IN VIVO MEASUREMENTS OF INTRA- AND EXTRACELLULAR Na^+ AND WATER IN THE BRAIN AND MUSCLE BY NUCLEAR MAGNETIC RESONANCE SPECTROSCOPY WITH SHIFT REAGENT

HIROAKI NARITOMI,* MASARU KANASHIRO,‡ MASAHIRO SASAKI,* YOSHIKAZU KURIBAYASHI,* AND TOHRU SAWADA§

Cerebral Circulation Laboratory, ‡Nuclear Magnetic Resonance Laboratory, and §Department of Neurology, National Cardiovascular Center, 5-7-1 Fujishiro-dai, Suita, Osaka 565, Japan

ABSTRACT The introduction of new paramagnetic shift reagents in the nuclear magnetic resonance (NMR) method has made it possible to distinguish intra- and extracellular ions in tissues or organs in vitro. We measured the intra- and extracellular ^{23}Na and ^{1}H in vivo in the gerbil brain and skeletal muscle by NMR spectroscopy employing the shift reagent, dysprosium triethylenetetraminehexaacetate ($Dy[TTHA]^{3-}$). Without $Dy(TTHA)^{3-}$, the ^{23}Na and ^{1}H signals were seen only as single peaks, but gradual intravenous infusion of $Dy(TTHA)^{3-}$ separated these signals into two peaks, respectively. The unshifted peaks reflected the intracellular ^{23}Na and ^{1}H signals, while the shifted peaks reflected the extracellular signals. In the brain spectra, an additional small peak, which represented intravascular signals, was detected and its intensity increased after injection of papaverine hydrochloride. The present method is advantageous over the microelectrode technique because of its nondestructiveness and its capability for obtaining intra- and extracellular volume information from measurements of the ^{1}H spectra, the peaks of which reflect the intra- and extracellular water amounts. The intracellular Na^+ increase associating with increased cellular volume after ouabain in the muscle was clearly visualized by this method. The technique is clearly of use for physiological and pathophysiological studies of organs.

INTRODUCTION

Measurement of intra- and extracellular ions in vivo is important for understanding the physiological and biochemical nature of organs. The microelectrode technique is often employed for measuring the intracellular ion concentration in various organs in vivo. However, this technique is not only destructive but also difficult to apply in certain organs, such as the brain, in which the cells are very small.

In vivo NMR spectroscopy is a useful tool for investigating the nature of organs noninvasively. Although the conventional NMR method is unable to distinguish intra- and extracellular ions, the introduction of new paramagnetic reagents (Gupta and Gupta, 1982; Balschi et al., 1982; Pike and Springer, 1982) has made it possible to separate the intra- and extracellular signals. The shift reagents usually do not cross the normal cell membrane and cause a shift in signal position of the extracellular ions selectively. The intra- and extracellular signals are thus separated into two peaks, as demonstrated in vitro in cells (Gupta and Gupta, 1982; Balschi et al., 1982; Ogino et al., 1983; Gupta et al., 1985; Castle et al., 1986), tissues (Gupta and Gupta, 1982; Civan et al., 1983; Gullans et al., 1985; Rayson and Gupta, 1985), or perfused organs (Pike et al., 1985). Paramagnetic shift reagents however are generally toxic, and probably for such reasons, in vivo separation of intra- and extracellular ions in organs has not yet been successfully accomplished by NMR spectroscopy.

The toxicity of $Dy(TTHA)^{3-}$, one of the new shift reagents, is known to be moderate (Pike et al., 1985), although its shift effect is rather small (Chu et al., 1984). We found in a preliminary study that gradual intravenous infusion of $Dy(TTHA)^{3-}$ in experimental animals causes little suppressive effects on cardio-respiratory function. Employing this reagent, the intra- and extracellular ^{1}H and ^{23}Na were measured in the gerbil brain and leg muscles in vivo using an NMR spectrometer.

METHODS

37 mongolian gerbils, comprising 33 adults that weighed 70–80 g and four neonates (1 wk after birth) that weighed 7–9 g, were anesthetized by intraperitoneal injection of 40 mg/kg of pentobarbital (Nembutal;

Address all correspondence to Hiroaki Naritomi, M.D., Cerebral Circulation Laboratory, National Cardiovascular Center, 5-7-1 Fujishiro-dai, Suita, Osaka 565, Japan.

Abbott Laboratories, Irving, TX). PE-10 polyethylene catheters (Intramedic; Clay Adams, Div. of Becton, Dickinson & Co., Parsippany, NJ) were introduced into the right femoral artery and vein. The arterial blood pressure was monitored continuously on an RM-6000 polygraph (Nihon Kohden Co., Tokyo, Japan), and the arterial blood gases were measured before and 2 h after $Dy(TTHA)^{3-}$ infusion.

Stock solution of $Dy(TTHA)^{3-}$ (300 mM) was prepared from $DyCl_3 \cdot 6H_2O$ (Wako Pure Chemical Industries Ltd., Osaka, Japan) and H_6TTHA (Dojin Chemical, Kumamoto, Japan) according to the procedures described by Chu et al. (1984) as the sodium salt, $Na_3Dy(TTHA) \cdot 3NaCl$. The pH of the solution was adjusted to 7.35–7.40. At the beginning of the experiments, conventional ^1H and ^{23}Na spectral measurements were carried out on the brain or left femoral muscle. Thereafter, $Dy(TTHA)^{3-}$ was slowly infused from the right femoral vein (10 μl/min in adults, 1 μl/min in neonates) for 2 h, and the intra- and extracellular signals were separated. After 2 h, 0.5 μl/min of infusion was continued to maintain the $Dy(TTHA)^{3-}$ concentration constant in the adults.

In four animals, ^{31}P spectra of the leg muscle were obtained before and after the infusion to assess the effects of $Dy(TTHA)^{3-}$ on the muscular energy metabolism. In another four animals, the brain ^{31}P spectral measurements with electroencephalogram (EEG) recording by an OEE-7102 EEG trend monitor (Nihon Kohden Co., Tokyo, Japan) were carried out during the infusion to determine the effects of $Dy(TTHA)^{3-}$ on the cerebral energy metabolism and function. After separation of the intra- and extracellular signals, 0.6 mg/kg of ouabain (Uabanin, Takeda Pharmaceutical Co., Osaka, Japan), an Na^+-K^+-ATPase inhibitor, or 1 mg/kg of papaverine hydrochloride (Wako Pure Chemical Industries Ltd., Osaka, Japan), a potent vasodilator, was injected in 10 animals, and the changes in the spectra were investigated.

In vivo NMR spectra of the brain or leg muscles were obtained with a JEOL JNM-270 spectrometer (Nihon Denshi Co., Tokyo, Japan, 6.34 Tesla), by means of a 10-mm-diam surface coil in a 70-mm-diam probe with a Fourier transform mode, operating at 269.6 MHz for ^1H, 71.2 MHz for ^{23}Na, and 109.0 MHz for ^{31}P. The spectra were collected as the free-induction decays (FIDs) using quadrature phase detection, and were digitized and processed with a computer. ^1H spectra were obtained as 40 time-averaged FIDs, ^{23}Na spectra as 500 time-averaged FIDs, and ^{31}P spectra as 400 time-averaged FIDs using repetition times of 1.8, 0.6, and 2.0 s, respectively.

RESULTS

After the infusion of $Dy(TTHA)^{3-}$, no significant change occurred in the arterial PO_2, PCO_2, and pH. The blood pressure was reduced moderately from 72 ± 8 mmHg to 58 ± 12 mmHg after 2 h of infusion. $Dy(TTHA)^{3-}$ infusion produced some broadening of the ^{31}P spectra, however no change in the intensity of the phosphocreatine, ATP, and inorganic phosphate peaks was detected in the brain and leg muscle. The intracellular pH also remained unchanged after $Dy(TTHA)^{3-}$. EEG revealed no change throughout the infusion in all four animals undergoing EEG recording.

Before $Dy(TTHA)^{3-}$, the ^{23}Na (Figs. 1 *a* and 3 *a*) and ^1H (Figs. 2 *a* and 4 *a*) spectra exhibited merely a single peak. In the leg muscle, the ^{23}Na and ^1H peaks became separated into two peaks after $Dy(TTHA)^{3-}$; one remained unshifted, while the other shifted (Figs. 1 *b* and 2 *b*). The unshifted peak corresponded to the intracellular signals, and the shifted peak corresponded to the extracellular signals. In the ^{23}Na spectra, the intensity of the intracellular signal was far smaller than that of the extracellular signal (Fig. 1 *b*). On the other hand, in the ^1H spectra, the intensity of intracellular signal exceeded that of the extracellular signal, the ratio being ~6:4 (Fig. 2 *b*). Intravenous injection of ouabain caused an enormous increase in the intracellular ^{23}Na signal (Fig. 1 *c*). At the same time, the intracellular ^1H signal was increased to a small extent (Fig. 2 *c*).

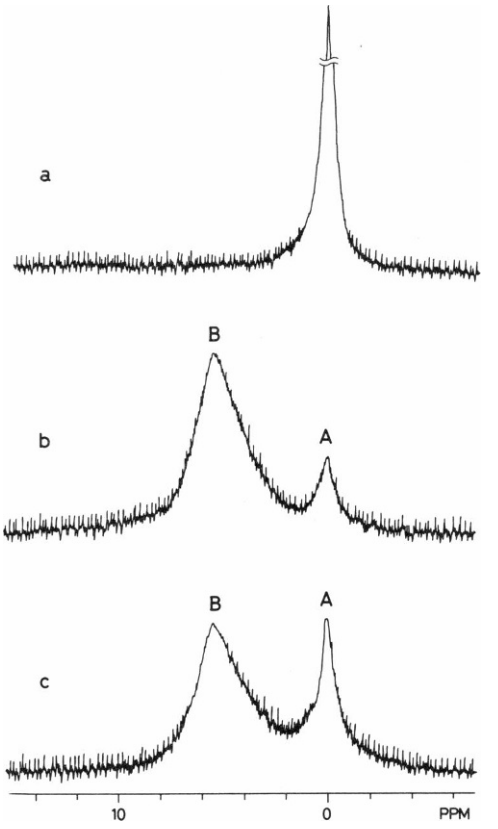

FIGURE 1 ^{23}Na-NMR spectra of leg muscles. (*a*) Before $Dy(TTHA)^{3-}$ infusion, only a sharp single peak was observed. (*b*) 2 h after $Dy(TTHA)^{3-}$ infusion, the signal was separated into unshifted (*A*) and shifted (*B*) peaks. The peaks *A* and *B* represent the intra- and extracellular Na^+. (*c*) 30 min after ouabain injection, the intracellular Na^+ (peak *A*) increased markedly.

In the brain spectra, the ^{23}Na (Fig. 3 *b*) and ^1H (Fig. 4 *b*) signals were separated into three peaks after $Dy(TTHA)^{3-}$; one was unshifted, another moderately shifted, and the third highly shifted. These three peaks were observed in both adults and neonates, although the two shifted peaks were somewhat smaller in the neonates. The unshifted peak undoubtedly reflected the intracellular ^{23}Na and ^1H signals. The highly shifted peak was small and probably reflected signals from the intravascular space, the $Dy(TTHA)^{3-}$ concentration of which is the highest. The intensity of the highly shifted peak increased markedly after papaverine hydrochloride injection (Figs. 3 *c* and 4 *c*). The moderately shifted peak probably reflected extravascular–extracellular signals. In the ^1H spectra of

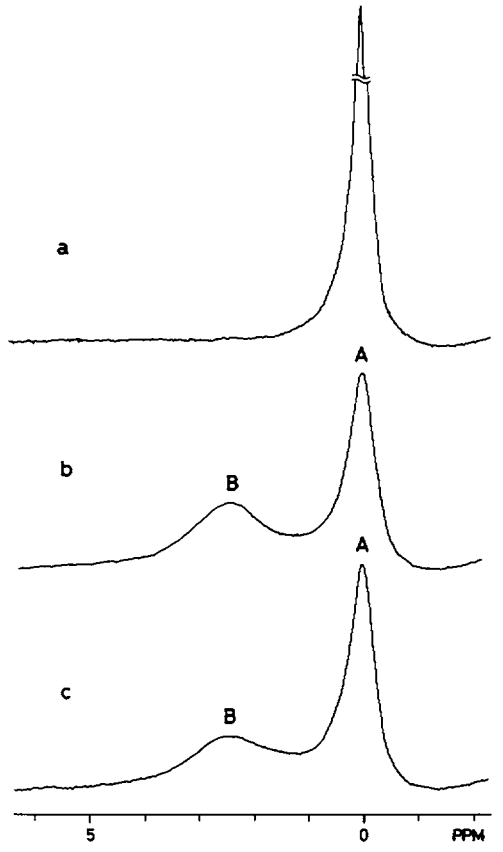

FIGURE 2 ^{1}H-NMR spectra of leg muscles. (*a*) Before Dy(TTHA)$^{3-}$ infusion. (*b*) 2 h after Dy(TTHA)$^{3-}$ infusion, the signal was separated into unshifted (*A*) and shifted (*B*) peaks. The peaks *A* and *B* represent the intra- and extracellular ^{1}H, which are almost equal to the intra- and extracellular water. (*c*) 40 min after ouabain injection, the peak *A* increased moderately, suggesting an increase in cellular volume.

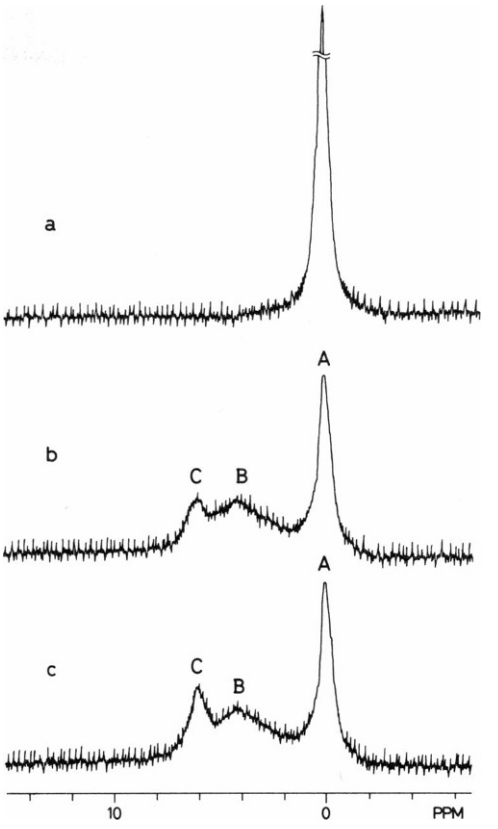

FIGURE 3 ^{23}Na-NMR spectra of the brain. (*a*) Before Dy(TTHA)$^{3-}$ infusion. (*b*) 2 h after Dy(TTHA)$^{3-}$ infusion. (*c*) 5 min after papaverine hydrochloride injection. The peaks *A*, *B*, and *C* represent the intracellular, extravascular–extracellular, and intravascular Na^{+}, respectively. 5 min after papaverine hydrochloride injection, the intravascular Na^{+} (peak *C*) increased markedly.

the brain (Fig. 4 *c*), the intensity ratio of the unshifted peak and two shifted peaks was ~8:2.

DISCUSSION

Although various paramagnetic shift reagents are employed in in vitro NMR studies to separate the intra- and extracellular signals, most of these reagents are toxic and cannot be used in in vivo experiments. In our preliminary study, intravenous infusion of shift reagents other than Dy(TTHA)$^{3-}$, such as dysprosium tripolyphosphate, was found almost always to result in rapid cardio-respiratory arrest of the animals. On the other hand, the toxicity of Dy(TTHA)$^{3-}$ is moderate, as reported by Pike et al. (1985). They separated the intra- and extracellular ^{23}Na and ^{39}K signals of perfused beating rat hearts by introducing this reagent into the perfusing medium. Dy(TTHA)$^{3-}$ entered all the extracellular space of the hearts and brought about a downfield shift in the extracellular signals without functional suppression.

In the present study, highly concentrated Dy(TTHA)$^{3-}$ was slowly infused intravenously for more than 2 h. This gradual infusion avoided suppression of the cardio-respira-

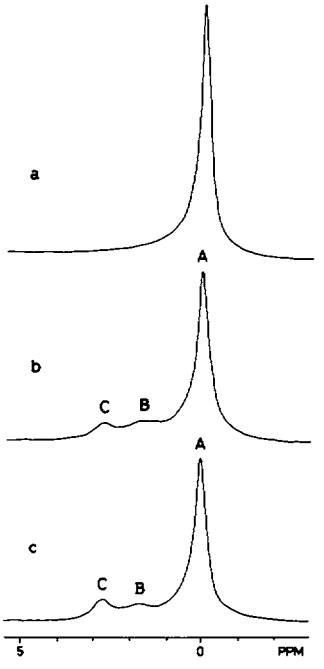

FIGURE 4 ^{1}H-NMR spectra of the brain. (*a*) Before Dy(TTHA)$^{3-}$ infusion. (*b*) 2 h after Dy(TTHA)$^{3-}$ infusion. (*c*) 15 min after papaverine hydrochloride injection. The peaks *A*, *B*, and *C* represent the intracellular, extravascular–extracellular, and intravascular water, which indicate the volume ratio of each space. 15 min after papaverine hydrochloride, the intravascular ^{1}H (peak *C*) increased markedly, suggesting an increase in blood volume.

tory function or impairment of the cerebral and muscular energy metabolism leading successfully to downfield shifts of the extracellular ^{23}Na and ^{1}H signals in vivo. Although the blood pressure decreased by ~14 mmHg after $Dy(TTHA)^{3-}$, this moderate decrease may be permissible for physiological and pathophysiological investigations of ionic homeostasis.

The separation of signals provided by such methods yields information concerning the relative amount of ^{23}Na or ^{1}H existing in the intra- and extracellular spaces, although the intra- or extracellular Na^+ concentration is not determined. In the ^{23}Na spectra, the intensity of the unshifted and shifted peaks essentially indicated the intra- and extracellular Na^+ amounts. In the present study, the proportion of the intra- and extracellular ^{23}Na signals was considerably smaller compared with that of the intra- and extracellular ^{1}H signals both in the muscle and brain spectra. This probably reflects the fact that the intracellular Na^+ concentration is far lower than the extracellular Na^+ concentration in these tissues (Walker and Brown, 1977). This result may be attributed partly to the invisibility of intracellular ^{23}Na, which is encountered characteristically in the NMR methods. With the NMR methods, the intracellular ^{23}Na in various tissues or cells is known to be partly invisible (Cope, 1967; Ogino et al., 1983; Gupta et al., 1985; Castle et al., 1986), while the extracellular ^{23}Na is totally visible. The degree of invisibility differs according to the cells and tissues. For instance, the degree of invisibility seems to be greater in the skeletal muscle than in the brain (Cope, 1967). This invisibility is usually explained by "bound" ^{23}Na (Cope, 1967) or quadrupolar interactions of the ^{23}Na nucleus (Berendsen and Edzes, 1973). The intracellular Na^+ amount may be somewhat underestimated by this method. In the present study, however, the intracellular ^{23}Na signal of the skeletal muscle was increased enormously after ouabain injection. This apparently indicates an accumulation of Na^+ within the cells due to inhibition of Na^+-K^+-ATPase activity. An intracellular Na^+ increase after ouabain was also demonstrated in in vitro ^{23}Na NMR studies by Rayson and Gupta (1985), Pike et al. (1985), and Gullans et al. (1985). Thus, although some problems do exist concerning intracellular ^{23}Na invisibility, the present NMR method reflects the changes in intra- and extracellular Na^+ amount, qualitatively.

In the case of the ^{1}H nucleus, the problem of NMR invisibility fortunately does not exist, so that ^{1}H spectral measurements provide more useful information than ^{23}Na spectral measurements. In the ^{1}H spectra, the unshifted and shifted peaks indicate the intra- and extracellular ^{1}H amount, which is almost equal to the intra- and extracellular water amount. The water amount in each space parallels the volume of space, so that intra- and extracellular fluid volume changes can be estimated from the ^{1}H spectra. This affords great advantages to the present NMR method, since no appropriate method for measuring the intra- and extracellular volume of organs in vivo presently exists. In the present study, a small but definitive increase in intracellular ^{1}H signal was detected after ouabain injection in the leg muscle. This indicates an increase in intracellular volume. It has been well documented in the physiological literature that inhibition of Na^+-K^+-ATPase activity causes not only an intracellular Na^+ increase, but also a cellular volume increase (Macknight and Leaf, 1977). This conclusion was reached by accumulating in vitro data alone. The present study has now confirmed a cellular volume increase after giving Na^+-K^+-ATPase inhibitor in vivo.

As mentioned above, semiquantitative information concerning the intra- and extracellular volume is obtained by separation of the ^{1}H signals. Thus, if the intra- and extracellular volumes estimated from the ^{1}H spectra are compared with those obtained by other methods, the reliability of the NMR method can be evaluated. The leg muscle spectra in the present study yielded a ratio of intra- and extracellular signals of ~6:4. Previously, the extracellular space of skeletal muscle was measured by several workers using radioactive tracers in vitro. The results obtained in such studies differed considerably depending on the tracers employed, the time for tracer equilibration, and animal species or circulatory condition at the time of measurement. The extracellular volume was found to be 20% of the total muscle volume in rats by Katz et al. (1970), 8–11% in rats by Reed (1981), and 12–52% in mice by Sheff and Zacks (1982). Compared with these values, the proportion of extracellular signal in the leg muscle ^{1}H spectra is somewhat larger. This is either due to overestimation of the extracellular volume in the present method because of penetration of $Dy(TTHA)^{3-}$ into the intracellular space, or to some other cause related to the conditions of measurement. The first possibility seems unlikely, since impermeability of $Dy(TTHA)^{3-}$ across the normal muscle cell membrane has been confirmed by Pike et al. (1985) in vitro. Also in the present study, the results for the ^{31}P spectra and the changes in ^{23}Na spectra after ouabain injection indicate that the cellular energy metabolism as well as the membrane activity of the leg muscle are maintained in a normal state which is unlikely to permit penetration of $Dy(TTHA)^{3-}$ into the intracellular space. It is known that the extracellular volume of skeletal muscle is greatly altered by circulatory changes. For instance, the extracellular volume of rat leg muscle was found to be increased enormously after venous congestion (Reed, 1981). The measurements of the muscle spectra in the present study were undertaken by tying the leg muscle tightly to the probe in order to avoid noise attributable to movement, so that a considerable degree of venous congestion must have resulted. The relatively greater value for the proportion of extracellular signal in the muscle ^{1}H spectra can probably be attributed to this situation. In any case,

the results for the leg muscle ^1H spectra in the present study suggest that $Dy(TTHA)^{3-}$ enters into the entire extracellular space of the muscle and causes the extracellular signals to shift sufficiently.

In the brain ^1H spectra, the proportion of intra- and extracellular signals was ~8:2. From the technical standpoint, measurement of the extracellular space of the brain is more difficult than that of other organs because of the existence of the blood–brain barrier (BBB), which prevents the entrance of various substances into the extravascular space. Thus far there are limited data concerning the extracellular volume of the brain. Bondareff and Narotzky (1972) estimated the extracellular space of rat brain by electron microscopy, and reported a value of 21%. Levin et al. (1970) measured the extracellular volume of the cerebral cortex in rabbits, dogs, cats, and monkeys in vitro by injecting radioactive tracers into the subarachnoid space. The extracellular volume obtained in their study was 17–20% of the total brain volume. Hossmann (1971) assessed the extracellular space of cat brain in vivo by the cortical specific impedance method and reported a value of 19%. Thus, the extracellular volume of the brain as measured by three different methods is broadly consistent. The proportion of extracellular signals in the brain ^1H spectra also demonstrates a very close agreement with these values suggesting that the in vivo separation of intra- and extracellular signals of the brain by the NMR method is highly reliable. Before the present study, we had some doubt regarding the permeability of $Dy(TTHA)^{3-}$ through the BBB. However, as discussed above, $Dy(TTHA)^{3-}$ seems to pass well through the BBB entering the extracellular space of the brain and causing a sufficient shift of the extracellular signals. Good permeability of $Dy(TTHA)^{3-}$ through the BBB is also indicated by a comparison of the brain spectra for adults and neonates. In neonates, the formation of the BBB is incomplete, so that $Dy(TTHA)^{3-}$ should be able to enter into the extravascular space rather freely. In fact, in the present study, the neonatal brain spectra revealed a considerable intensity of signal shift for both ^{23}Na and ^1H. However, the intensity of the shifted signals in the adult brain was greater than that of the neonatal brain. This apparently indicates that the existence of the BBB does not largely prevent the entrance of $Dy(TTHA)^{3-}$ into the extracellular space. The results for the adult and neonatal brain spectra probably indicate that the extracellular volume of the adult brain is essentially larger than that of the neonatal brain.

A brief comment should be made concerning the highly shifted small peak observed in the brain spectra, which probably reflects the intravascular ^{23}Na and ^1H signals. When the adult and neonatal brain spectra were compared, this peak was far more marked in the adult spectra. Furthermore, this small peak was never seen in the muscle spectra. Thus, manifestation of this small peak would seem to be related to the existence of the BBB or maturation of the BBB. It is assumed that in the adult brain, the BBB presents some limitation to the transport of $Dy(TTHA)^{3-}$ from the intra- to extravascular space producing a concentration gradient of $Dy(TTHA)^{3-}$ between the two spaces. This concentration gradient of $Dy(TTHA)^{3-}$ between the two spaces is likely to yield two shifted peaks in the adult brain spectra.

The present paper thus confirms that in vivo separation of intra- and extracellular ^{23}Na and ^1H signals by the NMR method is possible by the application of $Dy(TTHA)^{3-}$ infusion and provides useful information for physiological and biochemical studies. Unfortunately, the extent of intra- and extracellular signal discrimination obtained in the present experiments was by no means perfect because of the small shift effect of $Dy(TTHA)^{3-}$, and hence a quantitative analysis of the separated signals could not be carried out adequately. It is expected that the development of new paramagnetic shift reagents in the future will afford a better separation of intra- and extracellular signals and so increase the usefulness of the in vivo NMR method.

Received for publication 12 January 1987 and in final form 17 June 1987.

REFERENCES

Balschi, J. A., V. P. Cirillo, and C. S. Springer, Jr. 1982. Direct high-resolution nuclear magnetic resonance studies of cation transport in vivo. Na^+ transport in yeast cells. *Biophys. J.* 38:323–326.

Berendsen, H. J. C., and H. T. Edzes. 1973. The observation and general interpretation of sodium magnetic resonance in biological material. *Ann. NY Acad. Sci.* 204:459–485.

Bondareff, W., and R. Narrotzky. 1972. Age changes in the neuronal microenvironment. *Science (Wash. DC).* 176:1135–1136.

Castle, A. M., R. M. Macnab, and R. G. Shulman. 1986. Measurement of intracellular sodium concentration and sodium transport in *Escherichia coli* by ^{23}Na nuclear magnetic resonance. *J. Biol. Chem.* 261:3288–3294.

Chu, S. C., M. M. Pike, E. T. Fossel, T. W. Smith, J. A. Balschi, and C. S. Springer. 1984. Aqueous shift reagents for high-resolution cationic nuclear magnetic resonance. III. $Dy(TTHA)^{3-}$, $Tm(TTHA)^{3-}$, and $Tm(PPP)_2^{7-}$. *J. Magn. Res.* 56:33–47.

Civan, M. M., H. Degani, Y. Margalit, and M. Shporer. 1983. Observations of ^{23}Na in frog skin by NMR. *Am. J. Physiol.* 246:C213–219.

Cope, F. W. 1967. NMR evidence for complexing of Na^+ in muscle, kidney, and brain, and by actomyosin. The relation of cellular complexing of Na^+ to water structure and to transport kinetics. *J. Gen. Physiol.* 50:1353–1375.

Gullans, S. R., M. J. Avison, T. Ogino, G. Giebisch, and R. T. Shulman. 1985. NMR measurements of intracellular sodium in the rabbit proximal tubule. *Am. J. Physiol.* 249:F160–168.

Gupta, R. K., and P. Gupta. 1982. Direct observation of resolved resonances from intra- and extracellular sodium-23 ions in NMR studies of intact cells and tissues using dysprosium(III)tripolyphosphate as paramagnetic shift reagent. *J. Magn. Res.* 47:344–350.

Gupta, R. K., A. B. Kostellow, and G. A. Morrill. 1985. NMR studies of intracellular sodium ions in amphibian oocytes, ovulated eggs, and early embryos. *J. Biol. Chem.* 260:9203–9208.

Hossmann, K.-A. 1971. Cortical steady potential, impedance and excitability changes during and after total ischemia of cat brain. *Exp. Neurol.* 32:163–175.

Katz, J., A. Sellers, G. Bonorris, and S. Golden. 1970. Studies on the extravascular albumin of rats. *In* Plasma Protein Metabolism. M. A. Rothschild and T. Waldmann, editors. Academic Press, Inc., New York and London. 129–154.

Levin, V. A., J. D. Fenstermacher, and C. S. Patlak. 1970. Sucrose and inulin space measurements of cerebral cortex in four mammalian species. *Am. J. Physiol.* 219:1528–1533.

Macknight, A. D. C., and A. Leaf. 1977. Regulation of cellular volume. *Phys. Rev.* 57:510–573.

Ogino, T., J. A. den Hollander, and R. G. Schulman. 1983. ^{39}K, ^{23}Na, and ^{31}P NMR studies of ion transport in *Saccharomyces cerevisiae*. *Proc. Natl. Acad. Sci. USA.* 80:5185–5189.

Pike, M. M., and C. S. Springer, Jr. 1982. Aqueous shift reagents for high-resolution cationic nuclear magnetic resonance. *J. Magn. Res.* 46:348–353.

Pike, M. M., J. C. Frazer, D. F. Dedrick, J. S. Ingwall, P. D. Allen, C. S. Springer Jr., and T. W. Smith. 1985. ^{23}Na and ^{39}K nuclear magnetic resonance studies of perfused rat hearts. Discrimination of intra- and extracellular ions using a shift reagent. *Biophys. J.* 48:159–173.

Rayson, B. M., and R. K. Gupta. 1985. ^{23}Na NMR studies of rat outer medullary kidney tubules. *J. Biol. Chem.* 260:7276–7280.

Reed, R. K. 1981. Interstitial fluid volume, colloid osmotic and hydrostatic pressures in rat skeletal muscle. Effect of venous stasis and muscle activity. *Acta. Physiol. Scand.* 112:7–17.

Sheff, M. F., and S. I. Zacks. 1982. Interstitial space of mouse skeletal muscle. *J. Physiol. (Lond.).* 328:507–519.

Walker, J. L., and H. M. Brown. 1977. Intracellular ionic activity measurements in nerve and muscle. *Phys. Rev.* 57:729–778.