CARDIAC GLYCOSIDE-INDUCED ELEVATION OF INTRACELLULAR Na^+ ION CONCENTRATION IN HUMAN ERYTHROCYTES STUDIED BY ^{23}Na NMR SPECTROSCOPY: RELATIONSHIP BETWEEN INOTROPY SPEED AND ELEVATION RATE OF INTRACELLULAR Na^+ ION CONCENTRATION

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Elevation of intracellular sodium ion concentration in human erythrocyte induced by the cardiac glycoside, proscillaridin (**1**) , and its four derivatives (**2**-**5**) was measured using ^{23}Na NMR spectrometry. In this examination, there was a significant correlation between the time to half maximum inotropic effect and the time to maximum of Na^+ concentrations in human erythrocyte, determined by ^{23}Na NMR.

KEYWORDS cardiac glycoside; ^{23}Na NMR spectrometry; proscillaridin; inotropy speed; erythrocyte

Recently, intra- and extracellular sodium pools have been measured in biological systems without physically separating them by use of ^{23}Na NMR in combination with anionic paramagnetic shift reagents that do not cross plasma membrane.[2] Utilizing this technique, Na^+ ion transport facilitated by several ionophores and their derivatives have been investigated.[3] Cardiac glycosides such as proscillaridin (**1**) and ouabain are recognized to inhibit Na^+, K^+-ATPase, causing an increasing of intracellular Na^+ ion concentration that derives a Na^+, Ca^{2+} exchange system to develop the positive inotropic effect.[4] However, ^{23}Na NMR technique has seldom been applied to investigate concentration on change in intracellular Na^+ ion induced by cardiac ingredients.[5] Here we describe the relationship between inotropy speeds determined for isolated guinea-pig papillary muscle and the times required to reach maximum Na^+ concentration in human erythrocyte induced by proscillaridin (**1**) and its four derivatives, 20(*R*)-tetrahydroproscillaridin (**2**), 20(*S*)-tetrahydroproscillaridin (**3**), 21, 23-dihydroproscillaridin (**4**), and 22, 23-dihydroproscillaridin (**5**) measured by ^{23}Na NMR spectrometry.

MATERIALS AND METHODS

Materials Four derivatives of proscillaridin (**2**-**5**) were prepared by our described procedure.[6] The shift reagent, dysprosium (III) 1, 4, 7, 11-tetraazacyclododecane-*N*, *N'*, *N''*, *N'''*-tetramethylenephosphonate [$Dy(DOTP)^{5-}$], was prepared according to the reported manner.[7]

The Human Erythrocyte Suspension Human blood collected for clinical use and stored at 0°C for not more than 2 days was washed three times by centrifugation at 1800 *g* for 7 min with the medium containing 140 mM NaCl and 5 mM KCl at pH 7.4 adjusted by 5 mM Tris-HEPES buffer. Washed erythrocyte was separated from the plasma and buffy coat by aspiration and suspended again in the medium. The hematocrit value of the suspension was set to 0.4, and the suspension was stored at 0°C just

before ^{23}Na NMR measurement.

^{23}Na NMR Measurement The ^{23}Na NMR spectra were recorded using a JEOL EX-270 spectrometer and accumulated 128 times at 37°C, 71.32 MHz, a 90° pulse covering a sweep width of 7507.5 Hz. A concentric NMR tube combination (1 mm o. d. tube inside 5 mm o. d. NMR tube) was used in the experiments. The annular space between the inner and outer tubes contained 0.45 ml of human erythrocyte suspension, 0.05 ml of 50 mM $Dy(DOTP)^{5-}$ in water, and 5 μl of the test compound in dimethylsulfoxide (DMSO), while the inner tube containing 20 mM $Na_7Dy(PPPi)_2 \cdot 3NaCl$ solution was used as an external reference. The concentration of **1-5** was adjusted to pD_2 concentration. DMSO and $Dy(DOTP)^{5-}$ had no influence on $[Na^+_{in}]$. The ^{23}Na NMR in the presence of the test compounds (**1-5**) was measured at 10-minute intervals, and the ^{23}Na NMR measurements for each sample were carried out five times.[8] The intracellular Na^+ ion concentrations were calculated by using the previously described equation.[3d]

RESULTS AND DISCUSSION

After several preliminary examinations by ^{23}Na NMR under various concentrations of the test compounds (**1-5**), it was found that addition of **1-5** at the pD_2 concentration[9] at which those compounds induced half maximum of positive inotropic effect disclosed apparent differences in intracellular Na^+ ion concentration. Figure 1 shows the time course of variation of intracellular Na^+ ion concentration ($\Delta[Na^+_{in}]$). Although all compounds exhibited nearly the same maximum $\Delta[Na^+_{in}]$, there were apparent differences in the increasing rates of intracellular Na^+ ion concentration among the five tested compounds. In other words, the rate of elevation of intracellular Na^+ ion level tends to increase with decreasing T_{50} values,[9] the time developed to the half maximum positive inotropic effect after addition of the test compounds at the pD_2 concentration. Initial rise of $\Delta[Na^+_{in}]$ caused by **1-5** may be responsible for an inhibition of Na^+, K^+-ATPase located in erythrocyte membrane, and subsequent decrease in Na^+ ion through its maximum is probably due to the activation of pro-Na^+, K^+-ATPase induced by elevated intracellular Na^+ ion concentration .[10]

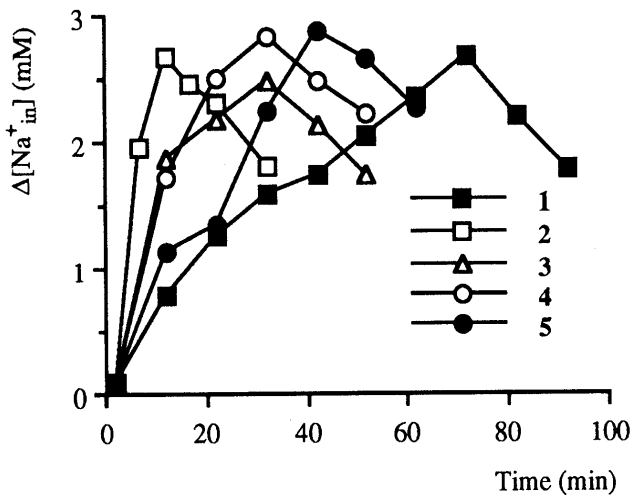

Fig. 1. Time Course of $\Delta[Na^+_{in}]$ Induced by Proscillaridin (**1**) and Its Derivatives (**2-5**) (n=5)

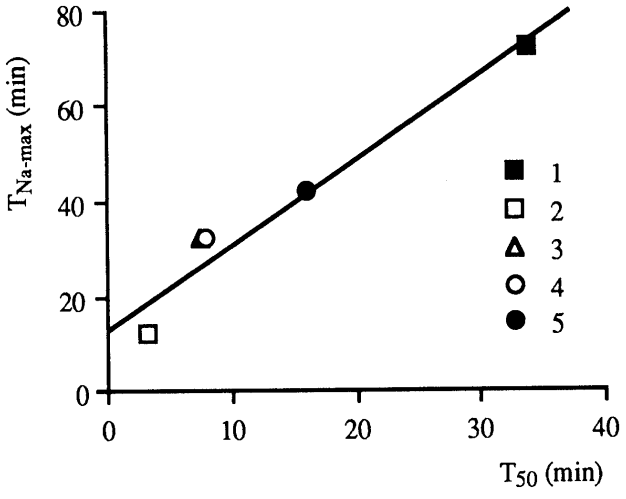

Fig. 2. Relationship between Inotropy Speeds (T_{50}) and the Time to Maximum $\Delta[Na^+_{in}]$ ($T_{Na\text{-}max}$)

Figure 2 shows the correlations between inotropy speeds (T_{50}) and the times to maximum $\Delta[Na^+_{in}]$ determined by ^{23}Na NMR spectrometry. There is a highly significant positive correlation between T_{50} values and the time required to maximum $\Delta[Na^+_{in}]$ ($T_{Na\text{-}max}$).

$$T_{Na\text{-}max} = 1.75\ T_{50} + 13.44 \quad (r = 0.974)$$

In conclusion, our present findings show that inotropy speeds of proscillaridin (**1**) and its derivatives (**2**-**5**) are physicochemically evaluated by ^{23}Na NMR spectrometry. We are currently working to extend this methodology to other cardiac ingredients.

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REFERENCES AND NOTES

1) Present address: Kyoto Pharmaceutical University, 5 Nakauchi-cho, Misasagi, Yamashina-ku, Kyoto 607, Japan.
2) a) R. K. Gupta and P. Gupta, *J. Magn. Reson.*, **47**, 344 (1982); b) M. M. Pike, J. C. Frazer, D. F. Dedrick, J. S. Ingwall, P. D. Allen, C. S. Springer, Jr., and, T. W. Swith, *Biophys. J.*, **48**, 159 (1985).
3) a) M. M. Pike, R. S. Simon, A. J. Balschi, and C. S. Springer, Jr., *Proc. Natl. Acad. Sci. USA*, **79**, 810 (1982); b). D. C. Buster, J. F. Hinton, F. S. Millett, and D. C. Shungu. *Biophys. J.*, **53**, 145 (1988); c) D. C. Shungu, D. C. Buster, and R. W. Briggs, *J. Mag. Reson*, **89**, 102 (1990). d) A. Nakamura, S. Nagai, T. Ueda, J. Sakakibara, Y. Hotta, and K. Takeya, *Chem. Pharm. Bull.*, **37**, 2330 (1989); e) A. Nakamura, S. Nagai, T. Ueda, N. Murakami, J. Sakakibara, H. Shibuya, and I. Kitagawa, *Chem. Pharm. Bull.*, **39**, 1726 (1991); f) A. Nakamura, S. Nagai, T. Takahashi, Renu Malhan (née Chopra), N. Murakami, T. Ueda, J. Sakakibara, and M. Asano, *Chem. Pharm. Bull.*, **40**, 2331 (1992).
4) a) T. Akera and T. M. Brody, *Pharmacol. Rev.*, **29**, 187 (1987): b) H. Flasch and N. Heinz, *Arch. Pharmacol.*, **304**, 37 (1978).
5) a) R. L. Ong and H. C. Cheung, *Biophys. Chem.*, **23**, 237 (1986); b) Y. Hotta, H. Ando, R. Eto, K. Takeya, M. Haruna, K. Ito, and J. Sakakibara, *Folia Pharmacol. Japon.*, **100**, 143 (1992).
6) J. Mori, S. Nagai, J. Sakakibara, K. Takeya, Y. Hotta, and H. Ando, *Chem. Pharm. Bull.*, **35**, 1839 (1987).
7) A. D. Sherry, C. R. Malloy, F. M. H. Jeffrey, W. P. Cacheris, and, C. F. G. C. Geralges, *J. Magn. Reson.*, **76**, 528 (1988).
8) Since the compound (**2**) had a large elevation rate of intracellular Na^+ ion level, the ^{23}Na NMR were obtained at 5-minute intervals for 20 minutes.
9) J. Sakakibara, J. Mori, S. Nagai, Y. Hotta, and K. Takeya, *Folia Pharmacol. Japon.*, **90**, 115 (1987). The pD_2 value of **1**-**5** are as follows: **1** (7.41), **2** (5.34), **3** (4.91), **4** (6.18), and **5** (5.81).
10) U. Sütterlin, K. -H. Gless, K. Schaz, M. Hüfner, V. Schütz, and W. Hunstein, *Klin. Wochenschr.*, **62**, 598 (1984).

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