Guanidine Uptake by HeLa Cells and Its Inhibition by Some Antiguanidine Agents

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SUMMARY

To understand better the action of guanidine and its antagonists on poliovirus replication, guanidine uptake by HeLa cells was studied. It was discovered that guanidine entered HeLa cells by at least two different mechanisms. At low concentrations (< 2 mM), it was transported mostly by a carrier-mediated, saturable mechanism with an apparent affinity constant of 1.26 mM-guanidine. About a third of uptake by this mechanism was sensitive to valinomycin and possibly dependent on membrane potential difference. At higher concentrations (5 to 10 mM), transport was predominantly by a non-saturable, low affinity process. The carrier-mediated transport mechanism was similar to the organic cation H+ exchanger-mediated excretion of organic cations from the kidney, because it was inhibited by organic cations and by high [Li+] . Physiological [Na+] caused less but significant inhibition of guanidine uptake by HeLa cells. Approximately 20% of total uptake could not be inhibited with organic cations and was probably due to diffusion. The antiguanidine agents choline, dimethylethanolamine and tetraethylammonium, but not methionine, inhibited guanidine uptake by HeLa cells. The inhibition caused by these agents depended on their concentration and more or less paralleled their reported ability to block guanidine inhibition of poliovirus replication. Even though choline inhibited guanidine uptake it appeared to reverse this inhibition of virus replication primarily by blocking the intracellular action of guanidine. Some unexplained previous observations on the action of guanidine and its antagonists were discussed in view of the results of this study.

INTRODUCTION

Guanidine in relatively low concentrations inhibits the replication of many picornaviruses (Caliguiri & Tamm, 1973), togaviruses (Friedman, 1970) and plant viruses (Varma, 1968; Dawson, 1976) without any apparent effect on cellular metabolism (Tamm & Eggers, 1963). It acts during the early period of poliovirus replication (Eggers et al., 1965) preventing the synthesis of virus-specific RNA species (Tershak, 1982) presumably by interfering with the formation of viral RNA polymerase rather than with the activity of this enzyme (Baltimore et al., 1963). There is evidence for (Cooper et al., 1970; Korant, 1977) and against (Cole & Baltimore, 1973) the involvement of poliovirus structural proteins in guanidine sensitivity. More recent reports, on the other hand, implicate the picornavirus non-structural protein 2C as the target of guanidine action (Anderson-Sillman et al., 1984; Pincus et al., 1986; Pincus & Wimmer, 1986). How guanidine interacts with the putative target and inhibits virus-specific RNA synthesis are unknown.

Numerous agents, of which a good many are methylated or ethylated amines and amino alcohols, are known to reverse guanidine inhibition of poliovirus replication (Lwoff, 1965; Loddo et al., 1966; Philipson et al., 1966). Several of these agents, like choline and methionine, contain methyl groups in their structure. The possibility that inactivation of guanidine, through methylation by methyl groups donated by guanidine antagonists, is the mechanism of their
action is ruled out by the findings that it is not methylated in KB cells by methionine or choline (Lwoff, 1965) and that S-adenosylmethionine (Philipson et al., 1966) and betaine (Loddo et al., 1966) are ineffective in reversing its inhibition of poliovirus replication. It has been shown that the action of antiguanidine agents is very much dependent on the host cell type (Philipson et al., 1966; Mosser et al., 1971).

It was recently observed that guanidine uptake by HeLa cells and guanidine inhibition of poliovirus replication in these cells were inversely related to [Na⁺] in the medium. In the presence of Na⁺-deficient medium there was both increased uptake and enhanced inhibition of virus replication (Nair, 1987). These findings suggested that monovalent cation concentration in the medium influences guanidine uptake and raised the possibility that some antiguanidine agents may act by inhibiting it. Therefore, the mechanism of guanidine uptake by HeLa cells and the effects of guanidine antagonists on it were investigated. The results revealed that diffusion and, more importantly, a carrier-mediated mechanism were involved in the uptake of guanidine by HeLa cells. Guanidine transport via the carrier-mediated mechanism was inhibited by organic cations that block guanidine inhibition of poliovirus replication.

METHODS

Cells and virus. These studies were carried out with monolayer cultures of HeLa cells (HeLa Ohio, Flow Laboratories) and poliovirus type 2. The cells were grown in the presence of MEM containing 10% calf serum and antibiotics. Virus growth was in the presence of MEM containing 5% calf serum. The HeLa cell line used in this study was free of mycoplasma contamination as judged by the Hoechst stain technique (Chen, 1977). [14C]Guanidine carbonate (sp. act. 116-0 mCi/mmole) and [14C]tetraphenylphosphonium bromide (sp. act. 19-2 mCi/mmole) were obtained from New England Nuclear. All other chemicals used in this study were of reagent grade or cell culture-tested and were purchased from Sigma.

Guanidine uptake. Replicate monolayer cultures in 35 mm Corning plastic dishes were washed with warm (36 °C) phosphate-buffered saline (PBS) and then with the appropriate non-radioactive uptake medium. The cultures were then incubated at 36 °C with warm, radioactive uptake medium containing 0.54 μCi/ml of [14C]guanidine. In some experiments preincubation with non-radioactive uptake medium for specified periods (see Results) preceded incubation with radioactive uptake medium. The concentration of guanidine in the uptake medium was 4.7 μM unless otherwise indicated. The nature and concentration of monovalent cations in the uptake medium and the duration of uptake varied for different experiments and are indicated in Results. Uptake of radiolabelled guanidine was terminated by quickly removing the radioactive medium and washing the cultures four times with cold (4 °C) PBS (2-5 ml/wash per culture) containing 5 mM-unlabelled guanidine. The entire washing procedure took 30 to 35 s per dish. To determine background radioactivity in each experiment, two cultures were processed exactly as the experimental cultures except that the radioactive uptake medium was added to them after they had been chilled to 4 °C and they were washed immediately. Chilling was necessary in order to minimize significant cellular uptake of guanidine which occurs rapidly at 36 °C. The background radioactivity so obtained varied from about 10 to 20% of total cell-associated radioactivity in different experiments. After solubilization of the cultures with alkali and neutralization of the solution, cell-associated radioactivity was determined by scintillation counting with a Triton X-100-toluene-based scintillation fluid.

Membrane potential difference (Δψ). Relative changes in Δψ of cultures preincubated with media varying in cation composition and concentration were monitored by measurement of the initial rate of uptake of tetraphenylphosphonium (TPP⁺) bromide (Lichtstein et al., 1979). Monolayer cultures in 35 mm dishes were preincubated with the appropriate media as indicated, and further incubated with the respective media containing 0-05 μCi/ml of TPP⁺ for 1 min at 36 °C. Uptake of TPP⁺ was terminated by removal of the radioactive medium and four rapid washes of the cultures with prewarmed PBS using 2-5 ml/wash per dish. Background radioactivity was obtained with cultures that were treated similarly except that the radioactive medium was added to and immediately removed from them. The background radioactivity accounted for about 10% of cell-associated radioactivity, which was determined by scintillation counting as described above.

RESULTS

Time course of guanidine uptake by HeLa cells

Since guanidine uptake by KB cells was not temperature-dependent and reached equilibrium rapidly, Lwoff (1965) concluded that it must occur by diffusion. A preliminary experiment indicated that uptake by HeLa cells was temperature-dependent. At 36 °C, the uptake was linear
Guanidine uptake and antiguanidine action

Fig. 1. Time course of guanidine uptake by HeLa cells. Monolayer cultures were incubated at 36 °C with MEM containing radioactive guanidine and buffered with 25 mM-HEPES in place of sodium bicarbonate. At intervals shown, the cultures were washed with PBS and cell-associated radioactivity was determined. See Methods for details of procedure. Each point represents the average of duplicate determinations.

Fig. 2. Effect of guanidine concentration in the medium on the initial rate of guanidine uptake by HeLa cells. Solutions containing radioactive guanidine and increasing amounts of unlabelled guanidine were prepared in HEPES-buffered MEM and the initial rate of uptake by monolayer cultures in the presence of each concentration of unlabelled guanidine was determined as described in Methods. (a) Each point except the last two on the graph represents the average of duplicate determinations. The last two points are averages of four determinations.

for about 2 min and did not approach equilibrium until about 10 min (Fig. 1). These results suggested that diffusion alone was not the mechanism of uptake by HeLa cells.

Effect of guanidine concentration on the initial rate of guanidine uptake by HeLa cells

To understand better the processes involved in guanidine transport, the effect of increasing concentration on guanidine uptake was studied. The results (Fig. 2a) indicated that uptake by HeLa cells was not linearly related to its concentration in the medium, confirming the suspicion that guanidine entry was not by diffusion alone. However, the uptake mechanism did not appear to become saturated with increasing guanidine concentration. A plot of the uptake rate (v) against v/guanidine concentration (Fig. 2b) suggested that two mechanisms were involved in uptake, one operating at low concentrations and the other becoming predominant at high concentrations. The correlation coefficient for the straight line representing the high affinity transport mechanism (Fig. 2b) was calculated to be 0.99. The apparent \( v_{\text{max}} \) (maximum velocity) and \( K_i \) (affinity constant) values for this transport mechanism were 4.14 nmol/mg protein/min and 1.26 mM, respectively. The low affinity transport process at high concentrations probably represented diffusion because the large excess of unlabelled guanidine would have prevented carrier-mediated transport of radioactive guanidine. If the diffusion component of transport were subtracted from the uptake rates in Fig. 2, \( v_{\text{max}} \) and \( K_i \) values different to those mentioned above would be obtained for the high affinity transport process. In the context of the antiviral action of guanidine, only the high affinity transport mechanism active at low guanidine levels would be relevant, since the useful range of virus inhibitory concentrations is 0.2 to 2 mM. Therefore, guanidine transport at high concentration was not further studied.
Influence of monovalent cations on guanidine transport and $\Delta \psi$

It was shown that incubation of HeLa cells with monovalent cation-deficient medium resulted in increased guanidine uptake and reduced intracellular [Na+] (Nair, 1987). To determine whether such treatment affected $\Delta \psi$, cultures were preincubated with media of various monovalent cation concentrations and compositions and guanidine or TPP$^+$ uptake by these cultures was determined. TPP$^+$ uptake was used as a measure of relative changes in $\Delta \psi$ (Lichtstein et al., 1979). The results are summarized in Table 1. Cultures incubated with medium of low total monovalent cation concentration (50 mM-Na$^+$, 5 mM-K$^+$) took up more than double the amount of guanidine and significantly more TPP$^+$ than cultures preincubated with medium containing physiological [Na$^+$]. Replacement of missing Na$^+$ with K$^+$ in the preincubation medium greatly reduced TPP$^+$ uptake as expected (Lichtstein et al., 1979) and caused some reduction in the uptake of guanidine. When choline was used instead of K$^+$ in Na$^+$-deficient preincubation medium, TPP$^+$ uptake was significantly reduced and guanidine uptake was reduced to a third. These results suggested that guanidine uptake by HeLa cells was inhibited by high levels of choline and Na$^+$, and was possibly sensitive to changes in $\Delta \psi$.

Organic cation-sensitive guanidine transport by HeLa cells and the relative involvement of different transport mechanisms

The results presented above suggested that the major component of guanidine transport by HeLa cells was sensitive to inhibition by choline which, like guanidine, is an organic cation. In this respect the transport mechanism resembled the mechanism by which organic cations are excreted through kidney tubular epithelium by the carrier cation H$^+$ exchanger (for review, see Ross & Holohan, 1983). Transport by this carrier can be competitively inhibited by organic cations including the drug cimetidine (Takano et al., 1985). Also, organic cation transport by renal brush border membrane vesicles is inhibited by 150 mM-Li$^+$ but not by Na$^+$ (Holohan & Ross, 1981). The role of $\Delta \psi$ in guanidine transport can be examined by eliminating $\Delta \psi$ with valinomycin and high [K$^+$] in the uptake buffer. It was therefore possible to confirm the involvement of an organic cation H$^+$ exchanger-like carrier in guanidine uptake by HeLa cells and to estimate the contribution of different transport mechanisms to total guanidine transport. This was achieved by determining uptake from high K$^+$ buffer in the presence or absence of valinomycin, choline or cimetidine and from high Na$^+$ or Li$^+$ buffer. The results (Table 2) revealed that about a third of total uptake was inhibited by valinomycin, whereas about 80% of uptake was inhibited by choline or cimetidine. The extent of inhibition of total uptake by choline or cimetidine was not increased by the simultaneous presence of valinomycin (C. N. Nair, unpublished results) suggesting that valinomycin-sensitive uptake was also inhibited by organic cations. The results also indicate strong inhibition by high [Li$^+$] and significant inhibition by high [Na$^+$] of guanidine uptake. Some inhibition could be observed with 10 mM-Li$^+$ but not with 10 mM-Na$^+$ (results not shown). The residual uptake which could not be inhibited by organic cations was probably due to diffusion.

Effect of antiguanidine agents on guanidine uptake

Choline is one of several agents that block guanidine inhibition of poliovirus replication (Lwoff, 1965; Loddo et al., 1966; Philipson et al., 1966). Like choline, some antiguanidine agents are organic cations whereas several others like methionine are not. In view of the inhibition of guanidine uptake by organic cations (Table 2) it was of interest to determine whether representative antiguanidine agents inhibited uptake in a concentration-dependent manner. Therefore, valinomycin-insensitive uptake was studied in the presence of various concentrations of the guanidine antagonists choline, tetraethylammonium (TEA), dimethylethanolamine (DMEA) and methionine. The results presented in Fig. 3 indicate that uptake was unaffected by 0.1 mM-methionine but was slightly elevated in the presence of 1 and 10 mM-methionine. On the other hand, choline, TEA and DMEA produced dose-dependent inhibition of guanidine uptake. Significant inhibition of uptake was caused by 0.1 mM-choline or DMEA, the latter being slightly more effective. Previous reports indicate that DMEA is more active than choline.
Fig. 3. Effects of inhibitory agents on valinomycin-insensitive guanidine uptake by HeLa cells. Monolayer cultures were preincubated at 36 °C with 25 mM-HEPES pH 7.3-120 mM-KCl buffer containing 20 µM-valinomycin for 5 min and guanidine uptake from the same buffer by these cultures was determined in the presence of increasing concentrations of methionine (●), choline (○), DMEA (■) or TEA (□) as described in Methods. Each point on the graph is the average of duplicate determinations. The residual uptake obtained in the presence of each concentration is expressed as a percentage of valinomycin-insensitive uptake, not of total uptake. Hence, the residual uptake with 10 mM-choline in this experiment is proportionately higher than in the experiment summarized in Table 2.

Table 1. Effect of the nature and concentration of monovalent cations in preincubation medium on guanidine or TPP+ uptake by HeLa cells

<table>
<thead>
<tr>
<th>Monovalent cations in preincubation medium*</th>
<th>Guanidine uptake† (pmol/mg protein/min)</th>
<th>Percent</th>
<th>TPP+ uptake† (pmol/mg protein/min)</th>
<th>Percent</th>
</tr>
</thead>
<tbody>
<tr>
<td>50 mM-Na+, 5 mM-K+</td>
<td>14.6 ± 0.8</td>
<td>100</td>
<td>115.9 ± 8.1</td>
<td>100</td>
</tr>
<tr>
<td>100 mM-Na+, 5 mM-K+</td>
<td>13.0 ± 0.2</td>
<td>89</td>
<td>ND§</td>
<td>–</td>
</tr>
<tr>
<td>145 mM-Na+, 5 mM-K+</td>
<td>6.3 ± 0.2</td>
<td>43</td>
<td>94.3 ± 4.1</td>
<td>81</td>
</tr>
<tr>
<td>50 mM-Na+, 100 mM-choline</td>
<td>4.5 ± 0.5</td>
<td>31</td>
<td>82.0 ± 3.2</td>
<td>71</td>
</tr>
<tr>
<td>50 mM-Na+, 100 mM-K+</td>
<td>11.5 ± 0.6</td>
<td>79</td>
<td>29.7 ± 1.3</td>
<td>26</td>
</tr>
</tbody>
</table>

* Preincubation was for 1.5 h with modified Eagle’s MEM prepared by mixing monovalent cations as shown and other ingredients including calf serum (final concentration 2-5%) and antibiotics.
† Uptake from the respective media containing radioactive guanidine or TPP+ expressed as mean ± S.D.
‡ Isotonicity was maintained with sucrose.
§ ND, Not determined.

Table 2. Organic cation- or valinomycin-sensitive and insensitive components of guanidine uptake by HeLa cells

<table>
<thead>
<tr>
<th>Uptake buffer*</th>
<th>Guanidine uptake† (pmol/mg protein/30 s)</th>
<th>Percent</th>
</tr>
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<tbody>
<tr>
<td>HEPES-KCl</td>
<td>12.2 ± 0.50</td>
<td>100</td>
</tr>
<tr>
<td>HEPES-KCl + 20 µM-valinomycin</td>
<td>7.76 ± 0.08</td>
<td>64</td>
</tr>
<tr>
<td>HEPES-KCl + 10 mM-choline</td>
<td>2.30 ± 0.00</td>
<td>19</td>
</tr>
<tr>
<td>HEPES-KCl + 10 mM-cimetidine</td>
<td>2.56 ± 0.34</td>
<td>21</td>
</tr>
<tr>
<td>HEPES-NaCl</td>
<td>7.48 ± 0.49</td>
<td>61</td>
</tr>
<tr>
<td>HEPES-LiCl</td>
<td>3.37 ± 0.32</td>
<td>28</td>
</tr>
</tbody>
</table>

* 25 mM-HEPES pH 7.3 with 120 mM-KCl, -NaCl or -LiCl.
† The cultures were preincubated for 5 min at 36 °C with the uptake buffer in the absence of radioactive guanidine, prior to determining uptake. The data represent mean values ± S.D.
Effects of cimetidine, choline or methionine on guanidine uptake and guanidine inhibition of poliovirus replication

The results summarized in Table 2 and Fig. 3 suggested that interference with guanidine transport may be involved in the action of cationic antiguanidine agents and conversely that agents blocking transport may reverse guanidine inhibition of virus replication. Because cimetidine was nearly as effective as choline in inhibiting uptake (Table 2), this compound was expected to block guanidine action on poliovirus replication. However, in the above experiments significant residual uptake was obtained even with a large excess of the cationic compounds (10 mM) despite the fact that the guanidine concentration in the medium was very low (4.7 \( \mu \text{M} \)). Therefore, it was possible that the organic cations may not adequately inhibit guanidine transport when a virus inhibitory concentration of guanidine is present in the medium. To examine these questions the effects of 10 mM-cimetidine and choline on guanidine uptake and guanidine inhibition of virus replication in the presence of 0.8 mM-guanidine were determined. Methionine was included as a control. The results (Table 3) revealed that both choline and cimetidine inhibited about 60% of uptake while methionine, as expected, had no effect. Under these conditions, choline and to a lesser extent methionine reversed inhibition of virus replication but, surprisingly, cimetidine failed to do so. It was separately determined that cimetidine itself had a slight inhibitory effect on virus replication. These results suggested that 10 mM-cimetidine or choline did not sufficiently inhibit the transport of 0.8 mM-guanidine to block guanidine action on virus replication. By inference, choline but not cimetidine was able to reverse inhibition of poliovirus replication after guanidine was transported into the cell.

DISCUSSION

A study of guanidine transport by cultured cells in the context of its antiviral action has not been reported even though it appears that KB cells take it up by diffusion alone (Lowff, 1965). The results presented in this paper revealed that in addition to diffusion a carrier-mediated, high affinity, saturable mechanism with a valinomycin-sensitive component was involved in the transport of relatively low concentrations (<2 mM) by HeLa cells. At higher concentrations, a low affinity, non-saturable mode of uptake, probably diffusion, was the major transport mechanism (Fig. 2b). The carrier-mediated transport process appeared to be analogous to the cation H+ exchanger-mediated excretion of organic cations from the kidney (Holohan & Ross, 1981; Takano et al., 1985), in terms of its inhibition by organic cations and by high [Li+] (Tables 1 and 2; Fig. 3). However, there were two apparent differences: unlike the transport mechanism in the kidney (Holohan & Ross, 1981; Sokol et al., 1985), the HeLa cell transport mechanism was partially inhibited by high [Na+] and was possibly dependent on \( \Delta \psi \) (Tables 1 and 2). Valinomycin inhibition of guanidine uptake from high K+ buffer (Table 2) was assumed to be due to dissipation of \( \Delta \psi \), but could have another explanation. Organic cation H+ exchanger-mediated transport in canine kidney brush border membrane vesicles is not dependent on \( \Delta \psi \) but is inhibited by a relatively high concentration (10 \( \mu \text{g/mg membrane protein} \)) of valinomycin (Sokol et al., 1985). Since the concentration of valinomycin used in the present experiments was >10 \( \mu \text{g/mg cell protein} \) it is quite possible that membrane alteration rather than dissipation of \( \Delta \psi \) by valinomycin was responsible for the observed inhibition of guanidine uptake. However, since in this study \( \Delta \psi \) changes resulting from monovalent cation alterations in the medium also appeared to affect its uptake (Table 1), an influence of \( \Delta \psi \) on guanidine transport cannot be excluded. Previous studies on the organic cation H+ exchanger employed kidney membrane vesicles from non-human animal species, and it is possible that this protein is not identical in human-derived HeLa cells. Also, other forms of this protein with different properties may exist in different tissues. An alternative or additional explanation for the differences between the present and previous results may be the use of intact viable cells in this study as opposed to...
Table 3. Effects of cimetidine, choline and methionine on guanidine inhibition of poliovirus replication in and guanidine uptake by HeLa cells

<table>
<thead>
<tr>
<th>Addition to medium</th>
<th>Virus yield* (% of control)</th>
<th>Relative guanidine uptake†</th>
</tr>
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<tbody>
<tr>
<td>0.8 mM-guanidine</td>
<td>0.02</td>
<td>100</td>
</tr>
<tr>
<td>0.8 mM-guanidine + 10 mM-cimetidine</td>
<td>0.01</td>
<td>44</td>
</tr>
<tr>
<td>0.8 mM-guanidine + 10 mM-choline</td>
<td>0.97</td>
<td>39</td>
</tr>
<tr>
<td>0.8 mM-guanidine + 10 mM-methionine</td>
<td>0.25</td>
<td>93</td>
</tr>
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</table>

* Monolayer cultures were infected with virus at a m.o.i. of 200 p.f.u./cell, extensively washed with PBS, and incubated with HEPES-buffered MEM containing 2.5% calf serum. At 1.5 h post-infection, guanidine alone, or guanidine plus cimetidine, choline or methionine was added to the cultures as indicated. After 6 h incubation at 36 °C, virus yields in the cultures were determined by plaque titration and expressed as percentages of the yield in the culture that was incubated with control medium.

† With a parallel set of cultures, the initial rate of guanidine uptake (1 min) was determined in the presence of the various agents shown in the table, as described in Methods.

membrane vesicles in previous investigations. At any rate, this is the first demonstration of carrier-mediated organic cation transport in cultured cells.

According to the results of this study, the inhibitory action of agents that are organic cations was related to their ability to inhibit guanidine transport (Fig. 3). On the other hand, the action of methionine must be by an unrelated mechanism since it did not inhibit guanidine uptake (Fig. 3, Table 3). These results support the previous conclusion that there are two classes of such agents (Mosser et al., 1971). Cationic agents may block guanidine action by inhibiting its transport and/or by an intracellular mechanism. The importance of inhibition of guanidine transport in antiguanidine action could not be ascertained since cimetidine and choline equally inhibited transport but only choline was able to reverse guanidine action (Table 3). Because significant residual uptake occurred even in the presence of 10 mM-cimetidine or choline (Table 3), it would appear that the inhibitory effect of choline resulted from its ability to block the intracellular action of guanidine. Conversely, the inability to neutralize the intracellular action of guanidine may explain the failure of cimetidine to reverse it. Agents like cimetidine which merely inhibit transport may reverse the inhibition of virus replication by guanidine only when its concentration in the medium is not much higher than the minimum virus inhibitory concentration, because at higher concentrations residual uptake will be sufficient to inhibit virus growth.

These results help understand the following unexplained observations concerning the action of guanidine and its inhibitors. (i) Some pairs of guanidine antagonists such as methionine and choline act synergistically (Philipson et al., 1966; Mosser et al., 1971) whereas others like choline and DMEA show no synergism (Mosser et al., 1971). The synergism between choline and methionine may be explained by the fact that these two agents antagonize guanidine action by separate mechanisms, choline by a mechanism related to its ability to block uptake, and methionine by a separate unknown mechanism. Similarly, the reason why choline and DMEA do not act synergistically must be because they both act by the same mechanism which is probably reflected in their ability to inhibit uptake (Fig. 3). (ii) The effective concentration of guanidine and its antagonists are cell type-dependent (Philipson et al., 1966; Mosser et al., 1971). This dependence of guanidine action can be explained in terms of differences among cell types in their transport mechanisms. To produce the same intracellular concentration, a higher medium concentration would be needed if guanidine entered the cell by diffusion alone than if it were actively transported by a carrier as well. Therefore, cell types lacking the guanidine transporter will be less sensitive to guanidine action than those which possess the carrier. On the other hand, the reported inverse correlation of guanidine sensitivity and antiguanidine (choline) sensitivity of cell lines (Mosser et al., 1971) cannot be explained in terms of differences in transport mechanisms since the presence or absence of the organic cation transporter must similarly affect the transport of guanidine and choline. (iii) The inhibition of poliovirus
replication caused by 0.4 mM-guanidine is fully reversed by 1 mM-choline whereas that caused by 1 mM-guanidine is not completely reversed even by 10 mM-choline (Mosser et al., 1971). According to the results in Table 3 it is clear that even 10 mM-choline would inhibit no more than about 60% of total guanidine uptake from medium containing 0.8 mM-guanidine. When the concentration in the medium is high, the residual uptake in the presence of choline will be great enough to inhibit virus replication. (iv) Guanidine uptake by HeLa cells is enhanced in the presence of Na⁺-deficient medium (Nair, 1987). Physiological [Na⁺] was found to inhibit uptake significantly (Tables 1 and 2). Therefore, lowering [Na⁺] in the medium would have reduced Na⁺ inhibition of uptake. Besides, incubation of cells in the presence of low Na⁺ medium was shown to cause an increase in Δψ (Table 1), and could have resulted in the activation of a Δψ-dependent component of transport (Tables 1 and 2). However, direct evidence for the involvement of Δψ in transport remains to be obtained.

Choline blocks guanidine action even when added after guanidine (Mosser et al., 1971; C. N. Nair, unpublished results) and does not enhance guanidine efflux from HeLa cells (C. N. Nair, unpublished results), but it reversed inhibition of virus replication in the presence of a virus-inhibiting level of guanidine in HeLa cells (Table 3). Therefore, it would appear that choline competes with guanidine for an intracellular target as well as for transport into the cell. We have hypothesized that guanidine acts by interfering with a Na⁺-dependent viral function such as the correct folding and processing of a viral precursor polypeptide (Nair et al., 1979). According to this view, binding by Na⁺ or choline to critical negative charges on the precursor would facilitate while binding by guanidine would interfere with its processing. An explanation for the observation that high [Na⁺] significantly inhibited guanidine uptake (Table 2) is that Na⁺ weakly competes with guanidine for binding to the transport protein. Similar competition between guanidine and Na⁺ may occur also for binding to the putative intracellular target of guanidine.

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