ELSEVIER

Biochimica et Biophysica Acta 1240 (1995) 18–24

BBA Biochimica et Biophysica Acta

Paraquat^{2+}/H$^+$ exchange in isolated renal brush-border membrane vesicles

Stephen H. Wright *, Theresa M. Wunz

Department of Physiology, College of Medicine, University of Arizona, Tucson, AZ 85724, USA

Received 16 February 1995; accepted 30 June 1995

Abstract

The mechanism(s) by which paraquat (1,1'-dimethyl-4,4'-bipyridinium), a divalent organic cation (OC) and proximal tubule nephrotoxicant, crosses renal cell membranes is unclear. The structurally-related monovalent OC, 1-methyl-4-phenylpyridinium (MPP$^+$), crosses the renal brush border via OC/H$^+$ exchange using the same pathway by which tetraethylammonium (TEA) is transported. We examined whether paraquat shares the TEA(MPP$^+$)/H$^+$ exchanger by examining ^{14}C-paraquat transport in rabbit renal BBMV. Compared to a pH equilibrium condition (pH 7.5_{in}:7.5_o), an H-gradient (pH 6_{in}:7.5_o) stimulated the 5 s and 60 s uptakes of 230 μM paraquat by 51% and 108%, respectively, and this stimulation was blocked by both 20 mM unlabeled paraquat and TEA. Pre-loading BBMV with 2 mM unlabeled TEA (under conditions of pH equilibrium) stimulated by 3-fold the 60 s uptake of 120 μM paraquat and by 5 min produced a transient intravesicular accumulation of paraquat that exceeded equilibrium (2 h) uptake by 45%. The presence of 200 μM paraquat in the extravesicular solution competitively inhibited H-gradient-stimulated transport of ^{14}C-TEA in renal BBMV, increasing the apparent K_t for TEA transport from 169 μM to 379 μM, without significantly influencing the J_{max} (16.0 vs. 15.4 nmol mg^{-1} min^{-1}). The calculated K_i for paraquat (presumably equal to its K_t for transport) after transport was between 160 and 220 μM (depending upon the method of estimation). Significantly, the K_t for MPP$^+$/H exchange is 12 μM, suggesting that the affinity of the exchanger is profoundly influenced by the presence on paraquat of a second positive charge. We conclude that renal transport of paraquat involves the OC/H$^+$ exchanger of proximal cell luminal membranes and that this pathway may play a role in the renal secretion of polyvalent organic cations.

Keywords: Transport; Organic cation; Kidney; 1-Methyl-4-phenylpyridinium (MPP$^+$); Tetraethylammonium (TEA)

1. Introduction

The epithelial cells of the proximal tubule secrete a wide variety of organic cations and bases (collectively, OCs) and thereby play a central role in the regulation of blood levels of these compounds. The current model of proximal tubule secretion of OCs has the solute enter the cells from the blood across the peritubular membrane by a process of carrier-mediated facilitated diffusion [1]. This process is electrogenic [2–4] and thus uses the inside negative electrical potential of proximal cells as the driving force for accumulation of these compounds. Alternatively, OCs may enter proximal cells by carrier-mediated countertransport for intracellular OCs [4,5], although this probably simply represents a different mode of action of the aforementioned electrogenic pathway [1]. The active step in transepithelial secretion of OCs is believed to reside at the luminal membrane and to entail the carrier-mediated exchange of H$^+$ for the OC [2,6,7]. An inwardly-directed electrochemical gradient for H$^+$ is maintained across the luminal membrane through the activity of the Na$^+$/H$^+$ exchanger and serves as the driving force to move the cationic organic substrate out of the negative interior of the cell into the tubule lumen.

Previous studies of the luminal OC/H$^+$ exchanger have focused almost entirely on the interaction of monovalent substrates with this transport process. The exchange of OC for H$^+$ is a one-to-one process [2]. This has important physiological implications because it means that the mediated efflux of OC is electrically silent and therefore elimi-

* Corresponding author. E-mail: shwright@ccit.arizona.edu. Fax: +1 (520) 6262383.

SSDI 0005-2736(95)00166-2

nates the need to have a large inwardly-directed chemical gradient for H^+ across the luminal membrane in order to support net secretion of OC. However, it is not evident to what extent the luminal OC/H^+ exchanger serves as an avenue for flux of polyvalent OCs.

The interaction of polyvalent OCs with the peritubular transporter has received some attention. In their extensive study of the molecular determinants associated with the interaction of OCs with proximal tubule OC transporters, Ullrich and his colleagues measured the inhibitory interaction of a series of divalent OCs with the peritubular transport process that handles N^1-methylnicotinamide (NMN) and tetraethylammonium (TEA) [8,9]. They found that divalent OCs could produce a modest inhibition of NMN transport, consistent with the degree of hydrophobicity imparted by their molecular structures (hydrophobicity being the most important parameter in dictating substrate–transporter interaction [8,9]).

In the present study we sought to determine whether a polyvalent OC could serve as a substrate for the luminal OC/H^+ exchanger. We elected to use the herbicide, 1,1′-dimethyl-4,4′-bipyridinium (paraquat), as a model substrate for two reasons. First, it has a chemical structure very similar to that of 1-methyl-4-phenylpyridinium (MPP^+), a known substrate of the luminal OC/H^+ exchanger [10]. Second, paraquat is an environmentally significant agent known to express nephrotoxicity [11–13]. Although the mechanism of paraquat transport in lung (e.g., [14,15]) and intestinal (e.g., [16,17]) tissues has received considerable attention, the mechanism(s) by which it is transported in kidney cells is not known. We found that paraquat is a potent inhibitor of MPP^+ and TEA transport via the OC/H^+ exchanger in renal brush border membrane vesicles. Moreover, paraquat was shown to be a substrate for transport by the luminal OC/H^+ exchanger. We conclude that the luminal OC/H^+ exchanger can handle both monovalent and divalent OCs and thereby may serve as a pathway for the secretion of an even wider variety of xenobiotic agents than previously acknowledged.

2. Materials and methods

2.1. Preparation of brush-border membrane vesicles

BBMV were prepared from cortices isolated from kidneys of New Zealand White rabbits using a Ca^{2+}-Mg^{2+} precipitation procedure [18]. Compared to the initial homogenate, these vesicles are routinely enriched approx. 10-fold in trehalase and alkaline phosphatase activity, and 1-fold or less in Na,K-ATPase and K-dependent *p*-nitrophenylphosphatase activity [18]. BBMV were used within 24 h of preparation if held on ice, or within 2 weeks if stored in liquid nitrogen. There was no significant decrease in transport activity compared to fresh vesicles when stored in these ways.

2.2. Measurement of transport

Uptake of [^{14}C]TEA was measured using a rapid filtration procedure [19]. Briefly, the transport reaction was started by rapidly mixing 10 μl of the BBMV suspension with 90 μl of transport buffer containing salts and radiolabeled substrate (see figure legends for a description of solutions used in individual experiments). The transport reaction was terminated by adding 1 ml of an ice-cold 'stop' solution, which typically was identical in composition to the BBMV suspension solution. 1 ml of the stopped reaction mixture was filtered under vacuum through a 0.45 μm filter (type HAWP; Millipore). The trapped BBMV were rinsed with 4 ml of stop solution and the radioactivity left on the filter was measured using a liquid scintillation counter (Beckman model LS3801). Samples were corrected for variable quench. Each sample was also corrected for non-specific binding of [^{14}C]TEA to the filters or vesicles by subtracting the number of counts remaining on the filters following filtration and washing of vesicles that were exposed to a mixture of transport buffer and ice-cold stop solution. Uptakes were expressed as moles of labeled substrate accumulated per milligram of membrane protein (Bio-Rad; γ-globulin standard). Experimental observations were performed at room temperature (21–23°C).

2.3. Chemicals

[^{14}C]Paraquat (13 mCi mmol^{-1}) was purchased from Sigma (St. Louis, MO). [^{14}C]TEA (56 mCi mmol^{-1}) was prepared by Wizard Laboratories (Davis, CA). [^{3}H]MPP^+ (80 Ci mmol^{-1}) was purchased from American Radiolabeled Chemicals (St. Louis, MO). All other chemical were purchased from Sigma or other routine suppliers and were the highest grade available.

3. Results

3.1. Paraquat uptake – effect of H^+ gradients

Transport of monovalent organic cations, including tetraethylammonium (TEA), across the luminal brush border membrane typically involves a carrier-mediated exchange of OC for H^+. Therefore, it was of interest to determine the effect of an H^+ gradient on the uptake of the divalent OC, paraquat, into renal BBMV. As shown in Fig. 1, an outwardly-directed H^+ gradient (pH_{in} of 6.0 vs. pH_{out} of 7.5) stimulated the accumulation of 230 μM [^{14}C]paraquat compared to the pH equilibrium condition ($pH_{in} = pH_{out} = 7.5$). Uptake at each time point through 60 s was increased 50% to 150% by the pH gradient condition. Uptake at 1 h did not differ for the two conditions. Under conditions of an outwardly-directed H^+ gradient, the addition of 20 mM TEA, a substrate for the OC/H^+ exchanger [2], or 20 mM unlabeled paraquat

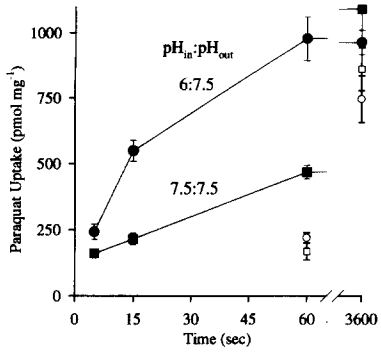

Fig. 1. The influence of an outwardly-directed pH gradient on the time course of [^{14}C]paraquat transport in renal BBMV. Vesicles were preequilibrated in a solution containing (in mM) 150 KCl, 5 Hepes (buffered to pH 6.0 or 7.5 with KOH), and 300 mannitol. The transport buffer contained (final concentration, in mM) 150 KCl, 5 Hepes-KOH (pH 7.5), and ~230 μM [^{14}C]paraquat (osmolality balanced as needed with mannitol). In some cases, under the pH gradient condition, 20 mM TEA (open squares) or 20 mM unlabeled paraquat (open circles) was also included in the transport buffer. Each point is the means ± S.E. of uptakes measured in three separate membrane preparations.

reduced the 60 s uptake of [^{14}C]paraquat to a common level, implicating the OC/H$^+$ exchanger as the probable pathway for carrier-mediated paraquat uptake. The outwardly-directed H$^+$ gradient did not, however, support the transient accumulation of paraquat to a level exceeding that noted at equilibrium (i.e., vesicle content at 1 h). Therefore, these data, although consistent with the activity of paraquat/H$^+$ exchange, are not sufficient to conclude that paraquat uptake involved countertransport.

The failure of an imposed H$^+$ gradient to support development of an 'overshoot' of paraquat accumulation in renal BBMV (Fig. 1) was probably a consequence of a relatively slow rate of paraquat transport, compared to the rapid collapse of an H$^+$ gradient in renal BBMV. The half time for the collapse of a 10-fold H$^+$ gradient in rabbit BBMV is ~15 s [20]. Thus, in the 60 s required to raise the intravesicular paraquat concentration to that of the external medium, the H$^+$ gradient present at time zero would have largely dissipated.

3.2. Effect of trans-TEA gradient on paraquat uptake

The OC/H$^+$ exchanger also supports OC/OC exchange [21] and TEA has been shown to support the concentrative transport of several other OCs via carrier-mediated exchange (e.g., [10,22]). *Trans*-membrane gradients of a solute like TEA are also probably sustained for a substantially longer period of time than H$^+$ gradients are, as suggested by the time course of *trans*-stimulation of [^{14}C]TEA transport driven by gradients of TEA [21]. Therefore, as an alternative test of the hypothesis that paraquat transport in renal BBMV involved the OC/H$^+$ exchanger, we examined the effect on paraquat accumulation of an outwardly-directed gradient of TEA. When BBMV were preloaded with 2 mM TEA, the uptake of [^{14}C]paraquat over a 2.5 min period was stimulated by up to 2.8-fold (Fig. 2), compared to the zero-*trans* condition. Moreover, within 60 s the intravesicular concentration of paraquat significantly exceeded that occurring at equilibrium (2 h). The only known mediated pathway for TEA transport in renal BBMV is the OC/H$^+$ exchanger [1]. Thus, in the light of the observations presented in Figs. 1 and 2, we concluded that paraquat uptake into renal BBMV includes a carrier-mediated exchange with TEA and H$^+$.

3.3. Effect of paraquat on transport of TEA

The evidence presented in Figs. 1 and 2 indicate that paraquat and TEA interact at a common transport site. To determine if that interaction is purely competitive in nature, we examined the influence of paraquat on the kinetics of TEA/H$^+$ exchange in renal BBMV. H-gradient-driven transport of TEA (pH_{in} of 6.0 vs. pH_{out} of 7.5) was adequately described by the Michaelis-Menten equation (see [23]). In the absence of paraquat, the J_{max}^{TEA} for TEA transport was 16.0 ± 1.0 nmol mg^{-1} min^{-1} with a K_t^{TEA} of 169 ± 20 μM ($n = 3$; analysis by non-linear regression). In the presence of 200 μM paraquat, the apparent K_t for TEA transport ($K_{t\text{-app}}^{TEA}$) was increased to 379 ± 17 μM ($P < 0.001$) while the J_{max}^{TEA} remained unchanged (15.4 ± 1.2 nmol mg^{-1} min^{-1}). This is graphically supported by the paired Woolf-Augustinsson-Hofstee plots (J vs. $J/[S]$) presented in Fig. 3; the presence of paraquat increased the slope of this relationship (i.e., increased the apparent K_t for TEA/H$^+$ exchange) without influencing the intercept (J_{max}^{TEA}). These data suggest that paraquat is a true competitive inhibitor of TEA/H$^+$ exchange.

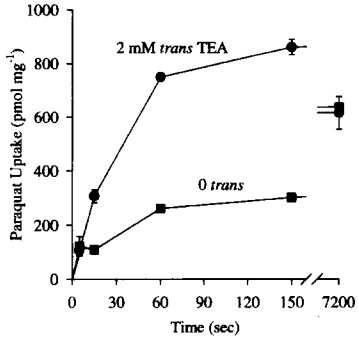

Fig. 2. The influence of an outwardly-directed gradient of TEA on the time course of [^{14}C]paraquat transport in renal BBMV. Vesicles were preequilibrated in a solution containing (in mM) 150 KCl, 5 Hepes-KOH (pH 7.5), 300 mannitol, with or without 2 mM TEA as indicated. The transport buffer contained (final concentration, in mM) 150 KCl, 5 Hepes-KOH, 300 mannitol, ~120 μM [^{14}C]paraquat, and 50 μM TEA for the control condition (to match the extravesicular carryover of TEA occurring when vesicles were preloaded with TEA). Each point is the means ± S.E. of uptakes measured in three separate membrane preparations.

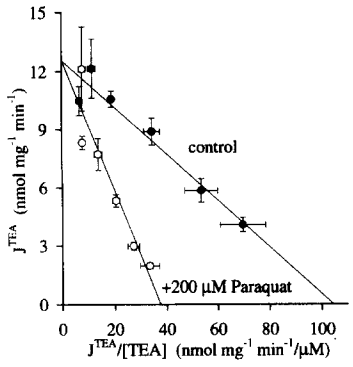

Fig. 3. Woolf-Augustinsson-Hofstee plot showing the influence of extravesicular paraquat on the kinetics of TEA transport via the luminal OC/H^+ exchanger. Vesicles were preequilibrated in a solution containing (in mM) 150 KCl, 5 Hepes-KOH (pH 6.0). The transport contained (final concentration, in mM) 130–150 KCl, 5 Hepes-KOH (pH 7.5), ~60 μM [^{14}C]TEA, 0–20 unlabeled TEA, with or without 200 μM paraquat, as indicated. Each point is the means ± S.E. of 3 s uptakes measured in three separate membrane preparations.

3.4. Reciprocal inhibitory interactions between paraquat and 1-methyl-4-phenylpyridinium

Further evidence that paraquat transport across renal brush borders is limited to its interaction with the OC/H^+ exchanger arises from the mutual, reciprocal interaction between the transport of paraquat and its closely related structural analog, MPP^+. H-gradient supported paraquat transport in renal BBMV was blocked by increasing concentrations of extravesicular (*cis*) MPP^+ (Fig. 4A). The relationship between *cis* MPP^+ concentration and paraquat transport was adequately described by the equation for competitive inhibition of a Michaelis-Menten mediated process:

$$J^{Para} = \frac{J_{max}^{Para}[Para]}{K_t^{Para}\left(1 + \frac{[MPP^+]}{K_i^{MPP^+}}\right) + [Para]} + D[Para] \quad (1)$$

where J^{Para} is the rate of paraquat transport from a paraquat concentration of [Para], J_{max}^{Para} is the maximum rate of paraquat transport, K_t^{Para} is the paraquat concentration resulting in half-maximal transport, K_i^{MPP+} is the inhibitor constant for MPP^+ (the Michaelis constant for MPP^+ transport *via* the paraquat transport pathway), and D is a first-order constant that reflects the non-saturable component of paraquat transport (some combination of diffusion and non-specific binding). When Eq. (1) is rearranged the relationship between MPP^+ concentration [MPP^+] and transport of [^{14}C]paraquat is apparent:

$$J^{14C-P} = \frac{J_{app}^{Para}[^{14}C-P]}{K_{app}^{MPP} + [MPP^+]} + C \quad (2)$$

where J^{14C-P} is the transport of [^{14}C]paraquat from a concentration of [^{14}C-P], J_{app}^{Para} is a constant consisting of $J_{max}^{Para}(K_i^{MPP+}/K_t^{Para})$, K_{app}^{MPP} is a constant consisting of $K_i^{MPP^+}\left(1 + \frac{[^{14}C-P]}{K_t^{Para}}\right)$ and C is a constant equal to (D[^{14}C-P]). According to this relationship, the K_{app}^{MPP} derived from three experiments was 39 ± 11 μM, from which a true $K_i^{MPP^+}$ of 15 μM was calculated. This estimate for the MPP^+ inhibitory constant is in reasonable agreement with the Michaelis constant of 11.6 μM for MPP^+/H^+ exchange determined in a previous study [10].

Fig. 4B shows the relationship between cis paraquat and H-gradient supported transport of MPP^+. The relationship was adequately described by Eq. (2), resulting in a K_{app}^{Para} of 227 ± 2 μM (n = 2), or a 'true' K_i for paraquat of 218 μM. This value is in reasonable agreement with an independent estimate of 163 μM for the K_t^{Para}, discussed below. The similarity between the respective values for apparent K_i values and K_t values for these two substrates supports the conclusion that paraquat transport across the renal brush border is limited to an interaction with the OC/H^+ exchanger.

3.5. Indirect assessment of the kinetics of paraquat / H^+ exchange

The low specific activity of commercially-available paraquat precluded accurate, direct experimental measurement of the kinetics of paraquat transport. However, values for the K_t and J_{max} for H-gradient driven paraquat transport in renal BBMV were estimated from the available data. The interaction between paraquat and TEA appears to

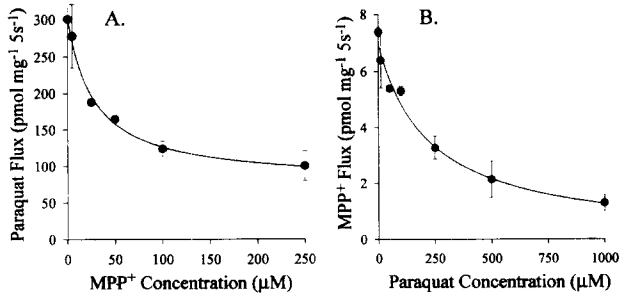

Fig. 4. (A) The effect of increasing concentrations of extravesicular MPP^+ on the transport of [^{14}C]paraquat into renal BBMV. Vesicles were preequilibrated in a solution containing (in mM) 150 KCl, 5 Hepes-KOH (pH 6.0), and 300 mannitol. The transport buffer contained (final concentration, in mM) 150 KCl, 5 Hepes-KOH (pH 7.5), 280 μM [^{14}C]paraquat, and 0–250 μM MPP^+ (osmolarity balanced with mannitol as needed). Each point is the means ± S.E. of uptakes measured in a representative membrane preparation. The line was based upon Eq. (2) (see text) and was fit to the data using a non-linear regression algorithm (Sigmaplot for Windows 1.1). (B) The effect of increasing concentrations of extravesicular paraquat on the transport of [^{3}H]MPP^+ into renal BBMV. Vesicles were preequilibrated in a solution containing (in mM) 150 KCl, 5 Hepes-KOH (pH 6.0), and 300 mannitol. The transport buffer contained (final concentration, in mM) 150 KCl, 5 Hepes-KOH (pH 7.5), < 1 μM [^{3}H]MPP^+, and 0–1 mM paraquat (osmolarity balanced with mannitol as needed). The line was based upon Eq. (2) (see text) and was fit to the data using a non-linear regression algorithm (Sigmaplot for Windows 1.1). Each point is the means ± S.E. of uptakes measured in a representative membrane preparation.

be competitive, so we assumed that the apparent K_t for TEA, measured in the presence of paraquat, K_{app}^{TEA}, is related to the true TEA K_t by the following relationship:

$$K_{app}^{TEA} = K_t^{TEA}\left(1 + \frac{[Para]}{K_i^{Para}}\right) \quad (3)$$

Rearrangement of this relationship permits calculation of the K_i for paraquat's inhibition of TEA/H^+ exchange: 163 ± 23 μM. The K_i for paraquat's interaction with TEA at the transport site should be equal to the K_t for paraquat transport via the OC/H^+ exchanger (see [23]). Given the estimate of 218 μM for K_t^{Para}, derived from the experiments described in Fig. 4, we suggest that the Michaelis constant for H-gradient supported paraquat transport via the OC/H^+ exchanger lies between 160 and 220 μM.

An estimate for the maximal, H-gradient supported transport of paraquat (J_{max}^{Para}) was calculated by the following method. Fitting Eq. (2) to the data presented in Fig. 4A results in calculation of the parameter J_{app}^{Para}, which is equal to $J_{max}^{Para}\left(\frac{K_t^{MPP^+}}{K_t^{Para}}\right)$. In three experiments J_{app}^{Para} was 43.5 ± 13.7 pmol mg^{-1} 5 s^{-1}. Using this value and the range of values noted above for K_t^{Para} and $K_i^{MPP^+}$ suggests that maximal paraquat transport ranges from 5.7 to 10.4 nmol mg^{-1} min^{-1}.

3.6. On the stoichiometry of paraquat^{2+}/H^+ exchange

The exchange of TEA, a monovalent OC, for H^+ has been shown to have a stoichiometry of 1:1 and to be an electroneutral process [2]. Because paraquat has a valence of +2, it was of interest to determine if an electrical gradient could influence paraquat/H^+ exchange. As shown in Fig. 5, under pH equilibrium conditions (pH 7.5 in and out) an inside-negative electrical potential difference had no effect on either the initial rate of paraquat transport or the maximal accumulation of paraquat.

Although these data suggest that paraquat^{2+}/H^+ exchange is an electroneutral process, such a conclusion may not be justified. The effect that an electrical driving force has on the rate of an electrogenic transport process can be quite small depending on such factors as the net charge of the fully loaded exchanger (see [24]). Thus, the failure of an imposed PD to influence paraquat accumulation may reflect our inability to adequately resolve a small effect, under the experimental conditions employed. Therefore, in a separate set of experiments, we examined the effect of varying transmembrane H^+ gradients on the equilibrium distribution of paraquat using the static-head approach introduced by Kinsella and Aronson [25] and Turner and Moran [26]. The results of a control set of experiments that examined the influence of imposed H^+ gradients on the distribution of TEA, agreed with those reported previously [2]: an apparent static head (i.e., a balance of the opposing forces in the imposed H^+ and TEA gradients) was achieved with opposing 10-fold chemical gradients, consistent with a 1:1 ratio of TEA/H^+ exchange (data not shown). However, in three experiments that examined the influence of imposed H^+ gradients on the distribution of paraquat, the results were not readily interpretable: there was no clear relationship between the rate of paraquat efflux from preloaded BBMV and the size of the imposed H^+ gradient. A likely basis for the equivocal nature of these results was the comparatively slow rate of paraquat flux (compared to TEA); the H^+ gradients employed in these studies (as large as 1.5 pH unit) have a half-time on the order of 15 s [20] and probably dissipated before their effects could influence the equilibrium distribution of paraquat. Therefore, because of the ambiguities associated with interpreting the results of the static-head experiments and the previously discussed experiments on the effect of membrane potential on paraquat transport, we found that we could draw no clear conclusion about the stoichiometry of paraquat^{2+}/H^+ exchange in renal BBMV.

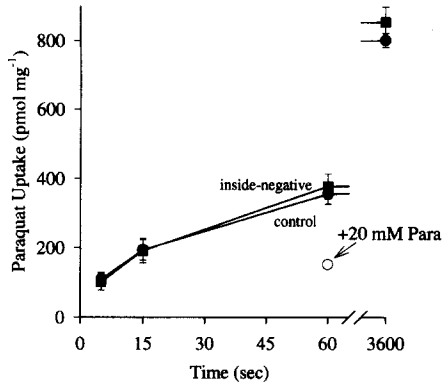

Fig. 5. The effect of an inside-negative membrane potential on the time course of [^{14}C]paraquat uptake in renal BBMV. Vesicles were preequilibrated in a solution containing (in mM) 150 KCl, 5 Hepes-KOH (pH 7.5), and 300 mannitol. The transport buffer contained (final concentration, in mM) 150 KCl or 150 NaCl, 5 Hepes-KOH (pH 7.5), 20 μM valinomycin, 30 μM FCCP, 100–300 μM [^{14}C]paraquat, and 20 mM unlabeled paraquat as indicated (osmolarity balanced with mannitol as needed). Each point is the means ± S.E. of uptakes measured in 3 separate membrane preparations (uptake in the presence of 20 mM unlabeled paraquat was from a single experiment).

4. Discussion

The present results show that paraquat can be transported across the luminal membrane of renal proximal tubules by a carrier-mediated process. The ability of oppositely-oriented gradients of H^+ and TEA to stimulate paraquat transport in isolated BBMV implicates the well-characterized TEA/H^+ exchanger [2] in the luminal transport of paraquat. Indeed, the ability of a TEA gradient to support the concentrative accumulation of paraquat argues that TEA and paraquat must share a common transport pathway. Furthermore, the profile of inhibitory interactions between the transport of paraquat, TEA and MPP^+, sug-

gests that paraquat flux across the luminal membrane of proximal cells is probably limited to the OC/H^+ exchanger.

Less clear from the present data set is the issue of the stoichiometry of coupling between the exchange of H^+ for the divalent (+2) paraquat. Three lines of evidence argue that the exchange of H^+ for monovalent organic cations is 1:1. First, on kinetic grounds, it appears that the transporter has only one binding site for the organic substrate on each face of the membrane: the relationship between rate of substrate (e.g., TEA) transport and substrate concentration is hyperbolic, rather than sigmoidal [2,21]; and the relationship between the rate of substrate transport and the intravesicular concentration of exchangeable substrate is also hyperbolic, rather than sigmoidal [21]. Second, an inside-negative membrane potential fails to support the concentrative transport of monovalent cations into luminal membrane vesicles [2]. Third, static-head experiments suggest that there is a obligatory exchange of 1 TEA molecule for one proton ([2]; see also [27]). In the light of these observations, paraquat transport via the OC/H^+ exchanger might be expected to display a different profile of response to parameters such as membrane potential or H^+ gradients. This was not, however, the case; neither membrane potential nor systematic alterations in H^+ gradients produced a profile of paraquat transport that clarified the issue of the stoichiometry of coupling between H^+ and paraquat. Although the failure of an inside-negative membrane potential to stimulate uptake of paraquat may reflect an electrically-silent mode of transport (e.g., 1 paraquat^{2+}:2 H^+), this need not be the case. As discussed by Turner [24], factors such as the net charge of the substrate/exchanger complex can influence the extent to which membrane potential influences the rate of transport. Indeed, depending on the assumptions employed, the rate of an electrogenic transport process can be independent of membrane potential [24]. Similarly, it is difficult to draw any conclusions from the results of the static-head experiments because the rate of paraquat flux under the conditions defined by this protocol was slower than the anticipated rate of collapse of the several H^+ gradients employed [20]. Therefore, we cannot discern whether paraquat/H^+ exchange is one-for-one (and therefore electrogenic) or one-for-two (and electrically silent).

It is useful to consider what impact the stoichiometry of luminal paraquat/H^+ exchange could have on the energetics of tubular secretion of this divalent cation. The electrically silent exchange of monovalent organic cations for H^+ is the basis of the active secretion of OCs by proximal tubules. The principal mode of entry of OCs into proximal cells across the peritubular membrane probably involves electrogenic facilitated diffusion [2–4]. The inside-negative electrical potential of proximal cells thus serves to concentrate OCs to levels substantially greater than that in the blood (although the very high intracellular concentrations observed in some studies has led to suggestions than an alternative/additional driving force may be involved, as well [28,29]). The electrogenic exit of OCs from proximal cells is energetically unfavorable. However, the exchange of one H^+ for one OC, via the luminal OC/H^+ exchanger, renders OC efflux electrically silent and allows monovalent OCs to move down their chemical gradient from the cytoplasm into the lumen. The most favorable mode of paraquat flux across the luminal membrane would be the exchange of 2 H^+ for 1 paraquat molecule; such a mode of exchanger activity would not only permit paraquat efflux to be electrically silent but also would augment the chemical energy in the transluminal membrane H^+ gradient by raising its effective size to the square of the chemical gradient [30]. If, however, paraquat/H^+ exchange is 1:1, paraquat efflux would be electrogenic and involve a net efflux of positive charge from the cell, an energetically unfavorable situation. Net paraquat secretion could still occur if the peritubular entry step for paraquat were to involve electrogenic facilitated diffusion, a situation which would favor the development of a concomitantly large intracellular concentration of this divalent molecule. It is, however, interesting to speculate that an energetically unfavorable luminal efflux step for paraquat could lead to the accumulation of this compound in proximal renal cells. This could be a contributing factor in the observed nephrotoxicity produced by paraquat [11–13].

In several cell types paraquat transport has been found to involve one or more transport processes for polyamines. In Chinese hamster ovary cells, at least two pathways for uptake of polyamines have been observed, although paraquat transport appears to be limited to an interaction with only one of these [31]. In the lung, uptake of paraquat into Type I and Type II alveolar cells is blocked by a variety of polyamines and diamines [14,15]. Significantly, in both cell types, the transporters displayed much higher affinities for the endogenous polyamines than for paraquat, with apparent K_t values for the former compounds in the 2–10 μM range vs. K_t values (or K_i values) for paraquat in the 75–250 μM range [14,15,31]. In renal cells, endogenous polyamines display a very limited interaction with the luminal OC/H^+ exchanger [32], which our data suggest is the principal route of luminal paraquat transport. Paraquat transport in intestinal cells, however, displays several characteristics which are similar to those observed here for renal proximal cells. In a recent study examining paraquat uptake in rat intestinal brush border membrane vesicles [16], transport was observed to be saturable and independent of membrane potential. Moreover, paraquat transport was found to be inhibited by the monovalent cations, tetramethylammonium and choline (both of which interact with the renal OC/H^+ exchanger [33]), but not by putrescine. Although the authors of this study examined the effect of a Na^+ gradient on paraquat transport (and found none), they did not examine the effect of an H^+ gradient on transport of paraquat. Interpretation of these results, and the potential similarity between the character-

istics of paraquat transport in renal and intestinal brush borders is complicated by several factors. First, there is no evidence supporting the presence in the intestine of the renal OC/H^+ exchanger. There is an exchanger for guanidine and H^+ [34], but this process is inhibited by polyamines and shows very little interaction with TEA. The authors of the study on paraquat transport in intestinal BBMV suggest that uptake may involve a choline transport pathway, but the characteristics of intestinal choline transport process remain poorly understood. In renal proximal tubules, luminal choline transport involves two pathways, the OC/H^+ exchanger and a very specific, carrier-mediated facilitated diffusion process, with the latter process producing a net reabsorption of choline under physiological conditions [33].

In conclusion, paraquat was found to be a potent inhibitor of the luminal OC/H^+ exchanger of proximal tubule cells and to be a substrate for this process, as well. Although it was not clear whether mediated exchange of $paraquat^{2+}$ for H^+ is an electrogenic process, the fact that it can traverse the luminal membrane by the OC/H^+ exchanger does make it likely that the proximal tubule is capable of secreting paraquat. The observation that the luminal OC/H^+ exchanger can handle both monovalent and divalent OCs implicates this process in the secretion of an even wider variety of potentially xenobiotic agents than previously recognized.

Acknowledgements

This work was supported by NIH grants PO1 DK41006, RO1 DK49222, and P30 ES06694.

References

[1] Pritchard, J.B. and Miller, D.S. (1993) Physiol. Rev. 73, 765–796.
[2] Wright, S.H. and Wunz, T.M. (1987) Am. J. Physiol. 253, F1040–F1050.
[3] Montrose-Rafizadeh, C., Mingard, F., Murer, H. and Roch-Ramel, F. (1989) Am. J. Physiol. 257, F243–F251.
[4] Sokol, P.P. and McKinney, T.D. (1990) Am. J. Physiol. 258, F1599–F1607.
[5] Dantzler, W.H., Brokl, O. and Wright, S.H. (1989) Am. J. Physiol. 256, F290–F297.
[6] Holohan, P.D. and Ross, C.R. (1981) J. Pharmacol. Exp. Ther. 216, 294–298.
[7] Wright, S.H. (1985) Am. J. Physiol. 249, F903–F911.
[8] Ullrich, K.J., Papavassiliou, F., David, C., Rumrich, G. and Fritzsch, G. (1991) Pflugers Arch. 419, 84–92.
[9] Ullrich, K.J., Rumrich, G. and Fritzsch, G. (1992) Pflugers Arch. 420, 29–38.
[10] Lazaruk, K.D.A. and Wright, S.H. (1990) Am. J. Physiol. 258, F597–F605.
[11] Ecker, J.L., Hook, J.B. and Gibson, J.E. (1975) Toxicol. Appl. Pharmacol. 34, 178–186.
[12] Oreopoulous, D.G., Soyannwo, M.A.O., Sinnah, R., Fenton, S.S.A., McGeown, M.G. and Bruce, J.H. (1968) Br. Med. J. 1, 749–750.
[13] Clark, G.D., McElligot, T.F. and Hurst, E.W. (1966) Br. J. Industr. Med. 23, 126–132.
[14] Hoet, P.H.M., Lewis, C.P.L., Demedts, M. and Nemery, B. (1994) Biochem. Pharmacol. 48, 517–524.
[15] Smith, L.L., Lewis, C.P.L., Wyatt, I. and Cohen, G.M. (1990) Environ. Health Perspect. 85, 25–30.
[16] Nagao, M., Saitoh, H., Zhang, W.D., Iseki, K., Yamada, Y., Takatori, T. and Miyazaki, K. (1993) Arch. Toxicol. 67, 262–267.
[17] Grabie, V., Scemama, J.-L., Robertson, J.B. and Seidel, E.R. (1993) Toxicol. Appl. Pharmacol. 122, 95–100.
[18] Wright, S.H. and Wunz, T.M. (1987) Am. J. Physiol. 253, F432–F439.
[19] Wright, S.H., Hirayama, B., Kaunitz, D.D., Kippen, I. and Wright, E.M. (1983) J. Biol. Chem. 258, 5456–5462.
[20] Ives, H.E., Chen, P.-Y. and Verkman, A.S. (1986) Biochim. Biophys. Acta 863, 91–100.
[21] Wright, S.H. and Wunz, T.M. (1988) J. Biol. Chem. 263, 19494–19497.
[22] Sokol, P.P., Holohan, P.D. and Ross, C.R. (1987) J. Pharmacol. Exp. Ther. 242, 152–157.
[23] Segel, I.H. (1975) Enzyme Kinetics, John Wiley and Sons, New York pp. 1.
[24] Turner, R.J. (1981) Biochim. Biophys. Acta 649, 269–280.
[25] Kinsella, J.L. and Aronson, P.S. (1982) Biochim. Biophys. Acta 689, 161–164.
[26] Turner, R.J. and Moran, A. (1982) J. Membr. Biol. 70, 37–45.
[27] Sokol, P.P., Holohan, P.D. and Ross, C.R. (1985) J. Pharmacol. Exp. Ther. 233, 694–699.
[28] Brändle, E. and Greven, J. (1991) J. Pharmacol. Exp. Ther. 258, 1038–1045.
[29] Schäli, C., Schild, L., Overney, J. and Roch-Ramel, F. (1983) Am. J. Physiol. 245, F238–F246.
[30] Aronson, P.S. (1981) Am. J. Physiol. 240, F1–F11.
[31] Byers, T.L., Kameji, R., Rannels, D.E. and Pegg, A.E. (1987) Am. J. Physiol. 252, C663–C669.
[32] Sokol, P.P. and Gates, S.B. (1990) J. Pharmacol. Exp. Ther. 255, 52–58.
[33] Wright, S.H., Wunz, T.M. and Wunz, T.P. (1992) J. Membr. Biol. 126, 51–65.
[34] Miyamoto, Y., Tiruppathi, C., Ganapathy, V. and Leibach, F.H. (1989) Am. J. Physiol. 256, F540–F548.