LETTER TO THE EDITOR

Validity of In Vivo Nuclear Magnetic Resonance Methods in Measurement of Intracellular Water and Sodium

Dear Sir:

We would like to thank Dr. D. Burstein for her comments on our recent work (1). In her letter to the editor, Dr. Burstein demonstrates changes in proton or sodium spectra of perfused frog heart after application of $Dy(TTHA)^{3-}$ and argues that the following changes should occur, if shift reagent fully penetrates into tissue. In proton spectra, a single resonance, instead of intra- and extracellular resonances, should be observed in a position intermediate to the true intra- and extracellular positions. In sodium spectra, the intracellular resonance should shift to a considerable extent, such as 1.2 ppm, due to bulk susceptibility effects. To the contrary, in our experiments (1), the proton spectra always exhibited two (or three) resonances at unshifted and shifted positions. In the sodium spectra, a small shift of the intracellular resonance was observed. However, this shift was very small (0.20–0.45 ppm) so that it was neglected in the paper (1) to distinguish the intra- and extracellular signals as unshifted and shifted resonances. Thus, the results demonstrated in Dr. Burstein's experiments and those obtained in our experiments seemingly show a large dissociation. However, we consider that this dissociation is attributable to the difference of models as mentioned as follows.

In our experiments, NMR measurements were performed in the brain or skeletal muscle in vivo, in which the size of intra- and extracellular spaces was comparable, such as 4:1 or 3:2 (1). On the other hand, in the experiments of Dr. Burstein, the measurements were probably undertaken employing the perfusing system described in her previous work (2). In this system, the frog heart is perfused in a large NMR tube which is filled with perfusate with or without shift reagent. This perfusing system, thus, consists of a very small intracellular space (the intracellular space of the heart) and a huge extracellular space (the extracellular space of the heart plus extra-tissue space). Because of such a tremendous difference of the intra- and extracellular volumes, the intracellular sodium resonance is so small compared with the extracellular resonance that the intracellular one is invisible, unless the spectrum around 0 ppm is scaled up vertically by a factor of 25 (Fig. 1 *b* in her letter) or 200 (2). In Fig. 1 *a* in Dr. Burstein's letter, the proton spectra obtained after $Dy(TTHA)^{3-}$ seem to show no intracellular resonance at unshifted position. However, this failure to demonstrate the intracellular resonance may be attributable to the small intracellular volume. Provided the spectra around 0 ppm are expanded vertically by a factor of 200 as done in her previous study (2), the intracellular resonance may be clearly visualized. The large shift of intracellular sodium resonance (1.2 ppm) shown in Fig. 1 *b* may be also attributable to such huge difference of intra- and extracellular volumes. It has been found in our experiments that the extent of intracellular signal shift due to bulk susceptibility effects correlates with the concentration of $Dy(TTHA)^{3-}$ and the size of extracellular space. Presumably, in the experiments of Dr. Burstein, the presence of huge amount of $Dy(TTHA)^{3-}$ in the extracellular space may be responsible for such a large shift of the intracellular resonance.

Dr. Burstein has pointed out that the separated signals in the paper of Naritomi et al. (1) may not necessarily represent pure intracellular or pure extracellular resonance. We agree with this view. In in vivo experiments, shift reagents may not fully penetrate into tissue, particularly in the brain. It is considered likely that the unshifted signal, which is actually showing a very small shift, represents the intracellular resonance plus small extent of extracellular resonance from regions of no $Dy(TTHA)^{3-}$ penetration, whereas the shifted signal represents pure extracellular resonance. Accumulation of similar in vivo studies employing shift reagents may be needed to assign the separated resonances more definitively.

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REFERENCES

1. Naritomi, H., M. Kanashiro, M. Sasaki, Y. Kuribayashi, and T. Sawada. 1987. In vivo measurements of intra-and extracellular Na^+ and water in the brain and muscle by nuclear magnetic resonance spectroscopy with shift reagent. *Biophys. J.* 52:611–616.
2. Burstein, D., and E. T. Fossel. 1987. Nuclear magnetic resonance studies of intracellular ions in perfused frog heart. *Am. J. Physiol.* 252:H138–H146.

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