

## Spectral Editing in $^{31}\text{P}$ NMR Spectra of Human Brain

KEVIN M. BRINDLE, MICHAEL B. SMITH,\* BHEESHMA RAJAGOPALAN,†  
AND GEORGE K. RADDA

*Department of Biochemistry, South Parks Road, Oxford OX1 3QU, England*

Received October 29, 1984

There are a number of NMR experiments designed to simplify complex spectra. For example, spin-echo techniques have been used in  $^1\text{H}$  NMR of intact cells and tissues to suppress broad background resonances from proteins and allow observation of resonances from small metabolite molecules (1-3). In this case discrimination between resonances is based on differences in  $T_2$ . In other experiments differences in spin-spin coupling have been exploited to obtain spectral editing and these have been applied in both  $^{13}\text{C}$  and  $^1\text{H}$  NMR (see, for example, 4-6). Spectral simplification is particularly important in  $^1\text{H}$  NMR *in vivo* where the large number of protons and narrow chemical-shift range of the nucleus results in crowded and poorly resolved spectra. This has not been a significant problem in  $^{31}\text{P}$  NMR *in vivo* since for this nucleus the relatively large chemical-shift range and small number of observable resonances has produced well resolved spectra using conventional high-field instruments (7). However, the development of relatively low-field (e.g., less than two tesla) wide-bore whole-body magnets, which are currently being used to investigate the clinical potential of NMR spectroscopy (8), has introduced a resolution problem into the  $^{31}\text{P}$  NMR experiment. This problem is illustrated by the human brain spectrum shown in Fig. 1a in which the inorganic phosphate and phosphocreatine resonances are partly obscured by resonances from phosphomonoesters and phosphodiester. These latter resonances have been assigned in guinea pig brain by comparing spectra of extracts with spectra obtained *in vivo* (11).

In a spin-echo experiment on the human brain the relatively short  $T_2$  values of the phosphomono and diesters resulted in significant suppression of these resonances when compared with the resonances of phosphocreatine and inorganic phosphate (Fig. 1b). The ATP resonances appear to have much shorter  $T_2$  and are eliminated from the spectrum. In addition the underlying broad resonances visible in the spectrum from the one-pulse experiment are also removed. The spin-echo experiment shown in Fig. 1b was initially performed on a phantom consisting of three plates 2 cm thick containing pyrophosphate and inorganic phosphate at two different pH values. The plate containing pyrophosphate was placed immediately adjacent to the coil and the plates containing inorganic phosphate were placed next to this. The

\* Present address: Department of Neurology, Henry Ford Hospital, 2799 West Coast Boulevard, Detroit, Mich. 48202.

† Clinical Magnetic Resonance Unit, John Radcliffe Hospital, Oxford, England.

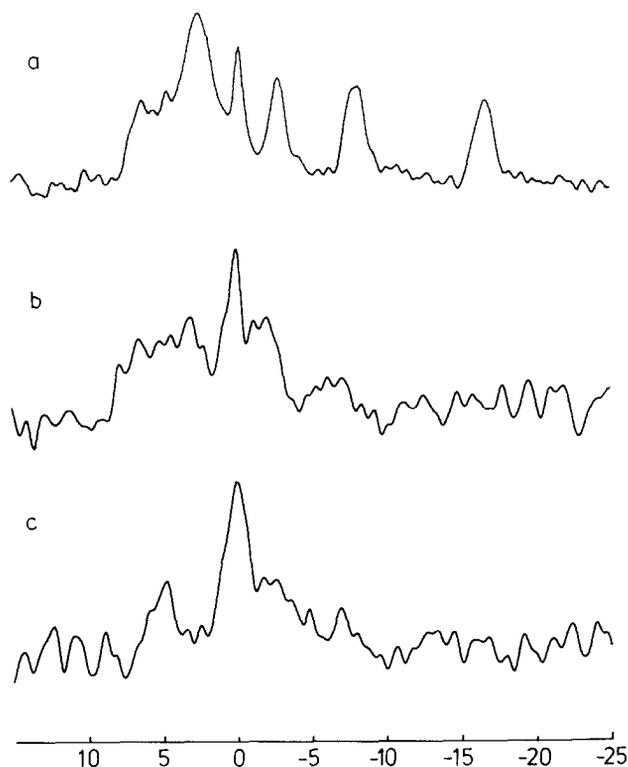


FIG. 1.  $^{31}\text{P}$  NMR spectra of human brain. Spectra were obtained using a TMR 32/600 (Oxford Research Systems) spectrometer with a 1.6 T 60 cm bore superconducting magnet, at 27.5 MHz for  $^{31}\text{P}$  and 67.9 MHz for protons. The probe was a 15 cm surface coil modified by the insertion of a slotted disk of copper foil with a central aperture of 7 cm diameter. Inductive coupling between the driven outer coil and the inner disk gave greater penetration of the rf field at the central aperture, compared with a conventional surface coil of 7 cm diameter. The coil was placed on the side of the subject's head. Exponential multiplication applied to the FID gave line broadening of 15 Hz. The chemical-shift scale is in ppm referred to phosphocreatine. (a) Spectrum obtained using a composite  $180^\circ$   $^{31}\text{P}$  pulse (10), which in phantom experiments minimized signal from regions close to the coil, i.e., surface muscle on the head. (b) Spectrum obtained using a spin-echo sequence with  $\tau = 30$  ms; 1024 scans collected in 35.6 min. (c) Spectrum obtained using the heteronuclear  $^{31}\text{P}/^1\text{H}$  spin-echo pulse sequence described in the text ( $\tau = 30$  ms). The coil was double tuned to  $^{31}\text{P}$  and  $^1\text{H}$  (9). The  $180^\circ$   $^1\text{H}$  pulse was applied immediately preceding the  $180^\circ$   $^{31}\text{P}$  pulse. A dummy delay equal in length to the  $^1\text{H}$  pulse was inserted in the pulse sequence following the  $180^\circ$   $^{31}\text{P}$  pulse to avoid phase distortions. The spectrum is the sum of 1024 scans collected in 35.6 min.

pulse lengths in the spin-echo sequence were adjusted so that the signal obtained from pyrophosphate was minimal compared to that from the two inorganic phosphate solutions (see Fig. 2). The ratio of the pulse lengths was set at 1:3 to suppress signal from the third harmonic close to the coil (12). The coil and pulse lengths determined on the phantom were then used in experiments on the human brain. It was assumed that the pulse lengths would be the same as on the phantom. Further suppression of the phosphomonoester and diester resonances was obtained by using a heteronuclear  $^{31}\text{P}/^1\text{H}$  spin-echo experiment. This experiment is related

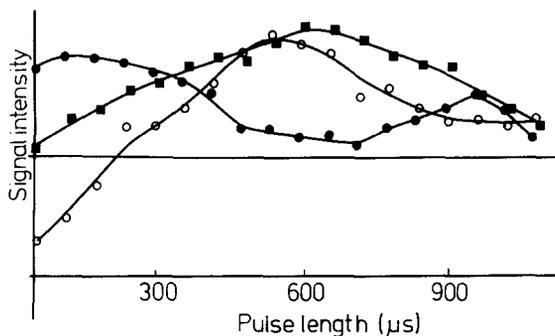


FIG. 2. Determination of the volume detected in the spin-echo experiment. The coil, the same as that used in experiments on the brain, was placed next to a three-compartment phantom. The first compartment was a 2 cm thick, 17 cm diameter glass plate containing 50 mM pyrophosphate, pH 7.0. The next two plates, 2 cm thick and 12 cm in diameter, contained 100 mM inorganic phosphate at pH 9 and pH 5, respectively. Signal intensity is plotted as a function of the length of the first pulse. The ratio of the pulses was 1:3 (see text). The symbols represent signal from the first (●), second (○), and third (■) compartments, respectively.

to one described by Freeman *et al.* (13) to observe heteronuclear spin coupling and to experiments which have been used to detect  $^{13}\text{C}$  and  $^{15}\text{N}$  labels in  $^1\text{H}$  and  $^{31}\text{P}$  spectra (14–17). In the heteronuclear spin-echo experiment used here a  $180^\circ$   $^1\text{H}$  pulse is applied at the same time as the  $180^\circ$   $^{31}\text{P}$  pulse in the  $90^\circ-\tau-180^\circ-\tau$ -acquisition  $^{31}\text{P}$  pulse sequence. The  $^1\text{H}$  pulse results in phase modulation of the  $^1\text{H}$ -coupled  $^{31}\text{P}$  resonances. On a high-field instrument with a homogeneous  $B_1$  field application of this pulse sequence to a mixture of inorganic phosphate, phosphocreatine,  $\alpha$ -glycerophosphorylcholine, D-glucose-6-phosphate, and adenosine triphosphate (ATP) produced the spectrum shown in Fig. 3. In the presence of the  $^1\text{H}$  pulse, modulation of the proton-coupled phosphomonoester (D-glucose-6-phosphate) and diester ( $\alpha$ -glycerophosphorylcholine) resonances results in their being nulled at  $\tau = 30$  ms due to overlap between components with positive and negative phase (Fig. 3b). Phase modulation due to proton coupling can also be observed in the  $\alpha$ -phosphate resonance of ATP. In addition to phase modulation of  $^1\text{H}$ -coupled resonances, significant NOEs were observed in the presence of  $^1\text{H}$  irradiation; for example, the intensity of the inorganic phosphate resonance increased by approximately 30% on addition of the  $^1\text{H}$  pulse. Application of this experiment to the human brain using a surface coil double tuned to  $^{31}\text{P}$  and  $^1\text{H}$  is shown in Fig. 1c. Significant suppression of the phosphomono and diester resonances is obtained in the presence of the  $^1\text{H}$  pulse, thus allowing clearer observation of the phosphocreatine and inorganic phosphate resonances. As with the  $^{31}\text{P}$  pulses, the  $^1\text{H}$  pulse length was optimized using a phantom. The phantom in this case contained glycerolphosphate and the  $^1\text{H}$  pulse length was set so as to achieve maximum suppression of the phosphorus resonance in the spin-echo experiment. The signal to noise ratio in the spin-echo experiment is clearly less than that observed in the one-pulse experiment. These preliminary experiments were performed using a double-tuned coil in which some loss of sensitivity is inevitable (9). Although this coil could probably be improved the experiment may be better performed using two singly

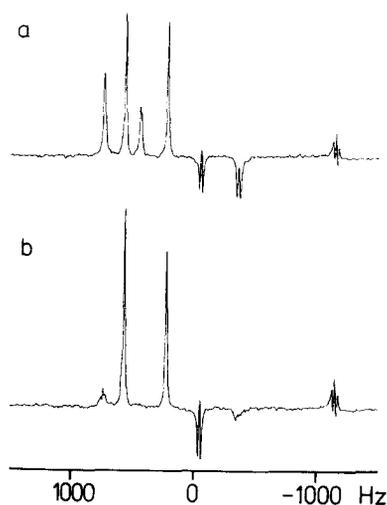


FIG. 3. Demonstration of the heteronuclear spin-echo experiment in an *in vitro* system. The sample contained approximately 20 mM  $\alpha$ -glycerophosphorylcholine (obtained as a cadmium chloride complex), glucose-6-phosphate, creatine phosphate, inorganic phosphate, and ATP in 100 mM Hepes buffer, pH 6.8, containing 40 mM EDTA. The spectra were obtained using a 4.3 T magnet operating at 73.8 MHz for  $^{31}\text{P}$  and 182.4 MHz for  $^1\text{H}$ . The probe, described previously (18), consisted of an inner three-turn solenoidal coil tuned to  $^{31}\text{P}$  and an outer saddle-shaped coil tuned to  $^1\text{H}$ . The observed  $^{31}\text{P}$  resonances are from (in order from the downfield end of the spectrum) glucose-6-phosphate, inorganic phosphate,  $\alpha$ -glycerophosphorylcholine, phosphocreatine, and the  $\gamma$ ,  $\alpha$ , and  $\beta$  phosphates of ATP. (a) Spectrum obtained with a homonuclear spin-echo sequence ( $\tau = 30$  ms). (b) Spectrum obtained using the heteronuclear spin-echo pulse sequence described in the text. The  $180^\circ$   $^1\text{H}$  pulse was applied using the outer  $^1\text{H}$  coil.

tuned coils (19). This has the added advantage that a larger  $^1\text{H}$  coil could be used thus ensuring that the effective  $^1\text{H}$   $B_1$  field extends over the entire volume detected in the  $^{31}\text{P}$  spin-echo experiment.

In conclusion, we have described the application of a heteronuclear spin-echo experiment to obtain spectral editing in  $^{31}\text{P}$  NMR spectra of human brain. Suppression of resonances from phosphomono and diesters results in clearer observation of the inorganic phosphate and phosphocreatine resonances thus allowing a more reliable determination of pH from the chemical shift of the phosphate resonance. In addition the phosphocreatine/inorganic phosphate ratio, which is an index of the energy status of the brain, is readily obtained, although with the caveat that this will be affected by the  $T_2$  values of the resonances. The sensitivity of the spin-echo experiment is clearly less than that of the one-pulse experiment as a result of the  $T_2$  dependent decay of the magnetization in the spin-echo sequence. Low intrinsic sensitivity and relatively short  $T_2$  may limit application of spin-echo techniques in  $^{31}\text{P}$  NMR *in vivo*. The technique described here may not be applicable in other tissues where the  $T_2$  might be expected to be much shorter, e.g., in liver where the measured  $T_1$  values in the perfused organ have been found to be much shorter than in other tissues (20).

## ACKNOWLEDGMENTS

We thank the British Heart Foundation, the Medical Research Council, and the Department of Health and Social Security for financial support.

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