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SHORT COMMUNICATIONS

Metabolic effects of ethyl adenosine 5′-carboxylate in Ehrlich ascites tumor cells *in vitro**

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Adenosine 5′-carboxylate and several of its esters and amides are potent coronary vasodilators [1, 2]. It is believed that these compounds act as adenosine analogs whose effects are prolonged because they are not phosphorylated and only slowly deaminated [3]. Neither adenosine 5′-carboxylate nor several derivatives inhibited commercial calf intestinal adenosine deaminase or rabbit muscle adenylate deaminase [1, 2].

We have considered the possibilities that ethyl adenosine 5′-carboxylate might still inhibit adenosine deaminase or adenylate deaminase in intact mammalian cells and that it might affect other aspects of purine metabolism. The results of studies of its effects on several aspects of purine ribonucleotide biosynthesis, interconversion and catabolism in Ehrlich ascites tumor cells are reported here.

Sources of most materials, methods of tumor cell preparation and incubation, and procedures for the separation and measurement of radioactivity in purine bases, ribonucleosides and ribonucleotides have been reported previously [4, 5]. Ethyl adenosine 5′-carboxylate was a gift of Abbott Laboratories, North Chicago, Ill.

Experiments were conducted to measure the effect of ethyl adenosine 5′-carboxylate on purine ribonucleotide synthesis and interconversion. Ehrlich ascites tumor cells were incubated in calcium-free Krebs–Ringer medium containing 25 mM phosphate for 20 min with 1 mM ethyl adenosine 5′-carboxylate; radioactive precursor (100 μM [^{14}C]adenine, 83 μM [^{14}C]hypoxanthine or 97 μM [^{14}C]adenosine; the sp. act. of each was *ca.* 50 mCi/mmole) was then added, and incubation was continued for an additional 30 min. Radioactivity in adenylate, ADP, ATP, NAD, inosinate, xanthylate, guanylate, GDP, GTP, inosine, hypoxanthine, adenosine and adenine was measured [4, 5]. These compounds are clearly separated from other purine derivatives.

Ethyl adenosine 5′-carboxylate did not affect total nucleotide synthesis from adenine, hypoxanthine or adenosine, incorporation of radioactivity into the acid-insoluble fraction, any aspect of purine ribonucleotide interconversion or catabolism, or the ratios of radioactivity in nucleoside triphosphates to that in nucleoside diphosphates. The deamination of the exogenous adenosine was also not obviously affected.

Other experiments were conducted to measure the effect of ethyl adenosine 5′-carboxylate on the processes of adenine nucleotide catabolism, when catabolism was accelerated by incubation with 2-deoxyglucose. Under such conditions the rate of deamination of adenylate is considerably increased, as is that of adenylate dephosphorylation to adenosine; the intracellular adenosine so formed is rapidly removed both by deamination to inosine and by rephosphorylation to adenylate [6, 7].

Tumor cells were first incubated with [^{14}C]adenine to produce radioactive ATP. Unused [^{14}C]adenine was removed by centrifugation and resuspension in fresh medium. Concentrations of radioactive metabolites were measured in cells incubated an additional 30 min with: (a) glucose ("control"), (b) a mixture of deoxyglucose plus glucose to induce a moderate degree of ATP catabolism, and (c) deoxyglucose plus glucose plus ethyl adenosine 5′-carboxylate. Details are given in the legend of Table 1.

Representative results are presented in Table 1. Incubation with the mixture of deoxyglucose plus glucose led to a decrease in radioactive ATP, total adenine nucleotides and total nucleotides, together with an increase in inosine and hypoxanthine. (There is very little radioactivity in

Table 1. Effect of ethyl adenosine 5′-carboxylate on deoxyglucose-induced ATP catabolism*

	Radioactive metabolites (nmoles/g cells)									
Conditions	ATP	ADP	AMP	Adenine nucleotides	IMP	XMP	Total nucleotides	Inosine	Hypo-xanthine	Adenosine
Control	1133	262	61.3	1457	7.01	5.36	1497	74.7	39.8	12.7
Deoxyglucose + glucose	462	214	88.8	765	21.6	7.19	815	428	296	11.9
Ethyl adenosine 5′-carboxylate + deoxyglucose + glucose	571	222	83.2	876	10.7	8.12	913	317	253	44.0

* Two ml of 2% (v/v) Ehrlich ascites tumor cell suspensions in calcium-free Krebs–Ringer medium containing 25 mM phosphate and 5.5 mM glucose was incubated in 10-ml Erlenmeyer flasks at 37° with shaking, with an air atmosphere. After 20 min, [^{14}C]adenine was added to a final concentration of 100 μM, and incubation was continued for 30 min to synthesize [^{14}C]ATP. Unutilized [^{14}C]adenine was then removed by centrifugation and resuspension of the cells twice in fresh, warmed medium containing glucose. Control cells were then incubated for 30 min with 5.5 mM glucose; the same results were obtained in the presence or absence of ethyl adenosine 5′-carboxylate. To induce the catabolism of ATP, cells were finally resuspended in medium containing 4.81 mM deoxyglucose plus 0.69 mM glucose, with and without 1 mM ethyl adenosine 5′-carboxylate, and incubated for 30 min. Values reported are averages of duplicate measurements and are representative of results obtained in four experiments. Within each experiment, average deviation of individual analyses from the mean was less than 7 per cent.

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guanine nucleotides under these conditions.) In the presence of ethyl adenosine 5'-carboxylate, there was a smaller decrease in radioactive ATP, total adenine nucleotides and total nucleotides, and a smaller increase in radioactive hypoxanthine and inosine. However, there was a 3.7-fold increase in the accumulation of radioactive adenosine.

These results are similar to those observed when deoxyglucose-induced ATP catabolism was studied in the presence of coformycin [6], a potent and specific inhibitor of adenosine deaminase in these cells [8]. The inhibition of this enzyme led to increased accumulation of adenosine and decreased formation of inosine and hypoxanthine during ATP catabolism; however, considerable inosine and hypoxanthine still were formed by the pathway adenylate → inosinate → inosine [6]. Some of the adenosine, which accumulated when adenosine deaminase was blocked, was rephosphorylated to ATP, whose concentration consequently increased. Inhibition of adenosine kinase or of adenylate deaminase, in the absence of concomitant inhibition of adenosine deaminase, would not produce the same results, as any adenosine that might tend to accumulate would be deaminated [6].

We conclude that ethyl adenosine 5'-carboxylate, like coformycin, may inhibit adenosine deaminase when relatively low concentrations of adenosine are generated intracellularly during nucleotide catabolism. However, because this effect is not observed when cells are incubated with relatively high concentrations of exogenous adenosine, and is not observed in studies with purified enzymes [1, 2], it would seem to be a very weak inhibitor. The relation, if any, of this presumably weak inhibition of adenosine deaminase by ethyl adenosine 5'-carboxylate to its vasodilatory action is not known. No other aspect of purine metabolism was affected by this adenosine analog. It seems likely that the potent physiological effects of this compound are exerted through a mechanism unrelated to inhibition of specific aspects of intracellular purine metabolism.

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Changes in liver microsomal cytochrome P-450 induced by dietary proteins and lipid material

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Nutritional status can influence the activity of the drug-metabolising enzymes located in rat liver microsomes [1, 2]. Protein-deficient diets lower the concentration of cytochrome-P-450 in liver microsomes[3] and reduce the *in vivo* metabolism of drugs [4]. Lipid materials are also necessary for the normal synthesis of microsomal cytochrome P-450 and hydroxylating enzymes [5]. Two types of lipid material (unsaturated fatty acids and oxidized sterols) can fulfil this function, presumably by acting as permissive agents in the induction of microsomal hydroxylation enzymes by substances such as phenobarbitone [5, 6]. The experiments described in this note were designed to extend our knowledge of these inter-relationships and in particular to examine the 'lipid effect' in the presence of different dietary proteins.

Male Wistar rats of initial body weight 60–80 g (CFHB strain, Carworth Europe, Alconbury, Herts) were caged in groups in mesh-floored cages and given a powdered synthetic diet with the following composition (g): Maize starch 700, protein 200, corn oil 50, salt mixture (Glaxo Research Ltd.) 10, choline chloride 0.2, vitamin mixture 0.3; the vitamin mixture had the following composition (mg): riboflavin 160, thiamine hydrochloride 160, pyridoxine 160, calcium pantothenate 400 , cyanocobalamin 0.5, nicotinamide 2000, folic acid 100, biotin 12. All animals received a weekly supplement of 0.5 ml cod liver oil and 0.3 ml of a 1% solution of vitamin K.

The rats received the appropriate diet for 21 days. The proteins used were casein, gluten, soya, zein and soya, with 1% (DL) methionine added, all obtained from BDH Ltd., Poole, Dorset. Rats given the zein and unsupplemented soya diet did not grow during the experimental period. Hexo-barbiturate sleeping time was measured after an intraperitoneal dose of sodium hexabarbiturate (100 mg/kg body wt), liver microsomes were prepared by differential centrifugation; cytochrome P-450 was determined as described by Omura and Sato [7] with an assay concentration of 1–2 mg microsomal protein/cuvette and cytochrome b_5 by the method of Baron and Tephly [8] using a Perkin–Elmer Hitachi 124 double beam spectrophotometer fitted with an external recorder.

Significant increases were found in the total liver cytochrome P-450 in rats given gluten and soya diets when compared with the casein diet; gluten, zein and soya-supplemented diets produced lower microsomal cytochrome b_5 concentrations (Table 1). No obvious explanation exists for the 10-fold difference in cytochrome b_5 concentrations. The presence is implied of an inducer of cytochrome b_5 in the soya and casein diets. Poorer-quality proteins (gluten and soya) unexpectedly perhaps, produced an increase in the total cytochrome P-450.

The substitution of aged (oxidized) corn oil for fresh oil elicited different responses in two diets. Aged oil gave an increase in the microsomal cytochrome P-450 in the