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Sodium Valproate Inhibits Glucose Transport and Exacerbates Glut1-Deficiency In Vitro

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Abstract Anticonvulsant sodium valproate interferes with brain glucose metabolism. The mechanism underlying such metabolic disturbance is unclear. We tested the hypothesis that sodium valproate interferes with cellular glucose transport with a focus on Glut1 since glucose transport across the blood-brain barrier relies on this transporter. Cell types enriched with Glut1 expression including human erythrocytes, human skin fibroblasts, and rat astrocytes were used to study the effects of sodium valproate on glucose transport. Sodium valproate significantly inhibited Glut1 activity in normal and Glut1-deficient erythrocytes by 20%–30%, causing a corresponding reduction of V_{max} of glucose transport. Similarly, in primary astrocytes as well as in normal and Glut1-deficient fibroblasts, sodium valproate inhibited glucose transport by 20%–40% ($P < 0.05$), accompanied by an up to 60% downregulation of GLUT1 mRNA expression ($P < 0.05$). In conclusion, sodium valproate inhibits glucose transport and exacerbates Glut1 deficiency in vitro. Our findings imply the importance of prudent use of sodium valproate for patients with compromised Glut1 function. J. Cell. Biochem. 96: 775–785, 2005.

Key words: glucose; Glut1; Glut1 deficiency syndrome; anticonvulsant; sodium valproate; epilepsy

Glucose is the principal fuel for mammalian cell metabolism. In brain, the blood-borne glucose provides over 98% of the energy for sustaining neuronal function [Pardridge and Boado, 1993]. The movement of glucose across the tissue barriers depends on different glucose transporters [Ho et al., 2002; Joost et al., 2002]. Glut1 located at the blood-brain barrier, lining the surfaces of the brain endothelium and astrocytes is responsible for glucose transport into the brain [Yu and Ding, 1998; Duelli and Kuschinsky, 2001].

It is conceivable that Glut1-inhibiting compounds may expose the brain to increased risks of energy failure and should be avoided by patients with predisposed glucose transport deficiencies. However, the impacts of anticonvulsants on glucose transport of the brain cells are mostly unknown.

Sodium valproate is a monocarboxylic acid commonly used for epilepsy control with a broad spectrum of action and efficacy [Davis et al., 1994]. Its anti-epileptic mechanisms remain elusive. Depression of local brain glucose utilization has been reported in epileptic and normal humans receiving valproate [Leiderman et al., 1991]. It is suggested that the interference of valproate on brain glucose metabolism contributes to the anti-epileptic action of the drug [Bolanos and Medina, 1997].

Compromised glucose transporter function has been associated with brain disorders such as the Glut1 deficiency syndrome (Glut1DS). Glut1DS is featured by infantile seizures, acquired microcephaly, developmental delay, and motor disorders. Biochemical hallmarks of Glut1DS include hypoglycorrhachia with normoglycemia and low to normal cerebrospinal fluid lactate value [De Vivo et al., 1991; Brown, 2000; Klepper and Voit, 2002]. The molecular

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defects of Glut1DS involve heterozygous *GLUT1* gene mutations impairing glucose transport across the blood-brain barrier [De Vivo et al., 1991, 2002a,b]. Due to the refractory seizures associated with Glut1DS, before a confirmed diagnosis is made, patients are often exposed to different anticonvulsants, including sodium valproate. In this study, the hypothesis that sodium valproate interferes with cellular glucose transport was tested.

EXPERIMENTAL PROCEDURE

Chemicals

2-deoxy-D-glucose (2-DOG), 3-*O*-methyl-D-glucose (3-OMG), phloretin, mercuric chloride, sodium valproate, HEPES, and Triton X-100 were purchased from Sigma (St. Louis, MO). All reagents used in cell culture including high-glucose (4,500 mg/L) DMEM, low-glucose (1,000 mg/L) DMEM, fetal bovine serum, and penicillin-streptomycin were purchased from Gibco (Carlsbad, CA).

Solutions

Sodium valproate was solublized in PBS (pH 7.4). All experiments were performed covering the range of the therapeutic plasma concentrations of sodium valproate as previously reported [Warner et al., 1998]. ^{14}C-labeled 3-OMG (56.4 mCi/mmol stock) was purchased from PerkinElmer Life Science, Inc. (Boston, MA). ^{3}H-labeled 2-DOG (1 mCi/ml) was purchased from Amersham Biosciences (Buckinghamshire, England). The stop solution containing 100 μM mercuric chloride and 50 μM phloretin in ice-cold PBS was prepared immediately before use. Cell lysis buffer contained 0.1% Triton X-100.

Human Tissue Samples

Erythrocytes were obtained from normal and diagnosed Glut1DS subjects [Fujii et al., 2005] with informed consent or from local blood bank. Diagnosis of Glut1DS was made based on hypoglycorrhachia, impaired glucose transport into the erythrocytes, and molecular analysis. Blood samples were preserved at 4°C in citrate-phosphate-dextrose anticoagulant solution. Blood specimens (2–4 ml) were washed twice with ice-cold PBS and centrifuged at 800 rcf for 4 min at 4°C. After washing, erythrocytes were incubated with PBS on wet ice for 15 min to achieve zero-trans (no intracellular glucose) condition, followed by centrifugation and the removal of supernatant [Klepper et al., 1999]. Erythrocytes were then re-suspended in PBS to the original blood-sample volume. Fifty microlitres of aliquots were taken for immediate assay and 10 μl were used for cell count. Erythrocytes prepared from different batches of blood samples gave similar results.

Primary fibroblast cultures from two Glut1DS patients (Patient 1 #1825 and Patient 2 #1818) were purchased from the University of Miami Brain and Tissue Bank for Developmental Disorders. Since this tissue bank did not have the corresponding parental control cell lines, Hs68 human foreskin fibroblasts purchased from ATCC (American Type Culture Collection) were used as a normal control cell line in this study. Cells were maintained in high-glucose DMEM containing 10% fetal bovine serum and 100 U/ml penicillin G sodium plus 100 μg/ml streptomycin sulfate. The cultures were kept at 37°C under a humidified 5% CO_2 atmosphere. In previous studies, the glucose uptake level of the Glut1DS fibroblasts was approximately 50% of the parental control level [Seidner et al., 1998]. Glucose uptake levels were comparable among Hs68, #1818, and #1825 cell lines used for this study.

Rat Primary Astrocytes

Astrocytes in primary culture were dissected from the forebrains of 2-day old SD rats (Animal House, CUHK, HKSAR, China) as previously described [Chang et al., 2001]. Briefly, areas of dissection were cleaned with 70% ethanol. Rats were decapitated with a pair of sterile operating scissors. Dissection started from cutting the base to the mid-eye area along the midline of the head followed by the removal of the brain from the skull. Olfactory lobes and meninges were removed. The hemisphere were collected in sterile PBS and diced into ~1 mm diameter pieces. Tissues were incubated in PBS containing 0.25% (v/v) trypsin in a 15 ml centrifugation tube in a 37°C water bath for 15 min with inversion every 3 min. Cells were then centrifuged for 5 min followed by the removal of the excessive solution. The trypsinized cells were re-suspended in DMEM with 50% fetal bovine serum and triturated through needles with 3 mm internal diameter syringes. Finally, dispersed cells were past through a 70 micron nylon mesh filter (Rancho Dominguez, CA) in filter holder (Millipore, Billerica, MA) by a

syringe and seeded in tissue culture flasks. Cells were incubated in a humidified incubator with 5% CO_2 at 37°C in high glucose DMEM with 20 mM HEPES, 10% fetal bovine serum and 100 U/ml penicillin G sodium plus 100 μg/ml streptomycin sulfate. Medium was changed every 72 hours. On day 7, cells were shaken for 8 h at 220 rpm on a rocker at 37°C for astrocyte selection. Detached cells were removed immediately. Remaining astrocytes were trypsinized from the flask and re-seeded into new tissue culture dishes or plates for experiment.

Zero-Trans Influx of 3-OMG Uptake in Erythrocytes

The Lowe and Walmsley techniques were modified as previously described [Lowe and Walmsley, 1986]. Time course zero-trans influx of ^{14}C-labeled 3-OMG was conducted over a period of 30 s. Equilibrium uptake of ^{14}C-labeled 3-OMG was measured after 25 min incubation. For each reaction, a 50 μl blood aliquot was incubated with 100 μl hot/cold 3-OMG mixture (H/C solution) containing 1 μCi/ml ^{14}C-3-OMG and 0.6 mM of cold 3-OMG in the presence or absence of sodium valproate. To evaluate the dosage effects of sodium valproate, the uptake reactions with ^{14}C-labeled 3-OMG were allowed to proceed for 15 s at 4°C. 3-OMG influx was terminated by rapid addition of 1 ml ice-cold stop solution. After centrifugation for 20 s, the erythrocyte pellet was washed twice with 1 ml ice-cold stop solution. Cell pellet in each tube was then digested with 250 μl solublizing agent containing quaternary ammonium hydroxide (Soluene-350, Packard, Meriden, CT) and isopropyl alcohol (in the ratio of 2:1 v/v). At room temperature, the samples were bleached with 250 μl of 30% hydrogen peroxide (BDH Laboratory, Dorset, England). The mixture was swirled until all foaming subsided. Four milliliters per sample of OptiPhase HiSafe 2 scintillation fluid (PerkinElmer Life Sciences, Inc., Boston, MA) was added, and radioactivity in aliquots was determined by a Beckman LS 6500 multipurpose scintillation counter (Beckman Coulter, Inc., Fullerton, CA).

Glucose Uptake Into Fibroblasts and Astrocytes

Fibroblasts were seeded in BD Falcon™ 12-well plates (BD Biosciences, Franklin Lakes, NJ) and allowed to grow until post-confluent [Seidner et al., 1998]. During the pre-incubation period, cells were cultured in low-glucose DMEM containing 10% fetal bovine serum for 24 h and then switched to low-glucose DMEM containing 0.5% fetal bovine serum for 4–6 h followed by incubation with low-glucose DMEM containing 10% fetal bovine serum for 24 h for enhanced Glut1 expression [Hiraki et al., 1988]. On day 3, cells were exposed to medium without glucose for 1 h to achieve a zero-trans condition followed by glucose transport study in which cells were incubated with 300 μl H/C mixture containing 1 mM ^{14}C-labeled 3-OMG (3 μCi/ml). The uptake reactions were allowed to proceed for 15 min at 37°C and were stopped by adding 3 ml/well ice-cold 0.9% sodium chloride solution. Time course studies indicated that 3-OMG transport does not saturate within 25 min under the experimental conditions of this study (data not shown).

To evaluate the prolonged effects of sodium valproate on glucose transport, fibroblasts were exposed to different concentrations of experimental compounds during the courses of both pre-incubation and glucose transport assays. Following the glucose transport assays, each well was washed by ice-cold sodium chloride solution twice. The washing procedures were performed on wet ice to prevent the efflux of 3-OMG.

For astrocyte studies, 1×10^5 cells/well were seeded in 12-well plates and allowed to grow for 48 h (to confluent). Cells were treated the same as the fibroblasts on day 1 and day 2. On day 3, cells were incubated for 1 h in Hank's buffer to achieve a zero-trans condition. For glucose uptake experiments, 1 ml H/C mixture was added including 0.5 μCi/ml ^{3}H-2-DOG, 0.1 mM of 2-DOG and sodium valproate [Nagamatsu et al., 1996]. Time course studies indicated that 2-DOG transport does not saturate within 60 min (data not shown) under the experimental conditions of this study. The uptake reaction was allowed to proceed in the presence of different amount of sodium valproate for 10 min at 37°C and was stopped by adding 2 ml/well ice-cold PBS twice.

For both fibroblasts and astrocytes, after stopping the glucose uptake reactions, each well was washed by the same ice-cold sodium chloride solution twice. The washing procedures were performed on wet ice to prevent the efflux of the glucose analogue. Then, 500 μl of lysis buffer was added and followed by freeze and thaw cycles to lyse the cells. Four hundred microliter samples plus 4 ml/sample of Opti-

Phase HiSafe 2 scintillation fluid was mixed and radioactivity in aliquots was determined by a Tri-carb 2900TR scintillation counter (Packard Bioscience Company, Meriden, CT) while 10 μl sample was used for protein concentration determination by the Bio-Rad Protein Assay Kit (Bio-Rad Laboratories, Hercules, CA). The amount of glucose influx was normalized by the cell protein concentration.

RNA Extraction and Reverse Transcription Reaction

Cells were seeded in 100-mm dishes and allowed to grow until confluent. The experimental treatment was the same as described in "Glucose uptake in fibroblasts and astrocytes" except that RNA was extracted after the 1-h glucose starvation on day 3 using 1 ml TRI reagent per dish (Molecular Research Center, Inc., Cincinnati, OH). RNA integrity was confirmed by resolving samples on a 1% agarose gel by electrophoresis followed by ethidium bromide staining showing the signal intensity ratio of 18S:28S ribosomal RNA bands at approximately 1:2. RNA purity was determined by OD A_{260}/A_{280} with a ratio greater than 1.7. RNA concentration was quantified by spectrophotometry at 260 nm. Total RNA extracts were used as templates for producing cDNA using RevertAid^{TM} H Minus M-MuLV Reverse Transcriptase (Fermentas, Hanover, MD). Reverse transcription (RT) reactions were performed using 5 μg of total cellular RNA/reaction in a total reaction volume of 20 μl following the manufacturer's protocols. For the subsequent semi-quantitative PCR and quantitative real time PCR reactions, 1 and 1.5 μl RT reaction products were used, respectively.

Semi-Quantitative PCR

Reactions were performed to study *GLUT1*, *GLUT3*, and *GLUT5* gene expression in control and sodium valproate treated cells using gene-specific primer sets (see table below for details). The expression of glyceraldehyde-3-phosphate dehydrogenase (*GAPDH*) gene served as the control gene.

GLUT1:	Forward: 5′-ATGGAGCCCAGCAGCAAGAAGCT-GACGGGTCGC-3′ Reverse: 5′-TCACACTTGGGAATCAGCCCCCA-GGGGATGGAA-3′ Annealing temperature/cycles: 55°C/24 Product size: 1500 bp
GLUT3:	Forward: 5′-GCTTTGGCAGGCGCAATTCAA-3′ Reverse: 5′-GGGGCCTGGTCCAATTTCAAA-3′ Annealing temperature/cycles: 58°C/24 Product size: 800 bp
GLUT5:	Forward: 5′-TAGGGCAAGCTTCTGAAGTGTAC-CCGGAAAAGG-3′ Reverse: 5′-TAGGGCGCGGCCGCGAAAAGTG-ATCAGGTTACT-3′ Annealing temperature/cycles: 55°C/33 Product size: 700 bp
GAPDH:	Forward: 5′-ACCACAGTCCATGCCATCAC-3′ Reverse: 5′-TCCACCACCCTGTTGCTGTA-3′ Annealing temperature/cycles: 55°C/18 Product size: 450 bp

Cycling conditions comprised an initial denaturation of 5 min at 95°C, followed by 18–33 cycles with denaturation at 95°C for 45 s, annealing at 55–58°C for 30 s and elongation at 72°C for 30–90 s. The PCR conditions were optimized by varying the annealing temperature and the number of cycles to allow for the detection of the amplified products without saturation. The PCR products were resolved by 1% agarose gel electrophoresis followed by visualization and documentation of the results by ImaGoGel Doc system and ImaComm data archiving software (B&L System, Maarssen, Netherlands) under UV illumination. The expression levels of the target genes were quantified by the Image J program (Research Services Branch, National Institute of Mental Health, Methesda, MD).

Quantitative Real Time PCR

Reactions were performed in a 20 μl reaction mixture containing 1.5 μl cDNA obtained from the above described reverse transcriptase reaction, 7.5 μl H_2O, 10 μl of 2× Taqman Universal PCR Master Mix, 1 μl of 20× Assays-on-Demand Gene Expression probes of human or rat GLUT1 using the ABI PRISM 7700 Sequence Detection System (Applied Biosystems, Foster City, CA). The control genes *GAPDH* (for fibroblasts) and rat β-actin (for astrocytes) were used as loading controls. An initial setup step at 50°C for 2 min is required for optimal enzyme activity when using Taqman Universal PCR Master Mix. Cycling conditions comprised an initial denaturation of 10 min at 95°C, followed by 40 cycles with denaturation at 95°C for 15 s and annealing/elongation at 60°C for 1 min. The fluorescence emitted in each thermal PCR cycle was measured by the ABI PRISM 7700 Sequence Detection System. After the reactions, quantitative results were generated using the sequence detection software. The fold change of *GLUT* gene expression (target gene) relative to the loading control was analyzed using the $2^{-\Delta\Delta Ct}$ method previously described

where $\Delta\Delta Ct = (C_{tTarget} - C_{tLoading\ control})_{treated} - (C_{tTarget} - C_{tLoading\ control})_{non\text{-}treated}$ [Livak and Schmittgen, 2001].

Statistics

Data are expressed as mean ± SE (standard error) unless otherwise indicated. A statistical significance was determined at a $P < 0.05$ level by Mann–Whitney test.

RESULTS

Effects of Sodium Valproate on Glucose Transport

Figure 1A shows that sodium valproate significantly inhibited glucose transport in erythrocytes at concentrations of 0.25 mM and above. In the time course study (Fig. 1B), significant inhibition of Glut1 activity was observed starting from 15 s with sodium valproate incubation. The relative glucose transport activity was calculated from taking the percentage ratio of the slope of the linear-regression line of the sodium valproate-treated-group (−0.0084) over the slope of the linear regression line of the control group (−0.00107). Sodium valproate resulted in a 21% inhibition of Glut1 activity (Fig. 1B, inset).

When the effects of sodium valproate were tested on Glut1DS erythrocytes, inhibition of glucose transport velocity was observed. As shown in Figure 2, 0.5 mM sodium valproate significantly inhibited the V_{max} of Glut1 in both control and Glut1DS samples by 20%–30%. Notice that the Glut1DS sample started with a substantially lower (~55%) V_{max} as compared to the maternal control. The lowered V_{max} of the Glut1DS sample was further compromised with the addition of sodium valproate.

Since astrocytes are the major cell type forming the structure of the blood-brain barrier where Glut1 is highly expressed [Yu and Ding, 1998; Duelli and Kuschinsky, 2001], we next determined the effects of sodium valproate on *GLUT1* gene expression and glucose transport in rat primary astrocytes. Prolonged exposure to sodium valproate significantly inhibited astrocyte glucose transport by up to 40% in a dose-dependent manner (Fig. 3). The same treatment also downregulated GLUT1 mRNA expression by approximately 60% (Fig. 3, inset).

Similarly, therapeutic concentrations of sodium valproate inhibited glucose transport into control and Glut1DS fibroblasts in a dose-dependent manner (Fig. 4A). A corresponding repression of GLUT1 mRNA expression by 20%–30% was observed in 0.5 mM sodium valproate treated fibroblasts (Fig. 4B).

DISCUSSION

Concerns over preserving glucose supply to the brain have driven the studies of the effects of anticonvulsants on glucose transport. Reported Glut1 inhibitors include methylxanthine, barbiturates, tyrosine kinase inhibitors, Genistein, GTP analogs, tricyclic antidepressants, general anesthetics, and ethanol [Motais et al., 1980; Salah et al., 1982; Wellner et al., 1993; Krauss et al., 1994; Honkanen et al., 1995; Vera et al., 1996, 2001; Klepper et al., 1999, 2003; Pinkofsky et al., 2000; Stephenson et al., 2000; Ho et al., 2001; Johannessen et al., 2001]. Consistent with the in vitro studies, clinical observations have found barbiturates ineffective in controlling Glut1DS-associated seizures and methylxanthines exacerbating haploinsufficiency in Glut1DS patients [Klepper et al., 1999; Ho et al., 2001]. It is conceivable that avoiding the consumption of Glut1-inhibiting compounds would facilitate seizure control in Glut1DS patients.

In previous studies, sodium valproate was shown to reduce brain glucose utilization in both human and rodents. Positron emission tomography demonstrated that sodium valproate treatment inhibited human cerebral glucose metabolism [Leiderman et al., 1991]. In addition, acute exposure to sodium valproate causes reduction of TCA cycle activity and ATP production in mice [Johannessen et al., 2001]. In this study, we have presented evidence that sodium valproate interferes with glucose transport by inhibition of Glut1 activity and mRNA expression, providing a novel mechanism for sodium valproate-induced disturbances of cellular glucose homeostasis.

The erythrocyte Glut1 is identical in molecular mass and antigenic properties to the 55 kDa form of Glut1 expressed in cerebral microvessels [Kalaria et al., 1988]. It has been suggested that erythrocyte Glut1 is functionally reflective of the Glut1 expressed in the brain endothelial cells [Drewes, 1998; Hruz and Mueckler, 2001]. The absence of nuclei and the abundance of Glut1 in the erythrocyte membranes provide a sensitive and easily accessible model for studying the acute post-translational effects of sodium valproate on Glut1 activity. However, the high Glut1 expression also presents limits

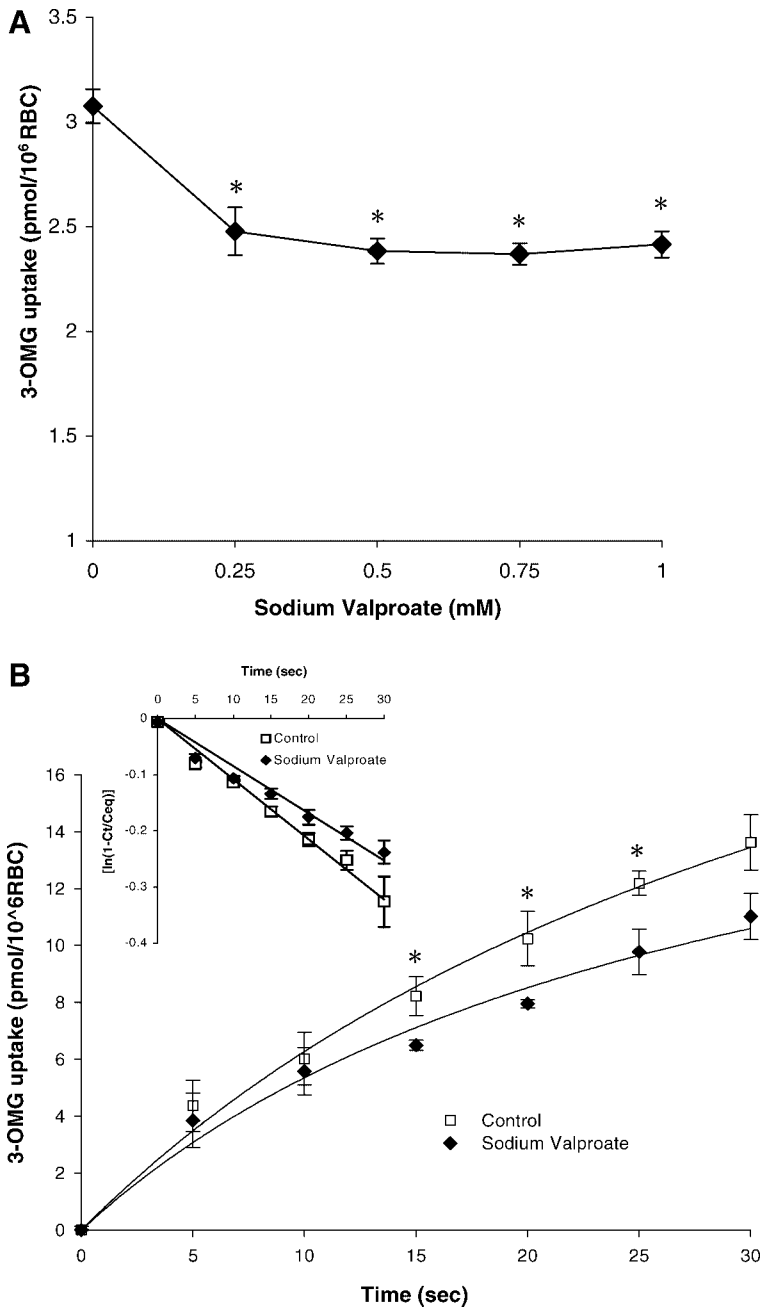
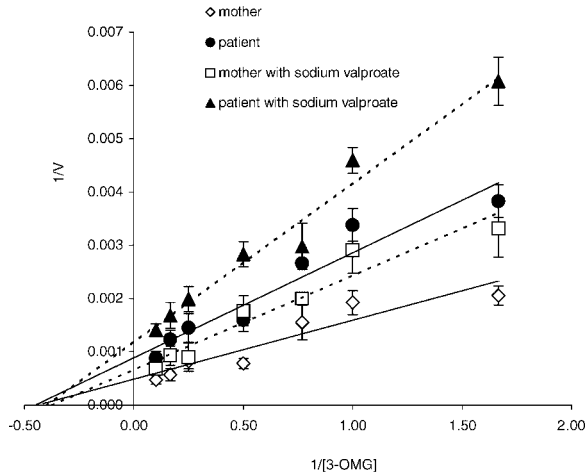


Fig. 1. Sodium valproate inhibits erythrocyte 3-OMG uptake. **A**: Effects of sodium valproate concentration on erythrocyte zero-trans 3-OMG uptake. Each data point represents the mean of three separate experiments in six-replicate set up. 3-OMG influx was measured for 15 s in cells exposed to different concentrations of sodium valproate. **B**: Time course study of sodium valproate effect on zero-trans 3-OMG uptake. Cells were incubated in PBS (control) or 0.5 mM sodium valproate followed by 3-OMG influx reactions for the indicated time periods. Open squares: PBS control, closed diamonds: sodium valproate treatment. Each data point represents the mean of three independent experiments in duplicate set up. Inset: Linear regression plot of the natural logarithm of 1-Ct/Ceq (Ct: intracellular radioactivity measured at individual time point during the zero-trans influx; Ceq: intracellular radioactivity measured at equilibrium time point 25 min). * Significant difference between control and sodium valproate treated groups ($P < 0.05$).

to the erythrocyte model since the 3-OMG transport is too rapid to be determined at 37°C. The previously established model measures Glut1-mediated glucose transport into erythrocytes at 4°C. We have adopted the low-temperature assay system to evaluate the effects of sodium valproate on Glut1 activity [Klepper et al., 1999a,b, 2003; Ho et al., 2001;



	K_m(mM)		V_{max} (fmol/s/million RBC)	
	-valproate	+valproate	-valproate	+valproate
mother	2.2±0.2	2.6±0.1	2000±500	1429±179*
patient	2.2±0.22	2.5±0.3	1111±111	833±164*

Fig. 2. Kinetic study of sodium valproate inhibition of 3-OMG transport in normal and Glut1DS erythrocytes. Erythrocyte glucose transport was measured by the zero-trans influx of ^{14}C-labeled 3-OMG (four determinations/data point) for 15 s in the absence or presence of 0.5 mM sodium valproate. The data is presented as the double-reciprocal plot of the 3-OMG transport velocity (V) versus 3-OMG concentration (0–10 mM). Open diamonds: maternal control sample without sodium valproate treatment; open squares: maternal control sample with sodium valproate treatment. Closed circles: patient sample without sodium valproate treatment; closed triangles: patient sample with sodium valproate treatment. Solid lines: without sodium valproate treatment. Dashed lines: with sodium valproate treatment. * Significant difference in V_{max} between control and sodium valproate treated groups ($P < 0.05$).

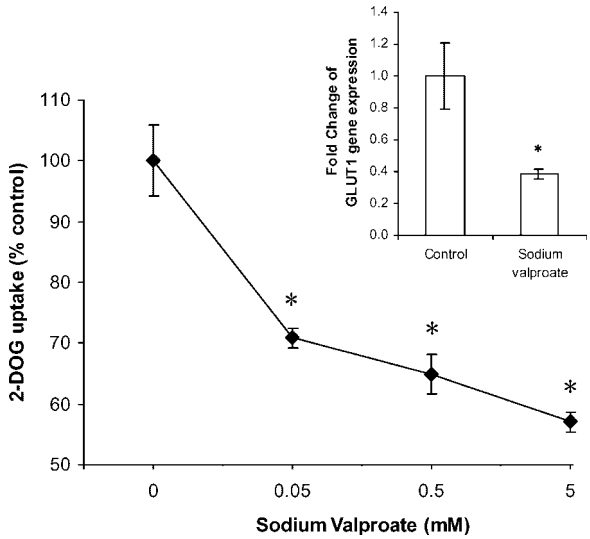


Fig. 3. Sodium valproate inhibits astrocyte glucose transport and GLUT1 mRNA expression. Sodium valproate inhibits zero-trans 2-DOG influx into primary astrocytes. Inset: Quantitative analysis of *GLUT1* gene expression by real-time PCR in control and sodium valproate (0.5 mM) treated cells. Each data point represents the mean of two independent experiments in triplicate set up. The relative GLUT1 expression level of cells without sodium valproate treatment is set at 1. * Significant difference between control and sodium valproate treated groups ($P < 0.05$).

Wong et al., 2005] so that the results of the current study may be compared with previous observations.

In erythrocytes, sodium valproate elicits acute inhibition of zero-trans glucose uptake measured within 30 s. The inhibition was observable as soon as 5 s with sodium valproate treatment, such effect became more potent as the incubation time increases and reached statistic significance from 15 to 25 s. At 30-s incubation, the inhibition was marginally significant ($P = 0.054$), likely due to the initiation of glucose efflux as the uptake reached saturation. Kinetic studies showed that sodium valproate inhibition of Glut1 activity was associated with a significant reduction of V_{max} but not K_m, indicating a non-competitive mode of inhibition.

Existing evidence indicate that sodium valproate can elicit biological activities by direct interaction with cell membrane through flip-flop across the lipid bilayers and thus altering membrane fluidity and stability. It is thought that the immediate effect of sodium valproate in some seizure models may be explained by its effect on these "extracellular sites" [Kessel et al., 2001]. Our observation that sodium valproate exerts acute inhibition on erythrocyte glucose transport is consistent with this notion. The alteration of membrane fluidity provides a possible explanation to the non-competitive inhibition of erythrocyte glucose transport by sodium valproate. However, the precise correlation between sodium valproate-induced change of membrane fluidity and cellular glucose transport remains to be established.

The inhibitory effect of sodium valproate on glucose transport expands beyond erythrocytes. In astrocytes and fibroblasts, Glut1 is the predominant glucose transporter [Miele et al., 1997; Yu and Ding, 1998]. We have shown that prolonged exposure to sodium valproate suppresses glucose transport in a dose-dependent manner and is associated with the downregulation of GLUT1 mRNA expression in both cell types. Such observations are of important relevance due to the long-term clinical use of

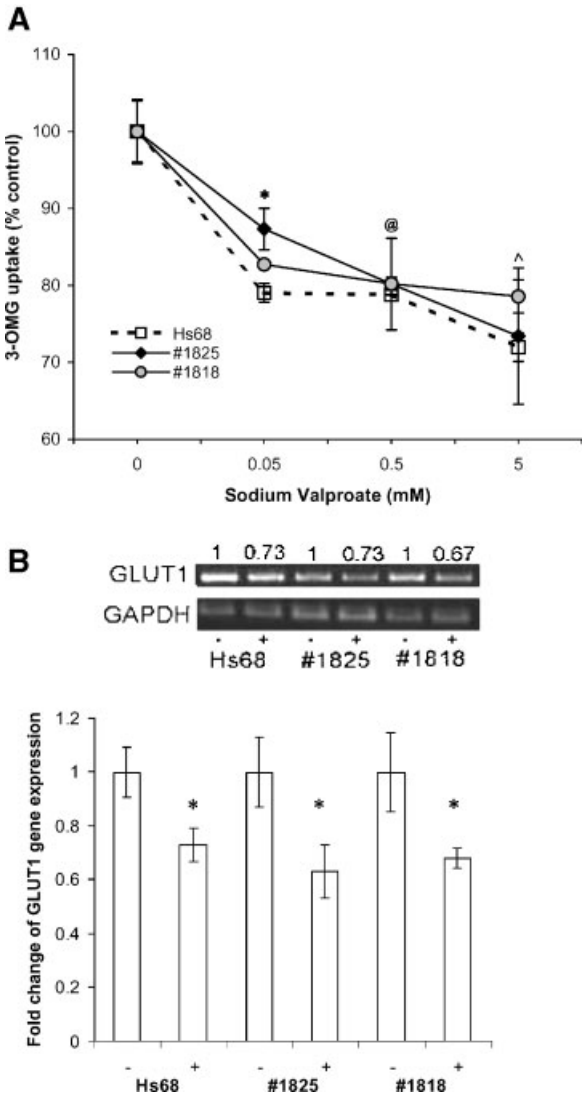


Fig. 4. Sodium valproate inhibits fibroblast glucose transport and GLUT1 mRNA expression. **A**: Sodium valproate inhibits zero-trans 3-OMG influx into fibroblasts. Open squares: HS68 fibroblasts, closed diamonds: Glut1DS fibroblasts clone # 1825, gray circles: Glut1DS fibroblasts clone # 1818. Each data point represents the mean of two separate experiments in quadruplicate set up. The 3-OMG-uptake level of cells without sodium valproate treatment is set at 100%. ^, @, * indicate significant differences between control (no sodium valproate) and sodium valproate treated groups ($P < 0.05$) observed in Hs68 (^) and the two clones of Glut1DS fibroblasts (@ clone 1818, * clone 1825). **B**: Analysis of *GLUT1* gene expression in cells without (−) or with (+) 0.5 mM sodium valproate treatment by real-time PCR (bar chart) and RT-PCR (gel photo). The relative GLUT1 expression level of cells without sodium valproate treatment is set at 1. Numbers on top of the gel photo indicate the relative GLUT1 mRNA expression levels after normalizing the expression of the control gene *GAPDH*. * $P < 0.05$.

this medication. Kessel et al. [2001], proposed that the late anticonvulsant effect of the drug can be explained by the slow diffusion of sodium valproate across the plasma membrane of the neuron and its action on intracellular sites. Although the mechanisms involved in sodium valproate-mediated inhibition of GLUT1 mRNA expression remain elusive, one of these "intracellular sites" could be regarded as the down-regulation of GLUT1 mRNA expression in fibroblasts and astrocytes upon prolonged exposure to sodium vaproate. In addition, carnitine status has been reported to affect glucose transporter activities, including Glut1 [Mesmer and Lo, 1990; Caviglia et al., 2004]. The possibility that the depression of glucose transport and GLUT1 expression in fibroblasts and astrocytes observed in this study is associated with a sodium valproate induced carnitine deficiency state should be considered.

Aside from Glut1, Glut3, and Glut5 having substrate specificity to glucose and fructose, respectively, are also expressed in fibroblasts and microglia cells [Brown, 2000]. Modulation of Glut3 and Glut5 activities has implications in sugar transport to neurons and microglial cells, respectively. Since Glut5 primarily transports fructose, this transporter is unlikely to have a major impact on cellular glucose uptake. We have measured the mRNA of GLUT3 and GLUT5 by real-time PCR analyses and found that sodium valproate does not affect gene expression of these transporters (data not shown). These results indicate that sodium valproate inhibition of glucose transporter mRNA expression is GLUT1 specific.

The Glut1, Glut3, and Glut5 transporters have distinct tissue distribution profiles, substrate specificities, transport kinetics, and regulation patterns. For example, erythrocytes and astrocytes predominantly express Glut1 whereas Glut3 is primarily expressed in neurons. While Glut1 and Glut3 primarily transport glucose, Glut5 is a fructose transporter [Ho et al., 2002]. Such complicated organization reflects sophisticated control and coordination of these genes and their products to fulfill the specific metabolic needs of individual organs.

The distinct tissue distribution profiles of Glut1, Glut3, and Glut5 implies differential regulation of these gene promoters in a tissue-specific manner. Specific induction of GLUT1 but not GLUT3 transcription by v-src further supports the notion that distinct regulatory

mechanisms control the expression of the two transporters [Wagstaff et al., 1995]. Thus, although the transcription regulation of the human GLUT promoters are not well understood, existing evidence indicate that tissue-specific factors as well as control elements unique to each promoter both contribute to the differential expression of these genes. It is likely that sodium valproate also specifically downregulate GLUT1 promoter activity without altering GLUT3 and GLUT5 expression through similar mechanisms. The precise mechanisms underlying the differential expression of individual glucose transporters to sodium valproate require further investigation. Also, it is important to notice that the decrease in GLUT1 mRNA could be the result of inhibition of gene expression or increased mRNA degradation and does not necessarily translate into a decrease in Glut1 protein expression.

The extent of sodium valproate inhibition of cellular glucose transport is similar in normal and the Glut1DS samples, raising the possibility that the compound exacerbates Glut1DS in vivo. Aside from Glut1DS, compromised Glut1 activity has been reported in diabetes and Alzheimer's disease [Kalaria and Harik, 1989; Hu et al., 2000]. It is noteworthy that Glut1 is also highly expressed in placenta responsible for glucose delivery to the fetus. Consistent with the Glut1-inhibiting actions of the compound, sodium valproate consumption during pregnancy has been associated with an increased risk of infantile hypoglycemia [Ebbesen et al., 2000].

To our knowledge, this is the first study to demonstrate both the acute and prolonged glucose transport responses to sodium valproate at the cellular level. This is also the first report showing the effects of sodium valproate at both Glut1 function and gene expression levels. Disturbed glucose transport was found in peripheral cells, that is, erythrocytes and fibroblasts, as well as in brain astrocytes.

In conclusion, the results here support the hypothesis that sodium valproate inhibits cellular glucose transport. Our in vitro data argue that exposure to sodium valproate imposes a risk of disturbing cellular glucose supply through the inhibition of Glut1 activity and mRNA expression. These results justify further substantiation of the observations in vivo. As for clinical implications, prolonged sodium valproate exposure has been associated with undesirable side effects including disturbed glucose homeostasis and ATP production [Bolanos and Medina, 1997]. Together with the Glut1-inhibiting properties of sodium valproate found in this study, current evidence indicate that the integrity of Glut1 function should be taken into consideration before the compound is prescribed to patients for epilepsy treatment. With regards to seizure control in Glut1DS, it is important to emphasize that these patients respond well to the ketogenic diet, which provides an alternative energy source to the brain.

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