SUCCINATE INHIBITION OF THE ETHANOLAMINE TO CHOLINE CONVERSION[1]

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During the course of metabolic studies with ethanolamine it became desirable to ascertain if the presence of oxidizable substrates, which lead to the formation of high energy phosphate derivatives, would accelerate the conversion of ethanolamine to phosphatidyl choline. During this study it was observed that succinate inhibited the in vitro formation of choline. It is the purpose of this paper to present data on this inhibition phenomenon and possible mechanisms.

EXPERIMENTAL

Preparation and Incubation of Liver Slices. The liver of non fasted male rats was immediately excised after killing the animals and placed in ice-cold, 0.9 per cent sodium chloride. Liver slices[2] totaling 500 mg wet weight were incubated aerobically at 37°C for two hours. The reaction was stopped by addition of 2 ml of a 3:1 ethyl alcohol-ethyl ether mixture and raising the temperature of the incubator bath to 75-80°C.

Respiration Measurements. Three hundred mg, wet weight[3], of rat liver slices were prepared as previously mentioned and incubated in a normal aerobic atmos-

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2 Experimental conditions which will permit the formation of choline from ethanolamine in a broken cell suspension or in a purified enzyme system are not known (Pilgeram et al., 1957).

3 Wet weight equals the weight of the tissues taken after gentle blotting on filter paper.

phere in the Warburg apparatus at 37°C for 2 hours. Oxygen consumption and $C^{14}O_2$ production were measured by conventional techniques; carbon dioxide was trapped in 0.2 ml of 10 per cent KOH in the Warburg flask center well, which was fitted with a fluted filter paper. The experiment was terminated by the addition of 4 N H_2SO_4 from the side arm of the incubation flask. Radioactivity determinations were made by preparing plates of barium carbonate from the alkali-trapped carbon dioxide.

Substrates. Five ml of Krebs-Ringer phosphate solution made as described by Umbreit et al. (1947), with the exception that the $CaCl_2$ was 0.0016 M, were used as the supporting media for the incubation experiments. Three ml of the Krebs-Ringer phosphate solution were used in the respiration experiments. Radioactive substrates included ethanolamine-1,2-C^{14} and $C^{14}H_3$-methionine.

Isolation of Choline. Following denaturation, the incubated mixture was hydrolyzed in 35 ml of 6 N HCl for 24 hours in the presence of carrier choline. The hydrolysate was filtered and taken to dryness in vacuo following which choline was isolated by use of a Dowex 50 cation exchange resin column (Pilgeram et al., 1957).

Analysis of Radioactive Choline. Dried choline chloride, as isolated from the column, was extracted with a minimum volume of water and was then precipitated by the addition of a 2 per cent Reineckate salt-methyl alcohol solution. The final volumes were 5 ml of choline-water solution and 10 ml of Reineckate salt-methyl alcohol solution. The choline reineckate was allowed to precipitate quantitatively by storing in the deep freeze for 6 hours. The precipitate was then plated on 4.25 cm Whatman No. 42 filter paper by the use of conventional procedures. Counting of the radioactive samples was carried out in a gas flow counter employing, as a gas phase, helium saturated with absolute ethyl alcohol at the temperature of an ice-water bath.

RESULTS AND DISCUSSION

Addition of succinate to incubation mixtures of ethanolamine-1,2-C^{14} and methionine reduced the choline yield by over 90 per cent (Table I). That this did not result from an increased oxidation of choline may be concluded from the

Table I

Effect of Succinate, Cyanide, and Glycine upon Choline Formation by Rat Liver Slices Incubated in the Presence of Ethanolamine-1,2-C^{14} and Methionine

Substrates*			Number of Experiments	Total Count in Choline per 100 seconds
Succinate	CN	Glycine		
0	0	0	11	1056
+	0	0	8	92
+	+	0	4	54
+	0	+	4	274

* Sodium succinate = 1.8×10^{-3} M, potassium cyanide = 1.0×10^{-3} M, glycine = 2.5×10^{-3} M, methionine = 7.5×10^{-4} M, ethanolamine-1,2-C^{14} = 3.8×10^{-4} M containing 239,500 ct/100 sec.

Table II

Effect of Succinate and Malonate upon O_2 Consumption and $C^{14}O_2$ Production by Ethanolamine-1,2-C^{14} and $C^{14}H_3$-Methionine in Rat Liver Slices

Substrates**				No. of Expts.	Endogenous O_2 Uptake	No. of Expts.	$C^{14}O_2$ activity	No. of Expts.	Unreacted Ethanolamine-1,2-C^{14}
Methionine	Ethanolamine	Succinate	Malonate						
					µl		cts/100 sec.		cts/100 sec.
+	+*	0	0	4	82	4	548	2‡	90,438
+	+*	+	0	4	50	4	324	2‡	98,212
+	+*	+	+	4	-20	4	300	-	----
+	+*	0	+	-	-18	3	532	-	----
+*	+	0	0	-	--	3	472	-	----
+*	+	+	0	-	--	3	406	-	----

* Radioactive.

** Methionine = 5.5×10^{-4} M, $C^{14}H_3$-methionine = 5.8×10^{-4} M containing 311,000 cts per 100 sec., ethanolamine = 3.8×10^{-4} M, ethanolamine-1,2-C^{14} = 3.8×10^{-4} M containing 96,000 cts per 100 sec., sodium succinate = 1.8×10^{-3} M, sodium malonate = 1.8×10^{-3} M.

‡ Ethanolamine-1,2-C^{14} = 239,500 cts per 100 sec. = amount of substrate at preincubation time.

Experiments performed with both succinate and glycine gave a greater yield of choline (Table I). This can not be explained on the basis of the formation of extra ethanolamine from glycine since this would result in a dilution of the radioactive ethanolamine with a consequent lowering of the radioactivity in the choline.

inhibiting effect of succinate on the oxygen consumption in this system (Table II) and the inhibition by succinate in the presence of cyanide. The latter, according to Mann and Quastel (1937), inhibits the activity of choline oxidase

under aerobic conditions[4]. Table II indicates that succinate actually caused a reduction of oxygen uptake in the presence of methionine and ethanolamine and a reduction in the radioactive CO_2 produced. Also less of the ethanolamine-1, 2-C^{14} substrate was used in the presence of succinate (Table II). The effect of succinate in reducing $C^{14}O_2$ production was also observed, but to a lesser extent, in preparations where $C^{14}H$-methionine was employed with unlabeled ethanolamine.

The possibility of attributing the inhibition of choline formation by succinate to an effect related to the oxidative cycle of succinate appears to be out of question in view of the fact that malonate, a competitive inhibitor of succinate oxidation, did not eliminate the inhibition of $C^{14}O_2$ production from the labeled ethanolamine by succinate. Malonate in the absence of succinate caused no decrease in $C^{14}O_2$ formation. However, little of the difference in the ethanolamine-1,2-C^{14} utilized or in the choline formed in the presence or absence of succinate can be accounted for by the oxidation to $C^{14}O_2$. This suggests that the effect of succinate is more closely related to some step in the intermediary metabolism of ethanolamine. Since approximately 40 per cent of the ethanolamine substrate or 98,000-100,000 cts per 100 seconds remained at the end of the incubation the effect of succinate does not stem from a lack of available ethanolamine for methylation to choline. The inhibition can not be the result of a lack of methionine methyl groups caused by oxidation since succinate reduced the oxidation of the $C^{14}H_3$ of methionine.

While a full explanation of the inhibitory action of succinate is not available, certain speculations are worthy of consideration. Stimulation of increased oxidation of choline or ethanolamine by succinate has been ruled out. It is not the result of an inhibition of lecithin formation and the accumulation of free choline since the choline measured represents phosphatidyl choline as well as free

4 Cyanide, at a concentration of 0.005 M, has been reported to cause a decrease in the activity of succinic oxidase to 40 per cent of the original after 2 hours of incubation (Tsou, 1951). However, since the concentration of cyanide in our experiments was 0.001 M and since succinic oxidase can be protected from inactivation by the presence of an adequate concentration of succinate it is to be expected that the activity of succinic oxidase was decreased but little, if any, by the presence of cyanide.

choline. A clue to the mechanism of the inhibition may be contained in the experiment showing a reduced degree of inhibition by glycine (Table I). This might be explained by competition of glycine with ethanolamine for succinate. For example, glycine is known to react with succinate to form α-amino-β-ketoadipic acid and finally δ-aminolevulinic acid (Shemin and Russell, 1953).

SUMMARY

Succinate inhibition of the *in vitro* conversion of ethanolamine-1,2-C^{14} to choline is reported. Agents preventing oxidation of choline and succinate respectively, i.e., cyanide and malonate, did not affect the inhibitory phenomenon thus indicating the apparent absence of accelerated choline oxidation or the oxidative cycle of succinate as a cause of the lack of appearance of radioactive choline. Glycine was observed to partially inhibit the succinate effect.

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