Intestinal Na^+/glucose cotransporter expressed in *Xenopus* oocytes is electrogenic

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ABSTRACT The cloned rabbit intestinal Na^+/glucose cotransporter was expressed in *Xenopus* oocytes, and transmembrane currents associated with this transporter were monitored using a two-electrode voltage clamp. Addition of D-glucose to a Na^+-containing solution bathing these oocytes generated a current which was blocked by phlorizin. Water-injected control oocytes did not exhibit any currents under these conditions. The magnitude and shape of the currents were dependent on the extracellular glucose and Na^+ concentrations and the membrane potential. At $V_{hold} = -50$ mV, the K_m values for glucose and Na^+ were 14 ± 2 ($N = 4$) μM and 17 ± 1 ($N = 3$) mM, respectively. These K_m values and i_{max} exhibited voltage dependence: increasing the membrane potential from −30 to −150 mV increased K_m^{Glc} and i_{max} threefold and decreased K_m^{Na} eightfold. The reversal potential (V_R) of the phlorizin-sensitive, glucose-dependent current varied with log Na_o^+ (slope 46 ± 6 [$N = 9$] mV). In the absence of sugar, a Na^+-dependent, phlorizin-sensitive ($K_i = 3 \pm 0.5$ μM) current was detected only in RNA-injected oocytes. The amplitude of this current at −50 mV was 6 ± 1% ($N = 13$) of the maximum current measured in the presence of *D*-glucose. The V_R of this sugar-independent current varied with log Na_o^+ (slope 63 ± 1 [$N = 4$] mV), indicating that the cotransporter may carry Na^+ in the absence of sugar. We conclude that the Na^+/glucose cotransporter is electrogenic and that investigations of currents associated with its operation can yield valuable insights into the mechanisms of solute translocation.

INTRODUCTION

Active transport of sugars across the intestine is driven by Na^+-coupled glucose transporters in the brush border membrane. Whereas it has long been established that this Na^+/glucose cotransporter is electrogenic (see Schell et al. 1983), technical difficulties have seriously hampered the measurement of carrier currents. For this reason, only one indirect estimate of the I/V relations of the cotransporter has been reported (Lapointe et al., 1986).

The cloning and expression of the rabbit intestinal Na^+/glucose cotransporter in *Xenopus* oocytes (Hediger et al., 1987; Ikeda et al., 1989) now makes it possible to measure the currents in a simple, direct manner. In this first study, we have expressed the cloned transporter in oocytes and examined current-voltage relations of the transport protein. We demonstrate that the cloned transporter is electrogenic, and that the membrane potential influences the apparent maximal velocity of the transporter and the apparent binding constants for Na^+ and glucose. A preliminary account of these results has been presented (Coady et al., 1988).

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METHODS

Plasmid pMJC424, which is Bluescript KS^+ containing a 2.2-kb insert coding for the rabbit intestinal Na^+/glucose cotransporter (Hediger et al., 1987), was cleaved with Not I and the linearized DNA was extracted with phenol/chloroform followed by ethanol precipitation. Capped mRNA was transcribed from the cleaved plasmid in vitro using T3 RNA polymerase and an RNA transcription kit (Stratagene, San Diego, CA).

Oocytes from adult *Xenopus laevis* frogs were treated with collagenase (2 μg/ml) for 1 h. The next day, 50 (± 2) nl of either water or mRNA in water (1 μg/μl) were injected. The oocytes were then incubated in Barth's medium plus 100 μg/ml gentamycin at 18°C for 6–9 d and were defolliculated in preparation for two-microelectrode voltage clamp recording (Umbach and Gundersen, 1987). Only cells with resting membrane potentials > −45 mV were used. Oocytes were held under voltage clamp at −50 mV and transmembrane currents were measured in response to changes in bath D-glucose (Glc), Na^+, and phlorizin concentrations. The standard bath solutions contained (in millimolar): NaCl (0–100), choline Cl (100–0), KCl (1), $NaHCO_3$ (2.4), $MgSO_4$ (0.82), $Ca(NO_3)_2$ (0.33), $CaCl_2$ (0.41), Hepes/Tris, pH 7.5 (5). In some experiments, Na^+ isethionate (50–90 mM) replaced equimolar amounts of NaCl in the bath solution. In others, the external K^+ was varied between 1 and 25 mM by replacing 25 mM choline Cl with KCl (NaCl = 75 mM). The SO_4^{--}, PO_4^{--}, and Ca^{++} levels in the external solutions were increased in a few experiments by adding either 0.82 mM Na_2SO_4, or 1 mM Na_2HPO_4, or 0.74 mM $CaCl_2$ to the standard solutions. Voltage ramps, generally from −150 to +50 mV in 1.5 s or −200 to +100 mV in 2 s, were applied to the oocyte before and after addition of D-glucose to the bath solution. The currents obtained in the presence and absence of D-glucose were subtracted to produce the I/V curve for the Na^+/glucose cotransporter. In general, oocytes were

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briefly exposed to D-glucose for no longer than 30 s and then were washed in sugar-free bath solutions for 5 min before testing another glucose concentration. With this protocol, repetitive testing of the same D-glucose concentration gave superimposable I/V curves and V_R values within 2 mV. This protocol minimized the accumulation of intracellular glucose during the experiment. In some experiments, the ramps were applied before and after the addition of phlorizin (500 μM) to bath solutions containing D-glucose.

Currents at each substrate concentration [S] and voltage were fitted to the equation

$$i = \frac{i^s_{max}[S]}{K^s_m + [S]}, \quad (1)$$

by nonlinear regression analysis (Leatherbarrow, 1987) to estimate the maximum currents (i^s_{max}) and the substrate concentrations giving half the i^s_{max} (K^s_m). Experiments were conducted at 100 mM NaCl while varying the D-glucose concentration to yield i^{Glc}_{max} and K^{Glc}_m, and at 1 mM D-glucose while varying the Na_o^+ concentration to yield i^{Na}_{max} and K^{Na}_m. In each individual experiment, i^s_{max} and K^s_m were estimated (±SEM) at 10-mV intervals and were plotted as a function of voltage. In the figures, symbols without error bars indicate that the error is smaller than the size of the symbol. Experiments were repeated on 3–16 oocytes and estimated parameters (K^s_m, I^s_{max}, V_R) are quoted in the text as the mean ± SEM with the number of oocytes in parentheses.

Intracellular glucose was assayed in groups of 25 oocytes which were homogenized in 150 μl and immediately deproteinized using $Ba(OH)_2$ and $ZnSO_3$ (Somogyi, 1945). The suspensions were centrifuged, and the supernatant was passed through a 0.45 μm filter. The filtrates were assayed for glucose using a glucose oxidase-peroxidase technique (Dahlqvist, 1964); 150 μl volumes of glucose standards were subjected to the same deproteinization and assay procedure.

Phloretin and phlorizin were both purchased from Sigma Chemical Co. (St. Louis, MO).

Experiments were conducted at 20–22°C.

RESULTS

After injecting oocytes with clone-derived mRNA encoding the Na^+/glucose cotransporter, the addition of D-glucose to the extracellular solution produced a large inward current (Fig. 1 *A*). This current developed fully within the time required to change the bath D-glucose concentration (10 s) and then declined (<15 nA/min). The inward current was blocked by phlorizin (500 μM, Fig. 1 *A*) and was absent after substitution of Na^+ with choline. Water-injected control oocytes showed no measurable current response (<1 nA) to bath application of either 1 mM D-glucose (N = 12) or 500 μM phlorizin (N = 8). This is consistent with the negligible expression of this cotransporter in native oocytes (Ikeda et al., 1989).

The I/V curves of the Na^+/glucose cotransporter were determined by applying a voltage ramp to the oocyte membrane. To eliminate the contribution of currents elicited by the voltage ramp which are not associated with the expressed cotransporter, ramps were either applied in

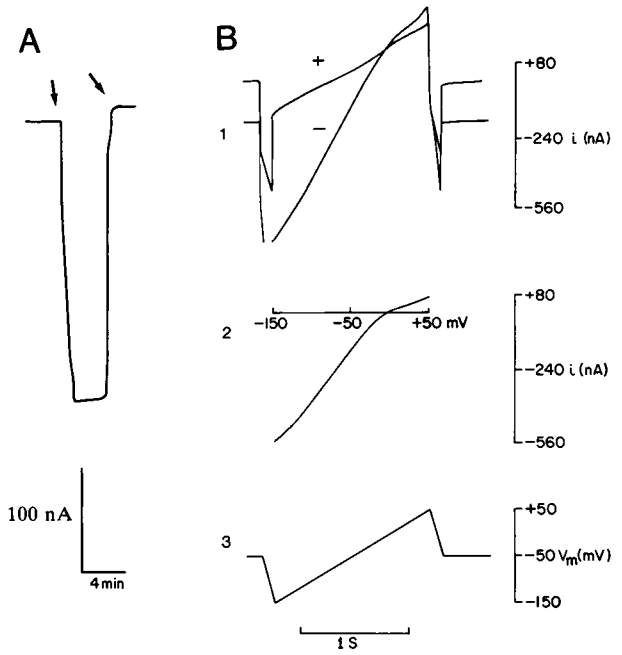

FIGURE 1 (*A*) Inward current associated with the Na^+/glucose cotransporter. A mRNA-injected ooycte was voltage clamped (V_{hold} = −50 mV) in a modified Ringer solution containing 100 mM NaCl, 0 mM glucose, and 0 mM phlorizin. At the first arrow, a similar solution containing 1 mM D-glucose was introduced. At the second arrow, 500 μM phlorizin (final concentration) was added to the glucose solution. Note that the baseline current before the addition of glucose differs from the level of current after the addition of phlorizin (see text for explanation). (*B*) Generation of a Na^+/glucose carrier I/V curve. (*1*) Superimposed current responses in a mRNA-injected oocyte elicited by identical voltage ramps in the presence (+) and absence (−) of 500 μM phlorizin. The extracellular solution contained 10 mM NaCl and 1 mM glucose, V_{hold} = −50 mV. See (*3*) for ramp protocol. (*2*) I/V curve associated with phlorizin-inhibitable currents in 10 mM Na and 1 mM glucose obtained by subtracting (+) and (−) records in *1*. (*3*) Voltage ramp protocol. The linear ramp from −150 to +50 mV lasted 1.5 s.

the presence and absence of D-glucose or in the presence of glucose before and after the addition of phlorizin (Fig. 1 *B 1*). These current records were then subtracted to produce either the D-glucose–dependent or phlorizin-sensitive currents. In the experiment illustrated in Fig. 1 *B*, where the bath D-glucose and NaCl concentrations were 1 and 10 mM, the phlorizin sensitive inward current was sigmoidal as a function of voltage between 0 and −150 mV. The current reversed at −2 mV, and a small outward current was recorded between 0 and +50 mV.

We generated I/V curves in the presence of 100 mM NaCl and four different D-glucose concentrations (Fig. 2 *A*). All four I/V curves were similar in that the V_R was +48 mV, the currents were linear from +50 to −25 mV, and then tended to saturate as the membrane potential

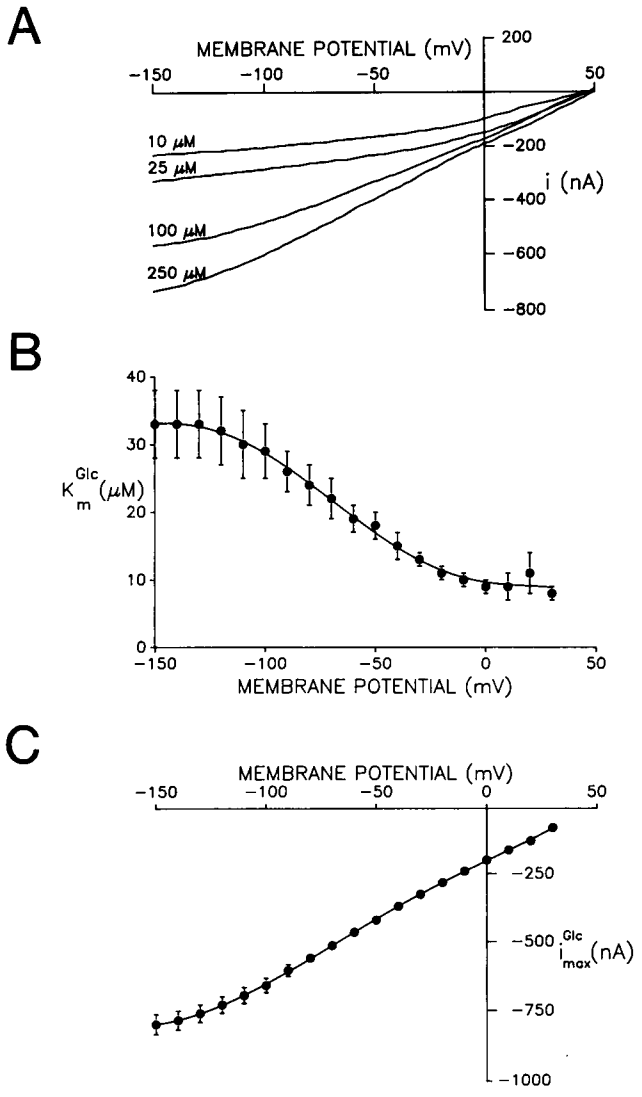

FIGURE 2 (*A*) Family of *I-V* curves with varying glucose$_o$. A family of *I-V* curves was generated by measuring the glucose-dependent currents in a mRNA-injected oocyte with varying external concentrations of glucose. The Na_o was 100 mM, and the glucose concentration ranged from 10–250 μM. (*B*) Effect of membrane potential on K_m^{Glc}. (*C*) Effect of membrane potential on i_{max}^{Glc}. i_{max}^{Glc} and K_m^{Glc} were estimated at 10-mV intervals by fitting the currents obtained at each glucose concentration (Fig. 2 *A*) to Eq. 1. The solid curves (*B* and *C*) are simply the best fit to the data, and they have no theoretical significance. The error bars were obtained from the linear regression analysis, and they reflect the goodness of fit of the data in *A* to Eq. 1.

increased towards −150 mV. V_R increased by +5 ± 4 (N = 6) mV when the D-glucose concentration was increased from 10 to 250 μM (the greatest dependence of V_R on Glc_o was observed in oocytes showing small carrier currents (<100 nA with Na_o^+ = 100 mM and Glc_o = 250 mM as compared to the relative independence of V_R on Glc_o with large [>500 nA] carrier currents).[1] At all voltages, the carrier-associated currents increased as the D-glucose concentration was raised from 10 to 250 μM. The currents (i) at each sugar concentration [S] and voltage were fitted to Eq. 1 to estimate i_{max}^{Glc} and K_m^{Glc}. i_{max}^{Glc} increased linearly from 70 nA at +30 mV to 650 nA at −100 mV and then appeared to saturate at 800 nA as the membrane potential approached −150 mV (Fig. 2 *C*). The K_m^{Glc} changed from 10 to 30 μM over this voltage range (Fig. 2 *B*). Similar results were obtained in three other oocytes. At the physiological membrane potential (−50 mV), K_m^{Glc} was 14 ± 2 μM, and i_{max}^{Glc} was 290 ± 70 (N = 4) nA. i_{max}^{Glc} was quite variable among oocytes, which reflects variations in the efficiency of expression of the cotransporter. By comparison, isotope flux experiments conducted within 2–3 d after injection of mRNA revealed that for the nonmetabolized sugar, α-methyl-D-glucopyranoside, the J_{max} varied between 200 and 1,500 pmol/(oocyte · h) and the K_m was 110 μM (Ikeda et al., 1989).

To determine the effect of Na^+ on the Na^+/glucose carrier currents, we varied Na_o^+ from 10 to 100 mM and measured the phlorizin-sensitive inward currents in the presence of 1 mM D-glucose, i.e., at a sugar concentration far in excess of the K_m^{Glc} (14 μM) at 100 mM NaCl. Fig. 3 shows the representative results obtained in one experiment. At 100 mM NaCl, the I/V curve obtained was very similar to that shown in Fig. 2 for 250 μM D-glucose. As the Na_o^+ was lowered to 10 mM (Fig. 3 *B*), smaller currents were measured at each membrane potential, and the V_R decreased from +50 to −5 mV. The V_R of the Na^+/glucose cotransporter is plotted as a function of the Na_o in Fig. 4. With 1 mM D-glucose, the semilog plot was linear with a slope of 46 ± 6 (N = 9) mV. At each voltage, the inward currents were fitted to Eq. 1 to estimate i_{max}^{Na} and K_m^{Na}. As shown in Fig. 3 *B* and *C*, i_{max}^{Na} increased linearly from 225 to 300 nA, and K_m^{Na} decreased from 40 to 5 mM as the membrane voltage was increased from −30 to −150 mV. Similar results were obtained from the analyses of two additional experiments. At physiological membrane potential (−50 mV), K_m^{Na} was 17 ± 1 mM, and i_{max}^{Na} was 290 ± 120 nA (N = 3). The K_m^{Na} was similar to that (32 mM) obtained for 50 μM α-methyl-D-glucopyranoside uptake (Ikeda et al., 1989).

In the absence of glucose, RNA-injected oocytes also expressed a Na^+-dependent inward current which could

[1] The 5-mV increase in V_r when Glc_o was increased from 10 to 250 mM was much less than expected (84 and 42 mV for n's of 1 and 2, see Eq. 2). In contrast, V_r varied with Na_o at fixed Glc_o as predicted (Fig. 4). The discrepancy between the observed as predicted changes in V_r with Glc_o is very puzzling, and we plan to reexamine reversal potentials under experimental conditions where we can confidently clamp the concentrations of Na^+ and Glc_o on each side of the plasma membrane.

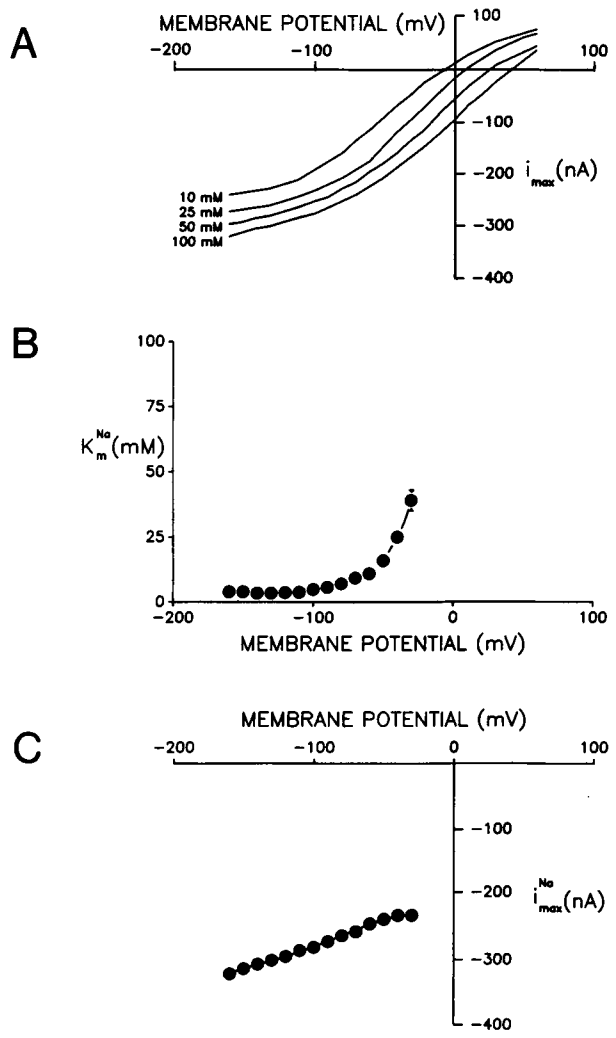

FIGURE 3 (*A*) Family of *I/V* curves with varying Na_o. A family of *I/V* curves was generated by measuring the phlorizin-sensitive currents as a function of Na_o in a mRNA injected oocyte. Currents were measured in 1 mM D-glucose as described in Fig. 1 *B*. (*B*) Effect of membrane potential on K_m^{Na}. (*C*) Effect of membrane potential on i_{max}^{Na}. i_{max}^{Na} and K_m^{Na} values were estimated at 10-mV intervals from −30 to −160 mV by fitting the currents obtained at each Na_o (*A*) to Eq. 1. The solid curves (Fig. 3, *B* and *C*) are simply the best fit to the data, and they have no theoretical significance. The error bars, when they are larger than the size of the symbol, were obtained from the linear regression analysis, and they reflect the goodness of fit of the data to Eq. 1.

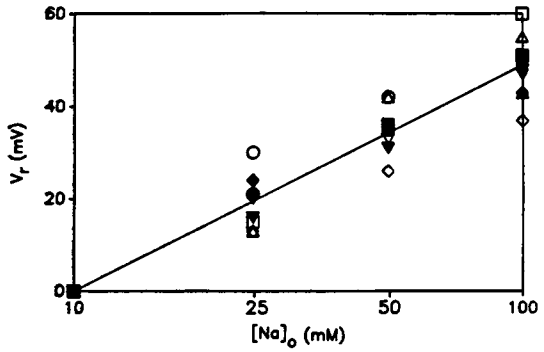

FIGURE 4 Reversal potential (V_R) of the Na^+/glucose current as a function of Na_o^+. In nine experiments, Na_o^+ was varied from 10 to 100 mM by replacing NaCl with choline Cl. The V_R of the phlorizin-sensitive current (see Fig. 3) was plotted against the log Na_o. Currents were measured in the presence of 1 mM D-glucose in the absence and presence of phlorizin (see Fig. 1 *B*). V_R values were plotted relative to the value obtained at 10 mM Na_o, and the slope was +46 ± 6 mV. At 10 mM Na, V_R was +11 ± 5 mV. The difference between the observed (46 mV) and the expected slope (59 mV, Eq. 2) may be in part related to the glucose-independent Na^+ current through the contransporter (see Fig. 6 and text).

be blocked by phlorizin. Analysis of this current was complicated by the presence of a background Na^+ conductance in oocytes (Dascal et al., 1984). In water-injected oocytes, a shift of Na_o^+ from 0–100 mM elicited a current (Fig. 5 *A*) with a V_R of +49 ± 1 mV (N = 4). A much larger current was observed in similar experiments with RNA injected oocytes (Fig. 5 *A*). Phlorizin abolished the difference in the Na^+-dependent current between water- and RNA-injected oocytes with a K_i of 3 ± 0.5 μM (Fig. 5 *C*). The aglycone, phloretin (100 μM), on the other hand, had no effect. This Na^+-dependent, phlorizin-sensitive current only accounted for 6 ± 1% (N = 13) of the total current measured in the presence of 1 mM D-glucose at a holding potential of −50 mV (Fig. 5 *B*). Parenthetically, the sugar-independent current accounts for the baseline shift observed when phlorizin was used to block the glucose-activated current (Fig. 1 *A*) and may account for the less than Nernstian slope (46 mV) of the V_R of the phlorizin-sensitive, glucose current (see Fig. 4).

The *I/V* curve of the sugar-independent current recorded in oocytes expressing the Na^+/glucose cotransporter exhibits anomalous behavior (Fig. 6 *A*). Phlorizin-sensitive current measured at 100 mM Na_o^+ reversed at −42 ± 2 mV in 16 oocytes. Neither the magnitude of the current nor the V_R was sensitive to variations in external K^+, Cl^-, Ca^{++}, SO_4^{--}, or PO_4^{--}. V_R did vary with external Na^+ in a Nernstian manner (Fig. 6 *B*). These experiments suggest that the phlorizin-sensitive, Na^+-dependent current observed in the absence of external sugar is a Na^+ current associated with the operation of the cotransport protein.

An unexpected feature of the sugar-independent, sodium-dependent current is that its V_R is −42 mV, which is far from the normal Na $V_R \geq +40$ mV in 100 mM Na_o^+ (Dascal, 1987). One clue to the origin of this negative V_R comes from experiments where we preloaded the oocytes with sugar, e.g., preincubating oocytes with 1 mM

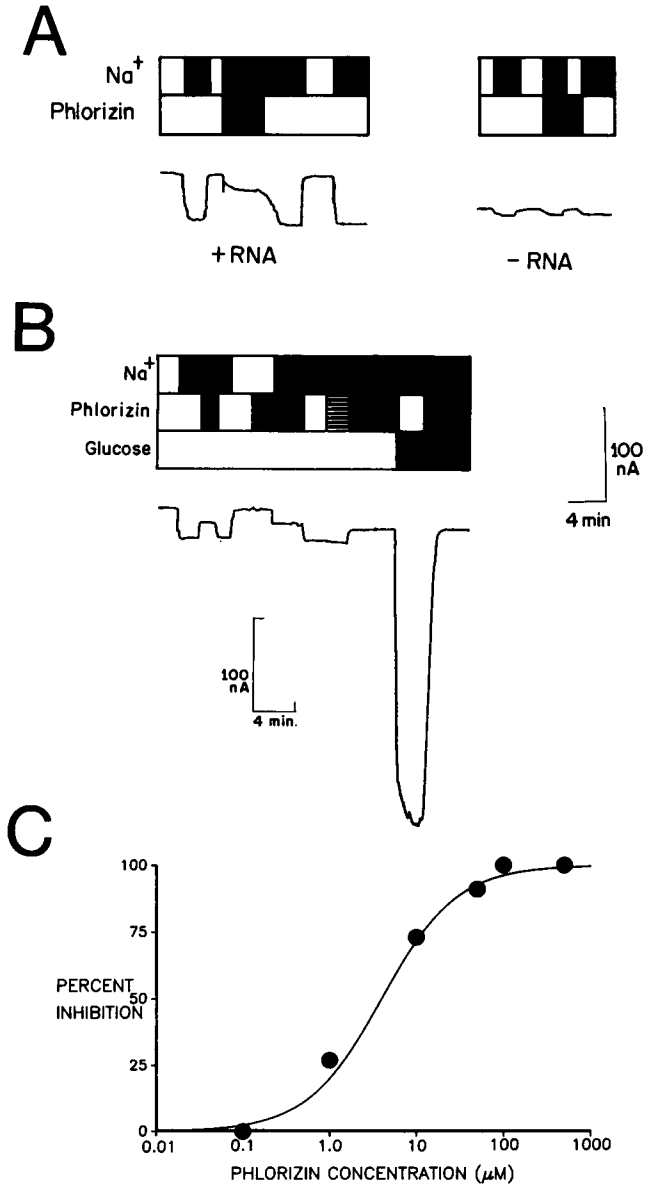

FIGURE 5 (*A*) Na^+ currents in control and mRNA injected oocytes. Currents were measured in oocytes from the same donor where either mRNA or H_2O was injected. Cells were clamped at −50 mV, amd the currents were recorded after changing the external Na (0–100 mM NaCl) and phlorizin (0–500 μM) in the absence of D-glucose. Solid bars represent the presence of Na^+ and phlorizin. (*B*) Demonstration of glucose-independent current. Currents across the membrane of a mRNA-injected oocyte were measured as a function of the external Na^+, glucose, and phlorizin concentrations. The box above the current trace shows the presence (*solid bars*) or absence (*open bars*) of 100 mM NaCl, 1 mM glucose, and 500 μM phlorizin in the bath. The striped box indicates the presence of 100 μM phloretin. (*C*) Phlorizin inhibition of glucose-independent current. The K_i for the inhibition by phlorizin of the glucose-independent current in mRNA-injected oocytes was examined by measuring the inhibition of this current by varying concentrations of phlorizin in the presence of 100 mM NaCl. The solid curve was that obtained for a nonlinear regression of the data using a competitive inhibition model, and this yielded a K_i of 3 ± 0.5 μM.

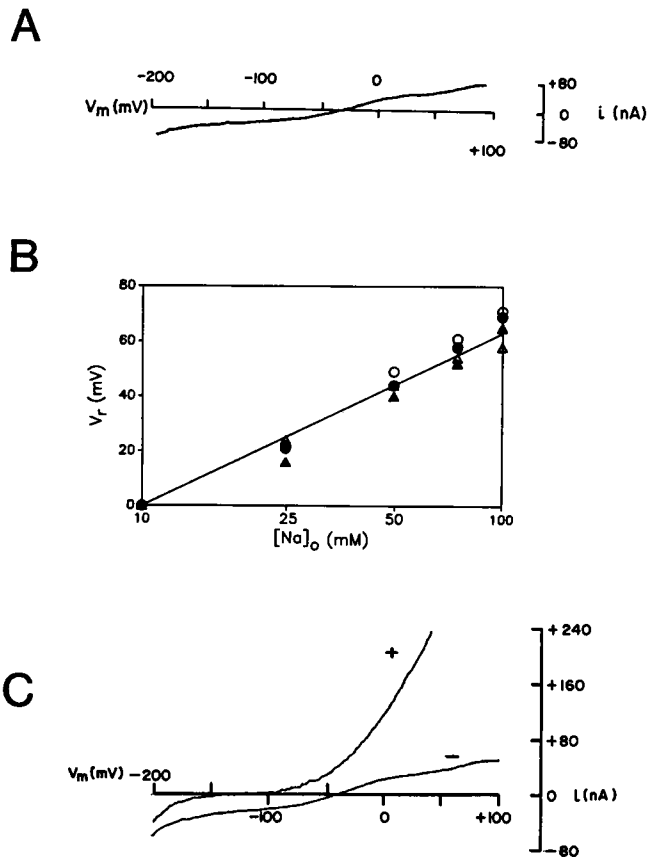

FIGURE 6 Glucose-independent currents. (*A*) *I/V* curve. In the absence of glucose, the current recorded in a mRNA-injected oocyte in response to a 2-s voltage ramp (−200 to +100 mV) from V_{hold} = −50 mV in the presence of 100 mM NaCl was subtracted from the current elicited with an identical ramp in the presence of 100 mM Na and 500 μM phlorizin to produce the *I/V* curve for this glucose-independent current. In this oocyte, the addition of 1 mM D-glucose at −50 mV generated an inward current of 350 nA. (*B*) Na^+ dependence of the reversal potential. In four mRNA-injected oocytes, phlorizin-sensitive currents were measured in the absence of sugar as a function of Na_o. Reversal potentials relative to those obtained at 10 mM Na_o are plotted against log Na_o. At 10 mM Na_o, V_R was 6 ± 1 mV, and the slope was 63 ± 1 mV. (*C*) Effect of intracellular sugar. Two oocytes from the same donor were incubated for 14–16 h in 10 mM NaCl, 90 mM choline chloride in the presence (+) or absence (−) of 1 mM α-methyl-D-glucopyranoside. This sugar is transported by the cotransporter, but is not metabolized (see Ikeda et al., 1989). Under these conditions, there should be no significant change in Na_i, but the intracellular concentration of the sugar should approach or exceed 1 mM. The oocytes were washed free of external sugar, and *I/V* curves were generated in 100 mM Na_o as described in *A*. Note the increase in the outward cotransporter current and the shift in the V_R in the oocyte preincubated with sugar. The *I/V* curve shown for the control oocyte was indistinguishable from that in *A*.

α-methyl-D-glucopyranoside in 10 mM Na^+ for 14 h. This increased the outward current and shifted V_R from −45 to −118 mV (Fig. 6 *C*). This result indicates that the level of intracellular sugar can affect the V_R of the Na^+ currents measured in the absence of external sugar.

In control oocytes, the level of intracellular glucose was not measureable in two experiments (limit of detection was 50 μM intracellular glucose, assuming 0.5 μl cytoplasmic volume per oocyte).

DISCUSSION

This investigation has revealed several features of the cloned intestinal Na^+/glucose cotransporter expressed in oocytes. First, our data provide direct evidence that the Na^+/glucose cotransporter is electrogenic. A current is associated with transporter operation in the oocytes, and the magnitude of this current is dependent upon the concentrations of extracellular glucose, Na^+, and phlorizin. At the physiological holding potential of −50 mV, the K_m values for glucose and sodium ($K_m^{Glc} = 14 \pm 2$ μM; $K_m^{Na} = 17 \pm 1$ mM) are consistent with those obtained for α-methyl-D-glucopyranoside uptake into oocytes and rabbit brush border membrane vesicles (Ikeda et al., 1989).

Our data show that operation of the cotransporter is voltage dependent. Inward current associated with the activated protein is enhanced by increasingly negative membrane potentials from +50 to −150 mV. The shape of the *I/V* curves measured in oocytes is similar to the *I/V* relations determined indirectly for the Na^+/glucose cotransporter in brush border membrane (Lapointe et al., 1986) and directly for the pancreatic Na^+/alanine cotransporter (Jauch et al., 1986). The cotransporter current in the oocytes tends toward saturation at −150 mV, and this indicates that a voltage-independent process becomes rate limiting at these highly negative potentials. Another corollary of these observations is that in future tracer flux experiments it will be necessary to clamp the membrane potential so that the driving forces for transport are precisely defined.

Current associated with the Na^+/glucose cotransporter reverses polarity at positive membrane potentials. This indicates that the cotransporter can function bidirectionally and suggests that there is a finite amount of Na^+ and perhaps glucose available to the internal face of the protein membrane during our measurements. To determine the Na^+/Glc coupling coefficient (n) from V_R, both the intracellular and extracellular concentrations of Na^+ and glucose must be known. The relationship between the V_R and these concentrations is described by the equation:

$$V_R = \frac{60}{n} \log \frac{Na_o^n Glc_o}{Na_i^n Glc_i} \qquad (2)$$

where the subscripts i and o refer to the internal and external concentrations. Eq. 2 indicates that the slope of the V_R is independent of n when varying Na. To determine n, it will be necessary to measure Na_i and Glc_i at V_R. Although Na_i^+ has been reported in control oocytes to be 6–22 mM (Dascal, 1987) and the intracellular glucose concentration in control oocytes is <50 μM, we have no information about the intracellular concentrations of substrates during our experiments. The abundance of Na/K pumps in oocytes suggests that Na_i^+ should remain fairly constant in the face of Na^+/glucose cotransport. Under resting conditions, the ouabain-sensitive ATPase activity in *Xenopus* oocytes has been found to exceed 2 pmol ATP hydrolyzed s^{-1} per oocyte (Gundersen, C., unpublished results). However, glucose will accumulate in the cell in the absence of significant metabolism. Given the H_2O volume of an oocyte (0.5 μl) and the expression of the cotransporter described here, we estimate that the rate of glucose accumulation may be as high as 6 μM s^{-1}. This means that in oocytes exhibiting the highest carrier currents Glc_i did not exceed 180 μM during the normal 30 s exposure of the cell to glucose. However, it is probable that the local concentration of glucose near the membrane may be much higher than this value. Indeed, such a local accumulation of glucose during our measurements may account for the unexpectedly small variation seen in V_R with changing Glc_o in 100 mM Na_o. It will pose a considerable challenge to design experiments where the concentrations of sugar and Na^+ at the membrane are controlled, especially with the potential for unstirred layer effects on each side of the plasma membrane.

Our experiments do provide preliminary information about the voltage dependence of Na^+/glucose cotransport kinetics. For the purpose of this discussion, we assume that the cotransporter can be repesented as a rapid equilibrium iso ordered bireactant system (see Turner, 1981; Kaunitz and Wright, 1984; Schultz, 1986; Jauch and Läuger, 1986). Although we are aware that these may not strictly be the conditions during our experiments, to keep the model simple, we will initially assume that the intracellular Na^+ and sugar concentrations are insignificant. The inward glucose dependent current is given by

$$i = \frac{i_{max}[Glc]_o}{K^{Glc}\left(1 + \frac{K^{Na}}{[Na]_o}\right) + [Glc]_o}, \qquad (3)$$

where at constant Na_o, the experimentally derived K_m^{Glc} is given by

$$K_m^{Glc} = K^{Glc}\left(1 + \frac{K^{Na}}{Na_o}\right). \qquad (4)$$

The apparent affinity constants (K^{Glc} and K^{Na}) are

related to the true affinity constants ($\underline{K}^{Glc}$, $\underline{K}^{Na}$) by terms relating to the translocation rates of the loaded (k_p) and unloaded (k'_p) carriers.

$$\text{e.g., } K^{Glc} = \underline{K}^{Glc}(k'_p/(k'_p + k_p)] \quad (5)$$

$$\text{and } i_{max} = \frac{[C]k_p \cdot k'_p}{(k_p + k'_p)}, \quad (6)$$

where C is the total number of cotransporters (see Kaunitz and Wright, 1984).

At saturating sodium concentrations (100 mM Na at membrane potentials more negative than −30 mV, Fig. 3 *B*), $K_m^{Glc} \sim K^{Glc}$, and $i_{max}^{Glc} \sim i_{max}$ (Eq. 4). Likewise, at saturating glucose concentrations (1 mM D-glucose, Fig. 2 *B*), $K_m^{Na} \sim K^{Na}$ and $i_{max}^{Na} \sim i_{max}$. Note that at −50 mV, we find i_{max}^{Glc} at 100 mM Na_o is not significantly different from i_{max}^{Na} at 1 mM Glc_o ($i_{max}^{Glc} = 290 \pm 70$ [$N = 4$] nA = $i_{max}^{Na} = 290 + 120$ [$N = 3$] nA).

There are only three steps in the transport cycle that may be voltage dependent:

(*a*) The translocation of the loaded or the unloaded cotransporter (k_p and k'_p). It is frequently assumed that the unloaded carrier is negatively charged (see Schultz, 1986), but direct evidence is lacking.

(*b*) Sodium binding (ion-well effects, Jauch and Läuger, 1986).

(*c*) Sugar binding. Because the sugar is electrically neutral, the effect of voltage on K_m^{Glc} may be due to conformational changes of the transporter in the vicinity of the sugar binding site.

Under the assumption that the intracellular substrate concentrations are vanishingly small, only voltage effects on the external binding sites need to be considered.

The results in Figs. 2 and 3 show that all kinetic parameters (i_{max}, K_m^{Na}, and K_m^{Glc}) are voltage dependent. i_{max}^{Glc} increased linearly from −30 to −100 mV and then approached saturation (Fig. 2 *C*). This suggests that the translocation of the carrier (either negatively charged unloaded or positively charged loaded) is a voltage-dependent process, and at higher potentials, another step appears to be rate limiting. K_m^{Glc} increased from 10 to 30 μM, from −30 to −150 mV. This effect may be related to the voltage dependence of k'_p or k_p, or to a conformational effect at the glucose binding site. The steep relationship between K_m^{Na} and voltage (Fig. 3 *B*) is similar to that described for both the Na^+/glucose, and the Na^+/alanine cotransporters (Kimmich and Randles, 1988; Jauch et al., 1986). This may be due in part to an effect on Na^+ binding or translocation rates (k'_p, k_p). The former corresponds to an ion-well effect where the external Na^+ binding site is some distance from the surface of the membrane (Jauch and Läuger, 1986; Schultz, 1986).

To make further progress in understanding the voltage dependence of the cotransporter, it will be necessary to determine the Na^+/glucose coupling coefficient and measure I/V relations under conditions where Na_i and Glc_i are varied between 0 and saturating concentrations. A coupling coefficient greater than 1 and the presence of intracellular Na^+ and glucose should not qualitatively alter our present conclusions, especially under conditions where the driving forces for inward transport exceed those for outward transport, i.e., $Na_o \gg Na_i$, and the membrane potential is between −30 and −200 mV. With the exception of one study on sugar uptake into isolated enterocytes where only K_m^{Glc} was affected by voltage (Restrepo and Kimmich, 1985), our results are consistent with those obtained by others on the effect of voltage on the kinetics of the Na^+/glucose cotransporter in brush border vesicles and cells (Brot-Laroche et al., 1987; Kimmich and Randles, 1988).

Current measurements can be used to calculate the number of cotransporters expressed in the oocyte membrane. At $V_{hold} = -50$ mV, inward current in the presence of saturating amounts of Na_o and Glc_o ranged between 250 and 560 nA. Using these values and a turnover number of 5 s^{-1} (Peerce and Wright, 1984) and $n = 1$, this yields a cotransporter density of 1.3–4.4 × 10^5 per μm^2 of surface membrane. This density of packing exceeds that seen for membrane proteins like the nicotinic ACh receptor at the neuromuscular junction and rhodopsin in rod outer segments (10^4 and 5 × 10^4 per μm^2 [Hille, 1984]), but is comparable to the Na^+/glucose cotransporter in intestinal brush border membranes (Peerce and Wright, 1984). These estimates indicate that the oocyte is capable of expressing a high density of these foreign proteins on its surface.

Our investigation also revealed the presence of a Na^+-dependent, glucose-independent current which was blocked by phlorizin, but not phloretin, in oocytes injected with clone-derived RNA. This observation suggests that the Na^+/glucose cotransporter may act as a current carrier in the absence of sugar. The small magnitude of this current relative to the amplitude of current in the presence of glucose implies that there may be some slippage in the coupling of Na^+ and glucose. Alternatively, this current may be a result of fragmented translation products. It will be interesting to obtain evidence of whether or not this glucose-independent current is a feature of the Na^+/glucose cotransporter in brush border membranes.

In this study we have exploited the advantage of the oocyte expression system to examine the electrical properties of the intestinal Na^+/glucose cotransporter. We anticipate that this expression system will provide unique information about the kinetic and electrical properties of this and other electrogenic co- and counter-transport systems for amino acids, neurotransmitters, carboxylic acids, and ions as they become cloned. Our strategy for

studying the electrophysiology of the Na^+/glucose cotransporter may be directly applicable to these other transporters.

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