 extracts from livers of each group of animals was used as the source of both synthetase activity and tRNA. Each tRNA was charged with [^{14}C]- or [^{3}H]-leucine by its homologous synthetase. The charged tRNAs were mixed and fractionated by reverse-phase chromatography (see *Methods*). Separation of the leucyl-tRNA species revealed a new isoaccepting peak for the tRNA derived from cortisone-treated animals, as compared to the normal controls (Fig. 1). When the tRNA species were reverse-labeled with the two leucine isotopes, the chromatographic profile of the mixed leucyl-tRNAs was the same (Fig. 2).

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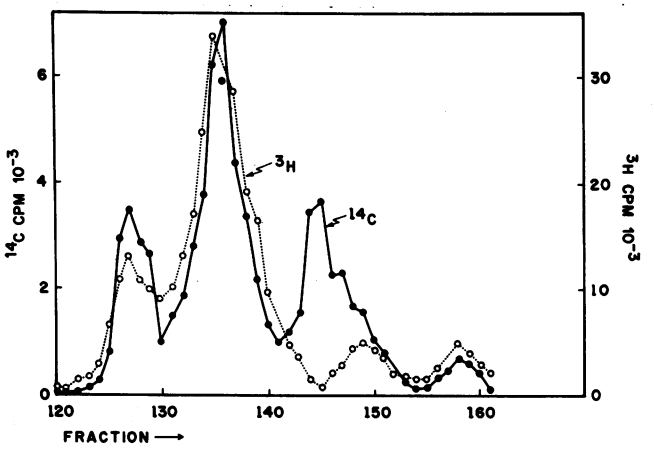

FIG. 1. Reverse-phase chromatography of rat-liver leucyl-tRNAs. [^{14}C]Leucyl-tRNA (●——●), prepared from tRNA isolated from animals treated with hydrocortisone 3 hr previously was charged by the homologous synthetase and mixed with [^{3}H]leucyl-tRNA (O- - -O) prepared with tRNA and homologous synthetase from saline-treated control rats; the mixture was chromatographed as described in *Methods*.

By contrast, profiles of tRNA prepared from control animals and esterified with leucine by the synthetase preparation from hormone-treated rats failed to demonstrate the new isoacceptor species of leucyl-tRNA (Fig. 3). Conversely, tRNA from cortisone-treated animals esterified with synthetase from control animals also failed to reveal this isoaccepting species (Fig. 4). These results support in part the

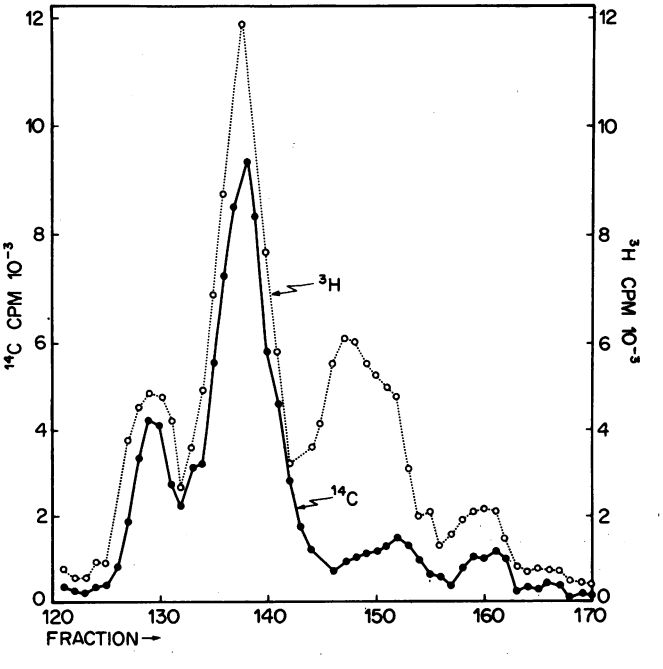

FIG. 2. Reverse-phase chromatography of rat-liver leucyl-tRNAs. [^{3}H]Leucyl-tRNA (O- - -O), prepared with the synthetase and tRNA isolated from animals treated 3 hr previously with hydrocortisone, was mixed with [^{14}C]leucyl-tRNA (●——●) prepared with the synthetase and tRNA derived from saline-treated control rats; the mixture was chromatographed as described in *Methods*.

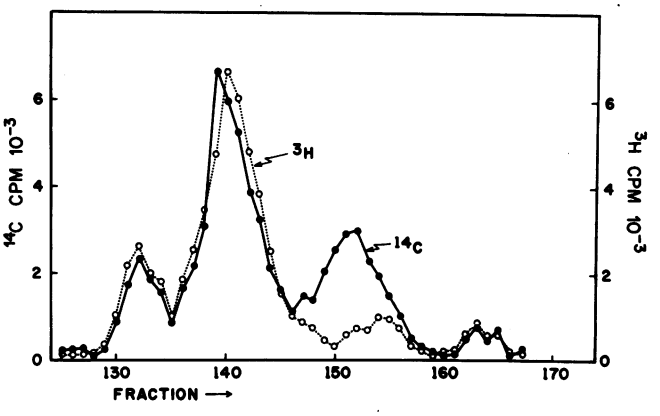

FIG. 3. Reverse-phase chromatography of hepatic leucyl-tRNAs. [^{14}C]Leucyl-tRNA (●——●) prepared from rats treated 3 hr previously with hydrocortisone and charged with the homologous synthetase was mixed with [^{3}H]leucyl-tRNA (O- - -O) prepared with tRNA from animals treated 3 hr previously with hydrocortisone and charged with the synthetase from saline-injected controls and chromatographed.

studies of Agarwal *et al.* (5), which demonstrated that "cortisone" tRNA and control tRNA have identical leucyl-tRNA profiles when esterified with the synthetase prepared from normal untreated animals.

Based on our own studies, we have concluded (*i*) that the hormone-treated liver contains a species of leucyl-tRNA that is undetectable in control animals and (*ii*) that this tRNA species can be acylated only by enzyme(s) prepared from hormone-treated liver, a result that suggests the formation of a new leucyl-tRNA synthetase activity after hydrocortisone treatment.

If the presence of the induced synthetase activity and tRNA is directly related to some aspect of hormone action, the kinetics of synthesis or degradation of these components

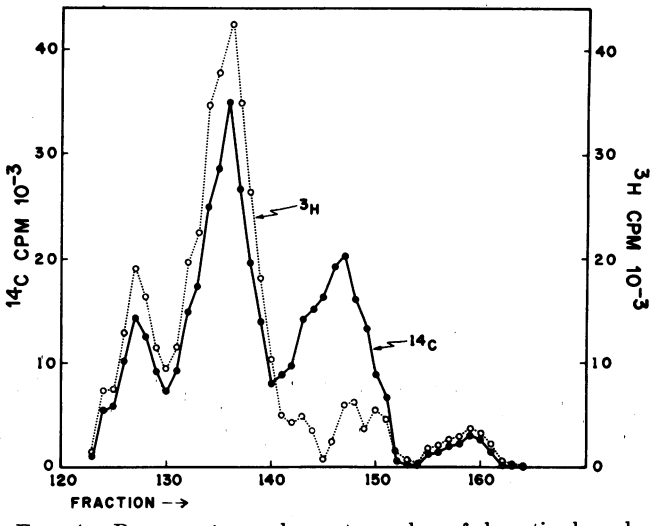

FIG. 4. Reverse-phase chromatography of hepatic leucyl-tRNAs. [^{14}C]Leucyl-tRNA (●——●) prepared with tRNA isolated from rats treated 3 hr previously with hydrocortisone and charged with homologous synthetase was mixed with [^{3}H]leucyl-tRNA (O- - -O) isolated from saline-injected controls and acylated by synthetase derived from animals treated 3 hr previously with hydrocortisone and the mixture was chromatographed.

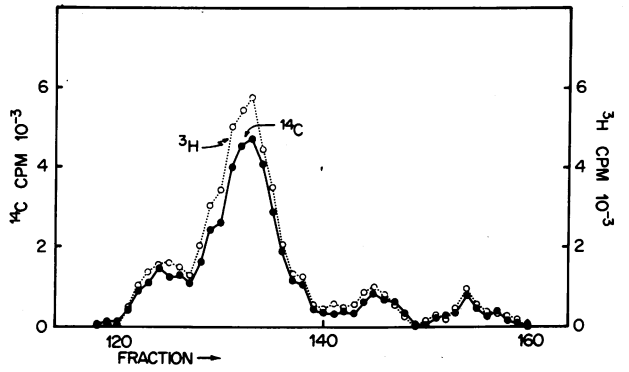

FIG. 5. Reverse-phase chromatography of rat liver leucyl-tRNAs. [^{14}C]Leucyl-tRNA (●——●) was prepared by acylation of tRNA obtained 12 hr after hydrocortisone administration with synthetase isolated 3 hr after hydrocortisone treatment. [^{3}H]Leucyl-tRNA (O- - -O) was prepared by acylation of tRNA obtained 3 hr after hydrocortisone administration with synthetase isolated 12 hr after hydrocortisone treatment. The two preparations were mixed and chromatographed.

might be similar to those of other macromolecules whose levels are responsive to steroid administration. This prediction could be examined, since other studies have shown that steroid-induced changes in the levels of several enzymes disappear within 8–10 hr after injection of a single dose of cortisone (6). We have performed experiments analogous to those presented above at both 3 and 12 hr after hydrocortisone treatment; tRNA and enzyme preparations from both times have been compared. The enzyme obtained 3 hr after hormone treatment aminoacylates the new tRNA species present in the homologous tRNA preparation from 3 hr after steroid administration; however, the same enzyme preparation does not elicit the new peak in the tRNA prepared from liver 12 hr after drug administration. Also, the synthetase that is obtained at 12 hr is not effective in acylating the new species of tRNA present in the tRNA prepared from liver 3 hr after hydrocortisone injection (Fig. 5).

The present results indicate that a new catalytic specificity in liver leucyl-tRNA synthetase arises after cortisone treatment; and that this change is accompanied by the appearance of a new leucyl-tRNA species; both the new enzyme and the new tRNA species are mutually specific. It is not clear if the induced synthetase activity and the tRNA are derived by *de novo* synthesis or by modification of preexisting molecules drawn from the cellular pool. However, preliminary data suggest that some *de novo* protein synthesis is required, since no new isoaccepting leucyl-tRNA is observed when tRNA isolated from rats treated with cortisone 3 hr previously is charged with synthetase derived from rats treated simultaneously with cortisone and actinomycin-D 3 hr before preparation of the synthetase.

These data may represent the first instance in which two leucyl-tRNA synthetases, one of which is specific for a different isoaccepting tRNA, have been observed in a single tissue; the coincident production of a specific synthetase activity and its isoacceptor tRNA species implies a highly sensitive control mechanism in protein synthesis. The hormone-induced changes in components related to protein synthesis are of considerable potential significance in understanding control mechanisms for specific macromolecule syntheses that occur, for example, in cellular differentiation and viral-induced transformation. Further experiments will be necessary to show whether other aminoacyl-tRNA species respond to regulatory stimuli in a similar way and whether other chromatographic procedures will reveal the same differences.

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1. Knox, W. E. & Greengard, O. (1965) *Advan. Enzyme Regul.* **3**, 247–313.
2. Greengard, O., Smith, M. A. & Acs, G. (1963) *J. Biol. Chem.* **238**, 1548–1551.
3. Greengard, O., Gordon, M., Smith, M. A. & Acs, G. (1964) *J. Biol. Chem.* **239**, 2079–2082.
4. Weiss, J. F. & Kelmers, A. D. (1967) *Biochemistry* **6**, 2507–2511.
5. Agarwal, M. K., Hanoune, J., Yu, F. L., Weinstein, I. B. & Feigelson, P. (1969) *Biochemistry* **8**, 4806–4812.
6. Csanyi, V., Greengard, O. & Knox, W. E. (1967) *J. Biol. Chem.* **242**, 2688–2692.