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# SHORT COMMUNICATIONS

## Effect of sodium and active sodium extrusion on quaternary amine uptake by mouse kidney cortex slices

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The naturally occurring organic cation choline and a great number of cationic drugs (quaternary and tertiary amines) are excreted by tubular secretion in the kidney [1]. The kidney cortex preparation has proven a suitable model *in vitro* for studying the cellular transport processes underlying the secretion since intracellular accumulation of organic cations in this tissue preparation displays phenomena, e.g. saturability and substrate interaction, which also characterize tubular transport *in vivo* [1]. The present study has been undertaken to establish whether $Na^+$ and active $Na^+$–$K^+$ exchange are factors of importance for accumulation of quaternary amines in kidney cortex cells. This problem was considered of interest since it has become evident that the $Na^+$-gradient across the cell membrane is of crucial importance for the capability of many cells to transport and accumulate a large number of organic solutes, e.g. amino acids and glucose [2].

Two transport substrates were used for the study. The monoquaternary amine [tetraethyl-1-$C^{14}$]ammonium bromide (TEA) from New England Nuclear Corp., Boston, U.S.A. (sp. act. 2.8 mCi/mM) and the polymethylene-bis-quaternary amine [methyl-$^{14}$C]decamethonium dibromide ($C_{10}$) from Radiochemical Centre, Amersham, England (sp. act. 15 mCi/mM). Paper chromatographic studies have previously shown that both $^{14}$C-labelled amines accumulate in mouse kidney cortex slices without undergoing metabolic transformation [3, 4]. Male albino mice (strain NMRI) weighing about 25 g were decapitated and exsanguinated. Kidney cortex slices were prepared and used as previously described [4]. Tissue from each animal (wet weight 50 mg) was immersed in a volume of 100 ml incubation buffer. Incubations were carried out in solutions of Krebs–Ringer bicarbonate buffer (37°, pH 7.4) with glucose (11 mM) under aeration with 95% $O_2$ and 5% $CO_2$. $^{14}$C-labelled amines were added to incubation media following a preincubation period of 10 min. At the end of the incubation slices were separated from media by filtration on nylon nets. Immediately after the separation procedure the tissue was transferred to previously weighed counting vials and weighed (wet wt). Tissue samples digested in 1 ml Soluene (Packard) and medium samples of 1 ml were completed for $\beta$-liquid scintillation counting by addition of 15 ml scintillation medium, Dimilume (Packard). Both types of samples were counted with identical efficiencies (internal standardization) in a liquid scintillation spectrometer (Beckman 250). Tissue concentration were calculated from cpm/g tissue (post incubation wet wt) using counting rates in the medium as reference. Addition of $^{14}$C-labelled TEA and $C_{10}$ to blank tissue and medium samples gave counting rates similar to those obtained in absence of tissue or medium.

Figure 1 shows the time course of TEA ($10^{-6}$ M) uptake by mouse kidney cortex slices incubated in solutions of varying ionic composition. TEA uptake increases rapidly towards a steady-state level around 10 nmole/g tissue when incubations are carried out under control conditions (145 mM $Na^+$ and 5 mM $K^+$). Initial as well as steady-state uptake values are halved in media where $Na^+$ has been substituted by equimolar $Li^+$ or isoosmotic sucrose. From the results it is obvious that a high $Li^+$ concentration in itself does not markedly impair TEA uptake since the effect is almost the same whatever $Li^+$ or sucrose are used as substitutes for $Na^+$. Substitution of $Na^+$ by $K^+$ (150 mM) leads to an almost complete depression of TEA uptake meaning that high $K^+$ concentrations *per se* have deleterious actions on uptake. Omission of $K^+$ (normally 5 mM) halves initial as well as steady-state uptake values. Additional experiments showed that neither $Li^+$ nor $Na^+$ (5 mM) were able to replace $K^+$, whereas this turned out to be the case with $Rb^+$. Figure 2 shows the time course of $C_{10}$ ($0.5 \times 10^{-6}$ M) uptake by mouse kidney cortex slices incubated in solutions of varying ionic composition. In contrast to the monoquaternary amine TEA (Figure 1) $C_{10}$ uptake increases almost linearly with time without reaching steady state within 1 hr incubation (control experiments in Figure 2). The time course of $C_{10}$ uptake in media where $Na^+$ had been replaced by sucrose (25 mM $K^+$ and isoosmotic sucrose replacing 125 mM NaCl) seems to be indistinguishable from that seen under control conditions meaning that $C_{10}$ uptake does not require the presence of $Na^+$ in the extracellular fluid. However, substitution of $Na^+$ (125 mM) by $Li^+$ or $K^+$ instead of isoosmotic sucrose leads to inhibition of $C_{10}$ uptake the effect being most pronounced with $K^+$ where a steady-state level as low as 1.5 nmole/g tissue is reached within a period of 30 min (Fig. 2). These results show that $C_{10}$ uptake is markedly impaired in the presence of high concentrations of $Li^+$* and especially $K^+$. The effect of $K^+$

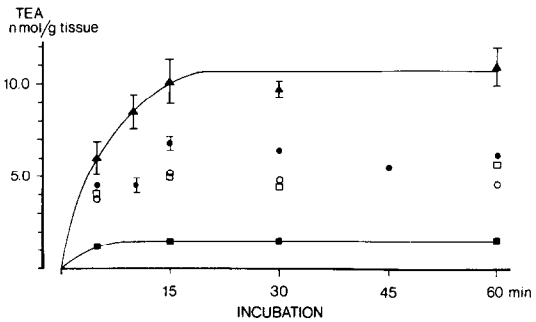


Fig. 1. Time course of [$^{14}$C]tetraethylammonium (TEA) uptake by mouse kidney cortex slices incubated in electrolyte media of varying composition. Concentration of TEA in external medium was $10^{-6}$ M. ▲ Control (5 mM $K^+$, 145 mM $Na^+$) or 25 mM $K^+$ and 125 mM $Na^+$, ○ (25 mM $K^+$, 125 mM $Li^+$), ● (25 mM $K^+$, isoosmotic sucrose replacing 125 mM NaCl), □ ($K^+$-free saline), ■ (150 mM $K^+$). Each symbol represents the mean with S.E.M. (vertical bars indicate values higher than 0.5 nmole/g tissue) of 3 experimental values (6–8 values in control experiments).

*$C_{10}$ uptake was depressed by $72 \pm 1$ per cent ($P < 0.001$; mean value $\pm$ S.E.M. of 9 paired comparison experiments) when substituting $Na^+$ by $Li^+$ instead of sucrose (25 mM $K^+$ plus $Li^+$ or sucrose).



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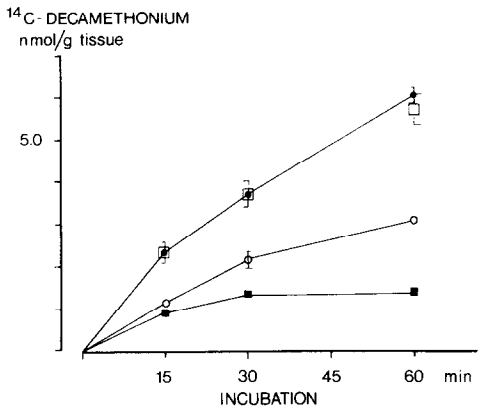


Fig. 2. Time course of $^{14}$C-decamethonium ($C_{10}$) uptake by mouse kidney cortex slices incubated in electrolyte media of varying composition. Concentration of $C_{10}$ in external medium was $0.5 \times 10^{-6}$ M. ● Control (5 mM $K^+$, 145 mM $Na^+$), □ (25 mM $K^+$ and isoosmotic sucrose replacing 125 mM $Na^+$), ○ (25 mM $K^+$, 125 mM $Li^+$) ■ (150 mM $K^+$). Each symbol represents the mean with S.E.M. of 3 experimental values (vertical bars for values higher than 0.5 nmole/g tissue).

omission on $C_{10}$ uptake was evaluated in experiments of paired comparison type [5]: tissue from each animal was incubated simultaneously under control and no-control (without $K^+$) conditions. The results demonstrated a statistically [5] ($P < 0.001$) significant depression of 1-hr $C_{10}$ uptake by 37$^+$-2 per cent in absence of $K^+$ (Mean ± S.E.M., N = 6). Normal uptake values were, however, obtained when $K^+$ was replaced by $Rb^+$, whereas Na was unable to replace $K^+$,

Active $Na^+$ extrusion in kidney cortex slices is markedly impaired in the absence of extracellular $K^+$ [6]. From studies on active $Na^+$ extrusion in erythrocytes it is known that $Rb^+$ can replace $K^+$ in the active $Na^+$–$K^+$ exchange [7]. The diminished capability of mouse kidney cortex slices to accumulate quaternary amines in the absence of these ions is therefore consistent with the assumption that part of the uptake process depends upon the function of the $Na^+$ pump. This concept finds additional support in the observation that $10^{-3}$ M g-strophantine (Merck), a potent inhibitor of active $Na^+$ extrusion in kidney slices [8], depresses TEA uptake (30 min) by 34 ± 2 per cent ($P < 0.001$) and $C_{10}$ uptake (1 hr) by 21 ± 5 per cent ($P < 0.01$; mean values ± S.E.M. from experiments of paired comparison type, N = 6).

Whittembury *et al.* (1961) reported a total depolarization of the electrical potential in the amphibian kidney slice (cell negativity 60 mV) in the presence of high $K^+$ concentrations [9]. Hence, it is possible that the observed effect of high $K^+$ concentrations results from a reduction of the electrical gradient which normally favours intracellular accumulation of the positively charged TEA and $C_{10}$ ions. The well-known toxic and swelling effect of high $K^+$ solutions on tissues should, however, also be taken into consideration as a possible mechanism.

Part of TEA uptake depends upon the presence of extracellular $Na^+$, whereas this is not the case with $C_{10}$ uptake. Furthermore, a high $Li^+$ concentration *per se* has a deleterious effect on $C_{10}$ uptake, at least in the absence of $Na^+$, whereas there seems to be no other effect of $Li^+$ on TEA uptake than that which can be accounted for by omission of $Na^+$.

It is a widely accepted hypothesis that $Na^+$ acts as a co-substrate in transport of many organic solutes thereby increasing affinity between substrate and transport site ('carrier') [2]. According to this theory the asymmetric distribution of $Na^+$ between the intracellular and extracellular spaces favours intracellular accumulation of the substrate (influx is stimulated by a high extracellular $Na^+$ concentration and efflux is similarly depressed by a low intracellular $Na^+$ concentration). A mechanism of this type may explain the influence of extra- and intracellular $Na^+$ concentrations on TEA uptake. The lack of any effect of external $Na^+$ on $C_{10}$ uptake indicates that such a mechanism cannot be active in $C_{10}$ transport. $C_{10}$ accumulation is, however, depressed when active $Na^+$ extrusion is inhibited. The latter observation is difficult to interpret but it might indicate a more specific relationship between $C_{10}$ uptake and the $Na^+$ pump, e.g. via $Na^+$-$K^+$ ATPase or that $C_{10}$ uptake in some other manner depends on the intracellular $Na^+/K^+$ concentrations. Previous *in vivo* and *in vitro* observations demonstrated on one hand large differences in the renal handling of monoquaternary and polymethylene-bisquaternary amines and on the other hand mutual substrate interaction phenomena between these two drug types during their renal transport [10]. In view of these results it seems justified to assume the existence of partially different transport mechanisms for these two quaternary amine types, however, with involvement of common steps. The observed difference between the monoquaternary amine TEA and the polymethylene-bisquaternary amine $C_{10}$ concerning requirement of external $Na^+$ for transport brings further support to this notion.

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