Vol. 137, No. 1, 1986 BIOCHEMICAL AND BIOPHYSICAL RESEARCH COMMUNICATIONS
May 29, 1986 Pages 579-584

PRELIMINARY STUDIES ON THE POTENTIAL OF *IN VIVO* DEUTERIUM NMR SPECTROSCOPY

Ian M. Brereton, Michael G. Irving, James Field and David M. Doddrell

School of Science, Griffith University, Nathan, 4111, Australia

Received April 18, 1986

Natural abundance deuterium NMR spectroscopy can be used to characterise *in vivo* ^2H signals arising from water and fat in mice, with acquisition times of less than two minutes. Administration of D_2O (10% V/V) in the drinking water enhances these signals so that excellent spectra can be obtained with one scan. Using these procedures the *in vivo* turnover of ^2H in water and fat in mice has been determined. This procedure may be of particular importance in studies of fat turnover in obesity.

Deuterium NMR spectroscopy has been used extensively in studies of oriented or partially oriented molecules including biological or model membranes and polymers and also in high-resolution studies of liquid systems (1). However, as far as we are aware there have been no reports of applications of deuterium NMR *in vivo*. The ^2H nucleus is quadrupolar with a spin quantum number (I) of 1. Its low gyromagnetic ratio and natural abundance (0.015%) lead to a detection sensitivity of 1.45×10^{-6} relative to that of ^1H (2). However, these unfavourable properties are offset to some extent by the shorter relaxation times due to the small electric quadrupole moment of the deuterium nucleus. Thus, more rapid accumulation of signal than for ^1H or ^{31}P is possible, particularly in *in vivo* experiments. Also, given that the total body water of animals is at least 70% of body weight, the concentration of HOD is of the order of 12mM.

We have investigated the potential of *in vivo* deuterium NMR spectroscopy using mice as test animals with a view to evaluating the potential of the technique in monitoring metabolic processes. Based on the above analysis we would expect deuterium signals to be readily observed, particularly in feeding experiments, and depending upon the magnitude of the T_1 and T_2 relaxation

0006-291X/86 $1.50

times, we would expect to observe the production of metabolic products. These expectations have been realized.

MATERIALS AND METHODS

^2H NMR spectra were obtained on a modified Bruker NMR system consisting of a 13 cm Oxford systems magnet operating at 4.7T field strength. A probe utilizing a horizontal solenoid coil, previously used in ^{13}C NMR *in vivo* studies (3) was tuned to the ^2H (30.7 MHz) excitation frequency.

Mice were positioned with the 1.5 cm wide coil around the upper abdomen, approximately at the level of the liver. Female Quakenbush mice (30-40 gm body weight) were used and anaesthetised (Ketamine, 100 mg and Diazepam, 3 mg/Kg body weight intraperitoneally) for all experiments. ^2H levels were increased by intubation of D_2O (0.4 ml), injection of D_2O (0.2 ml) into the tail vein or by the addition of D_2O to drinking water (10% V/V).

RESULTS AND DISCUSSION

The natural abundance ^2H NMR spectrum of a mouse obtained on our system is shown in Figure 1. This spectrum was recorded in 8 minutes. Although the ^2H chemical shift dispersion is only 15% of that of ^1H, the signal arising from the CHD-groups of fat tissue is readily resolved from the HOD signal. The linewidth of the HOD signal is typically 40-50 Hz.

In the case of the mice in which the level of ^2H had been artificially increased, a good signal was obtained in one scan, particularly from animals administered with D_2O in drinking water which produced a free induction decay

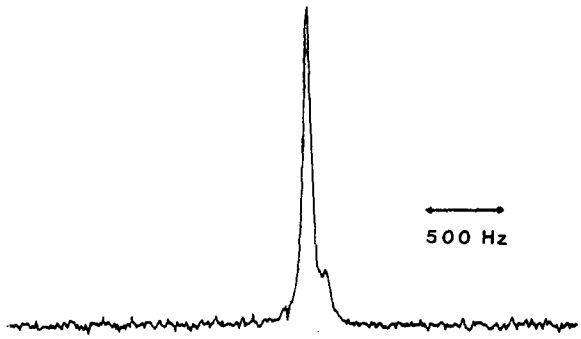

Figure 1. 30.7 MHz natural abundance ^2H NMR spectrum of the upper abdomen of a mouse. 1000 transients were accumulated into a 1K data base with a spectral width of 5000 Hz and recycle time of 0.5s.

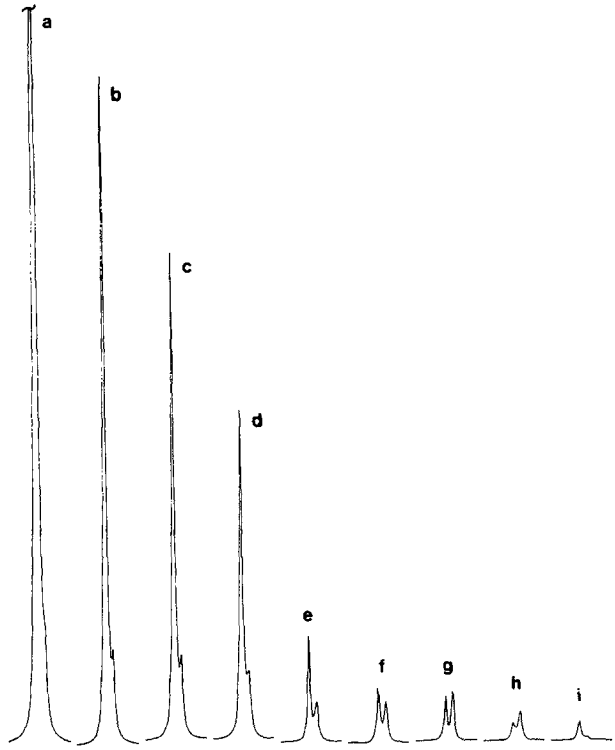

Figure 2. ^2H NMR spectra of a mouse following the removal of D_2O from drinking water. The spectra shown were recorded (a) 1, (b) 4, (c) 5, (d) 8, (e) 11, (f) 15, (g) 16 and (h) 21 days after resumption of normal drinking water. Spectrum (i) was recorded at natural abundance prior to administration of D_2O to drinking water.

intense enough to use for field shimming. This level of ^2H incorporation was achieved after only 12 hours exposure to drinking water containing 10% D_2O. The ^2H level in these animals equilibrated at a constant value after 3 to 4 days. Mice were exposed to 10% D_2O in their drinking water for 28 days before being given normal water, after which time the loss of deuterium from the body was monitored for a further 28 days. As depicted in Figure 2, the CHD-signal is initially unresolved but appears once the intensity of the HOD peak is reduced. Clearly there has been incorporation of deuterium into the fat tissue, the level after 28 days exposure having increased by 35 times the natural abundance level.

A plot of the signal intensity against time is shown in Figures 3a and b for the HOD and -CHD peaks, respectively. The loss of deuterium from fat tissue is significantly slower than from body water, with a half life of 8 to 9 days

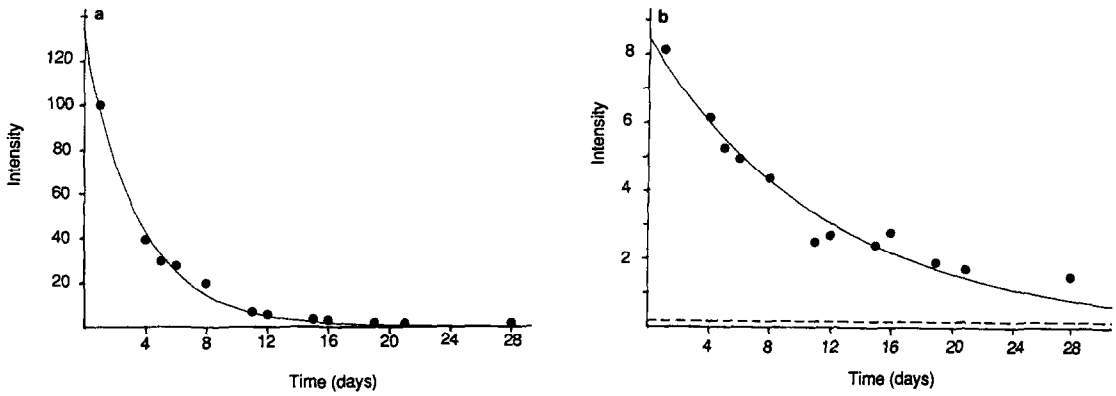

Figure 3. Plots of ^2H signal intensity against time for the (a) HOD and (b) -CHD-peaks. The smooth curves represent a fit of the data to a single exponential with time constants of (a) 0.278 s^{-1} and (b) 0.085 s^{-1} respectively. In (b), the dashed line indicates the -CHD-peak intensity at natural abundance.

compared to 3 to 4 days, respectively. This is demonstrated clearly in Figure 2 where the HOD signal actually becomes smaller than the CHD-signal after 16 days. This observation may represent a simple method for determining the rate of fat turnover *in vivo*. The determination of fat turnover *in vivo* is fraught with great difficulty because of uncertainty of triglyceride pool sizes. The turnover of ^2H associated with fat is currently being examined in this laboratory in terms of obesity in diabetic animals.

The measurement of ^2H T_1 relaxation times may also provide useful information, particularly if combined with a volume selection technique so that particular regions of the body may be monitored. Such a T_1 experiment is shown in Figure 4 of a mouse, 21 days after removal of D_2O from drinking water when the two signals are well resolved. As expected, the T_1 of the -CHD-group (typically 34±4 ms) is less than that of the more mobile HOD (228±3 ms).

The effect of increased body iron stores upon deuterium T_1 values is currently under investigation (4). Line broadening of ^2H signals caused by the presence of paramagnetic ions is reduced by a factor of $\gamma^2_{1H}/\gamma^2_{2H}$ (~42) compared to that induced in ^1H signals (5). Consequently, ^2H NMR spectroscopy is

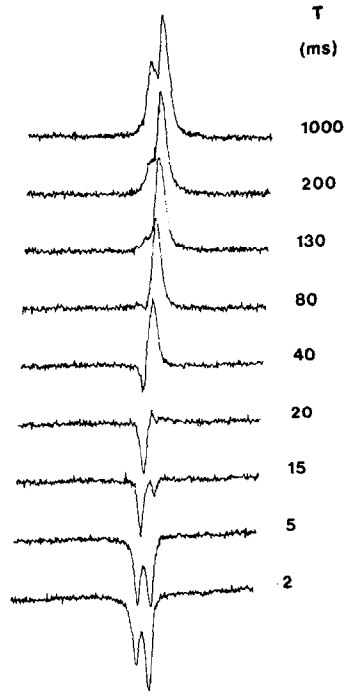

Figure 4. ^2H NMR spectra of a mouse 21 days after removal of D_2O from drinking water as a function of the delay time, τ, in the T_1 inversion recovery sequence $\pi[x]-\tau-\frac{\pi}{2}[x,\pm]$, rec±. 1000 scans per spectrum were acquired using a recycle time of 1.5 s.

possible when levels of iron in the animal are such that the ^1H and ^{31}P linewidths are too large to be observed. Because the observed T_1 relaxation times include a contribution from the species in contact with the iron, it is possible to measure the iron concentration in conditions of hepatic iron overload such as primary haemochromatosis.

We believe extension of the use of *in vivo* deuterium NMR spectroscopy to human subjects should be possible since it has been shown that man should readily tolerate concentrations of D_2O in body water of up to 10% (6). The level for prolonged exposure to D_2O has been recommended at 1% (7) and D_2O dilution techniques are used for measurement of body water in infants, pregnant women and obese subjects in clinical studies (8). On the basis of our results, these levels of deuterium incorporation should be more than adequate for the rapid measurement of deuterium NMR signals *in vivo*. The evaluation of the potential of deuterium NMR spectroscopy in monitoring other metabolic

processes by the administration of deuterated substrates, for example, CD_3CH_2OH is also proceeding.

ACKNOWLEDGEMENTS

This research was supported by grants from the John P. Kelly Mater Research Foundation, the Queensland Cancer Fund, and the Australian Research Grants Scheme.

REFERENCES

1. Mantsch, H.H., Saito, H., and Smith, I.C.P., (1977) Prog. NMR Spect. 11, 211-271.

2. Harris, R.K., (1978) in "NMR and the Periodic Table", Harris, R.K., and Mann, B.E., (eds), p.6, Academic Press, London.

3. Field, J., Irving, M.G., Brooks, W.M., and Doddrell, D.M., (1984) Biochem. Biophys. Res. Commun. 123, 882-887.

4. Irving, M.G., Brereton, I.M., Field, J., and Doddrell, D.M., (1986) Submitted Nature

5. Reuben, R. and Fiat, D., (1969) J. Amer. Chem. Soc. 91, 1242.

6. Förstel, H., (1978) Ber. Kernforschungsanlage Jülich, Jül 1539, 23 pp.

7. Peng, S-K., Ho, K-J., and Taylor, C.B., (1972) Arch. Path. 94, 81-89.

8. Coward, W.A., (1979) Lancet, 309.