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Voltage and substrate dependence of the inverse transport mode of the rabbit Na^+/glucose cotransporter (SGLT1)

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Abstract Properties of the cytoplasmic binding sites of the rabbit Na^+/glucose cotransporter, SGLT1, expressed in *Xenopus* oocytes were investigated using the giant excised patch clamp technique. Voltage and substrate dependence of the outward cotransport were studied using α-methyl D-glucopyranoside (αMDG) as a substrate. The apparent affinity for αMDG depends on the cytoplasmic Na^+ concentration and voltage. At 0 mV the K_M for αMDG is 7 mM at 110 mM Na^+ and 31 mM at 10 mM Na^+. The apparent affinity for αMDG and Na^+ is voltage dependent and increases at positive potentials. At 0 mV holding potential the outward current is half-maximal at about 70 mM. The results show that SGLT1 can mediate sugar transport out of the cell under appropriate concentration and voltage conditions, but under physiological conditions this transport is highly improbable due to the low affinity for sugar.

Key words: SGLT1; Inverse Na^+/glucose cotransport; Oocyte

1. Introduction

Expression of the Na^+/glucose cotransporter (SGLT1) in *Xenopus* oocytes and the measurement of steady-state and pre-steady-state currents related to SGLT1 has revealed many details of the transport mechanism ([1–6], for a review of earlier experiments see [7]), summarized in a six state model [3] with sequential binding of Na^+ and sugar [3,8]. Sodium binding and the translocation of the empty carrier are assumed to be voltage dependent. These previous studies were performed under conditions in which no [1–4] or incomplete [5,6] substrate control of the intracellular compartment was possible. In the present study the 'giant excised patch-clamp' technique [9–11] which allows access to the cytoplasmic side of the membrane was used to investigate the outwardly directed or inverse cotransport. The properties of the cytoplasmic binding sites for αMDG and Na^+ were determined by varying αMDG and Na^+ concentration and applying different holding potentials.

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Abbreviations: αMDG, α-methyl D-glucopyranoside; NMG, *N*-methyl D-glucamine; EGTA, ethylene-glycol-bis(2-aminoethyl)-*N*,*N*,*N*′,*N*′-tetraacetic acid; HEPES, *N*-[2-hydroxyethyl]piperazine-*N*′-[2-ethanesulfonic acid]; TEA, tetraethylammonium

2. Materials and methods

Oocytes were removed from *Xenopus laevis* frogs as described previously [11]. Stage V/VI oocytes were injected with 9.2–20 ng cRNA encoding SGLT1 [11] and incubated for 2–4 days at 16–18°C in a modified Ringer solution containing (in mM): 110 NaCl, 5 KCl, 2 $CaCl_2$, 1 $MgCl_2$, 5 HEPES at pH 7.4, 9.5 mg/l penicillin and 10 mg/l streptomycin sulfate. Details of the experimental procedure are described in [9–11]. Patch pipettes with tip diameters of 20–30 μm were made of borosilicate glass. The seal resistance was 1–10 GΩ. The pipette was then transferred into a glass tube (inner diameter 0.78 mm), where the patch was continuously superfused. Experiments were done at room temperature. To investigate the α-methyl D-glucopyranoside (αMDG) dependence, the bath solution contained (in mM): (0–100) αMDG, mannitol was used to replace αMDG isosmotically, 110 NaCl or (50 NaCl+60 N-methyl D-glucamine chloride (NMG-Cl)) or (10 NaCl+100 NMG-Cl), 20 tetraethylammonium chloride (TEA-Cl), 2 $MgCl_2$, 2 ethylene-glycol-bis(2-aminoethyl)-*N*,*N*,*N*′,*N*′-tetraacetic acid (EGTA), 10 *N*-[2-hydroxyethyl]piperazine-*N*′-[2-ethanesulfonic acid] (HEPES), pH 7.4. To investigate the sodium dependence the bath solutions contained (in mM): (0–130) NaCl, NaCl was isosmotically replaced by NMG-Cl, 100 or 300 αMDG, 20 TEA-Cl, 2 $MgCl_2$, 2 EGTA, 10 HEPES, pH 7.4. The pipette solution contained (in mM): 110 NMG-Cl, 2 $MgCl_2$, 5 HEPES, pH 7.4. Currents were recorded using an Axopatch 200A amplifier and pCLAMP 7 software (Axon Instruments, Foster City, CA, USA). To obtain the voltage dependence of SGLT1 currents between −100 and +50 mV voltage pulses of 450 ms duration were applied. Current records were averaged over the last 100 ms of the pulse. Current–voltage (*I*–*V*) relations were obtained from the difference of the averaged steady-state currents with and without sugar. The data were fitted by the Michaelis–Menten or the Hill equation with ORIGIN 5.0 (Microcal Software, Inc., Northampton, MA, USA). Experimental results are given as (mean ± S.E.M., *n*) where *n* is the number of experiments.

3. Results and discussion

Fig. 1 shows outward currents at 0 mV elicited by the application of different concentrations of αMDG on the cytoplasmic side of a membrane patch excised from a *Xenopus* oocyte expressing SGLT1. The current flow is outward and saturates at sugar concentrations ≥80 mM. These currents are due to the activity of expressed SGLT1 as no comparable currents were observed in uninjected control oocytes. Currents elicited by αMDG in control oocytes never exceeded 1 pA. Phlorizin, an inhibitor of inwardly directed cotransport [1], also inhibits αMDG-induced outward currents, if applied on the cytoplasmic side. Only partial inhibition (approx. 37%, $n = 2$) was obtained with a concentration of 2 mM phlorizin. Higher concentrations were not tested because of the low solubility of phlorizin in water. Although the affinity for phlorizin on the cytoplasmic side is much lower than on the extracellular side we suggest that the effect of phlorizin may

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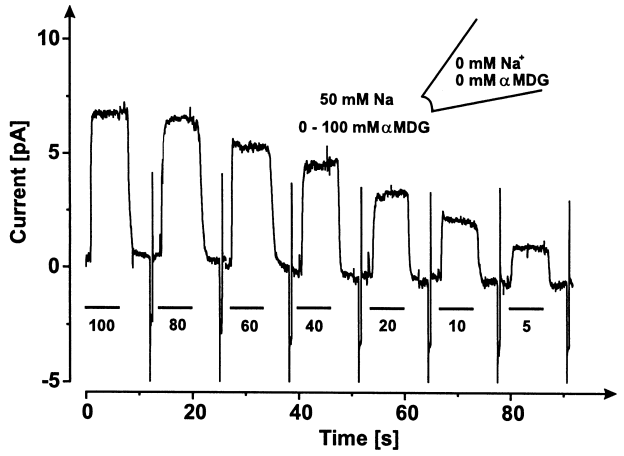

Fig. 1. Sugar-induced outward currents. Application of 100 to 5 mM αMDG to the cytoplasmic side of a giant membrane patch leads to outwardly directed currents. Bars indicate the application and concentration of αMDG (in mM). The pipette solution was free of Na^+ and αMDG, the bath solution contained 50 mM Na^+. The holding potential was 0 mV. After each application of αMDG a −10 mV voltage pulse was applied to determine the seal resistance, which was 3.4 ± 0.1 GΩ.

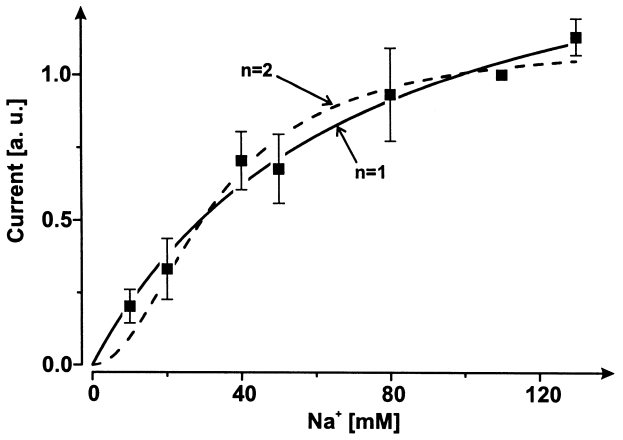

Fig. 3. Na^+ dependence of the outward currents at 100 mM αMDG. The holding potential was 0 mV. Currents were normalized to 110 mM Na^+ with mean currents of 32.6 ± 7.1 pA. The solid line shows a fit of the Michaelis–Menten equation, the dashed line a fit of the Hill equation with a Hill coefficient of $n = 2$.

be considered specific because the affinity for αMDG is also very low on this side.

Fig. 2 shows the αMDG dependence of the outward current at 0 mV and at three different cytoplasmic Na^+ concentrations. The apparent affinity for αMDG depends on the cytoplasmic Na^+ concentration: at 110 mM Na^+, $K_M^{\alpha MDG}$ is 7.0 ± 0.8 mM ($n = 3$), at 50 mM Na^+ $K_M^{\alpha MDG}$ is 15.4 ± 1.7 mM ($n = 3$) and at 10 mM it is 31.4 ± 2.9 mM ($n = 4$). The apparent affinity for αMDG at saturating Na^+ concentrations on the cytoplasmic side is 35 times lower than on the extracellular side (7 mM vs. 0.2 mM at 0 mV and 100 mM Na^+, see [2]). This is meaningful because it makes backward transport of sugar out of the cell highly improbable under physiological conditions. There is, however, similarity between inward and outward cotransport insofar as in both modes the apparent affinity for sugar decreases with decreasing sodium concentration on the same side [3]. This interdependence of substrates has been explained by binding of sodium preceding sugar binding on the extracellular side [3,8,12] and we assume that binding on the cytoplasmic side occurs in the same order.

The sodium dependence of the outward current was measured at 100 mM cytoplasmic αMDG and 0 mV membrane potential. This sugar concentration is saturating at Na^+ concentrations > 20 mM. As a change of the cytoplasmic Na^+ concentration by itself causes a shift of the holding current, experiments were performed in such a way that the Na^+ concentration was changed first and then αMDG was applied. The current activated by sugar was attributed to the transporter.

The data are satisfactorily described by the Michaelis–Menten equation with $K_M^{Na} = 69.5 \pm 13.1$ mM (Fig. 3). For comparison a fit with the Hill equation with $n = 2$ is included. In a second set of experiments the sodium dependence was determined at 300 mM αMDG (data not shown) extending

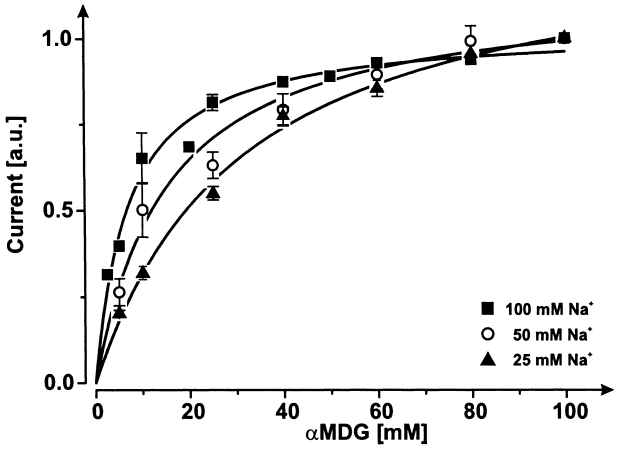

Fig. 2. αMDG dependence of outward currents at three Na^+ concentrations at a holding potential of 0 mV. The currents obtained from different patches are normalized to 100 mM αMDG with mean currents of 27.0 ± 10.8 (110 mM Na^+), 5.8 ± 0.4 (50 mM Na^+) and 12.0 ± 3.8 pA (10 mM Na^+). Solid lines are a fit of the Michaelis–Menten equation to the data (see text).

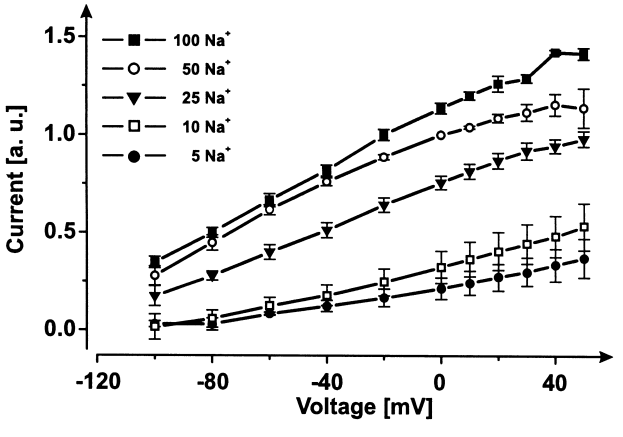

Fig. 4. Voltage dependence of currents obtained at different Na^+ concentrations. *I–V* relations determined for 10–130 mM Na^+ at 100 mM αMDG ($n = 6$). Currents were normalized to 110 mM Na^+ and a holding potential of 0 mV with mean currents of 30.2 ± 6.9 pA. The Na^+ dependence was determined for seven concentrations. For clarity only five are shown here.

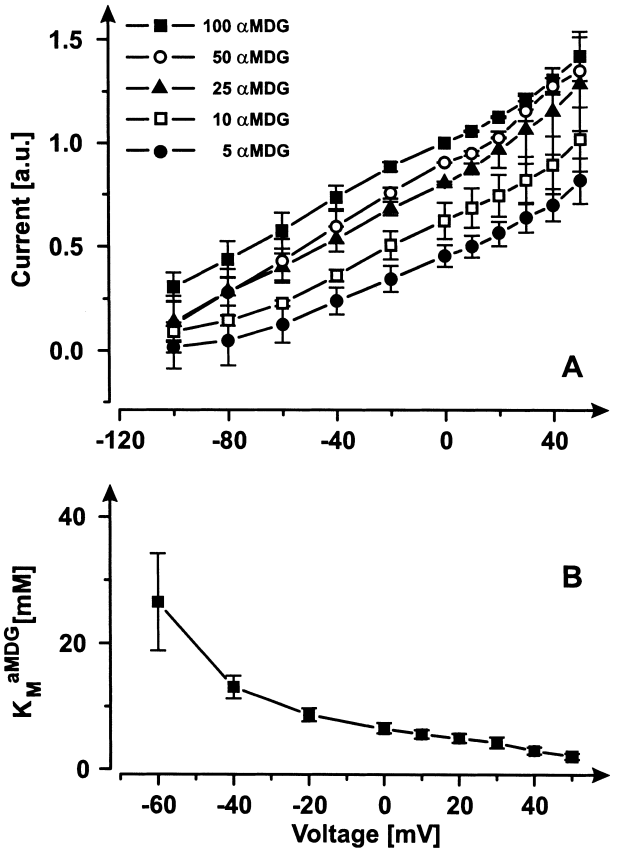

Fig. 5. *I–V* relations at different αMDG and Na^+ concentrations. A: *I–V* relations obtained for different αMDG concentrations at 110 mM Na^+ ($n=3$). The αMDG concentration range was 2.5–100 mM. For clarity, *I–V* relations at five out of 10 αMDG concentrations are shown. Currents were normalized to 100 mM αMDG and 0 mV with mean currents of 26.7 ± 9.8 pA. B: Voltage dependence of $K_M^{\alpha MDG}$. $K_M^{\alpha MDG}$ was determined from the *I–V* relations above at each holding potential. Between +50 and −60 mV, $K_M^{\alpha MDG}$ increases 12-fold.

the range of Na^+ concentrations at which αMDG is saturating below 20 mM Na^+. Under these conditions the Na^+ dependence can be described satisfactorily by the Michaelis–Menten equation with $K_M^{Na} = 54.3 \pm 7.8$ mM ($n=3$). The data show that the apparent affinity for Na^+ on the intracellular side is in the same range as on the extracellular side [2].

The voltage dependence of the outwardly directed substrate transport by rabbit SGLT1 was investigated by applying series of voltage steps with and without substrate and subtracting corresponding current records. *I–V* relations at sodium concentrations between 10 and 130 mM and 100 mM αMDG are shown in Fig. 4. The *I–V* relations show that outward transport by SGLT1 is voltage-dependent and increasing at positive potentials. It is also shown that voltage dependence is maintained up to 110 mM Na^+ which is close to saturation. This indicates that other voltage-dependent processes besides voltage-dependent Na^+ binding might be involved, e.g. a voltage-dependent translocation of the empty carrier, which is assumed to have two negative charges [2,12].

Fig. 5 shows a series of *I–V* relations obtained at different αMDG concentrations and 110 mM Na^+ (Fig. 5A). The outward current is slightly voltage-dependent, i.e. between −60 and +50 mV it increases by a factor 2.5 at 100 mM αMDG. The slope of the curves is not influenced by the αMDG concentration. The voltage dependence of the apparent affinity ($K_M^{\alpha MDG}$) was derived from the *I–V* curves in Fig. 5A. The $K_M^{\alpha MDG}$ increases at negative voltages (Fig. 5B). Because αMDG is not charged it is assumed that the effect of voltage on the apparent affinity for αMDG is indirect via voltage-dependent Na^+ binding or via a voltage-dependent shift of the occupation of states. If the voltage dependence of the apparent affinity for αMDG is exclusively due to the voltage dependence of Na^+ binding it is expected to disappear at saturating, i.e. higher Na^+ concentrations than those used here.

In conclusion, this paper shows that SGLT1 from rabbit mediates cotransport of sodium and αMDG from the intracellular to the extracellular phase under appropriate conditions. The apparent affinity of the inwardly directed sugar binding site is much lower than that of the outwardly directed site confirming earlier suggestions based on *trans*-inhibition experiments that the Na^+/glucose cotransporter is asymmetric [12]. This difference favors inward transport under physiological conditions, i.e. negative membrane potential and low intracellular sodium. In this respect it may be important that the intracellular affinity for sugar decreases at negative membrane potentials whereas the extracellular increases [2].

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