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Biochimica et Biophysica Acta 1193 (1994) 151–154

BBA Biochimica et Biophysica Acta

Transport of cycasin by the intestinal Na^+/glucose cotransporter

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Received 29 November 1993; revised manuscript received 9 March 1994

Abstract

The medicinal and food use of seed from the cycad plant (*Cycas spp.*), which contains the neurotoxin cycasin, is a proposed etiological factor for amyotrophic lateral sclerosis/Parkinsonism dementia complex (ALS/PDC), a prototypical neurodegenerative disease found in the western Pacific. Cycasin, the β-D-glucoside of methylazoxymethanol might enter neurons and other cells via a glucose transporter. Since the intestinal brush-border Na^+/glucose cotransporter plays a major role in the absorption of monosaccharides, the following studies were conducted to determine if cycasin, the β-D-glucoside of methylazoxymethanol, is a substrate for the transporter. We measured the ability of cycasin to (i) inhibit Na^+/glucose uptake into rabbit intestinal brush-border membrane vesicles, and (ii) to generate current by the cloned Na^+/glucose cotransporter (SGLT1) expressed in *Xenopus laevis* oocytes. The results show that cycasin inhibits Na^+-dependent sugar transport in the vesicles, and cycasin generates phlorizin-sensitive currents in oocytes. We conclude that cycasin is a substrate for the intestinal brush-border Na^+/glucose cotransporter, albeit with a lower affinity than D-glucose. This suggests that cycasin may be absorbed from the gut lumen by the cotransporter, and as a result either cycasin or the aglycone is presented to the blood-brain barrier for uptake into the brain.

Key words: Cycasin transport; Sodium-dependent glucose transport; Glucose transporter; Methylazoxymethanol; (Kidney); (Intestine)

1. Introduction

Cycasin (methylazoxymethanol β-D-glucoside) is a potent cycad plant toxin with teratogenic, mutagenic and carcinogenic properties [1–3] that induces in ruminants a poorly studied neuromuscular disorder characterized by 'ataxia' followed by hind limb weakness, muscle wasting, and long-tract damage. Cycasin is metabolized by plant and animal tissue β-glucosidases to the active agent, methylazoxymethanol (MAM), a rodent carcinogen. Cycasin is a major component (2–4% w/v) of cycad seeds, and epidemiological studies indicate that it is a candidate neurotoxin for a prototypical neurodegenerative disorder called western Pacific amyotrophic lateral sclerosis (ALS) and Parkinsonism-dementia complex (PDC). Recent studies demonstrating that cycasin induces selective neuronal degeneration in mouse cortical explants [4] suggest that the cycad toxin may have an important etiological role in western Pacific ALS/PDC.

Cycasin enters the brain of orally fed rats [5] and is taken up by astrocyte cultures and mouse cortical explants in a concentration-dependent manner [4]. This raises the possibility that cycasin is absorbed by the small intestine, transported across the blood-brain barrier, and taken up into neurons by glucose transporters: i.e., the Na^+/glucose cotransporter (SGLT1) and facilitated sugar transporters (GLUT2, GLUT5) of the brush-border and basolateral membranes in the intestine [6]; the blood-brain barrier GLUT1 transporter [7]; and the neuronal GLUT3 transporter [8].

The following studies examine cycasin transport by the intestinal and renal brush-border Na^+/glucose cotransporters. Since glycosides are substrates for the Na^+/glucose cotransporters [9], we reasoned that cycasin should be transported by these systems. On the basis of competition experiments with brush-border

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SSDI 0005-2736(94)00077-3

membrane vesicles and electrophysiological experiments with the cloned Na^+/glucose cotransporter (SGLT1) expressed in *Xenopus* oocytes, we confirm that cycasin may be transported across the intestinal (and renal) brush border by Na^+/glucose cotransporters.

2. Methods

Rabbit intestinal brush-border membranes were prepared and Na^+-dependent D-glucose transport activity was measured as described previously [10,11]. Briefly, brush-border membrane vesicles were prepared from the intestinal mucosa and renal cortex by a Ca^{2+} ion precipitation procedure, and enrichment of the vesicles (> 10-fold) was monitored by following the specific activity of brush-border marker enzymes (e.g., alkaline phosphatase). Vesicles were stored until use at liquid nitrogen temperature in 300 mM mannitol and 1 mM Tris-Hepes pH 7.4 buffer. Initial rates (3 s uptakes) of 50 μM D-[^{3}H]glucose transport into the brush-border vesicles were measured at 22°C by a rapid mix/filtration method. Na^+-dependent rates were taken as the difference between uptakes in the presence of 100 mM NaCl and uptakes in the presence of 100 mM KCl. Rates are expressed in pmol mg (membrane protein)$^{-1}$ s^{-1}. Since radioactive cycasin is not yet available, we measured the ability of cycasin (1–20 mM) to block D-[^{3}H]glucose uptake (see also Ref. [12]).

In a second series of experiments we measured the ability of cycasin to elicit current by the cloned Na^+/glucose cotransporter (SGLT1) expressed in *Xenopus* oocytes [13]. All substrates transported by SGLT1 generate inward currents [14]. SGLT1 cRNA was transcribed from plasmid pMJC424 and injected into oocytes, and cycasin-dependent currents were measured 3–5 days later using a two-electrode voltage-clamp method [13]. Briefly, current/voltage relations were recorded in the absence of cycasin, in the presence of cycasin, and the presence of cycasin and 100 μM phlorizin. Under each condition the membrane potential was held at the oocyte resting potential (−50 mV) and steady-state I/V relations were obtained by applying 25 ms square-wave voltage pulses across the membrane and recording the membrane currents. The membrane potential was varied between −150 and −30 mV. Each pulse was followed by a 1 s interpulse interval at the holding potential (−50 mV). In the same oocytes, we also measured currents produced by saturating concentrations (1 mM) of α-methyl-D-glucoside (αMDG). Control experiments with

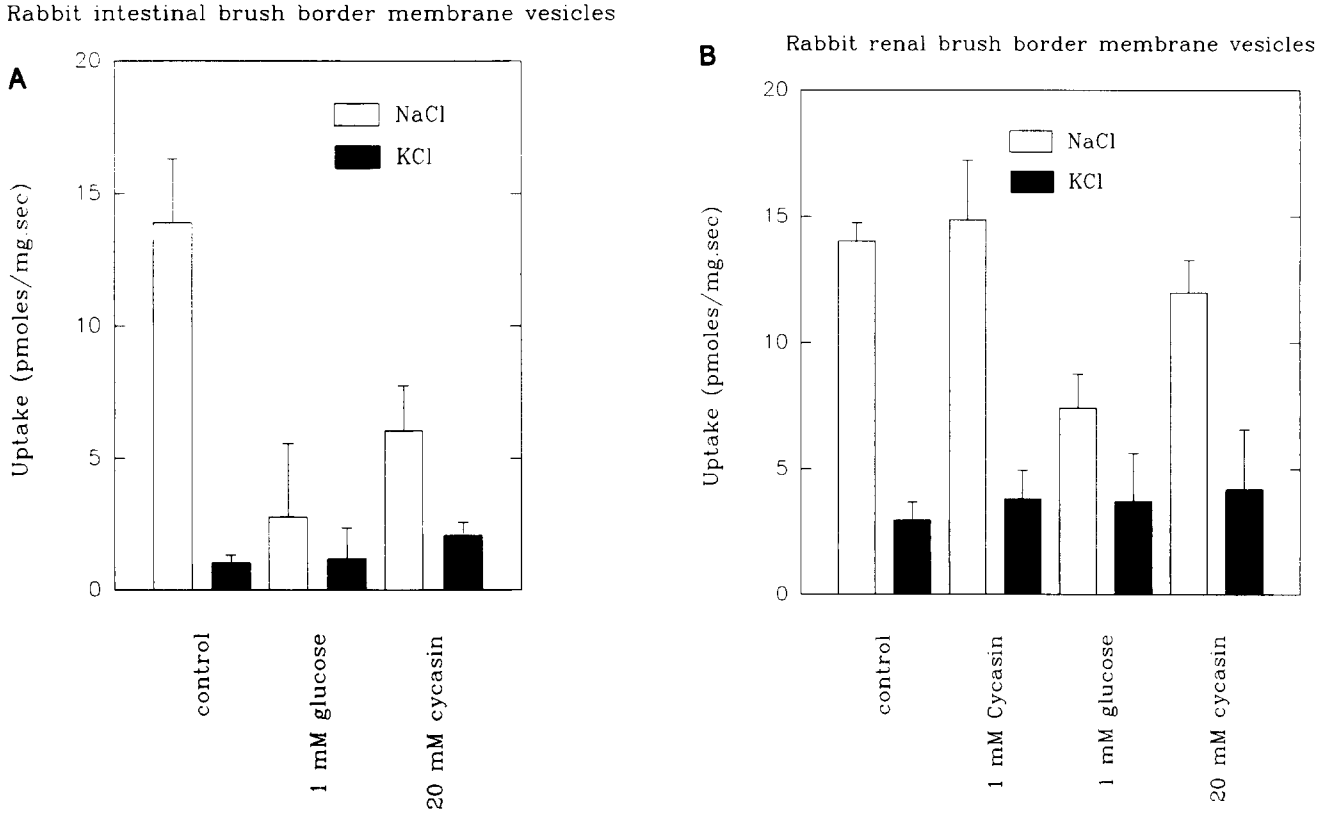

Fig. 1. Effect of cycasin on D-glucose uptake into rabbit (A) intestinal and (B) renal brush-border membrane vesicles. Initial rates of 50 μM D-[^{3}H]glucose uptake into membrane vesicles (pmol mg^{-1} s^{-1}) were measured in the presence of 100 mM NaCl and KCl in the absence and presence of 0–20 mM D-glucose or cycasin. Uptakes were measured in triplicate and are presented as the means ± S.D.

H_2O injected oocytes showed that endogenous sugar-induced currents were less than 1 nA, compared to 100–400 nA in SGLT1 RNA injected oocytes.

Cycasin was isolated from cycad seed kernels, purified and assayed as described previously [4]. Free D-glucose in the purified cycasin was less than 0.1%. All other sugars and reagents were from Sigma, St. Louis, MO, or were of reagent grade.

3. Results

D-Glucose uptake into rabbit intestinal brush-border vesicles is shown in Fig. 1A. Uptake of 50 μM D-[^{3}H]glucose with 100 mM NaCl in the incubation medium was 14 pmol $mg^{-1}s^{-1}$ and this was reduced to 1 pmol $mg^{-1}s^{-1}$ in the absence of Na^+ (see also Ref. [15], Fig. 1). The addition of 1 mM D-glucose to the incubation medium virtually abolished Na^+-dependent D-[^{3}H]glucose uptake (13–1.6 pmol $mg^{-1}s^{-1}$), while there was no significant effect of adding 1 mM cycasin (not shown). However, increasing the cycasin concentration to 20 mM produced >50% inhibition of D-[^{3}H]glucose uptake in the presence of NaCl, but produced no significant effect in the presence of KCl. This shows that cycasin competes with Na^+-dependent D-glucose uptake into rabbit intestinal brush-border membrane vesicles, but with a much lower affinity than D-glucose.

Similar experiments were carried out with rabbit renal cortex brush-border membranes (Fig. 1B). D-Glucose (1 mM) inhibited 50 μM D-[^{3}H]glucose uptake by 60% (from 14 to 3.7 pmol $mg^{-1}s^{-1}$, whereas 1 mM cycasin had no effect. Increasing the cycasin concentration to 20 mM inhibited Na^+-dependent uptake 30%, whereas 20 mM mannitol caused no inhibition (14 vs. 11 pmol $mg^{-1}s^{-1}$).

The ability of cycasin to generate inward currents in *Xenopus* oocytes expressing SGLT1 is shown in Fig. 2. The oocyte currents obtained in the absence of cycasin, in the presence of 30 mM cycasin, and in the presence of 30 mM cycasin and 100 μM phlorizin are presented in Fig. 2A. Oocytes were held at −50 mV and the oocyte, I/V curves were measured by voltage-clamping the membrane for 25 ms at potentials between −30 and 150 mV and recording the currents. At each potential 30 mM cycasin increased the inward steady-state (~25 ms) current, e.g., by 100 nA at −50 mV, and 100 μM phlorizin blocked this current. The I/V curves are shown for both cycasin and αMDG in the presence and absence of phlorizin in Fig. 2B, where it can be seen that the currents were linear over the voltage range tested. Extrapolation of the currents to zero nA indicates that both cycasin and αMDG depolarize the

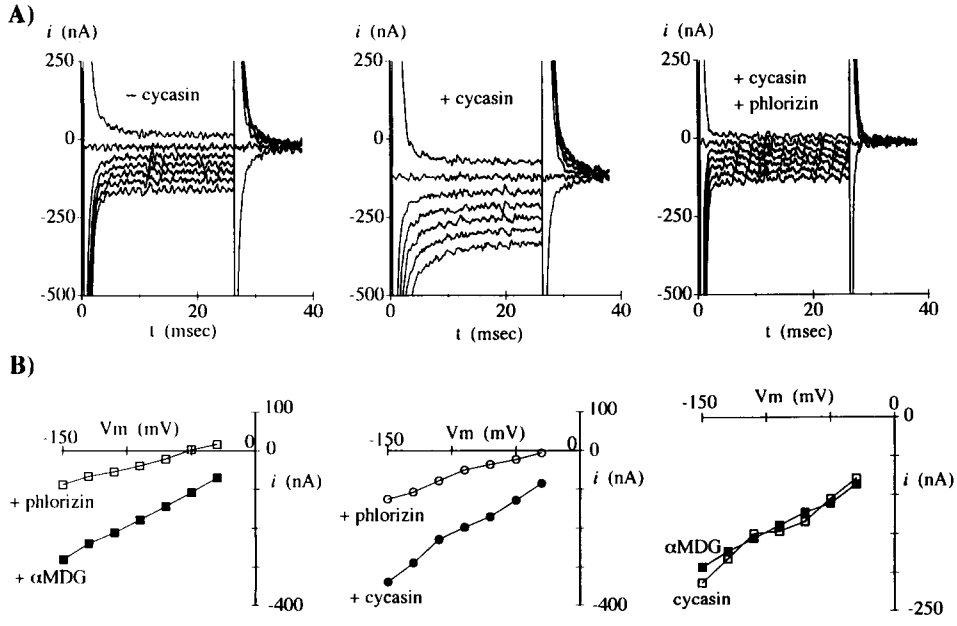

Fig. 2. Phlorizin-sensitive currents elicited by 1 mM αMDG and 30 mM cycasin in *Xenopus* oocytes expressing the intestinal Na^+/glucose cotransporter SGLT1. Steady-state currents were measured in an oocyte 3 days after injection of SGLT1 cRNA. (A) The currents generated by cycasin at voltages between −30 and −150 mV. (B) The I/V curves obtained from the records in the presence of cycasin and the absence or presence of 100 μM phlorizin. The I/V curves obtained in the same oocyte with αMDG, and the phlorizin-sensitive αMDG and cycasin currents are also shown.

resting oocyte membrane potential by 30–50 mV. The phlorizin-sensitive αMDG and cycasin currents are also plotted in Fig. 2B, and this shows that cycasin and αMDG produce similar currents over the −30 to −150 mV voltage range tested.

4. Discussion

The intestinal brush-border Na^+/glucose cotransporter has a wide specificity for sugars, especially α- and β-glucosides [9]. Methyl, ethyl, propyl, butyl and phenyl glucosides are readily transported, and recent studies with the cloned transporter expressed in oocytes shows that α-methyl-D-glucoside is transported with kinetics virtually identical to D-glucose [13,14]. The present study demonstrates that cycasin, methylazoxymethanol β-D-glucoside, is also transported by the Na^+/glucose cotransporter. In brush-border vesicles 20 mM cycasin inhibits D-glucose uptake by >50%, while in oocytes expressing the cloned transporter SGLT1 30 mM cycasin produces the same maximal phlorizin-sensitive current as saturating concentrations of αMDG. Phlorizin is a specific competitive inhibitor of Na^+/glucose cotransport with a K_i of 5–10 mM (see Ref. [12]). These results demonstrate that cycasin is transported by the intestinal brush-border Na^+/glucose cotransporter with affinity substantially less than D-glucose (~15 mM vs. 0.2 mM). This indicates that the cotransporter can play a role in the absorption of cycasin from the small intestine. Similar experiments with renal cortical brush borders also indicate that the cotransporter can play a role in the reabsorption of cycasin from the glomerular filtrate.

Given that enterocytes have the ability to absorb cycasin from the intestinal lumen via SGLT1, questions remain about the release of the toxin from the cell into blood and its uptake into the brain. Although cycasin has not yet been examined for transport by GLUT1–3, the literature suggests that GLUT1 and 2 have a low affinity for glucosides; methyl glucosides have no detectable effect on sugar transport by human erythrocytes [16] and rabbit intestinal basolateral membranes [17]. This suggests that cycasin does not exit enterocytes across the basolateral membrane via GLUT2 and does not cross the blood-brain barrier by GLUT1. The demonstration of detectable levels of cycasin in brain tissue of orally [5] and parentally [18] dosed rodents suggests that cycasin traverses the blood-brain barrier by an unknown mechanism. The recent identification of sodium-dependent glucose transporter in rodent brain endothelial cells [19] suggests that cycasin may be transported across the blood-brain barrier by such a mechanism. Alternatively, cycasin could be cleaved by intestinal glucosidases to the aglycone, methylazoxymethanol (MAM), which then could diffuse into blood and then into brain. Recent studies confirm that the aglycone readily crosses the rodent blood-brain barrier [18]. Additional studies are needed to determine if glucose transporters are involved in the transport of cycasin across the blood-brain barrier and into brain tissue. Results from such studies may provide additional clues about the role of this cycad toxin in western Pacific ALS/PDC.

Acknowledgements

We thank Ms. Valerie Nottingham and Mr. Johanns Kabel for technical assistance. Supported by U.S.N.I.H. grants NS-09666 and DK19567 and NS-19611.

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