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Biochemical Pharmacology 65 (2003) 911–916
Biochemical Pharmacology
www.elsevier.com/locate/biochempharm

Calpain inhibitor (BSF 409425) diminishes ischemia/reperfusion-induced damage of rabbit heart mitochondria

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Received 22 August 2002; accepted 21 November 2002

Abstract

Calpains are involved in ischemia/reperfusion-induced changes of myocard. To obtain information on the action of calpain on mitochondria, the effect of a new developed calpain inhibitor (CI) BSF 409425 on the ischemia/reperfusion-induced damage of rabbit heart mitochondria was investigated. Rabbit hearts were subjected to 45 min of global ischemia followed by 60 min of reperfusion in the presence or absence of 10 nM CI. Mitochondrial properties were characterized by skinned fiber technique with pyruvate + malate as substrates. In the presence of CI, the decrease of state 3 respiration and the increase of state 4 respiration after ischemia and reperfusion were clearly smaller than without CI resulting in significantly smaller changes of respiratory control index, too. Ischemia/reperfusion-caused leaks in mitochondrial inner and outer membranes were diminished by CI. It is concluded that mitochondria are a target of calpain which reinforces the damage of oxidative phosphorylation and mitochondrial membranes during ischemia/reperfusion.

Keywords: Calpain inhibitor; Ischemia; Reperfusion; Rabbit; Heart; Respiration; Mitochondria; Skinned fibers

1. Introduction

Calpains, calcium-dependent proteases, play an important role in both physiological and pathological processes of the cell, including ischemic injury, cell death, and degenerative diseases [1,2]. Activation of calpains are involved (i) in the mechanism of ischemia/reperfusion injury of liver [3–5] or heart [6–10], (ii) in initiating the mitochondrial membrane permeability transition [4,5], and (iii) in necrotic and apoptotic cell death [3,11]. It was shown that pre-ischemic administration of a CI caused reduced myocardial infarct size in ischemic/reperfused rat heart [7]. Calpains cause proteolysis-induced impairments of contractile proteins [6] and protein kinases [8]. Moreover, calpains cleave BAX and Bid to shorter fragments, which mediate specifically cytochrome *c* (Cc) release from mitochondria [11]. On the other hand, it is known that mitochondria swell during ischemia/reperfusion [12,13] causing disruption of the outer mitochondrial membranes with a subsequent unspecific release of Cc [13–15]. Those disturbances together with decreased respiratory control index (RCI) are detectable at the level of isolated mitochondria [13,16] and at early stages of ischemic damage in skinned muscle fibers [14,15]. The activity of calpains is regulated by calcium concentration and endogenous inhibitors as calpastatin [1,2,11,17]. With the new developed CIs it is now possible to investigate how calpains contribute to ischemic impairment of mitochondria, and it may be possible to find suitable drugs for protection of ischemic tissues. One of these CIs, a phenylalanine ketoamide BSF 409425 was provided by Abott Laboratories and described by Lubisch and Behl [18].

Thus, the aim of our work was to investigate, whether or not the administration of CI affects the ischemia/reperfusion-induced damage of the function of cardiac mitochondria. We utilized a model of global ischemia (Langendorff

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Abbreviations: CI, calpain inhibitor BSF 409425; RCI, respiratory control index; Cc, cytochrome *c*; AA, antimycin A; At, atractylate.

0006-2952/02/$ – see front matter
doi:10.1016/S0006-2952(02)01610-6

technique). Rabbit hearts were perfused with and without CI. After 45 min of ischemia and 60 min of reperfusion, mitochondrial function was investigated using the skinned fiber technique and high resolution respirometry with protocols successfully used before for detection of acute [15,19,21,22] and mutation-caused mitochondrial dysfunction [20,23]. Our studies show, that CI diminishes the ischemia/reperfusion-induced impairment of rabbit heart mitochondria.

2. Materials and methods

2.1. Animal model

Isolated rabbit hearts were perfused according to Langendorff preparation as described before [19] in an open system at constant pressure (80 mmHg) and temperature (37 °) with Krebs–Henseleit buffer solution in the presence or absence of 10 nM CI. After a steady-state period of 15 min, coronary perfusion was stopped, and hearts were subjected to 30 or 45 min of global ischemia followed by 60 min of reperfusion. For analysis of mitochondrial function small specimens (ca. 50 mg) were taken from the right ventricle of the same heart at both states: (1) before ischemia and at the end of the ischemia or (2) before the reperfusion and at the end of reperfusion.

2.2. Skinned fiber technique

Skinned fiber technique and high resolution respirometry were used as described previously [15,19,20–23]. Heart muscle fibers were roughly dissected and stored less than 24 hr until measurements in storage buffer at 0–4°. The long temporal stability of mitochondrial function was recently demonstrated for rabbit heart muscle fibers [19]. Immediately prior to oxygraphic measurements, fibers were permeabilized for 30 min with 50 μg saponin/mL of medium consisting of 8.1 mM K-EGTA, 1.9 mM Ca-EGTA buffer (free Ca^{2+} concentration 0.1 μM), 9.5 mM $MgCl_2$, 3 mM KH_2PO_4, 20 mM taurine, 5.2 mM ATP, 15 mM PCr, 49 mM K-Mes, 20 mM imidazole (pH 7.1) as described previously [20].

2.3. Oxygraphic measurements

Bundles of fibers (2–5 mg sample weight) were transferred into the Oroboros® oxygraph chambers [24]. The measurements were performed at 30° in 1.42 mL incubation medium consisting of 75 mM mannitol, 25 mM sucrose, 100 mM KCl, 10 mM KH_2PO_4, 0.5 mM Na_2EDTA, 5 mM $MgCl_2$, 20 mM Tris–HCl, and 1 mg/mL BSA, pH 7.4, using 10 mM pyruvate + 2 mM malate as substrates. The oxygen concentration in the air-saturated medium was considered to be 200 nmol O_2/mL at 95 kPa barometric pressure [20]. The weight-specific oxygen consumption was calculated as the time derivative of the oxygen concentration (DatGraph Analysis software, Oroboros®). Data are given as means ± SD of N hearts. From every heart at least three independent determinations were performed. Statistical analysis was performed using Student's *t*-test for unpaired variables. $P < 0.05$ was considered as the level of significance.

3. Results

As shown in Table 1, impairment of mitochondrial function was clearly dependent on the time of ischemia. After 30 min ischemia, the state 3 respiration rate was only slightly affected (−21%), whereas 45 min ischemia caused a significantly decreased state 3 respiration rate (−40%) in comparison to the controls. During reperfusion, a further decrease of state 3 occurred. Thirty minute ischemia did not affect the atractyloside-insensitive respiration, but after 45 min ischemia state 4 was significantly increased (+80%), indicating an impairment of mitochondrial inner membrane. As a result the RCI after 45 min ischemia was significantly decreased before (−67%, $P < 0.05$) and after reperfusion (−76%, $P < 0.05$), whereas the RCI was only tendentiously reduced by −26 and −36%, respectively, after 30 min ischemia. The difference between the RCI after 30 and 45 min ischemia was significant too. The RCIs of succinate-dependent respiration after 45 min ischemia (−45%) as well as after reperfusion (−53%) were significantly diminished also (data not shown) but to a less extent. From these results it can be concluded that under the experimental conditions used 45 min ischemia are

Table 1
Ischemia/reperfusion-induced changes of mitochondrial function in saponin-skinned fibers

	N	State 3 (%)	State 4 (%)	RCI (%)
Control	11	5.8 ± 1.1 (100)	0.5 ± 0.1 (100)	13.5 ± 4.3 (100)
30 min ischemia	6	4.6 ± 1.3 (79)	0.5 ± 0.2 (100)	10.0 ± 5.0 (74)
Reperfusion after 30 min ischemia	5	4.3 ± 1.5[a] (74)	0.6 ± 0.2 (120)	8.0 ± 6.0 (59)
45 min ischemia	8	3.5 ± 1.4[a] (60)	0.9 ± 0.3[a,b] (180)	4.4 ± 2.5[a,b] (33)
Reperfusion after 45 min ischemia	4	2.6 ± 1.3[a] (45)	0.9 ± 0.3[a] (180)	3.2 ± 2.1[a] (24)

Model of ischemia/reperfusion as described in Section 2. Mitochondrial respiration was measured with 10 mM pyruvate and 2 mM malate in the presence of 5 mM ADP (state 3) and in the presence of 1.8 mM atractyloside (state 4). Respiratory rates are given as nmol O_2/min/mg sample weight ± SD. Data are presented as means of 4–11 experiments ± SD measured as duplicates or triplicates. Time of reperfusion was 60 min.

[a] Significant differences from controls.

[b] Significant differences from ischemia (30 min).

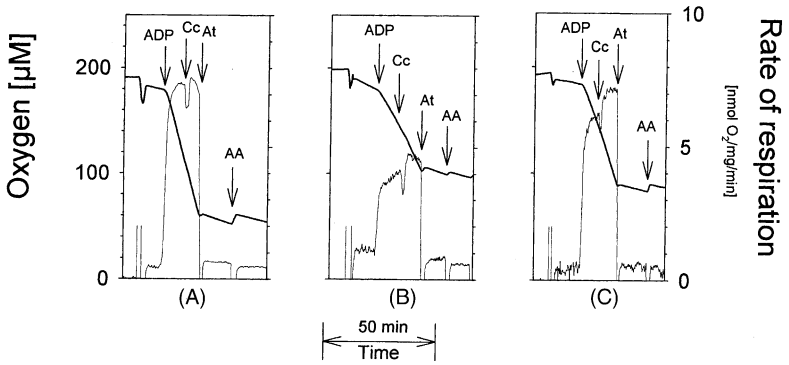

Fig. 1. Effect of ischemia and of CI on functional properties of rabbit heart mitochondria as measured in saponin-skinned fibers. Original traces of respirometric measurements of 4.2 mg fibers from control hearts (left), 2.5 mg fibers after 45 min ischemia (middle), and 2.6 mg fibers after 45 min ischemia in the presence of 10 nM CI (right). Substrates were 10 mM pyruvate + 2 mM malate. Further additions: ADP, 5 mM ADP (state 3); Cc, 32 µM cytochrome *c*; At, 1.8 mM atractylate (state 4); AA, 45 µM antimycin A. Oxygen concentration (left axis, thick line). The (negative) time derivative of this signal indicates the rate of respiration (right axis, thin line). Leak respiration as calculated from the difference of atractylate- and antimycin A-dependent respiration is given in Table 2. Leaks in the mitochondrial outer membrane as detected by the percentage of stimulation of state 3 respiration after the addition of cytochrome *c* are given in Table 2.

necessary to generate significant changes in functional properties of rabbit heart mitochondria. Therefore, we used this protocol to test the action of CI on ischemic impairment of heart mitochondria. In the presence or absence of 0.1 nM CI, isolated hearts were subjected to 45 min ischemia followed by 60 min of reperfusion. The experimental protocol of respirometric investigations is shown in Fig. 1A. Maximal pyruvate/malate-dependent respiration (state 3) was measured after the addition of 5 mM ADP. In all control hearts, this rate was 6.4 ± 1.1 nmol O_2/min/mg sample weight (Table 2). Then the intactness of mitochondrial outer membrane was checked by addition of Cc during state 3 respiration. In control mitochondria the rate of respiration increased by about 6% (Table 2). After inhibition of the AdN-translocator by actractyloside, the state 4 respiration was measured. This rate includes leak respiration and unspecific or non-enzymatic oxygen consumption. The latter can be measured after addition of antimycin A (AA), an inhibitor of complex III of the respiratory chain. The difference between atractylate (At)- and AA-dependent respiration indicates leaks in the mitochondrial inner membrane (Table 2). Fig. 1B shows a typical respirometric experiment of heart fibers after 45 min ischemia. State 3 respiration was clearly decreased. Cc test revealed a substantial stimulation of state 3 respiration, indicating an impairment of mitochondrial outer membrane. Fig. 1C shows the effect of CI on functional properties of ischemic mitochondria. The diminishing of state 3 respiration was less than without CI, but leaks in the outer membrane was still detectable.

The data of all experiments are collected in Table 2. Administration of CI had no effect on mitochondrial parameters from control muscle fibers of hearts (without ischemia/reperfusion). After 45 min ischemia, a clear and significant decrease in state 3 respiration rate from 6.4 to 3.5 nmol O_2/min/mg sample weight was observed.

Table 2
Effect of CI BSF 409425 on impairment of mitochondrial function due to ischemia/reperfusion

Substrate	N	State 3	State 4	RCI	Leak respiration	Stimulation by cytochrome *c*
−CI						
Control	4	6.4 ± 1.1	0.5 ± 0.1	12.5 ± 2.7	0.15 ± 0.07	6.0 ± 10.0
45 min ischemia	8	3.5 ± 1.4*	0.9 ± 0.3*	4.4 ± 2.5*	0.32 ± 0.14*	10.0 ± 6.0
60 min reperfusion	4	2.6 ± 1.3*	0.9 ± 0.3*	3.2 ± 2.1*	0.43 ± 0.29	28.0 ± 16.0
+CI						
Control	4	6.8 ± 1.3	0.6 ± 0.1	12.4 ± 1.1	0.12 ± 0.06	n.d.
45 min ischemia	9	5.0 ± 0.8*,#	0.6 ± 0.2	8.2 ± 2.3*,#	0.20 ± 0.14	16.0 ± 9.0
60 min reperfusion	5	4.2 ± 1.2*	0.7 ± 0.2	6.4 ± 2.7*	0.26 ± 0.24	15.0 ± 13.0

Mitochondrial function was investigated as shown in Fig. 2 with 10 mM pyruvate and 2 mM malate in the presence of 5 mM ADP (state 3), in the presence of 1.8 mM atractyloside (state 4), and 45 µM antimycin A. RCI was calculated as ratio of state 3 and state 4 respiration. The leak respiration was calculated as difference of At and antimycin A respiration. Respiratory rates are given as nmol O_2/min/mg sample weight ± SD. Intactness of mitochondrial outer membrane was detected by stimulation of pyruvate-dependent respiration by cytochrome as shown in Fig. 2. Changes of maximal respiratory rate (in percent) were calculated as ratio of state 3 respiration before and after the addition of 32 µM cytochrome *c*. Data as means of 4–9 experiments ± SD measured as duplicates or triplicates.

* Significant differences from representing controls of each group, $P < 0.05$.

Significant differences from ischemia (45 min) without pre-treatment with 10 nM CI, $P < 0.05$.

Pre-administration of CI prior to ischemia suppressed this damage of mitochondrial function as indicated by the significantly less decreased state 3 respiration (5.0 nmol O_2/min/mg). Also the increase of state 4 respiration by ischemia/reperfusion was diminished by CI. As a consequence, the RCI was significantly increased (+86%) after ischemia compared to the untreated group. During reperfusion, the state 3 respiration rate was further decreased by 59% in comparison to the controls, whereas with CI the decrease was only 38%. As a result after the reperfusion period, the RCI in the presence of CI was twice of that without inhibitor. As shown in Table 2, the leak respiration was increased after ischemia (+113%, $P < 0.05$) and the subsequent reperfusion (+186%) indicating that the inner membrane became more permeable for protons. CI clearly decreased this effect (+66 and +116%). Also stimulation of pyruvate-dependent state 3 respiration by Cc was tendentiously lower in the presence of CI. Without inhibitor, state 3 respiration after reperfusion was stimulated by 28% but only by 15% in the presence of CAL 425.

4. Discussion

To identify mitochondria as a possible target of calpains, we investigated the action of CI on state 3 and leak respiration of heart mitochondria and on the intactness of mitochondrial outer membrane. For that purpose, we used the skinned fiber technique, which is suitable for a sensitive characterization of acute [19,21,22] and mutation-caused [20,23] impairments of mitochondria. This technique is useful for such kind of studies because of the following reasons: (1) it requires very limited amounts of material; (2) practically all mitochondria present in the sample are accessible for investigation avoiding any possible pre-selection of mitochondrial subpopulations during preparation; (3) isolation artifacts are excluded, and mitochondria are studied in their natural surroundings. Forty-five minute ischemia was chosen because under these conditions the damage of mitochondria was much severer than after 30 min of ischemia in our model. These results confirm earlier results from Kay *et al.* [14].

In this study we used BSF 409425 (CI), a new developed water-soluble phenylalanine ketoamide carrying styrylbenzyl amines with a molecular mass of 422. The substance inhibits calpains with a K_i of 13 nM [17]. In previous experiments with concentrations from 0.1 to 1000 nM CI we found a bell-shaped dose–response relationship with a maximum at 10 nM, resulting in a 100% recovery of heart function during reperfusion after a 45 min global myocardial ischemia (results not shown). Therefore, experiments of this work were performed with a concentration of 10 nM CI.

For the first time, using the skinned fiber technique, we showed that CI substantially diminished ischemia/reperfusion-caused injury of mitochondrial function. The most important finding was that in hearts, treated with CI prior to ischemia/reperfusion, markedly improved mitochondrial parameters were detectable: in the CI group the ischemia/reperfusion-induced decrease of state 3 respiration was less pronounced (−26 and −38%) than in the group without inhibitor (−45 and −59%). Furthermore, CI increased the RCI 2-fold in both states after ischemia and after reperfusion as compared to the group without CI. It is also important to note that CI did not alter respiration rates of control fibers. Thus, unspecific effects of CI could be excluded.

At present, there are no data on the relationship between the calpain activation during ischemia/reperfusion and the damage of cardiac mitochondria. However, there are reports, that pre-ischemic administration of a CI was effective in reducing myocardial infarct size and the DNA damage of the myocytes in ischemic/reperfused rat heart [7] as well as in reducing of proteolysis [8–10]. In addition, there is some evidence on liver mitochondria that calpain proteases play a role in warm ischemia/reperfusion injury through modulation of apoptosis and necrosis: animals, pre-treated with a CI showed decreased apoptosis [3] in ischemic rat liver injury. It was assumed that the activation of mitochondrial calpain-like protease activity can function as a cytolytic trigger initiating the mitochondrial membrane permeability transition [4]. Our data support the conclusion that CI prevents the inner mitochondrial membrane to become permeable, as can be seen from slightly decreased state 4 respiration rate as well as from diminished leak respiration.

The ischemia/reperfusion-caused increase of leak respiration observed in our experiments probably is caused by two main mechanisms. One is the increase of free fatty acids causing a partial uncoupling of mitochondria [25,26] and promoting mitochondrial permeability transition [26]. The second possibility is the induction of permeability transition caused by an increased calcium-uptake into the mitochondria [27,28]. The mechanism of the CI action is unclear but due to its established inhibition of proteolytic calpain activity it can be assumed that components of the calpain cascade act on mitochondrial membranes. On the other hand, it cannot be ruled out that CI acts on other proteins too. For instance, an inhibition of the mitochondrial calcium-uniporter by CI could prevent an overload of mitochondria. Further work is necessary to clarify the mechanism of calpain action on mitochondria.

Another observation is that CI has also effects on the intactness of the mitochondrial outer membrane which decreases normally during ischemia/reperfusion. We tested this by the addition of exogenous Cc to mitochondria respiring in the state 3. This technique was successfully used for investigation of the intactness of mitochondrial outer membrane [14,15]. A slight stimulation of state 3 respiration by Cc was detectable even in controls, confirming our earlier results [15] and those from others [14], and a tendentious increase after ischemia and reperfusion was detectable in agreement with data from others [14]. CI,

however, preserved the intactness of outer membrane during ischemia/reperfusion.

For the release of mitochondrial Cc, at least three mechanisms are in discussion [28]: (i) Bax/Bak acts with VDAC to induce the Cc release, while BcL_2/BcL_{XL} closes this channel [29], (ii) Bid/Bik can open an up to now unidentified channel, and (iii) after rupture of the outer membrane Cc can non-specifically leave the mitochondria [15]. The rupture of the outer membrane could be also caused by swelling of the matrix space as one result of permeability transition due to activation of calpain [4].

In the presence of CI, rupture of the outer membrane as well as impairment of the inner membrane were decreased. Therefore, it can be concluded that calpain possibly contributes to stimulation of permeability transition and to Cc release through the outer membrane. The swelling of mitochondria during ischemia/reperfusion *per se* causes ruptures of the mitochondrial outer membrane [12,13], which causes a loss of Cc [15] and diminishes the action of ADP-shuttles [30]. Both effects together with fatal consequences of a decreasing concentration of adenine nucleotides [31] contribute to energetic depression of the heart [32]. Both, energetic depression and the release of apoptotic signals contribute to ischemic-caused impairment of tissue. Our studies suggest that CI diminishes ischemia/reperfusion-induced damage of rabbit heart mitochondria. The data demonstrate the protective effects of CI against ischemic myocyte injury as a result of preserved mitochondrial function.

Acknowledgments

The authors thank the Deutscher Akademischer Austauschdienst (DAAD) and the Martin-Luther-University for supporting S.T. We thank the DFG for financial support (SFB 583), Kultusministerium Sachsen-Anhalt for a Grant No. 3017A and the Deutsche Gesellschaft für Muskelkranke (DGM) for financial support.

References

[1] Sorimachi Y, Harada K, Saido TC, Ono T, Kawashima S, Yoshida K. Downregulation of calpastatin in rat heart after brief ischemia and reperfusion. J Biochem (Tokyo) 1997;122:743–8.
[2] Suzuki K, Sorimachi H, Yoshizawa T, Kinbara K, Ishiura S. Calpain: novel family members, activation, and physiological function. Biol Chem Hoppe-Seyler 1995;376:523–9.
[3] Kohli V, Madden JF, Bentley RC, Clavien PA. Calpain mediates ischemic injury of the liver through modulation of apoptosis and necrosis. Gastroenterology 1999;116:168–78.
[4] Aguilar HI, Botla R, Arora AS, Bronk SF, Gores GJ. Induction of the mitochondrial permeability transition by protease activity in rats: a mechanism of hepatocyte necrosis. Gastroenterology 1996;110: 558–66.
[5] Gores GJ, Miyoshi H, Botla R, Aguilar HI, Bronk SF. Induction of the mitochondrial permeability transition as a mechanism of liver injury during cholestasis: a potential role of mitochondrial proteases. Biochim Biophys Acta 1998;1366:167–75.
[6] Gao WD, Atar D, Liu Y, Perez NC, Murphy AM, Marban E. Role of troponin I proteolysis in the pathogenesis of stunned myocardium. Circ Res 1997;80:393–9.
[7] Iwamoto H, Miura T, Okamura T, Shirakawa K, Iwatate M, Kawamura S, Tatsuno H, Ikeda Y, Matsuzaki M. Calpain inhibitor-l reduces infarct size and DNA fragmentation of myocardium in ischemic/reperfused rat heart. J Cardiovasc Pharmacol 1999;33: 580–6.
[8] Urthaler F, Wolkowitz PE, Digerness SB, Harris KD, Walker AA. MDL-28170, a membrane-permanent calpain inhibitor, attenuates stunning and PKC epsilon proteolysis in reperfused hearts. Cardiovasc Res 1997;35:60–7.
[9] Izuka K, Kawaguchi H, Yasuda H, Kitabatake A. The role of calcium activated protease on myocardial cell injury in hypoxia. Jpn Heart J 1992;33:707–15.
[10] Gao G, Dou QP. N-terminal cleavage of bax by calpain generates a potent proapoptotic 18-kDa fragment that promotes bcl-2-independent cytochrome *c* release and apoptotic cell death. J Cell Biochem 2000; 80:53–62.
[11] Wang KK, Posmantur R, Nadimpalli R, Nath R, Mohan P, Nixon RA, Talanian RV, Keegan M, Herzog L, Allen H. Caspase-mediated fragmentation of calpain inhibitor protein calpastatin during apoptosis. Arch Biochem Biophys 1998;356:187–96.
[12] Jennings RB, Herdson PB, Sommers HM. Structural and functional abnormalities in mitochondria isolated from ischemic dog myocardium. Lab Invest 1969;20:548–57.
[13] Di Lisa F, Menabo R, Canton M, Petronilli V. The role of mitochondria in the salvage and the injury of the ischemic myocardium. Biochim Biophys Acta 1998;1366:69–78.
[14] Kay L, Rossi A, Saks V. Detection of early ischemic damage by analysis of mitochondrial function in skinned fibers. Mol Cell Biochem 1997;174:79–85.
[15] Gellerich FN, Trumbeckaite S, Opalka JR, Seppet E, Rasmussen HN, Neuhoff C, Zierz S. Function of the mitochondrial outer membrane as a diffusion barrier in health and disease. Biochem Soc Trans 2000; 28:164–9.
[16] Piper HM, Noll T, Siegmund B. Mitochondrial function in the oxygen depleted and reoxygenated myocardial cell. Cardiovasc Res 1994;28: 1–15.
[17] Kawasaki H, Kawashima S. Regulation of the calpain-calpastatin system by membranes. Mol Membr Biol 1996;13:217–24 (review).
[18] Lubisch W, Behl B. Discovery of phenyl alanine derived ketoamides carrying benzoyl residues as novel calpain inhibitors. Bioorg Med Chem Lett 2002;12:1335–8.
[19] Trumbeckaite S, Opalka JR, Neuhof C, Zierz S, Gellerich FN. Different sensitivity of rabbit heart and skeletal muscle to endotoxin-induced impairment of mitochondrial function. Eur J Biochem 2001;268:1422–9.
[20] Sperl W, Skladal D, Gnaiger E, Wyss M, Mayr U, Hager J, Gellerich FN. High resolution respirometry of permeabilized skeletal muscle fibers in the diagnosis of neuromuscular disorders. Mol Cell Biochem 1997;174:71–8.
[21] Gellerich FN, Trumbeckaite S, Opalka JR, Chen Y, Gellerich JF, Chen Y, Neuhoff C, Redl H, Werdan K, Zierz S. Mitochondrial dysfunction in sepsis: evidences from bacteraemic baboons and endotoxaemic rabbits. Biosci Rep 2002;22:99–103.
[22] Opalka JR, Gellerich FN, Kling L, Müller-Beckmann B, Zierz S. Only mild impairments of mitochondrial function by RO-28-2653—a new matrix metalloproteinase inhibitor. Biochem Pharmacol 2002;7057: 1–8.
[23] Gellerich FN, Deschauer M, Chen Y, Müller T, Zierz S. Functional impairment of mitochondria in skinned fibers of CPEO patients with single and multiple deletions of mt-DNA correlate with heteroplasmy. Biochim Biophys Acta 2002;1556:41–52.

[24] Haller T, Ortner M, Gnaiger E. A respirometer for investigating oxidative cell metabolism: toward optimization of respiratory studies. Anal Biochem 1994;218:338–42.
[25] Cocco T, DiPaola M, Papa S, Lorusso M. Arachidonic acid interaction with the mitochondrial electron transport chain promotes reactive oxygen species generation. Free Radic Biol Med 1999; 27:51–9.
[26] Schonfeld P, Bohnensack R. Fatty acid-promoted mitochondrial permeability transition by membrane depolarisation and binding to the ATP/ADP carrier. FEBS Lett 1997;420:167–70.
[27] Suleiman MS, Halestrap AP, Griffiths EJ. Mitochondria: a target for myocardial protection. Pharmacol Ther 2001;89:29–46.
[28] DiLisa F, Menabo R, Canton M, Barile M, Bernardi P. Opening of the mitochondrial permeability pore causes depletion of mitochondrial and cytosolic NAD^+ and is a causative event in the death of myocytes in postischemic reperfusion of the heart. J Biol Chem 2001;276:2571–5.
[29] Tsujimoto Y, Shimizu S. VDAC regulation by the Bcl-2 family of proteins. Cell Death Differ 2000;7:1174–81.
[30] Gellerich FN, Khuchua ZA, Kuznetsov AV. Influence of the mitochondrial outer membrane and the binding of creatine kinase to the mitochondrial inner membrane on the compartmentation of adenine nucleotides in the intermembrane space of rat heart mitochondria. Biochim Biophys Acta 1993;1140:327–34.
[31] Schild L, Gellerich FN. Effect of the extramitochondrial adenine nucleotide pool size on oxidative phosphorylation in isolated rat liver mitochondria. Eur J Biochem 1998;252:508–12.
[32] Gellerich FN, Chen Y, Deschauer M, Müller T, Zierz S. Energetic depression caused by mitochondrial impairments. Eur Cytokine Netw (in press).