Rapid Publications

Galactose Neuropathy

Structural Changes Evaluated by Nuclear Magnetic Resonance Spectroscopy

RICHARD H. GRIFFEY, R. PHILIP EATON, CHARLES GASPAROVIC, AND WILMER SIBBITT

SUMMARY

We examined the hypothesis that the polyol accumulation resulting from chronic galactose supplementation in the diet produces endoneurial edema that can be prevented by inhibition of aldose reductase. We explored the potential of nuclear magnetic resonance (NMR) spectroscopy to quantitate and characterize the water accumulation in the sciatic nerve in this "galactose neuropathy." The data demonstrate a 16% increase in gravimetrically determined total water content of nerve in the galactose-fed rat after 8 mo of this diet and a 50% increase in the T1 relaxation time for nerve water as determined by NMR spectroscopy. Prolongation of the T1 relaxation time reflects increased rotation of water in a magnetic field, consistent with an extracellular site of the additional water. Simultaneous feeding of sorbinil to inhibit aldose reductase resulted in normalization of both total nerve water and of the prolongation of T1 relaxation time. These data define the NMR-spectroscopic state of endoneurial edema in the galactose-fed rat and suggest specific application to the investigation of the role of aldose reductase in human diabetic neuropathy. *Diabetes* 36:776–78, 1987

Distal symmetric sensorimotor peripheral neuropathy is a common complication in diabetes, yet the pathogenesis of this disorder remains unclear (1). Structurally, one of the early changes observed in rats with the induction of experimental insulin deficiency with streptozocin is the accumulation of water within the sciatic nerve (2). It has been proposed that this endoneurial edema is secondary to polyol accumulation (3), and the classic model for investigation of this metabolic abnormality is the galactose-fed rat (4).

As recently reviewed by Myers and Powell (5), osmotically active galactitol formed by the action of aldose reductase accumulates within the endoneurial compartment. Subsequently, polyol-induced edema leads to increased endoneurial pressure (6) and reduced nerve blood flow (5). Reduced motor nerve conduction develops in parallel (7), and axonal degeneration and demyelination occur with time (8).

To examine the participation of endoneurial edema as a dominant structural change of galactose neuropathy, we evaluated the nerve water content by dehydration and by the T1 relaxation time as determined by nuclear magnetic resonance (NMR) spectroscopy. Because inhibition of aldose reductase is reported to reduce galactitol accumulation in this model (9), we further examined the effect of the inhibitor sorbinil on both the total nerve water content and the T1 relaxation time of nerve water. The data demonstrate marked accumulation of endoneurial free water in the galactose-fed rat, with complete normalization after aldose reductase inhibition. We demonstrate the applicability of NMR spectroscopy to investigate polyol neuropathy.

MATERIALS AND METHODS

Four sets of three male Wistar rats were grown for 8 mo on a control diet of rat chow, a diet of chow supplemented with 30% galactose, and a galactose diet with 0.7% sorbinil (Pfizer, New York). The rats were killed, and a 3-cm section of the sciatic nerve was excised. The tissue was immediately placed within a tared 2.5-mm glass tube, wet weight determined gravimetrically, and then analyzed in a 7-T General Electric NMR spectrometer. Each T1 relaxation time measurement was started exactly 20 min after removal of the tissue, after optimization of field homogeneity. The standard inversion-recovery pulse method was employed, and four scans were summed for each time point to cancel random variations. Ten time points on the inversion-recovery curve were sampled for each nerve, and the data were fitted with a log-linear least-squares solution. Measurement of the T1

From the Center for Non-Invasive Diagnosis (R.H.G., C.G.) and the Department of Medicine (R.P.E., W.S.), University of New Mexico School of Medicine, Albuquerque, New Mexico.
Address correspondence and reprint requests to Dr. R. Philip Eaton, Dept. of Medicine, The University of New Mexico School of Medicine, Albuquerque, NM 87131.
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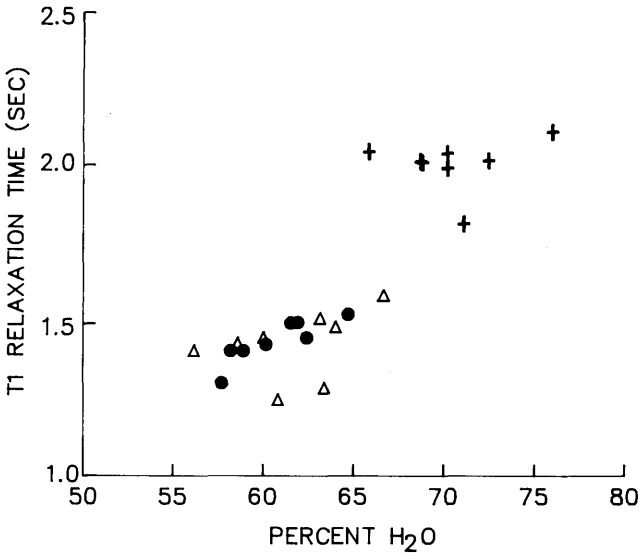

FIG. 1. Rat sciatic nerve hydration. Regression analysis of T1 relaxation time of resonance of water with gravimetrically determined total water content of rat sciatic nerve is highly significant (r = .87, P < .001). Data include analyses of 24 nerves from control (●), galactose-fed (+), and sorbinil-treated galactose-fed (△) animals.

relaxation time of the lipid resonance was then evaluated in an analogous manner. Dehydrated weights were determined by drying to constant weight at 105°C.

RESULTS

When killed, control animals weighed 429 ± 36 g, galactose-fed animals weighed 411 ± 20 g, and sorbinil-treated galactose-fed animals weighed 404 ± 30 g. All galactose-fed rats developed cataracts by 4–6 wk of feeding, whereas sorbinil-treated galactose-fed rats were completely free of cataract formation.

The water content of the sciatic nerves taken from control animals was 60.7 ± 2.6% wet wt and was significantly elevated by 16% in galactose-fed rats to 70.5 ± 3.9% (P < .001). In sorbinil-treated galactose-fed rats, the water content was normal at 61.6 ± 5.0% (P < .01), compared with galactose-fed animals.

The T1 relaxation time for the sciatic nerve water from normal rats ranged from 1.30 to 1.45 s, with an average of 1.35 ± 0.05 s. With 8 mo of galactose feeding, the T1 relaxation time was markedly increased by 50% to 2.02 ± 0.04 s (range 1.96–2.04 s; P < .001). In the sorbinil-treated galactose-fed rats the T1 relaxation time was normal, averaging 1.34 ± 0.08 s (range 1.26–1.45 s; P < .001 compared with galactose-fed rats). As shown in Fig. 1, the gravimetrically determined water content and the T1 relaxation time of the water resonance were highly correlated (r = .86, P < .001). The T1 relaxation time of the lipid resonance consistently fell in a range from 0.520 to 0.550 s, with no alteration introduced by either galactose feeding or by sorbinil in the diet.

DISCUSSION

The results of our studies are consistent with a significant increase in water content of the sciatic nerve by 8 mo of galactose feeding and can be completely normalized by sorbinil treatment. Investigators have previously reported that this drug will normalize nerve galactitol accumulation (9). Our demonstration of a simultaneous correction in nerve water content is consistent with a participation of endoneurial hydration in the pathophysiology of galactose neuropathy.

Published studies, by microscopic analysis, have documented that increased interstitial space is present throughout galactose nerve sections, with marked perivascular empty space and separation of myelinated fibers (6,7). It has been proposed that this empty space represents edema in the endoneurial compartment (6). This hypothesis is consistent with our observations by NMR spectroscopy, where the increase in T1 relaxation time of the water resonance is considered to represent more water in an extracellular environment. The resonance of free water (extracellular only) has an ideal T1 relaxation time of 4.0 s. In contrast, the T1 relaxation time of intracellular water reflects the effect of environmental interactions on its rotational motion in a magnetic field and can be expected to be reduced to ~1.4 s. Our observed T1 time of 2.02 s in the galactose-fed rats would be consistent with ~15% of the total nerve water residing within the interstitium, whereas <1% is so disposed in the control and sorbinil-treated rat nerves.

These studies demonstrate the utility of NMR proton spectroscopy to quantitate the state of water within the nerve, with close correlation to the gravimetrically determined water content. NMR is sensitive to the state and quantity of water and may be ideally suited for noninvasive evaluation of the hydrated state secondary to the occurrence of polyol accumulation in diabetes (10). The application of NMR to evaluate the effect of nerve hydration on extracellular edema in diabetic rats and humans is under evaluation.

The demonstration of an unaltered T1 relaxation time of the lipid resonance of the rat sciatic nerve suggests that no detectable demyelination has occurred in our 8 mo of galactose feeding. This is consistent with the reported microscopic data suggesting that 22–25 mo of galactose feeding may be required to develop demyelination (8). The finding of a prolonged T1 relaxation time for water resonance but not for the lipid resonance may serve to differentiate polyol edema from Wallerian degeneration. In the latter process, as observed after nerve transection, prolongation of the T1 relaxation time for both resonance peaks is expected (11; L. O. Sillerud, L. F. Kirsch, G. Miller, B. V. Griffey, and R. H. Griffey, unpublished observations).

Other metabolic events that could participate in the altered hydration of the nerve include *myo*-inositol deficiency, nonenzymatic protein glycosylation, and impaired axoplasmic transport, in addition to the polyol accumulation (1,10). Regardless of the pathogenesis, we propose that NMR provides a simple, sensitive, and noninvasive method for early detection of the endoneurial edema characteristic of polyol neuropathy. The response to aldose reductase inhibition is readily assessed by NMR, which has significant potential for the evaluation and monitoring of human diabetic peripheral neuropathy.

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