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EFFECT OF CHOLINE ON TETRAETHYLAMMONIUM TRANSPORT IN MOUSE KIDNEY CORTEX SLICES

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Abstract—Choline inhibits the uptake of [^{14}C]tetraethylammonium (TEA) by mouse kidney cortex slices incubated in Krebs–Ringer bicarbonate buffer (37°, pH 7.4)), aerated with O_2–CO_2 95:5 v/v%. Inhibition seemed to be of a competitive type. Stimulation of unidirectional [^{14}C]TEA exit occurred when choline was added to the wash-out medium, and approached a maximum value with increasing choline concentrations. Enhancement of TEA exit by choline could be demonstrated in the presence of high external K^+ concentrations suggesting that it was not secondary to unspecific changes in the membrane potential. Initial [^{14}C]TEA uptake was increased in slices preloaded with choline. This argues against the possibility that choline stimulates exit by displacing [^{14}C]TEA from intracellular binding sites. Preloading the tissue with choline did not reduce relative [^{14}C]TEA exit which indicates that stimulation of initial TEA uptake after choline preloading is due to an increase of TEA influx rather than to an inhibition of TEA efflux. The results suggest that choline and TEA share a common transport mechanism. Transfer of TEA across the membrane in both directions depends on the trans-concentration of choline. The stimulation phenomenon may be an example of accelerative exchange diffusion.

Renal transport *in vivo* and *in vitro* of nitrogenous organic cations is characterized by the occurrence of mutual transport inhibition phenomena among structurally analogous compounds [1]. Enhancement of organic cation transport *in vivo* was recently demonstrated in the chicken kidney where several quaternary amines had biphasic effects on the excretion of ^{14}C-labelled choline and acetylcholine [2–5]. Infusion of organic cations at low loads was thus shown to increase tubular secretion of these labelled compounds whereas infusion of the same organic cations at high loads resulted in an inhibition of the secretion process.

We have previously reported a transport interaction phenomenon of a stimulatory type associated with renal organic cation transport *in vitro* [6–9]. At low external concentrations a number of monoquaternary amines, e.g. choline and tetraethylammonium (TEA) enhanced the uptake of the polymethylene-bis-quaternary amine [^{14}C]decamethonium into mouse and rat kidney cortex slices, whereas the same amines at higher concentrations depressed the uptake. Increase of initial decamethonium uptake could be demonstrated in the absence of monoquaternary amines if the tissue had been preincubated with a monoquaternary amine before transfer to a [^{14}C]-decamethonium containing medium. This finding indicates that stimulation in some way is related to the presence of monoquaternary amines in the tissue. Enhancement of decamethonium uptake was not due to competitive inhibition of decamethonium efflux by intracellularly located monoquaternary amines since no efflux of decamethonium could be demonstrated in wash-out experiments. Hence, unidirectional influx of decamethonium was stimulated by the presence of monoquaternary amines in the tissue.

The purpose of this study was to investigate possible transport interaction phenomena between the monoquaternary amines [^{14}C]TEA and choline in mouse kidney cortex slices. The former compound was chosen as transport substrate since it accumulates in mouse kidney cortex slices without undergoing metabolic transformation [10]. The possibility that any effect of choline on [^{14}C]TEA transport should be secondary to changes in [^{14}C]TEA metabolism can therefore be disregarded.

MATERIALS AND METHODS

Tetraethyl-1-[^{14}C]ammonium bromide specific activity 2.8 mCi/mM) was supplied by New England Nuclear Corp., Boston, MA. Tetraethylammonium and choline were obtained as chloride salts from Merck.

Male albino mice (25 g) of a single strain (NMRI) were received from Moellegaard A/S Scanbur Ejby, Denmark.

Incubation procedure. Cortical slices from mouse kidneys were prepared and used as previously described [6]. Immediately after preparation the slices were placed in Erlenmeyer flasks (3–4 slices weighing 50 mg in each flask), containing ice cold Krebs–Ringer bicarbonate incubation buffer (100 ml) with 11 mM glucose [11]. The tissue was stored 1/2 hr under these conditions until start of incubation.

At start of incubation the flasks were closed with rubber stoppers perforated with polyethylene tubings through which a gas phase consisting of oxygen and carbon dioxide (95:5) was bubbled into incubation medium (pH adjusted to 7.4). The flasks were shaken gently (60 oscillations/min) in a water bath (37°). The tissue was preincubated (30 min) before addition of [^{14}C]tetraethylammonium (TEA). When necessary, choline was added to the medium at the start of preincubation. The external concentration of [^{14}C]TEA was kept constant during the entire incu-

bation period owing to the relatively large volume of buffer. Incubations were terminated by separating tissue from medium on nylon nets. If necessary, slices were carefully transferred to other media by means of a forceps. Otherwise, the tissue was placed in pre-weighed counting vials and weighed (wet weight).

Measurement of radioactivity. Tissue samples digested (48 hr at room temperature) in 1 ml tissue solubilizer 'Soluene-350' (Packard Instrument) and medium samples of 1 ml were completed for β-liquid scintillation counting by addition of 15 ml scintillation fluid 'Dimilume' (Packard Instrument). Both types of samples were counted with identical efficiencies (internal standardization) in a liquid scintillation spectrometer (Beckman 250) using a combined ^{14}C-^{3}H window. Tissue concentrations of [^{14}C]TEA were calculated from cpm/g tissue (post incubation wet weight) using counting rates in the medium (cpm/ml medium) as reference. Addition of [^{14}C]TEA to blank tissue and medium samples gave counting rates similar to those obtained in absence of tissue or medium.

RESULTS

Effect of choline on TEA uptake. Figure 1 shows the time course of TEA uptake at two external TEA concentrations (1 μM and 500 μM) in the absence or presence of 5 mM choline. TEA uptake is markedly inhibited by choline, the inhibition, however, being less pronounced at the high TEA concentration.

Steady-state uptake of TEA was measured at varying external TEA concentrations in the absence (control) or presence of 5 mM choline. Data from these experiments are depicted in a double reciprocal plot (Fig. 2) which shows steady state TEA uptake as a function of the external TEA concentration. The upper line represents experiments with choline addition while the lower one represents control experiments. Both lines seem to converge towards a common intersection point on the ordinate. The inserted plot in Fig. 2 contains data from similar studies dealing with the effect of choline on the initial uptake of TEA at varying external TEA concentrations. Both lines seem to converge towards a common intersection point on the ordinate also in this plot.

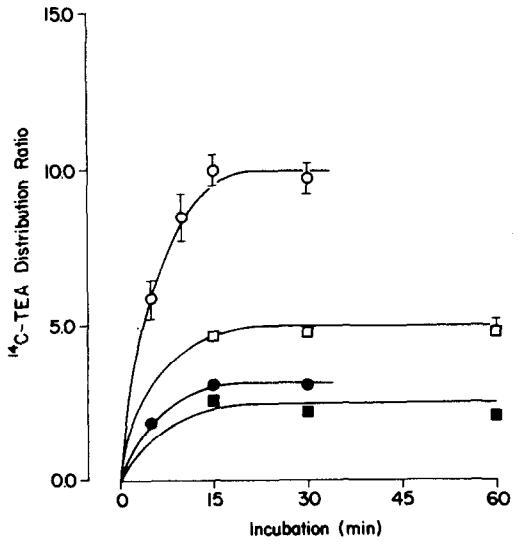

Fig. 1. Effect of choline (5 mM) on time course of [^{14}C]-TEA uptake by mouse kidney cortex slices (^{14}C-tissue-medium distribution ratio = cpm/g tissue/cpm/ml medium). The [^{14}C]TEA concentration of the medium was 1 μM (○) or 500 μM (□). The corresponding filled symbols represent experiments in which choline was added to the medium. Each point is the mean of 5–6 experimental values with S.E.M. (vertical bars for values higher than 0.3).

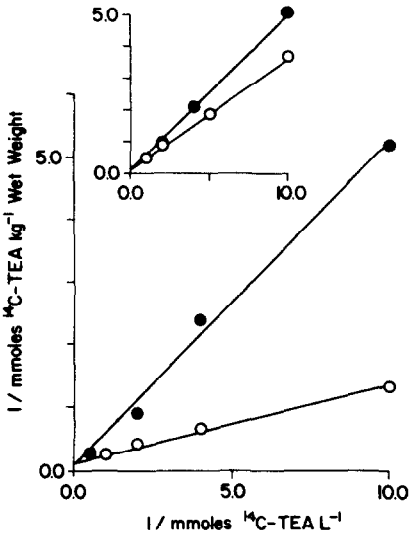

Fig. 2. Double reciprocal Lineweaver–Burk plot of TEA uptake in absence (○) or presence (●) of 5 mM choline. Each symbol is the mean of 3–4 experimental values (S.E.M. within circles). Upper figure shows the initial (5 min) uptake of [^{14}C]TEA while lower figure shows the steady state (30 min) uptake of [^{14}C]TEA.

Effect of external choline on TEA exit. Figure 3 shows time course of [^{14}C]TEA exit in wash-out experiments. Isotope washed out re-entered the tissue in negligible amounts owing to the dilution effect of the large volume of external medium (isotope concentration in the wash-out solution did not exceed background counting). This means that any observed effect on exit cannot be ascribed to changes in a parallel re-uptake of isotope from the wash-out solution. TEA exit was determined under steady-state conditions in control experiments (concentration of unlabelled TEA in the wash-out solution was equimolar to that of labelled TEA in the pre-equilibrating medium) meaning that no net transport of TEA occurred between tissue and medium. The loss of [^{14}C]TEA from the tissue was initially rapid, almost half part of the initial content being washed out during the first 5 min. This was followed by a much slower exit, and 10 per cent of the initial [^{14}C]TEA content was thus still remaining in the tissue after 30 min wash-out. Addition of 5 mM choline to the wash-out solution enhanced [^{14}C]TEA exit, the initial exit, however, being relatively most stimulated.

Table 1 shows the effect of increasing external choline concentrations on [^{14}C]TEA exit (5 min wash-out). Choline at a concentration of 0.5 mM or higher significantly stimulated [^{14}C]TEA exit. Figure 4 shows relative stimulation of [^{14}C]TEA exit depicted as a function of the external choline concentration

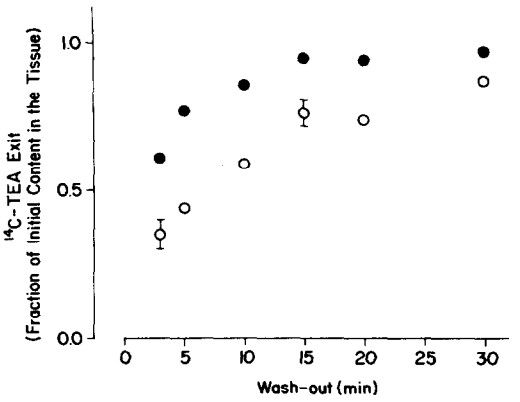

Fig. 3. Time course of [^{14}C]TEA exit from mouse kidney cortex slices in the presence (●) or absence (○) of external choline (5 mM). Tissue from each animal was pre-equilibrated (30 min) with 1 μM [^{14}C]TEA. Part of the tissue was removed for ^{14}C measurements while the remainder was divided into a control group (transferred to a choline-free wash-out medium) and an experimental group (transferred to a wash-out medium with choline). The concentration of unlabelled TEA in both wash-out media was 1 μM. At times indicated tissue slices were removed and ^{14}C-activity determined. [^{14}C]TEA exit was expressed as a fraction of the initial [^{14}C]TEA content per mass unit tissue according to the formula: $C_0 - C_t/C_0$, where C_0 represents the initial [^{14}C]TEA content after pre-equilibration and C_t the [^{14}C]TEA content after wash-out. Each point is the mean of 5–8 experimental values with S.E.M. (vertical bars for values higher than 0.03).

(data from Table 1). The curve seems to conform to a Michaelis–Menten hyperbola approaching a maximum value around 70 per cent at choline concentrations above 1 mM.

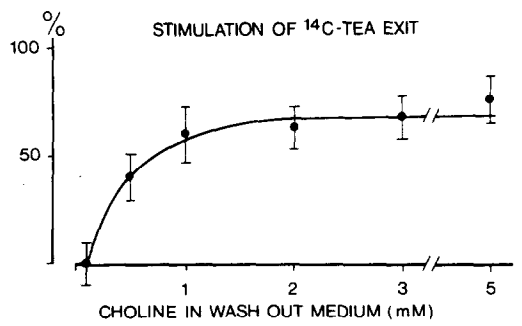

Fig. 4. Percentage stimulation of [^{14}C]TEA exit (5 min) from mouse kidney cortex slices plotted as a function of the choline concentration in the wash-out medium (paired comparisons). Data from Table 1.

Initial TEA uptake and exit in slices preloaded with choline. Experiments were performed to investigate the effect of choline preloading on [^{14}C]TEA uptake. Table 2 shows that initial uptake (5 min) of 1 μM [^{14}C]TEA was stimulated in slices that had been preincubated with choline (0.5 and 5 mM) prior to [^{14}C]TEA exposure.

Table 3 shows the results of experiments in which slices were preincubated with [^{14}C]TEA (1 μM) in the absence or presence of 0.5 mM choline. Relative [^{14}C]TEA exit (5 min wash-out) was the same whether or not the tissue had been preincubated with choline.

Effect of external choline on TEA exit in high K^+ solutions. Figure 5 shows the time course of [^{14}C]TEA exit in wash-out solutions containing high K^+ concentrations (125 mM). When comparing control data from Fig. 3 (○) to those of Fig. 5 (□) it

Table 1. Effect of external choline on [^{14}C]TEA exit (5 min wash-out) from mouse kidney cortex slices

Choline conc. (mM)	No. of exp.	[^{14}C]TEA exit (fraction of initial content)		Difference (%)
		Control	With choline	
0.1	8	0.46 ± 0.03	0.46 ± 0.05	+1 ± 10
0.5	9	0.44 ± 0.03	0.56 ± 0.04	+40 ± 11, P < 0.01
1.0	7	0.42 ± 0.03	0.65 ± 0.03	+60 ± 13, P < 0.005
2.0	8	0.45 ± 0.04	0.71 ± 0.02	+63 ± 10, P < 0.001
3.0	6	0.44 ± 0.03	0.72 ± 0.02	+68 ± 10, P < 0.005
5.0	6	0.44 ± 0.02	0.77 ± 0.01	+76 ± 11, P < 0.001

Results are mean values ± S.E.M. Student's *t* test on paired differences.
For details cf. legend to Fig. 3.

Table 2. Initial uptake of [^{14}C]TEA by mouse kidney cortex slices preloaded with choline

Conc. of choline (mM) in preloading medium	[^{14}C]TEA tissue-medium distribution ratio after 5 min incubation		Difference (%)
	Control	Preloaded with choline	
0.5	4.4 ± 0.2	6.0 ± 0.2	+39 ± 6, P < 0.001
5.0	4.6 ± 0.4	5.8 ± 0.4	+30 ± 5, P < 0.001

Slices from each animal were divided in two groups which were incubated 30 min with (preloaded) or without (control) choline before transfer to another medium containing 1 μM [^{14}C]TEA but not choline. Results are mean values ± S.E.M. (n = 9). Student's *t* test on paired differences.

Table 3. [^{14}C]TEA exit (5 min wash-out) from mouse kidney cortex slices preloaded with choline

[^{14}C]TEA exit (fraction of initial content)		
Control	Preloaded with choline	Difference (%)
0.44 ± 0.03	0.42 ± 0.04	0 ± 11

Slices from each animal were divided in two groups which were pre-equilibrated with 1 μM [^{14}C]TEA in the absence (control) or presence of choline (0.5 mM). Both groups were thereafter subjected to a procedure similar to that described for the control experiments in legend of Fig. 3. Results are mean values ± S.E.M. (n = 8). Student's t test on paired differences.

is seen that high external K^+ concentrations *per se* stimulate TEA exit. Addition of 5 mM choline to the wash-out solutions gave, however, rise to a further increase of TEA exit (Fig. 5). The stimulatory effect of choline on [^{14}C]TEA exit in high K^+ solutions was highest initially: 32 ± 7 per cent ($P < 0.005$, n = 8) and 19 ± 3 per cent ($P < 0.001$, n = 8) after 3 and 5 min wash-out respectively (data from Fig. 5).

DISCUSSION

Choline inhibits the uptake of [^{14}C]TEA by mouse kidney cortex slices (Fig. 1), and the inhibition seems to be of a competitive type (Fig. 2). Addition of choline to the wash-out solution increased the unidirectional exit of [^{14}C]TEA (Fig. 3), and the curve relating relative stimulation of [^{14}C]TEA exit to the external choline concentration conformed to a Michaelis–Menten hyperbola (Fig. 4). Enhancement of TEA exit in the presence of external choline may be due to an increased permeability of the cell membrane to TEA or to a rise in the intracellular level of freely diffusible TEA. The intracellular concentration of diffusible TEA would increase if choline displaced TEA from intracellular binding sites. According to the latter hypothesis a decrease of TEA uptake was to be anticipated in slices where these binding sites were pre-occupied by choline. However, the finding that initial TEA uptake was actually stimulated in slices preloaded with choline disagrees with this idea (Table 2). The increase of initial TEA uptake in choline preloaded slices may result from a decrease of TEA efflux (e.g. choline and TEA competing for a common efflux mechanism) or from an actual increase of TEA influx. The latter possibility seems to be the more likely one in view of the unchanged [^{14}C]TEA exit from tissue preequilibrated in the presence of choline (Table 3). The lack of any effect of intracellular choline on TEA exit is somewhat puzzling in view of the competitive inhibition of TEA uptake by extracellular choline. This might, however, be the result of an asymmetric affinity of choline for the transport system, i.e. that choline has a much lower affinity (lower inhibitory potency) for the intracellular than for the extracellular side of the transport system.

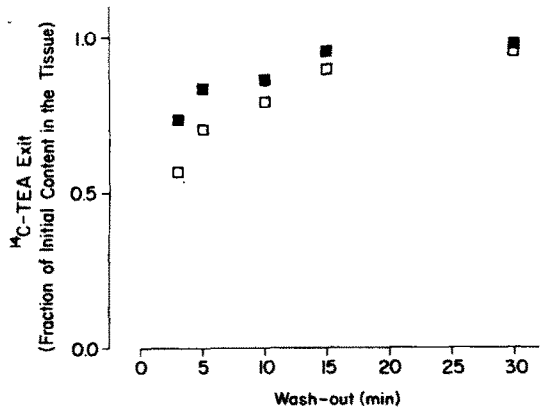

Fig. 5. [^{14}C]TEA exit from mouse kidney cortex slices into high K^+ media (120 mM Na^+ replaced by equimolar K^+) in the presence (■) or absence (□) of 5 mM external choline. Tissue from each animal was pre-equilibrated in a standard medium (30 min) with 1 μM [^{14}C]TEA. Part of the tissue was removed for ^{14}C measurements while the remainder was divided into a control group (transferred to a choline-free high K^+ medium) and an experimental group (transferred to a high K^+ medium with choline). For further details cf. Fig. 3.

At high concentrations, i.e. 100 mM choline was shown to be just as effective as K^+ in depolarizing the membrane potential of amphibian kidney slices, whereas choline at a low concentration, i.e., 5 mM had no effect [12]. The external choline concentrations which stimulated TEA exit in the present study were not exceeding 5 mM. It is, therefore, unlikely that stimulation of TEA exit should be secondary to a depolarization of the membrane potential produced by external choline. The observation that external choline enhanced TEA exit even in high K^+ solutions where the tissue is depolarized does also agree with this assumption (Fig. 5).

The present data suggest that the monoquaternary amines choline and TEA share a common transport mechanism in mouse kidney cortex cells. The transport of TEA across the cell membrane depends on the transconcentration of choline (trans-side: the side towards which the transport is directed). There is a resemblance between the present trans-stimulation phenomena and previous observations concerning transport of hexoses and amino acids. Efflux of [^{3}H]glucose from human erythrocytes approached a maximum value with increasing concentrations of unlabeled glucose or galactose in the efflux bath [13]. Preloading mouse ascites carcinoma cells with unlabeled glycine or *N*-methylglycine increased influx of [^{14}C]glycine, whereas addition of *N*-methyl-glycine to the external solution inhibited [^{14}C]glycine uptake [14]. The accelerative exchange diffusion or substrate facilitated transport model advanced to explain the latter phenomena implies that the binding of trans-substrate to the carrier facilitates re-orientation of carrier sites towards the opposite side (*cis*-side) and thereby enhances transport of substrate from *cis* to *trans*-side. On the basis of this model the data presented here suggest that choline influx increases TEA efflux while choline efflux increases TEA influx.

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