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Co^{2+} AND Mn^{2+} UPTAKE BY CRAB NERVE FIBERS IN RESTING STATE AND POTASSIUM DEPOLARIZATION

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Transition metal ions, Mn^{2+} and Co^{2+}, are incorporated into nerve fibers when they are applied externally. For nerve fibers in the resting state, however, extracellular and intracellular water may be distinguished by applying transition metal ions externally. NMR spectra of water protons from nerve fibers in high potasssium media, which contain transition metal ions, consist of three or more components, reflecting a complex distribution of these ions around the nerve membranes. In the case of Co^{2+}, three components may be identified.

Transition metal ions, Mn^{2+} and Co^{2+}, are regarded as Ca-channel blockers when applied externally.[1] Biochemical roles of Mn^{2+} are also well known.[2] In nuclear magnetic resonance (NMR) spectroscopy, these paramagnetic ions have been used as shift reagents to modify extracellular signals so that intracellular signals are distinguished from extracellular ones. Water content change during potassium depolarization in frog sciatic nerves was studied using this method.[3,4] However, the conclusion of NMR study[3] appeares to be contrary to what is observed in crustacean nerve fibers during potassium depolarization by volume measurement. Namely, the water content of crustacean nerve fibers increased during potassium depolarization, whereas a water content reduction was reported by NMR method.

Because these transition metal ions have biological effects, it is unlikely that interactions between these ions and the biological membranes can be ignored. We studied distribution of Co^{2+} and Mn^{2+} inside and outside of the crab nerve fibers in the resting state and during potassium depolarization by radioisotope uptake and water proton signal of nuclear magnetic resonance spectroscopy. We found that a considerable uptake of Co^{2+} and Mn^{2+} takes place by crab nerve

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fibers, and the interpretation of water proton signal in NMR spectra is not as simple as has been considered.

MATERIALS AND METHODS

Nerve fibers were dissected from claws of the crab, Collinectes sapidus. The nerve fibers were cleaned of blood vessels and gross connective tissues under a dissection microscope. The dissected nerve fibers were about 45 mm long, and they weighed 60 mg on the average.

The control external medium contained 423 mM NaCl, 9 mM KCl, 9.3 mM $CaCl_2$, 22.9 mM $MgCl_2$, 25.5 mM $MgSO_4$ and 10 mM Tris-HCl buffer (pH 7.5). Co^{2+}- and Mn^{2+}-added medium were prepared by dissolving $CoCl_2$ and $MnCl_2$, respectively, with the control medium. Stimulation threshold and conduction velocity of action potentials in the crab nerve fibers were not altered in the medium which contained 5 mM $CoCl_2$ or 5 mM $MnCl_2$ for at least 2 hours. Other solutions, e. g., 0.53 M KCl, 0.45 M KCl plus 50 mM $CaCl_2$, and 0.48 M KCl plus 50 mM $MgCl_2$, used as external media in the experiment, also contained 10 mM Tris-HCl buffer (pH 7.5).

Radionuclides, ^{54}Mn and ^{57}Co were purchased from New England Nuclear. Both were carrier free and were in 0.5 M HCl solutions. These solutions were neutralized by adding equal volumes of 0.5 M NaOH solution and were then added to external media. Radioisotope uptake experiment was carried out in media of activity 0.5 μ Ci/ml.

Crab nerve fibers were weighed and then incubated in media which contained ^{54}Mn or ^{57}Co. The nerve fibers were then washed with 0.81 M sucrose solution for 10 s to remove extracellular ions, and cut into three parts, the center part being half of the entire length. Activities of the central and end segments were counted separately. The activity values thus obtained were normalized with respect to the initial nerve mass as well as to the activity of the external media. The values given in the Table 1 are for central sections and were calculated as follows:

$$\frac{(\text{nerve count per min.})/(\text{initial nerve mass})}{(\text{solution count per min.})/(\text{solution mass})} \times 100. \qquad (1)$$

Water proton NMR spectra were taken at 500 MHz on a Nicolet NT-500 spectrometer operating in the Fourier transform mode. Typical spectra were obtained by using 8- μ s pulse, a sweep width of $\pm$ 5 kHz, an aquisition time of 2.55 s. The nerve fibers were tied to a capillary (outer diameter 1 mm) and then immersed in external media, which contained 5 mM $CoCl_2$ or $MnCl_2$. For each spectroscopic measurement capillary with nerve fibers was lifted from external media and placed in a glass NMR tube (internal diameter 4 mm). The external solutions contained sodium 3-trimethylsilyl propionate(TSP) as internal reference, and the capillary contained benzene as external reference. The external reference was used to determine the amount of water in the preparations.

The temperature was maintained between 22° to 24° during the experiment.

Results and Discussion

Mn^{2+} and Co^{2+} uptake

Table 1 shows that the normalized ^{54}Mn uptake calculated according to formula (1) was highly dependent on cold $MnCl_2$ concentration in the external media. When cold $MnCl_2$ concentration was 5mM, ^{54}Mn uptake during potassium depolarization was not sensitive to Ca^{2+} concentration. The difference in the uptake by the resting and potassium depolarized nerve fibers was large. When cold $MnCl_2$ concentration was

Table 1. Mn^{2+} and Co^{2+} uptake by crab nerve fibers (1 hr).*

^{54}Mn uptake				
cold $MnCl_2$ concentration	control	0.53 M KCl solution	0.45 M KCl +50 mM $CaCl_2$	0.48 M KCl +50 mM $MgCl_2$
5 mM	6 ± 1	54 ± 1	54 ± 4	62 ± 8
0.5 mM	14 ± 3	62 ± 8	52 ± 6	110 ± 20
< 1 nM	55 ± 23	154 ± 54	90 ± 8	160 ± 1

^{57}Co uptake			
cold $CoCl_2$ concentration	control medium	0.53 M KCl solution	0.45 M KCl +50 mM $CaCl_2$
5 mM	33 ± 1	84 ± 1	66 ± 10

*These values are normalized to the activity of the external media using formula (1) in Materials and Methods.

lower, ^{54}Mn uptake during potassium depolarization was highly dependent on external Ca^{2+} concentration: less uptake took place when Ca^{2+} concentration was higher. When Mn^{2+} concentration was less than 1 nM, Mn^{2+} uptake was sensitive to Ca^{2+} concentration also for nerve fibers in the resting state. In 0.53 M NaCl solution, a value 96 ± 10 was obtained for 1 hour Mn^{2+} uptake, to be compared with 55 ± 23 in the control medium, which contained divalent cations including 9.3 mM $CaCl_2$. Mg^{2+} was less effective than Ca^{2+} in reducing the uptake.

The ratio of Mn^{2+} uptake by K-depolarized nerve fibers to that by resting nerve fibers was smaller when Mn^{2+} concentarion was lower. This observation may suggest adsorption of Mn^{2+} ions by external tisses. To examine this possibility, time course of the uptake was studied. Uptake of Mn^{2+} did not level off in three hours. This observation is consistent with the interpretation that these ions entered into axons, and not adsorbed by external tissues.

^{57}Co uptake showed a trend similar to ^{54}Mn uptake. However, the ratio of Co^{2+} uptakes by potassium-depolarized nerve fibers to the uptake by resting nerve fibers was less than that for Mn^{2+}.

The normalization formula (1) needs an adjustment when mass of nerve fibers are changed during the uptake experiment. For this reason we monitored mass of nerve

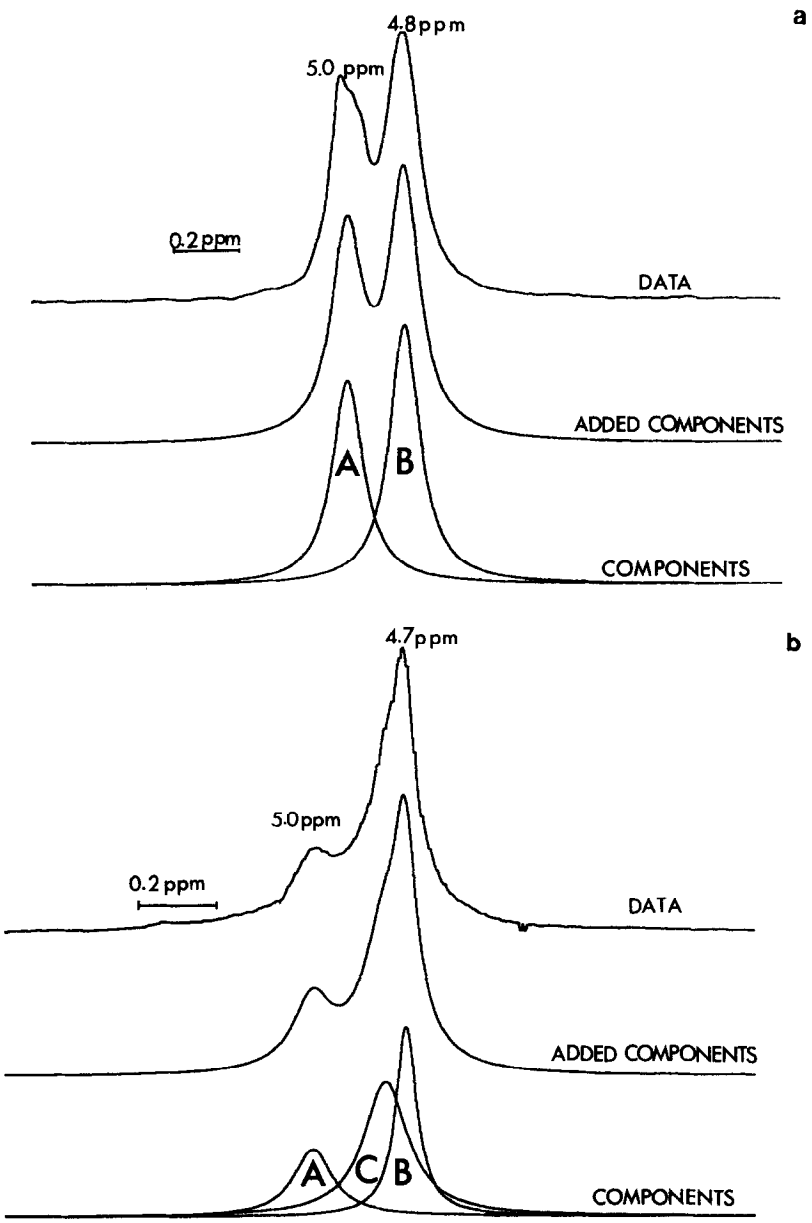

Fig. 1. Water proton signal from crab nerve fibers incubated in media which contained 5 mM $CoCl_2$.

1a: In control medium at 38 min. Curve fitting was tried with two Lorentzians A and B. Two peak positions are shown in ppm from TSP.

1b: In high-K medium (0.53 M KCl) at 32 min. Curve fitting was tried with three Lorentzians A, B, and C. Two peak positions are shown in ppm from TSP.

fibers. Since we could not remove extracellular water completely, we considered extracellular water as included in mass of nerve fibers. We estimated from NMR measurements that about 30% of the mass was extracellular. While mass of nerve fibers did not change in the control medium whether or not the medium contained 5 mM $CoCl_2$ or 5 mM $MnCl_2$, for more than three hours, the nerve mass increased in potassium-rich media. In 0.53 M KCl solution, it increased 60 % at 60 min. Addition of 5 mM $MnCl_2$ or 5 mM $CoCl_2$ to the KCl solution did not change amount of swelling. We did not observe any effect by isoosmolar replacement of a part of potassium chloride by 50 mM $CaCl_2$ or 50 mM $MgCl_2$. Thus, we can conclude that the large difference (about 3 fold or more) in Mn^{2+} and Co^{2+} uptake by nerve fibers in resting state and in K-depolarization cannot be attributed to volume changes per se.

NMR spectroscopy

Water proton signal from nerve fibers, which were incubated in Co^{2+}-containing media, are shown in Figure 1. Whereas there are only two lines in water spectra from nerve fibers incubated in control medium, three lines appeared from ones in high K-medium.

In the control medium, two lines (A and B) seem to represent intra- and extracellular water. Since the line A is shifted downfield, the part of water which give rise to the signal contains higher concentration of $CoCl_2$. Thus the line A is related to extracellular water. The line B, therefore, should represent intracellular water, which contains lower concentration of Co^{2+}. From this assignment we estimated external water to be (40 ± 10)% of the total. The intensities of these lines remained constant for 1 hr.(Fig. 2), but line B shifted downfield with time, indicating increased Co^{2+} concentration in the cells.

In high-K media, however, water signal consists of three Lorentzians (A, B, and C). Whereas the relative intensity of the two lines (A and B) of water signals from resting nerve fibers did not change with time, the intensity ratio of the two lines (B and C) from potassium depolarized nerve fibers changed (Fig. 2): the component C intensified with time in expense of B. The intensity of line A relative to the total water signal intensity did not change.

From this observation we may assign line A as from extracellular water, which contains highest concentration of Co^{2+}, line B as from water deeper inside cells,

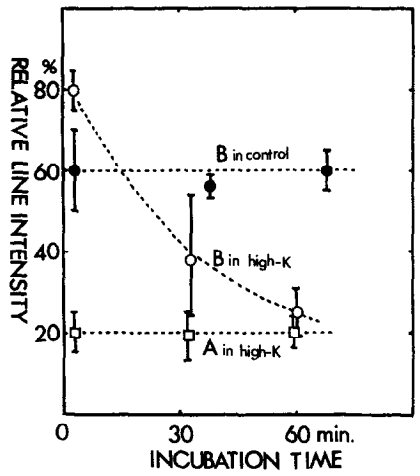

Fig. 2. Time course of line intensities relative to the total water signal. Lines A and B are obtained by curve fitting as shown in Fig. 1 (See text).

because line B shows least downfield shift. Line C represents a part of water which contains intermediate concentration of the paramagnetic ions. Since Co^{2+} uptake and intensity of line C increases with time, it is likely that peak C represents intracellular water near cell surface.

While the total water signal intensity remained constant for nerve fibers incubated in the Co^{2+}-containing control medium over an hour, the signal intensity increased 60% for nerve fibers incubated in 0.53 M KCl solution. This value is consistent with the one obtained by mass measurement described earlier.

Water signals from nerve fibers incubated in Mn^{2+}-containing media were also examined. As in the case of Co^{2+}-incubated nerve fibers, two Lorentzians sufficed to fit the water signal from nerve fibers in the control medium, but three Lorentzians were needed to fit the data from K-depolarized nerve fibers to the same degree. The signal from resting nerve fibers (i. e. in control medium) can be interpreted as consist of extracellular and intracellular water. The fraction of the extracellular water estimated by this assumption was (35 ± 5)%, which agrees with the value (40 ± 10)% obtained with Co^{2+}. However, we could not assign water signal from K-depolarized nerve fibers because of line broadening due to Mn^{2+}, in contrast to Co^{2+} which shifts but does not significantly broaden signals. Indeed the total water signal intensity evaluated from spectral area increased only 20% in

1 hour, whereas 60% increase was expected from mass measurement, indicating a difficulty in identifying and evaluating broadened signal intensities.

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