

GLUT8 Is Dispensable for Embryonic Development but Influences Hippocampal Neurogenesis and Heart Function

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Received 13 January 2006/Accepted 3 March 2006

GLUT8 is a glucose transporter isoform expressed at high levels in testis; at intermediate levels in the brain, including the hippocampus; and at lower levels in the heart and several other tissues. GLUT8 is located in an intracellular compartment and does not appear to translocate to the cell surface, except in blastocysts, where insulin has been reported to induce its surface expression. Here, we generated mice with inactivation of the *glut8* gene. We showed that expression of GLUT8 was not required for normal embryonic development and that *glut8*^{-/-} mice had normal postnatal development, glucose homeostasis, and response to mild stress. Adult *glut8*^{-/-} mice showed increased proliferation of hippocampal cells but no defect in memory acquisition and retention. Absence of GLUT8 from the heart did not alter heart size and morphology but led to an increase in P-wave duration, which was not associated with abnormal Nav1.5 Na⁺ channel or connexin expression. Thus, absence of GLUT8 expression in the mouse caused complex but mild physiological alterations.

GLUT8 is a recently characterized member of the glucose transporter (SLC2) family (4, 8). Functional studies of *Xenopus* oocytes revealed that GLUT8 is a high-affinity glucose transporter (K_m , ~2 mM) and that it can also transport fructose and galactose. GLUT8 mRNA is expressed at the highest levels in the testis, where the protein is found in type I spermatocytes; at intermediate levels in the brain; and at lower levels in several other tissues, including the heart, adrenals, and liver (4, 8). In the brain, GLUT8 is found in hippocampal excitatory and inhibitory neurons; the dentate gyrus, amygdala, and primary olfactory cortex; some hypothalamic nuclei; and the nucleus of the tractus solitarius, as well as at high levels in the supraoptic-hypophyseal tract (7, 15). During embryonic development, GLUT8 is found in blastocysts and translocates from an intracellular compartment to the cell surface in response to insulin (1). Suppression of GLUT8 expression in blastocysts by antisense oligonucleotides leads to an increased rate of apoptosis, suggesting that the transporter may be important for embryonic development (12).

GLUT8 is located in an as yet poorly characterized intracellular compartment, where it is retained by a dileucine motif present in its amino-terminal cytoplasmic tail. When expressed in adipocytes, PC-12 cells, or hippocampal neurons, no stimuli could be identified that trigger its cell surface expression (17, 22); the insulin-induced translocation described in blastocysts is thus a unique situation. Therefore, the physiological function of GLUT8 is still uncertain, and the available data do not allow us to conclude whether GLUT8 functions as a glucose transporter at the plasma membrane or whether it has a specific function as an intracellular glucose transporter. The latter hy-

pothesis appears unlikely, as there is usually no intracellular free glucose, because high-affinity hexokinases rapidly phosphorylate glucose into glucose-6-phosphate. An alternative possibility is that GLUT8 functions primarily as a transporter for another, as yet unidentified, substrate.

Here, to evaluate the possible physiological function of GLUT8, we inactivated its gene in embryonic stem (ES) cells and generated mice with suppressed GLUT8 expression. We show that GLUT8 is not required for embryonic development and that *glut8*^{-/-} mice have no impairment in growth or glucose homeostasis. A significant increase in BrdU labeling of the hippocampus was measured, but no changes in learning or memory retention could be observed. The absence of GLUT8 from the heart did not lead to any morphological or histological modification. Analysis of electrocardiograms (ECGs), however, revealed a small but significant increase in the P-wave duration, suggesting a possible involvement of GLUT8 in the control of atrial contraction.

MATERIALS AND METHODS

Preparation of *glut8*^{-/-} mice. The *glut8* gene was isolated as a 10-kb SacI fragment from a 129 mouse λ bacteriophage genomic library. LoxP sites (14) were introduced into an NcoI and an NdeI site present, respectively, in the 5' and 3' introns flanking exon 10 (Fig. 1). The orientations of the *loxP* sequences were verified by sequencing. The gene for neomycin resistance, placed under the control of the phosphoglycerate kinase promoter and flanked by *frt* sites (5), was cloned into a blunt-ended EcoRI site 1 kb downstream of exon 10 and downstream of the polyadenylation site. A 5.7-kb gene fragment was subcloned upstream of the proximal *loxP* site, and a 3.5-kb fragment was subcloned downstream of the neomycin gene. The thymidine kinase gene placed under the control of a herpes simplex virus promoter was inserted downstream of the 3' homologous region. The vector was subcloned into a Bluescript plasmid (Stratagene, La Jolla, California), linearized with SalI, and electroporated into GS-1 ES cells, as described previously (16). G418-resistant colonies were expanded and genotyped by Southern blot analysis of HindIII-digested DNA using exon 3 as a probe. The wild-type allele was revealed as a 14-kb fragment, and the recombined allele as a 10-kb fragment. Confirmation of the integrity of the targeted allele was performed using a 300-bp SacI/KpnI fragment located at

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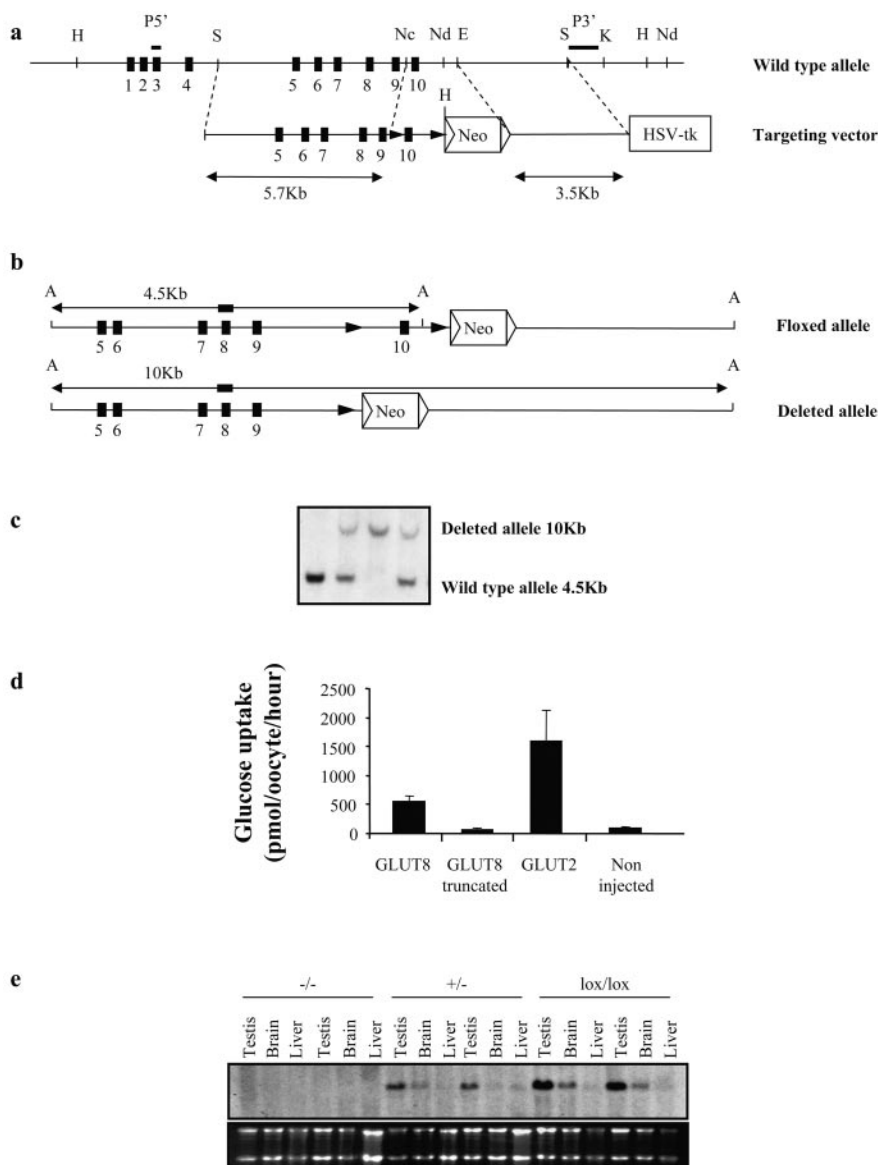


FIG. 1. Transgenic construct and gene targeting. (a) Targeting construct used to generate *glut8 loxP* mice. Arrowheads, *loxP* sites; open arrowheads, *fit* sites, Neo, neomycin selection marker; P3', probe 3'; P5', probe 5'. Other restriction sites shown: S, SacI; Nd, NdeI; Nc, NcoI; E, EcoRI; K, KpnI; H, HindIII. (b) ApaI (A) restriction sites were used in Southern blotting to identify targeted and wild-type alleles using a probe corresponding to exon 8. (c) Representative Southern blot of ApaI-digested genomic DNA revealing wild-type and recombined alleles. (d) Glucose uptake measurements carried out in *Xenopus* oocytes with GLUT8, truncated GLUT8, GLUT2, and the uninjected oocytes. The dileucine motif of GLUT8 was mutated to dialanine for cell surface expression. The truncated form of GLUT8 failed to transport glucose. The error bars indicate standard deviations. (e) Northern blot analysis of mRNA present in testis, brain, and liver of *glut8*-null ($-/-$), heterozygous ($+/-$), or *loxP*-containing mice (lox/lox). GLUT8-null mice did not express GLUT8 mRNA, whereas heterozygous mice showed decreased GLUT8 expression compared to *loxP* mice.

the 3' extremity of the targeting vector, which revealed a 5-kb wild-type allele or 7-kb mutated allele (data not shown). Two ES cell clones with homologous recombination were injected into blastocysts of C57BL/6 mice. Chimeric mice were bred with C57BL/6 mice, and germ line transmission was confirmed by Southern blot analysis.

To generate *glut8^{-/-}* mice, *glut8^{lox/+}* mice were crossed with NesCreI transgenic mice, which express the CRE recombinase under the control of the nestin promoter (19). Since the *NesCreI* transgene is active in the germ line, breeding of these mice with *glut8^{lox/+}* mice generated *glut8^{-/+}* mice, which were intercrossed to generate *glut8^{-/-}* mice. Genotyping was carried out by PCR using the following primers: sense (5'-GGAGGAGACAGACAAACC-3'), wtAS (5'-GT ATGTAGGAATAGCTCAC-3'), and KOAS (5'-GAAAGAACCTGTAAGT

GATG-3'). PCR conditions were as follows: 1 cycle at 94°C for 5 min; then, 30 cycles each at 94°C for 30 seconds, 55°C for 30 seconds, and 72°C for 30 seconds; and finally, 1 cycle at 72°C for 7 min. Wild-type and mutant alleles were diagnosed as 400-bp and 510-bp amplified products, respectively. Results were confirmed by Southern blot analysis of ApaI-digested DNA using exon 8 as a probe, which revealed a 4.5-kb wild-type allele and a 14-kb mutant allele. The presence of the Cre recombinase was identified by PCR with the following primers: cre1 (5'-CAGGGTGTATAAGCAATCCC-3') and cre2 (5'-CCTGAAAATGCT TCTGTCCG-3').

Glucose uptake in *Xenopus* oocytes. Stage V and VI oocytes were injected with 25 ng of RNA prepared from GLUT8(LL-AA) and a truncated GLUT8(LL-AA) cDNA in which the 3' extremity, corresponding to the last C-terminal 49 amino

acids of the protein, was deleted. Both were cloned into pSD5 as described previously (6). Glucose uptake assays were performed 3 days following injection with groups of 8 to 10 oocytes incubated in the presence of 2 mM glucose and 10 μ Ci of D-[³H]glucose (NEN Life Science) for 15 min. The oocytes were then washed with ice-cold MBS [NaCl, 85 mM; KCl, 1 mM; NaHCO₃, 2.4 mM; MgSO₄, 1 mM; Ca(NO₃)₂, 0.33 mM; CaCl₂, 0.55 mM; HEPES, 4 mM] solution containing 1 mM HgCl₂. Individual oocytes were dissolved in 2% sodium dodecyl sulfate, mixed with scintillation fluid, and counted. Analysis of GLUT8 expression in oocytes was performed by pulse-labeling with [³⁵S]methionine and immunoprecipitation, as described previously (6).

Physiological measurements. All animal procedures were approved by the Service Vétérinaire Cantonal (Lausanne, Switzerland). Intraperitoneal glucose tolerance tests (1 mg/g of body weight) were performed in mice after an overnight fast. Glycemia was measured in tail vein blood using a glucometer (Roche Diagnostics, Rotkreutz, Switzerland). Isoproterenol was administered with a Mini-Osmotic pump (model 2002; 200 μ l, 0.5 μ l per hour, 14 days; ALZET). Plasma insulin was measured after a 3-h fast with the Ultrasensitive mouse insulin enzyme-linked immunosorbent assay kit (Merckodia AB, Uppsala, Sweden). Mild stress was induced by an intraperitoneal saline injection. Plasma corticosterone was evaluated by radioimmunoassay (18).

RNA analysis. Total RNA was extracted by the guanidinium thiocyanate method (2), separated on a 1.2% formaldehyde agarose gel, and transferred to a nylon membrane. For detection of GLUT8 and Nav1.5 expression, probes were a 1.2-kb NotI/BglII fragment of the rat GLUT8 cDNA and a 670-bp PCR-amplified fragment encoding a part of the loop between domains I and II of the Nav1.5 cDNA radioactively labeled by random priming.

Histology. Hearts and brains were rapidly excised from mice after sacrifice with CO₂. The organs were preserved in buffered formaldehyde. The tissues were embedded in paraffin, and 5- μ m sections were cut. Heart and hippocampus sections were stained with hematoxylin and eosin. Heart sections were also stained with Masson trichrome to examine interstitial fibrosis. For quantitative histomorphometry, heart sections were stained with periodic acid-Schiff. The cell area was measured with image J software (<http://rsb.info.nih.gov/ij/>; author, W. Rasband, Research Services Branch, National Institute of Mental Health, Bethesda, Maryland).

Cell proliferation analysis. Bromodeoxyuridine (BrdU) labeling was performed using the BrdU labeling and detection kit II (Roche). BrdU was injected four times at 2-h intervals for a total volume of 1.8 ml/100 g of body weight. Animals were sacrificed 24 h after the last injection by cervical dislocation, and the brains were excised, frozen in isopentane at -20°C, and left for 24 h at that temperature. Ten-micrometer sections were cut and treated following the kit instructions. Positive cells were counted, and the dentate gyrus area was measured for normalization (with image J software).

ECG recordings and analysis. Six-lead surface-limb ECG measurements (aVF, aVL, aVR, and the derivatives I, II, and III) were recorded for mice under halothane anesthesia. Ag/AgCl gel-coated ECG electrodes (Unomedical) were attached to the two front and the left rear paws of the mice. The electrodes were connected to a standard six-lead ECG amplifier module (EMKA Technologies, Paris, France), which included high- and low-pass filters (set to 0.05 Hz and 500 Hz, respectively) and a gain selection device (set to 1,000-fold). Signals were digitized continuously at 1 kHz and recorded by using the IOX data acquisition system (EMKA Technologies, France). The software ECGAuto (EMKA Technologies, France) was used to perform quantitative analysis of the data recorded. Quantitative analysis of interval durations and wave surfaces was carried out on a 30-s interval taken ~1 min after the beginning of each 20-min recording. Within the chosen 30-s period, electrical complexes were averaged in blocks of five to minimize background noise and to increase the wave recognition power of the software.

The P-wave duration was measured from the first deflection of the P wave to the point where it joined the isoelectric line. The latter was set at the first deflection of the P wave. The PR interval was measured from the beginning of the P wave to the beginning of the QRS complex. The QRS duration was measured from the first deflection of the Q wave (or the R wave when the Q wave was absent) to the negative peak of the S wave. The QT interval was measured from the beginning of the QRS complex to the end of the T wave. The end of the T wave was defined as the point where the negative part of the T wave returned to the isoelectric line. The RR interval was determined automatically by the software by averaging individual RR intervals for the 30-s period.

Heart lysate preparation and analysis. Mouse heart ventricles were transferred into lysis buffer (50 mM Tris, pH 7.5, 150 mM NaCl, 1 mM EDTA, 1 mM phenylmethylsulfonyl fluoride, and complete protease inhibitor cocktail [Roche]). The tissue was homogenized using a Polytron and a Teflon homogenizer. Triton X-100 was added to a final concentration of 1%, and the lysate was

TABLE 1. Numbers of offspring of different genotypes obtained by crossing heterozygous mice together or with male or female knockout mice^a

Genotype	+/- \times +/-			δ -/- \times ♀ +/-		δ +/- \times ♀ -/-	
	No. of offspring	%	Expected (%)	No. of offspring	%	No. of offspring	%
-/-	124	17.4 ^b	25	20	56	23	47
+/-	368	51.8	50	16	44	26	53
+/+	219	30.8	25				
Total	711			36		49	

^a +/-, heterozygous; -/-, knockout; δ , male; ♀ , female.

^b Chi-square test, $P < 0.003$ versus expected distribution.

agitated by rotation for 1 h at 4°C. The soluble fraction from a 15-min centrifugation at 13,000 \times g (4°C) was used for Western blot analysis, performed as described previously (21). Protein concentrations were measured using the Bradford assay with bovine serum albumin as a standard. For the detection of Nav1.5, a rabbit polyclonal antibody (ASC-005) from Alomone was used. For connexin 43 (Cx43), a rabbit polyclonal antibody (71-0700) was obtained from Zymed. The secondary horseradish peroxidase-conjugated donkey anti-rabbit antibody (NA934) was from Amersham. Secondary antibodies were revealed by enhanced chemiluminescence (Super-Signal West-Dura Extended Duration Substrate [Pierce]).

Immunofluorescence microscopy. Hearts were quickly frozen in 2-methylbutane precooled in liquid nitrogen and embedded in OCT medium (Miles Inc., IN), and 8- μ m-thick frozen sections were prepared. The sections were rinsed in phosphate-buffered saline (PBS), incubated for 30 min in a buffer containing 0.5% bovine serum albumin and 0.2% Triton X-100, and then exposed for 20 h to antibodies against either Cx43 (AB1728; Chemicon) or Cx40 (AB1726; Chemicon) diluted 1:500 in PBS. Primary antibodies were detected using secondary antibodies labeled with fluorescein isothiocyanate (Molecular Probes Inc.) and directed against rabbit immunoglobulin G. The sections were then rinsed in PBS, counterstained with Evans Blue, and mounted with a coverslip for microscopic examination with a Leica fluorescence microscope.

RESULTS

Generation of GLUT8 knockout mice. To generate mice with a floxed *glut8* allele, we constructed the targeting vector shown in Fig. 1a. This contained two *loxP* sites flanking exon 10 of the *glut8* gene and a neomycin cassette flanked by *frt* sites. The structure of the recombined floxed alleles, as well as the sizes of the *Apal* restriction fragments used for genotyping and a representative Southern blot, is presented in Fig. 1b and c. Exon 10 of *glut8* encodes the carboxy-terminal cytoplasmic tail of the transporter, corresponding to the last 49 C-terminal amino acids. To verify that deletion of exon 10 was sufficient to inactivate transport activity, an mRNA encoding the predicted truncated GLUT8 was injected into *Xenopus* oocytes. We demonstrated that the deleted mutant of GLUT8 failed to transport glucose (Fig. 1d). Oocytes injected with a GLUT2 mRNA or not injected served, respectively, as positive and negative controls for the uptake experiments.

For homologous recombination of the *glut8* gene, the targeting vector was electroporated into ES cells, and four independent correctly targeted clones were obtained. Two of these were injected into blastocysts of C57BL/6 mice. Chimeric mice that transmitted the floxed allele to their offspring were obtained. The *glut8*^{+/*lox*} mice were crossed with NesCre1 transgenic mice. The NesCre1 gene is imprinted and is active in somatic tissues (specifically in the central nervous system) only

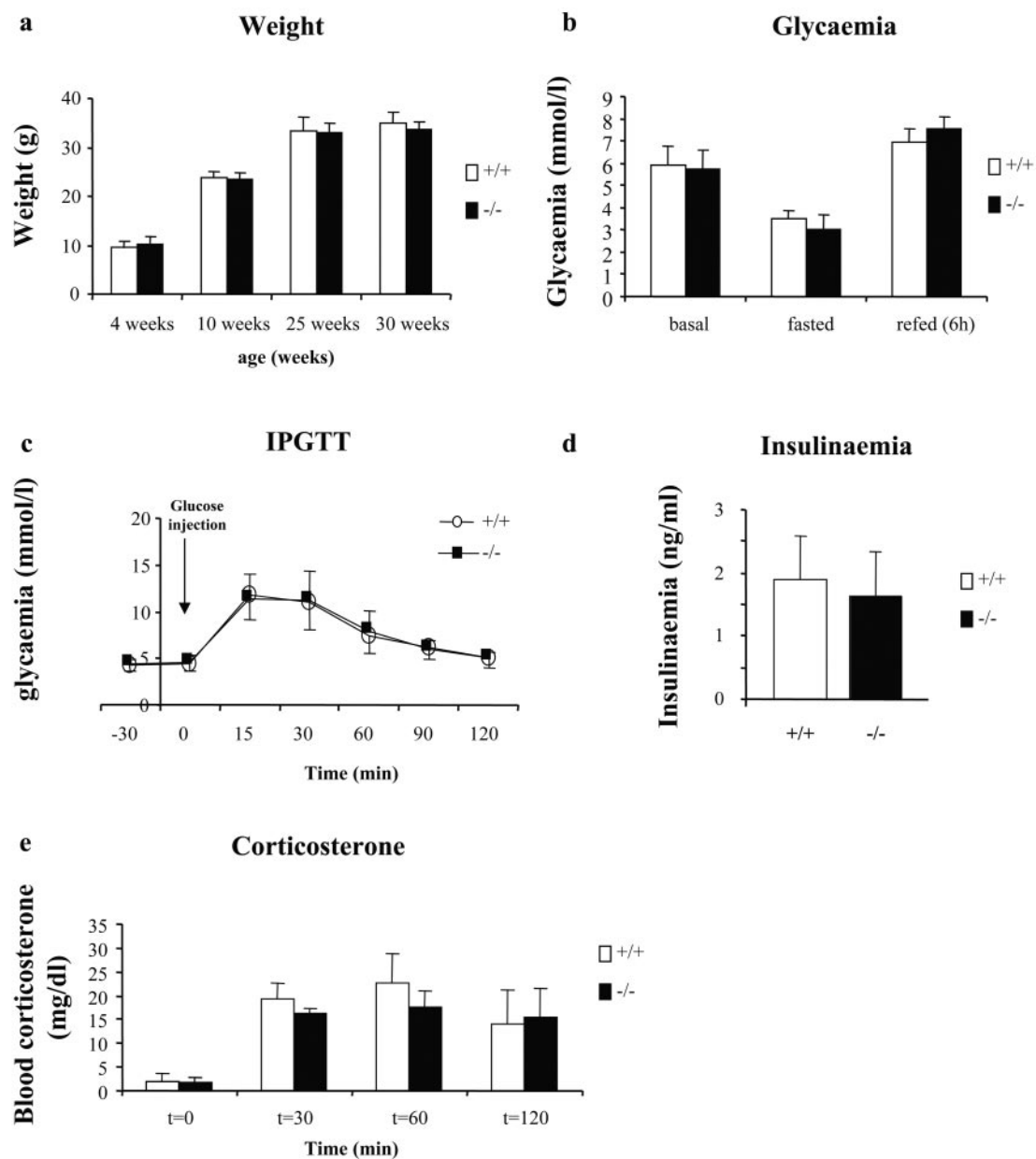


FIG. 2. Normal body weight and glucose homeostasis in *glut8*^{-/-} mice. (a) Body weights of *glut8*^{-/-} and wild-type mice at the ages of 4, 10, 25, and 30 weeks were identical ($n = 8$ for each genotype). (b) Random glycemia and glycemia in the fasted state and after 6 h of refeeding were identical in control and mutant mice ($n = 8$ for each genotype). (c) Glucose tolerance test results were identical in *glut8*^{-/-} and wild-type mice ($n = 7$ for each genotype). (d) Basal insulinemia measurements at 10:00 a.m. were the same in control and knockout mice ($n = 8$ for each genotype). (e) Blood corticosterone levels measured before and after mild stress induced by intraperitoneal saline injection showed normal basal and stress-induced corticosterone secretion in *glut8*^{-/-} mice compared to controls (wild type, $n = 5$; knockout, $n = 6$). The error bars indicate standard deviations.

when it is paternally inherited. However, it is active in the germ line irrespective of whether it is paternally or maternally inherited. F₂ mice of the *glut8*^{+/-} genotype were obtained and intercrossed to finally obtain six different genotypes: *glut8*^{+/+}, *glut8*^{+/-}, and *glut8*^{-/-}, all with or without the *Nes-cre1* transgene.

No mRNA for the *glut8* gene with exon 10 deleted was detected in brain, testis, or liver by Northern blot analysis using a full-length GLUT8 cDNA probe, and the GLUT8 mRNA level was reduced by approximately 50% in tissues from *glut8*^{+/-} mice (Fig. 1e), suggesting instability of the truncated mRNA.

GLUT8 was previously found to be expressed in mouse blastocysts and was proposed to play an important role in glucose utilization at this stage of development (12). Analysis of the offspring from crosses of heterozygous mice showed, among 711 births analyzed, that only 17.4% of the mice were homozygous for the inactivated *glut8* allele (instead of the expected ratio of 25%), 30.8% were wild type, and 51.8% were heterozygous (Table 1). These results suggest partial embryonic lethality of the mutation. However, male and female *glut8*^{-/-} mice were fertile, and when crossed together or with

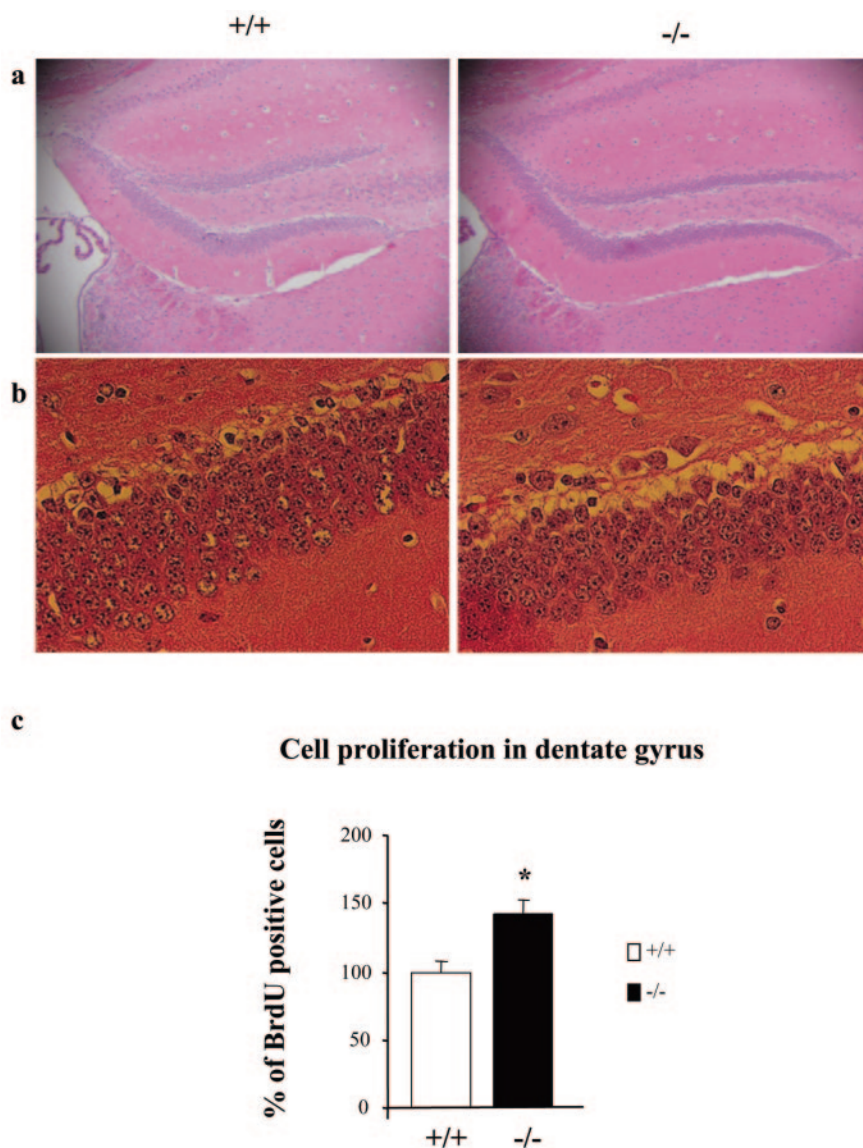


FIG. 3. Morphology and structure of the hippocampus. (a) Hematoxylin-eosin staining of the dentate gyruses of control (+/+) and *glut8*-null (-/-) mice. There was no difference in the morphology of the dentate gyrus in the *glut8*^{-/-} mice compared to controls. (b) Detail of the granule cell layer of the dentate gyrus showing no difference in the morphologies and numbers of nuclei in the *glut8*^{-/-} mice and controls. (c) BrdU staining in the dentate gyrus. The number of BrdU-positive cells normalized to the analyzed surface of the dentate gyrus was expressed as a percentage of that of control mice ($n = 6$ for each genotype). There was a statistically significant increase in the number of BrdU-positive cells in the *glut8*^{-/-} mice compared to the control mice (Student t test; *, $P < 0.001$). The error bars indicate standard deviations.

heterozygous mutant mice, they had a normal litter size and genotype distribution (Table 1). Thus, GLUT8 is dispensable for embryonic development and for germ cell function.

Body weight and glucose homeostasis. Homozygous mutant mice were born with normal body weight and gained weight normally (Fig. 2a). The random glycemia of 12-week-old mice, as well as the glycemia measured after 18 h of fasting or following 6 h of refeeding, was identical to that of control mice (Fig. 2b), and they did not show any impairment in glucose tolerance or alteration of the fasted plasma insulin levels (Fig. 2c and d). These results indicated that the absence of GLUT8 did not lead to any measurable defect in glucose homeostasis. *glut8*^{-/-} mice responded equally well to mild stress induced by

intraperitoneal saline injection. They showed the same plasma corticosterone levels when measured 30 min, 60 min, and 120 min after stress induction. Moreover, their basal blood corticosterone was normal (Fig. 2e).

Hippocampus histology and cell proliferation. GLUT8 is expressed in hippocampal neurons, where it resides in an intracellular compartment, and its physiological role in this structure is unknown. Histological examination of hematoxylin- and eosin-stained sections was performed on the brains of 12-week-old mice and showed no morphological abnormalities of the dentate gyrus (Fig. 3a and b).

Next, we evaluated whether the absence of GLUT8 expression could modify hippocampal cell proliferation. Mice were

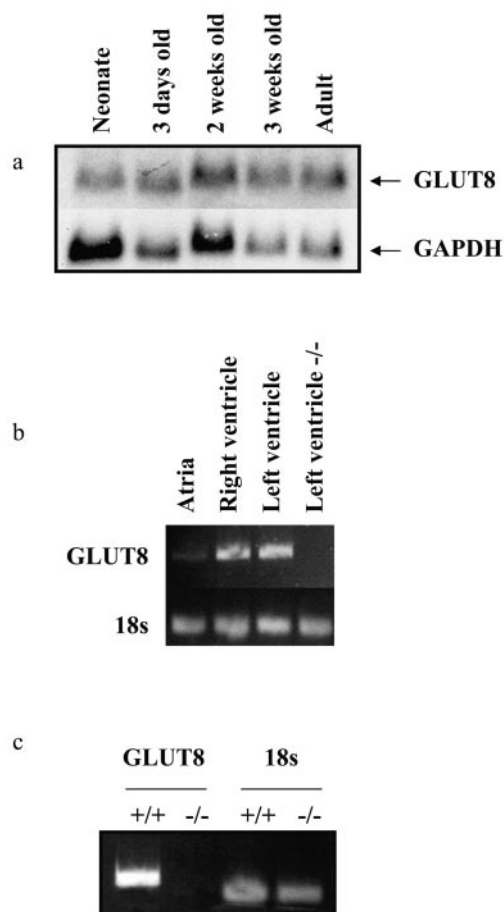


FIG. 4. GLUT8 expression in the heart. (a) Northern blot analyses performed on total mRNA isolated from neonate mice, 3-day-old mice, 2- and 3-week-old mice, and adult mice. GLUT8 mRNA was expressed at each time point. (b) RT-PCR analyses of GLUT8 mRNA expression performed on the different cardiac structures. GLUT8 was expressed at similar levels in the atrium and in both right and left ventricles. (c) RT-PCR analyses indicated that GLUT8 was expressed in isolated cardiomyocytes; 18S, amplified 18S RNA. +/+, control; -/-, *glut8*^{-/-}.

injected with BrdU, and its incorporation into cells of the dentate gyrus was evaluated by immunohistochemical analysis 1 day later. Absence of GLUT8 led to a 50% increase in BrdU-positive cells (Fig. 3c) in the dentate gyrus, suggesting increased cell proliferation.

Behavioral experiments performed in a Morris water maze (10) or in the homing board task, however, did not show any significant difference in memory acquisition and retention between control and knockout mice (data not shown).

Cardiac GLUT8 expression. GLUT8 protein is expressed in the mouse heart (1), and Northern blot analysis showed its mRNA to be expressed in the hearts of newborn mice and until the adult stage (Fig. 4a). GLUT8 mRNA was expressed in the atrium, as well as in the ventricles, as shown by reverse transcription (RT)-PCR analyses (Fig. 4b). At the cellular level, GLUT8 mRNA was found in isolated cardiomyocytes (Fig. 4c).

Cardiac weights and histology. The hearts from 30-week-old knockout and control mice were identical when normalized to either the body weight or tibia length (Table 2). Histological examination of hematoxylin- and eosin- and trichrome-stained sections was performed on the hearts of mice at 12 weeks of age (Fig. 5a and b). There were no morphological abnormalities of myocyte architecture, and analysis of trichrome-stained sections revealed no increase in interstitial collagen compared with controls (Fig. 5c). There was also no defect in glycogen storage in the knockout ventricles compared to the controls, as shown with periodic acid-Schiff staining. The sizes of the cells, measured on the periodic acid-Schiff-stained slides, were similar in the two groups (Fig. 5d).

Functional analysis of the heart. Electrocardiogram measurements were performed on 12-week-old mice. There was no significant difference in any intervals (P wavelength and morphology; PR, QT, and ST intervals; QRS complex; and heart rate) measured in mutant and control mice (data not shown). The same analyses were performed on 30-week-old mice. The P-wave duration, measured on the first Einthoven derivate, was increased by 12% in knockout mice compared to control mice (Table 3). The morphologies of the P wave, however, were not different in the two groups. There was no significant difference in the PR, QT, or ST interval length or in the QRS complex duration (Table 3).

To evaluate whether the absence of GLUT8 would lead to abnormal adaptation of the heart to an increased workload, mice were equipped with osmotic minipumps delivering isoproterenol continuously for a period of 2 weeks. Both groups of mice increased their heart rates to the same extent (Table 3). The QT segment was decreased by a diminution of the ST segment in both groups, as well as the PR segment. However, knockout mice showed a normalization of the P wavelength after isoproterenol administration, returning to the control values, whereas controls did not show any change in P wavelength after isoproterenol administration.

The increase in P-wave duration may be due to a defect in conduction in the atria of the *glut8*^{-/-} mice. Northern blot analyses were performed on whole-heart mRNA with a Nav1.5 sodium channel probe. There was no difference in expression of the messenger in the two groups of mice (Fig. 6a). At the protein level, Nav1.5, as well as connexin 43, showed the same expression level in *glut8*^{-/-} mice and in controls (Fig. 6b). To specifically look at the connexin pattern of expression, we performed immunofluorescence microscopy detection of connexins 40 and 43 in microscopic sections of the atria and ventricles of knockout and control mice. The distribution of connexin 40 showed a higher level of expression in the atria than in the ventricles, as expected, in both groups of mice (Fig.

TABLE 2. Heart weight measurements^a

Geno- type	<i>n</i>	Age (wk)	Heart wt (mg)	Tibia length (mm)	Body wt (g)	HW/TL ^b	HW/ BW ^c
+/+	9	32 ± 2	151.9 ± 22.1	19.1 ± 0.7	35.1 ± 3.2	7.9 ± 1.2	4.3 ± 0.4
+/-	9	31 ± 2	141.9 ± 10.5	19.6 ± 1.0	34.0 ± 3.0	7.2 ± 0.5	4.2 ± 0.3

^a Results are mean ± standard deviation.

^b HW, heart weight; TL, tibia length.

^c BW, body weight.

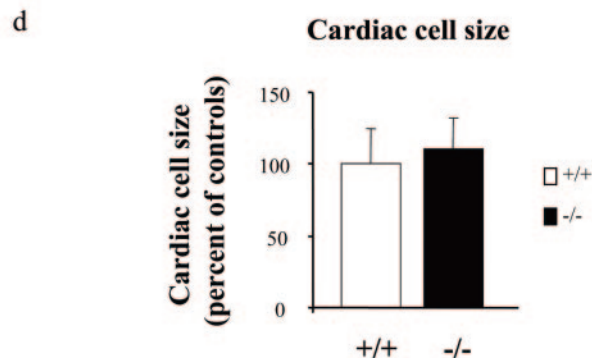
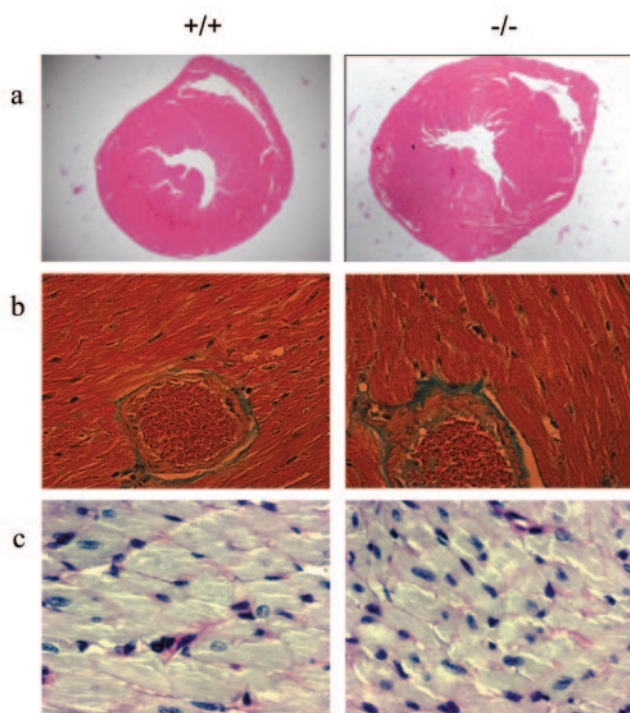


FIG. 5. Morphology of the *glut8*-null heart. (a) Transverse sections of wild type (+/+) and *glut8*-null (-/-) mouse hearts stained with hematoxylin-eosin. (b) Masson trichrome staining showed the absence of interstitial collagen. (c) Periodic acid-Schiff staining of sections from the left ventricles of control and knockout mice showed an absence of glycogen depots. These sections were used for the cell size measurements. (d) Cardiac cell sizes measured on periodic acid-Schiff-stained slides. There was no increase in the cell size of *glut8*^{-/-} mice compared to controls. The results are expressed as percentages of controls ($n = 80$ to 90).

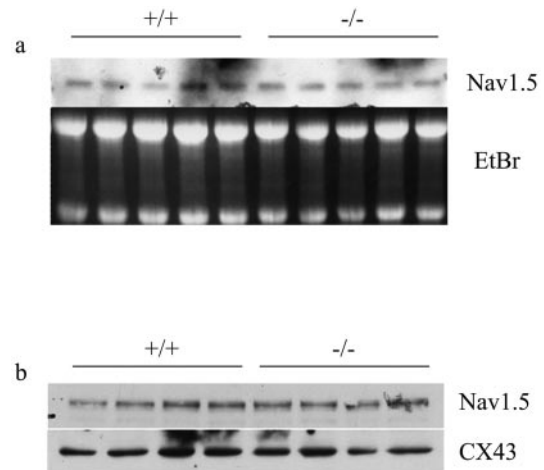


FIG. 6. Sodium channels and connexin expression analysis. (a) Northern blot analyses of heart mRNA with a Nav1.5 probe. There was no difference in Nav1.5 expression between the groups. (b) Western blot analyses of total proteins from whole hearts showed no difference in the expression of Nav1.5 and connexin 43 in the knockout mice and control mice.

7a and b). The distribution of connexins, however, was not different in knockout and control mice.

DISCUSSION

The present study showed that inactivation of the *glut8* gene leads only to relatively mild phenotypic alterations. Previous studies had shown that GLUT8 is expressed in blastocysts, where insulin could stimulate its cell surface expression, and that antisense suppression of GLUT8 at this stage of embryonic development leads to increased apoptosis. Our present results showed that crossing heterozygous mutant mice together led to a lower than expected ratio of *glut8*^{-/-} mice, suggesting partial embryonic lethality of the mutation. However, crossing homozygous mutant mice together or with heterozygous mice led to homozygous or heterozygous mutant mice with the expected Mendelian frequency. This suggests that *glut8*^{-/-} mice, which survive embryonic development, may do so because of compensatory mechanisms that are transmitted to their offspring. These mechanisms are not known, nor, at present, is it known at which stage embryonic lethality occurs. GLUT8 is found in spermatocytes, but its absence clearly does not reduce fertility. Thus, taken together, our data show that

TABLE 3. Electrocardiogram measurements^a

Genotype	<i>n</i>	Interval			P-wave duration (ms)	QRS complex duration (ms)	Heart rate (Hz)
		PR (ms)	QT (ms)	ST (ms)			
+/+	17	45.4 ± 1.6	57.8 ± 1.3	41.1 ± 2.9	12.3 ± 0.4	14.7 ± 1.0	489.4 ± 10.5
-/-	17	43.0 ± 1.3	59.6 ± 2.0	45.5 ± 1.8	13.7 ± 0.4*	15.5 ± 0.6	486.3 ± 12.6
+/+ ISO ^b	4	42.2 ± 1.5	50.0 ± 0.4	35.8 ± 0.9	12.4 ± 0.8	14.0 ± 0.8	584.7 ± 8.9
-/- ISO ^b	4	41.4 ± 1.8	50.2 ± 4.0	37.0 ± 3.8	12.2 ± 0.9	13.2 ± 1.0	584.9 ± 11.5

^a PR, QT, and ST interval lengths, P-wave durations, and QRS complex duration results obtained from wild-type and *glut8*^{-/-} mice. The results are means ± standard errors of the mean; *, $P = 0.002$ versus controls.

^b ISO, isoproterenol administration.

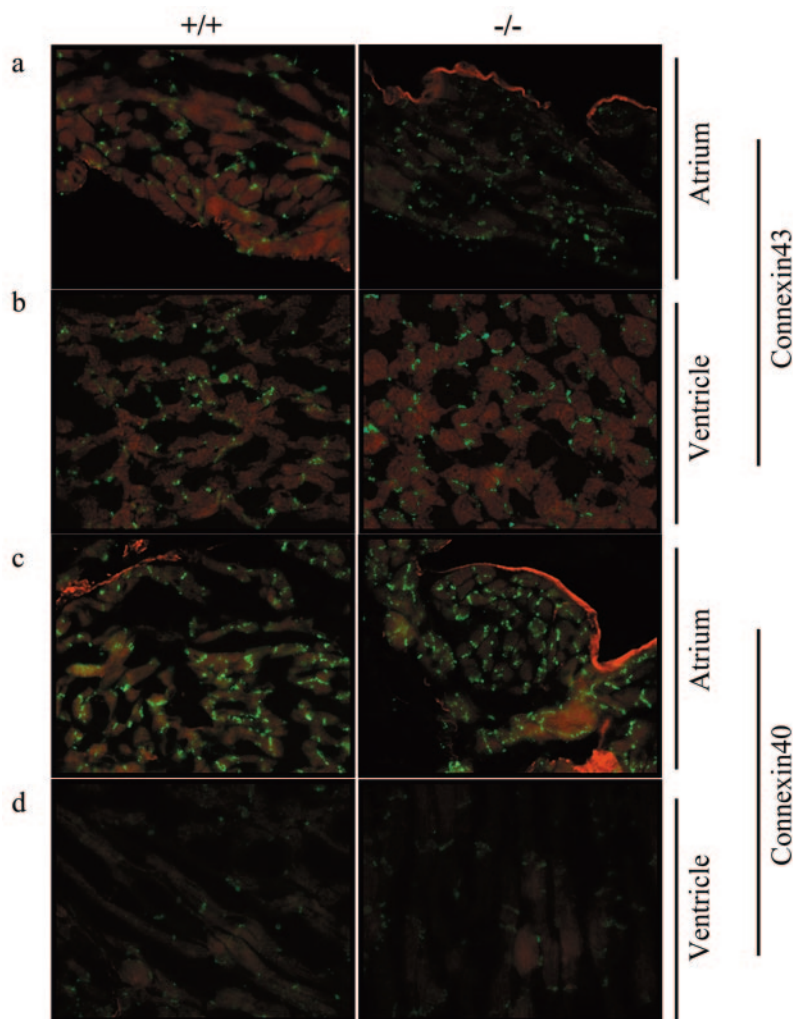


FIG. 7. Connexin distributions in atrium and ventricles. Immunofluorescence microscopy analyses of connexin 40 (a and b) and connexin 43 (c and d) expression in atria (a and c) and ventricles (b and d) of control and *glut8*^{-/-} mice. There was no difference in the distributions of the two connexins in the hearts of control and mutant mice.

GLUT8 is dispensable for spermatozoal function and embryonic development.

GLUT8 can transport glucose with relatively high affinity. However, as it is located in an intracellular location, and since it has not been found to translocate to the plasma membrane in any cell type studied except blastocysts, it is not known whether it can actively participate in the regulation of glucose homeostasis. Our data show that *glut8*^{-/-} mice have normal glycemic control, as assessed by measurement of blood glucose levels in randomly fed, fasted, or refed mice and also as measured by glucose tolerance tests and insulin plasma levels. In addition, the growth rate of the mutant mice is normal. There is thus no indication that this transporter plays a major role in the regulation of glucose or energy homeostasis.

GLUT8 is expressed by hippocampal neurons. In these cells, it is also present in an incompletely defined intracellular compartment, and so far, no stimuli have been identified that could induce its cell surface expression (22). Some studies, however, have shown that upon induction of hyperglycemia by streptoz-

ocin treatment, a redistribution of GLUT8 could be observed within hippocampal neurons, leading to an increased presence of the transporter in the endoplasmic reticulum (13). Although the significance of these observations is unclear, they suggest that GLUT8 may be sensitive to changes in the internal milieu. The hippocampus is known to play an important role in memory acquisition, and some studies have linked this with increased proliferation of neurons in the dentate gyrus (9, 11, 20). Our BrdU-labeling experiments showed that cell proliferation was increased in the hippocampus in mutant mice. However, when testing memory acquisition and retention in the Morris water maze or using a homing-board test, we could not demonstrate an increase or a decrease in memorization in the mutant compared to the control mice. Thus, the increased BrdU labeling observed in the absence of GLUT8 so far has unexplained functional consequences. It is also not clear why the absence of this transporter would lead to increased proliferation of these cells.

Expression of GLUT8 in the heart is detectable from the

time of birth. Absence of this transporter, however, does not lead to a noticeable change in heart size or morphological structure. Functional evaluation by electrocardiogram revealed a small but consistent reduction in P-wave duration. This reflects an impaired transmission of the electrical wave through the atrium, which could be related to changes in several ion channel activities or to changes in connexin expression. However, expression of the Nav1.5 channel or of connexin 40 or 43 was no different in control and mutant mice, and immunohistochemical localizations of the connexins were identical in control and mutant mouse atria. Thus, at present, it is not clear why the absence of GLUT8 causes a change in P-wave duration. Microarray analysis of genes differentially expressed in the hearts of control and mutant mice has not revealed any genes specifically regulated by the absence of GLUT8 (not shown). However, it should be noted that when these experiments were performed, the variability of the control mice made observation of significant changes difficult. Backcrossing the mice into a pure genetic background may significantly reduce the interindividual variability in gene expression and allow identification of minor changes in gene expression.

Taken together, our present data show that absence of GLUT8 causes only partial embryonic lethality and that the knockout mice that are born generate knockout offspring with the normal Mendelian frequency. This suggests that these knockout mice carry compensatory genes. After birth, *glut8*^{-/-} mice develop normally and have normal glucose homeostasis. An increase in proliferation of hippocampal neurons is observed, which is not linked to modified memory acquisition or retention. No change in heart structure or histology can be observed. However, the increase in P-wave duration in the ECGs of mutant mice may indicate a defect in atrium depolarization; this is nevertheless not linked to change in Nav1.5 channel expression or in connexin expression or distribution in the atrium. Elucidating how this phenotype, similar to common human conditions (3), is caused by the absence of GLUT8 will require further work.

ACKNOWLEDGMENTS

This work was supported by grant 3100-065219-01 from the Swiss National Science foundation to B.T.

We are grateful to F. Schenk for her help with behavioral tests and to A. Trumpp for providing the NesCre mice.

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