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REVERSED TRANSPORT OF AMINO ACIDS IN EHRLICH CELLS

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SUMMARY

Gramicidin induces a marked Na^+-dependent efflux of amino acids from Ehrlich cells. In absence of Na^+, gramicidin does not alter the efflux. In presence of gramicidin, glycine efflux is inhibited by methionine and less so by leucine. Glycine efflux caused by $HgCl_2$ is neither Na^+ dependent nor inhibitable by amino acids. Neither efflux of inositol which is transported by an Na^+-dependent route, nor efflux of several other solutes which are transported by Na^+-independent routes, is affected by gramicidin. The antibiotic appears to permit a reversal in the direction of the operation of the Na^+-dependent amino acid transport system. The increased efflux is partly, but not entirely, due to an increase in the cellular Na^+ concentration and a reduction of the electrochemical potential difference for Na^+.

INTRODUCTION

Several recent studies have led to the conclusion that the inhibitory action of some ionophore antibiotics on the transport of organic solutes in bacteria and in higher organisms is indirect and mediated by the action of these antibiotics on the dissipation of ion gradients and/or electrical potentials, which are required for the accumulation of an organic solute against its electrochemical gradient [1–4].

In our recent investigation on the action of some ionophore antibiotics, we have observed that in the presence of gramicidin there is an Na^+-dependent efflux of those amino acids whose transport is enhanced by Na^+.

The question of the degree of reversibility of the Na^+-dependent transport systems in mammalian cells has been an issue for intense discussion [5–21]. The Na^+-gradient hypothesis (for reviews see refs 8, 18 and 19) for the transport of organic solutes is based on the concept of a reversible system where the direction of the net flow of an organic solute against its chemical potential is determined by the direction of the electrochemical potential difference for Na^+. In epithelial tissues and other cells, such as the Ehrlich cells, intracellular Na^+ is probably the most important variable in determining the magnitude of the Na^+ electrochemical potential difference. Experimentally, it has been difficult to show marked Na^+-dependent efflux of organic solute(s) whether along or against its gradient, even when cellular Na^+ levels are elevated. When efflux of an organic solute against its concentration gradient has been observed [5, 9–11,

13, 14, 16], it has often been smaller than expected from the magnitude of the reversed Na^+ gradient. Moreover, in several instances where the Na^+ gradient appeared to have been reversed, uptake of the solute by the Na^+-dependent route, rather than efflux, persisted [12–17, 20, 21] suggesting that factors other than the Na^+ gradient had contributed to the vectorial operation of the transport systems.

This paper describes some recent studies which show that rapid and extensive Na^+-dependent efflux can be induced in Ehrlich cells. Yet net efflux of glycine against its chemical potential was not obtained when the direction of net Na^+-flow was reversed.

MATERIALS AND METHODS

Ehrlich ascites cells were maintained in Swiss white mice by weekly transfers and harvested as previously described [16]. Incubations were carried out in a Ringer medium containing 150 mM NaCl, 15 mM KCl, 1.45 mM $MgSO_4$, 2 mM KH_2PO_4 and buffered with *N*-2-hydroxyethylpiperazine-*N'*-2-ethane sulfonic acid (HEPES) at pH 7.4 (normal Ringer medium). Incubations were carried out in a shaking water bath at 37 °C, unless otherwise specified.

For measurements of uptake, the packed cell volume was one sixtieth of the total volume and 1-ml samples were analyzed, representing 30 ± 5 mg wet weight of tissue. For measurements of efflux, the packed cell volume was 1/120 of the total volume and 2-ml samples were assayed. In uptake studies, samples were washed with 10 times the sample volume, using isotonic choline chloride as wash medium.

The weight of the wet cell pellet and its dry weight were determined as described [22].

ATP estimations were carried out by the luciferase method of Stanley and Williams [23].

Measurements of uptake were initiated by adding the isotope to the incubation mixture. Efflux measurements were initiated by introducing the cells, containing isotope, into 120 times their volume of medium.

Other experimental procedures were described earlier [22].

All radioisotopes were purchased from New England Nuclear Corp., Waltham, Mass.

Gramicidin D was purchased from Sigma Chemical Co., St. Louis, Mo. and stored over a dessicant at 5 °C. All other reagents used were the best grade available commercially.

RESULTS

Effect of gramicidin on solute uptake

The addition of gramicidin D at 1–7 μg/ml of incubation medium (3–5 mg dry weight of tissue) causes a marked inhibition of amino acid uptake, a decrease in ATP levels and an elevation of cellular Na^+ (Fig. 1), as well as a decrease in cell K^+. Similar results on amino acid uptake have also been reported by Vidaver [4] and Christensen et al. [24]. The fall in ATP level from 2 mM to less than 0.1 mM with gramicidin that we obtained is much greater than that reported by Terry and Vidaver for avian red cells [4].

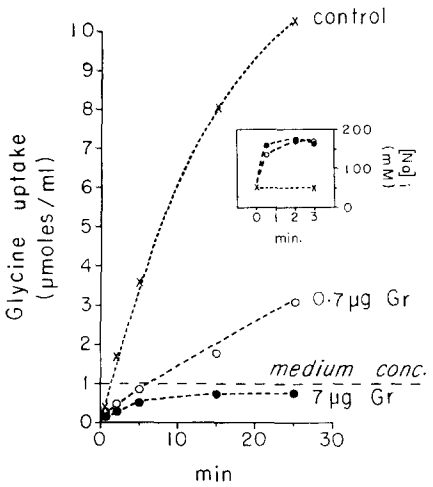

Fig. 1. Effect of gramicidin on initial rate of glycine uptake. The cells were incubated at 37 °C in a normal Ringer medium, buffered with HEPES at pH 7.4. Gramicidin, dissolved in 95 % ethanol, was added 2 min before the [^{14}C]glycine (final concentration 1 mM, 290 cpm · nmol^{-1}). The controls contained an equivalent volume of ethanol. The values of gramicidin given are per ml of medium. The data are representative and are expressed as µmol [^{14}C]glycine/ml cell water. The inset shows the change in cell Na^+ concentration with time in the same experiment. 1 ml cell water is equivalent to 274 mg dry weight. The ATP levels in presence of 7 µg/ml gramicidin dropped to 0.07±0.01 in 15–30 min, compared to control values of 2.0±0.5 mM.

Lowered ATP levels are observed in media of different composition, including isotonic mannitol and choline chloride, and therefore the fall in ATP level does not appear to depend on Na^+ pumping as suggested by Christensen et al. [24].

To ascertain whether solutes other than amino acids are affected by gramicidin, we examined *myo*-inositol which is also transported by an Na^+- and energy-dependent route in Ehrlich cells [25]. As shown in Table I, gramicidin blocks inositol uptake nearly completely.

To determine whether gramicidin affects all transport systems or just the Na^+-

TABLE I

ACTION OF GRAMICIDIN ON INOSITOL UPTAKE

myo-[U-^{14}C]Inositol was present at a concentration of 1 µM. All other conditions were as previously described (Fig. 1). This experiment is representative of two similar experiments. Incubation was at 37 °C and gramicidin was present at 7 µg/ml.

Time (min)	Inositol uptake (nmol · ml^{-1} cell water)		
	Control	Gramicidin	Na^+ free
2	0.11	0.08	0.10
10	0.42	0.17	0.19
15	1.00	0.16	–
30	2.40	0.25	0.56
45	4.00	0.41	0.56

TABLE II

ACTION OF GRAMICIDIN ON AMINO ACID EXCHANGE IN EHRLICH ASCITES CELLS

Cells were incubated without and with 10 mM aminocyclopentanecarboxylic acid for 30 min at 37 °C in the normal Ringer medium. Then the cells were chilled and centrifuged down. The medium was removed as completely as possible and the cells were resuspended in 1 ml fresh, cold incubation medium. The cell suspension was then introduced into 10 ml of incubation medium, containing 1.2 mM aminocyclopentanecarboxylic acid, specific activity 297 cpm · nmol^{-1}, and incubated at 25 °C. 1-ml samples were taken at intervals over a 15 min period. The values given are those after 10 min. The experiment is representative of three similar experiments. The results are expressed as μmol ^{14}C-labelled amino acid/ml cell water.

Amino acid used in preincubation	Gramicidin (7 μg/ml)	Aminocyclopentane-carboxylic acid (μmol · ml^{-1})	Δ due to aminocyclopentane-carboxylic acid prepacking (μmol · ml^{-1})
nil	—	4.5	–
Aminocyclopentane-carboxylic acid	—	10.0	5.5
nil	+	2.2	–
Aminocyclopentane-carboxylic acid	+	6.6	4.4

dependent systems, the effect of gramicidin on exchange diffusion of amino acids was examined, since exchange is known to operate in the absence of Na^+ or ATP [26, 27]. This system is often cited as the L-system according to the characterization of Oxender and Christensen [28]. It may be seen (Table II) that exchange diffusion is not markedly influenced by gramicidin. To measure exchange diffusion the temperature was reduced to 25 °C. It is known that interaction of gramicidin with the membrane is highly temperature dependent [29], therefore the possibility was considered that the lack of effect on exchange was due to the inability of gramicidin to interact with the cells at the lowered temperature. To test this possibility the action of gramicidin on Na^+ influx at different temperatures was measured. The activation energy for gramicidin interaction with the membrane (as measured by Na^+ uptake) was estimated to be 20 kcal/mol between 14 and 37 °C and appreciable Na^+ uptake was obtained at 25 °C. Hence, the lack of effect of gramicidin on exchange diffusion is not due to the lowered temperature.

Action of gramicidin on efflux

If gramicidin reduces accumulation of amino acids, it acts either by increasing efflux, or by decreasing influx, or both. The data in Fig. 1 show that influx of amino acid at early times is inhibited by gramicidin. However, since the uptake, even at early times, is not linear with time in the presence of gramicidin, it is not clear which flux is affected. Therefore the action of gramicidin directly on efflux was tested. The results in Fig. 2 show that efflux of amino acids from Ehrlich cells is markedly increased by gramicidin, efflux being greater at 37 °C than at 25 °C. Additional experiments show that, at 37 °C, efflux with gramicidin is first order until more than 70 % of the cellular amino acid is lost. The rate constant for glycine efflux rose from 0.017 to 0.05 min^{-1} and from 0.03 to 0.48 min^{-1} in the absence and presence of gramicidin, respectively,

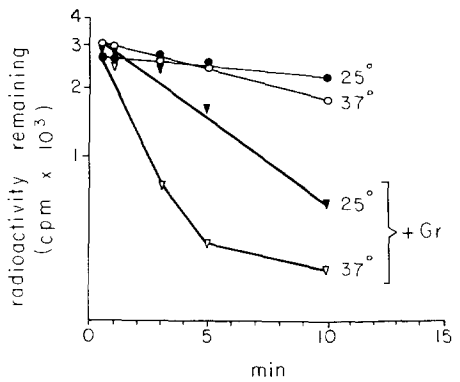

Fig. 2. Action of gramicidin on efflux of [^{14}C]glycine at 25 and 37 °C. The cells were preincubated with [^{14}C]glycine (1 mM, specific activity 290 cpm · nmol^{-1}) in a normal Ringer-HEPES medium, pH 7.4, for 30–35 min. The cells were then separated from the incubation medium by centrifugation for 2 min at 1400 × *g*. The cell pellet was resuspended with cold normal Ringer (1 ml final volume) and added directly to 11 ml of the normal Ringer-HEPES medium, which contained 7 μg · ml^{-1} of gramicidin (where indicated). Representative data are given. The experiments at the two temperatures are with the same preparation of cells. Cells were chilled and rapidly separated from the medium by centrifugation. The results are expressed as counts remaining per ml cell water.

between 14 and 37 °C. It may be noted that the normal value at 37 °C for k_e for glycine reported in this study is in line with previous reports using similar cells, namely, 0.04–0.05 min^{-1} [33, 34].

This 10-fold increase in k_e for glycine with gramicidin at 37 °C is observed only in an Na^+-containing medium. Little change in efflux is induced by gramicidin when choline chloride, $Tris^+$, Rb^+, K^+ or Cs^+ replaced Na^+ in the medium. A small increase in efflux is noted in Li^+ medium (Table III). Efflux of several amino acids is

TABLE III

k_e FOR GLYCINE IN DIFFERENT MEDIA

The determinations for k_e with and without gramicidin were done in paired experiments at 37 °C. All conditions are as in Fig. 2. The values in parentheses are the numbers of experimental observations ±S.D. With media other than the normal Ringer, the NaCl of the medium was replaced; all other components were unchanged.

Medium	k_e (min^{-1})	
	Without gramicidin	With gramicidin
NaCl (normal Ringer)	0.04±0.01 (20)	0.42±0.1 (10)
NaCl+L-methionine (20 mM)	0.02 (3)	0.045 (3)
NaCl+L-leucine (20 mM)	0.038 (3)	0.18 (3)
Na_2SO_4	0.07 (2)	–
KCl	0.08 (2)	0.09 (2)
LiCl	0.04 (2)	0.08 (2)
RbCl	0.04 (2)	0.03 (2)
CsCl	0.03 (2)	0.03 (2)
Choline chloride	0.04 (2)	0.04 (2)
Tris · Cl	0.05 (2)	0.05 (2)

TABLE IV

EFFLUX OF VARIOUS SOLUTES IN THE PRESENCE OF GRAMICIDIN

All values represent the radioactivity remaining at 10 min and are expressed as a percentage of the corresponding value at 1 min of incubation. Except where noted, efflux was determined in the normal Ringer medium at 37 °C. Samples were taken at four intervals over the 10 min period. Other conditions are as in Fig. 2.

Preloaded substrates	Percent of initial radioactivity	
	Control	+Gramicidin
Inositol	90	90
3-*O*-methylglucose	80	77
Ethanolamine	85	76
Phenylalanine	68	38
Histidine	73	27
Histidine (in choline chloride)	79	80
Aminocyclopentane-carboxylic acid	70	16

increased. These include glycine, α-aminoisobutyric acid, phenylalanine, histidine and aminocyclopentanecarboxylic acid, all of which are transported, at least in part, by Na^+-dependent routes [28, 30, 31] (Table IV). However, no increase in efflux is observed in Ehrlich cells with 3-*O*-methylglucose or ethanolamine, which are transported by Na^+-independent routes. Efflux of histidine from sheep reticulocytes* is not affected. The enhanced loss of cellular amino acid could be accomplished by two different mechanisms, (a) a failure to reaccumulate, hence increasing the net loss of amino acids, and (b) an increase in the rate constant for the efflux process itself. The data are clearly consistent with the latter, since (1) reaccumulation would be minimized in a choline medium, yet no increase in efflux is observed in an all-choline medium, and (2) methionine in excess would decrease reaccumulation [32] and increase the net loss. However, the data show that efflux is reduced by methionine, particularly in the presence of gramicidin (Table III). The data with methionine and leucine (Table III) show, in fact, that gramicidin-induced glycine efflux is sensitive to inhibition by amino acids, methionine being more potent an inhibitor than leucine as anticipated from studies on glycine uptake [28, 32]. Neither dimethylglycine nor ε-amino caproic acid affects efflux. $HgCl_2$ also induces increased efflux of glycine, but it was neither Na^+-dependent nor inhibited by methionine (data not given), showing, by contrast, that the gramicidin affects the carrier-mediated process.

According to a conventional interpretation of the Na^+-gradient hypothesis, the effects of gramicidin on amino acid uptake and efflux could be due to the elevated cell Na^+ level, which results in enhanced efflux thereby showing the inherent reversibility of Na^+-dependent transport systems. However, several lines of evidence indicate that such an interpretation is only partly correct and that the effects of gramicidin on uptake and efflux are more complex than mere elevation of cellular Na^+, although elevation of cellular Na^+ is required for gramicidin-induced efflux. The evidence for

* Sheep reticulocytes transport histidine by an Na^+-independent route (Benderoff, S., Blostein, R. and Johnstone, R. M. (1975) Can. Fed. Biol. Soc. 18, 116).

this conclusion is based on the following criteria.

(1) If cellular Na^+ is raised by other means, efflux, comparable to that seen with gramicidin, should occur at comparable cellular Na^+ levels. Similarly, enhanced efflux with gramicidin should occur only when cellular Na^+ levels are raised above the normal.

(2) Influx of amino acids should be unaffected by gramicidin in an Na^+-free medium.

(3) In the presence of gramicidin, alterations in cellular ATP levels should not affect accumulation if the cation distributions are not affected.

(4) All Na^+-dependent transport systems should behave in a like manner. These predictions were tested experimentally and found not to hold as indicated below.

Elevation of cellular Na^+ and Na^+ requirement for increased efflux

The data in Fig. 3 show the results of experiments with and without gramicidin, done over a number of years, in which the first order rate constant for efflux of glycine was measured and in which the cellular Na^+ was determined simultaneously. The experiments without gramicidin include data from a variety of experiments in which cell Na^+ was elevated by various procedures (see legend to Fig. 3). The experiments with gramicidin were carried out with media containing different Na^+ concentrations.

It is clear that there is a tendency for the rate constant for efflux to increase

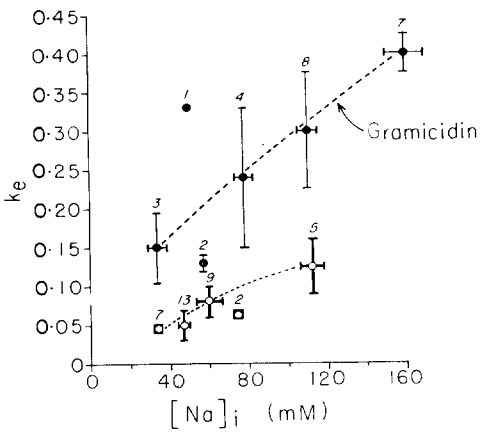

Fig. 3. Variation of k_e with cell Na^+ in the presence and absence of gramicidin. The experimental procedure for determining k_e was described in Fig. 2. The values of k_e were determined from the efflux over the time span between 30 s and 3 min with gramicidin or 30 s and 10 min for the controls when the experimental data showed that the slope was constant. All the data are drawn from experiments in which the efflux was measured as a function of time from 30 s to 30 min. The experiments with gramicidin were carried out with the sodium concentration in the medium varying from 10-150 mM to vary cellular Na^+. The replacement for Na^+ was always choline. All the experiments in presence of gramicidin in which $[Na]_i$ was 150 mM or greater were carried out with normal Ringer medium. The control values are derived from a variety of experiments. The higher cell Na^+ values (above 50 mM) come from experiments with ouabain, rotenone, monactin, nonactin, dinitrophenol and valinomycin or preincubation at 0 °C, but the medium was maintained at the normal Na^+ level. The values given are the means $\pm$S.D. of the $[Na]_i$ (horizontal bars) and the k_e (vertical bars). The number of observations at each point is given.

with increased $[Na]_i$. It is equally evident that with gramicidin, at any given cell Na^+ level, efflux is greater than at the corresponding cell Na^+ level without gramicidin. Moreover, it may be seen that at nearly normal $[Na]_i$ (30–50 mM), efflux is appreciably increased with gramicidin. Hence, gramicidin is having an action on efflux which cannot be due only to elevation of the cellular Na^+ level. Our earlier observation that rotenone and low cellular ATP levels increase efflux may be related to the elevated cellular Na^+ [22].

Actions of gramicidin on influx and role of ATP

If the effect of gramicidin on net amino acid uptake were due only to its action on efflux, gramicidin would not change the initial rate of uptake of amino acids in the absence of Na^+. The data in Fig. 4 show that gramicidin does reduce the initial glycine influx in an Na^+-free medium, and that rotenone and gramicidin have similar inhibitory effects, suggesting that the action of gramicidin on influx may be due partly to lowered ATP levels.

In normal Ringer medium addition of glucose in the presence of gramicidin permits some accumulation (about 2-fold) (data not given), whereas, in the absence of glucose, only equilibration is obtained with 7 μg gramicidin/ml. In these experiments the ion distributions were not affected by glucose, but the ATP levels were increased from 0.05 to 0.3 mM with glucose. Similar results were obtained with α-aminoisobutyric acid.

Action of gramicidin on other Na^+-dependent transport systems

Since Na^+-dependent *myo*-inositol transport is as well inhibited by gramicidin as is amino acid transport, the effect of gramicidin on inositol efflux was examined. The results in Table IV show that gramicidin does not alter efflux of inositol. Inositol is accumulated in the cell water as the free hexitol [25].

Since it is known that Na^+ may alter either K_m or V or both, of the transported solute [8], the kinetic parameter affected by Na^+ in the case of inositol was determined. It is known that Na^+ alters mainly the K_m for amino acid transport in Ehrlich

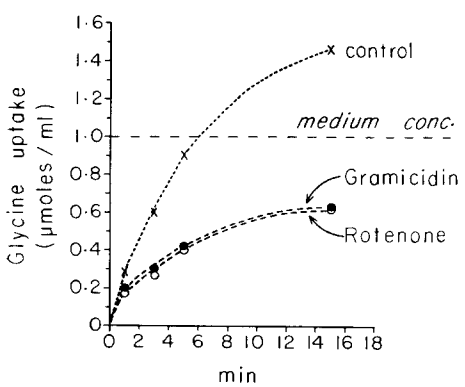

Fig. 4. Glycine uptake in Na^+-free medium. Action of rotenone and gramicidin. The cells were incubated in a medium in which choline chloride replaced the NaCl. Other incubation conditions are as in Fig. 1. Gramicidin was present at 7 $\mu g \cdot ml^{-1}$ and rotenone at 16 $ng \cdot ml^{-1}$. Uptake is expressed as μmol [^{14}C]glycine/ml cell water.

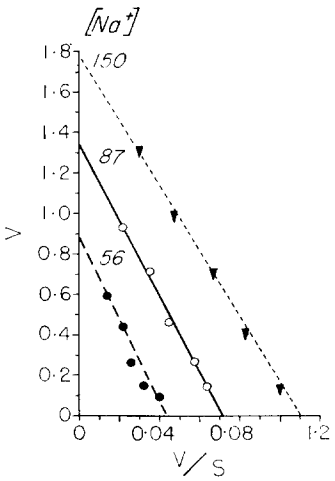

Fig. 5. The response of the *myo*-inositol transport system to Na^+ concentration in the medium. *myo*-[^{14}C]Inositol uptake was measured at three Na^+ concentrations (as given). Choline was used as replacement. Other experimental conditions are as in Fig. 1. The velocity is expressed as nmol inositol/ml cell water per 15 min. Preliminary experiments showed that the rate of uptake was constant for at least 30 min at 37 °C.

cells [7, 16, 28, 30, 31]. It was of interest to see whether the difference in response of amino acids and inositol to gramicidin-induced efflux could be associated with different effects of Na^+ on the transport system. The data in Fig. 5 show that the major effect of Na^+ on *myo*-inositol transport is to increase V for transport. Thus increased efflux induced by gramicidin may apply to systems where Na^+ affects the K_m for the transport system. We are currently testing this conclusion with other tissues.

For all the reasons cited above, the action of gramicidin cannot be fully ascribed to an elevation of cellular Na^+, causing increased efflux, nor that the increased efflux is entirely responsible for the decreased accumulation.

Reversed accumulation

Since gramicidin probably abolishes the electrochemical potential difference for cations, and efflux is greatly increased by gramicidin, we examined whether efflux of glycine against a gradient would occur with gramicidin if $[Na]_i > [Na]_o$. The cells were preincubated with [1-^{14}C]glycine in a normal Ringer medium with 1.0 μg gramicidin/ml or without gramicidin. After 30 min, the cellular [^{14}C]glycine level was estimated, the cells centrifuged down, and introduced into a medium containing the same [^{14}C]glycine concentration as that found in the cells but containing reduced Na^+ concentration (32 mM Na^+). While the Na^+ flowed out of the cell, the direction of isotope movement was measured. In three separate experiments (see Table V for a representative experiment) glycine moved into the cells against its concentration gradient despite the fact that medium Na^+ was less than cellular Na^+. Thus, although rapid efflux occurs with gramicidin, there is no reversed accumulation in its presence.

Action of other antibiotics

The effects described above were not observed with valinomycin under any

TABLE V

REVERSED GRADIENT AND GLYCINE UPTAKE

Cells were incubated either with 0.1 mM [1-^{14}C]glycine without gramicidin or with 2 mM glycine and 1 μg/ml of gramicidin for 30 min at 37 °C in a normal Ringer medium (150 mM Na^+). The [^{14}C]glycine and Na^+ concentrations in the cells were determined, the cells centrifuged down, the incubation medium removed and the cells resuspended and added to a medium containing the glycine concentration estimated to be in cells, i.e. 1 mM without gramicidin and 7 mM with gramicidin. The fresh medium contained 32 mM Na^+ (isotonicity was maintained with choline chloride). The buffer was HEPES pH 7.4 and the temperature 37 °C. [^{14}C]Glycine used in the second incubation had the same specific activity as for the preincubation, namely 258 cpm · nmol^{-1}. With gramicidin, the starting cellular Na^+ concentration was 90 mM.

Time (s)	No gramicidin		With gramicidin	
	$[Na]_i$ (mM)	$[Glycine]_i$ (mM)	$[Na]_i$ (mM)	$[Glycine]_i$ (mM)
15	22	1.19	64	9.8
30	22	1.17	68	10.3
60	20	1.30	58	10.0
120	20	1.70	58	12.4

conditions. With amphotericin β and nystatin, increased efflux of amino acids was observed in choline or K^+ media, and a further increase in the rate of loss could be obtained in an Na^+-containing medium.

DISCUSSION

The marked increase in efflux of amino acids seen with Ehrlich cells in the presence of gramicidin is clearly not due to the passage of these molecules through the gramicidin pore. Thus, the efflux of fairly bulky amino acids, such as phenylalanine, histidine and aminocyclopentanecarboxylic acid is accelerated, whereas the efflux of ethanolamine, 3-*O*-methylglucose and *myo*-inositol is not affected. The absolute Na^+ requirement for gramicidin-induced amino acid efflux, as well as the actions of methionine and leucine thereon, is also consistent with a specific effect of gramicidin on the transport mechanism for amino acids rather than efflux by a nonspecific route. Moreover, the pore formed with gramicidin is unlikely to accommodate molecules the size of amino acids, apart from any other considerations of shape, charge, etc. [35].

The enhanced efflux of amino acids seen with gramicidin is primarily due to an increase in the rate constant for efflux. In normal Ringer medium, the increased efflux with gramicidin is an order of magnitude greater than the control value.

The overall effect of gramicidin on net amino acid uptake is probably due to two separate events: (1) a large increase in the backflux (increased efflux), and (2) a smaller effect directly on influx. It is unlikely that the overall action of gramicidin on net uptake is due solely to its action on efflux for the following reasons. (a) The decrease in net glycine uptake seen with gramicidin is greater than would be expected on the basis of increased efflux alone. (b) Inhibition of amino acid uptake by gramicidin is observed in Na^+-free medium where gramicidin does not increase efflux.

Calculations* show that influx must also be reduced by 50–70 % to account for the decrease in net uptake. This calculation is in line with experimental observations in Fig. 4 on the reduction of glycine uptake by gramicidin in Na^+-free media. The fact that gramicidin and rotenone inhibit uptake of glycine in Na^+-free media to about the same extent suggests that the decrease is due to a common factor, probably the decreased ATP level.

Although incubation with gramicidin causes an elevation of cellular Na^+, it is not possible to account for the observed increase in k_e on the basis of the cellular Na^+ alone. Values 3–5 times the normal k_e can be obtained with gramicidin when cellular Na^+ levels are as low as 30–50 mM, that is, within the normal range for Ehrlich cells. Many agents such as ouabain, rotenone and dinitrophenol elevate Na^+ and increase efflux, but none with the efficiency of gramicidin. At comparable cellular Na^+ levels efflux is always greater in presence of gramicidin. Three possibilities may be suggested which, in addition to cellular Na^+, might increase efflux: (1) diminished cellular ATP, (2) a decreased membrane potential (depolarization), and (3) a combination of both. Much of our data is consistent with the increased efflux being due to depolarization in addition to an effect of internal Na^+. Gramicidin, because of its relatively unselective effect on Na^+ and K^+ permeability, is likely to cause depolarization. This conclusion is supported by the studies on ^{36}Cl distribution in the presence of gramicidin (Johnstone, R. M., unpublished observations). Also, in Na_2SO_4, Aull has reported depolarization of Ehrlich cells [36] and my studies on ^{36}Cl distribution (Johnstone. R. M., unpublished) in Na_2SO_4 support this conclusion. Cell Na^+ is little changed in Na_2SO_4 but efflux is increased (Table III). A direct role for ATP on efflux is less clear. since agents which lower ATP levels may also cause depolarization.

Previous studies on elevation of cell Na^+ in Ehrlich cells and other systems have already shown that, as $[Na]_i$ increases, efflux of an organic solute may increase [20, 34, 37, 38], although a direct relationship between cellular Na^+ and k_e has not previously been shown unequivocally for Ehrlich cells. If one assumes that conventional Michaelis-Menten kinetics apply to Na^+-dependent efflux, K_m values for cell Na^+ of 100 and 400 for glycine efflux are obtained in the presence and absence of gramicidin, respectively. Although these values must be considered rough approximations, they are clearly different from the K_m for Na^+ at the external surface of about 20–30 mM [27, 38]. These data support the idea that Na^+-dependent amino acid transport is reversible.

The reason for the lack of effect of gramicidin on *myo*-inositol efflux is not clear. One possibility may be an inherent difference between Na^+-dependent K_m systems and V systems. V systems may lack binding sites (or have very low affinity) for Na^+ on the internal surface and hence will not show Na^+-dependent efflux. It is noteworthy in this regard that most successful attempts to operate transport systems backwards, against a chemical potential for the solute in question, have been reported with those systems where Na^+ affects K_m [5, 6, 9–11, 20].

Ignoring questions of the adequacy of energy from the Na^+ electrochemical potential to account for amino acid accumulation in Ehrlich cells, the present data are

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* Equation used in calculation: net uptake at time $t = C_0\ (k_{influx}/k_{efflux})\ (1 - e^{-k_{efflux}\,t})$, where C_0 is the medium concentration of glycine and $C_0 \cdot k_{influx}$ is the initial rate of uptake in normal cells using 1 mM glycine.

generally consistent with several of the fundamental ideas of the Na^+ gradient hypothesis, vis a vis the reversibility of carrier operation in presence of cellular Na^+. Recently Asghar et al. [3] and Komor et al. [39] have shown that, in bacterial preparations, agents which collapse the proton gradients cause an increase in the efflux of cellular amino acids, giving results similar to those reported here with gramicidin.

The failure to get outflow of glycine against its chemical potential in the presence of gramicidin when net Na^+ outflow occurs suggests that factors in addition to the direction of the Na^+ electrochemical potential difference influence net accumulation of amino acids. Experiments showing modest reversed accumulation of amino acids, which were reported previously, were carried out with cells loaded with Na^+ and transferred to a medium devoid of Na^+ so that only efflux could occur [5, 9–11, 13, 14, 16].

Thus, in the present work, despite the ability of the transport system to cause rapid outward movement of glycine down its electrochemical potential when the electrochemical potential difference for Na^+ is abolished, it was not possible to obtain reversed accumulation of glycine against its chemical potential when the electrochemical potential difference for Na^+ was reversed. Instead the cellular [^{14}C]glycine concentration increased slightly when net Na^+ movement was out of the cell. Further work is required to elucidate the mechanism(s) responsible.

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REFERENCES

1 Harold, F. M. and Baarda, J. (1968) J. Bacteriol. 96, 2025–2034
2 Harold, F. M., Pavlasova, E. and Baarda, J. R. (1970) Biochim. Biophys. Acta 196, 235–244
3 Asghar, S. S., Levin, E. and Harold, F. M. (1973) J. Biol. Chem. 248, 5225–5233
4 Terry, P. M. and Vidaver, G. A. (1973) Biochim. Biophys. Acta 323, 441–455
5 Crane, R. K. (1964) Biochem. Biophys. Res. Commun. 17, 481–485
6 Vidaver, G. A. (1964) Biochemistry 3, 795–799
7 Eddy, A. A. (1969) Biochem. J. 108, 489–498
8 Schultz, S. G. and Curran, P. F. (1970) Physiol. Rev. 50, 637–718
9 Hajjar, J. J., Lamont, A. S. and Curran, P. F. (1969) J. Gen. Physiol. 55, 277–296
10 Chez, R. A., Palmer, R. R., Schultz, S. G. and Curran, P. F. (1967) J. Gen Physiol. 50, 2357–2375
11 Hajjar, J. J., Khuri, R. N. and Curran, P. F. (1972) J. Gen. Physiol. 60, 720–734
12 Kimmich, G. A. (1970) Biochemistry 9, 3669–3677
13 Vidaver, G. A. (1971) Biochim. Biophys. Acta 233, 231–234
14 Schafer, J. A. and Heinz, E. (1971) Biochim. Biophys. Acta 249, 15–33
15 Jacquez, J. A. and Schafer, J. A. (1969) Biochim. Biophys. Acta 193, 368–383
16 Potashner, S. J. and Johnstone, R. M. (1971) Biochim. Biophys. Acta 233, 91–103
17 Wheeler, K. P. and Christensen, H. N. (1967) J. Biol. Chem. 242, 1450–1457
18 Crane, R. K. (1962) Fed. Proc. 21, 891–895
19 Kimmich, G. A. (1973) Biochim. Biophys. Acta 300, 31–78

20 Goldner, A. M., Hajjar, J. J. and Curran, P. F. (1973) J. Membrane Biol. 10, 267–278
21 Tucker, A. M. and Kimmich, G. A. (1973) J. Membrane Biol. 12, 1–22
22 Johnstone, R. M. (1974) Biochim. Biophys. Acta 356, 319–330
23 Stanley, P. E. and Williams, S. G. (1969) Anal. Biochem. 29, 381–392
24 Christensen, H. N., de Cespedes, C., Handlogten, M. E. and Ronquist, G. (1974) Ann. N.Y. Sci. 227, 355–379
25 Johnstone, R. M. and Sung, C. P. (1967) Biochim. Biophys. Acta 135, 1052–1055
26 Johnstone, R. M. and Scholefield, P. G. (1962) Biochim. Biophys. Acta 94, 130–135
27 Potashner, S. J. and Johnstone, R. M. (1970) Biochim. Biophys. Acta 203, 445–456
28 Oxender, D. L. and Christensen, J. H. (1963) J. Biol. Chem. 238, 3686–3699
29 Szabo, G., Eisenman, G., Laprade, R., Ciani, S. M. and Krasne, S. (1973) in Membranes (Eisenman, G., ed.), Vol. 2, pp. 179–328, Marcel Dekker Inc., New York
30 Wheeler, K. P., Inui, Y., Hollenberg, P. F., Eavenson, E. and Christensen, H. N. (1965) Biochim. Biophys. Acta 109, 620–622
31 Jacquez, J. A. (1973) Biochim. Biophys. Acta 318, 411–425
32 Johnstone, R. M. and Scholefield, P. G. (1959) Cancer Res. 19, 1140–1149
33 Hempling, H. G. and Hare, D. (1961) J. Biol. Chem. 236, 2498–2502
34 Morville, M., Reid, M. and Eddy, A. A. (1973) Biochem. J. 134, 11–26
35 Myers, V. B. and Haydon, D. A. (1972) Biochim. Biophys. Acta 274, 313–322
36 Aull, D. (1967) J. Cell. Physiol. 69, 21–32
37 Gonda, O. and Quastel, J. H. (1962) Biochem. J. 84, 394–406
38 Eddy, A. A., Mulcahy, M. F. and Thomson, P. J. (1967) 103, 863–876
39 Komor, B., Komor, E. and Tanner, W. (1974) J. Membrane Biol. 17, 231–238