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THE RELATION BETWEEN FATTY ACID MOBILIZATION AND CONTRACTILITY IN THE ISOLATED, PERFUSED RAT HEART

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SUMMARY

The contractility of hearts from normal fed rats is decreased by 70% during perfusion with 50 µM chloroquine, which is a potent inhibitor of endogenous lipolysis. In triacylglycerol-rich hearts, obtained by feeding rats rapeseed-oil, chloroquine depresses lipolysis much less, while contractility was found to be inhibited only 30%. In both groups of hearts the effect of chloroquine was decreased by adding fatty acids, prostaglandin E_1, the Ca^{2+}/Mg^{2+} ionophore X-537A or more Ca^{2+} to the perfusion fluid. Norepinephrine and glucagon also stimulate chloroquine-depressed hearts. The conclusion is therefore reached that fatty acids act as Ca^{2+}-vehicles in heart cells and that chloroquine, by inhibiting lipolysis, decreases Ca^{2+}-transport by lowering unesterified fatty acid levels.

Alteration of the intracellular "free" ionic calcium concentration controls the contractile state of cardiac striated and vascular smooth muscle[1]. Therefore pharmacological, metabolic or hormonal modification of the inotropic state of these tissues may be caused by altered calcium transport through or binding to membranes such as the cell membrane, the sarcoplasmic reticulum and the inner mitochondrial membrane[1]. The Ca^{2+}-ionophoric properties of fatty acids have been concluded from earlier experiments carried out in our laboratory[2]. Medium- and long-chain fatty acids were found to have a positive inotropic effect when in vitro perfusions were carried out at low Ca^{2+}-concentrations and to increase the coronary flow rate.

The availability of ionic calcium to the contractile proteins may not only be determined by membrane processes involved in calcium uptake and release to the cytoplasm, but also by (intracellular) substances which influence the calcium transport from the sites of release to the sites of contraction. It may be questioned then if endogenous fatty acids, as products of intracellular lipolysis, are also involved in the contractile behaviour of the heart.

In a previous publication we[3] have shown that the weakly basic chloroquine which selectively accumulates in the lysosomes[4,5,6] and inhibits lysosomal degradative processes[4,7,8], inhibits basal- and hormone-stimulated lipolysis of isolated, perfused rat hearts from normal fed and rapeseed-oil fed rats.

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Inhibition in control hearts is complete, while in hearts from rapeseed-oil fed rats still some lipolysis was observed. It was noticed in the same study that the chloroquine-induced inhibition of lipolysis was accompanied by a depression of the contractility and that this depression was markedly less in hearts from rapeseed-oil fed rats. The present paper discusses the mechanism of chloroquine-induced reduction of myocardial contractility and suggests that fatty acids may act as calcium-vehicles during the excitation-contraction cycle.

METHODS

Male Wistar rats (200-300 g) were fed a standard laboratory chow (Purina pellets) or put on a 3-4 day diet containing 40% of the calories (40 cal%) as rapeseed-oil (RSO), containing 38% w/v erucic acid. The animals were anesthetized by an intraperitoneal injection of pentobarbital (70 mg/kg body weight), the hearts were quickly excised and perfused retrogradely as described previously[9]. Potassium palmitate (0.5 mM) was dissolved in distilled water of 70-80°C and added dropwise to a fatty acid-free bovine serum albumin (BSA) solution in a molar ratio of 1:1. Before use the fatty acid-containing BSA solution was passed through a, washed, 1.2 μ Millipore membrane filter. Glucagon and norepinephrine were infused at 10^{-7} M final concentration in the aortic canula. Contractility was revealed as apex displacement[10] and the coronary flow rate was determined. After a 15-20 min preperfusion the hearts showed a constant contraction amplitude and coronary flow rate. The values for contractility and coronary flow rate at the end of this stabilization period were taken as controls and changes in both parameters occurring during the subsequent perfusion were expressed in % of control. Basal coronary flow rates in hearts from rapeseed-oil fed rats differed significantly from flow rates in hearts from normal fed rats (7.34 ± 0.16 (n=10) vs 6.32 ± 0.09 (n=20), $P<0.001$)[11]. In the collected coronary effluents glycerol was determined fluorometrically[3,12]. The results are presented as mean values ± standard error of the mean (S.E.M.). n is the number of observations and significance was calculated with Students t-test (two tailed) $P>0.05$ was considered to be not significant.

RESULTS

Table I presents the depressive action of chloroquine upon the contractility of hearts from normal fed, and rapeseed-oil fed rats. Rapeseed-oil feeding leads to an increase of the endogenous triglyceride levels and lipolytic activities of the heart[3,13] and postheparin serum[14]. The, concentration-dependent, chloroquine-induced reduction of the contraction amplitude in hearts from rapeseed-oil fed rats was only about 30% while a 70% decrease was observed in hearts from normal fed rats. From Table II it can be observed that the detrimental effect of chloroquine could (partially) be overcome by the addition of octanoate, palmitate, prostaglandin E_1 or more Ca^{2+} (2.7 mM final concentration) to the perfusion medium. The effects of chloroquine and of the additions made during perfusion reached a steady state within 2-3 minutes and were completely reversible during subsequent control perfusion. Octanoate, palmitate, prostaglandin E_1

TABLE I

THE EFFECT OF CHLOROQUINE UPON CONTRACTILITY OF HEARTS FROM NORMAL FED, AND RAPESEED-OIL FED RATS

Perfusion	Cardiac contractility (% of control) normal (n)	rapeseed-oil (n)
control	100	100
" + 20 μM chloroquine	69.5 ± 2.4 (5)	n.d.[a]
" + 50 μM chloroquine	30.5 ± 3.9[b] (6)	70.7 ± 3.3[b] (4)

[a] n.d. = not determined.

[b] $P<0.001$ for hearts from normal fed rats vs hearts from rapeseed-oil fed rats.

or excess Ca^{2+} were not able to overcome the chloroquine-inhibition of endogenous lipolysis, as measured by the glycerol release into the perfusate (not shown). The stimulatory effect of 1 mM octanoate upon chloroquine depressed contractility was not due to enhanced formation of acetyl-CoA since 5 mM 3-hydroxybutyrate, added to the chloroquine containing perfusion medium instead, did not have any effect (not presented).

A complete restoration of the depressed contractility was reached by injection of 10 μl of 3 mg/ml Ca^{2+}/Mg^{2+} ionophore X-537A, in 50% ethanol ($^{w}/v$) in the aortic canula. 10 μl of 50% ethanol ($^{w}/v$) instead was without effect. These findings suggest that chloroquine limits the Ca^{2+} availability to the contractile apparatus of the cells.

It has been shown before[3] that chloroquine does not alter hormone-stimulated glycogenolysis in heart. The addition of 10^{-7} M norepinephrine or 10^{-7} M glucagon leads to an increase of both the basal and chloroquine-depressed contractile states in hearts of normal fed, and rapeseed-oil fed rats (Table III). The further addition of octanoate proved to be without significant stimulatory effect.

DISCUSSION

In a previous communication from this laboratory we reported the chloroquine-induced inhibition of basal- and norepinephrine-stimulated lipolytic activity in rat hearts and in adipocytes[3]. The inhibition of lipolysis was complete in hearts from rats fed control food and 75% in hearts from rapeseed-oil fed rats. Since the weakly basic chloroquine is selectively accumulated by the lysosomes[4], and thereby increases the intralysosomal pH[17], it is concluded that endogenous lipolysis might be of lysosomal origin.

TABLE II

THE EFFECTS OF FATTY ACIDS, PROSTAGLANDIN E_1, EXCESS CALCIUM, AND IONOPHORE X-537A, UPON BASAL AND CHLOROQUINE-DEPRESSED CONTRACTILITY IN HEARTS FROM NORMAL FED, AND RAPESEED-OIL FED RATS

Perfusion	Cardiac contractility (% of control)			
	normal	(n)	rapeseed-oil	(n)
control	100		100	
" + 50 µM chloroquine	30.5 ± 3.9	(6)	70.6 ± 3.3	(4)
" + 1 mM octanoate	105.6 ± 4.6[b]	(5)	n.d.[a]	
" + 50 µM chloroquine + 1 mM octanoate	65.7 ± 2.8[c]	(5)	80.6 ± 2.0[d]	(4)
" + 0.5 mM palmitate	94.2 ± 0.8	(3)	n.d.[a]	
" + 50 µM chloroquine + 0.5 mM palmitate	54.8 ± 1.4	(3)	n.d.[a]	
" + 10^{-7} M prostaglandin E_1	112.9 ± 1.6	(5)	n.d.[a]	
" + 50 µM chloroquine + 10^{-7} M prostaglandin E_1	55.7 ± 4.0[c]	(6)	83.2 ± 3.2[d]	(4)
" + 2.7 mM calcium	127.3 ± 4.4	(5)	n.d.[a]	
" + 50 µM chloroquine + 2.7 mM calcium	89.1 ± 2.6[c]	(4)	90.7 ± 2.3[e]	(4)
" + 50 µM chloroquine + 30 µg X-537A	94.0-102.4	(2)	n.d.[a]	

[a] n.d. = not determined

[b] not significant

[c] P 0.001 vs control + 50 µM chloroquine

[d] P 0.05 vs control + 50 µM chloroquine

[e] P 0.005 vs control + 50 µM chloroquine

As judged from the glycerol release, chloroquine induces a severe reduction of the long-chain fatty acid formation in hearts from control fed rats. It also will reduce fatty acid levels in hearts from rapeseed-oil fed rats, which have been found to be increased[18].

The present work demonstrates that chloroquine depresses the contractile state of hearts from control rats more severely when compared with hearts from rapeseed-oil fed rats. At all experimental conditions chloroquine inhibited glycerol release. Since chloroquine probably is not acting upon energy metabolism (no stimulation of nucleoside release from the hearts was observed; not shown) and does not influence hormone-sensitivity, it may be assumed that the intracellular fatty acid levels are related to the decrease in contractility. Increase of the intracellular fatty acid concentration, by the addition of 1 mM octanoate or 0.5 mM palmitate indeed restores the decreased contractile state to a large extent in both groups of hearts. The stimulatory effect of octanoate

TABLE III

THE EFFECTS OF NOREPINEPHRINE AND GLUCAGON (PLUS ADDITIONAL OCTANOATE) UPON CHLOROQUINE-DEPRESSED CONTRACTILITY IN HEARTS FROM NORMAL FED RATS

Perfusion	Cardiac contractility (% of control) (n)
control	100
" + 50 μM chloroquine	30.5 ± 3.9 (6)
" + 10^{-7} M norepinephrine	120.4 ± 1.1 (7)
" + 50 μM chloroquine + 10^{-7} M norepinephrine	71.8 ± 4.8 (5)
" + 50 μM chloroquine + 10^{-7} M norepinephrine + 1 mM octanoate	81.9 ± 2.2[a] (3)
" + 10^{-7} M glucagon	141.4 ± 2.7 (5)
" + 50 μM chloroquine + 10^{-7} M glucagon	102.3 ± 4.9 (3)
" + 50 μM chloroquine + 10^{-7} M glucagon + 1 mM octanoate	109.0 ± 6.1[a] (3)

[a] not significant *vs* control + 50 μM chloroquine + 10^{-7} M norepinephrine resp. glucagon.

in hearts from normal fed rats is more prominent than in hearts from rapeseed-oil fed rats. Also by increasing the availability of Ca^{2+} to the contractile apparatus (by the addition of prostaglandin E_1, of which the ionophoric properties have been shown[19,20], the Ca^{2+}/Mg^{2+} ionophore X-537A or by increasing the calcium concentration in the perfusion fluid) an increase of the contraction amplitude is observed in both groups of hearts, in the presence of chloroquine. Since, however, the recovery of the chloroquine-induced decrease in contractility is not completely overcome by these additions, the drug may have an additional, inhibitory action, which is possibly related to membrane phosphorylation. The norepinephrine- and glucagon-induced stimulation of myocardial contractility is probably mediated by cyclic AMP-dependent protein kinase activation, leading to increased phosphorylation of the cell membrane and sarcoplasmic reticulum[21]. Indeed, under conditions of elevated intracellular cyclic-AMP levels the chloroquine-induced decrease in contractility is less marked. Fatty acids do not activate the myocardial adenylcyclase system[2], but may still be involved in protein kinase activation if they serve as Ca^{2+}-vehicles in the cells, since Ca^{2+} stimulation of protein kinase is well documented. Therefore, it may be concluded that endogenous fatty acids possibly serve as Ca^{2+}-vehicles through membranes and as Ca^{2+}-shuttles between intracellular compartments, such as the sites of Ca^{2+}-storage and contractile proteins. This hypothesis implies that endogenous

lipolysis might be involved in the modulation (or regulation) of myocardial contractility.

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