Effect of a Polyunsaturated Fatty Acid-Enriched Diet on the State of the Ca-Transporting System of the Sarcoplasmic Reticulum in the Myocardium

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A long-term diet enriched with polyunsaturated fatty acids (PUFA), mainly docosahexaenoic and eicosapentaenoic acids, drastically reduces the occurrence and duration of arrhythmias during acute ischemia and reperfusion, restricts disturbances of the electrical stability of the heart, and lowers the probability of fibrillation in acute ischemia, reperfusion [4,8,9], infarction, and postinfarction cardiosclerosis [2,3,7], this resulting in a reduction of animal mortality for experimental myocardial infarction [2]. The mechanisms of such a cardioprotective effect are still to be clarified. Therefore, the aim of the present study was, first, to investigate the state of the Ca-transporting system in the sarcoplasmic reticulum (SPR) of the heart, which is responsible for the contractile activity of the cell, and, second, to study lipid peroxidation (LPO) in the myocardium under conditions of a PUFA-enriched diet.

MATERIALS AND METHODS

The experiments were carried out on male Wistar rats weighing 300 g. For 60 days the animals of the experimental group received eiconol added daily to the standard diet in a dose of 1.3 g a day. According to industrial data, eiconol (TU 400 SP "A"-1145-118-90, Trinita, Moscow) contains up to 18% eicosapentaenoic acid, up to 12% docosahexaenoic acid, and 18.1% oleic, 18.2% linoleic, and 18.3% linolenic PUFA, as well as saturated and monounsaturated fatty acids. The degree of eiconol unsaturation (iodine number) reaches 190. The hearts of the animals of the control and experimental (given PUFA) groups were excised, washed, and frozen in liquid nitrogen. Ca^{2+} transport was measured according to the rate of uptake of added Ca^{2+} by the SPR vesicles [10]. At the same time, accumulation of Ca^{2+} in the mitochondria was prevented by NaN_3, and Ca^{2+} uptake by the vesicles of the sarcolemma was absent due to the addition of K^+ oxalate not penetrating into these vesicles. The hearts were crushed in an Ultra-Turrox homogenizer with a 25N-10 blade during 30 sec at speed 8 in a medium containing 100 mM KCl, 20 mM imidazole (pH 7.8), and 25% glycerol; the tissue/medium ratio was 1:4. The rate of Ca^{2+} transport was determined over 5 min in thermostatically controlled cells with stirring, 50-200 μl of homogenate being added to 5 ml of the medium containing 100 mM KCl, 15 mM K^+ oxalate, 20 mM HEPES (pH 7.0), 4 mM $MgCl_2$, and 5 mM NaN_3. Before the measurements, ATP and Ca^{2+} were added in final concentrations of 4

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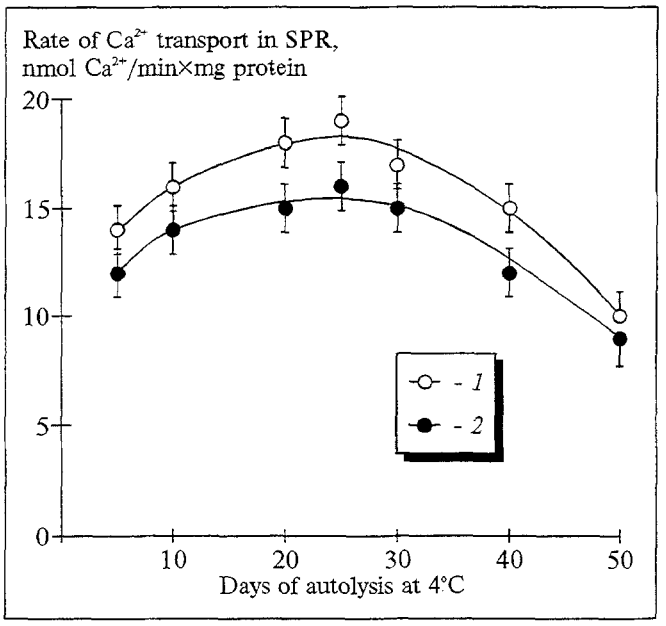

Fig. 1. Rate of Ca^{2+} transport in SPR of heart as a function of Ca^{2+} concentration in medium in control (*1*) and after a PUFA-enriched diet (*2*). The number of experiments in each series is 7. Here and in Figs. 2-5: mean values are presented.

mM and 2-20 mM, respectively. The rate of LPO was determined as the degree of accumulation of thiobarbituric acid(TBA)-reactive products in tissue homogenates after Ohkawa *et al.* [11]. LPO was induced by the system: Fe^{2+} (75 μM) + ascorbate (0.2 mM), and the kinetics of accumulation of oxidation products was recorded.

RESULTS

A PUFA-enriched diet markedly raised the level of LPO in the myocardium, this being previously recorded, for instance, in the liver, where this level was 18 times higher than in the control [2]. We showed that the initial level of oxidized products in the myocardium was 1.8-fold as much as in the control. Such a marked accumulation of the products of free-radical oxidation should lead to a sharp decline of the Ca-transporting function of the SPR. However, as is seen from Fig. 1, this did not occur. The initial rate of Ca^{2+} transport by the SPR after prolonged intake of the PUFA-containing diet did not reliably differ from that in the control group. At the same time, the kinetic analysis and determination of the rate of Ca^{2+} transport at various concentrations of the latter showed no differences between the control and experimental groups. It is evident that for both low and high Ca^{2+} concentrations the shape of the kinetic curves is similar to that obtained for the control animals and for those given high doses of PUFA, and, most important, the inhibition of Ca transport by low concentrations of Ca^{2+}, which was observed for stress-induced damage to the heart, is absent [5,6]. Thus, an increase of the Ca^{2+} concentration in the cell raises the efficacy of the Ca-transporting system both in the control and after a PUFA-containing diet; the inhibition of the process occurs in the same range of Ca^{2+} concentrations (Fig. 1) in which the transport is inhibited in the control too. Moreover, in the present case, even in the absence of direct changes in the transport and membrane permeability studied earlier [1,10], the autolysis test, which makes it possible to reveal slight alterations in the enzyme-membrane complex of the Ca pump, showed no differences in the resistance of the Ca-transporting system to endogenous damaging factors realizing their effect during autolysis (Fig. 2). Thus, a marked accumulation of LPO products, which resulted from the long-term PUFA-containing diet, was not attended by a pronounced drop of the activity of Ca transport by the SPR in the heart; moreover, the qualitative characteristics of the process remained the same as in the control, the resistance to endogenous damaging factors being preserved. This points directly to the increased resistance of the Ca-transporting system to oxidation processes after the animals were kept on a PUFA-enriched diet.

To assess this phenomenon, we made a straight comparison of the two processes: the accumulation of oxidized products of LPO and the reduction of the rate of Ca transport during the induction of LPO by the Fe^{2+}-ascorbate system in

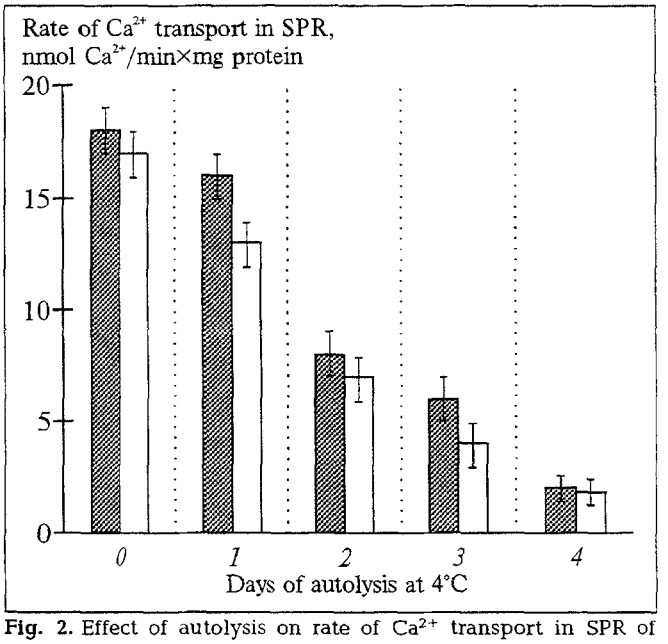

Fig. 2. Effect of autolysis on rate of Ca^{2+} transport in SPR of the heart in control (dark bars) and after a PUFA-enriched diet open bars).

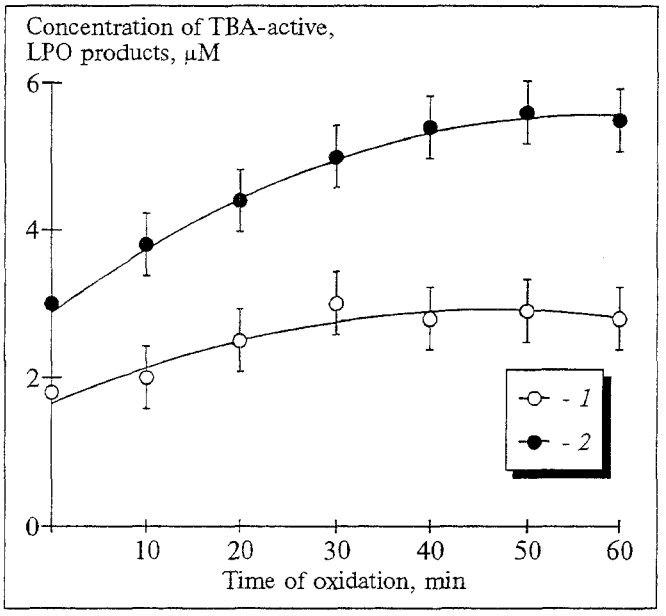

Fig. 3. Accumulation of LPO products for *in vitro* LPO induction in homogenates of myocardium in control (*1*) and after a PUFA-enriched diet (*2*).

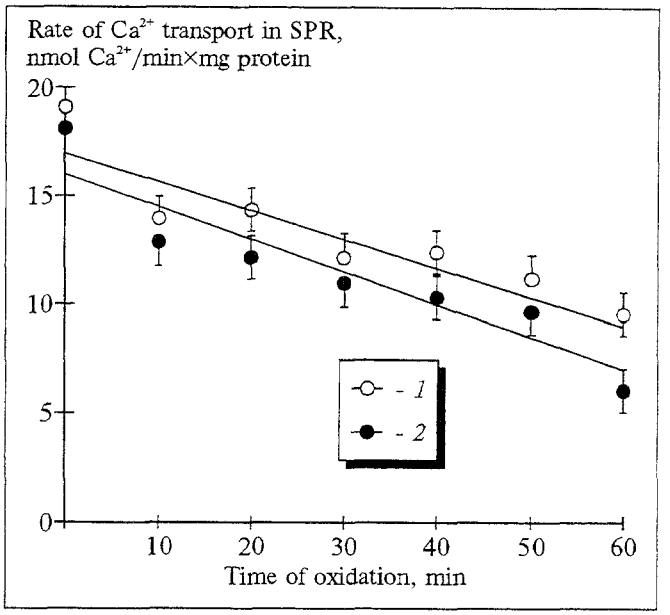

Fig. 4. Reduction of rate of Ca^{2+} transport in SPR of myocardium during LPO for its *in vitro* induction in control (*1*) and after a PUFA-enriched diet (*2*).

the myocardial homogenates of the control and experimental animals. As is seen from Fig. 3, the *in vitro*-induced accumulation rates of LPO products greatly differ. After the diet containing PUFA, which are the direct substrates of free-radical oxidation, the rate of their inducible accumulation increased more than 3-fold. This result is also significant for the following reason. The high level of oxidized products observed in the liver [5] and heart (in the present study) may be associated with several factors, the main ones being the addition of exogenous product, namely, oxidized products contained in eiconol per se, and direct activation of LPO in the organism. In this case, it is clear that, along with the possible presence of the former, the role of the latter is indubitable: in fact, LPO during its induction is far more intensive than after the animals have been kept on a PUFA-containing diet.

The next stage of assessment of the effect of oxidation processes on the cardiomyocytes for a PUFA-enriched diet was to determine the level of reduction of the activity of Ca^{2+} transport in the SPR during oxidation. As is seen from Fig. 4, this process does not reliably differ, either qualitatively or quantitatively, in the two groups. By comparing these two simultaneous processes (inhibition of Ca^{2+} transport and accumulation of LPO products during oxidation), we obtained the following results (Fig. 5). The dependence of the slowing of the rate of Ca^{2+} transport on the accumulation of LPO products greatly differs between the two groups. First, it is evident that for the accumulation of equal amounts of oxidized products the rate of reduction of Ca^{2+} transport is 3.5 times slower in the case of the PUFA-containing diet than in the control. Second, it is seen in Fig. 5 that the level of TBA-reactive products at which complete inhibition of Ca^{2+} transport in the SPR is achieved in the control is accompanied by a virtually complete preservation of the activity of the Ca pump at the initial level. Thus, the long-term PUFA-enriched diet apparently leads to a sharp increase of the

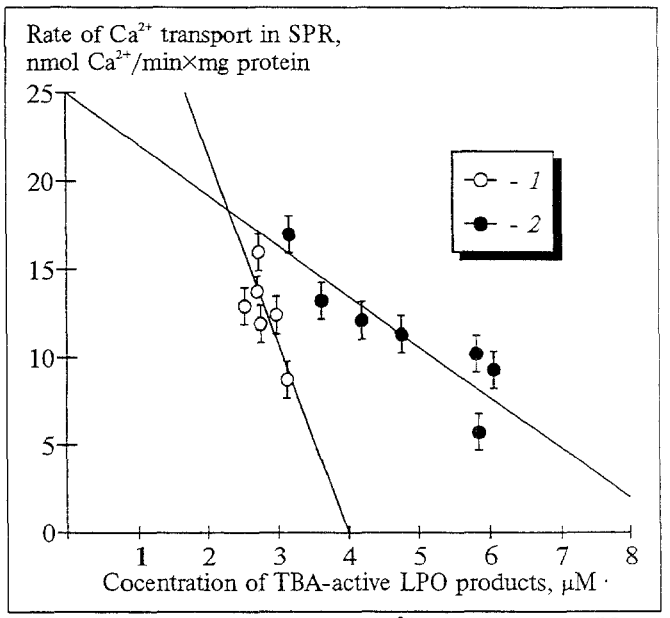

Fig. 5. Degree of inhibition of Ca^{2+} transport in SPR of myocardium as a function of level of accumulation of products of LPO for its *in vitro* induction in homogenates of myocardium in control (*1*) and after a PUFA-enriched diet (*2*).

resistance of the Ca-transporting system to free-radical oxidation. A fact of importance is that prolonged administration of PUFA, which causes a manifold compensated activation of LPO, results in an adaptive effect ensuring that the rate of Ca^{2+} transport is maintained on the necessary physiological level. These results agree well with our data on the effect of adaptation to periodic hypoxia on the Ca-transporting system of the myocardium [1]. These studies also showed a marked increase of the resistance of Ca^{2+} transport to LPO induction and to the accumulation of oxidation products as a response to a manifold induction of LPO for intermittent hypoxia, and a pronounced cardioprotective (antiarrhythmic and positive inotropic) effect of adaptation to periodic hypoxia was established.

Thus, the sharp increase of the resistance of the Ca-transporting system of the SPR in the myocardium to induction of free-radical oxidation and to a rise of the level of LPO products, which was shown in this study, may play an important role in the previously described increase of the electrical stability of the heart, since it is precisely damage to the membrane cationic pumps that causes its disturbance.

REFERENCES

1. Yu. V. Arkhipenko, T. G. Sazontova, I. I. Rozhitskaya, and F. Z. Meerson, *Kardiologiya*, № 6, 57-61 (1992).
2. F. Z. Meerson, L. M. Belkina, V. A. Isaev, *et al.*, *Ibid.*, № 3, 43-48 (1993).
3. F. Z. Meerson, L. M. Belkina, Fu Xiang Qun, and V. A. Isaev, *Byull. Eksp. Biol. Med.*, **115**, № 4, 343-345 (1993).
4. E. A. Prokhorovich, E. I. Zharov, A. I. Martynov, *et al.*, *Kardiologiya*, № 8, 99-102 (1990).
5. T. G. Sazontova, *Byull. Eksp. Biol. Med.*, **108**, № 9, 271-274 (1989).
6. T. G. Sazontova, V. I. Vovk, and F. Z. Meerson, *Ibid.*, **109**, № 2, 122-124 (1990).
7. M. L. Burr, *Clin. Exp. Hypertens.*, **14**, № 1-2, 181-192 (1992).
8. S. D. Kristensen, E. B. Schmidt, H. R. Andersen, and Y. Dyerberg, *Atherosclerosis*, **64**, № 1, H13-19 (1987).
9. P. L. McLennan, M. Y. Abeywardena, and J. S. Charnoci, *Amer. J. Clin. Nutr.*, **51**, 53-58 (1990).
10. F. Z. Meerson, T. G. Sazontova, and Yu. V. Arkhipenko, *Biomed. Sci.*, **1**, 373-378 (1990).
11. H. Ohkawa, N. Ohishi, and K. Yagi, *Analyt. Biochem.*, **95**, № 2, 351-358 (1979).