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TRANSPORT AND EXCHANGE OF TETRAETHYLAMMONIUM IN MOUSE KIDNEY CORTEX SLICES

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Abstract—Transport of ^{14}C-tetraethylammonium (TEA) was studied in mouse kidney cortex slices incubated in Krebs–Ringer bicarbonate buffer (37°, pH 7.4) aerated with O_2–CO_2, 95:5 v/v per cent. Unidirectional exit of ^{14}C-TEA was stimulated when unlabeled TEA was added to the wash-out medium. Stimulation reached a maximum value (50 per cent) at an external TEA concentration of 0.5 mM. Increasing inhibition of initial uptake of 1 μM ^{14}C-TEA was seen in slices preincubated with 0.1–5 mM TEA. However, initial uptake of 0.5 mM ^{14}C-TEA was stimulated in slices preincubated with the same concentration of unlabelled TEA. The phenomenon that ^{14}C-TEA under certain conditions is able to move faster across the membrane when TEA is present on the opposite side (*trans*-side) may resemble accelerative exchange diffusion. ^{14}C-TEA uptake was competitively inhibited by *d*-tubocurarine, decamethonium and physostigmine, which suggests that the latter organic cations and TEA share a common transport mechanism. ^{14}C-TEA exit was stimulated when *d*-tubocurarine was added to the wash-out medium, whereas addition of decamethonium or physostigmine at the concentrations tested had no effect.

Organic cations (quaternary amines) are known to be secreted in the proximal tubules of the kidney and to accumulate in kidney cortex slices [1]. These *in vivo* and *in vitro* transport processes are characterized by saturability and occurrence of mutual inhibition phenomena among structural analogues [1]. However, recent studies summarized below have shown that transport of organic cations in the kidney exhibits another important feature: transport stimulation in the presence of other organic cations.

(1) Quaternary amines had biphasic effects on the excretion of ^{14}C-labeled choline and acetylcholine in the chicken kidney. Infusion of organic cations at low loads was thus shown to increase tubular secretion of these labeled compounds, whereas the same organic cations infused at high loads depressed the secretion process [2–5].

(2) Several monoquaternary amines, e.g. choline and tetraethylammonium (TEA) stimulated influx of the polymethylene–bisquaternary amine ^{14}C-decamethonium into mouse and rat kidney slices. However, at higher external concentrations the same monoquaternary amines acted as inhibitors of influx. The enhancement of initial ^{14}C-decamethonium influx in slices preloaded with monoquaternary amines suggested a relationship between the stimulation phenomenon and the intracellular localization of monoquaternary amines [6–9].

(3) Choline inhibited ^{14}C-TEA uptake by mouse kidney cortex slices in a competitive manner. The inward and outward transport of ^{14}C-TEA in the tissue was moreover stimulated when choline was present on the *trans*-side, i.e. the side towards which transport is directed [10].

The main purpose of the present study was to establish whether *trans*-concentration of TEA affects inward and outward movements of ^{14}C-TEA in mouse kidney cortex slices. Experiments dealing with the effects of different types of organic cations on ^{14}C-TEA transport were moreover included in the study. No transport effect observed in the present study can be ascribed to changes in cellular metabolism of ^{14}C-TEA since this compound was shown to accumulate in mouse kidney slices without undergoing metabolic transformation [11].

MATERIALS AND METHODS

Tetraethyl-1-^{14}C ammonium bromide (sp. act. 2.8 mCi/mM) was supplied by New England Nuclear Corp., Boston, MA, U.S.A. The following unlabeled drugs were used: Tetraethylammonium chloride (Merck), decamethonium diiodide (Koch–Light), *d*-tubocurarine dichloride (Koch–Light) and physostigmine salicylate (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 [12]. The tissue was stored for 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 O_2–CO_2 (95:5) was bubbled into incubation medium (pH adjusted to 7.4). The flasks were shaken gently, 60 oscillations/min in a water bath at 37°. The tissue was preincubated for 30 min before addition of

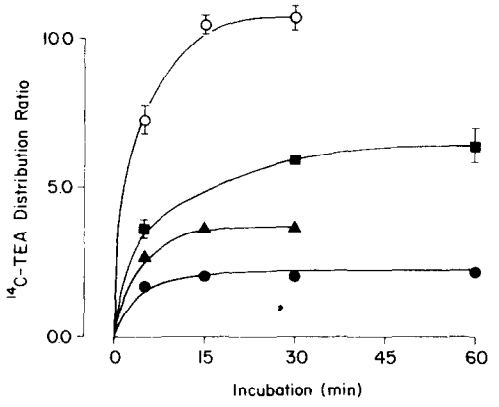

Fig. 1. Effect of amines on time course of ^{14}C-TEA uptake by mouse kidney cortex slices (^{14}C-tissue-medium distribution ratio) = (cpm/g tissue)/(cpm/ml medium).

The ^{14}C-TEA concentration of the medium was 1 μM. ○, (control in absence of amines), ● (0.23 mM *d*-tubocurarine), ▲ (0.2 mM physostigmine), ■ (1 mM decamethonium). Each point is the mean of five to six experimental values with S.E.M. (vertical bars for values higher than 0.3).

^{14}C-tetraethylammonium (TEA). When necessary, other drugs were added to the medium at the start of preincubation (Figs 1 and 2). The external concentration of ^{14}C-TEA was kept constant during the entire incubation 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 preweighed counting vials and weighed (wet wt).

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 wt) 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.

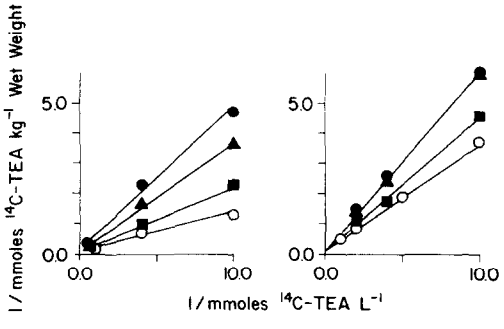

Fig. 2. Double reciprocal Lineweaver–Burk plot of ^{14}C-TEA uptake in absence (○) or presence of amines (filled symbols with the same meaning as in Fig. 1). Each symbol is the mean of three to four experimental values (S.E.M. within circles). Steady state (30 min) uptake of ^{14}C-TEA (left). Initial (5 min) uptake of ^{14}C-TEA (right).

RESULTS

Effect of external TEA on ^{14}C-TEA exit from slices. The effect of increasing external TEA concentrations on ^{14}C-TEA exit (5 min wash-out) from slices pre-equilibrated in the presence of 1 μM ^{14}C-TEA was studied (Table 1a). Isotope washed out reentered 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 back flux of isotope into the tissue. Table 1a shows that external TEA at a concentration of 0.1 mM or higher significantly stimulates unidirectional ^{14}C-TEA exit and that percentage stimulation of ^{14}C-TEA exit reaches a maximum value around 50 per cent at a TEA concentration of 0.5 mM. The experiments in Table 1a were performed under non steady state* conditions: decreasing tissue levels of TEA in control experiments (isotope exit represents a net loss of TEA from the tissue) and increasing levels in experiments where the wash-out media contained TEA (isotope was replaced by TEA taken up from an external concentration severalfold higher than that of ^{14}C-TEA in the pre-equilibrating medium).

Table 1b shows experiments where tissue preequilibrated (30 min) to steady state in the presence of 0.5 mM ^{14}C-TEA was washed out either in absence of TEA or in the presence of unlabelled TEA at a concentration (0.5 mM) equimolar to that of ^{14}C-TEA in the pre-equilibrating medium†. From Table 1b it is seen that the tissue has lost 35 per cent of its ^{14}C-TEA content after 5 min wash-out in absence of external TEA (non steady state conditions since isotope exit represents a net loss of TEA from tissue in absence of external TEA). However, the loss of ^{14}C-TEA increased to 50 per cent when TEA was present in the wash-out solution at a concentration of 0.5 mM which is equimolar to that of ^{14}C-TEA in the pre-equilibrating medium (steady state conditions since TEA influx from the medium counter-balances ^{14}C-TEA efflux i.e. the total TEA content in the tissue remains constant†).

Initial ^{14}C-TEA uptake by slices preloaded with TEA. Experiments were performed to investigate

* *Steady state* describes a situation where the TEA concentration (labeled and/or unlabeled) of the tissue after incubation at a given external TEA concentration has reached a final maximum level and remains constant upon further incubation (net transport of TEA into the tissue does not occur any more since efflux balances influx). *Non steady* state describes a situation where the TEA concentration of the tissue is changing.

† Preliminary experiments showed that a steady state level of ^{14}C-TEA was attained within 30 min in slices incubated in the presence of 0.5 mM ^{14}C-TEA. Transfer of tissue at that time to another medium also containing 0.5 mM ^{14}C-TEA did not produce any change in the steady state level.

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

	TEA conc. (mM)	No. of exp.	^{14}C-TEA exit (fraction of initial content) Control	With TEA	Difference (%)
a	0.1	5	0.46 ± 0.02	0.55 ± 0.03	+ 19 ± 6, P < 0.05
	0.5	6	0.41 ± 0.02	0.63 ± 0.01	+ 54 ± 8, P < 0.005
	1.0	10	0.44 ± 0.04	0.63 ± 0.02	+ 53 ± 12, P < 0.005
	5.0	5	0.46 ± 0.03	0.69 ± 0.05	+ 52 ± 9, P < 0.005
b	0.5	9	0.35 ± 0.02	0.05 ± 0.03	+ 43 ± 7, P < 0.001

Tissue from each animal was pre-equilibrated (30 min) with ^{14}C-TEA (a: 1 μM; b: 0.5 mM). Part of the tissue was removed for ^{14}C-measurements while the remainder was divided into a control group (transferred to a washout medium without TEA) and an experimental group (transferred to a wash-out medium containing the indicated concentration of TEA). Tissue slices were removed after 5 min wash-out 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: $C_o - C_t/C_o$, where C_o represents the initial ^{14}C-TEA content after pre-equilibration and C_t the ^{14}C-content after wash-out. Results are mean values ± S.E.M. Student's 't' test on paired differences.

the effect of TEA preloading on ^{14}C-TEA uptake. Increasing inhibition of the initial uptake of 1 μM ^{14}C-TEA (5 min) was seen in slices which prior to ^{14}C-TEA exposure were preincubated with increasing concentrations (0.1–5 mM) of TEA (Table 2a). These experiments were just as those of Table 1a; performed under non steady state conditions: increasing tissue concentrations of TEA in control experiments (isotope uptake represents a net transport of TEA into tissue preincubated in the absence of TEA) and decreasing concentrations in preloading experiments (tissue was pre-equilibrated with TEA concentrations many times higher than those of ^{14}C-TEA in the final incubation media).

Uptake experiments analogous to the exit experiments in Table 1b were performed with 0.5 mM ^{14}C-TEA (Table 2b). The results show a slight, but statistically significant increase of initial ^{14}C-TEA uptake at steady state (^{14}C-TEA influx counterbalances efflux from tissue pre-equilibrated with 0.5 mM TEA) as compared to the non steady state situation (isotope uptake represents a net transport of TEA into tissue not pre-equilibrated with TEA).

Effect of d*-tubocurarine physostigmine and decamethonium on* ^{14}C*-TEA uptake and exit.* Figure 1 shows the time course of ^{14}C-TEA uptake at an external ^{14}C-TEA concentration of 1 μM in the absence (control) or presence of other amines. The ^{14}C-TEA uptake is inhibited when other amines are present. The tertiary amine physostigmine (0.2 mM) and the bisquaternary amine *d*-tubocurarine (0.2 mM) seem to be almost equally potent inhibitors while the polymethylene–bisquaternary amine decamethonium (1 mM) is far less potent. Other experiments not presented showed relatively less inhibition of uptake in the presence of amines at high (0.5 mM) external TEA concentrations.

Steady state uptake of ^{14}C-TEA was measured at varying external ^{14}C-TEA concentrations in the absence (control) or presence of other amines. Data from these xperiments are depicted in a double reciprocal plot (Fig. 2, left) which shows steady state ^{14}C-TEA uptake as a function of the external ^{14}C-TEA concentration. The upper lines represent experiments with addition of amines, while the lower one represents control experiments. All lines seem to converge towards a common intersection point on the ordinate. Figure 2, right contains data from similar studies dealing with the effect of amines on the initial uptake of ^{14}C-TEA at varying external ^{14}C-TEA concentrations. All lines seem to converge towards a common intersection point on the ordinate also in this plot.

Table 3 shows the effect of external amines on ^{14}C-TEA exit. Neither physostigmine (0.2 mM) nor decamethonium (1 mM) had any effect on exit, whereas *d*-tubocurarine (0.2 mM) acted as a very potent stimulator.

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

	Conc. of TEA (mM) in preloading medium	No. of exp.	^{14}C-TEA tissue-medium distribution ratio* after 5 min incubation Control	Preloaded with TEA	Difference (%)
a	0.1	8	5.5 ± 0.3	5.3 ± 0.3	−1 ± 4
	0.5	9	5.0 ± 0.2	4.5 ± 0.2	−10 ± 6
	1.0	9	5.6 ± 0.3	4.8 ± 0.3	−14 ± 5, P < 0.05
	5.0	12	5.5 ± 0.2	4.0 ± 0.2	−26 ± 2, P < 0.001
b	0.5	9	2.4 ± 0.1	2.7 ± 0.1	+11 ± 3, P < 0.01

Slices from each animal were divided in two groups which were incubated 30 min with (preloaded) or without (control) TEA before transfer to another medium containing ^{14}C-TEA (a: 1 μM; b: 0.5 mM). Results are mean values ± S.E.M. Student's 't' test on paired differences.

* cpm/g tissue/cpm/ml medium.

Table 3. Effect of external *d*-tubocurarine, physostigmine and decamethonium on ^{14}C-TEA exit (5 min wash-out) from mouse kidney cortex slices

Drug	^{14}C-TEA exit (fraction of initial content) Control	With drug	Difference (%)
d-Tubocurarine (0.2 mM)	0.42 ± 0.04	0.63 ± 0.05	+ 66 ± 14, $P < 0.01$
Physostigmine (0.2 mM)	0.47 ± 0.03	0.49 ± 0.02	+ 5 ± 9
Decamethonium (1 mM)	0.43 ± 0.04	0.41 ± 0.04	− 3 ± 11

For details, cf. text to Table 1. Following pre-equilibration with ^{14}C-TEA (1 μM) tissue from each animal was transferred to wash-out solutions with or without (control) addition of drugs. Results are mean values + S.E.M. ($N = 6$). Student's '*t*' test on paired differences.

DISCUSSION

Addition of unlabeled TEA to the wash-out solution increased unidirectional exit of ^{14}C-TEA (Table 1). Maximum stimulation of exit was 50 per cent, which is a somewhat lower value than that (70 per cent) produced by external choline [10]. The initial uptake of 0.5 mM ^{14}C-TEA was stimulated in slices preloaded with equimolar TEA (Table 2b). Enhancement of isotope uptake in the latter experiments could either result from inhibition of isotope back flux to medium (TEA present in the preloaded tissue competes with ^{14}C-TEA for a common efflux mechanism) or from increase of isotope influx. The latter possibility seems to be the more likely one in view of the stimulated isotope exit from tissue with rising levels of unlabeled TEA (Table 1). Other results (Table 2a) showing depression of the initial uptake of 1 μM ^{14}C-TEA in slices preloaded with 0.1–5 mM TEA are contradictory to those of Table 2b. From the present data it is not possible to offer any definite explanation to the discrepancy between the results in Table 2a and Table 2b. However, one might suggest the hypothesis that TEA which in the experiments of Table 2a—in contrast to those of Table 2b, initially must be present in the extracellular fluid of the preloaded tissue at a concentration many times higher than that of ^{14}C-TEA (1 μM) competes with the latter, for transport into the cells. The initial uptake of ^{14}C-TEA is stimulated in slices preloaded with a choline concentration (0.5 or 5 mM) many times higher than that (1 μM) of ^{14}C-TEA [10]. However, this does not necessarily argue against the hypothesis since previous studies indicate that choline is a much weaker inhibitor of organic cation transport than is TEA, e.g. a pronounced inhibition (62 per cent) of ^{14}C-carbamoylcholine uptake by mouse kidney cortex slices occurred in the presence of 10^{-4} M TEA whereas no inhibition was seen with equimolar choline [8, 13].

Trans-stimulation phenomena, i.e. the transport of a substrate is stimulated by substrates present on the opposite side (*trans*-side) of the membrane have been extensively reviewed in the literature [14, 15]. Two different models have been offered to explain the phenomenon. The model of accelerated counterflow (accelerative exchange diffusion) implies that the loaded carrier moves faster than the empty one and that the flux is limited by the rate at which the carrier returns to the *cis*-side (the side from which the flux derives). The flux would be accelerated if the carrier could return loaded after exchanging the first substrate, (A) for another one, (B) from the *trans*-side. The model of competitive counterflow postulates that *trans*-stimulation is primarily due to a competitive inhibition on the *trans*-side meaning that the *trans*-stimulating substrate B inhibits back flux of the transported substrate A. Only the net flux (flux–back flux), not the unidirectional flux, is stimulated, whereas the accelerative exchange model implies a genuine stimulation of the unidirectional flux. The present experiments show a stimulation of the outward and in some cases the inward movement of ^{14}C-TEA when TEA is present on the *trans*-side. As discussed, several observations support the view that this is an acceleration of the unidirectional flux of ^{14}C-TEA. Hence, the data seem to resemble previous results from studies on transport of hexoses and amino acids interpreted as accelerative exchange diffusion. For example unidirectional efflux of [^{3}H]glucose from human erythrocytes approached a maximum value with increasing concentrations of glucose or galactose in the efflux bath, and preloading mouse ascites carcinoma cells with unlabeled glycine or *N*-methylglycine increased unidirectional influx of ^{14}C-glycine, whereas addition of *N*-methylglycine to the external solution inhibited ^{14}C-glycine uptake [16, 17].

The amines *d*-tubocurarine, physostigmine and decamethonium inhibit ^{14}C-TEA uptake by mouse kidney cortex slices (Fig. 1), and the inhibition seems to be of a competitive type (Fig. 2). These results suggest that TEA and the latter amines share a common transport mechanism. Added to the wash-out solutions at concentrations which inhibited ^{14}C-TEA uptake *d*-tubocurarine stimulated ^{14}C-TEA exit, whereas physostigmine and decamethonium did not (Table 3). These results do not exclude the possibility that the latter two drugs added at higher concentrations may stimulate exit, but at least they indicate that the dose–response relationship of exit stimulation differs from that of inhibition.

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