Myofibrillar Ca^{2+}-Stimulated Mg^{2+}-ATPase from Chronically Ischemic Canine Heart

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Abstract. Functional properties of myofibrils from chronically ischemic canine myocardium were evaluated. Ischemia was produced by tight stenosis of left anterior descending artery (LAD), followed by 40 min acute ischemia with prior preconditioning. Animals of the first group were sacrificed after 8 weeks. In the second group, angioplasty of LAD was performed after 8 weeks of ischemia and animals were kept alive for other 4 weeks. Control animals were sham operated.

Activity and kinetic parameters of myofibrillar Ca^{2+}-stimulated Mg^{2+}-ATPase were measured in myofibrils isolated from anterior and posterior parts of all hearts. We did not find any differences in maximal velocity (V_{max}), half-maximal activation constant for calcium ($K(Ca^{2+})_{50}$) and cooperativity coefficient (n_{hill}) of myofibrils from different experimental groups as compared to controls, either at pH 7, pH 6.5 (acidosis) or pH 7.5 (alkalosis). $K(Ca^{2+})_{50}$ increased in medium simulated acidosis (12.6–33.5 times) and n_{hill} decreased significantly in all groups as compared with values obtained at pH 7.

These results indicate that activity and Ca^{2+}-sensitivity of myofibrillar Mg^{2+}-ATPase remain unchanged despite deteriorated heart function 8 weeks after LAD obstruction. Experiments have confirmed that Ca^{2+}-stimulated Mg^{2+}-ATPase from canine heart myofibrils responded to pH decrease by a decreased sensitivity to Ca^{2+} and a decreased cooperativity. However, sensitivity of the enzyme to the pH changes is unaltered by 8 weeks of chronic ischemia.

Key words: Myofibrils — Mg^{2+}-ATPase — Ca^{2+}-sensitivity — Dog heart — Chronic ischemia

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Introduction

Functional properties of myocardial contractile apparatus has been found to be affected by different pathological conditions including acute or chronic myocardial ischemia (Schmidt-Ott et al. 1990; Gao et al. 1995, 1996). Acute myocardial ischemia results in postischemic myocardial dysfunction characterized by a decreased contractility lasting hours to days known as myocardial stunning. Recent studies have concluded that alterations responsible for the decreased contractility consist of a decrease in Ca^{2+}-sensitivity of myofilaments and of a reduction of maximal generated force activated by Ca^{2+} (Kloner et al. 1998). The decreased contractility in chronically ischemic myocardium could be related to the reduced Ca^{2+} responsiveness of myocytes, but the nature of this reduction is not yet clear (Heusch 1998).

The aim of this study was, therefore, to compare functional parameters of myofibrils isolated from chronically ischemic hearts with control values by measuring activity and kinetic parameters of Ca^{2+}-stimulated acto-myosin Mg^{2+}-ATPase.

Materials and Methods

Animal model of chronic ischemia

In the present study, transmural tissue samples from 14 mongrel dog hearts were used. Experimental protocol for animal instrumentation has been described elsewhere in details (Matejovicova et al. 1999). Briefly, three dogs were sham operated under similar conditions as experimental animals and served as controls. The remaining dogs ($n = 11$) were subjected to 8 weeks of chronic ischemia attained by an obstruction of the left anterior descending coronary artery (LAD). In all animals, after four episodes of preconditioning (5 min occlusion followed by 5 min of reperfusion), a prolonged total ischemia was kept by occlusion of the LAD for 40 min. After that period, the artery was reperfused through the tight stenosis. After 8 weeks, animals were either sacrificed or underwent a percutaneous transluminal coronary angioplasty (PTCA) of the LAD. Animals were followed up on post-surgery weekly basis. Left ventricular function was determined using two-dimensional transthoracic echocardiography. Regional myocardial blood flow under resting conditions and with vasodilator (coronary reserve) was determined using coloured microspheres. In chronically ischemic animals, hypokinesis of anterior wall developed early and was persisted up to 8 weeks after surgery (decrease in wall thickening by 59 % in comparison with baseline). Prompt improvement in the functioning was observed after the PTCA. A significant reduction in the resting transmural flow (a decrease by 29 % *vs* baseline) was found but the maximum vasodilatory capacity remained intact. The flow had normalized four weeks after PTCA. Samples obtained from the dog hearts at sacrifice were snap frozen in liquid nitrogen and stored at −80 °C until use.

Myofibrillar protein preparation

Myofibrils were isolated according to Murphy and Solaro (1990) from anterior (Ant) and posterior (Post) parts of the left ventricle of each heart. Powdered frozen tissue (approximately 1 g) was thawed and immediately homogenized with a Polytron PTA-10S probe, setting 6, for 3 × 5 s (with 30 s breaks) in ice-cold standard buffer containing in mmol/l: imidazole, 30 (pH 7.0); KCl, 60; $MgCl_2$, 2. Homogenates were filtered through cheesecloth and centrifuged at 12,000 × g for 15 min. Resulting pellets were resuspended in extraction solution containing in mmol/l: imidazole, 60 (pH 7.0); KCl, 14.4; $MgCl_2$, 8.2; EGTA, 10; ATP, 5.5; phosphocreatine, 12; 1% Triton-X 100 and creatine kinase (10 U/ml). Extraction was carried out for 30 min at 0°C and then the myofibrils were pelleted (1000 × g for 15 min). The pellets obtained were washed twice in the standard buffer and the final pellets were resuspended in 1–1.5 ml of the standard buffer, containing 50% glycerol. All solutions for isolation contained 0.1 μmol/l okadaic acid in order to inhibit endogenous phosphoprotein phosphatase A2 that could dephosphorylate regulatory myofibrillar proteins (Mumby et al. 1987). Samples were frozen in liquid nitrogen and were kept at −80°C until use. Protein concentration in myofibrillar samples was determined according to Bradford (1976) using bovine serum albumin as a standard.

Acto-myosin Mg^{2+}-ATPase assay

Myofibrillar Mg^{2+}-ATPase activity was determined from measurement of P_i liberated after incubation of the myofibrils with 2 mmol/l Mg-ATP in the following medium (in mmol/l): HEPES, 25 (pH 7.0 – physiological value, pH 6.5 – simulated acidosis or pH 7.5 – simulated alkalosis); KCl, 120; $MgCl_2$, 5; DTT, 2; EGTA, 4; $CaCl_2$, 0–4.18. Total $CaCl_2$ concentration was calculated using CABUF computer program (generously provided by Dr. G. Droogmans, Department of Physiology, University of Leuven, Belgium) so that the concentration of free Ca^{2+} varied from 0.1 μmol/l to 100 μmol/l. The reaction was started by addition of the myofibrillar protein (0.1–0.25 mg/ml) and kept at 30°C for 2 min. Phosphate liberated from the ATP was determined with Lanzetta reagent which was added to the medium at the end of the reaction (Lanzetta et al. 1979). Dependence of the Mg^{2+}-ATPase activity on free Ca^{2+} concentration in the reaction medium was plotted in semi-logarithmic coordinates and maximal velocity of the ATPase (V_{max}), half-maximally activating free Ca^{2+} concentration ($K(Ca^{2+})_{50}$) and cooperativity coefficient (n_{hill}) were derived by fitting the data to the Hill equation using computer program Origin (Microcal, Northampton, MA, USA).

Results

Myofibrillar Mg^{2+}-ATPase activation by Ca^{2+} was measured in isolated myofibrils from three groups of canine hearts: control, chronically ischemic for 8 weeks or chronically ischemic followed by PTCA. Myofibrils were prepared from two regions of each heart: anterior region perfused by stenotic artery (hypoperfused and

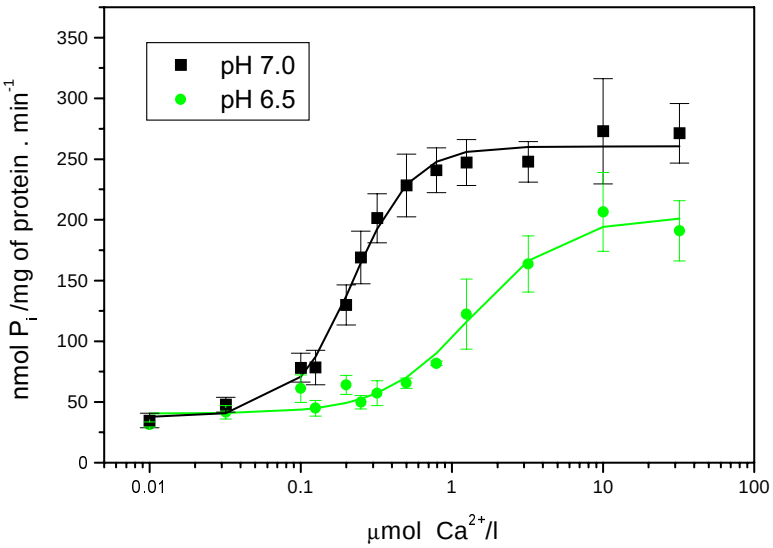

Figure 1. Ca^{2+}-activation of Mg^{2+}-ATPase of myofibrils from the ischemic group of hearts. ATPase activity was measured at 0.1–30 μmol/l Ca^{2+} in a medium with pH 7.0 and 6.5 buffered by 2 mmol/l EGTA.

hypokinetic part in the chronically ischemic group) and posterior region with normal perfusion and function maintained in all groups. In order to evaluate possible effect of chronic ischemia on responsiveness of myofibrillar proteins to acidosis or alkalosis, myofibrillar Mg^{2+}-ATPase activity was measured as a function of Ca^{2+} concentration at pH values 7.0, 6.5 and 7.5. Fig. 1 shows Ca^{2+}-dependent activation of Mg^{2+}-ATPase of myofibrils from the anterior part of the chronically ischemic hearts, measured at different pH values. As shown in the Fig. 2, there were no significant changes in kinetic parameters of myofibrillar Mg^{2+}-ATPase among the experimental groups, but a decrease both in Ca^{2+}-sensitivity (increased $K(Ca^{2+})_{50}$) and in cooperativity (decreased Hill coefficient n_{hill}) was found in myofibrils isolated from all tissue samples when measured at pH 6.5. A tendency for increased cooperativity was found in the control myofibrils at pH 7.5, excluding myofibrils from the ischemic hearts. This could indicate changed properties of regulatory thin fillament proteins (troponin complex). Maximal activity of myofibrillar Mg^{2+}-ATPase (V_{max}) was found to be affected netheir by chronic ischemia nor by pH changes of the reaction medium.

Basal Mg^{2+}-ATPase activity was measured in the absence of Ca^{2+}. At pH 7.0, an elevation of the basal activity was observed in the ischemic myofibrils obtained from both, Ant and Post regions, but the differences from the matching control were not significant according to t-test (Table 1). There were no differences in the basal Mg^{2+}-ATPase activity among other experimental groups.

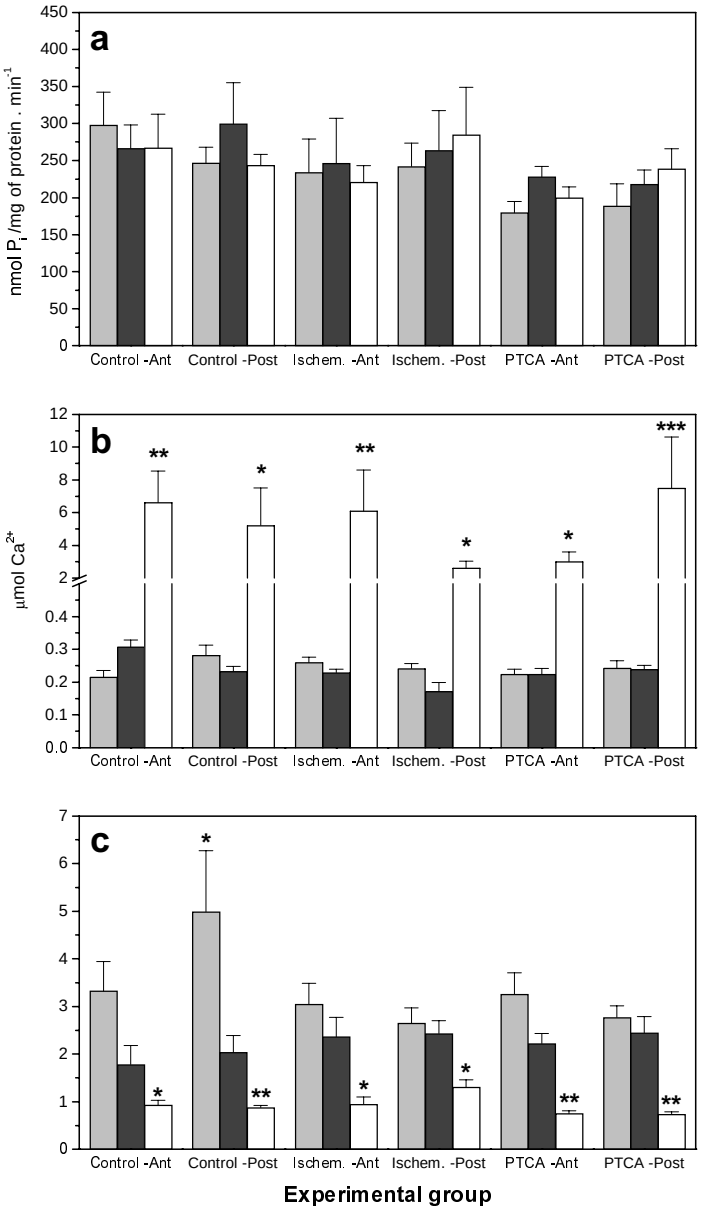

Figure 2. Kinetic parameters of myofibrillar Ca^{2+}-activated Mg^{2+}-ATPase from anterior (Ant) and posterior (Post) regions of control, ischemic (Ischem) and revascularized (PTCA) dog hearts. Panel a: maximal activity of Mg^{2+}-ATPase; Panel b: half-maximal activation constant for Ca^{2+}, Panel c: Hill cooperativity coefficient. Kinetic parameters obtained by measuring Mg^{2+}-ATPase activity at pH 6.5 (grey bars), pH 7.0 (black bars) and pH 7.5 (white bars). Data are expressed as means ± S.E.M.; * $p < 0.05$, ** $p < 0.01$, *** $p < 0.005$ according to t-test in comparison with values measured at pH 7.0.

Table 1. Myofibrillar Mg^{2+}-ATPase activity measured in the absence of Ca^{2+} and in the presence of 2 mmol/l EGTA

	Mg^{2+}-ATPase basal activity (Ca^{2+} independent) nmol P_i/mg of protein·min^{-1}		
Experimental group	pH = 6.5	pH = 7.0	pH = 7.5
Control-Ant	23.2 ± 2.68	28.2 ± 0.84	29.2 ± 5.98
Control-Post	31.8 ± 9.67	24.9 ± 5.71	35.8 ± 9.24
Ischem-Ant	30.0 ± 4.80	58.4 ± 12.35	41.8 ± 9.03
Ischem-Post	29.9 ± 5.96	39.2 ± 12.12	38.0 ± 4.34
PTCA-Ant	25.6 ± 5.14	36.3 ± 6.54	40.7 ± 4.12
PTCA-Post	27.5 ± 4.19	31.2 ± 2.65	35.2 ± 5.97

Discussion

Results of the present study show that chronic regional ischemia induced in dog heart by sequence of no-flow ischemia followed by hypoperfusion of LAD-dependent area did not influence Ca^{2+} activation of myofibrillar Mg^{2+}-ATPase, despite deteriorated cardiac function in the affected region. The maximal activity of acto-myosin ATPase is thought to be associated with heart performance (Pagani and Solaro 1984). However, in pig model of stunned myocardium, the maximal Mg^{2+}-ATPase activity as well as its calcium sensitivity was unchanged (Krause 1990; Bezstarosti et al. 1997) and maximal ATPase activity was found even elevated in globally stunned rabbit myocardium (Andres et al. 1991). Increased activity of myofibrillar Mg^{2+}-ATPase was also found at other types of pathology connected with depressed cardiac function, as in rat model of chronic peritoneal sepsis (Powers et al. 1998). Thus, it seems that uncoupling of ATP hydrolysis from the force development could play a role in the progress of myocardial dysfunction caused either by acute or by chronic ischemia as well as by other reasons. Such uncoupling of transport and ATP hydrolysis has been found in other ion transporting ATPases, e.g. in Ca^{2+}-ATPase of sarcoplasmic reticulum (Dhalla et al. 1985) and Na^{+},K^{+}-ATPase of sarcolemma (Vrbjar et al. 1994). However, mechanisms of ATP hydrolysis uncoupling should be yet elucidated.

One of the reasons of the changed myofilament Ca^{2+} responsiveness could be altered intracellular pH during ischemia. It has been shown that acidic pH causes desensitization of myofibrils to Ca^{2+} due to the changed affinity of myofibrillar regulatory proteins (troponin complex) (Fabiato and Fabiato 1978; Kimura et al. 1990). Moreover, the response of myofibrils to the acidic desensitization might be altered in the ischemic tissue. That is the reason why we tested Ca^{2+} activation of the myofibrillar Mg^{2+}-ATPase in a media buffered to yield normal, acidic or alkaline pH values. According to our expectations, the control samples responded to the acidification by rightward shift of the curve of Mg^{2+}-ATPase activity Ca^{2+} -dependence. However, responses of the myofibrils isolated from ischemic hearts

did not differ from the control group (Fig. 1). In both the ischemic group and the myofibrils from hearts after PTCA a rightward shift of the Ca^{2+}-sensitivity of the same extent as in the control myofibrils was observed (Fig. 2b,c).

By measuring Mg^{2+}-ATPase activity of myofibrils at pH 7.5, we observed differences in response of control and ischemic myofibrils to alkalosis. Control myofibrils showed increased cooperativity (increased n_{hill}) in comparison with physiological pH value, but no differences in cooperativity at pH 7.0 and 7.5 were found in ischemic myofibrils that would indicate changed properties of myofibrillar regulatory proteins in chronically ischemic hearts. However, this finding has probably no physiological significance for altered myofilament function during ischemia because lactic acidosis may occur during ischemia (Slezák et al. 1987; Heusch and Schulz 1998), but no evidence has yet been reported for alkalosis.

In conclusion, we have found that both the activity and Ca^{2+}-sensitivity of myofibrillar Mg^{2+}-ATPase remain unchanged in dog myocardium after 8 weeks of chronic regional ischemia despite depressed myocardial function and perfusion of the affected region. Myofibrillar Ca^{2+}-stimulated Mg^{2+}-ATPase from canine heart responds to acidosis by a decreased sensitivity to Ca^{2+} and by a decreased cooperativity of myofilament proteins. The myofibrillar Mg^{2+}-ATPase responsiveness to lowered pH values remains unchanged after 8 weeks of LAD occlusion. On the other hand, the differences in responsiveness to alkalosis of control and ischemic myofibrillar Mg^{2+}-ATPase indicate some changes in the properties of myofilaments, probably caused by chronic ischemia.

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