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Highly permeant anions and glucose uptake as an alternative for quantitative generation and estimation of membrane potential differences in brush-border membrane vesicles

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We have analyzed the combined utilization of highly permeant anions to induce membrane diffusion potentials and glucose uptake to probe the created potentials as a new approach to quantitative generation and estimation of membrane potential differences in vesicle studies. Rabbit jejunal brush-border membrane vesicles were used in our experiments so that membrane potential differences can be calculated from the Goldman-Hodgkin-Katz equation with the relative ion permeabilities recently reported for this preparation (Gunther, R.D., Schell, R.E. and Wright, E.M. (1984) J. Membrane Biol. 78, 119–127) or approximated by the Nernst potential for the anion. Iodide was selected as the highly permeant anion after showing its absence of effect on glucose uptake with equal concentrations of Na^+ inside and outside the vesicles and the membrane potential clamped to zero with gramicidin D. Membrane potential was varied by altering the intra- and extravesicular iodide concentrations while keeping isosmolarity and isotonicity constant by chloride replacement. In these conditions, glucose uptake was sensitive and correlated to the expected membrane potentials. Moreover, a linear relationship between the log initial rate of glucose transport and membrane potential differences could be established. This linear relationship was quite insensitive to inside replacement of choline by potassium and to pH variations in the incubation medium, thus showing the reproducibility and the versatility of the method and the adequacy of glucose uptake as a probe for membrane potentials. However, no information can be gained on the stoichiometry of the Na^+-glucose transporter as the slope of the straight line depends on both the charge carried by the fully loaded carrier and the point in the electric field at which the transition state of the carrier from *cis* to *trans* occurs. This new approach was compared with the more conventional one using valinomycin-induced K^+-diffusion potentials and the Nernst potential for potassium as means for creating and estimating membrane potential differences. Both techniques were not equivalent, as linear relationships showing smaller slopes and sensitivity to pH were recorded with the latter. These differences are compatible with a potassium permeability in the presence of valinomycin that is lower than generally assumed, at least when compared to the permeability of the other ions present in the incubation medium. it is concluded that our new procedure offers significant advantages over more classical approaches, particularly to test for membrane potential dependency of sodium cotransport systems in which cations have been implicated in the transport

Abbreviations: FCCP, carbonylcyanide *p*-trifluoromethoxyphenylhydrazone; Hepes, 4-(2-hydroxyethyl)-1-piperazineethanesulfonic acid; Mes, 4-morpholineethanesulfonic acid.

mechanism. This method can also be extended to other vesicle preparations by properly selecting an highly permeant anion. In the absence of knowledge upon the (relative) ion permeabilities of the membrane, an estimation of membrane potential differences by the Nernst potential for this anion should prove as good an approximation as the Nernst potential for potassium in the presence of valinomycin.

Introduction

Our recent studies on the characteristics of glutamic acid transport by rabbit jejunal brush-border membrane vesicles have led to the proposal of the existence of both protonated and unprotonated forms of the anionic amino acid carrier [1]. From the postulated model, one can thus predict that glutamate transport should be electrogenic, at least in some conditions. However, many conflicting results have been obtained in the fast few years when trying to answer the issue of the rheogenicity of glutamic acid transport. For example, electrophysiological studies in vivo [2] have demonstrated that tubular resorption of acidic amino acids by the rat kidney depolarized the brush border membrane. In contrast, in vitro studies with brush-border membrane vesicles isolated from the rat small intestine [3] or the rabbit kidney [4] have concluded to an electronetural Na^+-dicarboxylic amino acid cotransport. In these last studies, electrical potentials across the brush-border membrane have been generated by using K^+- or H^+ gradients and valinomycin or FCCP, respectively. However, using the same techniques, Burckhardt et al. [5] reached the opposite conclusion when using vesicles originating from rat renal proximal tubules. Besides the possibility of species and/or organ differences, a problem associated with these studies is that both K^+ and H^+, the two ions used to generate a membrane potential, are also substrates for the transport system [1,6]. A similar constatation was also made recently by Nelson et al. [7]. It thus appears that in vitro studies should be reevaluated by using other approaches.

Another way to induce membrane potentials in vesicle studies has classically been performed by imposition of salt gradients using anions of different lipophilicity [8,9], but this method suffers from the lack of knowledge upon the permeability coefficients of the different anions and thus can only give qualitative information concerning the membrane potential dependency of transport. However, since the report of Gunther et al. [10] who determined relative ion permeabilities of rabbit jejunal brush-border membrane vesicles, it appeared to us that salt-induced membrane potentials could be used for quantitative purposes. The high permeability reported for iodide as compared to chloride, sodium and potassium [10] makes it possible to consider the use of different concentrations of this anion inside and outside the vesicles in order to generate membrane potentials of different magnitudes. The values of these induced potentials can then be calculated by introducing the relative ion permeabilities into the Goldman-Hodgkin-Katz equation [10] and estimated with an appropriate probe.

This paper describes experiments designed to justify the utilization of iodide-induced membrane potentials for quantitative generation of electrical potentials across the brush-border membrane in rabbit jejunal vesicles. Uptake of glucose at low concentration (high-affinity pathway for glucose [11]) was chosen as a probe for quantification of the created membrane potentials. The method was also compared to the more classical utilization of valinomycin-induced K^+-diffusion potentials and proved to be of general application and, particularly, with systems in which K^+ participates to the transport mechanism.

Materials and Methods

Chemicals. All salts and chemicals for buffer preparation were of the highest purity available. Labeled compounds were from New England Nuclear Corporation as follows: D-[U-^{14}C]glucose (315 mCi/mmol) and D-[1-^{3}H(n)]mannitol (27.4 Ci/mmol). Ionophores, valinomycin and gramicidin D, were obtained from Sigma Chemical Company. Amiloride hydrochloride was a gift from Merck, Sharp and Dohme, Canada, Division of De Merck Frosst Canada Inc., Kirkland, Quebec.

Preparation of brush-border membrane vesicles.

The small intestine was removed from 2.0–2.5 kg male New Zealand white rabbits (Ferme cunicole Leonard, Ste-Cholastique, Quebec) and flushed with ice-cold 0.9% NaCl. The mucosa from the jejunum was scraped with a spatula on a cold glass plate. Brush-border membranes were purified by the calcium chloride precipitation method of Schmitz et al. [12] and vesicles were obtained by the method of Hopfer et al. [13] with slight modification as described recently [1]. Based on sucrase activity, enrichment factors in the range 13–18-fold over the homogenate were routinely obtained.

Transport studies. Uptake studies were carried out by the rapid filtration technique of Hopfer et al. [13] as described previously [1]. The freshly prepared vesicles were resuspended to a final protein concentration of 30–50 mg/ml with the final resuspension buffer, and an aliquot (0.3–0.5 mg protein) was added to the incubation medium kept at room temperature (20°C) to start the transport experiments. The composition of the final resuspension buffers for vesicles and the final concentrations in the incubation media will be indicated in the legends of the figures. At time intervals, aliquots were taken from the incubation mixture, poured in 1 ml quenched ice-cold stop-solution as in Ref. 1, filtered on a pre-wetted and chilled 0.45 μm nitrocellulose filter (Sartorium SM 11306) and washed with 4 ml of nonradioactive ice-cold stop-solution. Filters were then dissolved in mini-vials by 15-min incubation with 5 ml Filter Count (United Technologies Packard) and subsequent vortexing. ^{3}H and ^{14}C radioactivities were determined using a Minaxi Tri-Carb Series 4000, model 4450 scintillation counter (United Technologies Packard).

Results are expressed as nmol solute uptake/mg protein. Initial rates of uptake have been estimated from the 0.1 min time-point or by polynomial regression of the uptake time curve [11] and are expressed as nmol solute uptake/mg protein per min. Relative uptake values were calculated by normalization to 100 when membrane potential was set to zero. Regression analysis have been performed using an Apple IIe microcomputer and a curve-fitter program (P.K. Warme, Copyright 1980, Interactive Microware Inc.). Statistical analysis were done with Statcalc [14] on the same microcomputer.

Assays. Marker enzyme for the brush-border membrane, sucrase (EC 3.2.1.48), was routinely assayed by the method of Dahlqvist [15] as modified by Lloyd and Whelan [16]. Protein was measured according to Lowry et al. [17] with bovine serum albumin as standard.

Results

Absence of iodide effects on glucose transport

In order to use iodide-induced membrane potentials across the brush-border membrane vesicles and glucose uptake as a probe, one must first verify the absence of iodide effects per se on glucose uptake. This question was addressed in setting equal concentrations of Na^+ both inside and outside the vesicles and clamping the membrane potential to zero with gramicidin D. As shown in Table I, I^- and Cl^- behaved similarly as far as initial rates of glucose transport are concerned at each pH of the incubation medium.

TABLE I

ABSENCE OF IODIDE EFFECTS ON GLUCOSE TRANSPORT

Final resuspension buffers for vesicles: 50 mM Mes-Tris buffer (pH 6.0) or 50 mM Tris-Hepes buffer (pH 8.0), 0.1 mM $MgSO_4$, 100 mM NaCl plus 100 mM choline chloride or 100 mM NaI plus 100 mM choline iodide. Final concentrations in the incubation media (0.25 ml): 50 mM Mes-Tris buffer (pH 6.0) or 50 mM Tris-Hepes buffer (pH 8.0), 0.1 mM $MgSO_4$, 100 mM plus 100 mM choline chloride or 100 mM NaI plus 100 mM choline iodide, 0.01 mM D-[U-^{14}C]glucose, 0.5 mM amiloride and 0.016 mg gramicidin D. Values shown are the mean ± S.E. of two experiments with different preparations of vesicles. Initial rates of glucose uptake have been estimated by polynomial regression of the uptake time-course from 0.1 to 1.0 min (four points) as described in the text. One-way analysis of variance of the data performed as described in the text showed significant differences between uptakes at pH 8.0 and 6.0 but nonsignificant differences between the chloride and iodide values at both pH values.

pH	Anions	Initial rates of glucose uptake (pmol/mg protein per min)
6.0	Cl^-	15.4 ± 3.3
	I^-	14.1 ± 1.4
8.0	Cl^-	5.5 ± 1.4
	I^-	5.4 ± 1.4

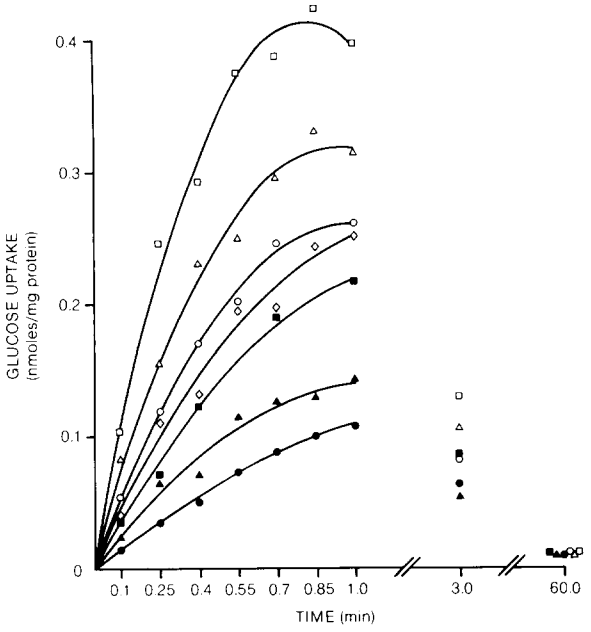

Fig. 1. Uptake time-course of glucose uptake at different ratios of intra- to extravesicular iodide concentrations. Final resuspension buffers for vesicles: 50 mM Tris-Hepes buffer (pH 7.5), 0.1 mM $MgSO_4$, and either 8 (□), 43 (△), 68 (○), 104 (◇), 138 (■), 163 (▲), or 198 mM (●) choline iodide (overall isotonicity and isosmolarity maintained constant to 208 mM with choline chloride). Final concentrations in the incubation media: 50 mM Tris-Hepes buffer (pH 7.5), 0.1 mM $MgSO_4$, 0.01 mM D-[U-^{14}C]glucose, 0.5 mM amiloride, 100 mM sodium chloride and/or iodide and 108 mM choline chloride and/or iodide to give iodide concentrations of 10 (●), 46 (▲), 71 (■), 104 (◇), 140 (○), 165 (△) and 200 mM (□). points shown are individual data-points from the same preparation of vesicles and are representative of the two experiments performed in the same conditions. The drawn curves have been obtained by polynomial regression as described in the text. Values for a_1 were 0.158 (●), 0.257 (▲), 0.368 (■), 0.416 (◇), 0.522 (○), 0.662 (△) and 1.096 (□) with coefficients of correlation of 0.998, 0.990, 0.998, 0.987, 0.999, 0.995 and 0.995, respectively.

Glucose uptake as a probe for membrane potential

In order to get a valuable probe for quantitative estimation of membrane potentials, one must find a molecule whose uptake is sensitive to the membrane potential and follows some kind of reproducible correlation with it. From the data of Kaunitz and Wright [18], showing a linear relationship between the log of initial rates of glucose uptake and the transmembrane potential differences in bovine intestinal brush-border vesicles, it appears that glucose transport could be such a probe. This possibility was analysed further in our system by studying the membrane potential dependency of glucose uptake, and the results are shown in Figs. 1 and 2. In these experiments, the glucose concentration was set to 0.01 mM, a condition which isolates the high-affinity pathway for glucose in rabbit jejunal vesicles [11].

Fig. 1 clearly shows that membrane potentials positive with respect to the vesicle interior decreased both the rate of glucose uptake and the maximum overshoot values, while the opposite polarity increased both parameters. Also, considering that membrane potentials have been generated by varying the iodide and chloride concentrations inside and outside the vesicles, it appears that iodide salts are effective in creating membrane potentials of different magnitudes.

Initial rates of glucose uptake have been estimated from the data presented in Fig. 1 by fitting Eqn. 1 to the progress curve up to 1 min [11]:

$$y = a_1 t + a_2 t^2 \qquad (1)$$

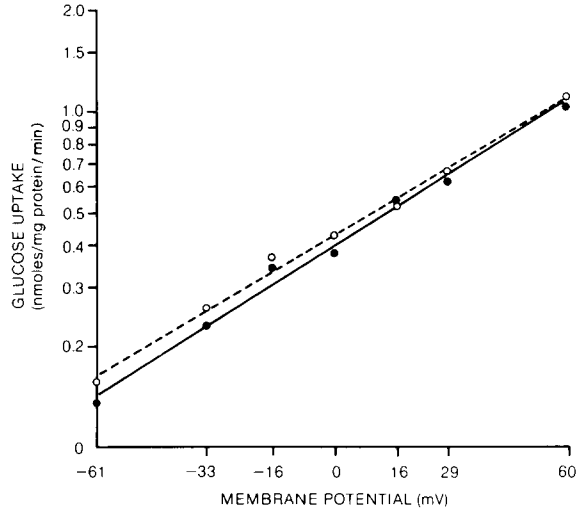

Fig. 2. Relationship between initial rates of glucose uptake and membrane potential differences. Initial rates of glucose uptake have been estimated from the data of Fig. 1 by polynomial regression (○) or by 10×0.1 min uptake values (●) as described in the text. Voltages are with respect to the vesicle exterior, calculated with the use of the Goldman-Hodgkin-Katz equation and relative ion permeabilities as reported in Ref. 10 (see text). Linear regression was performed as described under Materials and Methods and gave y intercepts of 0.4272 and 0.3956 nmol/mg protein per min, slopes of $6.78 \cdot 10^{-3}$ and $7.21 \cdot 10^{-3}$, and coefficients of correlation of 0.998 and 0.995 for (○) and (●), respectively.

In all cases, it should be noted that a coefficient of correlation equal to or higher than 0.987 was obtained and that the curves drawn in Fig. 1 represent the best fit to Eqn. (1). Values for a_1 have been plotted in Fig. 2 as the log initial glucose transport versus membrane potential differences across the brush-border membrane. For comparison, 10×0.1 min uptake values have been plotted in the same way on the same graph. It thus appears that the log of initial rates of glucose uptake rose linearly with the membrane potentials. However, one must consider that 0.1 min uptake values underestimated initial transport rates by an average of $7 \pm 2\%$ (S.E.) as compared to those determined through polynomial fitting but that this underestimation did not affect significantly the slope of the straight line obtained in Fig. 2. For this reason, we choose to use single (0.1 min) time-points as a reasonable approximation of initial rates in the following experiments.

Fig. 2 clearly demonstrates that a potential difference, vesicle exterior negative, inhibits sodium-coupled D-glucose transport, while the opposite polarity promotes transport, in agreement with previous qualitative [19,20] and quantitative [18] observations made with glucose transport in intestinal vesicles.

Versatility and reproducibility of iodide-induced membrane potentials

In the experiments reported in Fig. 3, we have compared the effect of replacing inside choline by potassium and determined the effect of pH on the relationship between glucose uptake and membrane potentials. Obviously, none of these modifications affected the system, thus showing that ion permeabilities relative to sodium were not modified by pH and that their values reported in Ref. 10 and used in the Goldman-Hodgkin-Katz equation are quite reliable. Also, considering the number of experiments reported in Fig. 3, it appears that our method is highly reproducible.

The data presented in Fig. 3 is compatible with a relationship between membrane potential and glucose uptake having the form of Eqn. 2:

$$J = J_0 e^{K\Delta\Psi} \qquad (2)$$

where J is the initial rate of glucose transport

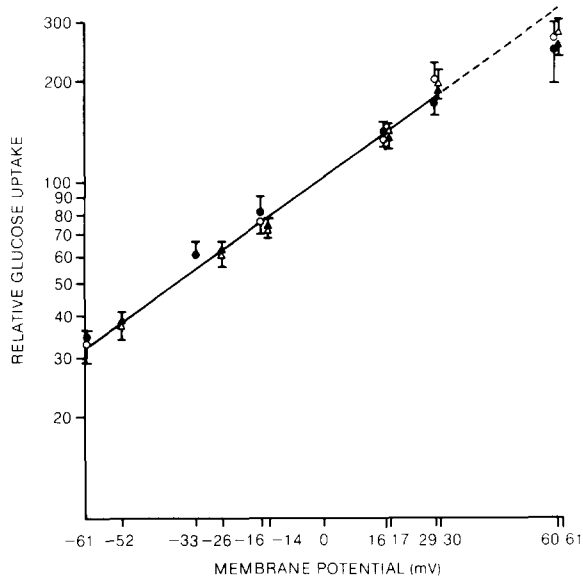

Fig. 3. Effect of replacing inside choline by potassium and varying pH in the incubation medium on the relationship between glucose uptake and membrane potential differences. Final resuspension buffers for vesicles as in Fig. 1 with 50 mM Tris-Hepes buffer (pH 8.0) (●, ▲) or 50 mM Mes-Tris buffer (pH 6.0) (○, △) and with inside replacement of 104 mM choline by 104 mM potassium (△, ▲) or not (○, ●). Final concentrations in the incubation media as in Fig. 1 (○, ●) with 50 mM Tris-Hepes buffer (pH 8.0) (●) or 50 mM Mes-Tris buffer (pH 6.0) (○). Final concentrations of chloride, iodide, sodium, glucose and amiloride in the incubation media for K^+-loaded vesicles as in Fig. 1, but with 50 mM Tris-Hepes buffer (pH 8.0) (▲) or 50 mM Mes-Tris buffer (pH 6.0) (△) and 104 mM choline plus 4 mM potassium (△, ▲). Points shown are the mean ± S.D. of two (○, ●) or three (△, ▲) experiments performed in duplicate on different preparations of vesicles. Initial rates of glucose uptake were estimated by single (0.1 min) time-point analysis. Voltages are as described in the legend of Fig. 2. Linear regression was performed as described in the text and gave a y intercept (0 potential) of 102, a slope of $8.16 \cdot 10^{-3}$ and a coefficient of correlation of 0.9946.

measured at different membrane potentials $\Delta\Psi$; J_0 is the transport rate when $\Delta\Psi = 0$; and K represents a constant determined by the slope of the straight line of Fig. 3. This equation is analogous to Eqn. 3 previously derived by Turner [21]:

$$J = J_0 e^{z\eta u} \qquad (3)$$

where z is the charge of the fully loaded carrier (in this case, the unloaded carrier is neutral), $u = F\Delta\Psi/RT$ (F, R and T have their usual meaning) and η characterizes the point in the electric field

at which the transition state of the carrier from form *cis* to *trans* occurs within the limits $1 \geqslant \eta \geqslant 0$ [21]. This last parameter can be obtained by comparison between Eqns. 2 and 3 and can be expressed as $\eta = KRT/zF$. With $K = 8.16 \cdot 10^{-3}$ (from Fig. 3) and assuming $z = +1$, $+2$ and $+3$, values of $\eta = 0.473$, 0.237 and 0.158 can be obtained, respectively. These values may be compared with $\eta = 0.24$ for sodium-succinate cotransport with renal vesicles [22] and $\eta = 0.09$ for sodium-glucose cotransport with intestinal vesicles [18].

Comparison of iodide-induced versus valinomycin-induced membrane potentials

In order to justify further the use of iodide induced membrane potentials across the rabbit jejunal brush-border membrane vesicles, we compared this new approach with the more classical use of valinomycin-induced K^+-diffusion potentials. In Fig. 4, the membrane potential was varied by altering the intra- and extravesicular KCl concentrations as described [18] and estimated by the Nernst potential for potassium. Also, the experiments have been performed at either pH 8.0 (closed symbols) or 6.0 (open symbols). The straight line obtained in Fig. 3 has been redrawn in Fig. 4 for comparison purposes. It thus clearly appears that both methods are not equivalent in their quantitative estimation of membrane potential values and that potassium permeability in the presence of valinomycin is variable with the pH of the incubation medium.

Three possibilities should be considered to explain these differences. First, an overestimation of iodide permeability relative to sodium as determined in Ref. 10 would lead to an overestimation of membrane potential values calculated by the Goldman-Hodgkin-Katz equation and thus to an underestimation of the slope in Fig. 3. Such an explanation would amplify the difference between the two methods noted in Fig. 4 and seems unlikely. Alternatively, the iodide permeability relative to sodium may have been underestimated by Gunther et al. [10]. The consequence can be checked directly by calculating the Nernst potential for iodide, i.e., assuming that iodide permeability greatly exceeds the permeability of all other ions present, and replotting the data for glucose

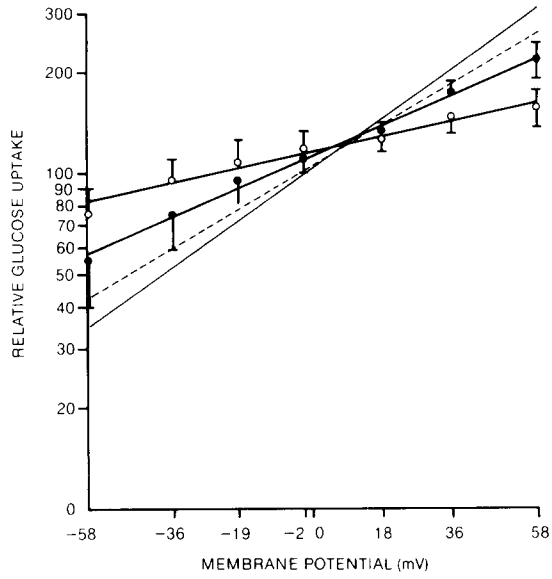

Fig. 4. Comparison of iodide-induced versus valinomycin-induced membrane potentials. Final resuspension buffers for vesicles: 50 mM Tris-Hepes buffer (pH 8.0) (●) or 50 mM Mes-Tris buffer (pH 6.0) (○), 0.1 mM $MgSO_4$, 300 mM D-mannitol, 0.003 mM valinomycin and either 100 mM KCl or 10 mM KCl plus 90 mM choline chloride. Final concentrations in the incubation media: 50 mM Tris-Hepes buffer (pH 8.0) (●) or 50 mM Mes-Tris buffer (pH 6.0) (○), 0.1 mM $MgSO_4$, 0.01 mM D-[U-^{14}C]glucose, 0.003 mM valinomycin, 0.5 mM amiloride, 100 mM NaCl, 80 mM mannitol, and 10–100 mM KCl (isotonicity with respect to KCl adjusted with choline chloride). Points shown are the mean ± S.D. of three experiments performed in duplicate on different preparations of vesicles. Initial rates of glucose uptake were estimated by single time point (0.1 min) analysis. Voltages are with respect to the vesicle exterior, calculated with the use of the Nernst equation as in Ref. 18. Linear regressions were performed as described in the text and gave y intercepts of 115 and 112, slopes of $2.53 \cdot 10^{-3}$ and $4.99 \cdot 10^{-3}$ and coefficients of correlation of 0.981 and 0.997 at pH 6.0 (○) and 8.0 (●), respectively. For comparison purposes, the data from Fig. 3 have been redrawn intact (———) or after recalculation of membrane potentials according to the Nernst potential of iodide (— — —). In this case, linear regression gave a y intercept of 104, a slope of $6.68 \cdot 10^{-3}$ and a coefficient of correlation of 0.994.

uptake in Fig. 3 according to these new potential values (dashed line in Fig. 4). Such a manipulation slightly reduces the slope difference between the two methods at pH 8.0 but not enough to give complete superposition of the two relationships. We are thus left with the third possibility, namely that the Nernst potential for potassium overestimates the true membrane potential difference

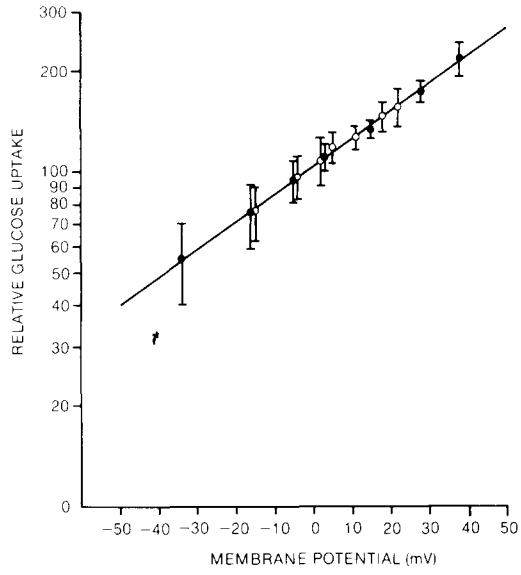

Fig. 5. Reconciliation of the data from Fig. 4 with those from Fig. 3. Valinomycin-induced K^+-diffusion potentials from Fig. 4 were recalculated using the complete Goldman-Hodgkin-Katz equation with ion permeabilities relative to sodium reported in Ref. 10 and K^+ permeabilities relative to sodium of 4 and 16 at pH 6.0 (○) and 8.0 (●), respectively. The drawn straight line corresponds to the regression line in Fig. 3.

across the brush-border membrane. Assuming different potassium permeabilities relative to sodium and using the Goldman-Hodgkin-Katz equation, one can estimate new membrane potential values for the data of Fig. 4. As shown in Fig. 5, a complete identity between the data of Figs. 3 and 4 can be obtained with relative potassium permeabilities of 16 (pH 8.0) and 4 (pH 6.0). It thus appears that this last explanation is compatible with both the slope difference between the two methods and the pH effect observed with K^+-valinomycin.

Discussion

The importance of membrane potentials in the energization and function of Na^+-cotransport systems is now firmly established (see Refs. 20, 23 and 24 for review) and can be demonstrated in studies using membrane vesicles [8,9,18–20,25]. In such studies, the general principle underlying the creation of membrane potentials of different magnitudes consists in the imposition of ion gradients that lead to the generation of diffusion potentials. Both cations in the presence of specific ionophores or anions with different lipophilicity have proved successful for this purpose (see Refs. 8, 9 and 24 for review). However, the precise determination of the magnitude of such diffusion potentials is impaired by the lack of knowledge upon the relative permeabilities for the ions used to establish the ion gradient and by difficulties associated with the utilization of several probes serving to estimate membrane potentials [8,9,24]. These considerations explain the paucity of kinetic data trying to quantify the effect of membrane potentials on Na^+-dependent transport systems. One approach has been attempted recently by Kaunitz and Wright [18] who analysed the effect of transmembrane potential differences on the uptake of D-glucose by bovine intestinal brush-border vesicles. In this paper, membrane potential was varied by altering the intra- and extracellular KCl concentration and estimated by calculating the Nernst potential for potassium, thus assuming that potassium permeability in the presence of valinomycin greatly exceeds the permeability of all other ions present. However, the method cannot be of general use, particularly with systems in which potassium has been found to participate in the reaction mechanism, like the anionic amino acid transport system [1,3–7]. In these systems, an inhibitory effect resulting from the valinomycin-induced dissipation of the internal K^+ gradient (used as a supplementary driving force for uptake) could reduce or even mask any stimulation produced by the so-created inside-negative membrane potential.

In this paper, we have designed experiments aimed at introducing an alternative method allowing quantitative estimation of membrane potentials generated by iodide, a highly permeant anion in rabbit jejunal brush-border vesicles [10]. Our results clearly show the validity of this new approach on the following grounds. First, iodide per se has no effect on glucose uptake (Table I). Next, varying the ratio of intra- to extravesicular iodine concentrations allows a linear relationship between the log of initial rates of glucose transport and membrane potential differences to be established (Fig. 2), in agreement with recently published results with bovine intestinal vesicles [18].

Moreover, it appears that the method is highly reproducible and versatile as it can be used in different ionic and pH conditions with the same efficiency (Fig. 3). It can also be concluded that glucose uptake is a good probe for quantitative estimation of membrane potential differences as it shows linearity over a large range of potential values independently of ionic and pH conditions. These results also show that membrane potential dependency of the Na^+-D-glucose cotransporter is pH-independent.

Finally, we compared our new approach with the use of valinomycin-induced K^+-diffusion potentials [18]. It clearly appears from Fig. 4 that both methods are not equivalent. However, the difference is easily explained by a K^+ permeability relative to the other ions present in solution which is lower in the presence of valinomycin than generally assumed. The instability of K^+-diffusion potentials in KCl solutions has been recognized by Wright during the calibration of membrane potentials by the dye, DiS-C_3-(5), and was attributed to the chloride permeability [26]. For the study of Na^+-cotransport systems, it also seems likely that the sodium permeability can interfere with the method and that replacing chloride by more impermeant anions as suggested [26] will only solve part of the problem. In this context, it is interesting to compare the data of Fig. 3 with those of Fig. 8 from the work of Kaunitz and Wright [18]. It thus appears that the glucose transporter from rabbit intestinal membranes is quite more sensitive to the membrane potential than the bovine carrier. Besides species differences, this observation could also be related to the techniques used to generate and estimate the potential differences across the membrane.

It should also be noted that no information can be gained on the stoichiometry of the Na^+-glucose transport in such studies, as the relationship between glucose uptake and membrane potential differences depends on both the charge carried by the fully loaded carrier (z) and the point in the electric field where the transition from *cis* to *trans* occurs (η). As calculated in the Results section, the data is compatible with any charge from 1 to 3 (or even more) being cotransported by the Na^+-glucose-carrier complex. A last comment concerns the linearity of the relationship observed between log glucose uptake and membrane potential differences. Such a result does not agree with the notion of 'saturating membrane potentials' [20,27] and with the expectations from theoretical analysis on either the effects of membrane potentials on the kinetics of solute cotransport [27,28] or the current-voltage relationships for cotransport systems [29], but is a very special case analysed by Turner [21]. In Fig. 3, the 61 mV point (interior negative) has not been included in the linear fit of the data-points, as this point significantly deviated by about 10% from the drawn straight line. Although this deviation could be the result of an underestimation of the initial rate of glucose transport by the single time-point procedure used in these experiments, the possibility that saturation of glucose transport takes place for membrane potentials higher than 60 mV should also be considered in the future by extending the range of membrane potentials and estimating true initial rates.

In conclusion, the new procedure described in this paper for quantitative creation and estimation of membrane potentials in rabbit jejunal brush-border membrane vesicles offers significant advantages over more conventional approaches. It is easier to handle than techniques using ionophores which require a solubilization step in ethanol, thus limiting the concentration of ionophore that can be used. It is insensitive to pH variations, a characteristic not shared by the K^+-diffusion potentials induced by valinomycin. Also, the high permeability of the membrane to iodide makes the method more insensitive to ionic replacement than the other one, at least for Cl^- and K^+, two ions which are used very often in vesicle studies. The utilization of sodium-dependent glucose uptake as a probe for membrane potentials circumvents the binding problems experienced with potential-sensitive dyes and the unknown mechanism of the dye response [8,9]. Finally, the method can be used with cotransport systems in which K^+ and/or H^+ have been shown to participate in the transport mechanism. This new method thus appears to be of more general use than methods with cations and ionophores. Moreover, the principle of this method should be applicable to other membrane vesicle systems if it is possible to find an anion with very high permeability. In the absence of

knowledge concerning the (relative) ion permeabilities in this new system, the Nernst potential for the selected anion should prove as good an approximation of membrane potential values as the Nernst potential for potassium in the presence of valinomycin.

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