Glucose Transporters in Rat Peripheral Nerve: Paranodal Expression of GLUT1 and GLUT3

Paolo Magnani, P. Varghese Cherian, Gwyn W. Gould, Douglas A. Greene, Anders A.F. Sima, and Frank C. Brosius III

Peripheral nerve depends on glucose oxidation to energize the repolarization of excitable axonal membranes following impulse conduction, hence requiring high-energy demands by the axon at the node of Ranvier. To enter the axon at this site, glucose must be transported from the endoneurial space across Schwann cell plasma membranes and the axolemma. Such transport is likely to be mediated by facilitative glucose transporters. Although immunohistochemical studies of peripheral nerves have detected high levels of the transporter GLUT1 in endoneurial capillaries and perineurium, localization of glucose transporters to Schwann cells or peripheral axons in vivo has not been documented. In this study, we demonstrate that the GLUT1 transporter is expressed in the plasma membrane and cytoplasm of myelinating Schwann cells around the nodes of Ranvier and in the Schmidt-Lanterman incisures, making them potential sites of transcellular glucose transport. No GLUT1 was detected in axonal membranes. GLUT3 mRNA was expressed only at low levels, but GLUT3 polypeptide was barely detected by immunocytochemistry or immunoblotting in peripheral nerve from young adult rats. However, in 13-month-old rats, GLUT3 polypeptide was present in myelinated fibers, endoneurial capillaries, and perineurium. In myelinated fibers, GLUT3 appeared to be preferentially expressed in the paranodal regions of Schwann cells and nodal axons, but was also present in the internodal aspects of these structures. The results of the present study suggest that both Schwann cell GLUT1 and axonal and Schwann cell GLUT3 are involved in the transport of glucose into the metabolically active regions of peripheral axons.

PERIPHERAL NERVE is a metabolically active, functionally excitable tissue in which specific metabolic substrate requirements, high energy needs, and specialized anatomic and cellular architecture impose complex requirements on metabolic substrate delivery.[1-3] The major source of metabolic energy for peripheral nerve is glucose.[1] However, to enter the axon, glucose must first traverse the highly developed perineurial diffusion barrier consisting of the multilayered perineurial epithelium and the blood-nerve barrier composed of nonfenestrated endoneurial microvascular endothelial cells interlocked by tight junctions.[3-5] In most mammalian cells, facilitative glucose transporters mediate the transport of glucose across plasma membranes, and several studies have demonstrated the expression of GLUT1 glucose transporters in cells of the perineurial and blood-nerve barriers,[6-10] supporting the role of these transporters in the delivery of glucose to the endoneurial compartment. To reach the energy-requiring axon of the nodal apparatus, glucose must also circumvene the paranodal ion channel and diffusion barrier[11-15] by traversing the paranodal Schwann cell that envelops the axon, as well as crossing the axolemma itself.

Axonal energy demands are highest in the region of the nodes of Ranvier. It is at these sites that Na^+/K^+-ATPase and sodium-channel molecules are concentrated.[13,15,16] In the paranodal region, the myelinated Schwann cell is in close apposition to the axolemma mediated by axoglial junctional complexes that constitute the paranodal diffusion barrier.[13,14,17-19] This barrier also effectively separates the axolemmal nodal Na^+ channels from the paranodal K^+ channels.[13,14,17] These anatomical relationships suggest that Schwann cell and axonal glucose transporters might be localized to the paranodal region. Previous studies have failed to demonstrate glucose transporters in native Schwann cells and axolemma, although GLUT1 has been demonstrated in Schwann cells in vitro,[9] and the presence of GLUT3 mRNA has been described in peripheral nerve.[10] In this communication, we demonstrate the presence of GLUT1 in the paranodal region and Schmidt-Lanterman incisures of Schwann cells in rat peripheral nerve and the presence of GLUT3 in the perineurium, endoneurial vessel walls, and paranodal Schwann cells and axolemma in aged rats, but not in young rats. These results suggest that both GLUT1 and GLUT3 are involved in the transport of glucose into the metabolically active nodal apparatus of the peripheral myelinated axon.

MATERIALS AND METHODS

Tissues

Sciatic nerves from three Sprague-Dawley rats weighing 200 to 250 g were used for Northern analysis and immunoblotting. Sural nerves from groups of four nondiabetic BB/W rats were used for immunocytochemistry, and sciatic nerves from the same animals aged 4, 6, 11, and 13 months were used for Northern analysis and immunoblotting.

From the Departments of Internal Medicine and Pathology and the Michigan Diabetes Research and Training Center, University of Michigan Medical Center, Ann Arbor; the Veterans Affairs Medical Center, Ann Arbor, MI; and the Department of Biochemistry, University of Glasgow, Glasgow, Scotland, UK.

Submitted September 27, 1995; accepted July 3, 1996.

Supported by National Institutes of Health Grants No. RO1-DK38304 (D.A.G.), and RO1-DK43884 (A.A.F.S.), by the Michigan Diabetes Research and Training Center (P60-DK20572, D.A.G. and A.A.F.S.) and Image Analysis and Molecular Biology Core Laboratories, and the University of Michigan Multipurpose Arthritis Center Molecular Biology Core Laboratory.

Present address: P.M., Universita Cattolica del Sacro Cuore, 1st Medicina Interna-Servizio di Diabetologia, 00168 Roma, Italy.

Address reprint requests to Anders A.F. Sima, MD, PhD, Department of Pathology, Wayne State University, 540 E Canfield Ave, Detroit, MI 48201.

0026-0495/96/4512-0004$03.00/0

GLUT cDNAs and Antisera

A full-length rat GLUT1 cDNA was cloned from a rat B2 insulinoma cell line cDNA library (gift from Dr B. Thorens), which by DNA sequencing and restriction mapping was identical to the cDNA reported by Birnbaum et al.[20] A partial rat GLUT3 cDNA was cloned from rat kidney using a reverse transcription–polymerase chain reaction (PCR) method as previously described[21] with two primers corresponding to nucleotides 1144 to 1163 and nucleotides 1582 to 1601 of the human GLUT3 cDNA.[22] The sequence of this PCR product was identical to the corresponding region of the rat GLUT3 cDNA reported by Nagamatsu et al.[23] Two antisera against a peptide corresponding to the 16 carboxy-terminal amino acids of GLUT1 were used (gifts from Dr B. Thorens [BT-1] and Dr Maureen Charron [R1B]). Human and rat GLUT1 sequences are identical in this region. An antiserum against a peptide corresponding to the 13 carboxy-terminal amino acids of murine GLUT3 was used for identification of GLUT3. This antiserum has been shown to react specifically with the rat GLUT3 antigen.[24] A second antimurine GLUT3 affinity-purified antiserum directed against the carboxy terminus of GLUT 3 (Charles River Pharmservices, Southbridge, MA) was used for 4-month-old BB/W rat samples only.

RNA Isolation and Northern Analysis

Total RNA was isolated from freshly excised rat sciatic nerves from 200 to 250-g Sprague-Dawley rats and from 4-, 6-, 11-, and 13-month-old nondiabetic BB/W rats. It was subjected to Northern analysis on 1% agarose/6% formaldehyde gels. After transfer of the RNA to nylon membranes, they were prehybridized for 30 minutes in 50% formamide, 5× SSC (1× SSC is 0.15 mol/L NaCl plus 0.015 mol/L sodium citrate), 5× Denhardt solution, 0.1% sodium dodecyl sulfate (SDS), and 100 μg/mL salmon sperm DNA, and were then hybridized overnight in the same buffer containing a full-length ^{32}P-labeled single-stranded antisense cRNA probe generated from the rat GLUT1 cDNA or a denatured uniformly ^{32}P-labeled double-stranded 457-bp rat GLUT3 cDNA probe.

Prehybridization, hybridization, and wash temperatures were 65°C for cRNA probes and 50°C for cDNA probes, to ensure detection of only the RNA for the specific isoform used as a probe.[21,25] Filters were washed in 0.1× SSC/0.1% SDS (15 minutes four times) and exposed to Xomat-AR film (Eastman Kodak, Rochester, NY) at −80°C with an intensifying screen.

RNAse Protection Assay

RNAse protection analysis was performed using a 229-bp antisense GLUT3 cRNA probe that encoded 161 bps of the rat GLUT3 cDNA (corresponding to nucleotides 1278 to 1438 of the rat GLUT3 cDNA sequence[23]) and 68 bps of the vector sequence. The probe was generated from 250 ng linearized plasmid and SP6 RNA polymerase. The GLUT3 antisense probe (10^5 cpm) was hybridized overnight at 50°C with 10 μg total sciatic nerve RNA, total brain RNA, or calf liver tRNA in 30 μL hybridization buffer (40 mmol/L 1,4-piperazinediethanesulfonic acid, pH 6.4, 0.4 mmol/L NaCl, 1 mmol/L EDTA, and 80% formamide).[26] The samples were digested in 350 μL 10-mmol/L Tris (pH 7.4), 300-mmol/L NaCl, and 5 mmol/L EDTA with 60 μg/mL RNAse A and 3 μg/mL RNAse T1 for 30 minutes at 30°C. The reactions were stopped by addition of 20% SDS (final dilution, 0.5%) and proteinase K (0.4 μg/mL) and incubated at 37°C for 15 minutes,[26] after which 10 μg calf liver tRNA was added and the samples were extracted with phenol-chloroform and precipitated with ethanol. The protection products were denatured for 5 minutes at 85°C, and separated by electrophoresis on denaturing 6% polyacrylamide/5-mmol/L urea sequencing gels. Gels were subsequently dried and exposed to Xomat-AR film at −80°C with an intensifying screen.

Immunoblotting

Frozen sciatic nerve samples from 200 to 250-g Sprague-Dawley rats and 4-, 11-, and 13-month-old BB/W rats were suspended in lysis buffer (1% Triton X, 0.1% SDS, 5 mmol/L *N*-ethylmaleimide, 2 mmol/L EDTA, and 2 mmol/L phenylmethylsulfonyl fluoride) and sonicated for 90 seconds. Lysates were tested for protein content by bicinchoninic acid assay (Pierce Chemicals, Rockford, IL) and were suspended in Laemmli sample buffer, separated on 10% SDS-PAGE gels, and blotted onto nitrocellulose filters. Filters were blocked with 5% nonfat dry milk in TBS (20 mmol/L Tris hydrochloride, pH 7.5, 150 mmol/L NaCl, and 1% Nonidet-P-40), probed with either of the antirat GLUT1 antisera (dilution, 1:800) or anti-GLUT3 antiserum (dilution, 1:200), washed in TBS, blocked again, incubated with ^{125}I-donkey antirabbit IgG (Amersham, Arlington Heights, IL), washed in TBS, and exposed to film. Peptide competition experiments were performed in an identical fashion except as follows. GLUT1 antisera were incubated with 5 μg/mL of the respective GLUT1 immunizing peptide or an equivalent concentration of nonspecific peptide for 15 minutes at room temperature and centrifuged at 13,000 × *g* for 15 minutes before incubation with the nitrocellulose filter. The GLUT3 antiserum was incubated with 40 μg/mL of the GLUT3 immunizing peptide or with an equivalent concentration of nonspecific peptide and processed similarly.

Immunocytochemistry

Light microscopy. Sural nerves from nondiabetic 6-, 11-, and 13-month-old BB/W rats were excised and immediately fixed in a mixture of 2% paraformaldehyde and 1% glutaraldehyde for 4 hours and processed for paraffin embedding. Six-micrometer sections were mounted on poly-L-lysine–coated slides. Sections were deparaffinized and hydrated through a descending series of ethanol to 30%, and then rinsed in distilled water. Tissue sections were then washed in phosphate-buffered saline ([PBS] pH 7.4) for 5 minutes at room temperature. Excess fluid was wiped off, and sections were incubated in normal goat serum for 30 minutes at room temperature to block nonspecific binding. Samples were then sequentially incubated in (1) one of the GLUT1 antisera (R1B, 1:100; BT-1, 1:200) or GLUT3 antiserum (1:100), (2) biotinylated goat antirabbit IgG (1:100), and (3) streptavidin-gold (1:40). All antibody incubations were performed for 1 hour at room temperature and were followed by three washes in PBS containing 1% bovine serum albumin. After the final wash, immunoreactivity was augmented by silver enhancement with the immunogold-silver staining method of De Waele et al.[27] For negative controls, specific peptide competition studies were performed by preincubating the antiserum with an excess of the peptide (5 μg/mL GLUT1 peptide and 40 μg/mL GLUT3 peptide), to which the antiserum was generated, followed by centrifugation. Tissue samples were incubated with the supernatant and processed in parallel with samples incubated with the noncompetition antisera. Further negative controls included sections incubated with preimmune serum or without secondary antibody.

Electron microscopy. Ultrastructural immunocytochemical localization of GLUT1 and GLUT3 in peripheral nerve was performed according to a previously reported immunogold method.[28] Briefly, samples were fixed as above, postfixed in 0.2% osmic acid in 0.1 mol/L cacodylate buffer (pH 7.4) for 30 minutes, and then processed for electron microscopy. Ultrathin sections (600 Å) were mounted on 200-mesh nickel grids, preincubated in 5% normal goat serum for 30 minutes, and then incubated with GLUT1

antisera (1:100 for R1B; 1:200 for BT-1) or GLUT3 antiserum (1:100) overnight at 4°C, and then for 1 hour at room temperature. After three washes in PBS solution containing 0.1% Tween 20, sections were incubated in a 1:40 dilution of goat antirabbit IgG conjugated to gold for 1 hour at room temperature. Sections were again washed three times for 5 minutes in PBS with Tween 20, rinsed twice in distilled water, stained with uranyl acetate and lead citrate, and examined electron-microscopically. Negative controls included grids incubated with equal amounts of preimmune serum in place of the primary GLUT1 or GLUT3 antiserum or antibodies preabsorbed with the GLUT1 or GLUT3 immunizing peptides (as for the light-microscopic experiments), or grids from which the secondary antibody was omitted from the incubation sequence.

RESULTS

Characterization of GLUT mRNAs in Rat Sciatic Nerve

Northern blot and RNAse protection analyses were used to identify glucose transporter transcripts in peripheral nerve. As previously shown by Muona et al,[9] a 2.8-kb GLUT1 mRNA was detected in Northern blots of RNA samples from rat sciatic nerve (data not shown). No GLUT3 mRNA was detected by Northern blotting of sciatic nerve total RNA from 200- to 250-g Sprague-Dawley rats (data not shown). However, RNAse protection analysis of this RNA demonstrated the presence of low levels of GLUT3 mRNA (Fig 1).

Characterization of GLUT Polypeptides in Sciatic Nerve

Immunoblots of rat sciatic nerve lysates indicated the presence of substantial amounts of GLUT1 polypeptide. A broad band at 46 to 53 kd was detected by both GLUT1 antisera, and the signal was eliminated when either antiserum was preincubated with the GLUT1 immunizing peptide (not shown). Similar experiments were performed with the GLUT3 antiserum. In 200- to 250-g Sprague-Dawley rats, little or no specific GLUT3 polypeptide was detected in lysates of rat sciatic nerve (data not shown). However, small amounts of GLUT3 polypeptide were detected by immunoblotting in 4-month-old nondiabetic BB/W rat sciatic nerve with the higher-affinity antiserum. Significantly larger amounts were detected in lysates derived from 11- and 13-month-old BB/W rats under identical conditions (Fig 2).

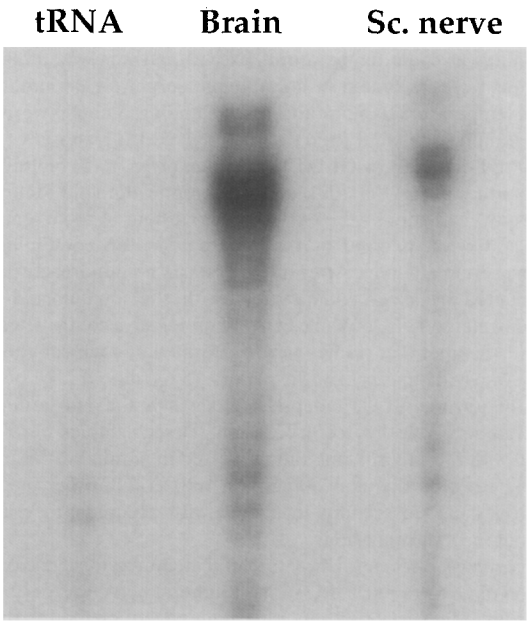

Fig 1. GLUT3 RNAse protection analysis of rat sciatic nerve and brain RNA. RNA (10 μg) from sciatic nerve (Sc. nerve) and brain, but not tRNA, protected the predicted 161-bp rat GLUT3 cRNA probe, demonstrating detectable GLUT3 mRNA expression in 200- to 250-g Sprague-Dawley sciatic nerve.

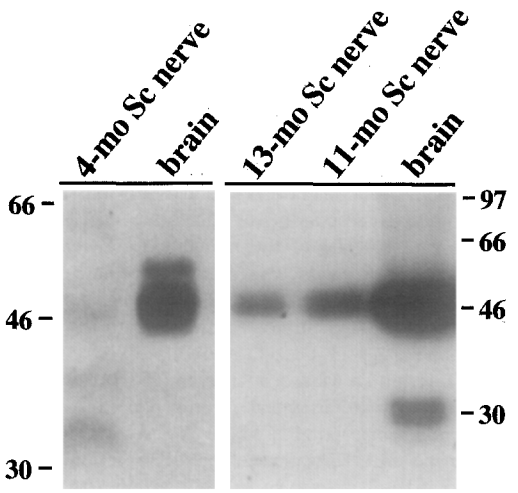

Fig 2. GLUT3 immunoblot of 4-, 11-, and 13-month-old BB/W rat sciatic nerve and rat brain lysates. Lysates from sciatic nerve (50 μg) and brain (20 μg) were electrophoresed, blotted, and incubated with a 1:200 dilution of the higher-affinity mouse GLUT3 antiserum. A faint band at ~46 kd was detected in 4-month sciatic nerve samples. This band was not detected with the other GLUT3 antiserum used in experiments in the right panel, but was specifically eliminated after preincubation of the higher-affinity antiserum with GLUT3 immunizing peptide (not shown). The lower bands in the 4-month samples were not eliminated after peptide preincubation and are therefore nonspecific. In the right panel, lysates from rat sciatic nerve (50 μg) and brain (50 μg) were electrophoresed, blotted, and incubated with the lower-affinity GLUT3 antiserum. In contrast to the results from 4-month-old BB/W rats, those from 11- and 13-month-old nondiabetic BB/W rats demonstrated readily detectable GLUT3 polypeptide corresponding to the band at 46 kd.

Immunocytochemical Localization of GLUT1 and GLUT3 Polypeptides in Sciatic Nerve

GLUT1 immunoreactivity was detected in several regions of rat sciatic nerve. Identical patterns of immunostaining were detected with each of the two GLUT1 antisera. GLUT1 immunostaining was present in the perineurial epithelium with a more intense staining of the inner perineurial laminae (Fig 3a). The vessel walls of endoneurial microvessels showed intense immunostaining for GLUT1 (Fig 3b). Myelinated nerve fibers showed positive immunoreaction products corresponding to nodes of Ranvier and Schmidt-Lanterman incisures (Fig 3c and d). In these regions, GLUT1 appeared to localize to the Schwann cell membranes and cytoplasm, with no discernible reaction products within axons (Fig 3c and d). The specificity of the reaction product was confirmed by the

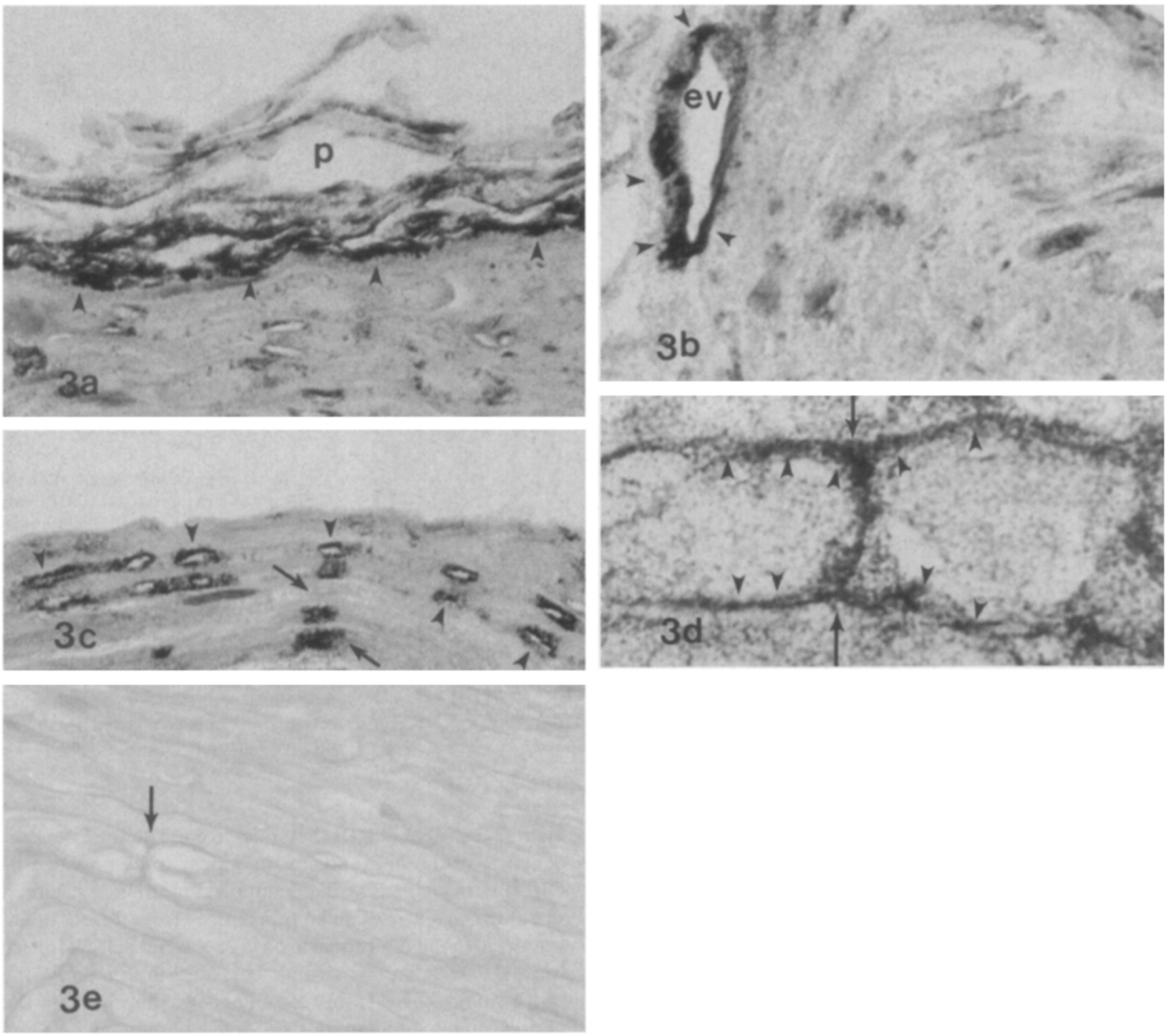

Fig 3. Micrographs of rat longitudinal sural nerve from 11- and 13-month-old BB/W rats showing immunolocalization of GLUT1. (a) Longitudinal section of sural nerve showing perineurium (p) with intense GLUT1 immunostaining (arrowheads) of the inner perineurial lamellae (original magnification ×300; 11-month-old BB/W rat). (b) Endoneurial vessel (ev) wall of rat sural nerve showing intense immunoreaction products (arrowheads) (original magnification ×560; 13-month-old BB/W rat). (c) Longitudinal section of a sural nerve showing positive GLUT1 immunoreactivity localized to the node of Ranvier (arrows). GLUT1 is also present at the Schmidt-Lanterman incisures (arrowheads) (original magnification ×320; 13-month-old BB/W rat). (d) Myelinated sural nerve fiber immunostained with GLUT1 antiserum. Node of Ranvier shows prominent GLUT1 immunostaining. Immunoreaction product is noted at the nodal gap (arrows) and the paranodal Schwann cell membranes (arrowheads) (original magnification ×1,250; 13-month-old BB/W rat). (e) Rat sural nerve incubated with GLUT1 antiserum (BT-1) subjected to peptide competition. This preincubation eliminated all GLUT1 immunostaining at the node of Ranvier (arrowhead) and the internodal region, confirming specificity of the immunoreaction demonstrated in Fig 3a to d (original magnification × 525; 13-month-old BB/W rat).

absence of immunoreactivity in sections incubated with antiserum preincubated with the immunizing peptides (Fig 3e). Ultrastructurally, GLUT1 was localized to the Schwann cell membrane and cytoplasm, often in close proximity to pinocytotic vesicles (Fig 4a). GLUT1 reaction products were also localized to the Schwann cell cytoplasm piercing through the internodal myelin sheath at the Schmidt-Lanterman incisures (Fig 4b). No ultrastructurally identifiable reaction products could be localized to axolemma or axoplasm.

Only faint immunostaining of GLUT3 was present in 6-month-old nondiabetic BB/W rats and appeared to be localized to the nodal and paranodal regions of myelinated fibers (Fig 5a). However, in 13-month-old nondiabetic BB/W rats, GLUT3 immunoreactivity was clearly discernible in the perineurium (Fig 5b) and in the endothelial cells of endoneurial microvessels (Fig 5c). In myelinated fibers of 13-month-old rats, GLUT3 was localized to the Schwann cell with increased reactivity of the paranodal Schwann cell (Fig 5d). In addition, GLUT3 appeared to decorate the internodal axolemma of myelinated fibers with increased immunostaining of the nodal and paranodal axolemma (Fig

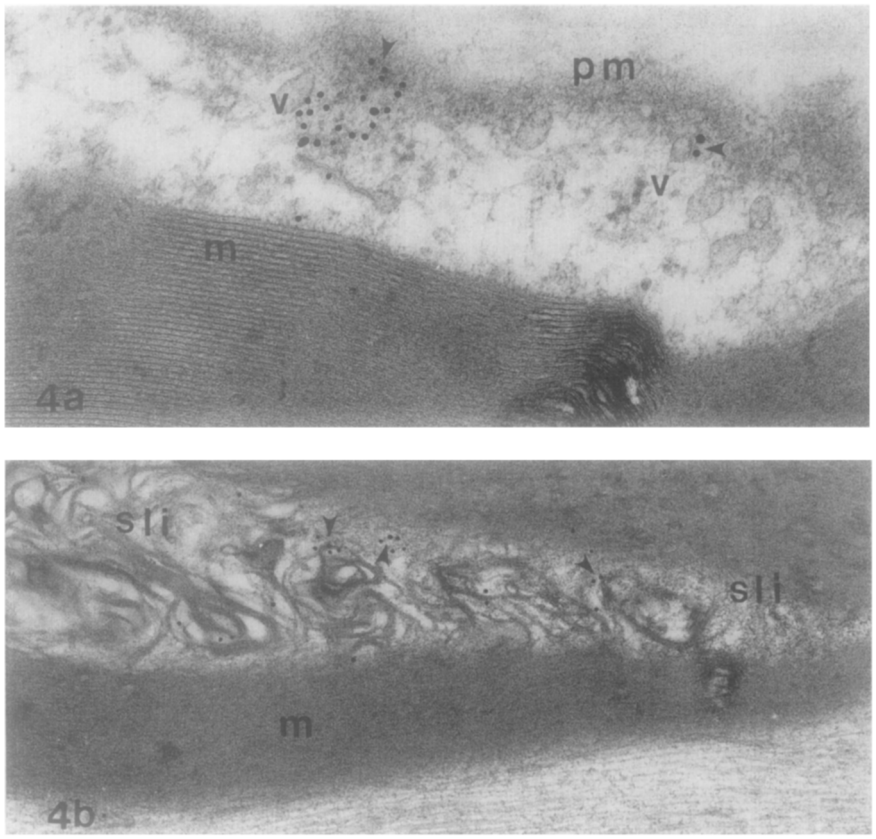

Fig 4. Ultrastructural localization of GLUT1 in rat sural nerve. (a) GLUT1 was localized to the Schwann cell plasma membrane (pm, arrowhead) and cytoplasm, but not to compacted myelin (m). Cytoplasmic GLUT1 was often associated with pinocytotic vesicles (v). (b) Electron micrograph of rat sural nerve showing a Schmidt-Lanterman incisure (sli) of the myelin sheath. GLUT1 was present within the cytoplasm of the Schmidt-Lanterman incisure, but not in compacted myelin (m) (original magnification ×50,000; 11-month-old BB/W rat).

5e and f). Antisera preincubated with the immunizing GLUT3 peptide revealed no immunoreaction (Fig 5g), nor did experiments in which tissue was incubated with preimmune serum or in which the secondary antibody was omitted (data not shown). Ultrastructurally, GLUT3 immunoreactivity was localized to the Schwann cell cytoplasm, axolemma, and axoplasm of myelinated fibers with increased reactivity of the paranodal axons (Fig 6a). No immunoreaction products were observed in sections incubated with immunoprecipitated serum (Fig 6b).

DISCUSSION

Highly dependent on glucose as a metabolic substrate,[29] peripheral nerve possesses facilitative glucose transport mechanisms that efficiently transfer glucose from the blood into peripheral nerve.[6] Previous studies have documented high-level expression of GLUT1 polypeptide in cells constituting the perineurial barrier and in endothelial cells of the endoneurial vessels responsible for the blood-nerve barrier.[9-10] In the present studies, these findings were extended by demonstrating GLUT1 polypeptide in Schwann cells in vivo and in the perineurium and endoneural vessels, and by detecting GLUT3 polypeptide in the same structures and in axons of aged, but not of young, adult animals.

The topographic distribution of GLUT1 as demonstrated in the present study gives credence to the concept that facilitated glucose transport is necessary not only to traverse the perineurial and blood-nerve barriers, but also to circumvent the paranodal diffusion barrier to meet the high-energy demands of the nodal axon. The lesser energy demands of the internodal axon appear to be provided by GLUT1 transporter at the Schmidt-Lanterman incisure, a site at which glucose can be transported from the endoneurial space to the internodal axon via Schwann cell cytoplasm without having to cross compacted myelin. Similarly, the expression of GLUT3 in old animals was localized to the perineurium, endoneurial vessel walls, paranodal Schwann cells, and axonal membranes, with a higher concentration in the nodal axolemma. These findings suggest that at least in old animals, GLUT3, in contrast to GLUT1, is also a neuronal transporter in peripheral nerve, similar to its function in the central nervous system.[30,31]

Only a few previous studies have examined GLUT3 expression in peripheral nerve. Muona et al[10] described the presence of GLUT3 mRNA in developing human nerve but did not determine polypeptide expression. Maher et al[31] and Haber et al,[32] in studies of rat and human peripheral nerve, respectively, found no evidence of GLUT3 polypeptide. However, neither study examined developing or older individuals. GLUT3 is the major neuronal glucose transporter in brain.[22,31,33,34] GLUT3 appears to be highly expressed in the neuropil and probably in axons and nerve cell bodies.[31,35] Thus, detection of GLUT3 in peripheral nerve axons is a logical extension of these findings. However, the absence of GLUT3 polypeptide in young rat nerves and expression in old rat nerves suggests age-mediated differences.

Muona et al[10] demonstrated the immunocytochemical

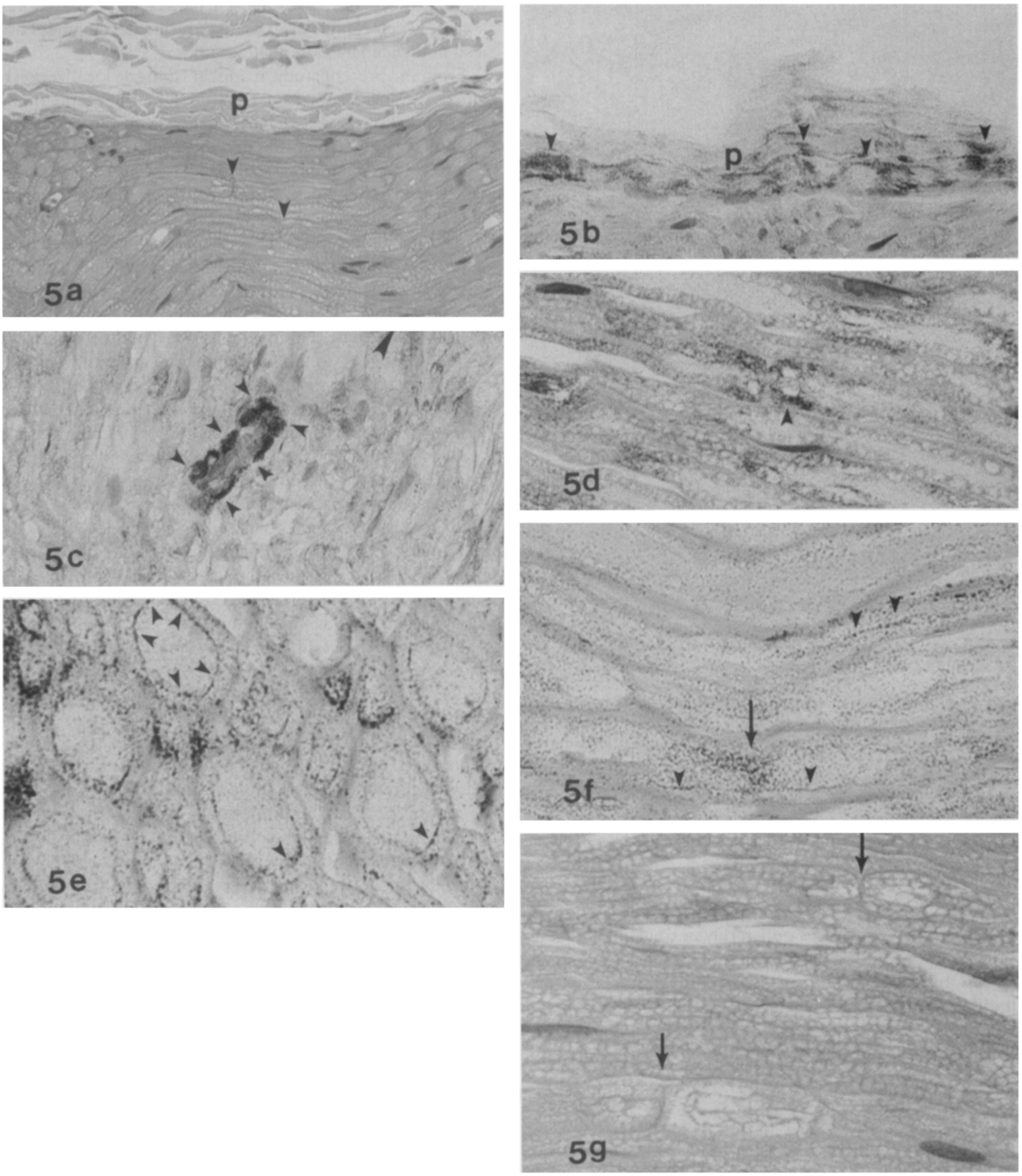

Fig 5. Light-microscopic immunostaining of GLUT3 in rat sural nerve. (a) Longitudinal section of sural nerve from a 6-month-old BB/W rat showing the perineurium (p) and nerve fibers. Only faint reactivity was present in the perineurium, nodes of Ranvier (arrowheads), and paranodal regions of myelinated nerve fibers (original magnification ×240). (b) Intense immunoreactivity of GLUT3 (arrowheads) observed in the perineurium (p) of a 13-month-old BB/W rat (original magnification ×300). (c) Endoneurial vessel wall showing immunoreactivity (arrowheads) of GLUT3 in a 13-month-old BB/W rat (original magnification ×460). (d) Longitudinal section of sural nerve showing GLUT3 reactivity of Schwann cells, exhibiting increased reactivity of paranodal Schwann cells (arrowhead). (original magnification ×450; 13-month-old BB/W rat). (e, f) GLUT3 appears to decorate the internodal axolemma of myelinated fibers (arrowheads) both in cross-section (original magnification ×720) and longitudinally (original magnification ×660). (Original magnification ×300; 13-month-old BB/W rat). (g) Lack of immunostaining in sural nerve sections incubated with immunoprecipitated GLUT3 antiserum. No immunostaining of GLUT3 was observed in nerve fibers and the node of Ranvier (arrow) (original magnification ×600; 13-month-old BB/W rat).

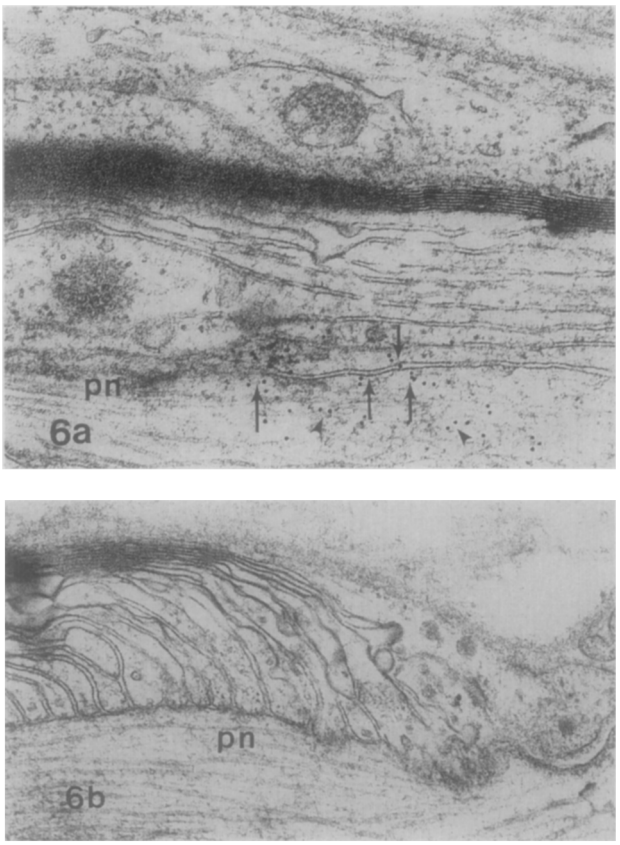

Fig 6. Ultrastructural localization of GLUT3 in rat sural nerve. (a) Immunoelectron-microscopic localization of GLUT3 in rat sural nerve. GLUT3 was localized to the paranodal (pn) axolemma (arrow) axoplasm (arrowhead). (Original magnifications ×50,000; 13-month-old BB/W rat). (b) Longitudinal section of a rat sural nerve immunostained after incubation with antiserum preabsorbed with the GLUT3 immunizing peptide. Note the lack of immunostaining of the paranodal (pn) axolemma and axoplasm (original magnification ×50,000; 13-month-old BB/W rat).

localization of GLUT1 to endoneurial capillaries in human fetal nerve, whereas it was only rarely positive in endoneurial capillaries in adult human nerves. These findings suggest that the expression of glucose transporters may vary at different developmental stages and ages in both humans and rodents. Recent reports have suggested that the expression of glucose transporters may be influenced by growth-regulatory cytokines and adhesion molecules,[36,37] the expression of which is highly programed during development and also influenced by pathologic conditions.[38,39]

From the present study, it appears that the localization of glucose transporters is associated with the topographic distribution of protective barrier systems that are mediated by adhesive molecule–dependent occluding junctions.[40] These undergo maturational processes and become less effective in senescence. In the rat, the perineurial barrier matures at about 4 weeks of age, when the blood-nerve barrier is already established.[41] The paranodal barrier matures with myelination, which reaches its peak at about 40 days of age in the rat[42] and shows a progressive deterioration in aging rats and humans.[13,43,44] One may therefore speculate that the expression of glucose transporters may be linked to the developmental or functional state of the protective barriers. Since GLUT1 does not seem to be localized to peripheral axolemma, it is possible that a yet unidentified glucose transporter is responsible for the bulk of glucose uptake by the axolemma, at least in young adult rats, as suggested by Muona et al.[10] Alternatively, GLUT3 epitopes may be "masked" in peripheral axons of young adult rats, as they may be in central neurons[31] and as are cell adhesive molecules localized to the node of Ranvier[40] (and Sima A.A.F., unpublished data, July 1995). However, this possibility is unlikely, since GLUT3 was not detected by immunoblotting in young adult rats.

The presence of GLUT1 in Schwann cells and GLUT3 in axons suggests that these transporters participate in the delivery of glucose to the axon. We propose that GLUT1 transporters are responsible for the transport of glucose across Schwann cells from the endoneurial space to the axon, and that GLUT3 molecules (and possibly other GLUTs) directly mediate uptake of glucose into the axon, at least in older rats. If this notion is correct, axonal glucose uptake and metabolic flux would be dependent in part on the expression of Schwann cell GLUT1 and axonal GLUT3 transporters, as well as their expression in perineurial and endoneurial endothelial cells.

ACKNOWLEDGMENT

We wish to thank Lisa Beyer and Yannan Liu for excellent technical assistance, Dr Bernard Thorens for the gift of GLUT1 antiserum (BT-1) and the B2 cDNA library, and Dr Maureen Charron for the gift of GLUT1 antiserum (R1B).

REFERENCES

1. Greene DA, Winegrad AI: In vitro studies of substrates and energy production and the effect of insulin on glucose utilization in the neural components of peripheral nerve. Diabetes 28:878-887, 1979

2. Ritchie JM: The oxygen consumption of mammalian non-myelinated nerve fibers at rest and during activity. J Physiol (Lond) 188:309-329, 1967

3. Thomas PK, Olsson Y: Microscopic anatomy and function of the connective tissue components of peripheral nerve, in Dyck PJ, Thomas PK, Lambert EH (eds): Peripheral Neuropathy, Vol 1. Philadelphia, PA, Saunders, 1975, pp 168-189

4. Sima AAF, Sourander P: The permeability of perineurium to peroxidase after early undernutrition. Acta Neuropathol 28:15-24, 1974

5. Bradbury MWB, Crowder J: Compartments and barriers in the sciatic nerve of the rabbit. Brain Res 103:512-526, 1976

6. Rechthand E, Smith QR, Rapoport SI: Facilitated transport of glucose from blood into peripheral nerve. J Neurochem 45:957-964, 1985

7. Froehner SC, Davies A, Baldwin SA, et al: The blood-nerve barrier is rich in glucose transporter. J Neurocytol 17:173-178, 1988

8. Gerhart DZ, Drewes LR: Glucose transporters at the blood-nerve barrier are associated with perineurial cells and endoneurial microvessels. Brain Res 508:46-50, 1990

9. Muona P, Sollberg S, Peltonen J, et al: Glucose transporters of rat peripheral nerve: Differential expression of GLUT-1 gene by Schwann cells and perineurial cells in vivo and in vitro. Diabetes 41:1587-1596, 1992

10. Muona P, Jaakkola S, Salonen V, et al: Expression of glucose transporter 1 in adult and developing human peripheral nerve. Diabetologia 36:133-140, 1993

11. Rosenbluth J: Intramembranous particle distribution at the node of Ranvier and adjacent axolemma in myelinated axons of the frog brain. J Neurocytol 5:731-745, 1976

12. Wiley CA, Ellisman MH: Rows of dimetric particles within the axolemma and juxtaposed particles within glia, incorporated into a new model for the paranodal glial-axonal junction at the node of Ranvier. J Cell Biol 84:261-280, 1980

13. Sima AAF, Lattimer SA, Yagihashi S, et al: "Axo-glial dysjunction": A novel structural lesion that accounts for poorly reversible slowing of nerve conduction in the spontaneously diabetic BB-rat. J Clin Invest 77:474-484, 1986

14. Cherian PV, Kamijo M, Angelides KJ, et al: Nodal Na^+-channel displacement is associated with nerve conduction slowing in the chronically diabetic BB/W-rat. Prevention by an aldose reductase inhibitor. J Diabetes Complications 10:192-200, 1996

15. Brismar T, Sima AAF: Changes in nodal function in nerve fibres of the spontaneously diabetic BB-Wistar rat. Potential clamp analysis. Acta Physiol Scand 113:499-506, 1981

16. Powell HC, Garrett RS, Kador PE, et al: Fine structural localization of aldose reductase and ouabain-sensitive K^+-dependent *nitro*-phenylphosphatase in rat peripheral nerve. Acta Neuropathol 81:529-539, 1991

17. Brismar T, Sima AAF, Greene DA: Reversible and irreversible nodal dysfunction in diabetic neuropathy. Ann Neurol 21:504-507, 1987

18. Waxman SG, Richie JM: Molecular dissection of the myelinated axon. Ann Neurol 33:121-136, 1993

19. Black JA, Waxman SG, Hildebrand C: Axo-glial relations in the retina–optic nerve junction of the adult rat: Freeze-fracture observations on axon membrane structure. J Neurocytol 14:887-907, 1985

20. Birnbaum MJ, Haspel HC, Rosen OM: Cloning and characterization of cDNA encoding a rat brain glucose transporter protein. Proc Natl Acad Sci USA 83:5784-5788, 1986

21. Brosius FC III, Briggs J, Marcus R, et al: Expression of the insulin-responsive glucose transporter (GLUT4) in renal microvessels and glomeruli. Kidney Int 42:1086-1092, 1992

22. Kayano T, Fukumoto H, Eddy RL, et al: Evidence for a family of human glucose transporter–like proteins. J Biol Chem 263:15245-15248, 1988

23. Nagamatsu S, Sawa H, Kamada K, et al: Neuron-specific glucose transporter (NSGT): CNS distribution of GLUT3 rat glucose transporter (RGT3) in rat central neurons. FEBS Lett 334:289-295, 1993

24. Gould GW, Brant AM, Kahn BB, et al: Expression of the brain-type glucose transporter is restricted to brain and neuronal cells in mice. Diabetologia 35:304-309, 1992

25. Charron MJ, Brosius FC, Alper SL, et al: A glucose transport protein expressed predominantly in insulin-responsive tissues. Proc Natl Acad Sci USA 86:2535-2539, 1989

26. Gilman M: Ribonuclease protection assay, in Ausubel FM, Brent R, Kingston RE, et al (eds): Current Protocols in Molecular Biology, vol 1. New York, NY, Wiley, 1992, pp 4.7.1-4.7.8

27. De Waele M, De Mey J, Reynaert P, et al: Detection of cell-surface antigens in cryostat sections with immunogold-silver staining. Am J Clin Pathol 85:573-578, 1986

28. Bendayan M, Roth J, Perrelet A, et al: Quantitative immunocytochemical localization of pancreatic secretory proteins in subcellular compartments of the rat acinar cell. J Histochem Cytochem 28:149-160, 1980

29. Greene DA, Winegard AI, Carpentier JL, et al: Rabbit sciatic nerve fascicle "endoneurial" preparations for in vitro studies of peripheral nerve glucose metabolism. J Neurochem 33:1007-1018, 1979

30. Nagamatsu S, Kornhauser JM, Burant CF, et al: Glucose transporter expression in brain. cDNA sequence of mouse GLUT3, the brain facilitative glucose transporter isoform, and identification of sites of expression by in situ hybridization. J Biol Chem 267:467-472, 1992

31. Maher F, Vannucci SJ, Simpson IA: Glucose transporter proteins in brain. FASEB J 8:1008-1011, 1994

32. Haber RS, Weinstein SP, O'Boyle E, et al: Tissue distribution of the human GLUT3 glucose transporter. Endocrinology 132:2538-2543, 1993

33. Gould GW, Hohman GD: The glucose transporter family: Structure, function and tissue specific expression. Biochem J 295:329-341, 1993

34. Vannucci SJ, Willing LB, Vannucci RC: Developmental expression of glucose transporters, GLUT1 and GLUT3, in postnatal rat brain, in Drewes LR, Betz AL (eds): Frontiers in Cerebral Vascular Biology: Transport and Its Regulation. New York, NY, Plenum, 1993, pp 3-7

35. McCall AL, Van Bueren AM, Moholt-Siebert M, et al: Immunohistochemical localization of the neuron-specific glucose transporter (GLUT3) to neuropil in adult rat brain. Brain Res 659:292-297, 1994

36. Pekala P, Marlow M, Heuvelman D, et al: Regulation of hexose transport in aortic endothelial cells by vascular permeability factor and tumor necrosis factor-alpha, but not by insulin. J Biol Chem 265:18051-18054, 1990

37. Gould GW, Bell GI: Facilitative glucose transporters: An expanding family. Trends Biochem Sci 15:18-23, 1990

38. Edelman G: Cell adhesion and the molecular processes of morphogenesis. Annu Rev Biochem 54:135-169, 1985

39. Rieger F, Pincon-Raymond M: The node of Ranvier in neurological mutants, in Federoff S (ed): Advances in Cellular Neurobiology, vol 6. New York, NY, Academic, 1984, pp 273-310

40. Rieger F, Daviloff JK, Pincon-Raymond M, et al: Neural cell adhesion molecules and cytotactin are colocalized at the node of Ranvier. J Cell Biol 103:379-391, 1986

41. Sima AAF, Sourander P: The effect of perinatal undernutrition on perineurial diffusion barrier to exogenous protein. Acta Neuropathol 24:263-272, 1973

42. Sima AAF: Studies on fiber size in developing sciatic nerve and spinal roots in normal, undernourished and rehabilitated rats. Acta Physiol Scand Suppl 406:1-55, 1974

43. Sima AAF: Can the BB-rat help to unravel diabetic neuropathy? Annotation. Neuropathol Appl Neurobiol 11:253-264, 1985

44. Sima AAF, Nathaniel V, Bril V, et al: Histopathological heterogeneity of neuropathy in insulin-dependent and non–insulin-dependent diabetes, and demonstration of axo-glial dysjunction in human diabetic neuropathy. J Clin Invest 81:349-364, 1988