EFFECT OF VARIOUS COMBINATIONS OF EXOGENOUS HIGHER FATTY ACIDS ON CARDIAC FUNCTION IN TRANSIENT CORONARY INSUFFICIENCY AND REPERFUSION

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The study of lipid metabolism in the myocardium has revealed two important facts. First, under aerobic conditions higher unsaturated fatty acids (HUFA) are one of the main sources of energy formation in cardiomyocytes [9]. Second, exogenous HUFA (arachidonic and linoleic) are precursors for prostaglandin (PG) synthesis and significantly reduce the frequency of cardiac arrhythmias and the degree of depression of contractility of the myocardium [5, 6] when it is damaged.

These facts served as the basis for the hypothesis that combinations of HUFA, containing precursors of PG synthesis, may exert a protective action during transient coronary insufficiency (TCI). A particular feature of TCI is that a period of local cessation of the coronary blood flow is followed by its resumption and by restoration of aerobic conditions in the previously ischemic region of the heart [1-3]. Accordingly, on the one hand, exogenous HUFA can serve as oxidation substrate in hyperfunctioning areas of the heart surrounding the zone of ischemia, where aerobic metabolism is preserved, and also in the reperfused zone of the myocardium, where aerobic conditions are restored, and on the other hand, HUFA such as arachidonic and linoleic acids, which are PG precursors, can facilitate their synthesis in the cardiomyocytes. Meanwhile, PG (A, E, F) formed in the myocardium, are known to exert an important protective and, in particular, antiarrhythmic effect [8, 10].

The aim of this investigation was to study the dynamics of the rhythm and contractile function of the heart during TCI after preliminary parenteral administration of HUFA combinations differing in their content of precursors for PG synthesis.

EXPERIMENTAL METHOD

Experiments were carried out on 77 noninbred male albino rats weighing 200 ± 10 g. TCI was produced, and the parameters of cardiac function determined and calculated by methods described previously [1, 2]. The duration of the period of myocardial ischemia (MI) in the different groups of animals was 10, 40, and 120 min respectively. During postischemic reperfusion (PRP) observations were made in the course of 40-60 min. HUFA, in the form of ethyl esters, were injected intramuscularly in a dose of 5 ml/kg body weight (or 20% of LD_{50}) 60 min before production of TCI. Two types of HUFA combinations were used: with high and low content of precursors for PG synthesis (arachidonic and linoleic acids) respectively. In the first version (experiment 1) the solution consisted of a combination of HUFA in proportion similar to those in lipid fractions of cardiomyocytes [12]. It contained 20.1% of ester of arachidonic acid, 34.9% of linoleic (altogether 55% of precursors for PG synthesis), 17.5% of oleic, 12% of palmitic, 6% of stearic, and 5.7% of linolenic. In the second version (experiment 2) the HUFA combination did not contain arachidonic acid, the direct precursor for PG synthesis. The proportions of HUFA were as follows: 46.8% of ester of linoleic acid, 16.1% of oleic, 26% of palmitic, 3.7% of stearic, and 6.9% of linolenic. Control animals received an intramuscular injection of 1.0 ml of physiological saline 60 min before production of TCI.

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TABLE 1. Frequency of Arrhythmias in Rats with TCI Treated with HUFA Combination Containing Precursors for Prostaglandin Synthesis (in %)

Parameter	Period of MI			Period of PRP								
				control			experiment 1			experiment 2		
				duration of MI before reperfusion, min								
	control	experiment 1	experiment 2	10	40	120	10	40	120	10	40	120
Total number of animals with arrhythmias	100	62,1	100	100	50	50	88,9	14,3	16,7	85,7	50	50
Including:												
with extrasystoles	0	77,8	13,6	17,4	50	82,6	87,5	100	100	83,3	100	75
with an extrasystole	17,4	22,2	81,9	17,4	50	17,4	12,5	0	0	16,7	0	25
PT + VF	82,6	0	4,5	82,6	0	0	0	0	0	0	0	0

Legend. PT + VF) Combined frequency of paroxysmal tachycardia and ventricular fibrillation.

EXPERIMENTAL RESULTS

In the course of the experiment three important facts were established: 1) both combinations of HUFA used had a significant antiarrhythmic action, which was observed not only in the period of MI, but also during PRP; 2) the HUFA combination containing arachidonic and linoleic acids as PG precursors gave a stronger antiarrhythmic effect than that without arachidonic acid; 3) the HUFA combination containing arachidonic acid lowered the degree of depression of parameters of cardiac contractility in the ischemic period of TCI compared with the control.

In the period of MI arrhythmias were observed in all control rats and in the animals in experiment 2 (Table 1). In experiment 1 arrhythmias developed in 62.1% of rats. The important point is that the character of the arrhythmias was changed in animals of the experimental series. For instance, whereas in the control paroxysmal tachycardia and ventricular fibrillation developed most frequently (in 82.6% of cases altogether), rats in experiment 2 developed an extrasystole (in 81.9%), and animals in experiment 1 had mainly single or grouped extrasystoles (in 77.8%).

During the period of PRP the frequency of arrhythmias differed depending on the duration of MI (Table 1). In the control, during PRP after a short period (10 min) of MI, cardiac arrhythmias were observed in all animals, and in 82.6% of them in the form of paroxysmal tachycardia and ventricular fibrillation. During PRP after a long period (40 and 120 min) of MI single extrasystoles or an extrasystole were observed in 50% of rats. Injection of HUFA in both experiment 1 and experiment 2 had the strongest antiarrhythmic effect during PRP after MI lasting 10 min. The effect was characterized by complete prevention of paroxysmal tachycardia and ventricular fibrillation. Arrhythmias in these series of experiments were manifested chiefly as single extrasystoles. Both versions of HUFA combinations prevented the development of an extrasystole during PRP following a longer period (40 min) of MI. Under these circumstances this effect in experiment 1 was 3.5 times greater than in experiment 2. During PRP after a very long period (120 min) of MI the HUFA combination not containing arachidonic acid had no antiarrhythmic action. Addition of arachidonic acid to the combination reduced the frequency of extrasystoles by two-thirds compared with the control.

The results of this stage of the investigation thus confirmed the hypothesis that HUFA combinations containing precursors for PG synthesis have a protective effect against arrhythmias both during MI and during PRP. One possible mechanism of this effect may be the less severe degree of damage to the cardiomyocyte membranes and enzymes (and, consequently, of the electrophysiological processes lying at the basis of pacemaker activity) by free radicals and lipid peroxidation products. The writers showed previously that during TCI these processes are considerably potentiated in the heart [2, 3]. Exogenous HUFA can serve as a "trap" for free radicals and can thus prevent their action on membrane lipids and proteins. This mechanism can also prevent labilization of lysosomal membranes and, consequently, can reduce the outflow of enzymes from them, hydrolyzing lipoprotein components of myocardial cells. It can also be supposed that some HUFA can act as "donors" for synthesis of phospholipids for damaged cardiomyocyte cell membranes.

TABLE 2. Dynamics of Parameters of CSH in Rats during TCI and Treatment by HUFA Combinations Containing Precursors for PG Synthesis (M ± m)

Parameter	Group of animals	Background	10 min of MI	40 min of PRP	Background	40 min of MI	40 min of PRP	Background	120 min of MI	40 min of PRP
Heart rate, beats/min	Control	339±2,0	376±8,0* (111)	348±8,0 (102)	348±5,4	336±10,0 (96,5)	322±6,0 (93)	340±21,0 (100)	249±170* (73)	216±9,0* (63)
	Experiment 1	347±16,6	336±23,0 (95,6)	311±17,0 (89,6)	358±25,0	345±21,0* (96,4)	230±12,0* (64)	378±16,0 (100)	175±12,0* (73)	228±23,0* (60)
	Experiment 2	354±6,0	374±24,0 (106)	303±17,0* (85,6)	344±16,0	355±23,0 (103)	323±12,0 (94)	377±28,0 (100)	250±16,0* (63)	233±25,0* (62)
Maximal rate of rise of pressure in left ventricle, mm Hg/sec	Control	1743±58	1693±80 (97)	2260±174* (129,7)	2206±117	1873±52* (84,5)	1632±115* (74)	1273±130 (100)	913±61* (72)	837±51* (66)
	Experiment 1	1766±191	1629±148 (92)	1536±153 (86,9)	1802±157	2006±171 (111)	1240±37* (68,8)	2083±150 (100)	1245±86* (67)	995±154* (48)
	Experiment 2	1899±160	1707±185 (90)	1519±184* (86,9)	1739±189	1865±206 (107,2)	1570±108 (90,3)	1844±104 (100)	1392±151* (75)	1091±112* (60)
Mean rate of fall of pressure in left ventricle, mm Hg/sec	Control	659±12	826±21* (125)	639±80 (97)	677±24	660±40 (97,5)	558±18* (82,4)	722±73 (100)	464±41* (64)	382±15* (53)
	Experiment 1	728±40	791±80 (108)	639±14* (87,4)	871±95	724±32 (81,3)	533±37* (61,2)	696±26 (100)	524±71* (75)	353±91* (51)
	Experiment 2	747±56	465±118* (62)	588±58* (78,7)	784±29	757±60 (96,5)	643±41* (81,9)	798±55 (100)	691±152* (87)	420±60* (53)
Integrative parameter of cardiac function ($1 \cdot 10^3$)	Control	27±0,3	32±0,7* (120)	29±0,9 (107)	30±0,8	27±1,5* (91,6)	24±0,5* (81,2)	27±2,3 (100)	19±1,9* (70,3)	16±0,7* (57)
	Experiment 1	32±2,0	32±2,7 (100)	24±1,1* (82,7)	32±2,0	31±2,1 (98,1)	20±0,6* (61,6)	34±2,4 (100)	22±2,4* (62,5)	17±3,0* (52)
	Experiment 2	29±1,4	37±3,2* (126)	26±1,2 (81,2)	31±1,7	30±1,2 (97,7)	27±1,6* (88,6)	33±2,4 (100)	26±1,6* (78)	19±2,2* (57)

Legend. *) Difference from background significant at $P < 0.05$ level. Percentage of background value, taken as 100, given in parentheses.

In the next stage of the investigation the effect of both versions of HUFA combinations on parameters of the contractile function of the heart (CFH) was studied (Table 2).

In the ischemic period of TCI the control animals and rats in experiment 2 developed tachycardia in the first 10-15 min of MI, the pressure in their left ventricle rose, and the integrative parameter of left ventricular function — the product of the heart rate and the pressure developed in the left ventricle — increased. Addition of arachidonic acid to the HUFA combination prevented these changes. If it is recalled that the integrative parameter of cardiac function indirectly reflects the level of work of the heart and its oxygen consumption, this fact can be interpreted as evidence of the protective action of arachidonic acid on the early stage of MI. Conversely, in animals receiving the HUFA combination without arachidonic acid, the integrative parameter of CFH after 10 min of MI was 26% higher than the background value. At the same time, a considerable (by 37.8% compared with the background) fall was recorded in the mean rate of fall of pressure in the left ventricle, possible evidence of more marked changes in the mechanisms responsible for myocardial relaxation when the HUFA combination without arachidonic acid was used.

Postischemic resumption of the coronary blood flow in the control animals was accompanied by restoration of the parameters of CFH to close to their initial levels, but only after a short period (10 min) of MI. PRP after longer periods (40 and 120 min) of MI was characterized by a progressive fall of all parameters of CFH studied. PRP after preliminary administration of HUFA combinations led to a further decline in some parameters of CFH in both experiment 1 and experiment 2. This applied mainly to the rate of systolic rise and diastolic fall of pressure in the left ventricle.

A protective action on parameters of CFH was thus observed after the use of an HUFA combination containing arachidonic acid. This effect was observed only in the ischemic period of TCI. During PRP both HUFA combinations aggravated the disturbance of CFH. This latter effect may be largely explained on the grounds that HUFA, which are ionophores, promote accumulation of cations during PRP in the mitochondria [13], with swelling of these organelles and spatial dissociation of enzymes, including tissue respiration enzymes. The situation may also be aggravated by the direct action of calcium ions, which accumulate in excess in the mitochondria during PRP, and which directly uncouple oxidation and phosphorylation [2, 3]. The ATP deficiency developing as a result of this in the zone of myocardial injury and, to a lesser degree, in surrounding areas of the heart, as the present writers and others showed previously [2, 7, 9, 11], leads mainly to depression of CFH, for about 90% of the total amount of energy synthesized in the cardiomyocytes is expended on the contractile process [7, 11]. Pacemaker activity may not be significantly impaired under these circumstances, for the expenditure of energy on it, on the one hand, is comparatively small and, on the other hand, it is provided mainly by energy of glycolysis and the pentose phosphate cycle [7, 11].

On the whole the results of this investigation are evidence of the essential antiarrhythmic action of the HUFA combination containing precursors for PG synthesis, during both MI and subsequent reperfusion. In addition, the HUFA combination including arachidonic acid reduces the degree of depression of the contractile process in the ischemic period of TCI compared with the control. These results may be interesting for clinical cardiology, for types of TCI reproduced in animals are experimental models of forms of ischemic heart disease widely distributed in man: angina, the intermediate coronary syndrome (so-called threatening myocardial infarction), and also states after revascularization of the myocardium in the acute period of infarction.

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CHANGES IN PLATELET STRUCTURE AND FUNCTION IN EXPERIMENTAL ATHEROSCLEROSIS

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The role of platelets in atherogenesis and the connection between platelet function and the pattern of lipid metabolism and its changes in atherosclerosis are widely familiar [7, 8, 12, 13]. What leads to changes in platelet function during atherogenesis? We know that the physiological activity of any cells, including platelets, largely depends on the physicochemical properties of their membranes. A dynamic regulatory role in biological membranes, which consists in particular of the control of fluidity, is played by cholesterol (Ch). A decrease in fluidity arising with an increase in the fraction of Ch prevents normal functioning of membrane-bound enzymes and disturbs some properties of the cells. It was shown previously [3] that incubation of platelets with low- and very-low-density lipoproteins, taken from patients with ischemic heart disease, enhances their aggregation, whereas incubation with high-density lipoproteins has the opposite effect. Considering the donor and acceptor character of these lipoproteins relative to Ch, it can be postulated that changes in platelet function are determined primarily by the quantity of this steroid in their membrane. We know that in hypercholesterolemia there is an increase in the Ch content in membranes of erythrocytes [1], affecting their function. It can be tentatively suggested that similar phenomena also take place in platelets, and that this is one cause of their hyperreactivity.

In the investigation described below changes in the physicochemical characteristics of platelet membranes and in the functional activity of the cells (ability to aggregate) were studied in rabbits with experimental atherosclerosis.

EXPERIMENTAL METHOD

Experiments were carried out on 35 Chinchilla rabbits. Experimental atherosclerosis was induced in 15 rabbits by feeding them daily for 3 months with 0.25 g Ch/kg body weight. The control group consisted of 20 rabbits. Blood was collected from the auricular vein into siliconized tubes containing 3.8% sodium citrate in the ratio of 9:1 by volume. Platelets were isolated by gel filtration from platelet-enriched plasma on Sepharose 2B. The content of Ch and phospholipids (PL) was determined after extraction of the lipids by Folch's method [6], by the methods of Abell and Vaskovsky [14]. The structure of the membranes was studied by the electron paramagnetic resonance (EPR) of spin probes method, using stearic acid derivatives with nitroxyl fragments in positions 5 (I) and 16 (II) relative to the carboxyl group. The appearance of the EPR spectra and the structural formulas of the probes used in rabbit platelets are shown in Fig. 1. The probes were introduced into the test samples in the form of ethanol solutions; their final concentration was 10^{-5} M per $6 \cdot 10^{8}$ cells/ml, and

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