SHORT COMMUNICATION

Drug Action and Aminoacyl RNA Ligases

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(Received February 15, 1966)

SUMMARY

The effects of insulin, testosterone, hydrocortisone, dibenz[*a,j*]acridine, stilbestrol, 2-ethylisothionicotinamide, tetracycline, D-4-amino-3-isoxazolidone, streptomycin, and Pactamycin on activation and transfer of glycine, valine, phenylalanine, histidine, methionine, glutamic acid, and lysine were tested in an *in vitro* system from calf liver. Aminoacylation by phenylalanine, histidine, methionine, and glutamic acid was greatly reduced in the presence of 2-ethylisothionicotinamide.

Recent experiments implicate transfer RNA (tRNA) and aminoacyl RNA ligases (EC 6.1.1) in the control of RNA and protein synthesis at the level of messenger RNA transcription (1–7). Hormones (8–12), antibiotics (13–15), carcinogens (16), and numerous other drugs (17) also affect RNA and protein synthesis. Since the effects of many of these compounds might result from their action on aminoacyl RNA ligases, we have investigated the action of insulin, testosterone, hydrocortisone, dibenz[*a,j*]acridine, stilbestrol, 2-ethylisothionicotinamide, tetracycline, D-4-amino-3-isoxazolidone, streptomycin sulfate, and Pactamycin (NSC 52947) on activation and transfer to tRNA of glycine, valine, phenylalanine, histidine, methionine, glutamic acid and lysine in *in vitro* systems prepared from calf liver.

Aminoacyl RNA ligases were prepared from calf liver by a modification of the method of Bergquist and Scott (18, 19). Total RNA was isolated by phenol extraction from frozen tissues. tRNA was recovered by column chromatography on diethylaminoethyl cellulose (20). Amino acid activation and transfer was determined by a micro assay technique using membrane filters and high specific activity ^{14}C-L-amino acids (18). Solutions containing individual drugs were added to the assay mixtures prior to incubation, and all assays were carried out in triplicate. In triplicate assays with each amino acid, variation in amino acid incorporation never exceeded 10% of the mean incorporation. A modification of the procedures of Sueoka and Yamane was used for fractionating radioactive tRNA's on columns of methylated bovine serum albumin (21). Radioactivity in individual fractions was determined following 5% trichloroacetic acid precipitation and Millipore filtration in a Packard Tri-Carb liquid scintillation spectrometer using double label counting techniques as previously described (22).

No significant differences from controls (less than 10%) in aminoacylation were detected in the presence of streptomycin sulfate (1 μg/ml), tetracycline (1 μg/ml), D-4-amino-3-isoxazolidone (25 μg/ml), Pactamycin (NSC 52947) (1 μg/ml), or stilbestrol (27 μg/ml). However, soluble insulin (83 μg/ml), testosterone (10 μl of a saturated solution), hydrocortisone (10 μg/ml), and dibenz[*a,j*]acridine (10 μl of a saturated solution) appeared to cause minor variations (exceeding 10% of controls) in aminoacylation with certain amino acids (see Table 1). The significance, if any, of the minor variations is being further evaluated.

2-Ethylisothionicotinamide (15 μg/ml)

TABLE 1
Effect of drugs on aminoacylation in a calf liver system[a]

Drug	Gly	Val	Phe	His	Met	Glu	Lys
1. Control	420[a]	362	390	630	710	464	924
2. Streptomycin sulfate	429	394	380	638	700	434	880
3. Tetracycline	490	400	402	610	800	429	953
4. D-4-Aminoisoxazolidone	426	360	400	638	740	440	895
5. Pactamycin	428	374	355	687	702	439	896
6. Soluble insulin	434	348	405	608	710	355	935
7. Testosterone	420	348	482	510	710	492	912
8. Hydrocortisone	408	348	390	500	608	376	926
9. Stilbestrol	424	358	412	644	644	464	935
10. Dibenz[*a,j*]acridine	362	362	390	450	680	450	1100
11. 2-Ethylisothionicotinamide	390	318	82	310	290	29	885

[a] Values: acid precipitable ^{14}C-amino acid (counts per minute). Mean of three assays (range less than ±10%). Abbreviations: Gly, glycine; Val, valine; Phe, phenylalanine; His, histidine; Met, methionine; Glu, glutamic acid; Lys, lysine.

caused major differences in aminoacylation when tested in the same system (Table 1, line 11). In earlier studies (19) two histidinyl-tRNA's were detected in liver tRNA. Histidinyl-tRNA was therefore aminoacylated in the presence and absence of 2-ethylisothionicotinamide using ^{3}H- and ^{14}C-histidine, respectively, and the aminoacylated tRNA's were twice extracted with phenol, mixed, and fractionated on columns of methylated bovine serum albumin (19, 21). The drug selectively inhibited aminoacylation of one histidinyl tRNA (Fig. 1).

2-Ethylisothionicotinamide is a bacteriostatic agent developed for the treatment of tuberculosis. Its mode of action is unknown. In our system it appears to specifically inhibit the activation and transfer of certain amino acids. The inhibition of activation and transfer of histidine to one histidinyl tRNA species suggests that the drug may have marked specificity in its action on protein biosynthesis. While the drug may directly inactivate certain specific aminoacyl RNA ligases, it is interesting to speculate that it may act as an inhibitor of chain initiation in protein synthesis. Since the drug is a substituted pyridine derivative it may antagonize vitamin B_6. Vitamin B_6 has recently been implicated in nucleic acid and protein biosynthesis through its role in the production of "active formaldehyde"

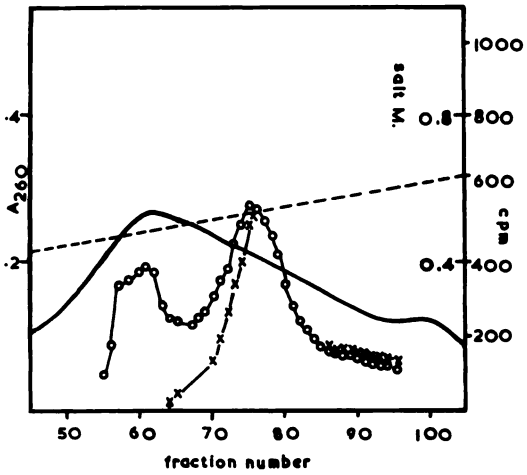

FIG. 1. *Fractionation on methylated bovine serum albumin columns of radioactive histidinyl transfer RNA*

Two aliquots of liver transfer RNA were aminoacylated with ^{14}C- and ^{3}H-histidine in the absence and presence of 2-ethyl isothionicotinamide, respectively. Transfer RNA's were isolated from each aliquot by phenol extraction, mixed, and fractionated on a column of methylated bovine serum albumin on Celite. Individual fractions (1.3 ml) were precipitated with carrier protein and 5% trichloroacetic acid and were Millipore filtered; radioactivity was measured. ———, Absorbance at 260 mμ; ○——○, ^{14}C-histidine radioactivity; ×——×, ^{3}H-histidine radioactivity; ------, salt gradient.

(23). It may also participate in the synthesis of chain-initiating *N*-formyl aminoacyl tRNA's by specific aminoacyl RNA ligases (24, 25).

We wish to thank May and Baker (N.Z.) Ltd. for the gift of 2-ethylisothionicotinamide and the Upjohn Co. (U.S.A.) for the sample of Pactamycin. We thank Drs. P. L. Bergquist and B. C. Baguley for their assistance with these studies and the Westfield Freezing Co. Ltd. for cooperation in supplying fresh tissue.

This work was supported in part by grants from the U.S. Public Health Service (AI-04973-01) and the N.Z. Cancer Society, Auckland Branch.

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Erratum

Vol. **1,** No. 3 (1965), in the article, "Metabolism of Trichloroethylene in Liver Microsomes. I. Characteristics of the Reactions," by Kenneth C. Leibman, pp. 239–246:

p. 245, in the legend to Fig. 2, the second sentence should read: "Numbers in parentheses represent millimolar concentration of the inhibitor."