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INTESTINAL ABSORPTION OF CHOLINE IN THE CHICK

GENE R. HERZBERG* and JOSEPH LERNER**

Department of Biochemistry, University of Maine, Orono, Me. 04473 (U.S.A.)

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SUMMARY

The intestinal absorption of choline in the chick has been studied in 1-min incubations using a tissue-accumulation method. There are two processes involved in choline absorption, a mediated route with an apparent K_m of 110 μM and a maximum velocity V of 25 nmoles/ml tissue water per min and a diffusion mechanism. Lowering the external Na^+ concentration leads to an increase in apparent K_m but to no change in V. Mediated choline entry is not sensitive to anaerobiosis, ouabain or oligomycin. The transport of choline can be competitively inhibited by structural analogs, the best inhibitors being hemicholinium-3 and *N,N*-dimethylethanolamine which also cause *trans*-inhibition. Choline influx, however, is independent of intracellular choline. Although the transports of choline and thiamine are mutually antagonistic, results of a kinetic test indicate that there probably is not a single common site for choline and thiamine absorption.

INTRODUCTION

The transport of choline has been studied in such diverse tissues as blood[1–4], brain[5–8], myocardial cells[9], hepatoma cells[10], diaphragm[11] and kidney[12–15]. In early studies, Vetper *et al.*[16] and Rohse and Searle[17] investigated the *in vivo* disappearance of choline from rat and dog intestine, respectively. Detailed analysis of choline transport in intestine, however, has not been initiated until recently. Sanford and Smyth[18,19] have investigated choline transfer in everted sacs of rat and hamster intestine in 1-h incubations and a preliminary report has been presented for choline absorption in guinea pig ileum[20].

We chose to investigate choline absorption in the chick intestine, because it is an essential dietary constituent. Further, our study has been performed under conditions in which choline entry is not subject to subsequent metabolism, a condition not met by other intestinal studies.

MATERIALS AND METHODS

Black, sex-linked male chicks, 8–15 days old, were used as a source of intestinal tissue. The animals were fed on a standard diet and fed *ad libitum*. The tissue handling

* Present address: Committee on Biochemistry, Bowdoin College, Brunswick, Me. 04011, U.S.A.

** To whom requests for reprints should be addressed.

and manipulations involved in the preparation of the intestinal segments have been previously described[21]. The tissue segments were incubated 1 min at 37 °C in 5 ml of previously oxygenated (O_2–CO_2; 95:5,v/v) Krebs–Henseleit buffer (pH 7.4) containing 0.3% glucose and the appropriate substrate in a 25-ml erlenmeyer flask. The incubation was terminated by pouring the contents of the flask onto a vacuum-evacuated Hirsch funnel. The segments were blotted, weighed and extracted by shaking for 2 h in 0.75 ml of 2.5% trichloroacetic acid. The extracts were centrifuged at 23000 × g for 15 min and 0.2-ml aliquots of the supernatant were added to 10 ml of scintillation solution[22] and counted in a Tri Carb liquid scintillation spectrometer (Packard). The counts were corrected for quenching by the channels ratio method. The uptake is expressed as the accumulation of the substrate in the tissue water inaccessible to inulin[23]. Except where otherwise noted, the uptake was also corrected for diffusion[1].

To test for metabolism of choline during the 1-min incubation period, tissue segments were incubated normally in buffer containing radioactive choline. The tissue was homogenized in cold distilled water and the extract heated to 100 °C to precipitate the protein. Paper chromatography of the clarified extract in n-butanol–ethanol–acetic acid–water (8:2:1:3, by vol.) revealed that 96–98% of the radioactive material had the same R_F as choline.

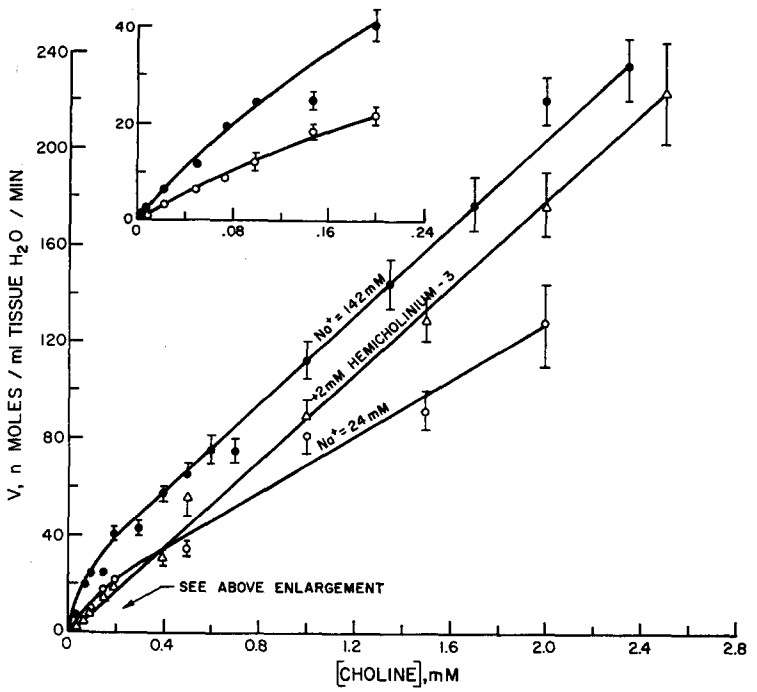


Fig. 1. Choline uptake as a function of choline concentration. The intestinal segments were incubated 1 min at 37 °C as described in the text. The low Na^+ concentration was attained by the isotonic substitution of the 118 mM NaCl in the Krebs–Henseleit buffer with L-lysine·HCl. The intestinal segments were preincubated for 15 min in the lysine-substituted buffer. The segments were then reincubated for 1 min in the low Na^+ buffer containing choline. Preincubation for 15 min in normal Krebs–Henseleit buffer had no effect on 1 min uptake. Each value represents the mean of 10 individual experiments. Typical variability is shown by the standard error of the mean. Uptake was not corrected for diffusion.

All compounds used were of the highest purity offered. [*Me*-^{14}C]Choline chloride, DL-[*Me*-^{14}C]carnitine and [thiazole 2-^{14}C]thiamine were obtained from Amersham/Searle. [*Me*-^{14}C]Betaine, *N*,*N*-dimethyl [1-^{14}C]glycine, [1-^{14}C]sarcosine and [*carboxyl*-^{14}C]inulin were purchased from New England Nuclear.

RESULTS AND DISCUSSION

Fig. 1 shows the rate of choline uptake as a function of choline concentration. As noted in a number of other systems[1,7,8,10,19,24], the data indicate that there are two processes involved in choline uptake, one a mediated mechanism which is saturated at relatively low choline concentration, the other, a simple diffusion mechanism. Further evidence in support of this two-component mechanism is the observation that 2 mM hemicholinium-3, a choline analog[25], abolishes the mediated component and the system shows diffusion kinetics over the entire concentration range. In normal Krebs–Henseleit buffer (Na^+ concentration 142 mM), the diffusion coefficient was found to be $0.09 \cdot 10^{-3}$/min. The mediated component was obtained by subtracting the diffusion process from the total uptake. Fig. 2 shows a Lineweaver–Burk plot of the saturable portion of choline uptake. In normal buffer the apparent K_m is 110 μM and the V is 25 nmoles/ml tissue water per min. The magnitude of this apparent Michaelis constant is nearly the same as that found for hamster intestine[19], giant axon of *Loligo*[24], mouse cerebral cortex[8], guinea pig synaptosomes[5] and rat brain synapto-

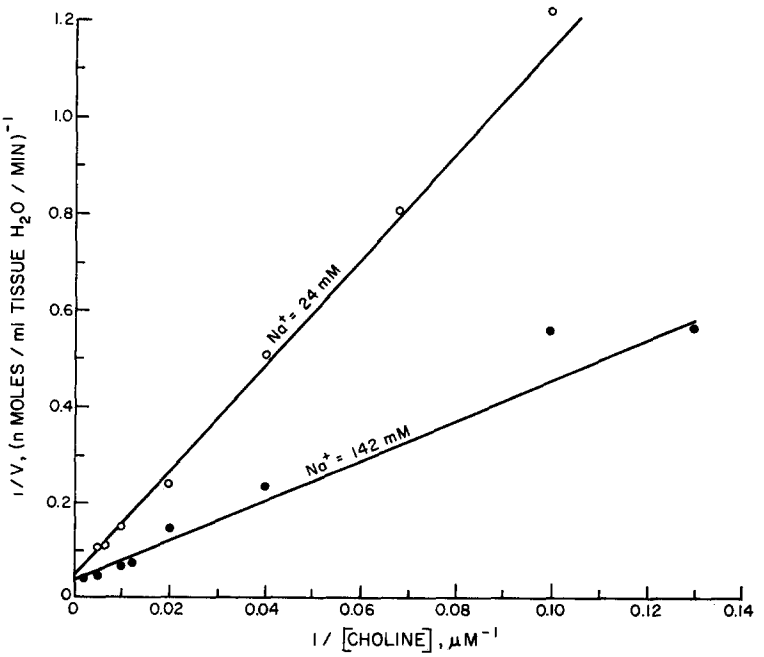


Fig. 2. Lineweaver–Burk plot of choline uptake in the presence of 142 mM Na^+ and 24 mM Na^+. The experimental conditions were the same as described in Fig. 1. The data have been corrected for diffusion. Each point represents the mean of 10 individual experiments.

somes[6]. Recently, Yamamura and Snyder[20] described the occurrence of a second choline receptor in rat brain synaptosomes which has an apparent K_m of 1 μM.

The rate of choline uptake in the presence of 24 mM Na^+ is reduced compared to the Na^+-replete condition (Fig. 1). This reduction is due to, in part, a decreased diffusion coefficient ($0.058 \cdot 10^{-3}$/min). The low Na^+ concentration was attained by the isotonic replacement of the NaCl of Krebs–Henseleit buffer with L-lysine·HCl which has no effect on choline uptake (Table I). Lysine·HCl was used, rather than mannitol, to retain a normal Cl^- concentration in the incubation medium. The non-diffusional uptake of choline under conditions of Na^+ depletion conforms to Michaelis–Menten kinetics with an apparent K_m of 220 μM and a V of 20 nmoles/ml tissue water per min. This V is not significantly different from the V obtained under Na^+-replete conditions. The change in K_m between these conditions may reflect a requirement for Na^+ in choline binding to the carrier. The difference in affinity also may be due to structural changes in the membrane caused by the reduced Na^+ concentration. Evidence for such structural changes is the decreased diffusion coefficient.

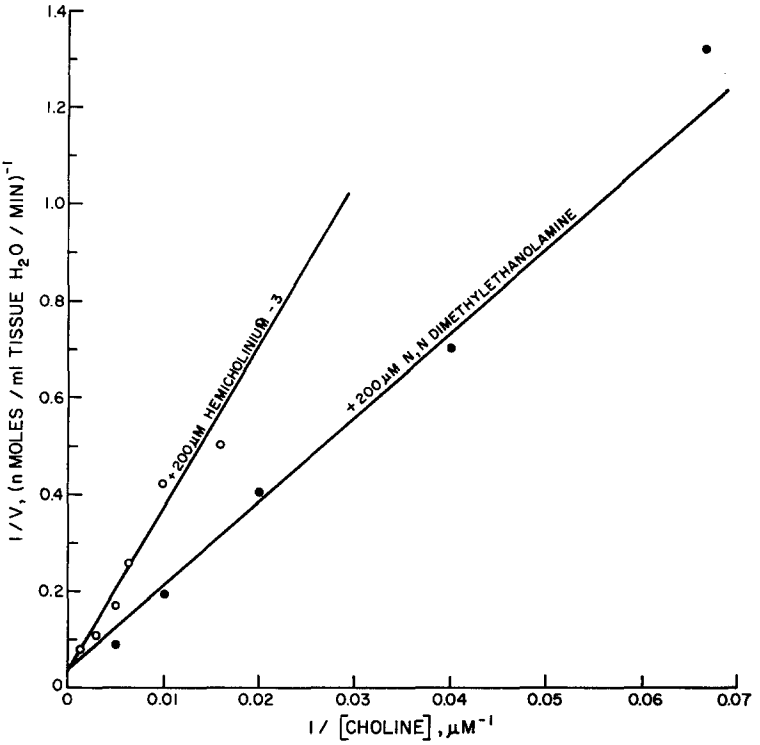
TABLE I

THE EFFECT OF VARIOUS COMPOUNDS ON THE UPTAKE OF 0.05 mM CHOLINE BY INTESTINAL SEGMENTS

Intestinal segments were incubated 1 min in Krebs–Henseleit buffer containing 0.05 mM choline and the indicated compounds. Except where noted the inhibitor concentration was 5 mM. Each value represents the mean ±S.E. of 10 individual experiments. The percent inhibition was found from the fraction uptake by paired samples.

Inhibitor	*Inhibition (%)*
Hemicholinium-3	100.0± 4.4
N,N-Dimethylethanolamine	100.0± 9.1
Atropine	83.0± 3.2
(+)-Tubocurarine	78.0± 6.6
Decamethonium bromide	77.7± 3.2
Thiamine	76.6± 3.2
Eserine	53.6± 8.3
Tetramethylammonium chloride	44.9± 5.1
Carbachol	39.3± 8.3
Ethanolamine	31.8± 9.1
Tetraethylammonium chloride	29.5± 7.6
Mannitol, 236 mM	10.7± 4.8
Betaine	9 2± 6.2
Hexamethonium bromide	8.3±10.7
N,N-Dimethylglycine	7.7± 9.2
L-Homoarginine	5.4± 5.4
L-Lysine·HCl, 118 mM	4.8± 3.6
DL-Carnitine, 10 mM	2.9± 4.6
L-Serine	1.1± 7.5
Sarcosine	0.2± 4.5
3-*O*-Methylglucose*	−13.0± 7.6
NaBr	−19.2±11.5

* Buffer for experimental and control experiment did not contain glucose.



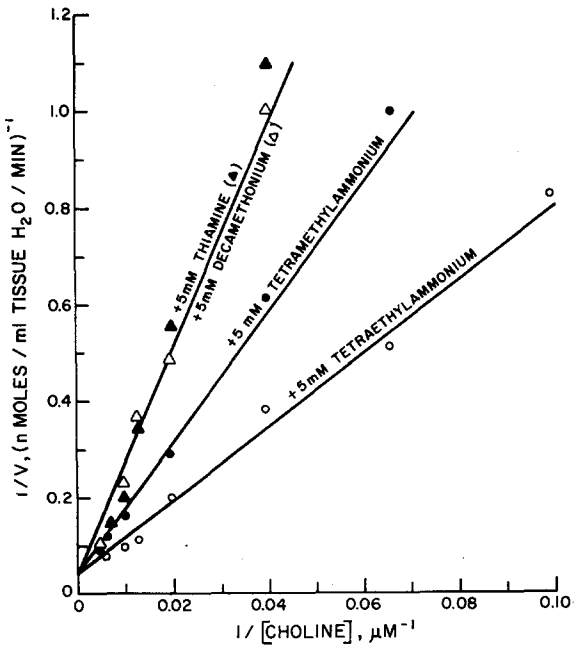


Fig. 3. Lineweaver–Burk plot of choline uptake in the presence of 200 μM hemicholinium-3 and 200 μM *N,N*-dimethylethanolamine. Experimental conditions as in Fig. 2.

Fig. 4. Lineweaver–Burk plot of choline uptake in the presence of 5 mM tetraethylammonium chloride, 5 mM tetramethylammonium chloride, 5 mM thiamine and 5 mM decamethonium bromide. Experimental conditions as in Fig. 2.

In terms of comparative physiology, Sung and Johnstone[12] found choline entry to be Na^+ dependent in rat kidney cortex slices and Yamamura and Snyder[20] have reported Na^+ dependence of choline uptake in guinea pig ileum. No requirement, however, was observed in hamster small intestine[19] or in the human red blood cell[1].

Table I illustrates the effect of a number of compounds on choline uptake. The strongest inhibitors possess either a quaternary or a tertiary nitrogen atom. As seen in Figs 3 and 4, the inhibitors appear to be of the competitive type with hemicholinium-3 and *N,N*-dimethylethanolamine being much more effective than the others. The relative effectiveness of the test inhibitors is similar to that found for the red blood cell[3] and for rat kidney slices[13]. Of special interest is the influence of thiamine on choline. In this respect, Rennick[14] demonstrated that the *in vivo* active excretion of choline by the chicken kidney is antagonized by thiamine, as was found by Sung and Johnstone[13] for the uptake of choline in rat kidney.

The presence of a carboxyl group in choline analogs abolishes their reactivity in choline absorption as evidenced by the lack of inhibition by betaine, carnitine, sarcosine and *N,N*-dimethylglycine (Table I). This finding is supported by investigations which showed that no interaction occurs between betaine and choline in rat kidney[13] or in hamster intestine[26] and is corroborated by data in Table II which illustrate that choline has no influence on the uptake of these analogs. Furthermore, the transport of choline does not appear to occur by pathways which are shared with either sugars or amino acids.

TABLE II

INHIBITION OF 0.05 mM ANALOG UPTAKE BY 5 mM CHOLINE

Experimental conditions are given in the text. Values for percent inhibition of uptake were found from the fraction uptake by paired samples. Each value represents the mean ± S.E. of 10 individual experiments. The uptake values used to calculate the percent inhibition were not corrected for diffusion.

Substrate	*Inhibition* (%)
Betaine	−4.6± 5.8
N,N-Dimethylglycine	−1.3± 6.7
Sarcosine	0.7± 6.4
DL-Carnitine	2.1±11.4
Thiamine	47.6± 4.8

Rindi and Ventura[27] have proposed that two processes, one active, the other passive, are involved in the absorption of thiamine from rat intestine. The velocity *versus* substrate concentration curve (not shown) suggests that a two component mechanism functions in the absorption of thiamine from chicken intestine as well, with a diffusion coefficient of $0.052 \cdot 10^{-3}$/min. Lineweaver–Burk plots for the saturable component of thiamine uptake, alone, and in the presence of choline are shown in Fig. 5. The apparent Michaelis constant is 75 μM and the V is 5 nmoles/ml tissue water per min. The observation that the inhibitory effect of choline is of the competitive type indicates that choline and thiamine interact at a common site. It should be

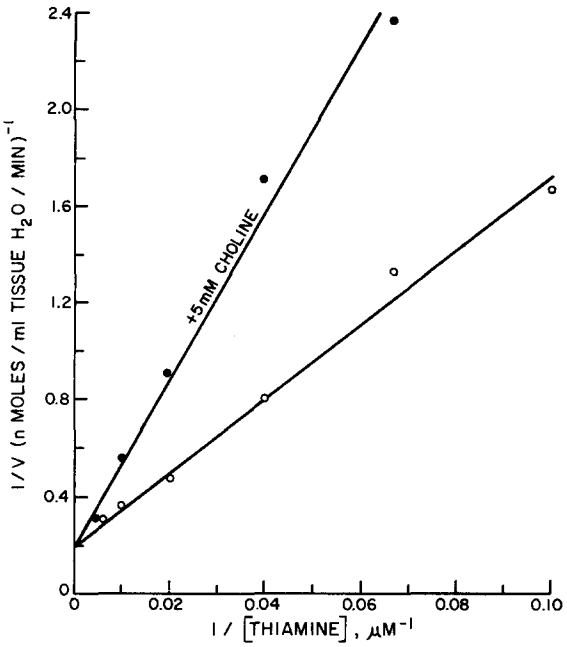


Fig. 5. Lineweaver–Burk plot of thiamine uptake in the presence and absence of 5 mM choline. Experimental conditions as in Fig. 2.

TABLE III

THE UPTAKE OF 0.05 mM CHOLINE FOLLOWING PREINCUBATION WITH VARIOUS ANALOGS

The intestinal segments were preincubated 1 min in the indicated medium. The segments were then removed, rinsed with 3 ml of Krebs–Henseleit buffer at 37 °C and reincubated in 5 ml of buffer containing 0.05 mM choline. In select experiments the second incubation was in 25 ml of buffer containing 0.05 mM choline. Each value represents the mean ±S.E. from 10 individual experiments. The percent of control values were obtained by dividing the average uptake of choline in the experimental condition by the average control uptake.

Preincubation media	*Incubation media*	*Choline uptake*	
		nmoles/ml tissue water per min	*Control (%)*
Control (no addition)	5 ml 0.05 mM choline	5.53 ± 0.63	—
	25 ml 0.05 mM choline	5.48 ± 0.36	—
1 mM choline	5 ml 0.05 mM choline	5.98 ± 0.56	108.1
	25 ml 0.05 mM choline	5.30 ± 0.48	96.7
1 mM hemicholinium-3	5 ml 0.05 mM choline	2.27 ± 0.56	41.0
	25 ml 0.05 mM choline	1.66 ± 0.35	30.0
1 mM *N,N*-dimethylethanolamine	5 ml 0.05 mM choline	3.68 ± 0.61	66.5
	25 ml 0.05 mM choline	2.47 ± 0.32	45.1
Control (no addition)	5 ml 0.05 mM choline	6.27 ± 0.34	—
1 mM betaine	5 ml 0.05 mM choline	6.50 ± 0.45	103.7
1 mM decamethonium bromide	5 ml 0.05 mM choline	5.95 ± 0.55	94.9
1 mM tetramethylammonium chloride	5 ml 0.05 mM choline	5.97 ± 0.47	95.2

noted, however, that there probably is not a single common mechanism for choline and thiamine entry, because the reciprocal inhibitory effects of choline and thiamine are much weaker than predicted on the basis of their respective transport affinities.

In Table III the effects of preloaded choline analogs on choline influx are demonstrated. Hemicholinium-3 and *N,N*-dimethylethanolamine, the most potent *cis*-inhibitors of choline uptake, elicit *trans*-inhibition of choline absorption. In select experiments, a 25-ml incubation volume was used to rule out external competition effects due to efflux of the preloaded substance. Choline, betaine, decamethonium and tetramethylammonium cause no *trans*-effects.

TABLE IV

THE EFFECT OF OLIGOMYCIN, OUABAIN AND ANAEROBIOSIS ON THE UPTAKE OF 0.05 mM CHOLINE

The intestinal segments were preincubated 15 min in Krebs–Henseleit buffer with the indicated additions. The segments were then reincubated for 1 min in the indicated incubation media. Each value represents the mean ± S.E. of 10 individual paired experiments.

Preincubation media	*Incubation media*	*Uptake (nmoles/ml tissue water per min)*
Control (no addition)	0.05 mM choline	7.32 ± 0.71
Under N_2–CO_2, no glucose	0.05 mM choline, under N_2–CO_2, no glucose	7.41 ± 0.72
10 μg/ml oligomycin	0.05 mM choline	7.11 ± 0.80
0.5 mM ouabain	0.05 mM choline	7.32 ± 0.71
No preincubation	0.05 mM choline	8.19 ± 0.43
No preincubation	0.05 mM choline + 10 μg/ml oligomycin	8.07 ± 0.34
No preincubation	0.05 mM choline + 0.5 mM ouabain	8.31 ± 0.21

Choline influx is not sensitive to anaerobic conditions, ouabain, or oligomycin (Table IV). These results show that the initial uptake of choline is not dependent on oxidative metabolism nor on the (Na^+–K^+)-ATPase[28–30]. Because a 15-min preincubation in ouabain or under N_2 greatly reduces the Na^+ gradient[31], choline influx would appear to be independent of the intracellular Na^+ concentration. This finding, the absence of a *trans*-choline effect on choline influx, and the fact that choline entry in the presence of 24 mM Na^+ occurs by way of a mechanism kinetically similar to that involved when the Na^+ concentration is 142 mM (with no change in V) suggests that the uptake of this substrate takes place by a mechanism similar to that proposed by Curran *et al.*[32] for alanine entry into rabbit ileum. Our results, moreover, are inconsistent with the model of Kimmich[31], which predicts that choline influx should be immediately inhibited by ouabain and oligomycin.

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