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EVIDENCE FOR THE EXISTENCE OF SEPARATE TRANSPORT MECHANISMS FOR CHOLINE AND BETAINE IN RAT KIDNEY

CHENG PO SUNG* AND R. M. JOHNSTONE
Department of Biochemistry, McGill University, Montreal, P Q. (Canada)
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SUMMARY

A small number of quaternary nitrogen compounds, namely tetraethylammonium, thiamine, benzoylcholine and the hemicholinium, HC-3 [4,4'-biphenylylene bis-(2-oxoethylene)] bis-[(2-hydroxyethyl) dimethyl bromide] inhibit the oxidation of choline to betaine in slices of rat kidney cortex, but are ineffective in kidney homogenates. These compounds do not interfere with the energy and sodium ion-dependent accumulation of betaine by rat kidney cortex slices. It is concluded that both choline and betaine are transported by rat kidney cortex slices and that the transport systems are independent of each other.

INTRODUCTION

In recent years evidence for choline transport across plasma membranes has been obtained in a variety of systems such as avian kidney[1–4], erythrocytes[5,6], squid axon[7], heart muscle[8], preparations of brain tissue[9–12] and mammalian kidney[13,14].

In several instances such as red blood cells[5,6], squid axon[7] and mouse brain slices[12] it has been demonstrated that choline movement may occur against its concentration gradient. In other systems quoted above, the evidence for a transport mechanism has been inferred from the behaviour of drugs, such as the hemicholinium, HC-3 [4,4'-biphenylylene bis-(2-oxoethylene)] bis-[(2-hydroxyethyl) dimethyl bromide][15] and other quaternary nitrogen compounds on the excretion or the transformation of choline by the tissue in question.

With rat kidney cortex slices, incubated with choline, no free choline is found accumulating in the tissue under aerobic conditions[14], the main product accumulating in both tissue and medium being betaine[14]. Since it has been reported that betaine may also undergo active transport in mammalian intestine by a mechanism shared by N-substituted amino acid derivatives[16], we investigated the possibility that in rat kidney cortex slices, choline is taken up by a mechanism common to betaine and other N-substituted amino acids. Our earlier observations[14] suggested that choline may be transported independently of betaine and our present findings confirm and extend these observations.

* Present address: Department of Pharmacology, University of Wisconsin, Madison, Wisc., U.S.A.

MATERIALS AND METHODS

The preparation of tissue slices, tissue homogenates and the conditions of incubation were carried out as previously described[14]. Unless otherwise stated, tissue slices were incubated at 37° in a Krebs–Ringer phosphate medium containing 3 mM sodium pyruvate with pure oxygen as the gas phase. Homogenates were prepared in 0.25 M sucrose incubated in a phosphate buffer (pH 7.4) as described before[14].

Betaine was separated from choline using Dowex-50 resin (H^+) and identified chromatographically as before[14]. After incubation with [*Me*-^{14}C]betaine only a single component was found in the tissue extracts and the medium which co-chromatographed with betaine in two solvent systems during paper chromatography and behaved like betaine on a Dowex-50 resin with respect to adsorption and elution[14]. To estimate the intracellular to extracellular ratio of [*Me*-^{14}C]betaine and other amino acids, the procedure outlined by KIPNIS AND PARRISH[17] was followed.

[*Me*-^{14}C]Choline was obtained from the Radio Chemical Centre, Amersham, England. [*Me*-^{14}C]Betaine and other ^{14}C-labelled amino acids were obtained from New England Nuclear Corp., Boston, Mass., U.S.A.

RESULTS

We have previously reported that the oxidation of choline to betaine in rat kidney cortex slices is inhibited by dinitrophenol and the absence of Na^+, neither of which interferes with choline oxidation to betaine in homogenates of kidney tissue. Data of this type led us to conclude that the rate of choline oxidation in rat kidney slices is limited by an energy and Na^+-dependent transport system[14]. To provide additional evidence for a transport mechanism, we now examined a variety of choline analogues and inhibitors of choline oxidation to establish whether certain compounds affect choline oxidation to betaine in tissue slices without affecting the oxidation in homogenates. Since no free choline is found in the tissue, the rate-limiting step in choline oxidation in slices is clearly the rate of uptake of choline, regardless of the mechanism. If a choline-transport system exists, certain choline-like substances would be expected to interfere specifically with the transport of choline which would be reflected in a decreased oxidation of choline in slices but not in homogenates.

The data in Table I show that of the 19 compounds examined, 4 (benzoylcholine, tetraethylammonium, thiamine and hemicholinium) interfere with betaine formation from choline in slices but not in homogenates. Of the four mentioned, three (tetraethylammonium, thiamine and hemicholinium) have been reported by other investigators to inhibit choline transport in other systems using different approaches to study transport[2–9,18].

Strong evidence for a carrier-mediated active transport system is obtained if it can be established that the movement of the substance in question is against its concentration gradient. In kidney slices the movement of choline is usually along the concentration gradient because conversion of choline to betaine is rapid, no free choline being found in the tissue. By using a variety of amines[19], we attempted to suppress choline oxidation, hoping thereby to determine whether choline movement can occur against its concentration gradient in rat kidney slices. The data in Table I show that choline oxidation to betaine in homogenates is inhibited by several amines

TABLE I

CONVERSION OF CHOLINE TO BETAINE IN RAT KIDNEY CORTEX SLICES AND HOMOGENATES

Conditions of incubation. Tissue slices were incubated in a Krebs–Ringer medium with O_2 as the gas phase for 60 min. Sodium pyruvate, 3 mM, was present throughout. 1 ml of a 10% homogenate prepared in 0.25 M sucrose was used. The buffer was 10 mM sodium phosphate (pH 7.4) [*Me*-^{14}C]Choline chloride, specific activity, 1200 counts/min per nmole at a concentration of 0.2 mM was used. The incubation volume was 3 ml. The control values for choline oxidation in tissue slices were 450 ± 20 S.D. nmoles/100 mg wet weight tissue per h and in the homogenate 225 ± 20 S.D. nmoles/h. Each value for the inhibitor quoted is based on duplicate determinations of at least two separate experiments (4 values) where the variation from the mean value was never greater than ± 5%.

Additions	*Inhibition (%) of choline conversion to betaine*	
	Slices	*Homogenates*
Glycine (4 mM)	Nil	—
L-Arginine (4 mM)	Nil	—
L-Aspartic acid (4 mM)	Nil	—
Thiamine (0.2 mM)	22	Nil
Betaine (5 mM)	5	23
N,N'-Dimethylglycine (5 mM)	10	22
N-Methylglycine (5 mM)	13	Nil
Succinylcholine chloride (0.5 mM)	Nil	Nil
Propionylcholine iodide (0.5 mM)	5	13
Benzoylcholine chloride (0.5 mM)	75	Nil
Ethanolamine (20 mM)	15	38
Triethanolamine (1 mM)	Nil	Nil
2-Dimethylaminoethanol (1 mM)	20	87
Tyramine hydrochloride (0.5 mM)	Nil	73
Benzedrine sulfate (0.5 mM)	36	84
Phenylephrine hydrochloride (0.5 mM)	22	44
β-Phenylethylamine hydrochloride (0.5 mM)	40	70
Eserine (0 5 mM)	50	20
Carnitine (0 5 mM)	Nil	Nil
Tetraethylammonium bromide (0.5 mM)	67	8
Hemicholinium HC-3 (0.04 mM)	40	Nil

such as ethanolamine, 2-dimethylaminoethanol, tyramine, benzedrine, phenylephrine, and β-phenylethylamine. In kidney slices, however, these compounds are decidedly less inhibitory. Therefore, this approach did not permit us to establish whether choline is transported against its concentration gradient. In passing, it is interesting to note that none of the compounds examined affects choline oxidation to the same extent when examined with homogenates and tissue slices. Some are more effective with slices, the others more effective in homogenates.

Since betaine is the major product of choline oxidation found accumulating in both tissue and medium, we examined whether choline and betaine interfere with each other's uptake. Our earlier observations indicated that the accumulation of [*Me*-^{14}C]-betaine by kidney slices is less inhibited by $5 \cdot 10^{-5}$ M 2,4-dinitrophenol, than is the conversion of [*Me*-^{14}C]choline to betaine[14]. However, with 10^{-4} M 2,4-dinitrophenol, a substantial reduction of accumulation of [*Me*-^{14}C]betaine is observed. The results in Table II also show that Na^+ is required in the incubation medium to obtain an accumulation of [*Me*-^{14}C]betaine. In a normal Krebs–Ringer medium containing 3 mM sodium pyruvate and 0.2 mM [*Me*-^{14}C]betaine, the distribution ratio of

TABLE II

BETAINE UPTAKE BY RAT KIDNEY CORTEX SLICES

Conditions of incubations were as in Table I. Incubation time was 60 min. [*Me*-^{14}C]Betaine specific activity, $8.6 \cdot 10^6$ counts/min per μmole, was used at a concentration of 0.2 mM. Each value given is the mean of at least two separate determinations carried out in duplicate. The variation from the mean value was always less than 10%.

Conditions	*Accumulation of [Me-^{14}C]betaine (counts/min per 100 mg wet wt. of tissue)*	*Concn. ratio* $\frac{\text{counts/min per ml cell water}}{\text{counts/min per ml medium}}$
Control	32 500	2.9
2,4-Dinitrophenol (10^{-4} M)	11 000	1.1
Na^+ free (sucrose Ringer)*	9 400	0.9
Choline (1 mM)	32 400	2.9
Hemicholinium HC-3 (10^{-4} M)	31 200	2.8
Tetraethylammonium bromide (1 mM)	31 000	2.8
Benzoylcholine chloride (1 mM)	26 000	2.3
Succinylcholine chloride (1 mM)	32 300	2.9
Lysine (1 mM)	30 500	2.7
Arginine (1 mM)	25 400	2.3
Carnitine (1 mM)	29 000	2.6
Ethanolamine (1 mM)	30 600	2.7
N,N'-Dimethylglycine (1 mM)	34 300	3.0
N-Methylglycine (1 mM)	28 300	2.5

* In these incubations, the NaCl of the Ringer medium was replaced by isosmolar sucrose, all other components remaining unchanged.

[*Me*-^{14}C]betaine between tissue water and medium is approx. 3.0 after 60-min incubation. When most of Na^+ is replaced by sucrose or when 2,4-dinitrophenol is added, the ratio is reduced to unity (±10%). The energy and Na^+ requirements for betaine accumulation are similar to those reported for the accumulation of a variety of organic compounds against a concentration gradient in a number of mammalian tissues[20–28].

None of the compounds capable of reducing choline oxidation in slices (except possibly benzoylcholine) affects betaine accumulation. It is evident that the effect of benzoylcholine (at 1 mM) on betaine accumulation is much smaller than its effect on choline oxidation (at 0.5 mM) in slices. Of the 11 compounds examined, only arginine and benzoylcholine show a small effect on betaine accumulation when used at a concentration 5 times greater than that of betaine. It is clear that hemicholinium and tetraethylammonium ions at concentrations which markedly reduced choline oxidation in kidney cortex slices have no effect on betaine accumulation.

Many amino acids are known to be transported by kidney slices[29–31]. We therefore examined the possibility that amino acids and choline share a transport system. The amino acids selected for this study were arginine, glycine and aspartic acid. None alters the rate of oxidation of choline by kidney slices (Table I). Moreover, neither choline (at 10–20 times the concentration of the amino acid) nor the hemicholinium affects the uptake of any of the 3 amino acids examined.

DISCUSSION

Choline has three major routes of metabolism. (1) It may be converted to acetylcholine and become a neurotransmitter substance; (2) it may become esterified to

phosphocholine and become a precursor for phospholipid synthesis; and (3) it may become oxidized to betaine and act as a source of methyl groups. This study and those of other workers[1–14,18] provide evidence that in some tissues choline is transported across the cell membrane and that the rate of its transformation to its ultimate metabolic fate is regulated by the rate at which it moves into the cell. The evidence for choline transport in rat kidney slices may be summarized as follows: choline oxidation in homogenates occurs at a rate about 50 % of that observed with slices per unit fresh weight of tissue. The oxidation in homogenates is unaffected by 2,4-dinitrophenol (10^{-4} M), lack of Na^+, presence of hemicholinium (0.08 mM), tetraethylammonium bromide (0.5 mM), benzoylcholine (0.5 mM), and thiamine (0.2 mM). In slices of rat kidney cortex the oxidation of choline to betaine is inhibited by 75 % by 2,4-dinitrophenol ($5 \cdot 10^{-5}$ M) (ref. 14), 40 % by hemicholinium (0.04 mM), 67 % by tetraethylammonium bromide (0.5 mM), 75 % by benzoylcholine (0.5 mM), 48 % in low sodium media (ref. 14) and 22 % by thiamine (0.2 mM).

If the effects of the inhibitors in slices were due to some indirect effect on choline oxidation due to an alteration in the intracellular steady-state conditions, it would be anticipated that homogenization would cause a greater decrease in oxidation than any of the inhibitors. It must be expected that homogenization would cause a much greater disruption of the steady-state conditions than any of the inhibitors added. Such is clearly not the case (see above). The previous[14] and present data are, however, consistent with a transport system for choline.

The specificity of the choline-transport system is highlighted by the fact that those compounds which affect choline uptake appear to act primarily on the latter process and do not markedly affect other aspects of choline metabolism. Thus it has been shown that tetraethylammonium ions and hemicholinium have little effect on choline oxidation (ref. 14, and this paper) choline phosphorylation[14] or acetylcholine synthesis in cell-free preparations[9,11,18] whereas one or both are reported to be effective inhibitors of choline transport in squid axons[7], avian kidney[2,4], brain slices[12], rat kidney cortex slices[14], and red blood cells[5,6]. Thiamine also inhibits choline transport (ref. 3, this paper) and is not known to inhibit other aspects of choline metabolism, such as oxidation. Of the compounds which have been shown to inhibit choline uptake in the present report, only benzoylcholine has not previously been reported to act in this manner in other tissue preparations. Close structural analogues of choline, such as dimethylaminoethanol, betaine and carnitine, do not affect choline transport in rat kidney slices even when used in large excess relative to choline (2.5–25 times excess). Carnitine transport, however, has been reported to be inhibited by both choline and betaine in a microbial system[32].

Evidence for the active transport of betaine in the kidney slices is provided by the facts that betaine is accumulated against its concentration gradient, the process is inhibited by 10^{-4} M 2,4-dinitrophenol, and requires the presence of Na^+. Two lines of evidence indicate that betaine transport occurs independently of choline transport in rat kidney cortex slices. (a) A large excess of betaine (Table I) has no effect on choline oxidation with intact cell preparations, and the presence of choline in excess (Table II) has no effect on betaine accumulation. (b) Compounds such as hemicholinium, tetraethylammonium, and benzoylcholine which reduce choline uptake (50 % or more at the concentrations used) are without appreciable effect on betaine accumulation.

The present report also shows that compounds which affect choline oxidation

in cell-free preparations may be without effect on intact cell preparations. Several potent choline oxidase inhibitors[19] are ineffective in the tissue slices. This indicates that the presence of the plasma membrane limits the rate or extent of penetration of these compounds so that their potential inhibitory action is not apparent.

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