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EFFECTS OF L-2-PYRROLIDONE-5-CARBOXYLATE ON HEPATIC ADENOSINE TRIPHOSPHATE LEVELS IN THE ETHIONINE-TREATED RAT*

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Abstract—L-2-Pyrrolidone-5-carboxylate, the lactam of L-glutamate, when given at the same time as DL-ethionine to female rats largely prevents the decrease in hepatic ATP brought about by this methionine analogue. Pyrrolidone carboxylate does not interfere with the "trapping" of ATP by ethionine, since the levels of *S*-adenosylethionine are similar to those receiving ethionine alone. The effect of pyrrolidone carboxylate was found to be primarily on the synthesis *de novo* of adenine nucleotides. This compound was found to produce a 70 per cent increase in this biosynthetic pathway as measured by the incorporation of 2-^{14}C-glycine. The increase in ATP concentration was largely prevented by hadacidin. Furthermore, pyrrolidone carboxylate exerted no effect on the ATP levels several hours after ethionine administration. At this time the levels of ATP are decreased 80–85 per cent and are too low to support the *de novo*-synthetic pathway.

FEMALE rats treated with DL-ethionine develop a profound ATP deficiency (80 per cent decrease) which persists for periods of at least 24 hr.[1,2,3] This deficiency becomes maximal after 2 hr and is followed at 3 hr by a 90–95 per cent inhibition of RNA synthesis.[4] and at 4 hr by an 80–95 per cent inhibition of protein synthesis.[5] This inhibition of protein synthesis obtains *in vitro* as well as *in vivo*. The hepatic polyribosomes lose integrity and function in a time course similar to that for the inhibition of protein synthesis.[6] All of these biochemical lesions are readily prevented and may be reversed by adenine or methionine or a combination of the two.[7] The male rat treated with ethionine exhibits a decrease in hepatic ATP which is both temporally and quantitatively similar to the female rat. However, in contrast to the females the males have intact hepatic polyribosomal patterns and undiminished protein synthesis for at least 8 hr after treatment with ethionine.[8]

In a search for factors which might account for the sex differences in protein synthesis and ribosomal integrity in rats given ethionine acutely, pyrrolidone carboxylate was investigated. This compound, the lactam of glutamic acid, was tested because it bears a chemical similarity to tenuazonic acid, a potent inhibitor of protein synthesis *in vivo* and *in vitro*.[9] In addition, it requires ATP and an energy source for one of its biosynthetic pathways from glutamic acid in the liver.[10] It was found that pyrrolidone carboxylate did largely protect the hepatic protein synthesis *in vivo* and polysome structure of female rats treated with ethionine. This protective effect rendered by pyrrolidone carboxylate on the protein synthetic apparatus of the liver was found to

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be mediated through ATP. The mechanism by which pyrrolidone carboxylate exerts its effect on the hepatic ATP concentrations of ethionine-treated rats is the subject of this report.

MATERIALS AND METHODS

Female rats of the Wistar strain weighing between 170–200 g were used. The rats were fasted overnight before the experimental procedures were carried out.

ATP determinations. Sections of liver frozen in tongs at the temperature of liquid nitrogen were obtained from anesthetized animals as described by Bucher and Swaffield.[11] ATP was determined on neutralized perchloric acid extracts of these sections by the luciferin-luciferase enzymic assay.[12] The determinations were not modified by concentrations of DL-ethionine or pyrrolidone carboxylate of 0·01 M.

AEt determinations.* The concentration of AEt was determined on the Spinco model 120B amino acid analyzer using a 0·4 N perchloric acid extract of the liver as described by Shull *et al.*[2]

Synthesis of total adenine de novo. Rats received intraperitoneally 0·2 ml of a solution containing 25 μc of 2-^{14}C-glycine (4·92 mc/m-mole, New England Nuclear). Total adenine was determined as previously described.[2] A labeling-period of 1 hr was chosen since at longer time intervals such as 4 hr there is a great deal of synthesis and interchange of purines among tissues.[13] For this reason rates of adenine nucleotide synthesis may be ambiguous if measured over long time periods after administration of the radioactive precursor.[14] The ^{14}C-pulse was given at 60 min since this is the earliest that synthesis *de novo* is inhibited after this dose of ethionine.[2]

Chemicals. Hadacidin (*N*-formylhydroxyaminoacetic acid) was a gift of Merck Institute for Therapeutic Research through the courtesy of Dr. H. T. Shigeura. L-2-Pyrrolidone-5-carboxylate was purchased from Aldrich Chemical Co. Ethionine and pyrrolidone carboxylate were administered intraperitoneally. Hadacidin was given subcutaneously.

All experimentally determined values listed in the Tables are means $\pm$ standard errors of the means.

RESULTS

Table 1 shows the results of administering pyrrolidone carboxylate at the same time as ethionine on the hepatic ATP and AEt levels. This regimen involving these 2 compounds largely prevents the fall in hepatic ATP. Comparable levels of AEt in the ethionine and ethionine + pyrrolidone carboxylate-group indicate that this is not due to interference with the trapping of adenosine in AEt. The results with hadacidin indicate rather that pyrrolidone carboxylate probably facilitates the synthesis *de novo* of adenine nucleotides, since when this inhibitor of purine synthesis *de novo*[15] is given along with pyrrolidone carboxylate to ethionine-treated rats the protective effect of this compound is largely abolished. This lends credence to the site of action of pyrrolidone carboxylate being at the level of synthesis *de novo* of purines since hours after ethionine-treatment synthesis *de novo* of purines is largely inhibited, while at short intervals such as an hour or less after administration of this compound synthesis *de novo* of purines is accelerated.[2] Again, here as in the experiment where ethionine and pyrrolidone carboxylate were given together, the AEt levels in the 2 groups are

* Abbreviation used: AEt, S-adenosylethionine.

TABLE 1. EFFECTS OF PYRROLIDONE CARBOXYLATE AND HADACIDIN ON THE HEPATIC ATP AND S-ADENOSYLETHIONINE LEVELS OF ETHIONINE-TREATED RATS

Treatment	ATP (μmoles/g liver)	AEt (μmoles/g liver)
Control (10)*	2·937 ± 0·175	
Ethionine (10)†	0·678 ± 0·084	2·05 ± 0·152
Ethionine + pyrrolidone carboxylate (10)‡	1·679 ± 0·277	1·90 ± 0·079
Ethionine + pyrrolidone (10) Carboxylate + hadacidin§	0·929 ± 0·092	1·86 ± 0·05

* Number of rats in parentheses.

† At time zero 1mg/g body weight of DL-ethionine was given. Rats sacrificed time 4 hr.

‡ At time zero 0·5 mg/g body weight of L-2-pyrrolidone-5-carboxylate was given; 0·125 mg/g body weight was given at 1, 2 and 3 hr. Rats sacrificed at time 4 hr.

§ At times 0, 1, 2 and 3 hr 0·25 m-moles of sodium hadacidin was given per rat. Rats sacrificed at time 4 hr.

similar showing that there is no interference with the trapping of adenosine by ethionine to form AEt. When pyrrolidone carboxylate was given to untreated rats the hepatic ATP levels were unaffected.

To obtain additional independent data of the effect of pyrrolidone carboxylate on the synthesis of adenine nucleotides *de novo*, incorporation of 2-^{14}C-glycine was employed as a measure of this synthesis. The values listed in Table 2 indicate that this compound increases the synthesis *de novo* of hepatic total adenine by approximately 70 per cent. This increase is brought about during the incorporation interval of 60–120 min after ethionine; during this time period synthesis *de novo* of purines has been largely inhibited in rats receiving only ethionine.[2] These findings corroborate those with hadacidin and indicate a direct effect of pyrrolidone carboxylate on the synthesis of ATP *de novo*.

TABLE 2. LABELING *in vivo* OF TOTAL ADENINE BY 2-^{14}C-GLYCINE IN LIVERS OF RATS TREATED WITH ETHIONINE AND ETHIONINE PLUS PYRROLIDONE CARBOXYLATE*

Treatment	Incorporation interval (min)	Radioactivity in liver total adenine (counts/min/g)
Ethionine (5)†	60–120	12,000 ± 1400
Ethionine + pyrrolidone carboxylate (5)‡	60–120	20,200 ± 3100

* All rats were given 25 μc of 2-^{14}C-glycine at time 1 hr and sacrificed at time 2 hr. Number of rats in parentheses.

† At time zero 1 mg of DL-ethionine/g body wt. was given.

‡ At time zero 0·5 mg of L-2-pyrrolidone-5-carboxylate was given and 0·25 mg at 1 hr.

TABLE 3. EFFECT OF L-2-PYRROLIDONE-5-CARBOXYLATE ON THE HEPATIC ATP AND AEt LEVELS OF RATS TREATED 4 HR EARLIER WITH ETHIONINE

Treatment	ATP (μmoles/g liver)	AEt (μmoles/g liver)
Saline (4)*	2·27 ± 0·05	
Ethionine (5)†	0·18 ± 0·06	2·24 ± 0·35
Ethionine + pyrrolidone carboxylate (5)‡	0·19 ± 0·02	2·12 ± 0·43

* Number of rats in parentheses.

† At time zero rats were treated with 1 mg of DL-ethionine/g body weight and sacrificed 7 hr later.

‡ At time 4 hr rats were given 100 mg of L-2-pyrrolidone-5-carboxylate, 25 mg at 5 hr, 6, and 6·5 and sacrificed at time 7 hr.

The results presented in Table 3 indicate that pyrrolidone carboxylate is unable to increase the ATP levels of rats treated 4 hr previously with ethionine. These results are similar to those reported previously[2] in which the synthesis *de novo* of ATP was largely inhibited 4 hr after ethionine treatment. Presumably when the ATP levels reach 15–20 per cent of control values the synthesis *de novo* of adenine nucleotides is shut down. This follows since ATP is required in this synthetic pathway of these nucleotides. Once again the data in Table 3 indicate that pyrrolidone carboxylate does not interfere with the formation or turnover of AEt.

DISCUSSION

The presumptive evidence indicates that pyrrolidone carboxylate partially prevents the lowering of hepatic ATP by ethionine *in vivo* by stimulating the synthesis of this nucleotide *de novo*. This is demonstrated by the increased synthesis of adenine nucleotides as measured by incorporation of 2-^{14}C-glycine and by the prevention of the protective effect by hadacidin, an inhibitor of the synthesis of AMP *de novo*, when pyrrolidone carboxylate is given at the same time as ethionine to female rats. The failure of hadacidin to completely abolish the protective effect of pyrrolidone carboxylate on hepatic ATP levels after ethionine treatment (see Table 1) may indicate the participation to a small extent of a mechanism not involving the *de novo*-synthetic pathway. Ring opening with the formation of glutamate or glutamine was ruled out as a possible means for the protective effect of pyrrolidone carboxylate, since neither of these amino acids produced any effect on the ATP levels when administered along with ethionine. In a like manner neither do any of the other compounds required in the synthesis of purines *de novo* afford any protection.[16]

Although the effect of pyrrolidone carboxylate on the ATP levels of ethionine-treated rats appears to be centered primarily on the *de novo*-biosynthetic pathway of this nucleotide, the exact enzymic reactions affected have not as yet been elucidated. L-Asparagine has been reported to partially prevent the decrease in hepatic ATP after ethionine administration, but no mechanism of this phenomenon was given.[17] In the case of pyrrolidone carboxylate, one possible explanation of its effect might be as a positive allosteric effector at one or more of the enzymic reactions in the pathway

de novo of purine synthesis. Allosteric activators like inhibitors often bear no structural similarity to the substrates of the reactions that they modify.[18] It is also possible that some of the carbons and the nitrogen of pyrrolidone carboxylate are used directly in the *de novo*-pathway at a point beyond glycinamide ribotide. This would in effect conserve ATP through by-passing some of the steps that are energy requiring. An alternate step to the Buchanan–Greenberg pathway for the formation of 5-phosphoribosylamine has been reported.[19]

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