Magnetic Resonance in Medicine 42:461–466 (1999)

Anisotropic and Restricted Diffusion of Water in the Sciatic Nerve: A ^{2}H Double-Quantum-Filtered NMR Study

Yoshiteru Seo,[1] Hadassah Shinar,[2] Yasushi Morita,[3] and Gil Navon[2]*

The signals of water in the different compartments of rat sciatic nerve are resolved in the ^{2}H double-quantum-filtered NMR spectrum, due to their different quadrupolar splittings and relaxation rates. This resolution allowed the independent measurement of the water diffusion coefficients in the different compartments. The water diffusion in all three compartments, the endoneurium, the epineurium and the axon was found to be anisotropic. Parallel to the nerve fiber the average intraxonal water diffusion coefficient was 1.11×10^{-5} cm^2/sec, while in the perpendicular direction the diffusion is heavily restricted. The average perpendicular diffusion coefficient ranged from 0.29×10^{-5} cm^2/sec to 0.05×10^{-5} cm^2/sec for diffusion times of 7 msec and 50 msec, respectively. Assuming restricted diffusion in nonpermeable cylinders, intra-axonal mean diameters of 6.0, 7.4 and 9.0 μm were obtained for nerves taken from three different rats. Magn Reson Med 42:461–466, 1999. © 1999 Wiley-Liss, Inc.

Key words: sciatic nerve; vagus nerve; anisotropic diffusion; restricted diffusion; ^{2}H DQF NMR

The NMR measurement of water diffusion coefficients in various tissues, as well as in the central nervous system, has proven to be of clinical value (1,2). The pulsed gradient spin echo (PGSE) method provides, in addition to the diffusion coefficients, also information of the dimension and geometry of the compartment under study (3–6). Recently, it has been clearly demonstrated that the apparent water diffusion coefficients in the central and peripheral nervous systems are orientation dependent (7–10).

The nerve fiber of peripheral nerves consists of an axon surrounded by a phospholipid bilayer membrane and wrapped by many layers of the myelin sheath. Bundles of nerve fibers are held together by the collagen network of the endoneurium. A semipermeable membrane, the perineurium, which serves as the blood-nerve barrier, encloses hundreds of nerve bundles. Several of these are separated from the rest of the tissue by an outer layer of collagen fibers and fibrocytes—the epineurium.

In isolated nerves, the transverse magnetization decay curve (11–13), as well as the diffusion magnetization decay curves (13–16), measured by the PGSE sequence, are multi-exponential, representing water populations in different, noninteracting compartments. By resolving the magnetization decay curve, Seo et al. (15) have found three anisotropic water diffusion coefficients in rat sciatic nerve. The major part of the signal which remained after the addition of $MnCl_2$, showed both anisotropic and restricted diffusion and was assigned to the intra-axonal water. For optic nerve, it was assumed that the water component with the longest T_2 represents the intra-axonal water (16). The apparent diffusion coefficient measured for diffusion parallel to the nerve tract was found to be significantly larger than that for diffusion perpendicular to nerve axis.

Double-quantum-filtered (DQF) NMR spectroscopy is a sensitive technique which can reveal otherwise undetectable anisotropic motion of quadrupolar nuclei in biological systems (17). Anisotropic motion of water molecules has been detected by ^{2}H DQF measurements in a variety of biological tissues such as blood vessels (18), rat brain (19), skin and connective tissues such as cartilage and tendon (20,21). In blood vessels, the variations in the magnitudes of the residual quadrupolar splittings and the creation times of the second rank tensors enabled to differentiate between water in the different layers of the vessel's wall and to assess the strain exerted on them (22).

We have recently shown that the ^{2}H DQF NMR spectrum of isolated rat sciatic nerve, equilibrated in deuterated saline, is composed of three quadrupolar-split water signals (23). On the basis of the time course of their shift by Co-EDTA^{2-} and $CoCl_2$, the signals with quadrupolar splitting of approximately 120, 470 and 9 Hz were assigned to water in the epineurium, endoneurium and the intra-axonal compartment, respectively. The signal of the bulk water, which experience isotropic motion, was eliminated by the DQF pulse sequence.

Multiple quantum filtered diffusion measurements were previously performed in anisotropic systems. Martin et al. (24) have studied the translational diffusion of dichloromethane in various liquid crystals by DQF diffusion measurements. The authors have stressed the fact that the method is superior to analogous techniques, based on the single quantum echoes because the double-quantum coherence experience twice the applied field gradient. Zax and Pines (25) have extended the method to higher coherences. The diffusion of lithium ions, interacting with the macroscopically ordered collagen fibers of bovine Achilles tendon, was measured by triple quantum filtered diffusion measurements and found to be both anisotropic and restricted (26). Recently we have shown (27) that ^{2}H DQF diffusion measurements of ^2H_2O are an efficient method for looking at water interacting with ordered structures in cartilage. The same method was used by Assaf and Cohen (28) for the diffusion measurements of different water population in brain tissue. DQF diffusion measurements can also be performed in isotropic media in the presence of J coupling. This was first demonstrated by Kay and Preste-

[1]Department of Physiology, Kyoto Prefectural University of Medicine, Kyoto, Japan.

[2]School of Chemistry, Tel Aviv University, Tel Aviv, Israel.

[3]Department of Orthopedic Surgery, Kyoto Prefectural University of Medicine, Kyoto, Japan.

Portions presented at the 4th Annual Meeting of the International Society for Magnetic Resonance in Medicine, New York, NY, April 1996.

Grant sponsors: German-Israel Foundation for Scientific Research Development; Japanese Ministry of Education, Culture and Science.

*Correspondence to: Professor Gil Navon, School of Chemistry, Tel Aviv University, Tel Aviv 69978, Israel. E-mail: navon@post.tau.ac.il

Received 30 November 1998; revised 14 June 1999; accepted 16 June 1999.

gard (29) for a fluorinated compound in viscous medium. Sotak (30) used this method in in-vivo measurement of apparent diffusion coefficients of lactic acid in the presence of large concentrations of water and interfering lipid resonances. The same method, but this time utilizing the heteronuclear DQ coherences of a $^{1}H/^{31}P$ system in a phosphorous-containing solute, was used by Kuchel and Chapman (31). A 3.5-fold reduction in magnetic field gradient, relative to the PGSE experiment, was achieved.

In the present study, we take advantage of the fact that the water ^{2}H DQF NMR signals in the different compartments of sciatic nerve are resolved. Thus the apparent diffusion coefficients of each of the water populations can be measured independently.

MATERIALS AND METHODS

Wistar Hamamatsu rats (250–350 g, 7–13 weeks) were anesthetized with sodium pentobarbital (50 mg/kg body wt intraperitoneally). Sciatic nerves were isolated and the outer coat of adipose and connective tissue was carefully removed. Each nerve was placed in a 100 µL capillary tube and positioned with its long axis parallel to the magnetic field. All samples were equilibrated in 150 mM NaCl D_2O solution, for 40 min prior to the NMR measurements.

NMR measurements were carried out on Bruker AMX300 WB, AMX360 WB and ARX500 NMR spectrometers. The first two spectrometers were equipped with a micro-imaging accessory and a 200 G/cm gradient unit. The ARX500 NMR spectrometer, was equipped with 20 G/cm z-gradient unit.

^{2}H DQF spectra were measured with the conventional pulse sequence

$$90°\text{-}\ \tau/2\text{-}\ 180°\text{-}\ \tau/2\text{-}\ 90°\text{-}\ t_{DQ}\text{-}\ 90°\text{-}\ \text{Acq} \qquad \text{(PS1)}$$

where τ is the creation time of the second rank tensors and t_{DQ} is the evolution time of the DQ coherences. In the DQF diffusion measurements, two gradient pulses were introduced during the DQ evolution time, before and after the 180° refocusing pulse:

$$90°\text{-}\ \tau/2\text{-}\ 180°\text{-}\ \tau/2\text{-}\ 90°\text{-}\ t_3\text{-}\ G\text{-}\ t_4\text{-}\ 180°\text{-}\ t_5\text{-}\ G\text{-}\ t_6\text{-}\ 90°\text{-}\ \text{Acq} \qquad \text{(PS2)}$$

where G denotes a gradient pulse of strength g and duration δ. The signal intensity as a function of the gradient strength is given by (24)

$$\ln [I(g)/I(0)] = D_{app}\gamma^2 g^2 \delta^2 p^2 (\Delta - \delta/3) \qquad [1]$$

where I(g) and I(0) are the signal intensity in the presence and absence of the gradient, respectively. The diffusion time Δ is the time interval between the gradient pair ($t_4 + \delta + t_5$), the time to echo is $TE = t_3 + t_4 + t_5 + t_6 + 2\delta$, γ is the gyromagnetic ratio, p is the coherence (2 for DQ), and $t_3 + t_4 = t_5 + t_6$. The apparent diffusion coefficient D_{app}, is obtained from the semi-logarithmic plot of the signal intensity as a function of g^2, keeping both Δ and δ constant.

The gradient strength was calibrated using the known diffusion coefficients of benzene and water at 22.5°C, i.e., 2.17×10^{-5} cm^2/sec and 2.14×10^{-5} cm^2/sec, respectively (32). The mean apparent diffusion coefficient of D_2O under the same conditions was $1.71 \pm .01 \times 10^{-5}$ cm^2/sec, in good accordance with the value reported in the literature (32).

RESULTS

The ^{2}H DQF NMR spectra of rat sciatic nerve equilibrated in deuterated saline, at three values of creation times, τ, are given in Fig. 1. Three pairs of satellites with splittings of 500, 150 and 10 Hz are apparent. Their assignment was described in our previous publication (23) to be the water in the endoneurium (a), the epineurium (b) and the axon (c), respectively. As is evident from the spectra, the intensity of each of the signals has a different dependence on the creation time, τ (PS1). Thus, by controlling this time interval, each of the signals can be observed individually. We utilized this fact in measuring the diffusion coefficients of water in the three compartments independently. In the DQF-PGSE sequence (PS2), the diffusion sensitizing gradients are placed during the DQ evolution time. In our previous study of rat sciatic nerve (23) we have found that the DQ relaxation time is long relative to the single quantum (SQ) relaxation time: 41 msec as compared to 3.6 msec for the endoneurium water, 200 msec as compared to 9 msec for the water in the epineurium and 200 msec as compared to 70 msec for the intra-axonal water. This makes it feasible to perform diffusion measurements at relatively long diffusion times, in order to detect possible restricted diffusion. Another advantage of the DQF-PGSE diffusion measurements is that during the DQ evolution time, due to the double rate of the phase evolution the effective gradient strength is doubled (24).

Representative ^{2}H DQF spectra of water in rat sciatic nerve, at a creation time of 0.6 msec, as a function of the gradient strength, applied parallel to the nerve axis, are given in Fig. 2. The quadrupolar-split signals of the water in the endoneurium and the epineurium are observed. The signal of the intra-axonal water is exclusively detected at longer creation times (see Fig. 1). The measurements were performed with the nerve immersed in its equilibrating

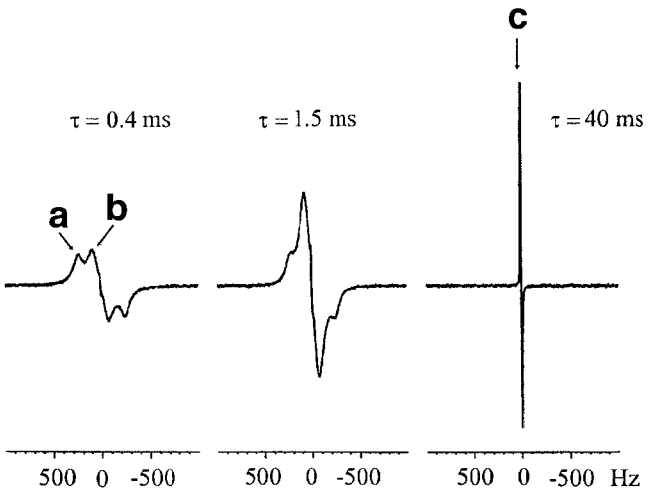

FIG. 1. ^{2}H DQF NMR spectra at 46.0 MHz of an excised rat sciatic nerve equilibrated in deuterated saline at three values of the creation time τ. **a:** Signal of the water in the endoneurium. **b:** Water in the epineurium. **c:** Intra-axonal water. Number of accumulations = 128.

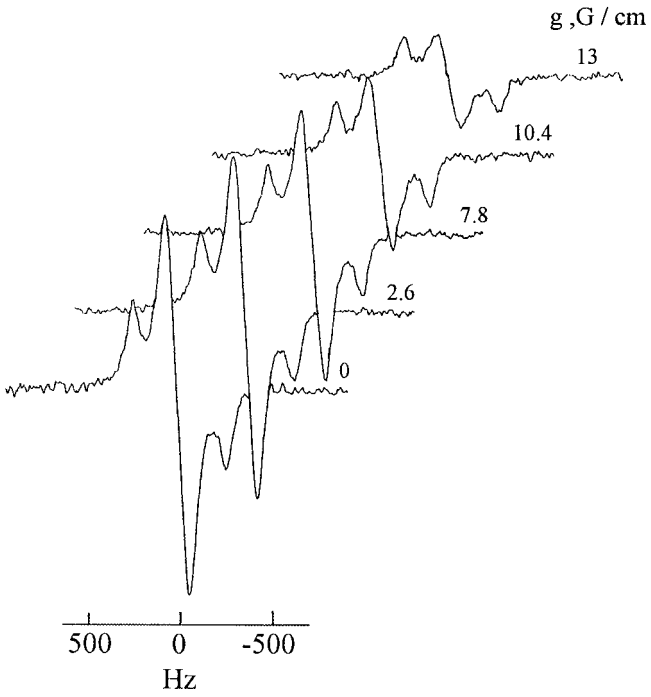

FIG. 2. ^2H DQF NMR diffusion-weighted spectra at 76.7MHz of an excised rat sciatic nerve as a function of the gradient strength. The gradients were applied along the axis of the nerve. Creation time, τ = 1 msec; gradient pulse duration, δ = 2 msec; diffusion time, Δ = 6 and time to echo,TE = 10 msec. Number of accumulations = 256. At this value of τ, only the signals of the water in the endoneurium and the epineurium can be found.

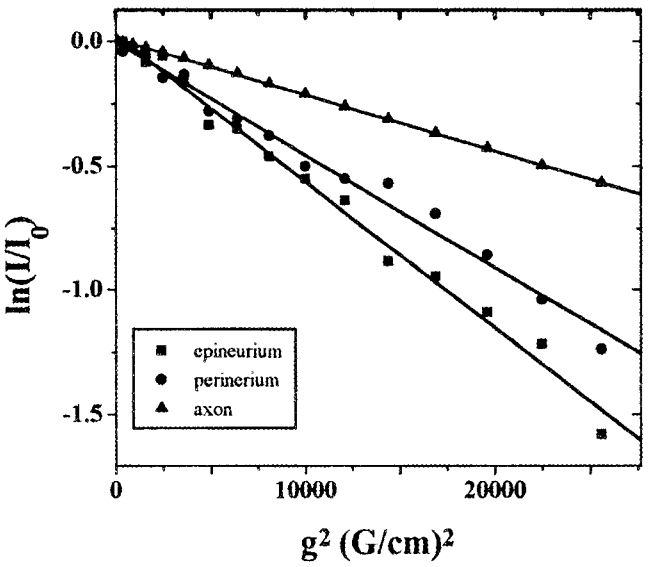

FIG. 3. ^2H diffusion-weighted DQF NMR signal intensity at 46.0 MHz as a function of g^2. The gradients were applied along the axis of the nerve. Gradient pulse, δ = 3 msec; diffusion time Δ = 10 msec, and time to echo, TE = 20 msec. Number of accumulations was 128. For the endoneurium and the epineurium, creation time τ = 0.6 msec, and for the intra-axonal water τ = 30 msec.

solution, but the DQ filter eliminated the strong signal of the bulk water. The gradient time, δ, and the time to echo, TE, were kept constant for the whole set of measurements. The apparent diffusion coefficients for each diffusion time, Δ, were calculated from the attenuation of the echoes which were linear in the semi-logarithmic plot (Fig. 3).

In Fig. 4a and b the apparent diffusion coefficients obtained from another set of measurements, performed both parallel, D_0, and perpendicular, D_{90}, to the long axis of the nerve are given. As can be seen the water populations of the endoneurium, epineurium as well as the intra-axonal water exhibits anisotropic diffusion. For a diffusion time of 9 msec, the parallel and perpendicular diffusion coefficients, D_0 and D_{90} were 0.78×10^{-5} cm^2/sec and 0.38×10^{-5} cm^2/sec for the water in endoneurium and 1.09×10^{-5} cm^2/sec and 0.51×10^{-5} cm^2/sec the water in the epineurium. The anisotropic diffusion factor is 2.05 for the endoneurium water and 2.13 for the water in the epineurium. The diffusion coefficients remained the same, within the experimental error, for diffusion times in the range of 9–24 msec, indicating only a slightly hindered diffusion in these two compartments.

The intra-axonal water exhibited much greater diffusion anisotropy (Fig. 4a and b). Moreover, for this compartment, the apparent diffusion coefficient perpendicular to the nerve axis had a strong dependence on the diffusion time, indicating heavily restricted diffusion. To interpret the results obtained for the intraxonal water, we have used a model for restricted diffusion in cylinders (33,34) where the perpendicular diffusion coefficient D_{90} is given by

$$D_{90} = 2[\Sigma[2D_f\alpha_m^2\delta - 2 + 2\exp(-D_f\alpha_m^2\delta) + 2\exp(-D_f\alpha_m^2\Delta) - \exp(-D_f\alpha_m^2(\Delta-\delta)) - \exp(-D_f\alpha_m^2(\Delta+\delta))]/[D_f^2\alpha_m^6(R^2\alpha_m^2-1)]]/[\delta^2(\Delta-\delta/3)] \quad [2]$$

where R is the radius of the cylinder and α_m are the roots of the derivative of the Bessel function of the first kind, order one, $J'_1(\alpha_m R) = 0$. Both D_f and D_{90} are obtained from the fitting procedure. The results obtained for D_f, were found to be the same as the average value obtained from the measurement of the diffusion coefficients parallel to the nerve fiber as a function of Δ. The experiment was performed on three sciatic nerves taken from three different rats. The results of the fitting were 6.0, 7.4 and 9.0 μm for the intra-axonal diameter with $D_f = 1.20 \times 10^{-5}$, 0.97×10^{-5}, and 1.17×10^{-5} cm^2/sec, respectively, yielding average values of 7.5 μm for the diameter and 1.11×10^{-5} cm^2/sec for D_f. The set of experiments for one of the sciatic nerves is described in Fig. 4.

A similar experiment was performed on the nonmyelinated vagus nerve of the rat. Here again, the diffusion of the intra-axonal water was found to be anisotropic. The apparent diffusion coefficients for the parallel and perpendicular orientations, as a function of the diffusion time are given in Fig. 5. As can be seen from the figure, the diffusion perpendicular to the fibers is restricted yielding an intraxonal diameter of 5.8 μm with D_f of 1.34×10^{-5} cm^2/sec.

DISCUSSION

In the present work the water diffusion coefficients, in the different compartments of rat sciatic nerve were deter-

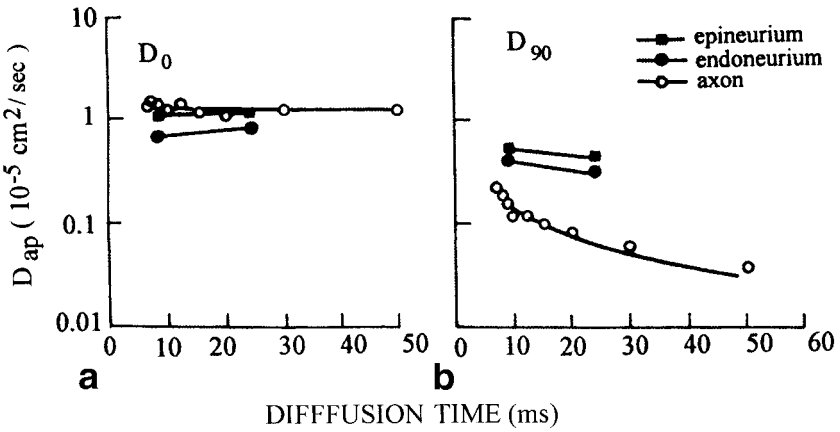

FIG. 4. Diffusion coefficients of deuterated water in an excised rat sciatic nerve equilibrated in deuterated saline measured at 46.0 MHz as a function of the diffusion time Δ. Parallel to the nerve axis (**a**) and perpendicular to the nerve axis (**b**). Eight gradient strengths, between 10 –150 G/cm were taken for each measurement. The gradient duration time, δ, was 3 msec, and the number of accumulations was 128. For the axon, creation time τ = 30 msec and time to echo TE = 60 msec. For the endoneurium and epineurium, τ = 0.6 msec and TE = 16 msec. The solid line for the axon in **b** is a fitted curve based on Eq. [4] with a diameter of 6.0 μm and $D_f = 1.20 \times 10^{-5}$ cm²/sec.

mined by the ^{2}H DQF diffusion sequence and were found to be orientation dependent. The diffusion along the nerve fiber is faster than the diffusion across the fiber, in all three compartments. In the endoneurium and epineurium, the thick collagen fibers and the myelinated axons may cause anisotropic diffusion of water. These structures hinder the diffusion of the water perpendicular to the axis of the fibers.

For the intraxonal water, the diffusion perpendicular to the fiber was found to be heavily restricted. A model for restricted diffusion in cylinders was used to interpret the results (33,34). This is a simplified model, which assumes short gradient pulses and impermeable barriers. The last assumption is valid in our case since we observe distinct signals from different compartments, each characterized by a distinct relaxation time (23). From the restricted diffusion of the intra-axonal water the average inner diameter of the axon, for three different rats, was estimated as 7.5 μm. The outer diameter of the axons in rat sciatic nerve varies from 3 to 12 μm (35). The calculated effective diameter of the assumed cylindrical diffusion barrier (7.5 μm) is in good agreement with the mean inner diameter of the axons 6 ± 1 μm (35,36).

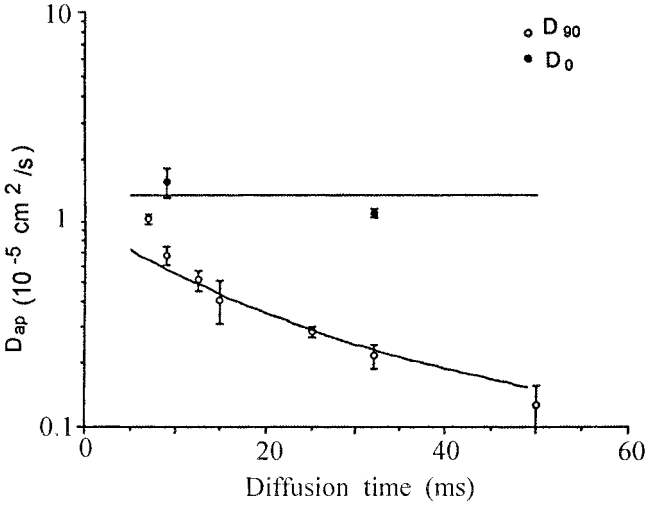

FIG. 5. Diffusion coefficients of deuterated water in the intra-axonal compartment of an excised rat vagus nerve equilibrated in deuterated saline, measured at 46.0 MHz as a function of the diffusion time Δ parallel (●) and perpendicular (○) to the nerve axis. Eight gradient strengths, between 10 –150 G/cm were taken for each measurement. The creation time τ, was 14 msec, the gradient duration time, δ, was 3 msec, and the number of accumulations was 512. The solid line for the perpendicular direction is a fitted curve based on Eq. [4] with a diameter 5.9 μm and $D_f = 1.34 \times 10^{-5}$ cm²/sec.

In our previous assignment of the different ^{2}H DQF signals in rat sciatic nerve, we did not find any evidence of a signal that can be assigned to the water in the myelin (23). This is probably because the amount of water in the myelin is very small and the resonances, because of the enhanced relaxation times, are expected to be very broad. The estimation of the ratio of the relative amount of water in the different compartments, based on single pulse experiments, rules out the possibility that one of the signals arises from the water in the myelin. The fact that the diffusion distance is larger than 7 μm, while the distance between the layers of the myelin is of the order of 0.003 μm, is in favor of the above conclusion.

Beaulieu and Allen (14) have measured the apparent water diffusion coefficients in the myelinated optic and trigeminal nerves as well as in the nonmyelinated olfactory nerve of the garfish. For the three garfish nerves, the diffusion was found to be anisotropic indicating that myelin is not a necessary determinant of diffusion anisotropy in axons. We have also obtained similar restriction of water diffusion in the myelinated sciatic and the nonmyelinated vagus nerves. It should be pointed out that the nonmyelinated axon is not a naked axon. The axon has its own plasma membrane and is ensheathed by a pair of membranes of the cytoplasm of the Schwann cells (neurilemma). Therefore, there are three plasma membranes between the interstitial space and the intra-axonal space. In addition, no water channel was reported on the peripheral nervous system, in contrast to the central nervous system. However, in the latter, the water channel (Aquaporin-4) is highly localized in the astrocyte's membrane in direct contact with the capillaries and ependymal layer, and is not observed in neural cells and axons (37,38). Thus we suggest that the neurilemma of the nonmyelinated axon is an effective diffusion barrier.

In previous ^{1}H NMR experiments, the major water signal remaining after the equilibration of rat sciatic nerve with $MnCl_2$ was assigned to the intra-axonal water (15). Diffusion anisotropy factors of 2.9 and 4.3 at diffusion times of 5 and 25 msec, respectively, were obtained. For frog sciatic nerve (39,40), diffusion anisotropy factors of 2 and 5.3 at diffusion times of 2 and 28 msec, respectively, were

obtained. Henkelman et al. (13) have recently reported multi-component diffusion echo attenuation with significant anisotropy for bovine optic nerve and white matter. Stanisz and Henkelman (16) have used a diffusion-CPMG experiment (41) to analyze the diffusion characteristics of different T_2 relaxation components in bovine optic nerve. The long T_2 component, assigned to the intra-axonal water was found to have an anisotropy factor of 2.3 at a diffusion time of 17 msec. The anisotropic diffusion factors obtained from our results for the intra-axonal water in rat sciatic nerve are in the range of 3–20 at diffusion times of 8–50 msec indicating a very strong dependency on the diffusion time. The reason for this disparity between our results and the measurements cited above (13,15,16,39,40) probably arises from the fact that in the ^1H measurements the observed intra-axonal water signal is susceptible to contamination from water in other compartments. We have shown that the water in the epineurium and the endoneurium are only slightly restricted. Thus contamination from water signals of the epineurium and the endoneurium is expected to reduce the measured restriction for the intra-axonal water.

The DQF method enabled us to measure the diffusion constants of water in each of the compartments separately. This is done on the basis of the different dependencies of the DQF spectra on the creation time and the different quadrupolar interactions experienced by the water in the epineurium, endoneurium and axon. To the best of our knowledge, this is the first report of independent diffusion measurement of the different water population in nerves. Each of the diffusion decay curves as a function of the gradient strength was mono-exponential (Fig. 3), indicating contribution from a single compartment exclusively.

As has been mentioned in the introduction, the ^2H DQ relaxation time T_{DQ} is long relative to the SQ relaxation time. This enables the measurements of the diffusion coefficients at relatively long diffusion times, so that restricted diffusion could be observed and analyzed. Also, since the signal of the bulk water was filtered out, the measurements could be performed with the nerve immersed in aqueous solutions that preserve the physiological environment and also enable perfusion experiments.

ACKNOWLEDGMENTS

The authors thank Mr. H. Okawara from the National Institute of Physiological Sciences, Okazaki, Japan, for technical assistance.

REFERENCES

1. Moseley ME, Cohen Y, Mintorovitch J, Chileuitt L, Shimizu H, Kucharczyk J, Wendland MF, Weinstein PR. Early detection of regional cerebral ischemia in cats: comparison of diffusion- and T2-weighted MRI and spectroscopy. Magn Reson Med 1990;14:330–346.
2. Hossman KA, Hoehn-Berlage M. Diffusion and perfusion MR imaging of cerebral ischemia. Cerebrovasc Brain Metab Rev 1995;7:187–217.
3. Basser PJ. Inferring microstructural features and the physiological state of tissues from diffusion weighted images. NMR Biomed 1995;8:333–344.
4. Le-Bihan D. Molecular diffusion, tissue microdynamics and microstructure. NMR Biomed 1995;8:375–386.
5. Callaghan PT. Pulsed-gradient spin-echo NMR for planar, cylindrical and spherical pores under conditions of wall relaxation. J Magn Reson A 1995;113:53–59.
6. Soderman O, Jonsson B. Restricted diffusion in cylindrical geometry. J Magn Reson A 1995;117:94–97.
7. Moseley ME, Cohen Y, Kucharczyk J, Mintorovitch J, Agri HS, Wendland MF, Tsuruda J, Norman D. Diffusion-weighted MR imaging of anisotropic water diffusion in cat central nervous system. Radiology 1990;176:439–445.
8. Sakuma H, Nomura Y, Takeda K, Tagami T, Nakagawa T, Tamagawa Y, Ishii Y, Tsukamoto T. Adult and neonatal human brain: diffusional anisotropy and myelination with diffusion-weighted MR imaging. Radiology 1991;180:229–233.
9. Howe FA, Filler AG, Bell BA, Griffiths JR. Magnetic resonance neurography. Magn Reson Med 1992;28:328–338.
10. Pierpaoli C, Jezzard P, Basser P, Barnett A, Chiro GD. Diffusion tensor MR imaging of the human brain. Radiology 1996;201:637–648.
11. Menon,RS, Rusinko MS, Allen PS. Proton relaxation studies of water compartmentalization in a model neurological system. Magn Reson Med 1992;28:264–274.
12. Fenrich FRE, Allen PS. Transverse relaxation rate distributions for water protons in myelinated and unmyelinated nerves. In: Proceedings of the ISMRM 4th Annual Meeting, San Francisco, 1991. p 693.
13. Henkelman RM, Stanisz GJ, Kim JK, Bronskill MJ. Anisotropy of NMR properties of tissues. Magn Reson Med 1994;32:592–601.
14. Beaulieu C, Allen PS. Determinants of anisotropic water diffusion in nerves. Magn Reson Med 1994;31:394–400.
15. Seo Y, Morita Y, Kusaka Y, Steward MC, Murakami M. Diffusion of water in rat sciatic nerve measured by ^1H pulsed field gradient NMR: compartmentation and anisotropy. Jpn J Physiol 1996;46:163–169.
16. Stanisz GJ, Henkelman RM. Diffusional anisotropy of T_2 components in bovine optic nerve. Magn Reson Med 1998;40:405–410.
17. Eliav U, Shinar H, Navon G. The formation of a second rank tensor in ^{23}Na double quantum filtered NMR as an indicator for order in biological tissues. J Magn Reson 1992;98:223–229.
18. Sharf Y, Akselrod S, Navon G. Measurement of strain exerted on blood vessel walls by double-quantum-filtered ^2H NMR. Magn Reson Med 1997;37:69–75.
19. Assaf Y, Navon G, Cohen Y. In vivo observation on anisotropic motion of brain water using ^2H double quantum filtered NMR spectroscopy. Magn Reson Med 1997;37:197–203.
20. Sharf Y, Eliav U, Shinar H, Navon G. Detection of anisotropy in cartilage using ^2H double quantum filtered NMR spectroscopy. J Magn Reson B 1995;107:60–67.
21. Shinar H, Eliav U, Knubovetz T, Sharf Y, Navon G. Measurement of order in connective tissues by multiple quantum filtered NMR spectroscopy of quadrupolar nuclei. Q Magn Reson Biol Med 1995;2:73–82.
22. Sharf Y, Seo Y, Eliav U, Akselrod S, Navon G. Mapping strain exerted on blood vessel walls using deuterium double-quantum-filtered MRI. Proc Natl Acad Sci U S A 1998;95:4108–4112.
23. Shinar H, Seo Y, Navon G. Discrimination between the different compartments in sciatic nerve by ^2H double-quantum-filtered NMR. J Magn Reson 1997;129:98–104.
24. Martin JF, Selwyn LS, Vold RR, Vold RL. The determination of translational diffusion constants in liquid crystals from pulsed field gradient double quantum spin echo decays, J Chem Phys 1982;76:2632–2634.
25. Zax D, Pines A. Study of anisotropic diffusion of oriented molecules by multiple quantum spin echoes. J Chem Phys 1983;78:6333–6334.
26. Eliav U, Knubovets T, Navon G. An NMR study of lithium diffusion in bovine Achilles tendon. In: Proceedings of the SMR 4th Annual Meeting, New York, 1996. p 259.
27. Shinar H, Eliav U, Schneiderman R, Maroudas A, Navon G. ^2H and ^{23}Na multiple quantum filtered NMR spectroscopy and diffusion measurements of human articular cartilage, In: Proceedings of the SMR 3rd Annual Meeting, Nice, France, 1995. p 432.
28. Assaf Y, Cohen Y. Detection of different water populations in brain tissue using ^2H single- and double-quantum-filtered diffusion NMR spectroscopy. J Magn Reson B 1996;112:151–159.
29. Kay LE, Prestegard JH. An application of pulse-gradient double-quantum spin echoes to diffusion measurements on molecules with scalar coupling. J Magn Reson 1986;67:103–113.
30. Sotak CH. A method for measuring the apparent self-diffusion coefficient of *in vivo* lactic acid using double-quantum coherence-transfer spectroscopy. J Magn Reson 1990;90:198–204.
31. Kuchel PW Chapman BE. Heteronuclear double-quantum-coherence selection with magnetic-field gradients in diffusion experiments. J Magn Reson A 1993;101:53–59.

32. Mills R. Self-diffusion in normal and heavy water in the range of 1–45 degrees. J Phys Chem 1973;77:685–687.
33. van Geldern P. Diffusion techniques for *in vivo* NMR (Ph.D. thesis). Delft, The Netherlands: Delft University Press; 1993.
34. van Gelderen P, DesPres D, van Zijl PCM, Moonen CTW. Evaluation of restricted diffusion in cylinders. Phosphocreatine in rabbit leg muscle. J Magn Reson B 1994;103:255–260.
35. Hidebrand C, Mustafa GY, Bowe C, Kocsis JD. Nodal spacing along regenerated axons following a crush lesion of the developing sciatic nerve. Brain Res 1987;429:147–154.
36. Bergman RA, Afifi AK. Nervous tissue. In: Atlas of microscopic anatomy. Philadelphia: W. B. Saunders; 1974. p 107–150.
37. King LS, Agre P. Pathophysiology of the aquaporin water channels. Rev Physiol 1996;58:619–648.
38. Nielsen S, Nagelhus EA, Amiry-Moghaddam M, Bourque C, Agre P, Ottersen OP. Specialized membrane domains for water transport in glial cells: High resolution immunogold cytochemistry of aquaporin-4 in rat brain. J Neurosci 1997;17:171–180.
39. Beaulieu C, Allen PS. An in vitro evaluation of the effects of local magnetic susceptibility-induced gradients on anisotropic diffusion in nerve. Magn Reson Med 1996;36:39–44.
40. Beaulieu C, Does MD, Snyder RE, Allen PS. Changes in water diffusion due to Wallerian degeneration in peripheral nerve. Magn Reson Med 1996;36:627–631.
41. van Dusschoten D, Moonen CTW, de Jager PA, Van As H. Unraveling diffusion constants in biological tissue by combining Carr-Purcell-Meiboom-Gill imaging and pulsed field gradient NMR. Magn Reson Med 1996;36:907–913.