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Short communication

Myocardial contractility after infarction and carnitine palmitoyltransferase I inhibition in rats

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Abstract

Inhibition of carnitine palmitoyltransferase I with etomoxir increases sarcoplasmic reticulum Ca^{2+}-transport and V_1 isomyosin expression. To test whether etomoxir attenuates contractile dysfunction after myocardial infarction, we compared the contractility of papillary muscles from etomoxir- and placebo-treated rats 6 weeks after infarction. Etomoxir induced cardiac hypertrophy in animals with small infarctions, and enhanced compensatory heart growth at large infarct size. Contractile function of papillary muscles from etomoxir-treated rats was improved particularly in animals with small infarctions. Thus, induction of mild cardiac hypertrophy by etomoxir in rats with small infarctions may be beneficial for myocardial performance.

Keywords: Heart, Rat; Contractile function; Carnitine palmitoyltransferase I inhibition; Etomoxir; Hypertrophy; Infarct size

1. Introduction

Altered cardiac contractility and compensatory hypertrophy of the non-infarcted myocardium are considered as risk factors for increased cardiovascular morbidity and mortality after myocardial infarction (Pfeffer et al., 1992). On the other hand, we could demonstrate that post-infarction hypertrophy is associated with increased resistance of the heart to oxidative stress due to adaptive changes in Ca^{2+} handling, creatine kinase isoenzymes and antioxidant enzymes (Wagner et al., 1998). After infarction, we also found alterations of isometric twitch characteristics which were attenuated by angiotensin-converting enzyme inhibition and β-adrenoceptor blockade in the surviving rat myocardium (Wagner et al., 1997).

Clinical trials have shown that inhibition of mitochondrial carnitine palmitoyltransferase I with etomoxir (2(6(4-chlorophenoxy)hexyl)oxirane-2-carboxylate) improved cardiac contractile function in patients with heart failure (Schmidt-Schweda and Holubarsch, 1997). The beneficial action of etomoxir may involve a metabolic shift from fatty acid oxidation to glucose utilisation (Rupp et al., 1992). Etomoxir promotes cardiac growth and increases myosin isoenzyme V_1 and sarcoplasmic reticulum Ca^{2+} ATPase activities in the heart (Rupp et al., 1992; Vetter and Rupp, 1994). We have recently shown that the rates of contraction and relaxation of rat papillary muscle and sarcoplasmic reticulum Ca^{2+}-transport were impaired in hypertrophied hearts after experimental infarction (Wagner et al., 1998). In this study, we examined whether long-term treatment with etomoxir would preserve contractile function of the surviving myocardium after infarction.

2. Material and methods

2.1. Induction of myocardial infarction

The investigation conforms with the Guide for the Care and Use of Laboratory Animals published by the US National Institute of Health (ISBN 0-309-05377-3, revised

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1996). Myocardial infarction was induced by ligation of the left coronary artery under chloral hydrate anaesthesia (400 mg/kg) in 10–15-week-old male Wistar rats weighing between 250 and 280 g (Wagner et al., 1997). For sham operation ($n = 22$), ligations were placed beside the coronary artery. Surviving rats (about 50%) with infarction were randomised on the first post-operative day and treated for 6 weeks either with placebo ($n = 16$) or with etomoxir ($n = 28$). To avoid acute drug effects, treatment was stopped 36 h before the start of the experiments. Etomoxir was added to the drinking water. The water intake was adjusted and monitored to assure a daily dose of 10 mg etomoxir/kg body weight. A comparable oral dose of etomoxir has been shown to inhibit carnitine palmitoyltransferase I in rats as indicated by modified lipid metabolism (Rupp et al., 1992; Vetter and Rupp, 1994). Six weeks after coronary artery ligation, the rats were anaesthetised by intraperitoneal injection of ketamine hydrochloride (75 mg/kg) plus xylazine hydrochloride (7.5 mg/kg), and the hearts were excised. Left ventricular papillary muscles were dissected, and both heart chambers were weighed separately. Visible transmural infarction size was estimated by measuring the percentage of left ventricular endocardial circumference replaced by scar tissue. Small non-transmural infarctions were identified by light microscopical examination. Since small infarct size (< 25% of the ventricular area) did not induce significant hypertrophy in untreated animals, the preparations were divided into placebo- and etomoxir-treated subgroups with small (< 25%) and large infarct size (26–40%) of the left ventricular area.

2.2. Experimental protocol for papillary muscles

Posterior left ventricular papillary muscles were mounted horizontally in a tissue bath. The bathing solution which was oxygenated with pure O_2 (pO_2 about 80 kPa) at 31°C, had the following composition (mM): NaCl 140.0, KCl 5.0, $CaCl_2$ 1.5, $MgCl_2$ 1.1, Tris–HCl 10.0, glucose 11.1 (pH 7.43). Field stimulation with 0.5 Hz and isometric force measurement were achieved using the stimulator module, the force transducer and the bridge amplifier of the Plugsys system 603 (Hugo Sachs Elektronik, March-Hugstetten, Germany). Data acquisition, analysis of the mechanogram and statistics were performed on a computer using software developed in our laboratory. Preload was adjusted to a value producing half-maximal load-dependent peak force (PF). Under these conditions, force development was nearly stable for more than 2 h. Biphasic rectangular impulses of 7-ms duration and a current 30% above threshold were applied. To ensure adequate oxygen supply to the preparations, the stimulation frequency was fourfold increased for several minutes without affecting contractile function. To avoid the build-up of a hypoxic core in the papillary muscles, the preparations were allowed to equilibrate for about 25 min at the low stimulation frequency of 0.25 Hz at 0.75 mM $CaCl_2$ and low preload of 1 mN. PF, maximum dF/dt (dF/dt_{max}), the time to peak force (TPF), minimum dF/dt (dF/dt_{min}) and the relaxation time for decline of force by 50% (RT50) and by 97% (RT97) were determined. In addition, $(dF/dt)_{max}/PF$ and $(dF/dt)_{min}/PF$ were calculated. Plotting of $(dF/dt)/F$ vs. F resulted in a partly hyperbolic force–velocity relationship. Extrapolation of the hyperbolic segment to $F = 0$ provided a "V_{max}-equivalent" as an assessment for the rate of sarcomeric shortening (Gülch, 1990).

2.3. Statistical analysis

Values are expressed as means ± S.E.M. The Wilcoxon test for unpaired or paired samples was used for the non-normally distributed data. $P < 0.05$ was considered statistically significant.

3. Results

Body weights were comparable in all groups (Table 1). In the placebo group, at the small infarct size of 6.8 ± 3.2%, the values for left and right ventricular weight-to-body

Table 1
Effect of postinfarct treatment with the CPT-1 inhibitor etomoxir on body and heart weights of rats with small and large myocardial infarction

	Without infarct	Small infarct		Large infarct	
	Sham operated ($n = 22$)	Placebo ($n = 9$)	Etomoxir ($n = 11$)	Placebo ($n = 7$)	Etomoxir ($n = 17$)
Body weight (g)	378.3 ± 6.0	360.0 ± 8.5	370.3 ± 5.5	345.5 ± 13.0	351.1 ± 9.2
Ventricular weights (mg)	981 ± 30	1002 ± 40[a]	1200 ± 30[a,b,c]	1242 ± 60[a,b]	1381 ± 72[a,b]
Left ventricular weight/body weight (mg/g)	2.12 ± 0.03	2.18 ± 0.06[a]	2.57 ± 0.06[b,c]	2.75 ± 0.15[a,b]	2.82 ± 0.12[b]
Right ventricular weight/body weight (mg/g)	0.55 ± 0.02	0.59 ± 0.06[a]	0.68 ± 0.03[a,b]	0.86 ± 0.09[a,b]	1.11 ± 0.03[a,b,c]
Papillary muscle cross-sectional area (mm^2)	0.95 ± 0.09	0.94 ± 0.17	0.84 ± 0.12[a]	1.17 ± 0.11	1.21 ± 0.11[a,b]

Values are means ± S.E.M. Rats with an infarct size < 25% were grouped in the "small infarct" group; a infarct size range of 25–40% was used to group the rats into the "large infarct" group.

[a] $P < 0.05$ significant difference between the respective value of the small and large infarct group.

[b] $P < 0.05$ vs. sham operation.

[c] $P < 0.05$, significantly different from respective placebo value.

weight ratio were close to those in sham operated animals. Only in the placebo group, at the large infarct size of $34.9 \pm 1.9\%$, higher values of both ratios compared to sham operation indicate hypertrophy of left and right ventricles. In comparison to sham operation, the higher values of both ratios in etomoxir-treated animals with small infarct size of $10.1 \pm 3.3\%$ group indicate cardiac hypertrophy which was not seen in placebo-treated rats at a similar infarct size. In etomoxir-treated animals at the large infarct size of $33.2 \pm 1.0\%$, the right ventricular weight-to-body weight ratio was significantly increased and higher than in the respective placebo group. The cross-sectional areas of the papillary muscles as a measure for hypertrophy after infarction (Wagner et al., 1998) were significantly enlarged in etomoxir-treated animals with large infarction vs. sham operation (in the placebo group with large infarction, the value of P for significance was 0.08).

3.1. Contractile function

Isometric PF development per cross-sectional area was not significantly different between the experimental groups (Table 2). Rates of contraction and relaxation of the non-hypertrophied myocardium in the placebo group with small infarction were clearly decreased compared to sham operation.

Long-term treatment with etomoxir induced cardiac hypertrophy at low infarct size leaving the course of contraction in this group largely unchanged vs. sham operation. In particular, etomoxir treatment completely normalised the course of relaxation as indicated by the nearly identical $(dF/dt_{max})/(dF/dt_{min})$ ratios in etomoxir-treated animals with small infarction and in sham operated rats. Increasing the stimulation frequency from 0.5 to 1 Hz produced a typical negative inotropic effect in etomoxir-treated animals with small infarction ($PF_{1\ Hz}/PF_{0.5\ Hz}$: 0.93 ± 0.02, $P < 0.05$ vs. 1.00, $n = 10$) as after sham operation ($PF_{1\ Hz}/PF_{0.5\ Hz}$ 0.95 ± 0.01, $P < 0.05$ vs. 1.00, $n = 22$). However, negative inotropy in placebo-treated animals with small infarction gradually disappeared at the 20th twitch ($PF_{1\ Hz}/PF_{0.5\ Hz}$: 1.00 ± 0.02) which is not seen in normal rat myocardium (Lammerich et al., 1995).

After onset of infarction-induced hypertrophy in placebo-treated animals with large infarction, the rate of contraction was lower than after sham operation, whereas the rate of relaxation was normal as indicated by the lower $(dF/dt_{max})/(dF/dt_{min})$ ratio.

Interestingly, the V_{max}-equivalent as a measure for the maximum velocity of unloaded sarcomere shortening, which is thought to correlate with the myosin–ATPase activity (Gülch, 1990), was significantly reduced in the hypertrophied papillary muscles in placebo-treated animals with large infarction compared to small infarction, to sham operation, and also to the respective etomoxir-treated group. In the placebo group, a negative correlation between the "V_{max}-equivalents" and the cross-sectional area of papillary muscles ($r = -0.72$; $n = 16$; $P < 0.01$) was found. In etomoxir-treated animals with large infarction, along with the development of hypertrophy the decrease in the "V_{max}-equivalent" was less pronounced. Accordingly, a significant correlation between the "V_{max} equivalents" and the cross-sectional area of papillary muscles was not found ($r = -0.35$; $n = 28$).

Other parameters for the rate of contraction were similar in both groups with large infarction. In etomoxir-treated animals with large infarction, the relaxation rate of the hypertrophied papillary muscles was not significantly different from that in the respective placebo group.

Table 2
Effect of postinfarct treatment with etomoxir on isometric twitches of isolated papillary muscle of sham-operated rats without myocardial infarction and animals with small and large infarction

	Without infarct	Small infarct		Large infarct	
	Sham operated ($n = 22$)	Placebo ($n = 9$)	Etomoxir ($n = 11$)	Placebo ($n = 7$)	Etomoxir ($n = 17$)
PF/cross-sectional area, mN/mm^2	4.3 ± 0.7	4.0 ± 1.2	3.3 ± 0.9	4.0 ± 1.5	4.6 ± 1.2
TPF, ms	143.3 ± 3.4	161.3 ± 6.4^{a}	$146.9 \pm 4.4^{b,c}$	157.1 ± 1.9^{a}	$155.6 \pm 2.7^{a,c}$
(dF/dt_{max})/PF, 1/s	13.4 ± 0.3	11.5 ± 0.4^{a}	$12.7 \pm 0.4^{b,c}$	12.1 ± 0.3^{a}	$12.1 \pm 0.2^{a,c}$
$(dF/dt_{max})/F$, 1/s	29.0 ± 0.5	25.7 ± 0.9^{a}	27.0 ± 0.9^{a}	25.9 ± 0.6^{a}	26.2 ± 0.4^{a}
V_{max}-equivalent, 1/s	154.1 ± 4.3	$142.7 \pm 6.6^{a,c}$	148.9 ± 5.0^{c}	$122.9 \pm 6.4^{a,c}$	$138.0 \pm 2.3^{a,b,c}$
RT50, ms	80.3 ± 2.2	$99.1 \pm 8.9^{a,c}$	81.6 ± 4.5^{b}	79.7 ± 2.1^{c}	85.1 ± 3.0
RT97, ms	168.3 ± 4.8	$208.4 \pm 16.9^{a,c}$	176.7 ± 10.2	165.7 ± 3.3^{c}	173.1 ± 5.6
(dF/dt_{min})/PF, 1/s	8.6 ± 0.2	$7.5 \pm 0.3^{a,c}$	8.4 ± 0.4^{b}	8.9 ± 0.3^{c}	8.3 ± 0.3
$(dF/dt_{min})/F$, 1/s	13.9 ± 0.5	$11.6 \pm 0.8^{a,c}$	13.6 ± 0.8	14.6 ± 0.7^{c}	13.9 ± 0.5
$(dF/dt_{max})/(dF/dt_{min})$	1.56 ± 0.03	$1.66 \pm 0.09^{a,c}$	1.54 ± 0.06	$1.38 \pm 0.04^{a,c}$	1.46 ± 0.04

Values are means ± S.E.M. Rats with an infarct size < 25% were grouped in the "small infarct" group; an infarct size range of 25–40% was used to group the rats into the "large infarct" group. PF, peak force; dF/dt_{max}, maximum dF/dt; TPF, time to PF; dF/dt_{min}, minimum dF/dt; RT50(97), relaxation time for the decline of PF by 50% (97%).

[a] $P < 0.05$ vs. sham operation.

[b] $P < 0.05$ significantly different from the respective placebo value.

[c] $P < 0.05$ significant difference between the respective value of the small and large infarct group.

4. Discussion

This study demonstrates that chronic application of a low dose of etomoxir almost completely restores the mechanical function of the rat myocardium after myocardial infarction, particularly at a small infarct size.

In contrast, myocardial contraction and relaxation were disturbed in placebo-treated animals after infarction (Wagner et al., 1998). Isometric contraction and relaxation depend upon myosin isoenzyme activity and Ca^{2+} homeostasis (Bers, 1997). It is likely, therefore, that a shift from the "fast" V_1 to the "slow" V_3 myosin isoenzyme (Mercadier et al., 1981) and a reduced sarcoplasmic reticulum Ca^{2+}-transport (Zarain-Herzberg et al., 1996; Wagner et al., 1998) may at least in part account for the contractile dysfunction in the placebo group after infarction. This idea is supported by the prolonged TPF and the delay especially of the early relaxation, which may also indicate an impaired Ca^{2+} removal in the placebo group with small infarction. In the etomoxir-treated group with small infarction, isometric twitch characteristics and negative frequency inotropism suggest that the hypertrophic response to etomoxir was associated with preserved function of the myofilaments and undisturbed Ca^{2+} homeostasis. These findings are consistent with the stimulatory effect of etomoxir on the expression of the "fast" V_1 myosin isoform and the sarcoplasmic reticulum Ca^{2+} ATPase (Rupp et al., 1992; Vetter and Rupp, 1994).

At large infarctions in the placebo group, the strongest decrease in the "V_{max}-equivalent" is possibly due to upregulation of the "slow" V_3 myosin isoform in the surviving hypertrophied rat myocardium (Mercadier et al., 1981). This interpretation is supported by the negative correlation between "V_{max}-equivalents" and the cross sectional area of the papillary muscles in the placebo group.

Taken together, the beneficial effects of etomoxir on the contractility of the surviving myocardium after infarction may be attributed to its combined action on cardiac growth and contractile function. Particularly at small infarct size, normal contraction and relaxation are almost completely restored by a relatively small dose of etomoxir. In conclusion, etomoxir could be of potential therapeutic value in the treatment and prevention of contractile dysfunction after MI.

References

Bers, D., 1997. Ca transport during contraction and relaxation in mammalian ventricular muscle. Basic Res. Cardiol. 92 ((Suppl. 1)), 1–10.

Gülch, R.W., 1990. Principles of assessment of cardiac mechanics. In: Jacob, R. (Ed.), Evaluation of Cardiac Contractility. Gustav Fischer Verlag Stuttgart, New York, pp. 47–60.

Lammerich, A., Günther, J., Pfitzer, G., Storch, E., Vetter, R., 1995. Alterations of cardiac contractile function are related to changes in membrane calcium transport in spontaneously hypertensive rats. J. Hypertens. 13, 1313–1324.

Mercadier, J.J., Lompré, A.M., Wisnewsky, C., Samuel, J.L., Bercovici, J., Swinghedauw, B., Schwartz, K., 1981. Myosin isoenzymic changes in several models of rat cardiac hypertrophy. Circ. Res. 49, 525–532.

Pfeffer, M.A., Braunwald, E., Moyé, L.A., Basta, L., Brown, E.J., Cuddy, T.E., Davis, B.R., Geltman, E.M., Goldman, S., Flaker, G.C., Klein, M., Lamas, G.A., Packer, M., Rouleau, J.L., Rutherford, J., Wertheimer, J.H., Hawkins, C.M., 1992. Effect of captopril on mortality in morbidity in patients with left ventricular dysfunction after myocardial infarction: results of survival and ventricular enlargement trial. N. Engl. J. Med. 327, 669–677.

Rupp, H., Elimban, V., Dhalla, N.S., 1992. Modification of subcellular organelles in pressure-overloaded heart by etomoxir, a carnitine palmitoyltransferase I inhibitor. FASEB J. 6, 2349–2353.

Schmidt-Schweda, S., Holubarsch, C., 1997. First clinical trial with etomoxir in patients with chronic heart failure NYHA II–III. J. Mol. Cell. Cardiol. 29 (A55), (Abstract).

Vetter, R., Rupp, H., 1994. CPT-1 inhibition by etomoxir has a chamber-related action on cardiac sarcoplasmic reticulum and isomyosins. Am. J. Physiol. 267, H2091–H2099.

Wagner, K.D., Theres, H., Born, A., Strube, S., Wunderlich, N., Pfitzer, G., Baumann, G., Günther, J., 1997. Contractile function of papillary muscle from rats with different infarct size after β-adrenergic blockade and ACE-inhibition. J. Mol. Cell. Cardiol. 29, 2941–2951.

Wagner, K.D., Geil, D., Schimke, I., Stauss, H.M., Lammerich, A., Theres, H., Pfitzer, G., Vetter, R., Günther, J., 1998. Decreased susceptibility of contractile function to hypoxia/reoxygenation in chronic infarcted rat hearts. J. Mol. Cell. Cardiol. 30, 2341–2353.

Zarain-Herzberg, A., Afzal, N., Elimban, V., Dhalla, N.S., 1996. Decreased expression of cardiac sarcoplasmic reticulum Ca^{2+}-pump ATPase in congestive heart failure due to myocardial infarction. Mol. Cell. Biochem. 163–164, 285–290.