Values of the constants of interaction of Ca^{++} with $O_2^{\cdot}$ in X–XO and PMS–NADH systems, calculated by equation (3), have values of $5.5 \pm 0.7 \times 10^4$ and $3.0 \pm 0.4 \times 10^4$ $M^{-1} \cdot sec^{-1}$, respectively, i.e., they agree sufficiently closely. Considering that the value obtained for $K_{Ca^{++}}$ was close to the value of K_{TNTB}, it can be concluded that interaction of Ca with O_2 is not catalytic but stoichiometric in character.

This effect of Ca^{++} is specific, for other bivalent cations (Mg^{++} and Zn^{++}) have no such action on $O_2^{\cdot}$ (Table 1).

Considering that the intracellular Ca^{++} concentrations vary between 10^{-7} and 10^{-5} M, and the extracellular Ca^{++} concentrations are between 10^{-4} and 10^{-3} M, and also considering values for the constant of interaction between Ca^{++} and $O_2^{\cdot}$ obtained in the present experiments, it can be concluded that Ca^{++} ions may regulate the concentration of $O_2^{\cdot}$ – a product of single-electron reduction of oxygen *in vivo*.

LITERATURE CITED

1. V. E. Kagan, V. M. Savov, V. V. Didenko, et al., Byull. Éksp. Biol. Med., No. 3 (1983).
2. I. Fridovich, in: Free Radicals in Biology [Russian translation], Vol. 2, Moscow (1979), pp. 272-310.
3. C. Beauchamp and I. Fridovich, Anal. Biochem., 44, 276 (1971).
4. B. H. J. Bielski and H. W. Richter, J. Am. Chem. Soc., 99, 3019 (1977).
5. B. H. J. Bielski and A. O. Allen, J. Phys. Chem., 81, 1048 (1977).
6. A. M. Michelson et al. (editors), Superoxide and Superoxide Dismutases, London (1977), p. 873.

PROTECTION OF SARCOPLASMIC RETICULAR MEMBRANES AGAINST DAMAGE BY FREE FATTY ACIDS BY VITAMIN E

L. V. Tabidze, V. B. Ritov, V. E. Kagan, and Yu. P. Kozlov

UDC 616.74-008.932.95-02:615.356:577.161.3

KEY WORDS: Ca^{++}-dependent ATPase; vitamin E; free fatty acids; sarcoplasmic reticulum; temperature inactivation.

Free fatty acids (FFA) are present in small quantities in membranes of the sarcoplasmic reticulum (SR) of heart and skeletal muscles [9, 11]. The FFA content rises during the development of certain pathological states (ischemia, stress) up to a level where it disturbs the transport function of SR membranes [10, 11]. It is therefore important to seek ways of protecting the Ca^{++}-pump of SR membranes against damage by FFA. The latter increased the passive permeability of SR membranes for Ca^{++} and essentially reduced the resistance of Ca^{++}-dependent ATPase to thermal denaturation [5]. It was shown previously that α-tocopherol reduces the passive permeability of membranes for Ca^{++}, in agreement with the widely held view that vitamin E plays a stabilizing role in biological membranes [3, 8].

The object of this investigation was to study the protective action of α-tocopherol against temperature inactivation of the Ca^{++} pump of SR membranes in the presence of arachidonic acid (AA).

EXPERIMENTAL METHOD

Fragments of SR from rat skeletal muscles were isolated by differential centrifugation from a homogenate of hind limb muscles [12]. A highly purified fraction of SR membranes from rabbit muscles was isolated from white muscles of the hind limbs by the method described in [7]. ATPase activity and efficiency of Ca^{++} transport by SR membranes were determined by pH-

Laboratory of Physical Chemistry of Biomembranes, M. V. Lomonosov Moscow University.

(Presented by Academician of the Academy of Medical Sciences of the USSR S. E. Severin.) Translated from Byulleten' Éksperimental'noi Biologii i Meditsiny, Vol. 96, No. 11, pp. 48-50, November, 1983. Original article submitted April 6, 1983.

0007-4888/83/9611-1548$07.50

© 1984 Plenum Publishing Corporation

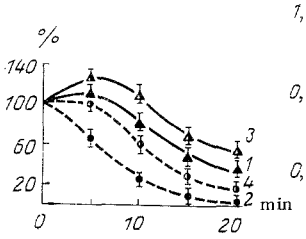

Fig. 1

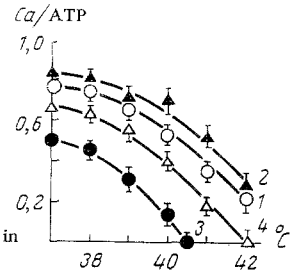

Fig. 2

Fig. 1. Effect of AA on temperature-inactivation of Ca^{++}-dependent ATPase of rat SR membranes. 1, 2) Control; 3, 4) diet rich in α-tocopherol. 2-4) Temperature inactivation + AA (20 μg/mg protein). Abscissa, time of temperature inactivation (in min); ordinate, Ca^{++}-dependent ATPase activity (in %).

Fig. 2. Effect of α-tocopherol, AA, and human serum albumin on temperature inactivation of Ca^{++}-pump of rat SR membranes. 1) Control; 2) control + human serum albumin; 3) control + AA (20 μg/mg protein); 4) control + AA (20 μg/mg protein) + α-tocopherol (30 μg/mg protein). Abscissa, temperature of preincubation of samples for 10 min (in °C); ordinate, parameter Ca/ATP.

metry [6]. Thermal denaturation of SR membranes was carried out in medium containing 5% sucrose (for rat SR) or 5% glycerol (for rabbit SR) and 50 mM phosphate buffer (pH 6.8, 20°C). The protein concentration was 5 mg/ml. In some experiments ATP and $MgCl_2$, in a concentration of 2 mM, were added to the temperature denaturation medium. A solution of AA (from Sigma, USA) and α-tocopherol (from Serva, West Germany) in ethanol was added to a suspension of SR membranes in medium for temperature inactivation and the mixture was incubated at 37°C for 10 min (the final ethanol concentration did not exceed 2%). In experiments *in vivo* Wistar rats were kept on a diet with α-tocopherol in a dose of 50 mg/kg body weight once every 2 days for 3 weeks. Control rats were kept on the standard laboratory diet.

EXPERIMENTAL RESULTS

In the experiments of series I the resistance of Ca^{++}-dependent ATPase to temperature inactivation in SR membranes isolated from control rats was compared with that in rats kept on a diet rich in vitamin E. Membranes isolated from animals of the experimental and control groups were practically indistinguishable as regards specific Ca^{++}-ATPase activity (4.0 ± 0.5 μmoles P_i/min/mg protein at 37°C). During temperature inactivation of the SR membranes essential differences were found between experiment and control. Preincubation of SR membranes at 48°C led to inactivation of Ca^{++}-ATPase (Fig. 1). Weak activation of the enzyme in the initial period was due to increased membrane permeability for Ca^{++}, for when ATPase activity was measured in the presence of the Ca^{++}-ionophore A23187 (3 μg/ml) activation of the enzyme was not observed. Inhibition of ATPase by 50% in SR membranes isolated from muscles of the control animals was observed after incubation at 48°C for 15 min. As Fig. 1 shows, Ca^{++}-ATPase in SR membranes isolated from animals kept on a diet rich in α-tocopherol had higher resistance to heat. The stabilizing effect of α-tocopherol became even more marked on temperature inactivation in the presence of FFA. For instance, addition of AA accelerated temperature inactivation of ATPase in the SR membranes of the control animals by a greater degree than in the SR membranes of animals receiving vitamin E, probably due to the increased α-tocopherol concentration in SR membranes of rats kept on a diet rich in vitamin E [13].

Insertion of α-tocopherol into the structure of isolated SR membranes from muscles of the control rats led to an increase in resistance of their Ca^{++}-pump to temperature inactivation in the presence of AA. For the added α-tocopherol to exhibit its stabilizing effect, incidentally, the SR membranes must have an increased FFA content. Addition of α-tocopherol to freshly isolated preparations of SR membranes (20-100 μg/mg protein) did not alter the kinetics of temperature inactivation of the transport function in ATPase activity. The protective

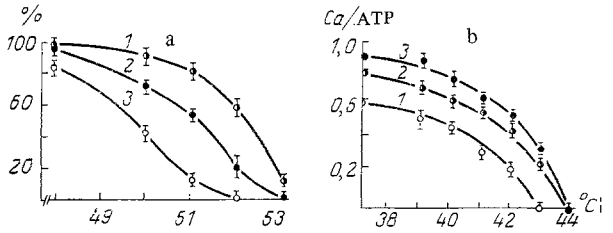

Fig. 3. Effect of α-tocopherol and AA on temperature inactivation of Ca^{++}-dependent ATPase and Ca^{++}-pump of rabbit SR membranes. SR membranes incubated 10 min in presence of Mg-ATP. a: 1) Control, 2) control + AA (12 μg/mg protein), 3) control + AA (12 μg/mg protein) + α-tocopherol (60 μg/mg protein). b: 1) Control + AA (12 μg/mg protein), 2) control + AA (12 μg/mg protein) + α-tocopherol (20 μg/mg protein), 3) control + AA (12 μg/mg protein) + α-tocopherol (60 μg/mg protein). Abscissa, temperature of preincubation for 10 min (in °C); ordinate: a) activity of Ca^{++}-dependent ATPase (in %), b) parameter Ca/ATP.

effect of α-tocopherol was demonstrated on preparations of SR membranes kept for a long time, in which products of phospholipid hydrolysis by phospholipase had accumulated. The protective effect of α-tocopherol was abolished after treatment of the membranes with human serum albumin, specially freed from FFA beforehand: this treatment, moreover, increased the heat resistance of the membranes (Fig. 2). Addition of AA caused the temperature inactivation curve of the transport function to be shifted into the region of lower temperatures, but insertion of α-tocopherol weakened the action of AA.

Similar experiments (series II) were conducted on highly purified SR membranes from rabbit skeletal muscles. They showed that α-tocopherol, if added to a suspension of SR membranes, had no protective action regardless of whether these membranes were freshly isolated or "old," and with or without added AA. However, the protective effect of α-tocopherol was exhibited if membranes with added AA were incubated for 10 min in the presence of Mg-ATP (2 mM). It follows from the results that α-tocopherol protects the transport function and stabilizes the ATPase activity of rabbit SR membranes during temperature inactivation aggravated by the action of AA (Fig. 3). This effect of ATP was evidently linked with a change in the structure of Ca^{++}-ATPase [2]; it facilitates insertion of α-tocopherol into the microenvironment of the enzyme, as was shown previously for FFA [1].

The results as a whole thus indicate that vitamin E can protect the Ca^{++}-pump of SR membranes against damage by FFA. It must be noted that SR membranes are sites of concentration of α-tocopherol in larger quantities than in other membrane structures of muscle cells [4, 13].

LITERATURE CITED

1. É. A. Avakyan, V. B. Ritov, O. A. Azizova, et al., Biokhimiya, 46, 809 (1981).
2. O. A. Azizova, L. G. Artemova, Yu. A. Vladimirov, et al., Dokl. Akad. Nauk SSSR, 246, 214 (1979).
3. Yu. V. Arkhipenko, S. K. Dobrina, V. E. Kagan, et al., Dokl. Akad. Nauk SSSR, 43, 1525 (1977).
4. V. E. Kagan, V. A. Pisarev, Yu. V. Arkhipenko, et al., Biokhimiya, 47, 7 (1983).
5. M. K. Murzakhmetova, V. B. Ritov, and O. V. Esyrev, Biokhimiya, 44, 2161 (1979).
6. V. B. Ritov, Biokhimiya, 36, 393 (1971).
7. V. B. Ritov, M. K. Murzakhmetova, and N. S. Shcherbakova, Dokl. Akad. Nauk SSSR, 263, 483 (1982).
8. A. T. Diplock, Acta Vitaminol. Enzymol., 4, 303 (1982).
9. W. Fiehn, J. B. Peter, J. F. Mead, et al., J. Biol. Chem., 246, 5617 (1971).
10. A. Katz, J. Dunnett, D. Repke, et al., FEBS Lett., 67, 207 (1976).
11. A. M. Katz and F. C. Messineo, Circ. Res., 1, 1 (1981).
12. A. Martonosi, J. Biol. Chem., 243, 71 (1968).
13. D. I. Murphy and R. D. Mavis, Biochim. Biophys. Acta, 663, 390 (1981).