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Comparison of a Na^+/D-glucose cotransporter from rat intestine expressed in oocytes of *Xenopus laevis* with the endogenous cotransporter

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Epithelial Na^+/D-glucose cotransport was incorporated into the plasma membrane of *Xenopus* oocytes after microinjection of poly(A)$^+$-mRNA from rat intestine tissue and was detected by measurements of uptake of [^{14}C]AMG (methyl α-D-glucopyranoside). In mRNA-injected oocytes, the rate of AMG uptake exceeds the rate of endogenous Na^+/AMG cotransport by a factor of up to 30. It is demonstrated that the additionally expressed transport differs qualitatively from the endogenous transport with respect to several parameters which is a prerequisite for the demonstration of expression of a foreign transporter: (1) The expressed system is more sensitive to external glucose or AMG and to the specific inhibitor phlorizin, (2) it is less sensitive to external Na^+ and to changes in membrane potential, and (3) it is susceptible to inhibition by monoclonal antibodies, known to bind specifically to Na^+/glucose cotransporters and to modulate the cotransport in kidney and intestine. The use of the antibodies allows one to distinguish between endogenous Na^+/AMG cotransport and foreign cotransport expressed by injection of foreign mRNA. The expression of the foreign transport leads to transport rates that are high enough to detect the electrical current generated by the Na^+/glucose cotransport. This allows future characterization of the cotransport system under voltage-clamp conditions by analyzing membrane current.

Introduction

Na^+/glucose cotransport has been assumed to be present only in certain epithelial cells including those in kidney and intestine (see, for example, Ref. 1). But cotransport with qualitatively similar characteristics can also be detected in the oocytes of *Xenopus laevis* [2,3]. The oocytes have been introduced by Gurdon et al. [4] as an expression system for foreign mRNA. After injection of mRNA encoding for intestine Na^+/glucose cotransport, increased glucose transport has been demonstrated by increased Na^+-dependent uptake of radioactively labeled glucose [2,5,6] or increased membrane conductance [7]. In these investigations, the presence of endogenous glucose transport may have been underestimated. Previously, we have demonstrated [3] that the endogenous Na^+/glucose cotransport can exhibit maximum uptake rates of about one order of magnitude higher (25 pmol/h per oocyte) than estimated by Hediger et al. [2] and Ikeda et al. [6] for the endogenous transport (< 0.25–4.5 pmol/h per oocyte). Large variability in endogenous transport characteristics is typical for the *Xenopus* oocytes and most likely reflects developmental and/or seasonal variations. Therefore, if a foreign transport protein is to be expressed in the oocytes by injection of mRNA one ought to prove that the mRNA injection indeed leads to expression of the foreign transporter rather than to stimulation of the endogenous transporter (see, for example, Ref. 8).

Here we present data which demonstrate that injection of poly(A)$^+$-mRNA obtained from rat intestine leads to increased Na^+-dependent transport of glucose and methyl α-glucopyranoside (AMG). Two types of Na^+/glucose cotransport have been described, one that accepts AMG as substrate, the other one is insensitive to AMG [9,10]. The AMG transporter expressed after injection of the mRNA shows kinetic properties distinctly different compared to the endogenous cotrans-

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port. In addition, the expressed cotransport could be inhibited by monoclonal antibodies against Na^+/D-glucose cotransport of porcine kidney [11]. Stimulation of Na^+/glucose cotransport results in electrical inward current carried by the Na^+ ions. After injection of mRNA, the electrogenic contribution of the elevated transport system is large enough to be detected, and the glucose-dependent current can be analyzed in voltage-clamp experiments. Parts of the results have been reported previously [12].

Materials and Methods

Preparation of mRNA

Poly(A)$^+$-mRNA was prepared from small intestine of female Wistar rats. 2 g of scraped intestinal mucosa was homogenized in 20 ml of 6 M guanidine hydrochloride, 100 mM 2-mercaptoethanol and 20 mM sodium citrate (pH 7.0), and total RNA was isolated as described by Braell and Lodish [13] with modifications described by Oberleithner et al. [14]. The poly(A)$^+$-mRNA was purified by oligo(dT)-cellulose chromatography, and analyzed on 1% agarose minigels containing 2.2 M formaldehyde as described by Maniatis et al. [15]. Poly(A)$^+$-mRNA was then precipitated with ethanol, solved in water and stored frozen in small fractions. About 50 to 90 ng of poly(A)$^+$-mRNA were injected per oocyte (see Grygorczyk et al. [16]).

Monoclonal antibodies

In this study two monoclonal antibodies (R5A3, T4B2) were used which were obtained after immunization of mice with brush-border membrane proteins of porcine kidney [11]. R5A3 (IgG_3, kappa) and T4B2 (IgM, kappa) bind to polypeptides of porcine kidney with apparent molecular weights of 75 and 47 kDa; these polypeptides have been identified previously as components of the renal Na^+/D-glucose cotransporter [17]. In pig kidney, binding of R5A3 and T4B2 to proteins of brush-border membranes depends on the presence of D-glucose and inhibits Na^+-dependent D-glucose uptake into vesicles [11]. Furthermore, high-affinity binding of phlorizin to brush-border membranes of porcine kidney was inhibited by R5A3, and in rat kidney specific binding to the luminal side of proximal tubules could be demonstrated [18]. R5A3 and T4B2 crossreact with a Na^+/D-glucose cotransporter from intestine since in Western blots of intestinal brush-border membranes from pig, rabbit and rat the antibodies again bind to polypeptides with molecular weights of 75 kDa and 47 kDa. In brush-border membrane vesicles of intestine, R5A3 and T4B2 stimulated Na^+-dependent D-glucose uptake ([19] and unpublished data). These data indicate that the antibodies specifically bind to the Na^+/D-glucose cotransporter of epithelial membranes and can specifically modulate the activity of the cotransport; depending on the preparation, glucose transport can either be inhibited or stimulated [19].

The hybridoma cell lines R5A3 and T4B2 were grown in mass culture. To obtain antibody or control solutions, the hybridoma supernatant of R5A3 and T4B2 or culture medium that contained fetal calf serum were 10-fold concentrated over YM-100 filters (Amicon) and frozen at −80 °C. Before use, the antibody or control medium was dialysed extensively against oocyte Ringer's solution (ORi, see below), and the protein concentration was adjusted to 20 mg/ml. The concentration of R5A3 and T4B2 in the antibody media was determined by radioimmune assays [11]. Mouse myeloma IgG_3 and IgM was obtained from Sigma.

Uptake experiments

The methods to obtain oocytes of the clawed toad *Xenopus laevis*, to measure glucose uptake, and to perform electrophysiological measurements were identical to those described in the previous paper by Weber et al. [3]. Oocytes were incubated for 1 to 3 h in solution containing the radioactively labeled substrate D-[^{14}C]glucose or D-[^{14}C]AMG at 148 kBq or 370 kBq per 500 µl incubation solution, respectively (Amersham Buchler, Braunschweig, F.R.G.). After the incubation period, oocytes were washed and radioactivity taken up by single oocytes was determined by liquid szintillation counting. Most uptake experiments were performed in the standard bath solution (ORi) with the following composition (in mM): 110 NaCl, 3 KCl, 2 $CaCl_2$, and 5 *N*-2-hydroxyethylpiperazine-*N'*-ethanesulfonic acid (Hepes, adjusted to pH 7.6). If lower Na^+ concentrations were used, the Na^+ was replaced by tetramethylammonium (TMA). All experiments were performed at room temperature.

To describe concentration dependencies, the following equation was fitted to the data shown in Figs. 3 and 4:

$$k = k_{max} \frac{[X]^n}{(K_{1/2})^n + [X]^n} \quad (1)$$

where k represents either rate of transport or degree of transport inhibition, and [X] is the respective substrate or inhibitor concentration. Characteristics of the expressed transport were determined from data that have been corrected for the endogenous contribution to total flux. Since the data in Fig. 2 suggest two types of glucose binding sites, the $K_{1/2}$ value for high affinity was calculated from the uptake rates at low glucose concentrations fitting the data by Eqn. 1; with the obtained parameters, the $K_{1/2}$ value for low affinity was estimated by fitting the whole set of data by the sum of two components using the $K_{1/2}$ value and n for the low-affinity component as fit parameters.

To investigate the effects of antibodies on glucose transport, oocytes were incubated for 1 h in ORi to which control or antibody medium was added. The total protein concentration was 2 mg/ml, the concentrations of R5A3 and T4B2 were 10 and 25 μg/ml, respectively. In control experiments, nonspecific IgG_3 or IgM antibodies were added to the control solution. After incubation with the antibodies or control solution, [^{14}C]AMG was added and uptake by the oocytes was measured.

Electrophysiological measurements

Voltage-clamp was performed by conventional two-microelectrode techniques. For determination of current-voltage relations, steady-state current was measured during the last 100 ms of 500-ms rectangular voltage pulses to different potentials. These pulses were applied from the respective resting potential at a frequency of 0.25 Hz.

Results

In a previous report [3], it was shown that at D-glucose concentrations below 0.25 mM most of the endogenous glucose uptake in ORi is mediated by Na^+/glucose cotransport; the fraction of Na^+-independent uptake is less than 25%. Whether this Na^+-independent glucose uptake is carrier-mediated has not been clarified, components have been described that are inhibited by [20] or resistant to cytochalasin B [3]. To detect the Na^+-driven cotransport, in the experiments described in this paper substrate uptake was determined as the phlorizin-sensitive component, or AMG was used as substrate. AMG is accepted by most Na^+/glucose cotransporters as substrate but not by Na^+-independent glucose carriers [21] (for the oocytes see Weber et al. [3]).

At 40 μM AMG, the rate of substrate uptake by the endogenous cotransporter is below 1 pmol/h. Two days after injection of mRNA, the uptake rate may be elevated by more than one order of magnitude (Fig. 1A). Glucose transport is even further increased three days after the injection (Fig. 1B). Longer incubation led to a slight further increase (about 30%); nevertheless, the experiments described below were always performed two or three days after mRNA injection. As has been shown also for uninjected oocytes [3], Na^+-dependent glucose uptake is inhibited by more than 98% in the presence 1 mM phlorizin (Fig. 1B).

To elucidate possible differences between Na^+/glucose cotransport in control and in mRNA-injected oocytes, several of the following figures, demonstrating transport characteristics, will show in addition to the data obtained for injected oocytes also the corresponding data for uninjected control oocytes some of them we already presented previously [3].

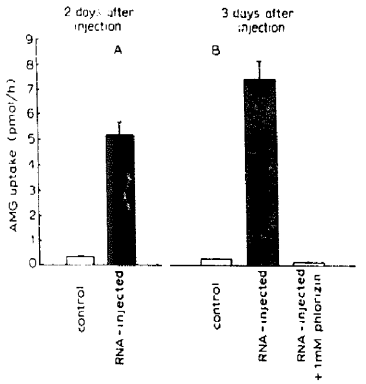

Fig. 1. Rate of uptake measured with ^{14}C-labeled AMG in uninjected control oocytes and in oocytes injected with 50 ng/oocyte poly(A)$^+$-mRNA from rat intestine. Flux measurements were performed 2 (A) and 3 (B) days after the mRNA injection. The AMG concentration in the bath solution was 40 μM. Phlorizin concentration to inhibit Na^+/glucose cotransport was 1 mM. Values are averages (±S.E.) of 10 oocytes.

Dependence of AMG uptake on AMG concentration

Fig. 2 shows the dependence of rate of AMG uptake on AMG concentration. As was found already for the endogenous Na^+/AMG cotransport, also the AMG transport in the injected oocytes seems to be composed of two saturating components. To calculate $K_{1/2}$ values, the data were fitted by the sum of two components of the type described by Eqn. 1; values of about 0.08 and 1.11 mM were obtained. These values are lower then those obtained in control oocytes (see legend to Fig. 2) and demonstrate higher sensitivity for AMG in

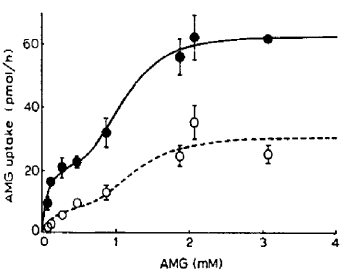

Fig. 2. Dependence of rate of AMG uptake on AMG concentration. Filled symbols represent data (±S.E.) from mRNA-injected oocytes. A fit of Eqn. 1 to the first four data points representing high-affinity uptake yielded $K_{1/2} = 0.08 \pm 0.02$ mM, $k_{max} = 16.2$ pmol/h, and $n = 1$. If the whole set of data were fitted by the sum of two transport components using the above parameters for the high-affinity component, $K_{1/2} = 1.11 \pm 0.08$ mM, $k_{max} = 46.3$ pmol/h, and $n = 4$ are obtained. The open symbols with the dotted line were taken from Weber et al. [3] and represent the AMG dependence of endogenous Na^+/glucose cotransport. The corresponding fit parameters were for the high-affinity component: $K_{1/2} = 0.19$ mM, $k_{max} = 10$ pmol/h, $n = 1$; and for the lower-affinity component: $K_{1/2} = 1.25$ mM, $k_{max} = 16$ pmol/h, $n = 4$.

TABLE I

Characteristics of Na^+/AMG cotransport

Data obtained from measurements with D-glucose as substrate were presented with brackets.

	Endogenous transport	Expressed transport	
		rat intestine	rabbit intestine
$K_{1/2}$ (high affinity) AMG	0.2 mM [3]	0.035 mM [a]	0.11 mM [6]
K_{12} (low affinity)	1.25 mM [3]	1.09 mM [a]	
Maximum rate	25 pmol/h [3]	100 pmol/h [a]	200–1500 pmol/h [6]
$K_{1/2}$ (Na)	27.9 mM	57.8 mM	32 mM [6]
Hill coefficient	2	2	1.5–1.7
K_I (phlorizin)	(0.91 μM) [3] 1.0 μM	(0.19 μM) [a] 0.015 μM	9 μM [6]
R5A3	ineffective [3]	inhibition [a]	
Depolarization	inhibition [3]	inhibition [7] [a]	inhibition [29]
Polarization for e-fold change	52 mV [3]	103 mV [a]	87 mV [29]

[a] This work.

injected oocytes. Particularly at higher substrate concentrations the endogenous component can no longer be neglected. If the endogenous transport is subtracted from the transport measured in injected oocytes, the 'two-component' dependency is nevertheless maintained. This correction yields $K_{1/2}$ values of 0.035 and 1.09 mM for the two saturating components of the exogenous transporter (see Table I).

Dependence of AMG uptake on Na^+ concentration

The dependence of the rate of AMG uptake on external Na^+ concentration was measured at an AMG concentration of 80 μM. At this substrate concentration the component with the higher affinity is nearly saturated, and more than 60% of the cotransport is mediated by the component expressed after mRNA injection (see Fig. 2). Fig. 3 suggests that the endogenous AMG transport is more sensitive to Na^+ than the expressed AMG transport. While in control oocytes about 28 mM Na^+ gives 50% stimulation of AMG uptake, 58 mM is necessary in the injected oocytes. In control as well as in injected oocytes, the Na^+ dependence can be described satisfactorily by a Hill coefficient of $n = 2$ (see Fig. 3). This suggests that, at this concentration of external AMG, more than one Na^+ ion is necessary for activation of one transport cycle.

Since transport activity is effected by changes in membrane potential (see below), the interpretation of the results on Na^+ dependence is only valid if alterations in external Na^+ concentration do not effect the membrane potential. This, in fact, is not the case;

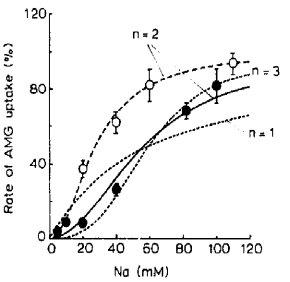

Fig. 3. Dependence of rate of AMG uptake on external Na^+ with 80 μM AMG in the bath solution. Symbols represent normalized average values (±S.E.) from 18 mRNA-injected oocytes (filled symbols), and 30 non-injected control oocytes (open symbols); for symbols without error bar S.E. is smaller than the size of the symbol. The solid and broken lines represent fits of Eqn. 1 to data points with $n = 2$. The fitted $K_{1/2}$ values are for injected cells 57.8 mM and for control cells 27.9 mM. For comparison, the dotted lines represent fits of Eqn. 1 to the data of mRNA-injected oocytes using $n = 1$ or $n = 3$, respectively. 100% of the rate of uptake corresponds to 1.4 pmol/h for non-injected and 6.4 pmol/h in injected oocytes.

reduction of extracellular Na^+ concentration leads to hyperpolarization, primarily through stimulation of the Na^+/K^+ pump [22]. The polarizations show large variabilities among oocytes from different batches (−50 to −100 mV), but are similar in control and mRNA-injected oocytes. Therefore the parameters calculated above (see also Table I) are only apparent values but, nevertheless, they demonstrate qualitative differences between the endogenous and the additionally expressed transporter.

Dependence of AMG uptake on phlorizin concentration

For further comparison of the endogenous and the additionally expressed AMG transport, dependence of transport inhibition on phlorizin concentration was measured. Fig. 4 shows that the endogenous transport has a much lower sensitivity for phlorizin; in 80 μM

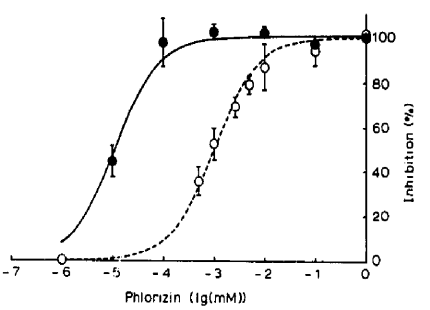

Fig. 4. Degree of inhibition by phlorizin of the Na^+-dependent AMG uptake. AMG concentration in the bath solution was 80 μM. Data represent averages from 18 mRNA-injected (filled symbols) and control oocytes (open symbols), bars indicate S.E., symbols without error bar are single measurements. The lines represent fits of Eqn. 1 to the data points with $n = 1$. The fitted $K_{1/2}$ values are for injected cells 0.015 μM and for control cells 1.00 μM.

AMG nearly two orders of magnitude lower concentrations are necessary to give 50% inhibition of the expressed uptake of AMG. This further supports that the endogenous and the additionally expressed transporter are different membrane proteins.

Inhibition of AMG uptake by antibodies

Koepsell et al. [11] have raised several monoclonal antibodies which interact with Na^+/D-glucose cotransport in kidney and intestine [19]. If non-injected oocytes were incubated with 10 μg/ml of R5A3 (IgG_3) no effect on AMG uptake could be detected (Table II). For oocytes injected with mRNA of rat intestine, on the other hand, significant (57%) inhibition of glucose uptake can be demonstrated (Table II). Significant inhibition of AMG uptake was also observed with this antibody if oocytes were injected with mRNA from rat kidney (not shown). Orientating experiments with another monoclonal antibody (T4B2) also showed inhibition (about 30%) in oocytes that were injected with mRNA from rat intestine. If AMG-uptake experiments were performed in solution containing non-specific IgG_3 or IgM antibodies, no effect could be detected neither in control nor in mRNA-injected oocytes. These data suggest structural differences of the endogenous and expressed AMG transporter.

Voltage dependence of the cotransport

The results described above demonstrate that injection of the poly(A)$^+$-mRNA leads to expression of a glucose transporter that differs in several respects from the endogenous Na^+/glucose cotransporter. The dependence on external Na^+ and phlorizin, on the other hand, suggests that also a cotransport system is expressed. If the Na^+ is cotransported, stimulation of glucose transport should lead to inward current of positive ions. Indeed, if 10 mM glucose or 5 mM AMG is added to the bath solution, depolarization of the membrane potential by more than 10 mV can be detected; this polarization is not seen if 1 mM phlorizin is present. The rate of electrogenic transport can be expected to depend on membrane potential since membrane

TABLE II

Effect of the monoclonal antibody R5A3 on rate of AMG uptake in pmol/h ± S.E. (n = 18)

The incubation with antibody R5A3 [11] or control antibody were performed as described in Materials and Methods. Uptake was measured with 40 μM AMG in the bath solution.

	Rate of AMG uptake	
	uninjected oocytes	mRNA-injected oocytes
Control	1.07 ± 0.06	7.41 ± 0.57
R5A3	0.99 ± 0.09	3.28 ± 0.33

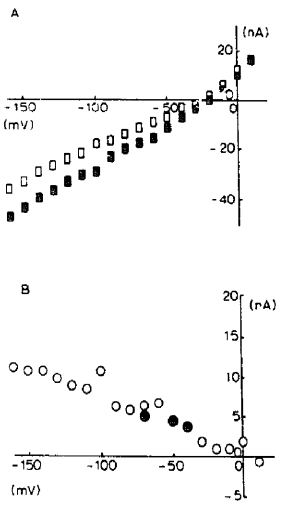

Fig. 5 (A) Voltage dependence of the membrane current without (open squares) and in the presence of 5 mM AMG (filled squares) in the bath solution in mRNA-injected oocytes. To block passive K^+ conductances, 5 mM $BaCl_2$ and 20 mM tetraethylammonium chloride were added. (B) Open circles show the inward current component mediated by Na^+/AMG cotransport. Filled circles are data obtained from measurements of [^{14}C]AMG uptake in oocytes that were kept by two-microelectrode voltage clamp at three different membrane potentials. For the presentation, the flux rates from three batches of oocytes were normalized to the current at −70 mV.

potential directly acts as driving force on the charge translocating steps in the reaction cycle. We have demonstrated for the endogenous Na^+/glucose cotransport that depolarization, counteracting the inward movement of the Na^+ ions, reduces the rate of uptake of ^{14}C-labeled glucose [3]. In the mRNA-injected oocytes, the transport rate is large enough to detect AMG- or glucose-stimulated inward current under voltage-clamp conditions. This cotransport-mediated current is assumed to be the difference current measured with and without substrate in the bath medium (see Fig. 5A). An example for the voltage dependence of the difference current is presented by the open symbols in Fig. 5B. As already demonstrated in the flux measurements for endogenous cotransport, the current-voltage curve shows that depolarization reduces cotransport also in the injected oocytes though the slope is less pronounced; while 52 mV polarization give an e-fold change of the endogenous transport, 103 mV polarization is necessary to give the same change for the expressed transport from rat intestine (see Table I). A similar voltage dependence of glucose transport in mRNA-injected oocytes is obtained if AMG uptake is determined by tracer flux measurements; the data represented by the filled circles in Fig. 5B were obtained from oocytes that were kept for 30 min under voltage clamp.

Discussion

Elevated Na^+-dependent uptake of D-glucose can be detected after injection of poly(A)$^+$-mRNA from rabbit and rat intestine into oocytes of *Xenopus laevis* [2,7]. A polypeptide with a molecular weight of 74080 Da has been cloned, and injection of the cRNA increases Na^+-dependent AMG uptake in the oocytes [5,6]. Since endogenous Na^+/glucose cotransport has been characterized in the oocytes recently [3], the question arises whether endogenous and expressed transporters are different or not. The situation is further complicated since: (a) More than one type of Na^+/D-glucose cotransporters may exist and (b) the functional Na^+/D-glucose cotransporter is supposed to be an oligomeric protein which contains low- and high affinity substrate binding sites [23]. Furthermore, in jejunum of guinea pig two Na^+-dependent, phlorizin-inhibitable D-glucose transport systems have been described which are regulated independently [9,10,24]. Only one of these cotransport systems accepts AMG as substrate. In outer cortex and outer medulla of the kidney, high and low affinity substrate activation sites for Na^+/D-glucose cotransport [25] and high- and low-affinity Na^+-dependent phlorizin binding sites have been reported [23]. In renal outer cortex as well as in renal outer medulla both types of D-glucose activation sites and of Na^+-dependent phlorizin binding sites were observed [23,26], and a stoichiometry of one was obtained for the Na^+-dependent phlorizin binding sites [23]. Therefore, it cannot be decided whether also in kidney two transporters are present and/or whether the functional renal transporter contains two substrate binding sites.

In the present study, the endogenous Na^+-dependent AMG transport in the oocytes of *Xenopus laevis* is compared with the AMG transport after injection of non-fractionated poly(A)$^+$ mRNA from rat intestine. The characteristics of the AMG transporter expressed from rat intestine are also compared with those of an AMG transporter that has recently been cloned from rabbit intestine and expressed in the oocytes [5,6].

Dependence on AMG concentration

For the endogenous as well as for the expressed AMG transport we observed low- and high-affinity substrate activation sites. Table I shows that the $K_{1/2}$ values for the high-affinity sites differ by nearly one order of magnitude. In the injected oocytes, high-affinity AMG transport is about six times more sensitive than in non-injected cells. At variance, the low affinity of the expressed transport shows sensitivity similar to the endogenous component. Since non-fractionated poly(A)$^+$-mRNA was injected into the oocytes and we do not know whether the oocytes contain one or two endogenous AMG transporters, we cannot distinguish whether the observed two components reflect two distinct transport proteins or two substrate sites with different affinity on the same transporter as discussed by Koepsell et al. [23]. Ikeda et al. [6] investigated the substrate dependence of AMG uptake of oocytes after injection of cloned cRNA. They only observed one saturable component with a $K_{1/2}$ value of 0.11 mM which they assigned to a low-affinity transporter. Comparison of the data in Table I rather suggests that primarily the high-affinity component was investigated, and that the low-affinity component may not have been detected due to limited resolution of the data.

For a further comparison of endogenous and expressed AMG transport, concentration dependencies of transport activation by Na^+ and inhibition by phlorizin were investigated. The above described AMG-dependencies of endogenous and exogenous transport suggest differences in the high rather than in the low-affinity transport. The measurements were, therefore, performed under conditions where the high-affinity site was nearly saturated and low-affinity AMG transport could be neglected.

Dependence on Na^+ concentration

Since changes in extracellular Na^+ concentration cause changes in membrane potential (see above), the analysis of the transport data is obscured. Therefore, the fitted parameters for the Na^+ dependence and those reported by others (see Table I) allow only qualitative comparison. Nevertheless, the results demonstrate that the endogenous AMG transport has a sensitivity to Na^+ similar to the AMG transport expressed after injection of the cRNA from rabbit intestine [6]. For the cotransporter expressed after injection of intestinal mRNA from rat we obtained a 2-fold lower sensitivity for Na^+.

Dependence on phlorizin concentration

For the concentration dependence of inhibition by phlorizin of the high-affinity AMG transport, we found that the K_I value for the endogenous transport (1 μM) was nine times lower than the K_I value obtained for the transporter from rabbit intestine [6]. The K_I value for AMG transport expressed after injection of rat intestinal mRNA (0.015 μM) was nearly two orders of magnitude lower than the K_I value of the endogenous transporter.

These data show only small differences between the endogenous AMG transport of the oocytes and the transport expressed after injection of the cRNA cloned by Hediger et al. [5] from rabbit intestine. At variance, the AMG transport obtained after injection of non-fractionated mRNA from rat intestine was distinctly different to the endogenous transport. Thus, it is highly probable that after injection of mRNA from rat intestine a Na^+/AMG cotransporter, distinct from the

endogenous cotransporter, is expressed in the oocytes after injection of poly(A)$^+$-mRNA.

Effects of monoclonal antibodies

The monoclonal antibodies R5A3 and T4B2 against epithelial Na^+/glucose cotransporter modulate the rate of transport. In vesicles of brush-border membranes from pig kidney the antibodies inhibit Na^+-dependent D-glucose uptake [11], and in vesicles from rat intestine they stimulate the uptake [19]. After injection of the mRNA from rat intestine the expressed AMG transport is reduced by R5A3 and T4B2. Since it was found that antibodies against the Na^+/D-glucose cotransporter which alter the cotransport in kidney have also an effect on intestinal transport, it can be assumed that the antibodies bind to both the renal and the intestine transporter. Whether binding leads to inhibition or stimulation of glucose uptake may depend on differences of in the transporters of the respective preparation [19]. The finding that the transport is differently affected depending on whether the uptake is measured in intestine membrane vesicles or in oocytes injected with intestine mRNA could suggest differences in the assembly of transporter components, in posttranslational modifications, in proteolytic degradations, or in transporter microenvironment. Our finding that the endogenous transporter of *Xenopus* oocytes is neither inhibited nor stimulated by R5A3 and T4B2 suggests that the intestine transporter expressed in the oocytes after injection of the mRNA is structurally different from the endogenous transporter.

Dependence on membrane potential

Potential dependence of glucose cotransport has been demonstrated by flux measurements in epithelial preparations [27,28], for the intestinal cotransport expressed in *Xenopus* oocytes [29], and for the endogenous cotransport in the oocytes [3]. In all cases, depolarization reduces the rate of transport (see Table I), and is interpreted in terms of a reaction cycle with positive charges moving inwardly during Na^+/glucose translocation and/or negative charges moving outwardly during the reorientation of the unloaded carrier. For injected oocytes, voltage-dependent cotransport could be demonstrate by measurements of AMG-induced current (Fig. 5). The results again show reduction of transport by depolarization, but the dependence on membrane potential is much less pronounced than for the endogenous transport but comparable with the voltage dependence of the transport from rabbit intestine expressed in the oocytes (see Table I). This demonstrates that the additionally expressed cotransport has qualitatively similar electrical properties as the endogenous cotransport but differs quantitatively.

In conclusion, we have demonstrated that different Na^+-dependent AMG transport can be detected in the oocytes of *Xenopus laevis*. After injection of non-fractionated mRNA from rat intestine, AMG transport becomes expressed that differs from the endogenous AMG cotransporter with respect to substrate dependencies and inhibition by phlorizin, antibodies and depolarization.

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