THE EFFECTS OF HADACIDIN AND INOSINE ON HEPATIC PROTEIN SYNTHESIS AND ADENOSINE TRIPHOSPHATE LEVELS IN ETHIONINE-TREATED RATS.*

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Previous work in this laboratory has shown that the hepatic ATP levels are profoundly depressed in rats treated with ethionine. It was further shown that there is a striking positive correlation between the ATP levels and protein synthesis (measured either in vitro or in vivo) in ethionine-treated female rats. Maximal depression of ATP levels occurs two hours after ethionine treatment while maximum inhibition of protein synthesis appears one to two hours later. Adenine, adenosine and the adenine nucleotides 5'-AMP, 5'-ADP, and 5'-ATP as well as methionine prevent both the drop in hepatic ATP and inhibition of protein synthesis when given at the same time as ethionine (Shull, 1962; Villa-Trevino et al., 1963). Since inosine is a precursor of adenine nucleotides through the intermediate formation of inosinic and adenylic acids (Schulman, 1961), it was of interest to observe whether this nucleoside would effectively counteract the effects of ethionine upon the ATP levels and protein synthesis. In addition, since hadacidin appears to be a specific inhibitor of the conversion of inosine to AMP, (Shigeura and Gordon, 1962), the possible effect of this compound upon the efficiency of inosine was determined. The results of these experiments and their probable implications are the subject of this report.

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Methods. ATP was determined in neutralized perchloric acid extracts by the luciferin-luciferase reaction (Strehler and McElroy, 1957). Protein synthesis was measured by the in vivo uptake of L-leucine-C^{14} into liver proteins. Three μc of uniformly labeled L-leucine-C^{14} was given intraportally to each rat 15 minutes before sacrifice. Hadacidin (N-formylhydroxyaminoacetic acid (Kaczka et al., 1962)) in the form of the monosodium dihydrate was generously supplied by Merck Sharp and Dohme Research Laboratories through the courtesy of Dr. Harold T. Shigeura.

Results. Table I lists the hepatic ATP levels and the amount of leucine-C^{14} incorporated into liver proteins in control and ethionine-treated female rats in two separate experiments. The influence of inosine and hadacidin on these two parameters is also given in this table. It is seen that inosine completely reverses the effects of ethionine on ATP levels and incorporation of leucine into protein. When hadacidin is given in addition to inosine to ethionine-treated rats these effects of inosine are almost completely blocked. Hadacidin alone did not significantly affect the ATP levels or protein synthesis. In both experiments there is a highly significant positive correlation between the ATP levels and the leucine incorporated (Exp. 1 $r=0.90$, $P<0.001$; Exp. 2 $r=0.88$, $P<0.001$). This is brought out very clearly in Experiment 1 where inosine alone caused enhancement in both ATP levels and protein synthesis compared to saline-treated rats and in Experiment 2 where ethionine-treated rats given both hadacidin and inosine show slight increases in both ATP levels and protein synthesis as compared to rats treated solely with ethionine.

Discussion. The first known effect produced in the liver by ethionine when given by injection is a rapid decrease in ATP concentration followed in order by inhibition of protein synthesis and accumulation of lipid in the form of triglycerides. The

TABLE I

The Effect of Hadacidin and Inosine on the Hepatic ATP Levels and the In Vivo Uptake of Leucine into Protein of Control and Ethionine-Treated Female Rats

	Treatment			ATP Levels	Leucine Incorporated
	Ethionine*	Inosine**	Hadacidin***		
				µmoles/g. liver	cpm/mg. protein
Experiment 1					
(4)	-	-	-	1.125 ± 0.082	145 ± 20
(5)	+	-	-	0.421 ± 0.027	23 ± 4
(4)	-	+	-	1.685 ± 0.109	218 ± 32
(4)	+	+	-	1.520 ± 0.228	227 ± 19
(3)	-	-	+	1.243 ± 0.041	151 ± 20
(4)	+	+	+	0.474 ± 0.007	37 ± 11
Experiment 2					
(4)	-	-	-	1.623 ± 0.035	262 ± 16
(5)	+	-	-	0.464 ± 0.009	40 ± 9
(6)	-	+	-	1.521 ± 0.084	242 ± 17
(5)	+	+	-	1.681 ± 0.061	246 ± 12
(6)	-	-	+	1.311 ± 0.038	243 ± 21
(6)	+	+	+	0.641 ± 0.065	106 ± 38

Number of rats in each group is indicated by the numbers in parentheses.

*-Saline treated

+1 mg. (6.1 µmole) per g. body wt. of DL-ethionine (saline solution containing 25 mg/ml) given intraperitoneally at time zero.

**-Saline treated

+0.32m mole per animal (aqueous solution containing 0.064 m mole/ml) given intraperitoneally at time 2 hrs.

***-Saline treated

+A total of 0.75 m mole per animal of sodium hadacidin dihydrate (aqueous solution containing 0.25 m mole/ml) given subcutaneously, 0.25 m mole given at time 2 hrs., time 3 hrs., and time 4 hrs.

Mean ± standard error of the mean. All animals were sacrificed 5 hours after the injection of ethionine or saline.

postulated mechanism for this deficiency in ATP is through trapping of the adenine moiety in S-adenosylethionine (SAE), a compound apparently only slowly metabolized. High levels of SAE are present in the liver even 24 hrs. after a single dose of ethionine (Farber & Castillo, 1963). It is further postulated that adenine exerts its protective effect on protein synthesis and ATP levels by being converted to adenylic acid via

the catalytic action of AMP phosphorylase with 5-phosphoribosylpyrophosphate (PRPP) and thence to ADP which in turn can be converted to ATP by oxidative phosphorylation. The present work adds credence to these views. Hadacidin has been shown to block the conversion of inosinic acid to adenylic acid by inhibiting reactions of inosinic acid with aspartate to form adenylosuccinate (Shigeura and Gordon, 1962). The present experiments strongly indicate that inosine exerts a protective effect on protein synthesis and ATP levels by virtue of being converted to adenylic acid and then to ATP. This is demonstrated by the almost complete block of these effects when hadacidin is given along with inosine.

The results of the present study also indicate that the overall rate of the de novo synthesis of adenine nucleotides from glycine, formate, glutamate, and PRPP and the rate of irreversible destruction of adenine nucleotides are probably relatively slow, since no change in the concentration of hepatic ATP occurred within 3 hrs. after the administration of hadacidin. The dosage used inhibited almost completely the conversion of inosine to adenine nucleotides in the same time interval. In contrast, from the results of this and the previous studies (Shull, 1962; Villa-Trevino et al., 1963), it is becoming apparent that the rates of synthesis of adenine nucleotides from preformed purines (adenine, hypoxanthine) is rapid.

Summary. It has been shown in this study that the administration of inosine, like adenine, is effective in counteracting the decrease in hepatic ATP concentration and protein synthesis induced by ethionine. This effect of inosine is probably due to its conversion to adenine nucleotides, since it is prevented by the simultaneous injection of hadacidin, a compound known to inhibit the conversion of inosinic acid to adenylic acid.

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