PATHOLOGICAL PHYSIOLOGY AND GENERAL PATHOLOGY

Impact of 13(S)-HPODE, a Lipoxygenase Product of Linoleic Acid, on Calcium Transport and Na,K-ATPase Activity in the Myocardial Sarcolemma

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In this *in vitro* study using a purified sarcolemmic fraction of guinea pig myocardium, the 13(S)-hydroperoxide of linoleic acid (13-HPODE) increased in a dose-dependent manner the permeability of myocardial sarcolemma to Ca ions in concentrations above 10 μmol/liter, stimulated Na/Ca exchange there in concentrations from 0.1 to 10 μmol/liter, and exerted a digitalis-like action on sarcolemmic Na,K-ATPase in concentrations between 0.1 and 100 μmol/liter (IC_{50} = 20 μmol/liter). The results indicate that the linoleic acid hydroperoxide may be an effective modulator of sarcolemmic Ca^{2+} transport and of membrane-bound enzymes.

Key Words: *hydroperoxylinoleic acid; sarcolemma; Ca^{2+} transport; Na,K-ATPase*

In recent years, increasing significance in the regulation of cardiac activity and in the impairment of cardiac function has been attached to processes activating membrane phospholipids and releasing polyunsaturated fatty acids, particularly arachidonic and linoleic acids. These acids account for a high proportion of all membrane acids and lipids in cardiac myocytes and are released in large amounts in pathological states of the myocardium [7].

Polyunsaturated fatty acids may play an important part in the regulation of intracellular processes [14]. The substances best studied in this respect are arachidonic acid and its metabolic products (prostanoids and leukotrienes), some of which are active regulators of vascular tone and myocardial contractility.

Information about the role of linoleic acid in the regulation of cardiac activity is extremely scarce. However, analysis of the relevant data obtained for cells other than cardiac myocytes indicate that both linoleic acid and its primary metabolic products exhibit a fairly wide range of biological activities and effects. Metabolic products such as hydroperoxylinoleic (HPODE) and hydroxylinoleic (HODE) acids are intensively produced by endothelial cells [4] and leukocytes [2,13]. Examples of biological effects are inhibition of thrombin-induced thromboxane release [12], alteration in the responsiveness of smooth muscle to biologically active substances, and the ability to damage vascular endothelium [17] and to inhibit certain enzymes [10].

There are indications that metabolic products of linoleic acid are able to modify fundamental characteristics of cellular membranes. Its oxidized prod-

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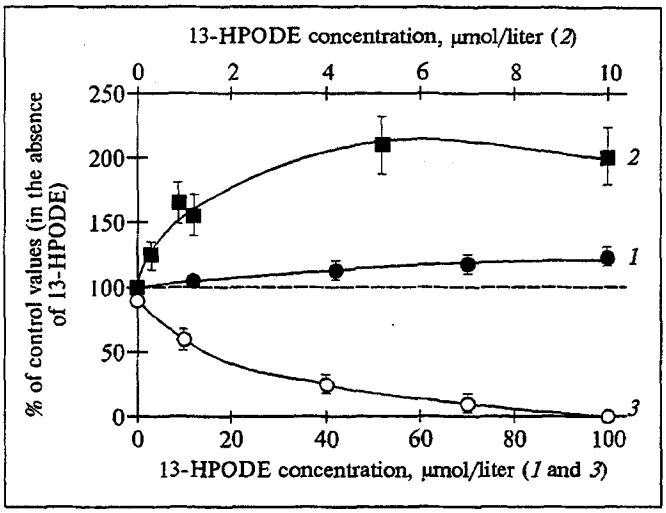

Fig. 1. Effects of 13(S)−HPODE on passive calcium permeability (*1*), Na/Ca exchange (*2*), and Na,K−ATPase activity (*3*) in the myocardial sarcolemma.

ucts can act as calcium ionophores [11], modulate the calcium-transporting system of the sarcoplasmic reticulum [5], and, under certain conditions, be sources of free radicals [16]. Since the impact of fatty acid hydroperoxides on the myocardium has been studied inadequately, we evaluate here the direct effects of 13(S)-HPODE, a lipoxygenase derivative of linoleic acid, on the permeability of myocardial sarcolemma to calcium and to the sarcolemmic Na/Ca exchange and Na,K-ATPase activity.

MATERIALS AND METHODS

For this *in vitro* study we used sarcolemmic vesicles obtained from the left ventricle of guinea pigs as previously described [1]. The resulting highly purified sarcolemmic fraction was frozen and stored in liquid nitrogen.

To determine the activity of Na/Ca exchange, sarcolemmic vesicles preloaded with sodium ions (160 mmol/liter) by passive diffusion were incubated with 13-HPODE in various concentrations, after which 30-fold diluted suspension of them was added to a medium containing 160 mmol/liter KCl or NaCl, Tris-20, 0.5 μmol/liter valinomycin, and 50 μmol/liter $^{45}CaCl_2$ (pH 7.4, 37°C). $^{45}Ca^{2+}$ accumulation was terminated after 10 sec by rapid filtration of the suspension via 0.45 μ Millipore membrane filters which were then washed with a solution containing (mmol/liter) 200 KCl, 10 $CaCl_2$, and Tris-20; after the filters had been dried, radioactivity was measured in a scintillation spectrometer. The true activity of Na/Ca exchange was calculated by subtracting the recorded $^{45}Ca^{2+}$ accumulation in the presence of a sodium gradient from that in its absence.

For the determination of calcium permeability, sarcolemmic vesicles were preloaded with $^{45}Ca^{2+}$ by passive diffusion for 1 min in the course of Na/Ca exchange, followed by the addition to their suspension of an equal volume of the following medium (mmol/liter): 160 KCl, 2 EGTA, Tris-20, and 13-HPODE (final concentrations 0.1-100 μmol/liter). In this way the accumulation of $^{45}Ca^{2+}$ was stopped and its exit against the concentration gradient was initiated. The latter process was interrupted after 2 min by means of rapid filtration, as described for the Na/Ca exchange.

Activity of the ouabain-sensitive sarcolemmic Na,K-ATPase was measured after incubating the vesicles at 37°C for 10 min in a medium composed of (mmol/liter) 100 NaCl, 10 KCl, 5 $MgCl_2$, 10 NaN_3, 1 EGTA, and 50 imidazole (pH 7.4) plus 13-HPODE (final concentrations 0.1-100 μmol/liter). The reaction was initiated by adding ATP, and the inorganic phosphorus produced in the ATPase reaction was measured spectrophotometrically. The activity of Na,K-ATPase was taken to be the fraction of its activity inhibited by 1 mmol/ouabain per liter.

The linoleic acid 13(S)-hydroperoxide (13-HPODE) was obtained from linoleic acid immobilized with soybean lipoxygenase, as previously described. The final product was purified using liquid chromatography and assayed by high performance liquid chromatography, ultraviolet spectrometry, infrared spectrometry, ^1H-nuclear magnetic resonance, and polarimetry.

The results were statistically analyzed with Student's test for paired comparisons.

RESULTS

Figure 1 (curve *1*) shows the effect of 13-HPODE on passive Ca^{2+} transport across the sarcolemmic vesicular membrane. It can be seen that the hydroperoxide had little or no effect on membrane permeability to Ca^{2+} in concentrations of up to 10 μmol/liter but led to a dose-dependent acceleration of calcium exit from the membrane at higher concentrations.

Curve *2* in Fig. 1 depicts the effect from pretreating the myocardial sarcolemma with hydroperoxylinoleic acid on Na/Ca exchange. In these tests, 13-HPODE concentrations of only up to 10 μmol/liter were used because higher concentrations might decrease the recorded Na_i-dependent $^{45}Ca^{2+}$ entry by increasing passive calcium permeability. 13-HPODE stimulated the Na/Ca exchange markedly at 0.1 μmol/liter (the lowest concentration used) and increased it by more than 100% in concentrations of

about 5 μmol/liter. 13-HPODE did not affect the Na_i-independent (passive) $^{45}Ca^{2+}$ entry.

13-HPODE inhibited, also in a dose-dependent manner, the catalytic activity of sarcolemmic Na,K-ATPase (Fig. 1, curve *3*), which fell virtually to zero at a concentration of 100 μmol/liter; the IC_{50} was about 20 μmol/liter. It should be noted that the hydroperoxide inhibited only the ouabain-sensitive Na,K-ATPase activity.

It follows from the above that 13(S)-HPODE, a primary lipoxygenase product of linoleic acid, exerts a digitalis-like action on myocardial sarcolemmic Na,K-ATPase, stimulates NA/Ca exchange in the sarcolemma, and renders this plasma membrane more permeable to calcium ions. The concentrations at which 13-HPODE exhibited these effects lie within the physiological (or pathophysiological) ranges observed for intracellular concentrations of nonesterified fatty acids and their primary derivatives. For example, intracellular concentrations of arachidonic acid in activated platelets reach 50-100 μmol/liter [8] and those of lipid hydroperoxide also attain high micromolar values [3]. The high linoleic levels and presence of lipoxygenase activity in cardiac muscle cells [6] suggest that linoleic acid metabolites are formed in these cells and act as endogenous modulators of the sodium pump and Na/Ca exchange and thus of intracellular Ca^{2+} levels and myocardial contractility.

Although Na,K-ATPase may be inhibited by nonesterified linoleic acid and by other fatty acids [9,15], effects comparable to those recorded in this study are attainable at much higher concentrations. Linoleic acid has been reported to inhibit microsomal Na,K-ATPase with an IC_{50} of 110 μmol/liter and to reduce ^{3}H-ouabain binding with an IC_{50} of 250 μmol/liter [15]. Using methods and materials similar to ours, Philipson and Ward [9] did not observe a significant inhibition of Na,K-ATPase activity by linoleic or arachidonic acid in a concentration of 30 μmol/liter.

Linoleic and other unsaturated acids are capable of stimulating Na/Ca exchange [9], but are much less active than 13-HPODE, which produced a strong effect in a concentration as low as 0.1 μmol/liter and the maximal effect in a concentration of about 5 μmol/liter, as compared to 30 μmol/liter in the case of linoleic acid.

The 13-HPODE effects described above, at least those on calcium permeability and Na,K-ATPase activity, may be due to the ability of this linoleic acid hydroperoxide to form free radicals, in particular hydroxyl radical [16]. Sakuma *et al.* [10] found, however, that the inhibitory action of 13-HPODE on hydroxyprostaglandin dehydrogenase is not associated with the formation of this radical.

The hydroperoxide of linoleic acid was found to be devoid of pronounced detergent properties [5], which perhaps explains why it reduces membrane permeability to Ca^{2+} to a lesser extent than does linoleic acid itself [9]. Since the increase in the rate of calcium transport after exposure to 13-HPODE in the "threshold" concentration (10 μmol/liter) was slight, this compound can apparently be classed among the weak ionophores.

That such an endogenous inhibitor of Na,K-ATPase and endogenous activator of Na/Ca exchange as the linoleic acid hydroperoxide can be formed may be of fundamental importance for the regulation of cardiac activity. Lipoxygenase products of linoleic acid metabolism may modulate the activity of Ca^{2+}-transporting systems in the myocardial sarcolemma and also, as shown by Kaduce *et al.* [4], in the myocardial sarcoplasmic reticulum, which eventually should be reflected in the state of calcium homeostasis and in myocardial contractility.

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