Effect of Endogenous and Exogenous Polyamines on Organic Cation Transport in Rabbit Renal Plasma Membrane Vesicles

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ABSTRACT

The effect of several polyamines on the transport of organic cations \([N^1-^3H]methylnicotinamide (NMN), [^3H]NMN and \(^2H\)-tetraethylammonium, ([^3H]TEA)\) in rabbit renal brush border (BBMV) and basolateral membrane vesicles (BLMV) was studied using a rapid filtration assay. Under pH-driven conditions in BLMV, \([^3H]NMN\) transport was \textit{cis} inhibited approximately 30\% by the naturally occurring polyamines (cadaverine, putrescine, spermine and spermidine) and nearly 90\% by methylglyoxal bis(guanylhydrazone) (MGBG), a synthetic polyamine, mepiperphenidol and cimetidine, classical organic cation transport inhibitors. In the absence of a pH gradient, the capability of these agents to block \([^3H]NMN\) transport was diminished. The capability of these compounds to translocate the membrane was assessed by examining the phenomenon of counterflow. TEA, mepiperphenidol and MGBG produced trans stimulation of TEA uptake, whereas putrescine did not. Kinetic studies revealed that both putrescine and MGBG affected the \(K_m\) value for TEA transport while having a minimal effect on the \(V_{max}\) value. These results are most consistent with competitive inhibition. The specificity of polyamine inactivation of organic cation transport was assessed by examining what effect they had on the transport of another brush border transporter, \(D\)-glucose. These agents did not inhibit \(D\)-glucose transport. In addition, the effect of polyamines on the transport of organic cations in BLMV was studied. Only MGBG, cimetidine and mepiperphenidol inhibited organic cation transport. The results indicate that endogenous polyamines \textit{cis} inhibited the organic cation transporter in BBMV competitively, whereas the exogenous polyamine MGBG \textit{cis} inhibited competitively and produced trans stimulation. In contrast in BLMV, only MGBG was an effective inhibitor.

The polyamines cadaverine, putrescine, spermine and spermidine are ubiquitous polycationic molecules that exist in nature (for reviews see Tabor and Tabor, 1984). Although these chemicals are necessary for cell growth and differentiation, their exact roles in mediating these processes are not known (Pegg and McCann, 1988; Seiler and Heby, 1988). Many cells are capable of transporting polyamines (for review see Pegg, 1987), and intracellular polyamine stores have been shown to increase during renal hypertrophy and renal failure (Kirschbaum, 1984; for review see Fine and Norman, 1989). Cells undergoing rapid division such as those found in tumors require polyamines for growth (Seppanen et al., 1980). Polyamines are also required for parasitic growth in disease states (Balana-Fouce et al., 1989). In fact, drugs that interfere with polycationic transport and/or intracellular polycationic pathways have shown promise as antiparasitic and antineoplastic chemotherapeutic agents (for review see Pegg, 1988; Pegg and McCann, 1988).

Recently, gentamicin, a polyamine antibiotic, has been demonstrated to interact with the renal brush border membrane organic cation transporter (Sokol et al., 1989a). The transport of organic cations in the kidney has been studied and recently reviewed (McKinney, 1988; Brater et al., in press). The transport across the basolateral membrane is electrogenic (Takano et al., 1984; Wright and Wunz, 1987) or can involve an organic cation/organic cation exchange mechanism (Montrose-Rafizadeh et al., 1989; Sokol and McKinney, 1990). The major mechanism of transport across the brush border membrane is via an electroneutral organic cation/H\(^+\) antiporter (Sokol et al., 1985). The H\(^+\) gradient provides the energy necessary to produce concentrative transport (Holohan and Ross, 1981).

Polyamines are also capable of inhibiting the intestinal organic cation transporter (Miyamoto et al., 1988). Although the transport of putrescine has been examined in the renal epithelial cell line LLC-PK1 (De Smedt et al., 1989; Van Den Bosch et al., 1990), whether there is any overlapping specificity with the organic cation transporter was not studied. Therefore, the

ABBREVIATIONS: BLMV, brush border membrane vesicles; BLMV, basolateral membrane vesicles; NMN, \(N^1\)-methylnicotinamide; TEA, tetraethylammonium; MGBG, methylglyoxal bis(guanylhydrazone); HEPES, 4-(2-hydroxyethyl)-1-piperazineethanesulfonic acid.
objective of this study was to determine whether endogenous polyamines share a common transport mechanism with organic cations in renal plasma membrane vesicles and to make a comparison with MGBG, a synthetic polyamine that is a blocker of endogenous polyamine transport (Janne et al., 1981; Gordonsmith et al., 1983; Sokol et al., 1989b).

**Methods**

Membrane preparation. BBMV and BLMV were isolated from the outer cortex of white New Zealand rabbit kidneys of both sexes (5-7 lb) by previously described methods (Grasal and Aronson, 1986; McKinney and Kunemann, 1985). The enzyme enrichment for the brush border membrane marker alkaline phosphatase, assayed by a kit (Sigma Diagnostics ALP, procedure no. 245) was approximately 8-fold with respect to the homogenate, whereas the enrichment for the basolateral membrane marker Na, K -ATPase was approximately 10-fold (McKinney and Kunemann, 1985). The level of cross-contamination of BBMV with BLMV and BLMV with BBMV was negligible inasmuch as the enrichment of alkaline phosphatase (brush border membrane) and Na,K-ATPase (basolateral membrane) in BLMV and BBMV was less than 10. Protein concentration was assayed using bovine serum albumin as a standard (Lowry et al., 1951). Both purified membrane fractions were suspended in a medium of 10 mM HEPES, 50 mM K+ gluconate and 200 mM mannitol, pH 7.5 or 6.0, via overnight dialysis to assure complete equilibration (Lipkowitz and Abramson, 1989) and stored at ~70°C until use. No difference was observed between the transport capacity of freshly prepared vesicles and those that were frozen.

**Transport measurements.** The transports of [3H]N1-methylnicotinamide and [3H]tetraethylammonium were assayed by a rapid filtration technique (Sokol et al., 1985; Sokol et al., 1986) using a 10-place filtering manifold (Hoefer Scientific Instruments, San Francisco, CA). The transport measurements of MN were conducted at 37°C whereas those of TEA were conducted at 25°C. The reason for using the different temperature is that the TEA/TEA exchange mechanism (McKinney and Kunnemann, 1987), inhibited 90%, and the transport of the radiolabeled substrate. It appears that there are two classes or levels of cis inhibitors, one group that is quite effective and the other group less so. The more potent class inhibited MN transport approximately 90%. MGBG, the synthetic polyamine, cis inhibited MN transport 87%; meipiperphenidol, the classical organic cation transport inhibitor, blocked transport 90% and cimetidine, a histamine H2-receptor antagonist that is also transported via the same mechanism (McKinney and Kunemann, 1987), inhibited 90%. The second class of cis inhibitors was less effective in inhibiting transport. It consists of the naturally occurring polyamines that cis inhibited pH-driven MN transport approximately 30% (cadaverine, 24%; spermidine, 30%; putrescine, 30%; and spermine, 31%). The inhibitions produced by these compounds, although statistically significant, were significantly less than that produced by the potent organic cation transport inhibitors meipiperphenidol, cimetidine and MGBG. The extent of cis

**Results**

**Cis inhibition.** The cis effect of several organic cations and polyamines on pH-driven MN transport in BBMV was examined (fig. 1). Cis refers to the chemical present on the same side of the membrane as the radiolabeled substrate. It appears that there are two classes or levels of cis inhibitors, one group that is quite effective and the other group less so. The more potent class inhibited MN transport approximately 90%. MGBG, the synthetic polyamine, cis inhibited MN transport 87%; meipiperphenidol, the classical organic cation transport inhibitor, blocked transport 90% and cimetidine, a histamine H2-receptor antagonist that is also transported via the same mechanism (McKinney and Kunemann, 1987), inhibited 90%. The second class of cis inhibitors was less effective in inhibiting transport. It consists of the naturally occurring polyamines that cis inhibited pH-driven MN transport approximately 30% (cadaverine, 24%; spermidine, 30%; putrescine, 30%; and spermine, 31%). The inhibitions produced by these compounds, although statistically significant, were significantly less than that produced by the potent organic cation transport inhibitors meipiperphenidol, cimetidine and MGBG. The extent of cis

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**Fig. 1.** Effect of organic cations and polyamines on pH-driven MN transport in BBMV. BBMV were suspended in 10 mM HEPES, 50 mM K+ gluconate, 200 mM mannitol, pH 6.0. The uptake of 50 μM [3H]HMN in the same medium, pH 7.5, was examined for 15 sec in the presence of 1.0 mM concentrations of the chemicals as indicated in the figure. The data are presented as percentages of the control transport (n = 5-8). Insert, representation of the experimental conditions. *: radiolabeled MN; †: organic cation or polyamine being tested. * P < 0.05 (experimental vs. control). § P < 0.05 (weak inhibitors vs. potent inhibitors).
inhibition of organic cation transport produced by the natural polyamines was quite similar to that found previously for gentamicin, a polyamine antibiotic, in dog BBMV (Sokol et al., 1989a). None of these agents altered the equilibrium uptake (60 min) of NMN, indicating that the effects did not result from a loss of vesicle integrity.

The effect of these agents on non-pH-driven NMN transport was also assessed (fig. 2). As seen above, evidently these agents represent two classes of interaction with the NMN transport system. MGBG, cimetidine and mepiperphenidol cis inhibited transport approximately 75% (MGBG, 72%; cimetidine, 72%; mepiperphenidol, 79%). Putrescine and cadaverine did not inhibit NMN transport significantly (15 and 19%, respectively), whereas spermine and spermidine did (23 and 36%, respectively). Unexpectedly, spermidine also had a major effect on the NMN equilibrium distribution (25%). Thus, unlike the other chemicals tested, the effects of spermidine on NMN transport cannot be clearly ascertained under these conditions. We attempted to increase the chemical concentrations of these compounds up to 10 mM in order to determine whether the cis inhibition of non-pH-driven NMN transport could be increased. Unfortunately, at this concentration every chemical had an effect on the NMN equilibrium distribution so the inhibitory patterns could not be clearly assessed. The range of inhibitions of equilibrium values was from a low of 13% for cadaverine to a high of 43% for spermidine. This observation is important for two reasons. First, it underscores the necessity of including an equilibrium time point inasmuch as effects on transport could be secondary to vehicle disruption. Second, it points out that using unphysiologically high concentrations of chemicals does not always lead to useful information about a biologic system.

Concentration dependence. Dose response curves for the potent inhibitors of NMN transport were constructed (fig. 3). All three inactivated transport nearly 80% over a 2-fold log range. Cimetidine and mepiperphenidol had identical IC50 values (8 ± 4 μM, 8 ± 2 μM, respectively), whereas MGBG was approximately 4-fold greater (30 ± 6 μM).

Countertransport. Although both naturally occurring and synthetic polyamines produced cis inhibition of organic cation transport, it is necessary to demonstrate that they produce trans stimulation if it is to be concluded that polyamines and organic cations are transported by the same system. Trans refers to the compartment toward which the radiolabel is moving. If a compound is a substrate for a given transport system,
then when vesicles loaded with the compound are diluted into medium containing the radioligand, there should be an acceleration of radiolabel transport into the vesicle. This is consistent with the saturated transporter being capable of reorienting more readily across the membrane than an unsaturated one. The effect of NMN preloading on NMN uptake into BBMV was examined (fig. 4). A trans stimulation of NMN transport was seen that was significant at the early time points (5 sec, 15 sec). However, there was no accumulation of NMN above equilibrium, in contrast to what has been shown for NMN in dog BBMV (Holohan and Ross, 1981). When TEA was used as a substrate (fig. 5), an overshoot of nearly 1.5 times the equilibrium value was obtained that peaked at 30 sec. The uptake was linear for 20 sec ($r = 0.99$). In addition, we found that NMN preloading stimulated TEA uptake 2 times the equilibrium value ($217 \pm 10\%$, $n = 3$), whereas TEA preloading produced a stimulation of NMN ($68 \pm 22\%$ vs. $24 \pm 4\%$ in the unloaded control) but no overshoot. Therefore, because TEA was a much better organic cation for demonstrating uphill transport, the trans effects of mepiperphenidol, MGBG and putrescine, chosen as a prototypic endogenous polyamine, on TEA transport were examined at 30 sec, the time corresponding to the peak TEA/TEA overshoot. TEA, mepiperphenidol and MGBG preloading produced trans stimulation of TEA uptake ($349 \pm 80$, $232 \pm 5$, $163 \pm 14\%$, respectively), whereas putrescine did not (fig. 6). Under these conditions only TEA was capable of producing an overshoot ($202 \pm 35\%$). The mepiperphenidol and MGBG trans stimulations were close to the equilibrium value ($100 \pm 14$, $1800 \pm 66\%$, respectively), whereas the control trans uptake was $49 \pm 5\%$ of the equilibrium value.

![Fig. 4. Effect of NMN preloading on NMN transport in BBMV. BBMV suspended in 10 mM HEPES, 50 mM K+ gluconate, 200 mM mannitol, pH 7.5, were loaded with 1.0 mM concentrations of NMN (C) or buffer control (B) for 3 hr. The uptake of 50 µM [3H]NMN in the same buffer, pH 7.5, was examined for 30 min (1800 sec). The data are presented as percentages of control transport ($n = 3$). Insert, representation of the experimental conditions. * radiolabeled NMN. *P < 0.05.](image1)

![Fig. 5. Effect of TEA preloading on TEA transport in BBMV. BBMV suspended in 10 mM HEPES, 50 mM K+ gluconate, 200 mM mannitol, pH 7.5, were loaded with 1.0 mM concentrations of TEA (C) or buffer control (B) for 3 hr. The uptake of 50 µM [3H]TEA in the same buffer, pH 7.5, was examined for 1 hr (3600 sec). The data are presented as percentages of control transport ($n = 3$). Insert, representation of the experimental conditions. * radiolabeled TEA.](image2)

![Fig. 6. Trans effect of organic cations and polyamines on TEA in BBMV. BBMV suspended in 10 mM HEPES, 50 mM K+ gluconate, 200 mM mannitol, pH 7.5, were loaded with 1.0 mM concentrations of TEA, mepiperphenidol (Mepi), MGBG and putrescine (Putr) for 3 hr. The uptake of 50 µM [3H]TEA in the same buffer, pH 7.5, was examined for 30 sec. The data are presented as percentages of control transport ($n = 3$). Insert, representation of the experimental conditions. * radiolabeled TEA; X, organic cation or polyamine being tested. *P < 0.05.](image3)

![Fig. 7. Kinetic evaluation of the effect of putrescine on TEA uptake in BBMV. Lineweaver-Burk transformations are presented for 5-sec TEA transport (pH = 6.0, pH0 = 7.5) in the presence (○) and absence (□) of 1 mM putrescine (n = 3).](image4)

**Table 1**

<table>
<thead>
<tr>
<th>Kinetic parameters for TEA transport</th>
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<tbody>
<tr>
<td>Transport of TEA (pH = 6.0, pH0 = 7.5) was examined for 5 sec in the presence of 1 mM concentrations of putrescine or MGBG (n = 3).</td>
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<tr>
<td></td>
<td>$K_m$ (µM)</td>
<td>$V_{max}$ (nmol/min x mg protein/milliliter)</td>
</tr>
<tr>
<td>Control TEA</td>
<td>80 ± 7</td>
<td>3.3 ± 0.4</td>
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<tr>
<td>+ Putrescine</td>
<td>100 ± 14</td>
<td>3.2 ± 0.7</td>
</tr>
<tr>
<td>+ MGBG</td>
<td>1800 ± 66*</td>
<td>2.4 ± 1.1</td>
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*P < 0.05 vs. control.

(116 ± 15, 80 ± 12%, respectively), whereas the control transport was 49 ± 5% of the equilibrium value.

**Kinetics.** Kinetic studies were undertaken to further clarify the inhibition of the organic cation transporter by putrescine and MGBG. Transport was linear for at least 10 sec so 5-sec uptakes were used to get initial rates. The effect of putrescine on TEA uptake is presented in the form of a Lineweaver-Burk plot (fig. 7). Although putrescine increased the $K_m$ value of TEA ($80 \pm 7$ vs. $100 \pm 14$ µM in the presence of putrescine), this difference was not significant ($P > 0.05$, table 1). The $V_{max}$ values for both were indistinguishable ($3.2 \pm 0.4$ vs. $3.2 \pm 0.7$ nmol/min x mg in the presence of putrescine). In addition, the kinetic parameters for TEA uptake in the presence of MGBG were determined. MGBG increased the $K_m$ value to 1800 ±
66 μM (P < 0.05) while producing a slight decrease in the $V_{\text{max}}$ value to 2.4 ± 1.1 nmol/min × mg (P > 0.05). These results are most consistent with putrescine and MGBG being competitive inhibitors of TEA transport.

**Specificity.** The specificity of the polyamine effect on organic cations was further examined by determining the effect of these agents on the activity of an independent brush border transport system, the electrogenic Na+-dependent D-glucose transporter (for review see Kinne et al., 1984). Organic anion transport was not examined inasmuch as rabbit BBMV lack an organic anion exchanger (Guggino and Guggino, 1989). A combination of an inwardly directed Na+ gradient and an inside negative PD produced a 3-fold overshoot (3.2 ± 0.4). This value represents the control transport ($n = 3$). Phloridzin, an inhibitor of the Na+-dependent D-glucose transporter (Moran et al., 1988; Turner, and George, 1984) inhibited transport 93%. None of the polyamines or organic cations inhibited transport of D-glucose. In fact, two polyamines (putrescine and cadaverine) produced a slight (115 ± 8, 116 ± 8%, respectively) but significant inhibition of D-glucose transport (P < 0.05). Although the magnitude of this stimulation is presently unclear, it could reflect an allosteric modification of the transporter. Such a conclusion is not unfounded inasmuch as polyamines are required for cell growth (Pegg and McCann, 1988). Activation of D-glucose transport would be important to maintain adequate energy reserves for cells.

**Basolateral membrane transport.** The effect of polyamines on basolateral organic cation transport was also examined (fig. 8). Cimetidine, spermine, MGBG and mepiperphenidol cis inhibited NMN transport by 20, 21, 32 and 40%, respectively. However, because spermine also had an effect on the equilibrium distribution of NMN (40%), its effect on transport could not be determined unequivocally. Recently, it has been shown that concentrative transport of organic cations in BLMV can be studied by following the TEA/TEA exchange mechanism (Montrose-Rafizadeh et al., 1989; Sokol and McKinney, 1990). Therefore, we examined the effect of these agents on TEA/TEA exchange (fig. 9). The results demonstrated that although the cis inhibitions produced by mepiperphenidol and MGBG were increased to 90 and 69%, respectively, the effect of the natural polyamines was still negligible.

**Discussion**

The objective of this study was to determine the mechanism by which endogenous polyamines (cadaverine, putrescine, spermidine and spermine) and an exogenous one (MGBG) interact with the renal transport mechanism for organic cations. All five polyamines tested herein cis inhibited NMN transport in BBMV. However, it appears that there are two classes of inhibition. The naturally occurring polyamines were weak inhibitors, whereas the synthetic polyamine MGBG, an inhibitor of endogenous polyamine transport (Janne et al., 1981), was a potent inhibitor of NMN transport. In fact, the IC50 value for MGBG inhibition of NMN transport was on the same order of magnitude as the IC50 values for mepiperphenidol and cimetidine, potent inhibitors of organic cation transport. The effect of the polyamines was specific for two reasons. First, there was no perturbation of the equilibrium values under most transport conditions using 1 mM concentrations with the exception of spermidine under non-pH driven transport. Second, the polyamines did not inhibit the transport of D-glucose, another renal brush border transport system. The counterflow studies demonstrated that only the potent cis inhibitor of NMN transport, MGBG, was capable of trans stimulating TEA transport, whereas the weak cis inhibitor, putrescine, did not. These experiments imply that under the extant conditions, MGBG was translocated across the membrane but putrescine was not. However, the kinetic studies revealed that both putrescine and MGBG are competitive inhibitors of organic cation transport. It is possible that putrescine, a weak competitive inhibitor, is translocated across the membrane albeit inefficiently to demonstrate a trans stimulation. The inability to demonstrate trans stimulation could be a limitation of vesicle technology when dealing with low affinity inhibitors. Nevertheless, putrescine may bind competitively to the substrate binding site and not be translocated. Although the latter situation appears to be remote, at this point it is not possible to discern between these two possibilities. In contrast to the BBMV, in BLMV only the synthetic polyanine MGBG inhibited organic cation transport. These results are consistent with endogenous polyamines being capable of interacting competitively with the renal brush border organic cation transporter, whereas the exogenous polyanine MGBG can inhibit organic cation transport in both BBMV and BLMV. Last, our findings have biologic relevance because the concentrations of polyamines that were used in these studies were not supraphysiological; polyamine concentrations in normal mammalian cells are approximately 1 mM (Russell, 1983) and in tubular fluid can reach several hundred micromolar in disease states (Russell, 1971).
Recently we demonstrated that the polyamine antibiotic gentamicin was a substrate for the renal organic cation transporter in BBMV and that the protective effect of Ca\(^{2+}\) channel blockers on gentamicin nephrotoxicity could be due to competition for a common transport mechanism across the brush border membrane (Sokol et al., 1989a). In that study, gentamicin was a weak cis inhibitor of NMN transport, whereas verapamil, a Ca\(^{2+}\) channel blocker, was a potent cis inhibitor. Both were capable of trans stimulating NMN transport and produced an overshoot over the equilibrium value, which is indicative of concentrative transport. In the present study, the naturally occurring polyamines produced a similar cis inhibition of NMN transport as was observed with gentamicin. The difference, however, is that only the synthetic polyamine MGBG produced trans stimulation of organic cations. Putrescine did not trans stimulate transport. In fact, at 30 sec MGBG and mepiperphenidol preloading did not produce a significant overshoot of TEA transport, whereas TEA did. Perhaps an overshoot might have been observed at a time point greater than 30 sec. Additionally, the lack of overshoot could be due to the carryover of the compounds during the dilution with the reaction solution resulting in some cis inhibition.

In this study, NMN preloading was not capable of producing an overshoot of NMN uptake in BBMV. In contrast, other investigators have found NMN preloading to produce an overshoot of NMN uptake. Holohan and Ross (1980) first demonstrated uphill transport of NMN in canine BBMV. The peak overshoot for NMN preloading was approximately 125% of the equilibrium value. The organic cation that produced the greatest overshoot was choline, with the uptake being approximately 180% of the equilibrium value. Rafizadeh et al. (1986) found that in rabbit BBMV, NMN preloading produced a peak overshoot of NMN around 180% and TEA preloading produced an overshoot of TEA around 305% of the equilibrium value. Similarly, Wright (1985) found NMN preloading to stimulate NMN uptake to around 150% of the equilibrium value. In addition, Rafizadeh et al. (1986) examined the effects of TEA preloading on NMN uptake and NMN preloading on TEA uptake. The former produced a significant overshoot (230%), whereas the latter produced a negligible one (130%). In contrast, we found the converse; NMN preloading produced an overshoot of TEA uptake (217%), whereas TEA preloading did not produce an overshoot of NMN uptake (68%). It is not clear why NMN preloading failed to produce an overshoot of NMN uptake in this study whereas TEA preloading was capable of producing an overshoot of TEA uptake. The difference was not due to BBMV being more permeable to NMN than to TEA inasmuch as mepiperphenidol was effective in blocking the transport of both compounds approximately 90%. Because NMN did trans stimulate its own uptake without producing an overshoot, we believe that the differences among the various laboratories studying organic cation transport are quantitative rather than qualitative. This does not rule out the possibility for both inter- and intraspecies differences responsible for the extant variations.

The IC\(_{50}\) values for the inhibition of NMN transport by mepiperphenidol and cimetidine were identical (8 ± 2 and 8 ± 4 \(\mu\)M, respectively); MGBG had a value of 30 ± 6 \(\mu\)M. All of these IC\(_{50}\) values are similar and agree quite well with the IC\(_{50}\) values obtained for other potent organic cation transport inhibitors in canine BBMV: verapamil, 20 \(\mu\)M; mepiperphenidol, 16 \(\mu\)M; MPP, 15 \(\mu\)M; acridine orange, 5 \(\mu\)M (Sokol et al., 1987; Sokol et al., 1989a; Sokol et al., 1990). It appears that despite some differences in NMN overshoot data discussed above, there are striking similarities in the IC\(_{50}\) values for potent organic cation transport inhibitors in canine and rabbit BBMV. Although interspecies variations in the renal handling of organic cations make it difficult to formulate unequivocal deductions from one species to the next, it appears that some general observations do hold, i.e., mepiperphenidol is a potent organic cation transport inhibitor.

MGBG, in contrast to the naturally occurring polyamines, is a potent inhibitor of organic cation transport. What could be the reason for this difference? Some insight can be gained by taking a look at the chemical structures. The endogenous polyamines putrescine, spermine and spermidine are structurally related in the following fashion. Putrescine (1,4-butanediamine) represents the backbone of this series because the succeeding members are formed by the addition of an aminopropyl group. The addition of this group to putrescine forms spermidine [N,N'bis(3-aminopropyl)-1,4-butanediamine] and the subsequent addition forms spermine [N,N,N',N''tetraakis-(3-aminopropyl)-1,4-butanediamine] (for review see Pegg and McCann, 1988). Cadaverine, on the other hand, differs from putrescine by a methylene group (1,5-pentanediamine). All four of these compounds that fall under the general category of alkylamines contain two primary amines. In addition, spermidine has one secondary amine group and spermine has two. MGBG differs in that it contains two guanyl groups and, thus, is classified as a tertiary amine. A rank order of amine interactions with the organic cation transporter is known. In general, quaternary amines are more potent inhibitors of organic cation transport than tertiary amines, etc. Thus, it appears reasonable on the basis of the chemical structures that MGBG, a tertiary amine, is a more potent inhibitor of organic cation transport than the naturally occurring polyamines, which are a combination of secondary and primary amines. It is also notable that a functional moiety of MGBG, guanidine, has been shown recently to be transported via an organic cation/H\(^+\) antiporter that is distinct from the NMN and TEA transport system in rabbit renal BBMV (Miyamoto et al., 1989). Similarly, cimetidine and amiloride, which are both guanidine-like molecules, are substrates for the organic cation transporter (Gisclon et al., 1987; Wright and Wunz, 1989). Perhaps this helps to explain why MGBG is such a strong organic cation transport inhibitor.

There is growing support for the multiplicity of organic cation transport systems in the kidney (Miyamoto et al., 1989; Sokol and McKinney, 1990). The inability of the endogenous polyamines to totally inhibit NMN transport raises the possibility that these compounds may interfere with a subclass of the organic cation transporters, e.g., guanidine, whose transport is distinct from the NMN and TEA system (Miyamoto et al., 1989). It is also possible that the endogenous polyamines have a separate system and the inhibition of organic cation transport seen here does not reflect the dominant transport pathway.

In recapitulation, the endogenous and exogenous polyamines are capable of inhibiting the transport of organic cations in renal BBMV. The synthetic polyanine MGBG was a potent competitive inhibitor of transport capable of trans stimulating organic cation uptake, whereas the naturally occurring polyanine putrescine was a weak competitive inhibitor incapable of trans stimulating organic cation uptake. It is possible that the effect of endogenous polyamines on organic cation transport is due to some overlapping specificities among amine transport.
systems and that these compounds, in fact, might be transported via a separate polyamine transport system.

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References


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