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ETHANOLAMINE PHOSPHOKINASE: ACTIVITY AND PROPERTIES DURING LIVER DEVELOPMENT

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SUMMARY

The conditions for maximal activity of ethanolamine phosphokinase from rat liver were determined. All of the activity was located in the supernatant fraction after centrifugation at 100 000 × *g* for 60 min. The enzyme activity had a pH optimum at 8.5 and an apparent K_m for ethanolamine of $1 \cdot 10^{-4}$ M at an ATP and Mg^{2+} concentration of 3.0 mM. The enzyme uses the Mg–ATP complex as substrate and is inhibited by free ATP. Choline inhibits ethanolamine phosphokinase. Maximal inhibition is obtained at choline concentrations of 0.4 mM. Treatment of the supernatant with Sephadex G-25 or by dialysis causes an increase in the maximal amount of inhibition obtained with choline. The inhibition by choline is non-competitive with ethanolamine and competitive with ATP. Ethanolamine phosphokinase is inhibited by *N*,*N*-dimethylethanolamine and *N*-methylethanolamine but not by betaine, phosphoryl choline, CDP-choline or phosphorylethanolamine.

The activity of ethanolamine phosphokinase is low in −5-day fetal liver. The activity increases from −5 days to −2 days and drops at 1 day after birth. An endogenous inhibitor of ethanolamine phosphokinase is present in all preparations but at different levels. The drop in activity in 1-day-old animals is due to the presence of higher amounts of inhibition by the endogenous inhibitor within the preparation. Evidence indicates that the endogenous inhibitor is choline.

INTRODUCTION

Ethanolamine phosphokinase catalyzes the formation of phosphorylethanolamine from ATP and ethanolamine. This is the initial reaction in the pathway for the *de novo* synthesis of ethanolamine phosphoglycerides[1]. The occurrence of such a reaction was first suggested by studies on the incorporation by brain minces of ^{32}P into phosphorylethanolamine[2]. However, relatively few studies have been reported on the activity and properties of the enzyme responsible for the reaction. Wittenburg and Kornberg[3] showed that choline kinase (ATP:choline phosphotransferase, EC 2.7.1.32)

from yeast would also catalyze the formation of phosphorylethanolamine, although at considerably lower rates. The phosphorylation of both ethanolamine and choline has been shown to occur under the same assay condition with a preparation from the mollusc *Helix lactea*[4]. The properties and conditions for maximum assay of ethanolamine phosphorylation were not studied in either of the above investigations. A more detailed study of the phosphorylation of ethanolamine by extracts from Ehrlich ascites cells has been performed[5]. These studies suggest that the phosphorylation of choline and ethanolamine are catalyzed by separate enzymes and that the phosphorylation of ethanolamine is inhibited by choline.

We have previously shown that the ability of liver slices to incorporate [1,2-$^{14}C_2$]-ethanolamine into ethanolamine phosphoglycerides changes significantly during functional development of the liver[6]. The incorporation by liver slices from −5-day fetal rats is about 20% of the incorporation by adult liver slices. The rate of incorporation of [1,2-$^{14}C_2$]ethanolamine into ethanolamine phosphoglyceride increases progressively with prenatal development so that by birth 80% of the adult levels are obtained. In attempting to explain these developmental changes we are investigating the activities and properties of the enzymes that are associated with the pathway for ethanolamine phosphoglyceride synthesis. In this paper we report the results of studies on the activity and properties of ethanolamine phosphokinase from the liver of developing rats.

MATERIALS AND METHODS

Rats, females 180–200 g and sperm positive pregnant, were obtained from Holtzman Company, Madison, Wisconsin. The beginning of gestation of the pregnant animals was determined by Holtzman Company with an accuracy of *plus* or *minus* 12 h.

The [1,2-$^{14}C_2$]ethanolamine, phosphoryl[1,2-$^{14}C_2$]ethanolamine and [γ-^{32}P]ATP were purchased from New England Nuclear Corporation. Phosphoenolpyruvate, pyruvate kinase (EC 2.7.1.40), lactate dehydrogenase (EC 1.1.1.27), ADP, ATP, CTP, CDP-choline, phosphorylethanolamine and phosphorylcholine were purchased from Sigma. *N*,*N*-Dimethylethanolamine, *N*-methylethanolamine and 2-amino-2-methyl-1-propanol were purchased from Aldrich Chemical Company.

Minced liver was homogenized in 4 vol. of 0.25 M sucrose. The homogenate was centrifuged at 100 000 × *g* for 1 h. The supernatant from the centrifugation contained all of the enzyme activity and was used in all subsequent experiments except where stated otherwise.

Enzyme activity was determined by measuring the production of radioactive phosphorylethanolamine from [1,2-$^{14}C_2$]ethanolamine. The incubation mixture contained, except where altered as noted in specific experiments, 20 mM glycylglycine buffer (pH 8.5), 0.5 mM ethanolamine, 0.35–0.40 μCi/ml [1,2-$^{14}C_2$]ethanolamine, 3 mM ATP, 3 mM $MgCl_2$ and 0.2 ml of supernatant (3–4 mg protein). The reaction was usually performed in a final volume of 1.0 ml. The reaction was incubated at 37 °C and was stopped by placing the assay tube in boiling water for 2 min followed by cooling in an ice bath. The protein precipitate was removed by centrifugation and an aliquot of the supernatant was passed through a small column (0.5 cm × 2 cm) of ion exchange resin, AG 1-X8, 100–200 mesh, acetate form. The column was washed four

times with 5 ml of water and the phosphorylethanolamine was eluted with 3.0 ml of 1 M HCl. The amount of radioactivity in the HCl eluate was determined in a liquid scintillation spectrometer. Either Bray scintillation fluid[7] or Aquasol scintillation mixture* was employed. Samples of phosphoryl [1,2-$^{14}C_2$]ethanolamine were carried through the entire procedure and recoveries of 90–100% were achieved. Zero-time blanks were routinely run and were always less than 5% of the assay values. Initially, samples of the HCl eluate were separated by paper chromatography on Whatman 3M paper with the solvent system ethanol–conc. NH_4OH–water, (61:29:10, by vol.). The majority of the radioactivity (95–97%) migrated similar to standard phosphorylethanolamine. The remaining 3–5% was ethanolamine. Phosphorylethanolamine production was measured using a paper chromatographic method in addition to an ion exchange column method. Both gave identical results. Experiments were performed where phosphoryl [1,2-$^{14}C_2$]ethanolamine was added to the incubation mixture in place of the radioactive ethanolamine. The usual concentration of ethanolamine was included. There was no evidence for the production of radioactive ethanolamine during a 15-min incubation and all of the phosphoryl [1,2-$^{14}C_2$]ethanolamine was recovered after the incubation.

Enzyme activity was measured in some experiments in the presence of an ATP-generating system. The reaction mixture consisted of 20 mM glycylglycine (pH 8.5), 0.5 mM ethanolamine, 0.35–0.40 μCi/ml [1,2-$^{14}C_2$]ethanolamine, 0.8–4.5 mM ATP, 3 mM $MgCl_2$, 0.4 mM phosphoenolpyruvate, 17 units/ml of pyruvate kinase and liver supernatant. Pyruvate kinase preparations were dialyzed against 200 vol. of 1 mM EDTA before use in the assay. The reaction was stopped by placing the tubes in boiling water. The amount of pyruvate formed during the reaction was determined by measuring the decrease of absorbance at 340 nm due to the oxidation of NADH as described by Bücher *et al.*[8]. The amount of phosphorylethanolamine formed during the reaction was determined as described previously.

Sephadex G-25 chromatography was performed with a column (2.5 cm × 33 cm) that was packed and eluted with 0.02 M potassium phosphate buffer (pH 7.0).

Choline was determined by the method described by Reid *et al.*[9]. This procedure uses a purified preparation of choline phosphokinase and [γ-^{32}P]ATP. The amount of [^{32}P]phosphorylcholine produced is a measurement of the amount of choline present in the assay. We used a purified preparation of choline phosphokinase from rat liver.

The quantitative determination of the amount of enzyme inhibition by choline was made by plotting $(V - v)/v$ *versus* the concentration of choline according to the transformed Michaelis–Menten equation for competitive inhibition,

$$\frac{V - v}{v} = I \frac{K_m}{SK_i} + \frac{K_m}{S}$$

V is the velocity of the reaction in the absence of choline and v the velocity in the presence of choline. The choline concentration where $(V - v)/v = 1$ is the concentration that gives 50% inhibition.

Protein was determined by the Lowry method[10].

* A product of New England Nuclear Corporation, Boston, Mass.

RESULTS

Characteristics of the enzyme reaction

The rate of phosphorylethanolamine production, catalyzed by the 100 000 × *g* supernatant from adult liver, was constant for 20 min but declined with longer incubations. The average initial rate of the reaction for 18 separate experiments was 0.46 ± 0.04 μmole/min per mg protein. This amounted to a conversion of about 7% of the ethanolamine to phosphorylethanolamine during the usual 15-min incubation. The rate of the reaction catalyzed by fetal liver preparation was constant for 60 min.

The pH optimum was found to be 8.5. The activity at pH 8.0 and pH 9.0 was 80% of the maximal activities.

The velocity of the reaction was determined at different concentrations of ethanolamine. The data gave a linear Lineweaver–Burk plot and an apparent K_m of $1 \cdot 10^{-4}$ M was obtained when the ATP and Mg^{2+} concentrations were 3.0 mM.

The relationship between Mg^{2+} concentration, ATP concentration and enzyme activity is shown in Fig. 1. These results indicate that the enzyme uses the Mg–ATP complex as substrate. ATP concentrations that are higher than the Mg^{2+} concentration produce inhibition of the enzyme activity. At Mg^{2+} concentrations of 1.5 and 3.0 mM the inhibition is apparently caused by free ATP. However, the enzyme activity appears also to be inhibited by the Mg-ATP complex since the activity with 6.0 mM ATP and 6.0 mM Mg^{2+} is much lower than the activity at 4.5 mM ATP and 6.0 mM Mg^{2+}.

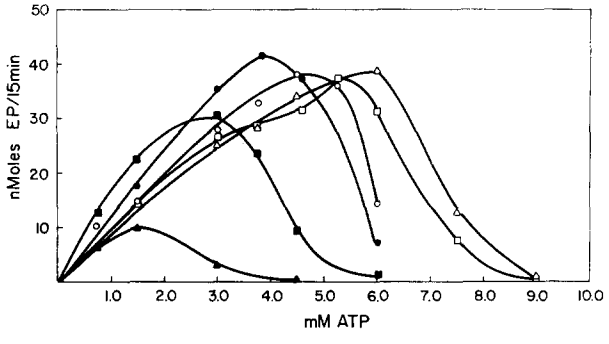

Fig. 1. The relationship between the concentrations of ATP and Mg^{2+} and the activity of ethanolamine phosphokinase. Each value is the average of 3 assays. Each curve represents a different concentration of Mg^{2+} in the assay. The Mg^{2+} concentrations are: ▲—▲, 1.5 mM; ■—■, 3.0 mM; ●—●, 4.5 mM; ○—○, 6.0 mM; □—□, 7.5 mM; △—△, 9.0 mM. EP = ethanolamine phosphate.

Enzyme activity was determined in the presence and absence of the ATP-generating system. At ATP concentrations below optimum, higher enzyme activity was obtained in the presence of the ATP-generating system, Table I. Maximum activity was obtained at 3.0 mM ATP and the same activity was obtained in the presence and in the absence of the ATP-generating system. Inhibition was still observed at ATP concentrations higher than the Mg^{2+} concentrations. The amount of pyruvate produced during the reaction, as a measurement of the total production of ADP, was determined with lactate dehydrogenase[8]. Since more pyruvate was pro-

TABLE I

ETHANOLAMINE PHOSPHOKINASE ACTIVITY IN THE PRESENCE AND ABSENCE OF THE ATP-GENERATING SYSTEM, PHOSPHOENOLPYRUVATE AND PYRUVATE KINASE

Enzyme activity is expressed as nmoles of phosphorylethanolamine produced in 15 min. Pyruvate production is reported as the nmoles produced in 15 min. The concentration of Mg^{2+} was 3.0 mM.

ATP (mM)	*Ethanolamine phosphokinase activity*		*Pyruvate*
	Without ATP-generating system	*With ATP-generating system*	*With ATP-generating system*
0	0	0	5
0.8	21	32	64
1.5	28	40	53
3.0	36	38	67
3.3	33	35	67
3.8	23	24	69
4.5	16	16	69

duced then predicted from the amount of phosphorylethanolamine produced, some ADP was formed by reactions other than the phosphorylation of ethanolamine.

Inhibition by choline

Choline inhibits the phosphorylation of ethanolamine catalyzed by liver supernatant preparation from fetal, postnatal and adult rats, Table II. Complete inhibition is not achieved in any preparation. Fetal preparations appear to be inhibited more than the 1-day and adult preparations. In several experiments adult supernatants were assayed in the presence of the ATP-generating system and the same amount of inhibition was observed. The inhibition with an adult preparation is non-competitive with ethanolamine and competitive with ATP, Fig. 2. A K_i for choline of 0.02 mM was calculated from the ATP experiment shown in Fig. 2.

TABLE II

THE CHOLINE INHIBITION OF ETHANOLAMINE PHOSPHOKINASE ACTIVITY IN LIVER FROM FETAL, NEWBORN AND ADULT RATS

A unit of enzyme activity is that amount of enzyme that produces 1 nmole of phosphorylethanolamine in 15 min. Each value is the average of 3 assays. The values of individual assays varied from the average by no more than 10%.

Choline concn (mM)	*Ethanolamine phosphokinase inhibition*							
	−3 days		*−1 day*		*+1 day*		*Adult*	
	Units	*% Inhibition*	*Units*	*% Inhibition*	*Units*	*% Inhibition*	*Units*	*% Inhibition*
0	24.0	—	19.4	—	17.6	—	39.4	—
0.01	21.0	11	14.8	18	14.5	18	39.0	0
0.10	7.6	68	6.0	69	11.9	32	25.8	32
0.40	5.8	76	4.5	77	8.8	50	22.5	43
1.20	5.0	79	5.2	73	10.6	60	21.1	46
1.60	4.9	80	5.6	71	9.4	53	20.8	47

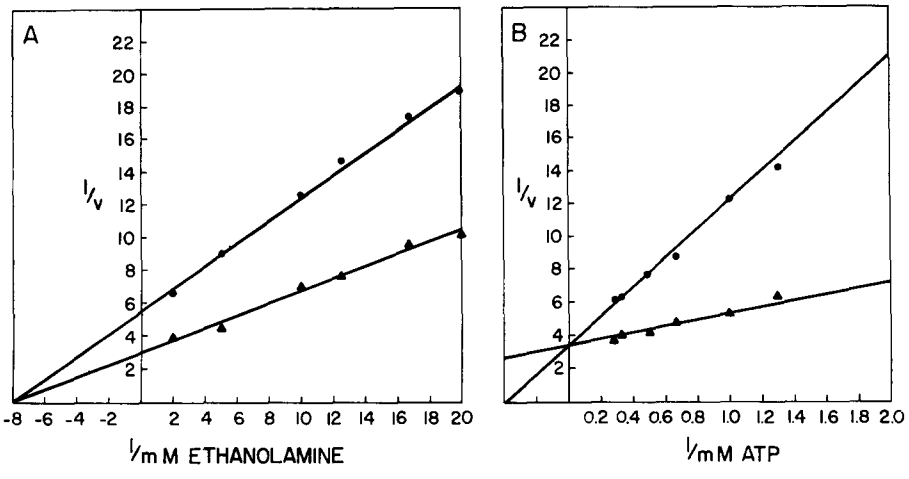

Fig. 2. The inhibition of ethanolamine phosphokinase by choline. ●—●, $3 \cdot 10^{-5}$ M choline; ▲—▲, no choline.

TABLE III

THE EFFECT OF SEPHADEX TREATMENT ON THE INHIBITION BY CHOLINE OF ETHANOLAMINE PHOSPHOKINASE FROM ADULT LIVER

See Table II for explanations.

Choline concn (mM)	*Ethanolamine phosphokinase activity*			
	Before Sephadex		*After Sephadex*	
	Units	*% Inhibition*	*Units*	*% Inhibition*
0	39	—	27	—
0.01	38	0	23	15
0.1	26	32	8	72
0.4	23	43	5	83
1.2	21	46	—	—
1.6	21	47	5	82

The maximal amount of choline inhibition that is observed with an adult preparation increases after passage of the preparation through a Sephadex G-25 column, Table III. This also occurs with fetal preparations, Table IV. This phenomena is apparently caused by the removal of low molecular weight materials from the preparation since dialysis of supernatant against either 20 mM phosphate buffer (pH 7.0) or against distilled water produces the same result as Sephadex treatment. Furthermore, dilution of the supernatant with 20 mM phosphate buffer or with 0.25 M sucrose does not cause a change in choline inhibition, nor does concentration of the diluted preparation by lyophilization.

The ability of compounds that are structurally or metabolically related to choline to inhibit the phosphorylation of ethanolamine was determined, Table V. A Sephadex-treated supernatant from adult liver was utilized. Betaine and cytidine-diphosphocholine had no inhibitory activity at the concentration employed. Phosphoryl-

TABLE IV

THE EFFECT OF SEPHADEX TREATMENT ON THE INHIBITION BY CHOLINE OF ETHANOLAMINE PHOSPHOKINASE FROM FETAL LIVER

A unit of activity is defined in Table II. Each value is the average of 3 assays.

Choline concn (mM)	*Ethanolamine phosphokinase activity*							
	−3 days				*−1 day*			
	Before Sephadex		*After Sephadex*		*Before Sephadex*		*After Sephadex*	
	Units	*% Inhibition*	*Units*	*% Inhibition*	*Units*	*% Inhibition*	*Units*	*% Inhibition*
0	19.4	—	12.9	—	24	—	20.6	—
0.01	14.8	18	10.6	18	21.0	11	21.3	0
0.1	6.0	69	1.3	90	7.6	68	2.6	87
0.4	4.5	77	0	100	5.8	76	0.3	99
1.2	5.2	73	0.3	97	5.0	79	0	100
1.6	5.6	71	0	100	4.9	79	0	100

TABLE V

THE SPECIFICITY OF THE INHIBITION OF ETHANOLAMINE PHOSPHOKINASE

All compounds were added to the standard assay at a final concentration of 0.1 mM. The Sephadex-treated supernatant from adult liver was used as a source of ethanolamine phosphokinase.

Compound	*% Inhibition*
Choline	81
Phosphorylcholine	29
Cytidinediphosphocholine	0
Betaine	0
Phosphorylethanolamine	8
Dimethylethanolamine	79
Monomethylethanolamine	34
2-Amino-2-methylpropanol	21

choline has some inhibitory activity while phosphorylethanolamine gave only slight, if any, inhibition. However, dimethylethanolamine was equally as inhibitory as choline, while monomethylethanolamine was considerably less inhibitory. 2-Amino-2-methylpropanol, a compound that has been shown to inhibit the incorporation of [1,2-$^{14}C_2$]ethanolamine into ethanolamine phosphoglycerides[11], also inhibits ethanolamine phosphokinase. However, the amount of inhibition is much less than that obtained with choline.

Ethanolamine phosphokinase activity during development

The activity of ethanolamine phosphokinase in the liver from fetal and young rats was compared to the activity in the livers from adult female rats, Fig. 3. The activity is expressed per g wet liver, per total mg of protein and per mg of protein in the 100 000 × *g* supernatant. The same relative developmental pattern is obtained regardless of how the activity is expressed. Low activity is observed in liver from −5-day-old fetuses. The activity increases about 2-fold from −5 days to −2 days. A large drop in activity occurs between birth and 1 day after birth. The activity

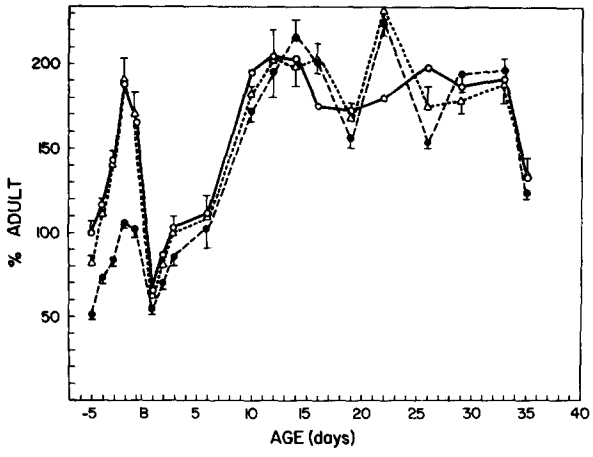

Fig. 3. Ethanolamine phosphokinase activity during the development of the liver. Each value is the average of 3–6 separate experiments. Experiments with fetal rats involved the combination of all livers from the fetuses from a single pregnant rat. The experiments with young rats involved the combination of the livers from 3 rats from a single litter. All assays were done in triplicate and averaged. The data is represented as: activity/g fresh liver, (●—●); activity/mg of total protein (△—△); and activity/mg of 100000 × *g* supernatant protein (○—○). The bar above or below the points represent 1 SE.

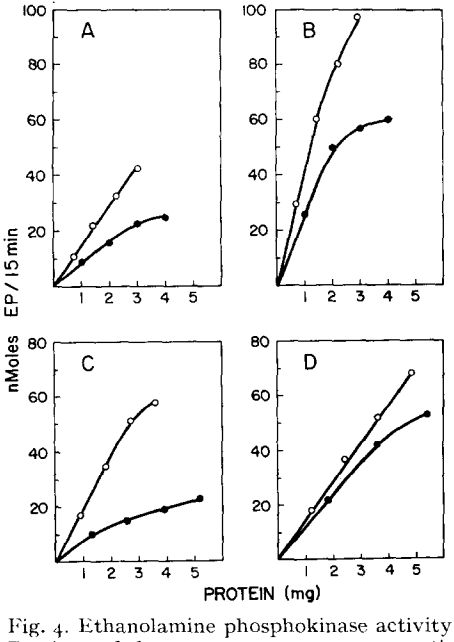

Fig. 4. Ethanolamine phosphokinase activity at different concentrations of supernatant protein. Portions of the same supernatant preparations were assayed before passage through Sephadex G-25 (●—●) and after Sephadex G-25 treatment (○—○). A, −4 days; B, −1 day; C, 1 day; D, adult. EP = ethanolamine phosphate.

increases at a rather uniform rate from 1 day to a peak value 200% of the adult at 14 days. The activity begins to drop toward adult levels at 33–34 days. The differences between the values expressed on a wet weight basis and the values expressed on a tissue protein basis are primarily due to differences in the amount of protein per weight of tissue.

The inhibition of ethanolamine phosphokinase by an endogenous inhibitor

A non-linear relationship between the amount of phosphorylethanolamine produced and the amount of supernatant used in the assay was observed, Fig. 4. This suggests that the 100 000 × *g* supernatant preparations contain an inhibitor of ethanolamine phosphokinase. The degree of inhibition of enzyme activity varied with the age of the animal. The amount of inhibition was slight in the adult preparations, Fig. 4D, but was considerable in −4-day fetal and 1-day-old preparations, Figs 4A and 4C. This inhibition could be eliminated by passage of the supernatant preparation through a Sephadex G-25 column, Fig. 4, or by dialysis.

When the enzyme activity was determined on Sephadex-treated preparations, a different developmental pattern of enzyme activity was obtained, Fig. 5. The activity was now near adult levels at −4 days and increased above adult levels as development progressed. The drop in activity 1 day after birth that was observed with untreated preparations did not occur but instead the enzyme activity continued to rise after birth. The peak values at 14 days were not significantly altered by the Sephadex treatment.

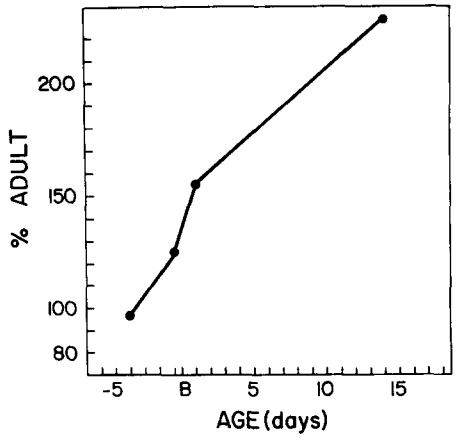

Fig. 5. The developmental change in ethanolamine phosphokinase that was determined with Sephadex G-25-treated preparations. The activity was expressed as units/g of fresh liver.

Characteristics of the endogenous inhibitor

A crude preparation of the inhibitor was obtained by passing liver supernatant from 1-day-old rats through a column of Sephadex G-25. The fractions from the column that were obtained between the last measurable amount of protein and the total column volume were combined and evaporated under vacuum. These preparations had considerable inhibitory activity. Equally as active preparations were obtained by dialysis of supernatant against distilled water and evaporation of the dialysate. The inhibition by such preparations is competitive with ATP (Fig. 6).

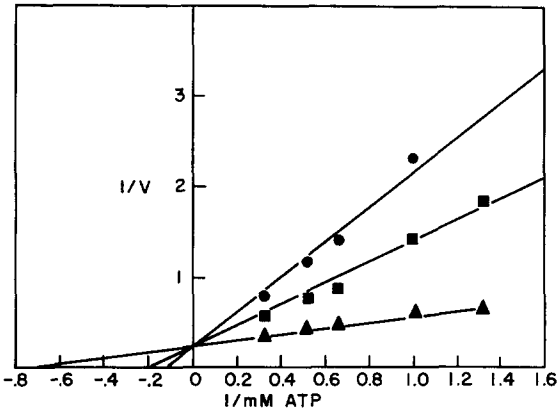

Fig. 6. The inhibition of ethanolamine phosphokinase by the endogenous inhibition: ●, 5 μl; ■, 2.5 μl; ▲, no inhibitor.

The chromatographic behavior of the inhibitor was investigated. The crude preparation was chromatographed on Whatman 3M paper. The chromatograms were sectioned and each section was extracted with 0.2 M HCl. The inhibitory activity moved identically with standard choline in the solvent systems 95% ethanol–conc. NH_4OH (95:5, v/v) and *n*-propanol–water (7:3, v/v).

The amount of choline was determined in a preparation that was obtained by dialysis of adult supernatant. This preparation had 0.46 μmole of choline per ml. The amount of inhibition that was obtained with this preparation was compared with the inhibition obtained with choline on the same enzyme preparation. By referring to the $(V - v)/v$ *versus* I plot for choline inhibition we predicted from the choline concentration of the inhibitor preparation that 2 μl would give 49% inhibition. We actually obtained 39% inhibition.

DISCUSSION

The conditions for maximal activity of ethanolamine phosphokinase from rat liver have been determined. The activity is recovered in the supernatant fraction after centrifugation at 100 000 $\times$ *g* for 60 min. A similar distribution of ethanolamine phosphokinase in Ehrlich ascites cells has been reported[5]. The relationship of enzyme activity with ATP and Mg^{2+} concentration indicates that the Mg–ATP complex is the substrate for the reaction and that ATP causes inhibition. The enzyme is not inhibited by ADP under our assay conditions since the same maximal activity and the same amount of inhibition with ATP was obtained with or without the ATP-generating system. The apparent K_m for ethanolamine is similar to the apparent K_m that was reported for preparations from Ehrlich ascites cells[5].

Sung and Johnstone[5] reported that ethanolamine phosphokinase activity in Ehrlich ascites cells was inhibited by choline. Ethanolamine phosphokinase activity in the spleen, kidney and brain of mice was also inhibited by choline. However, they found no inhibition by choline with enzyme preparations from mouse liver. We find that ethanolamine phosphokinase from rat liver is strongly inhibited by choline.

Furthermore, the inhibition is non-competitive with ethanolamine and is competitive with ATP. Thus, the inhibition is apparently not a result of competition between the structurally similar compounds, ethanolamine and choline, for a site on the enzyme. It is conceivable that choline is causing an apparent inhibition of ethanolamine phosphorylation through depletion of the ATP concentration and/or the production of ADP by choline phosphokinase activity. These possibilities are excluded by the observations that choline still inhibited ethanolamine phosphokinase when the assay was performed in the presence of the ATP-generating system. An interesting aspect of the choline inhibition is the observation that ethanolamine phosphokinase activity in the supernatant can not be completely inhibited by choline. This phenomenon was apparently also observed by Sung and Johnstone[5] with preparations from mouse spleen and kidney since they obtained the same amount of inhibition with 0.05 and 0.5 mM choline. Our observations that Sephadex G-25 treatment or dialysis increases the amount of maximal inhibition by choline suggests that the susceptability of ethanolamine phosphokinase to choline inhibition may be altered by the removal of some low molecular weight substances. One possibility is that ethanolamine phosphokinase exists in the supernatant in two forms. One form is inhibited by choline while the other is not inhibited. The removal of substances by either Sephadex G-25 treatment or by dialysis converts the non-inhibited form to the inhibited form. This phenomenon is under further investigation with a purified preparation of ethanolamine phosphokinase.

The 100 000 $\times$ g supernatant fraction at all ages contains an endogenous inhibitor of ethanolamine phosphokinase. The amount of inhibition changes with development and therefore affects the developmental pattern of apparent enzyme activity. All the evidence indicates that the endogenous inhibitor is choline. The inhibition by the endogenous inhibitor is competitive with ATP, as is the inhibition by choline. The inhibitory activity migrates on paper the same as choline in two solvent systems. Furthermore, the amount of inhibition that is obtained with a preparation of the endogenous inhibitor is correlated with the amount of choline in the preparation.

The inhibition by choline of ethanolamine phosphokinase may be an example of feedback control. Choline is synthesized in the liver by the methylation of ethanolamine phosphoglycerides[12]. Thus, a pathway for choline synthesis begins with the phosphorylation of ethanolamine by ethanolamine phosphokinase and proceeds through the synthesis of ethanolamine phosphoglycerides. Therefore, the concentrations of choline, the final product of the pathway, could regulate choline synthesis by altering the activity of ethanolamine phosphokinase.

The developmental pattern of ethanolamine phosphokinase in some ways is similar to the development pattern that was observed for the incorporation by liver slices of [1,2-$^{14}C_2$]ethanolamine into ethanolamine phosphoglycerides[6]. Both activities are low in -5-day fetal and increase with prenatal development. However, the ability of slices to incorporate [1,2-$^{14}C_2$]ethanolamine into ethanolamine phosphoglycerides is 20% of the adult at -5 days, while the ethanolamine phosphokinase activity is 50% of the adult levels. The ethanolamine phosphokinase activity reaches adult levels before birth and increases to two times adult levels by 14 days after birth, while the incorporation of [1,2-$^{14}C_2$]ethanolamine by liver slices reaches 80% of adult values before birth and does not increase above adult levels in the young rat. Thus, the

developmental changes in the activity of the total pathway for the incorporation of ethanolamine into ethanolamine phosphoglycerides, as measured previously with liver slices, is not solely due to changes in ethanolamine phosphokinase activity, particularly in the postnatal period.

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