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Stabilizing effects of coenzyme Q10 on potassium ion release, membrane potential and fluidity of rabbit red blood cells.

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Abstract

The effects of coenzyme Q10 (Co Q10) on potassium ion release, membrane potential and fluidity of rabbit red blood cells were studied. Co Q10 inhibited the increased potassium ion release induced by cetylamine or lysolecithin from the cells. Co Q10 slightly decreased the membrane potential monitored by changes in fluorescence intensity of cyanine dye, 3,3'-dipropyl-2,2'-thiodicarbocyanine iodide [diS-C3-(5)], and also slightly decreased the membrane fluidity measured by using 1,6-diphenyl-1,3,5-hexatriene (DPH). These effects of Co Q10 on the membrane are considered to be due to its membrane stabilizing activity by interaction with lipid bilayers of the membrane.

KEYWORDS: coenzyme Q_{10}, red blood cells, potassium release, membrane potential, fluidity.

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STABILIZING EFFECTS OF COENZYME Q_{10} ON POTASSIUM ION RELEASE, MEMBRANE POTENTIAL AND FLUIDITY OF RABBIT RED BLOOD CELLS

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Abstract. The effects of coenzyme Q_{10} (Co Q_{10}) on potassium ion release, membrane potential and fluidity of rabbit red blood cells were studied. Co Q_{10} inhibited the increased potassium ion release induced by cetylamine or lysolecithin from the cells. Co Q_{10} slightly decreased the membrane potential monitored by changes in fluorescence intensity of cyanine dye, 3, 3′-dipropyl-2, 2′-thiodicarbocyanine iodide [diS-C_3-(5)], and also slightly decreased the membrane fluidity measured by using 1, 6-diphenyl-1, 3, 5-hexatriene (DPH). These effects of Co Q_{10} on the membrane are considered to be due to its membrane stabilizing activity by interaction with lipid bilayers of the membrane.

Key words: coenzyme Q_{10}, red blood cells, potassium release, membrane potential, fluidity.

Co Q_{10} was isolated from the bovine myocardial mitochondria by Crane and others in 1957 (1), and its structure was elucidated by Lester and others (2). Its physiological activities have been reported as taking part in the mitochondrial electron transfer process (3) and the improvement of the mitochondrial oxidoreduction system in the myocardium (4–7). The stabilizing effects of Co Q_{10} on biomembranes and artificial membranes have been reported (8–10), and the physiological functions of Co Q_{10} as a membrane stabilizer are also considered important.

We examined the effects of Co Q_{10} on rabbit red blood cells and found that it inhibited the increase in K^+ ions induced by cetylamine or lysolecithin, and decreased the membrane potential and fluidity, thereby stabilizing the membrane.

MATERIALS AND METHODS

Fresh rabbit red blood cells were washed twice with a choline medium (150 mM choline chloride-10 mM Tris-HCl buffer, pH 7.4) at 4°C. Cholesterol was obtained from Nakarai Chemical Co. (Kyoto); lysolecithin from Sigma Chemical Co. and diS-C_3-(5) from the Japanese Research Institute for Photosensitizing Dyes

Co. (Okayama). Co Q_{10} was kindly donated by Eisai Co. (Tokyo). The other chemicals used were obtained from Katayama Chemical Co. (Osaka).

Measurement of K^+ ion release from rabbit red blood cells. Rabbit red blood cells ($1-2\times10^7$ cells/ml) in a choline medium (3 ml) were incubated with or without Co Q_{10} at 37°C for 0.5 min and then cetylamine or lysolecithin was added to the reaction medium. Pottassium ions released from the cells in the reaction medium were measured by a K^+-sensitive electrode (Electronic Instrument Ltd., Surrey, England; model GKN 33B) connected to a pH meter (Toa Electronic Instrument Ltd. model HM-20B, Tokyo). Total K^+ ions in the cells were determined by dissolving the cells with 5 μl of 0.05% Triton-X-100. The ethanol concentration used for dissolving lysolecithin and cholesterol in the reaction medium was less than 0.5% (V/V).

Measurement of membrane potential. DiS-C_3-(5) (0.16 μg/ml), a cyanine fluorescence dye, was used for the measurement of the membrane potential of red blood cells (2×10^7 cells/ml) in a choline medium at 37°C (11). The fluorescence intensity of the dye was recorded using a spectrofluorometer (Shimazu, Type RF-502) at an emission wavelength of 670 nm with an excitation wavelength of 622 nm.

Measurement of membrane fluidity. Membrane fluidity was measured by the fluorescence polarization technique of Shinitzky and Inbar (12) using DPH. In brief, a mixture of 1 volume of a red blood cell suspension (2×10^7 cells/ml) in the choline medium and 1 volume of 2×10^{-6}M DPH solution, was incubated at 25°C for 60 min, and the labelled cells were washed twice with 0.15 M KCl solution. Then, the cells were incubated with or without Co Q_{10} in 0.15 M KCl solution at 37°C for 15 min, washed with 0.15 M KCl solution again, and resuspended in the same solution. The fluorescence polarization of the reaction mixture was measured at 25°C using a spectrofluorometer (Shimazu, Type RF-502) equipped with polarizers and a thermostatic cell holder at the emission wavelength of 426 nm with the excitation wavelength of 360 nm. Microviscosity ($\bar{\eta}$) values were calculated.

RESULTS

Addition of cetylamine to the red blood cell suspensions resulted in increased K^+ ion release from the cells depending on the cetylamine concentration (Fig. 1). By the addition of 1.3×10^{-6} M of cetylamine, 6.3% of total K^+ ions in the cells was released about 7 min after the addition. With 1.3×10^{-5} M of cetylamine, 84.5% of total K^+ ions was released in about 1 min.

While the addition of 3.4×10^{-6} M of cetylamine resulted in release of 46.5% of total K^+ ions in the cells about 4 min after the addition, this increased K^+ ion release was inhibited by prior addition of Co Q_{10}; that is, inhibited to 31 5% with 1.7×10^{-5} M of Co Q_{10} and 9.5% with 1×10^{-4} M of Co Q_{10} (Fig. 2).

Lysolecithin (1.8 μg/ml) induced K^+ ion release to the extent of 41.6% of total K^+ ions from the cells in 3 min, while prior addition of Co Q_{10} (3.3×10^{-5} M) resulted in inhibition of K^+ ion release to 20.0%. Lysolecithin (1.2 μg/ml)

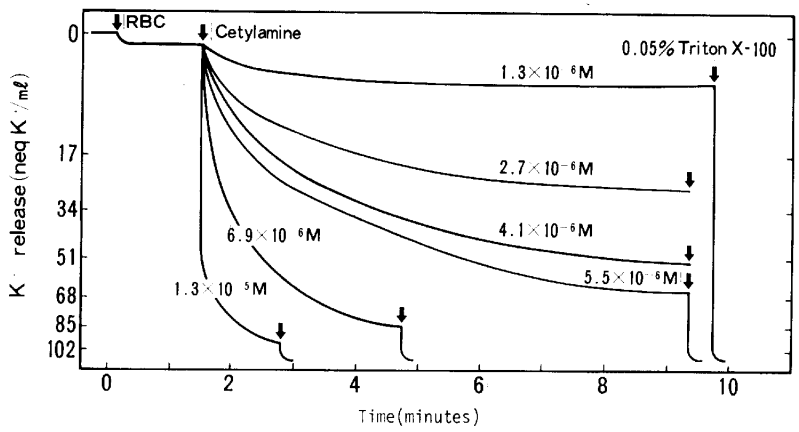

Fig. 1. K^+ ion release induced by cetylamine from rabbit red blood cells (2×10^7 cells/ml) in 150 mM choline chloride-10 mM Tris-HCl buffer (pH 7.4) at 37°C.

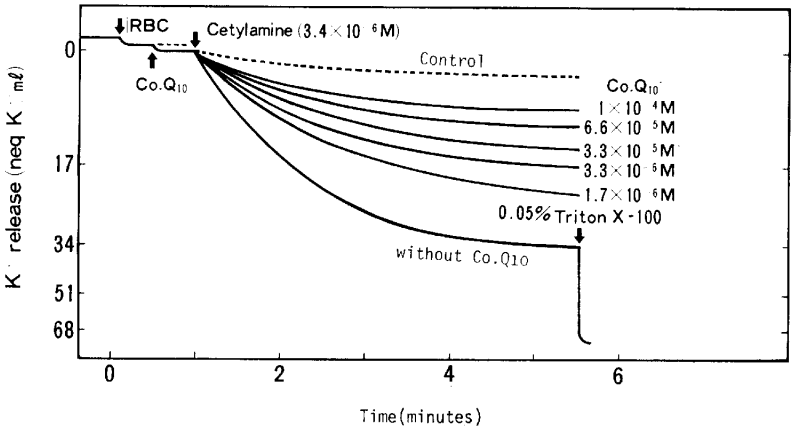

Fig. 2. Inhibitory effect of Co Q_{10} on K^+ ion release induced by cetylamine (3.4×10^{-6} M) from rabbit red blood cells (1.5×10^7 cells/ml) in 150 mM choline chloride-10 mM Tris-HCl buffer (pH 7.4) at 37°C.

induced K^+ ion release to 23.3% of total K^+ ions and prior addition of Co Q_{10} (3.3×10^{-5} M) resulted in inhibition of K^+ ion release to 6.6% (Fig. 3).

When diS-C_3-(5) was added to the red blood cells suspensions in a choline medium, a marked increase in fluorescence occurred. This fluorescence intensity decreased with time of incubation and reached a steady-state level after about 6 min (Fig. 4). Addition of Co Q_{10} at this steady-state induced a slight increase in fluorescence intensity depending on the concentration. This was thought to decrease the membrane potential (11).

Table 1 shows that the effect of Co Q_{10} on the fluorescence polarization of

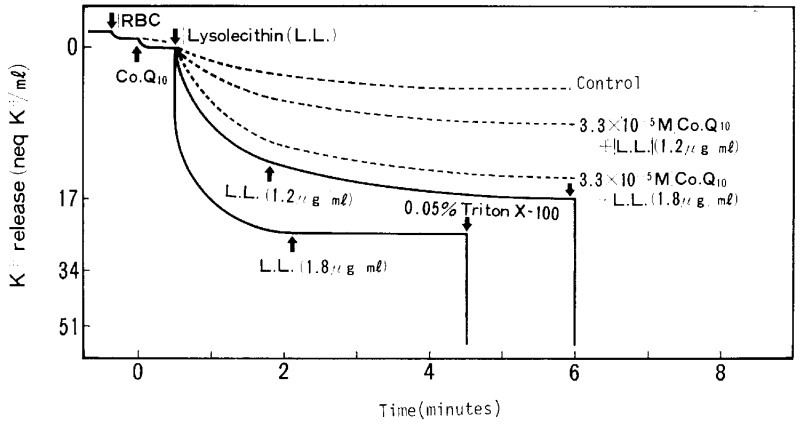

Fig. 3. Inhibitory effect of Co Q_{10} on K^+ ion release induced by lysolecithin from rabbit red blood cells (1.0×10^7 cells/ml) in 150 mM choline chloride-10 mM Tris-HCl buffer (pH 7.4) at 37°C.

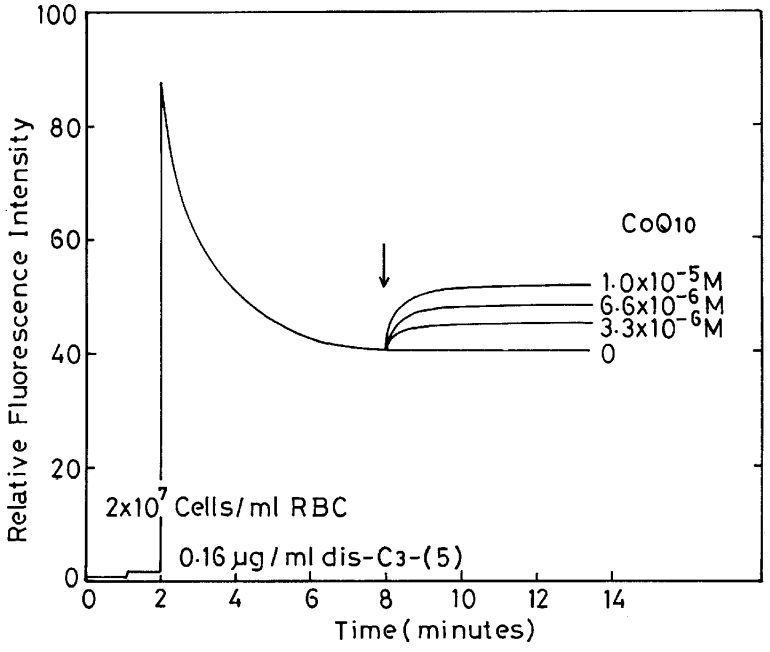

Fig. 4. Effect of Co Q_{10} on the fluorescence intensity of diS-C_3-(5) in rabbit red blood cells (2×10^7 cells/ml) in 150 mM choline chloride-10 mM Tris-HCl buffer (pH 7.4) at 37°C.

rabbit red blood cells. The microviscosity ($\bar{\eta}$) of rabbit red blood cells treated with Co Q_{10} (6.6×10^{-6} M) was lowered to 3.4 ± 0.3 compared to that of 3.6 ± 0.2 in the control red blood cells.

TABLE 1. EFFECT OF Co Q_{10} ON THE FLUORESCENCE POLALIZATION OR RABBIT RED BLOOD CELLS

Sample	n	Microviscosity ($\bar{\eta}$) at 25°C mean±SD
Red blood cells (2×10^7 cells/ml)	17	3.6±0.2
Red blood cells (2×10^7 cells/ml)+6.6×10^{-6} M of Co Q_{10}	9	3.4±0.3

DISCUSSION

One of the main functions of biomembranes is the regulation of the compartmentation of intracellular substances (13, 14). If biomembranes are damaged by drugs or detergents (15, 16), this compartmentation will be changed, resulting in instantaneous change of membrane potential (17, 18) and rapid release of low-molecular substances such as Ca^{2+} and K^+ ions. Therefore, the measurement of K^+ ions as a change in cellular compartmentation allows the examination of an extremely minute initial change.

Cetylamine, which possesses an intramolecular positive charge and a polar group, acts on membrane lipids, especially on phospholipids, perturbs the arrangement of lipid bilayers (19), and therefore may increase the K^+ release. Lysolecithin acts on membrane lipid bilayers and destroys the lipid structure by micellation, thereby increasing K^+ ion release and efflux of hemoglobin (15).

Co Q_{10} markedly inhibited the increased K^+ release from red blood cells induced by cetylamine and lysolecithin.

The fact that Co Q_{10} increased the fluorescence intensity of diS-C_3-(5) adsorbed on lipid bilayers of the membrane depending on membrane potential (11, 20), suggests that the drug had entered binding sites of diS-C_3-(5) and substituted for the dye. Co Q_{10} slightly decreased the fluidity of DPH-labelled red blood cells, so the drug may have stabilized the membrane by acting on lipid bilayers of the cell membrane and lowering the microviscosity of the cell membrane.

Lucy and Dingle reported that the membrane stabilizing function of vitamin E was physicochemical interaction between its isoprenoid side chain and membrane-constituting unsaturated fatty acids (21). Co Q_{10} has 10-isoprenoid side chains in its molecule, so it may act on lipid bilayers of the membrane and physically protect the double bond in higher unsaturated fatty acids in the membrane, like an arachidonyl residue of phospholipids (22), thereby stabilize the cell membrane.

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REFERENCES

1. Crane, F. L., Hatefi, Y., Lester, R. L. and Widmer, C.: Isolation of a quinone from beef heart mitochondria. *Biochim. Biophys. Acta* **25**, 220-221, 1957.
2. Lester, R. L., Crane, F. L. and Hatefi, Y.: Coenzyme Q : A new group of quinones. *J. Am. Chem. Soc.* **80**, 4751-4752, 1958.
3. Crane, F. L., Widmer, C., Lester, R. L., Hatefi, Y. and Fechner, W.: Studies on the electron trasport system. XV. Coenzyme Q (Q 275) and the succinoxidase activity of the electron transport particle. *Biochim. Biophys. Acta* **31**, 476-489, 1959.
4. Gale, P. H., Koniuszy, F., Page, Jr. A. C. and Folkers, K.: Coenzyme Q XXIV. On the significance of coenzyme Q_{10} in human tissues. *Arch. Biochem. Biophys.* **3**, 211-213, 1961.
5. Folkers, K., Littarru, G. P., Ho, L. and Runge, Th. M.: Evidence for a deficiency of coenzyme Q in human heart disease. *Internat. J. Vit. Res.* **40**, 380-390, 1970.
6. Lenaz, G., Daves, G. D. Jr. and Folkers, K.: Organic structural specificity and sites of coenzyme Q in succinoxidase and DPNH-oxidase systems. *Arch. Biochem. Biophys.* **123**, 539-550, 1968.
7. Ernster, L., Lee, I.-Y., Norling, B. and Persson, B.: Studies with ubiquinone-depleted submitochondrial particles. Essentiality of ubiquinone for the interaction of succinate dehydrogenase, NADH dehydrogenase, and cytochrome b. *Eur. J. Biochem.* **9**, 299-310, 1969.
8. Anderson, S. S., Lyle, L. G. and Paterson, R.: Electron transfer across membranes using vitamin K_1 and coenzyme Q_{10} as carrier molecules. *Nature* **259**, 147-148, 1976.
9. Maggio, B., Diplock, A. T. and Lucy, J. A.: Interactions of tocopherols and ubiquinones with monolayers of phospholipids. *Biochem. J.* **161**, 111-121, 1977.
10. Spisni, A., Masotti, L., Lenaz, G., Bertoli, E., Pedulli, G. F. and Zannoni, C.: Interactions between ubiquinones and phospholipid bilayers. A spin-label study. *Arch. Biochem. Biophys.* **190**, 454-458, 1978.
11. Sims, P. J., Waggoner, A. S., Wang, C. H. and Hoffman, J. F.: Studies on the mechanism by which cyanine dyes measure membrane potential in red blood cells and phosphatidyl choline vesicles. *Biochemistry* **13**, 3315-3330, 1974.
12. Shinitzky, M. and Inbar, M.: Difference in microviscosity induced by different cholesterol levels in the surface membrane lipid layer of normal lymphocytes and malignant lymphoma cells. *J. Mol. Biol.* **85**, 603-615, 1974.
13. Van den Berg, K. J. and Betel, I.: Early increase of amino acid stimulated lymphocytes. *Exp. Cell Res.* **66**, 257-259, 1971.
14. Inoue, M., Utsumi, K. and Seno, S.: Effect of concavalin A and its derivative on the potassium compartmentation of Ehrlich ascites tumor cells. *Nature* **255**, 556-557, 1975.
15. Utsumi, K., Miyahara. M., Inoue, M., Mori, M., Sugiyama, K. aud Sasaki, J.: Inhibition by cepharanthin of red blood cell potassium release induced by lead acetate and lysolecithin. *Cell Struct. Funct.* **1**, 133-136, 1975.
16. Shinozawa, S. and Araki, Y.: Comparative studies on the chemical modifications of Ehrlich ascites tumor cells membranes by hydrophobic drugs (cepharanthine, papaverine and cholesterol). *Folia pharmacol. Jpn.* **75**, 207-214, 1979 (in Japanese).
17. Miller, J. B. and Koshland, D. E. Jr.: Sensory electrophysiology of bacteria : Relationship of the membrane potential to motility and chemotaxis in *Bacillus subtilis*. *Proc. Natl. Acad. Sci. U. S. A.* **74**, 4752-4756, 1977.
18. Shinozawa, S., Araki. Y., Utsumi, K. and Oda, T.: Stabilizing effects of cholesterol on changes in membrane permeability and potential induced in red blood cells by lysolecithin. *Physiol. Chem. Physics* **11**, 161-167, 1979.
19. Gomperts, B. D.: Inducing ion permeability. In *Plasma Membrane*; *Models for Structure*

and Function. Academic Press, London, pp. 109-179, 1977.

20. Laris, P. C., Pershadsingh, H. A. and Johnstone, R. M.: Monitoring membrane potentials in Ehrlich ascites tumor cells by means of a fluorescent dye. *Biochim. Biophys. Acta* **436**, 475-488, 1976.
21. Lucy, J. A. and Dingle, J. T.: Fat-soluble vitamines and biological membranes. *Nature* **204**, 156-160, 1964.
22. Lucy, J. A.: Functional and structural aspects of biological membranes: A suggested structural role for vitamin E in the control of membrane permeability and stability. *Ann. N. Y. Acad. Sci.* **20**`, 4-11, 1972.