ATP-stimulated Tetraethylammonium Transport by Rabbit Renal Brush Border Membrane Vesicles*

T. Dwight McKinney† and Melanie A. Hosford
From the Nephrology Section, Department of Medicine and Medical Service, Veterans Affairs Medical Center, Indiana University Medical Center, Indianapolis, Indiana 46202-5116

These studies examined the ability of ATP to stimulate transport of the organic cation tetraethylammonium (TEA) into proximal tubular brush border membrane vesicles. ATP markedly enhanced TEA uptake, whereas other nucleotides did not. ATP-stimulated TEA uptake was saturable, temperature-dependent, and markedly reduced by the organic cations amiloride, quinidine, cimetidine, and verapamil, but only modestly reduced by the organic cations N'-methylnicotinamide and choline. Some inhibitors of other transport ATPases, including N-ethylmaleimide, N,N'-dicyclohexylcarbodiimide, and oligomycin, reduced the effect of ATP, whereas ouabain, vanadate, and azide did not. 4,4'-Dioctyloxacytatostilbene-2,2'-disulfonic acid also reduced TEA uptake in the presence of ATP. Vinblastine, but not actinomycin D and colchicine (all inhibitors of P-glycoprotein-mediated transport), reduced TEA uptake. The reduction of TEA transport by amiloride and cimetidine was most consistent with competitive inhibition, whereas the inhibition produced by N-ethylmaleimide and vinblastine evidently was not. ATP also stimulated uptake of N'-methylnicotinamide but not that of vinblastine. These studies have identified a previously unrecognized process by which ATP hydrolysis may directly energize the reabsorption of organic cations from the renal tubule lumen.

Multiple mechanisms have been identified in the renal proximal tubule for the mediated transepithelial transport of organic cations (reviewed in Refs. 1-3). Although net transport is generally in the secretory direction, net reabsorption of organic cations has also been demonstrated (1-4). Specific mechanisms, which might be responsible for organic cation transport across the apical membrane of the proximal tubule, have been discovered from experiments in brush border membrane vesicles (BBMV) prepared from dog, rat, rabbit, and human renal cortex include electroneutral organic cation/H+ exchange, organic cation/organic cation exchange, and conductive organic cation transport (5-17). In addition, there appear to be different classes of transporters in the apical membrane of the proximal tubule which, although they may utilize the same basic mode of transport such as organic cation/H+ exchange, have different substrate profiles (11,17). However, given the proton and/or organic cation gradients that might be expected to exist across the apical cell membrane in intact proximal tubules, whether these two mechanisms can fully account for transport of organic cations across this membrane is not certain (1,3). Additionally, other important mechanisms might be destroyed or otherwise inactivated, or co-factors or energy sources critical in the intact cell might be lost in preparation of the vesicles. The aforementioned mechanisms of organic cation transport represent secondary or tertiary active transport processes (1). For example, the H+ or organic cation gradients needed for operation of an organic cation/H+ exchanger resulting in secretion of the organic cation into the tubule lumen would be established by H+ secretion via Na+/H+ exchange, which is ultimately dependent on Na+/K+-ATPase (18) and by concentration of the organic cation inside the cell by transport across the basolateral membrane. Several substances such as sodium, potassium, calcium, protons, and chemotherapeutic agents may be transported into or out of renal epithelia and/or other cells by processes directly coupled to ATP hydrolysis. In order to determine whether organic cations could be transported across the apical membrane by a primary active process driven by ATP, we evaluated the uptake of tetraethylammonium (TEA) into BBMV prepared from rabbit renal cortex in the presence or absence of ATP and an ATP regenerating system. The results indicate that ATP stimulates uptake of TEA into the vesicles by a saturable, temperature-dependent process that is reduced by several other organic cations and inhibitors of transport ATPases.

MATERIALS AND METHODS

Brush border membrane vesicles were prepared from outer renal cortices of male New Zealand White rabbits by the method of Evers et al. (19) as employed previously (9,10). Vesicles were stored in 100 mM mannitol, 30 mM HEPES, pH 7.4, at -70 °C until used. For uptake studies BBMV were thawed, centrifuged, and resuspended in the desired transport buffer for 90 min.

Except where specifically noted, transport measurements were made at room temperature (23-25 °C) and pH 7.4 utilizing a rapid filtration method as employed previously (9,10,20). The transport buffers contained 10 mM HEPES, pH 7.4, and either 150 mM KCl, dicyclohexylcarbodiimide; [d-o-C2-(5)], 3,3'-diethylxadicarbocyanine iodide; NEM, N-ethylmaleimide; NMN, N'-methylnicotinamide; TEA, tetraethylammonium.
potassium gluconate, NaCl, sodium gluconate, or 300 mM mannitol. ATP stimulated TEA uptake in all these buffers, although when specifically tested, uptake appeared to be greatest in sodium gluconate buffer and least in mannitol buffer (data not shown). We employed the basic ATP regenerating system used by Horio et al. (21) in studies utilizing plasma membrane vesicles prepared from a human epidermoid carcinoma cell line. For uptake experiments 20 μl of BBMV (2 mg of protein/ml) were preincubated for 10 min with 30 μl of the appropriate intravesicular buffer with or without the ATP regenerating system consisting of 10 mM phosphocreatine, 100 μg/ml creatine phosphokinase, 10 mM MgCl₂, and variable amounts of ATP or other nucleotides and any potential inhibitors being studied. Uptake was initiated with the addition of 50 μl of buffer containing 2.5-5.0 μM [3H]TEA (13.5 Ci/mmol), 2.5 μM [3H]NMN (66 Ci/mmol) or [3H]vinblastine (18 Ci/mmol) (all obtained from Amersham Corp.), variable amounts of non-radioactive TEA, and NMN. The specific conditions employed in each experiment are described in the figure legends. Uptake was stopped at the desired time interval by diluting the reaction mixture into 3 ml of ice-cold transport buffer (without ATP or the ATP regenerating system) contained on a filtering manifold (Hoefer Scientific, San Francisco, CA). This mixture was immediately filtered through 0.22-μm Nuclepore polycarbonate filters (Costar Nuclepore Corp., Pleasanton, CA) at 440-550 torr. The filter containing the labeled compound and immediately filtered was subtracted from the experimental values. In each experiment the radioactivity associated with blank filters or with preincubated vesicles exposed to the H-labelled compound and immediately filtered was subtracted from the experimental values. In the trans efflux experiments BBMV were equilibrated for 2 h at room temperature in transport buffer containing [3H]TEA. Thereafter, they were diluted 20-fold into buffer with or without ATP and samples of this mixture removed at selected times and the amount of radioactivity remaining associated with the vesicles determined. In the cis efflux experiments vesicles were preloaded by a freeze-thaw method (22) in a buffer containing 150 mM sodium gluconate, 10 mM Hepes, and 3 mM MgCl₂, pH 7.4. Briefly, a solution containing 6 mg/ml BBMV ± 1.2 mM ATP, 12 mM phosphocreatine, 123 μg/ml creatine phosphokinase, and 5.7 μM [3H]vinblastine, 56 μM [3H]TEA, or no isotope was frozen in liquid nitrogen and then allowed to thaw in ice water for 60 min. At various times ice-cold samples containing 24 μg of protein were diluted 167-fold into buffer at room temperature, filtered, and rinsed as above. In some experiments, acridine orange was used to monitor changes in intravesicular pH (23) and fluorescence of the voltage-sensitive dye (dii-C₂-(5)) was used to detect changes in transvesicular potential difference (24, 25).

Protein retained on the filter was determined by the micro-BCA (bicinchoninic acid) assay (Pierce Chemical Corp.). Sodium metavanadate was obtained from Aldrich. Other chemicals were obtained from Sigma. Actinomycin D, verapamil, DCCD, and vinblastine were dissolved in ethanol; an equal concentration of ethanol was used in the control vesicles in experiments employing these compounds. In each experiment, all conditions were generally evaluated with quadruplicate samples, and a mean of these was calculated for that particular experiment. The results are presented as the mean ± S.E. for individual experiments or groups of similar experiments. Statistical significance was determined utilizing Student's t test for paired observations or analysis of variance followed by testing of means by Fisher's test with p < 0.05 being considered statistically significant.

RESULTS

A typical experiment demonstrating the effect of ATP and the ATP regenerating system compared to the regenerating system alone on TEA uptake is shown in Fig. 1. ATP was associated with a marked increase in TEA uptake compared to the absence of ATP, an effect that persisted for an hour or more. Membrane-associated radioactivity in the presence of ATP was reduced dramatically when vesicles were sonicated persisting for only a few minutes, at most, and with uptakes being linear for only a few seconds (7-10, 13, 16, 26-28).

To determine if TEA uptake occurred in the presence of ATP or other nucleotides and the absence of the regenerating system the experiments shown in Fig. 2 were performed. ATP without the regenerating system stimulated uptake at 30 min but not as much as when used in conjunction with the regenerating system at 2 h. GTP and ITP appeared to be almost

FIG. 1. Time course of TEA uptake. Uptake of 50 μM [3H]TEA was measured in sodium gluconate buffer at the indicated times in the presence of the ATP regenerating system and the presence or absence of 300 μM ATP. The results represent quadruplicate measurements from a representative experiment. * and § represent vesicles from the + ATP and - ATP groups, respectively, which were sonicated at the indicated times.

FIG. 2. Effect of various nucleotides and the ATP regenerating system (RS) on TEA uptake. Uptake of 50 μM [3H]TEA was measured in sodium gluconate buffer at the indicated times in the presence of 300 μM ATP and the ATP regenerating system, in the presence of 1000 μM ATP or other nucleotides and absence of the regenerating system or in the absence of both the regenerating system and nucleotides. The results represent the means of two experiments.
as effective as ATP in stimulating uptake at 30 min, whereas UTP, CTP, ADP, and AMP had little or no effect. At 2 h uptake was similar under all conditions in the absence of the regenerating system, but was higher in the presence of ATP and the regenerating system. In other experiments we observed that adenosine in concentrations up to 1 mM neither stimulated TEA uptake nor inhibited TEA uptake occurring in the presence of ATP (data not shown). Finally, the effect of AMP-PNP, a poorly hydrolyzable analogue of ATP, was examined. As shown in Fig. 3, this compound inhibited the effect of ATP to stimulate TEA uptake, whereas AMP-PNP alone only minimally stimulated TEA uptake.

TEA uptake at 4 min was a function of the ATP concentration in the medium (Fig. 4) with an apparent K_m for ATP around 50 μM in the experiment shown. Comparable results were obtained at 30 min and in other experiments.

To further characterize ATP-stimulated TEA uptake by these vesicles, we examined, in a dose-response fashion, the effect of several compounds that might be potential inhibitors of this process. Compounds were chosen on the basis of their ability to inhibit renal organic cation transport using other model systems or their ability to inhibit other ATP-dependent processes. In these experiments the test substance was added to the extravesicular medium or cis side of the vesicles, i.e. the same side to which TEA was added. Fig. 5 shows the effect of amiloride, a commonly prescribed diuretic and organic cation that is secreted into the urine by the proximal tubules. Amiloride was a potent inhibitor of ATP-stimulated TEA uptake. In concentrations of 50 and 100 μM amiloride reduced uptake to a value similar to that observed in the absence of ATP. Between 1 and 10 μM amiloride inhibited TEA uptake by 50%. Analogous experiments were performed with several other compounds. These results are summarized in Table 1. As in previous experiments evaluating organic cation transport in intact proximal tubules, renal epithelial cells in culture or in proximal tubular brush border membrane vesicles (9, 12, 14, 15, 28–32) the organic cations quinidine, cimetidine, verapamil, and amiloride were potent inhibitors of TEA transport, whereas the organic cation NMN was only a weak inhibitor. DCCD, oligomycin, and the sulfhydryl reagent NEM, inhibitors of other ATPases (see “Discussion”) were also potent inhibitors of ATP-stimulated TEA uptake, whereas ouabain was not. In one experiment sodium azide in a concentration of 5–1000 μM also failed to inhibit TEA transport in the presence of ATP. 4,4′-Diisothiocyanatostilbene-2,2′-disulfonic acid, which is generally used as an inhibitor of anion transport, but which also inhibits vacuolar type H^+−ATPase (33), reduced TEA uptake. Finally, the effects of vinblastine, actinomycin D, and colchicine, inhibitors of ATP-dependent transport occurring via P-glycoprotein (see “Discussion”), were examined. Although vinblastine was a potent inhibitor of TEA uptake, colchicine and actinomycin D were not. Compared to many of the substances examined, choline, an endogenous organic cation that may be both reabsorbed and secreted by proximal tubules, appeared to be a poor inhibitor of ATP-stimulated TEA transport (Fig. 6).

To obtain additional fundamental information about ATP stimulation of TEA transport and about the nature of the inhibition produced by some of the aforementioned compounds, we examined the kinetics of ATP-stimulated TEA uptake and the effect of some of the above inhibitors on kinetic parameters. Fig. 7 demonstrates that TEA uptake is a saturable function of the medium TEA concentration. Uptake in the absence of ATP (curve 3 in panels A and B) was linearly dependent on the [TEA] and was assumed to represent a passive process. When values calculated from the regression parameters describing, curve 3 were subtracted from total uptake (curve 1) the difference (curve 2) was inferred to represent mediated transport. As shown in panel C, the results demonstrated saturation and were well described by a Hanes-Woolf plot with apparent K_m and V_max as indicated. Under control conditions in five different experiments performed in this manner, the mean apparent K_m was 173.8 ± 17.8 μM and the mean apparent V_max was 202.9 ± 36.1 pmol/mg/min. As
Fig. 4. Effect of ATP concentration on TEA uptake. A, uptake of 55 
μM [3H]TEA was measured in KCl buffer at 4 min in the presence of the 
ATP regenerating system and varying concentrations of ATP. The results are 
from a representative experiment. B, Hanes-Woolf plot of the data in panel A. 
The line and corresponding numerical parameters describing it were derived 
from linear regression analysis.

indicated by the Hanes-Woolf plot (panel C), the inhibition 
of ATP-stimulated TEA uptake by amiloride was most con-
sistent with a competitive interaction. Additional experiments 
similar to this were performed with amiloride and selected 
other inhibitors. As shown in Table II, the inhibition of TEA 
transport by amiloride and cimetidine was most consistent 
with a competitive interaction, since the predominant effect 
of these compounds was on apparent \( V_{max} \). These results are, 
perhaps, not surprising as both these compounds are organic 
cations. In contrast to the effect of cimetidine and amiloride, 
the inhibitory effect on TEA transport produced by vinblas-
tine and NEM was predominantly on apparent \( K_m \), with, 
perhaps, some decrease in \( V_{max} \), suggesting a non-competitive 
or uncompetitive interaction (34).

Although many of the compounds which were found to 
inhibit ATP-stimulated TEA uptake are known to be trans-
ported by renal organic cation pathways, we did not determine 
in all instances whether their inhibition resulted from a 
competitive interaction with TEA for transport. To obtain 
additional preliminary information regarding whether two of 
these compounds may be transported in a manner similar to 
TEA, we examined the effect of ATP on uptake of NMN and 
vinblastine. As shown in Fig. 8, NMN membrane-associated 
radioactivity was stimulated by ATP, an effect reduced to the 
same level observed in the absence of ATP by 1 mM TEA.2 
However, ATP in the uptake solution had no effect on mem-
brane-associated vinblastine radioactivity (data not shown).

In addition to the above experiments, we examined the

\[ y = a + bx \\
\]

\[ a = 8.08 \\
\]

\[ b = .158 \\
\]

\[ r = .99 \\
\]

2 We note that the amount of [3H]NMN associated with the vesicles 
in the absence of ATP is higher than that observed for [3H]TEA in 
other experiments. We did not determine whether this might result 
in part from a higher level of binding of NMN to the membranes 
compared to TEA.
FIG. 5. Effect of amiloride on TEA uptake. Uptake of 50 μM [3H]TEA was measured in sodium gluconate buffer at 30 min in the presence of the ATP regenerating system, 300 μM ATP, and varying concentrations of amiloride or in the absence of ATP and amiloride (0 ATP). The data are from three different experiments. *, p < 0.01 compared to control values.

TABLE I
Effect of other compounds on ATP-stimulated TEA uptake
Thirty-minute uptake of 50 μM TEA was measured in Na-gluconate buffer as described in the legend to Fig. 5. The data represent results from two to three different experiments with each compound tested. NI, no inhibition.

<table>
<thead>
<tr>
<th>Compound</th>
<th>Concentration range examined</th>
<th>Concentration causing 50% inhibition of uptake</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>μM</td>
<td></td>
</tr>
<tr>
<td>Quinidine</td>
<td>1–50</td>
<td>&gt;1</td>
</tr>
<tr>
<td>Vinblastine</td>
<td>1–20</td>
<td>&gt;1</td>
</tr>
<tr>
<td>Cimetidine</td>
<td>1–50</td>
<td>&gt;1 &lt; 5</td>
</tr>
<tr>
<td>DCCD</td>
<td>1–50</td>
<td>&gt;1 &lt; 5</td>
</tr>
<tr>
<td>Verapamil</td>
<td>0.5–10</td>
<td>&gt;1 &lt; 5</td>
</tr>
<tr>
<td>Amiloride</td>
<td>1–100</td>
<td>&gt;1 &lt; 10</td>
</tr>
<tr>
<td>NEM</td>
<td>0.5–25</td>
<td>&gt;1 &lt; 10</td>
</tr>
<tr>
<td>Oligomycin</td>
<td>1–25</td>
<td>&gt;6 &lt; 12.5</td>
</tr>
<tr>
<td>DIDS</td>
<td>10–500</td>
<td>&gt;50 &lt; 100</td>
</tr>
<tr>
<td>NMN</td>
<td>250–1000</td>
<td>&gt;250 &lt; 500</td>
</tr>
<tr>
<td>Colchicine</td>
<td>250–1000</td>
<td>&gt;1000</td>
</tr>
<tr>
<td>Actinomycin D</td>
<td>1–30</td>
<td>NI</td>
</tr>
<tr>
<td>Ouabain</td>
<td>10–1000</td>
<td>NI</td>
</tr>
</tbody>
</table>

The preceding results notwithstanding, it is conceivable that in our experiments an H+-ATPase (33, 36–38) might be operative. In the presence of this type of transporter, acidification of the vesicles could occur, which might secondarily increase TEA uptake by TEA/H+ exchange. If this were the case, lowering the buffer concentration of the intra- and extravesicular medium should increase and increasing the buffer concentration should decrease ATP-stimulated TEA uptake. The results shown in Fig. 10 argue against this possibility. Additionally, when acridine orange was used to monitor changes in intravesicular pH, no intravesicular acidification was observed under conditions identical to those used to measure TEA transport (data not shown). Furthermore, although most studies have not identified conductive organic cation transport in renal BBMV (9, 12–14, 26, 39) (in contrast to basolateral membrane vesicles) (14, 28, 40, 41), because two reports suggested that organic cation uptake in these vesicles might have a conductive component (28, 42) we investigated whether ATP produced a transvesicular electrical potential difference as indicated by [diO-C2-(5)] fluorescence. No evidence for such a potential difference could be found (data not shown).

The effect of temperature on ATP-stimulated TEA uptake is shown in Fig. 11. At 4 °C TEA uptake in the presence of ATP was similar to that observed at 25 °C in the absence of ATP. Under both conditions uptake was dramatically less than that occurring at 25 °C in the presence of ATP. To further explore the effect of temperature on ATP-stimulated TEA uptake, we performed kinetic experiments similar to those described in Fig. 7 at temperatures ranging from 15 to 30 °C. These results are shown in the form of an Arrhenius plot (34) in Fig. 12. From these data an activation energy of 14,445 cal/mol was calculated. Between 20–30 °C a Q10 of 2.27 is computed. These results may be compared, for example, with an activation energy of 14,400 cal/mol for NMN and 6500 cal/mol for choline uptake into canine renal BBMV in the absence of an electrochemical gradient (16) and 19,700 cal/mol for TEA uptake in rat renal BBMV over approximately the same temperature range in the absence of an electrochemical gradient (14). These values are also comparable for other ATPases. For example, the activation energy for sarcoplasmic reticulum Ca2+-ATPase varied from 15,000–30,000 cal/mol over a temperature range of 5–37 °C (43). A Q10 of 4.6 was reported for H+-ATPase-dependent amine effect of vanadate, a potent inhibitor of several transport ATPases (see "Discussion") (21, 35, 36). As demonstrated in the experiment depicted in Fig. 9, vanadate, even in a concentration of 1 mM, did not reduce TEA transport. These results were confirmed in other experiments utilizing 100–1000 μM vanadate.
FIG. 6. Effect of choline on ATP-stimulated TEA uptake. Uptake of 50 μM [3H]TEA was measured in sodium gluconate buffer at the indicated times in the presence of the ATP regenerating system and 300 μM ATP and in the presence or absence of 1 mM choline or in the absence of ATP and choline (−ATP). The data are from quadruplicate determinations in one experiment.

FIG. 7. Kinetics of ATP-stimulated TEA uptake and effect of amiloride. Uptake of varying concentrations of [3H]TEA was measured in sodium gluconate buffer at 10 min in the presence of the ATP regenerating system and presence or absence of 300 μM ATP and 75 nM amiloride. A, control. Curve 1 represents uptake in the presence of ATP. Curve 3 represents TEA uptake occurring in the absence of ATP. The line and corresponding numerical parameters describing it were derived from linear regression analysis. Curve 2 represents mediated TEA uptake. These values were obtained by subtracting uptake occurring in the absence of ATP (calculated from the regression parameters describing curve 3) from that in the presence of ATP at each TEA concentration. B, amiloride. Experiments were performed as described in panel A except for the presence of amiloride. C, Hanes-Woolf plot of the data depicted in curve 2 of panels A and B. The lines and corresponding numerical parameters describing them were derived from linear regression analysis. The results represent quadruplicate determinations from one experiment.

TABLE II

Kinetics of inhibition of ATP-stimulated TEA uptake

<table>
<thead>
<tr>
<th>Compound</th>
<th>K,  μM</th>
<th>Vmax  pmol/mg/min</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Experimental</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Amiloride (75 nM)</td>
<td>165</td>
<td>267</td>
</tr>
<tr>
<td></td>
<td>211</td>
<td>196</td>
</tr>
<tr>
<td>Cimetidine (1 μM)</td>
<td>139</td>
<td>187</td>
</tr>
<tr>
<td></td>
<td>257</td>
<td>211</td>
</tr>
<tr>
<td>NEM (5 μM)</td>
<td>158</td>
<td>134</td>
</tr>
<tr>
<td></td>
<td>276</td>
<td>112</td>
</tr>
<tr>
<td>Vinblastine (1 μM)</td>
<td>152</td>
<td>112</td>
</tr>
<tr>
<td></td>
<td>306</td>
<td>104</td>
</tr>
</tbody>
</table>

uptake by chromaffin granules (44). In two experiments, we determined that, in contrast to TEA uptake, external (trans) ATP did not stimulate efflux of TEA from vesicles preloaded with this solute (data not shown). Furthermore, when we used both osmotic shock and freeze-thaw techniques (22, 45) to load vesicles with ATP and [3H]TEA or [3H]vinblastine, we could not demonstrate enhanced efflux of either solute by intravesicular (cis) ATP (data not shown). In freeze-thawed vesicles, however, external ATP stimulated TEA uptake as it did in vesicles not subjected to this maneuver, indicating that the freeze-thaw procedure did not result in permanent disruption of this transport process (data not shown). Finally, ATP addition to the intravesicular space by the freeze-thaw technique resulted in diminished ATP-stimulated TEA uptake by chromaffin granules (44).
Fig. 8. Effect of TEA on ATP-stimulated NMN uptake. Uptake of 50 μM [3H]NMN was measured in sodium gluconate buffer at the indicated times in the presence of the ATP regenerating system and 300 μM ATP and in the presence or absence of 1 mM TEA or in the absence of ATP and TEA (− ATP). The data are from quadruplicate determinations in one experiment.

Fig. 9. Effect of vanadate on ATP-stimulated TEA uptake. Uptake of 50 μM [3H]TEA was measured in sodium gluconate buffer at 30 min in the presence of the ATP regenerating system, 300 μM ATP, and varying concentrations of vanadate. The data are from quadruplicate determinations in one experiment.
ATP-stimulated Renal Organic Cation Transport

FIG. 10. Effect of buffer concentration on ATP-stimulated TEA uptake. Vesicles were pre-equilibrated for 2 h in KCl buffer with either 0.5, 10, or 50 mM HEPES, pH 7.4. Thereafter, uptake of 55 μM [3H]TEA was measured in the same buffers at the times shown in the presence of the ATP regenerating system and 300 μM ATP. The data are from quadruplicate determinations in one experiment.

FIG. 11. Effect of temperature on ATP-stimulated TEA uptake. Uptake of 52.5 μM [3H]TEA was measured in KCl buffer at the indicated times and temperatures in the presence of the ATP regenerating system and presence or absence of 300 μM ATP. The results represent quadruplicate determinations from one experiment.

DISCUSSION

The results of the present studies indicate that the organic cation TEA can undergo mediated transport across the apical membrane of the proximal tubule by a process evidently energized directly by ATP. Since little external ATP would be expected to penetrate the intravesicular space (45), and since the vast majority of BBMV have been shown previously to be oriented outside out (45, 47), we presume the effects of ATP observed in these experiments are due to a transporter with an ATP binding site located on the extracellular face of the membrane. We could not demonstrate an effect of intra-
or extrasynaptic ATP on efflux of TEA from the vesicles. Although numerous effects of extracellular ATP, resulting especially from activation of P2 type purinoceptors, are well recognized (48–51), to our knowledge, there have been few studies of potential effects of extracellular ATP on epithelial transport. Nevertheless, it has been observed, for example, that external ATP activates potassium channels and induces hyperpolarization of the cell membrane in Madin-Darby canine kidney cells, an effect which may subsequently stimulate chloride secretion. This effect appears to be mediated by increases in intracellular calcium (52). In contrast to many P2 type purinoceptors, which may be activated by ADP or poorly hydrolyzable analogues of ATP (48, 50), the stimulation of TEA transport in BBMV appeared to require ATP (or ITP or GTP) hydrolysis; no initial electrochemical gradients were present in these experiments and transport was not stimulated by CTP, ADP, AMP, or adenosine. In addition, AMP-PNP, a poorly hydrolyzable analogue of ATP, inhibited the effect of ATP but resulted in little stimulation of TEA transport by itself, results consistent with inhibition of ATP binding by AMP-PNP.

Several transport ATPases have been previously characterized. However, based on inhibitor profiles and nucleotide preferences, ATP-stimulated TEA transport in renal BBMV appears to represent a heretofore unrecognized process. ATP-stimulated TEA transport was not inhibited by vanadate or ouabain, differentiating it from Na⁺-K⁺-ATPase, H⁺/K⁺-ATPase, and Ca²⁺-ATPase. Similar to vacuolar type H⁺-ATPase, ATP-stimulated TEA transport was inhibited by DCCD and NEM. However, vacuolar type H⁺-ATPase is inhibited by this reagent.

The results of the present studies are in some respects reminiscent of those obtained in membrane vesicles prepared from a multidrug-resistant human epidermal carcinoma cell line (21). These cells contain high levels of P-glycoprotein and are resistant to killing by a variety of chemotherapeutic agents such as vinblastine. The latter phenomenon is due to the extrusion of the drugs from the cells via the P-glycoprotein transport system (also termed the multidrug-resistant transporter or MDR, gene product in humans), which utilizes ATP as an energy source for the transport process (21, 56). Furthermore, it has been demonstrated that P-glycoprotein is present in several normal cells including the apical membrane of mammalian proximal tubules (57). A variety of drugs that interact with the multidrug-resistant transporter share certain common characteristics including a lipophilic aromatic ring, linked to a hydrophilic N-alkyl group and positive charge at neutral pH (58, 59). Some of the drugs demonstrated to inhibit TEA transport in the present studies, including quinidine and vinblastine, possess these characteristics and have been shown previously to interact with the multidrug-resistant transporter (21, 58, 60, 61). Thus, it is possible in the present experiments that TEA was transported by the P-
lycoprotein system. However, a number of observations argue against this possibility. First, TEA does not possess the aforementioned features of compounds that interact with the multidrug-resistant transporter, i.e. it is a small non-aromatic hydrophilic compound. Second, transport via this system seems unlikely a priori based on experiments by others, in which it was shown that TEA (and tetramethylammonium) do not inhibit transport presumed to occur by the multidrug-resistant transport system (60, 61). Third, vanadate, which is known to undergo net reabsorption. Our results separate, but potentially related, transport systems.

The significance of our observations to the intact proximal tubule is not certain. As noted earlier, net transport of organic cations by the proximal tubule is generally in the secretory direction into the tubule lumen. However, some organic cations are known to undergo net reabsorption. Our results suggest that ATP may stimulate reabsorption of organic cations from the tubule lumen. For this to occur, ATP would be required to transport substrates out of cells, whereas the ATP-stimulated TEA transport identified in the present work would serve to transport substrates into cells. Finally, ATP-stimulated TEA transport was inhibited by vinblastine, but not in a competitive manner, and we could not demonstrate ATP-stimulated vinblastine uptake into (or efflux from) the vesicles in the present experiments. Therefore, although there are several functional similarities between the ATP-stimulated organic cation transporter we have identified in rabbit BBMV and the multidrug-resistant transport system, these appear to be separate, but potentially related, transport systems.

REFERENCES