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Biochimica et Biophysica Acta 1188 (1994) 373-379

BBA Biochimica et Biophysica Acta

Caffeine and Ca^{2+} stimulate mitochondrial oxidative phosphorylation in saponin-skinned human skeletal muscle fibers due to activation of actomyosin ATPase

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Received 28 June 1994

Abstract

The rate of mitochondrial oxidative phosphorylation of saponin-skinned human muscle fibers from m. vastus lateralis in the presence of glutamate, malate and ATP is reported to be sensitive to caffeine and to changes of free calcium ion concentration. An approximately twofold increase in respiration was observed by the addition of 15 mM caffeine, because of the efflux of calcium from sarcoplasmic reticulum. Direct addition of a Ca^{2+}/CaEGTA buffer, containing 1.5 μM free calcium ions had a similar effect. The ATP-splitting activity of skinned fibers was also stimulated by caffeine or calcium. These observations can be explained exclusively by the calcium-induced activation of actomyosin ATPase. (i) Thapsigargin, an inhibitor of the sarcoplasmic reticulum Ca^{2+}-ATPase, had no influence. (ii) In myosin-extracted 'ghost' fibers containing intact mitochondria and an intact sarcoplasmic reticulum caffeine had a negligible effect on oxidative phosphorylation. (iii) The caffeine-induced increase in rate of fiber respiration was concomitant with a decrease in mitochondrial membrane potential and a decrease in the redox state of the mitochondrial NAD system. (iv) The calcium ionophore A 23187 caused a stimulation of respiration and ATP-splitting activity, similar to caffeine. (v) The calcium dependencies of respiration and ATP splitting activity of saponin-skinned human muscle fibers were in experimental error identical. Therefore it is concluded that calcium efflux from sarcoplasmic reticulum affects oxidative phosphorylation in skeletal muscle mostly via the stimulation of actomyosin ATPase.

Keywords: Skeletal muscle; Saponin-skinned fiber; Oxidative phosphorylation; Caffeine; Calcium; (Human)

1. Introduction

The regulation of mitochondrial oxidative phosphorylation in muscle is still a matter of dispute. It is a well known fact that isolated mitochondria respond to externally added ADP with an increase in oxygen consumption [1]. Such energy consumer dependent changes of the rate of mitochondrial oxidative phosphorylation have been also observed applying reconstituted systems of isolated mitochondria with various ATPases [2–4]. The usefulness of these model systems for the description of the in vivo situation has been criticized on the basis of several arguments. (i) The contraction-relaxation cycle of skeletal muscle is regulated by the accumulation and release of Ca^{2+} from sarcoplasmic reticulum and calcium is known to affect not only the activity of actomyosin ATPase but also the activity of several mitochondrial dehydrogenases [5,6]. (ii) The load enzymes used behave differently to actomyosin ATPase and mitochondria seem to show in their natural environment different properties especially with respect to

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SSDI 0005-2728(94)00148-0

the diffusivity of nucleotides [7]. These differences could be introduced by destroying close contacts between mitochondria and ATP-utilizing enzymes so diminishing compartmentation effects occurring probably in the intact cell [8]. A model system to overcome some of these difficulties is the permeabilized muscle fiber [9,10].

Recently, we have reported [11] that caffeine addition to saponin-skinned fibers from human muscle vastus lateralis led to a stimulation of oxidative phosphorylation and ATPase activity. Due to the facts that those fibers skinned at low saponin concentrations contain not only intact mitochondria [9,10] but an intact sarcoplasmic reticulum (SR) [12,13], these effects were postulated to be the result of caffeine-induced calcium efflux from SR, leading to the stimulation of actomyosin ATPase. This efflux most probably occurs via the ryanodine receptor of skeletal muscle (RYR1) being the target for the action of caffeine [12–16]. However, alternative explanations for the effects of caffeine exist. It is possible that the direct action on thin filaments [17,18] or the calcium-dependent activation of mitochondrial dehydrogenases [5,6] is involved in the observed stimulation of oxidative phosphorylation.

In this work we provide evidence that the effects of caffeine on the velocity of mitochondrial oxidative phosphorylation in skinned human skeletal muscle fibers are caused by calcium efflux from sarcoplasmic reticulum and can be explained exclusively by the activation of actomyosin ATPase.

2. Materials and methods

2.1. Isolation of saponin-skinned muscle fibers

Bundles of fibers between 10 and 15 mg wet weight of m. vastus lateralis were obtained from otherwise healthy orthopaedic patients and treated with saponin essentially as described in Ref. [10].

2.2. Extraction of myosin from saponin-skinned skeletal muscle fibers

After saponin treatment the fibers were incubated 30 min at 4°C in a medium containing 0.9 M potassium chloride, 75 mM mannitol, 25 mM sucrose, 10 mM KH_2PO_4, 5 mM $MgCl_2$, 2 mg/ml bovine serum albumin, 5 mM ATP and 20 mM Tris-HCl (pH 7.4) [7,19]. Thereafter, the fibers were washed in the medium used for respiration measurements. This treatment led to a removal of myosin, tropomyosin and troponin from the skinned fibers, while the F-actin filaments remain essentially unaffected [19].

2.3. Respiration measurements

The respiration measurements were performed at 25°C using a Cyclobios-oxygraph (Anton Paar, Graz) in a medium consisting of 75 mM mannitol, 25 mM sucrose, 100 mM KCl, 10 mM KH_2PO_4, 2 mM $MgCl_2$, 10 mM glutamate, 5 mM malate and 20 mM Tris-HCl (pH 7.4).

2.4. Measurements of Ca^{2+} efflux

The calcium efflux from skinned muscle fibers was determined by measuring the 340 nm/380 nm ratio of Fura 2 fluorescence using a Sigma ZWS-11 dual wavelength spectrophotometer in the fluorimetric mode. In order to diminish the effects of calcium contamination, a medium containing 125 mM purified Ca^{2+}-free KCl, 10 mM K_2HPO_4, 2 mM $MgCl_2$, 20 mM Hepes (pH 7.2), 2 μM fura-2 and 20 μM thapsigargin was used. The calcium efflux was initiated by the addition of 15 mM caffeine. The signals were corrected for artificial caffeine-caused fluorescence changes, determined in the absence of fibers, and calibrated with known additions of $CaCl_2$.

Free Ca^{2+} concentrations were calculated using a computer program designed by Dr. D. Benevolensky on the basis of equations described by Fabiato and Fabiato [20] with the reported values of dissociation constants [21].

2.5. Measurements of myosin ATPase activity

The measurement of ATPase was performed at 25°C in a stirred thermostated cuvette of a Sigma ZWS-11 dual wavelength spectrophotometer at 340 nm–375 nm using the respiration medium (without glutamate and malate). Additionally, the medium contained 2 mM phosphoenolpyruvate, 2 mM ATP, 0.15 mM NADH, 2 U/ml lactate dehydrogenase, 2 U/ml pyruvate kinase, 2 μg/ml oligomycin, 10 μM ouabain and 1 μM rotenone.

2.6. Measurement of mitochondrial membrane potential

The changes of mitochondrial membrane potential ($\Delta\psi$) were recorded simultaneously with the oxygen consumption in a thermostated stirred oxygen chamber containing in addition to the Clark-type oxygen electrode a tetraphenylphosphonium (TPP^+)-sensitive electrode as described in Ref. [22].

2.7. Fluorescence measurements

For the measurements of fluorescence of NAD(P)H and of fluorescent flavoproteins between 2 and 5 mg wet weight skinned fibers were immobilized (by attach-

ment to glass wool) in a light screened quartz tube and perifused at 1 ml/min with the medium used for the oxygraphic determinations. The NAD(P)H and flavoprotein fluorescence measurements were performed essentially as described in Ref. [23] using a 325 nm HeCd laser or a 454 nm argon-ion laser for excitation, respectively, and a Shimadzu RF 5001 spectrofluorimeter for fluorescence detection.

2.8. Detection of myosin in 'ghost' fibers

The same experimental setup was used for the indirect immunofluorimetric assay of human myosin in 'ghost' fibers. Initially, the immobilized saponin-skinned fibers were labeled with a rabbit antiserum against human skeletal muscle myosin (Sigma) and stained by an anti-rabbit IgG FITC conjugate (Sigma). To obtain the 100% reference value the fluorescence emission of fluorescein at 520 nm using the 454 nm argon-ion laser excitation was measured. Thereafter, the fibers were treated with the 0.9 M KCl-containing buffer. The labeling procedure was repeated to account for a possible release of antibodies induced by the high ionic strength. The remaining amount of myosin after high salt treatment was calculated from the decrease in fluorescence emission of fluorescein.

3. Results

It is well known that muscle fibers chemically skinned with low saponin concentrations (50 μg/ml) contain an intact sarcoplasmic reticulum from which calcium is released by the action of caffeine leading to contraction [12,13]. We recently reported [11] that in the presence of ATP, caffeine addition also caused an approximately twofold stimulation of mitochondrial oxidative phosphorylation. In Fig. 1A the typical experimental protocol of the effect of caffeine on the rate of respiration of saponin-skinned human muscle fibers from vastus lateralis oxidizing glutamate + malate is shown. This effect was not sensitive to the irreversible inhibitor of the SR Ca^{2+}-ATPase thapsigargin. The addition of 1 mM EGTA led to an inhibition of the caffeine-induced stimulation of oxidative phosphorylation. Next, the rate of respiration of the saponin-skinned human skeletal muscle fibers can be stimulated with ADP and inhibited with carboxyatractyloside, indicating mitochondrial intactness. In Fig. 1B a similar experiment is shown in which the ATP splitting activity of saponin-treated fibers was recorded. Similarly, caffeine caused a substantial activation of ATPase which again was not affected by the addition of thapsigargin. However, 1 mM EGTA blocked the caffeine-induced stimulation of ATPase activity almost completely. The addition of 1.6 mM $CaCl_2$ then caused a maximal activation of ATPase of skinned fibers. These experimental data are consistent with our earlier proposal [11] that the caffeine-induced efflux of Ca^{2+} ions from sarcoplasmic reticulum (sensitive to ruthenium red) stimulates actomyosin ATPase. This in turn decreased the phosphorylation potential stimulating mitochondrial oxidative phosphorylation.

To further characterize the role of actomyosin ATPase, we extracted myosin from the skinned fiber preparation by treatment with high ionic strength [7,19]. Experiments analogous to Fig. 1A and B were performed with skinned fibers and myosin-extracted 'ghost' fibers (summarized in Table 1A and B). These 'ghost' fibers contain intact mitochondria as is visible by the normal respiration rates in the presence of 1 mM ADP and by being inhibited by carboxyatractyloside (not shown). However, a large decrease in the stimulation of respiration (Tab. 1A) and ATP-splitting activity (Tab.

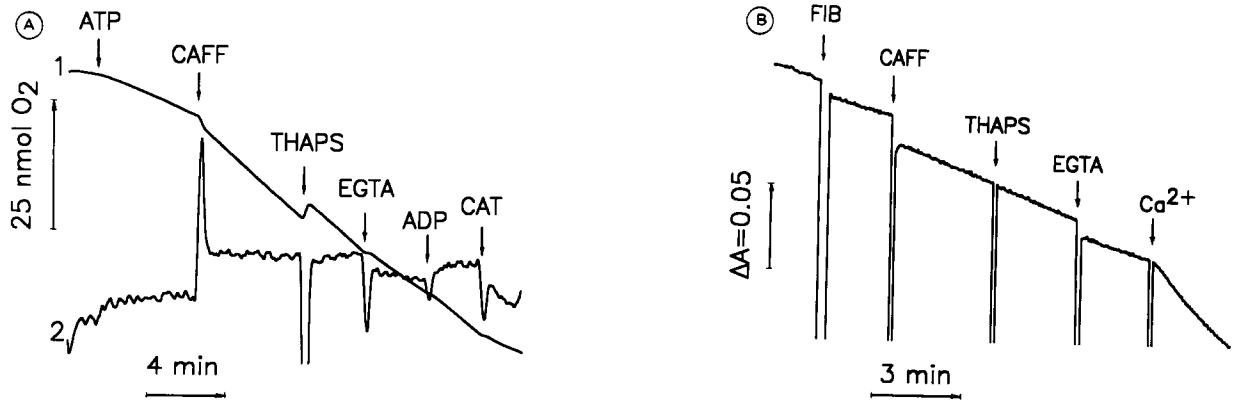

Fig. 1. Effects of caffeine and thapsigargin on respiration (A) and ATP-splitting activity (B) of saponin-skinned human muscle fibers. (A) 2.4 mg dry wt. skinned fibers in 1.5 ml of medium for respiration measurements. Curve 1, oxygen content; curve 2, rate of respiration. Additions: ATP, 2 mM; caffeine (CAFF), 15 mM; thapsigargin (THAPS), 26.7 μM; EGTA, 1 mM; ADP, 1.2 mM; carboxyatractyloside (CAT), 35 μM. (B) 3 ml of medium for ATPase measurements. Additions: fibers (FIB), 0.6 mg dry wt.; caffeine (CAFF), 15 mM; thapsigargin (THAPS), 26.7 μM; EGTA, 1 mM; $CaCl_2$ (Ca^{2+}), 1.67 mM.

Table 1A
The effect of caffeine on rate of respiration of saponin-skinned and 'ghost' human skeletal muscle fibers

	v_o	v_{ATP}	v_{caff}	v_{ADP}
Skinned fibers ($n = 8$)	1.35 ± 0.24	1.96 ± 0.19	4.25 ± 0.57	4.47 ± 1.33
'Ghost' fibers ($n = 5$)	0.98 ± 0.33	1.87 ± 0.17	1.98 ± 0.41	4.64 ± 1.54

The experiments were performed in the medium for respiration measurements (cf. Section 2) using between 1.5 and 2.5 mg dry wt. fibers. The rates are expressed in nmol O_2/min/mg dry wt. v_o, rate of respiration with glutamate + malate; v_{ATP}, rate after the addition of 2 mM ATP; v_{caff}, rate after the addition of 15 mM caffeine; v_{ADP}, rate after addition of 1.2 mM ADP (experimental protocol of Fig. 1A, except the thapsigargin addition).

Table 1B
The effect of caffeine on the ATP-splitting activity of saponin-skinned and 'ghost' human skeletal muscle fibers

	v_{ATP}	v_{caff}	v_{Ca}
Skinned fibers ($n = 5$)	2.4 ± 0.68	11.9 ± 0.4	21.2 ± 2.7
'Ghost' fibers ($n = 3$)	2.9 ± 0.51	4.52 ± 0.9	12.6 ± 1.7

The experiments were performed in the medium for ATPase measurements using between 1.0 and 2.0 mg dry wt. fibers. The rates are expressed in U/g dry wt. v_{ATP}, endogenous ATP-splitting activity; v_{caff}, rate after the addition of 15 mM caffeine; v_{Ca}, rate after addition of 1 mM EGTA and 1.67 mM $CaCl_2$ (experimental protocol of Fig. 1B, except the thapsigargin addition).

1B) on caffeine addition was detected in myosin-extracted fibers. Comparing the maximal rates of Ca^{2+}-dependent ATP-splitting activities in Table 1B we see that almost one half of this activity was removed by the high salt treatment. In agreement to these data we could show by applying an indirect immunoassay for human myosin (cf. Section 2) that 51.2 ± 4.8% of myosin is extracted by the treatment of saponin-skinned fibers with 0.9 M potassium chloride ($n = 3$).

To rule out possible effects of high salt treatment on sarcoplasmic reticulum function we directly measured the caffeine-induced calcium efflux using fura-2 fluorescence. In order to prevent Ca^{2+} reuptake these experiments were performed in the presence of 20 μM thapsigargin (cf. Section 2). We observed a liberation of 1.24 ± 0.11 nmol Ca^{2+}/mg dry wt. from the saponin-skinned fibers (four experiments) and of 1.25 ± 0.03 nmol Ca^{2+}/mg dry wt. from the myosin-extracted 'ghost' fibers (three experiments). Therefore, extraction of myosin by high salt treatment of fibers did not affect their ability to release calcium on caffeine addition.

To check the possibility of Ca^{2+}-dependent activation of mitochondrial dehydrogenases [5,6,24–26] being responsible for the caffeine-induced stimulation of oxidative phosphorylation, we investigated the effects of the caffeine caused Ca^{2+} efflux on the redox state of the mitochondrial NAD system. For this, we recorded the fluorescence of NAD(P)H and fluorescent flavoproteins of perifused saponin-skinned fibers [23]. In Fig. 2A the trace of NAD(P)H fluorescence of perifused skinned fibers is shown. The addition of glutamate + malate to the perifusion medium led to a strong reduction of pyridine nucleotides visible as increase in fluorescence at 450 nm. 2 mM ATP caused a small reoxidation of NAD(P)H (most probably due to its ADP contamination). The caffeine addition resulted in a decrease in NAD(P)H fluorescence under these circumstances. This is an indication that the stimulation of oxidative phosphorylation is caused by an increased load rather than by an increase in supply of reducing equivalents. In order to prove if this decrease in fluorescence at 450 nm is really caused by the reoxidation of mitochondrial NADH we performed similar experiments monitoring the flavoprotein fluorescence of the perifused fibers. This fluorescence signal is mainly caused by the oxidized form of α-lipoamide dehydrogenase being in redox equilibrium with the mitochondrial NAD system [23]. The result of a typical experiment is shown in Fig. 2B. The caffeine-induced Ca^{2+}

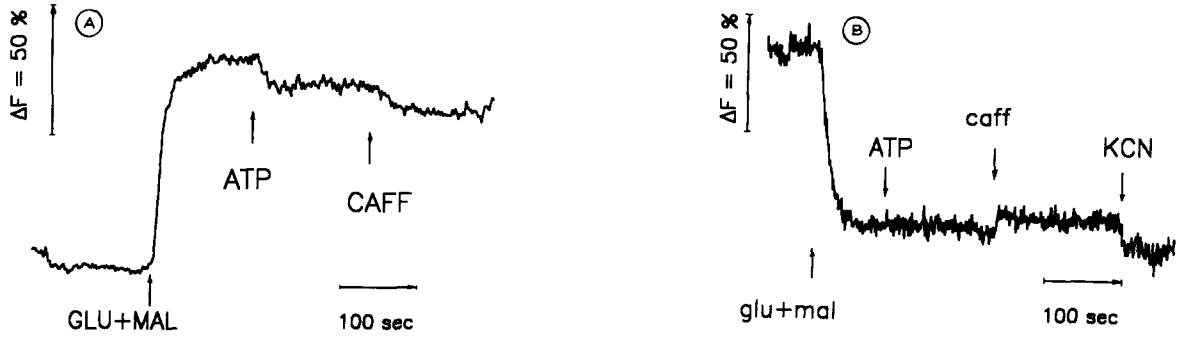

Fig. 2. Fluorescence responses of saponin-skinned human muscle fibers oxidizing glutamate + malate. (A) Time course of the NAD(P)H fluorescence. 4 mg wet wt. of fibers were excited at 325 nm and the emission at 450 nm was recorded. Additions: glutamate (GLU), 10 mM; malate (MAL), 5 mM; ATP, 2 mM; caffeine (CAFF), 15 mM. Representitive experiment of four. (B) Time course of the flavoprotein fluorescence. 3 mg wet wt. of fibers were excited at 454 nm and the emission at 520 nm was recorded. Additions as in A; cyanide (KCN), 4 mM. Representitive experiment of three. ΔF is the fluorescence calibrated on the signal difference between the fully oxidized state (in the absence of substrates) and the fully reduced state (in the presence of substrate and cyanide).

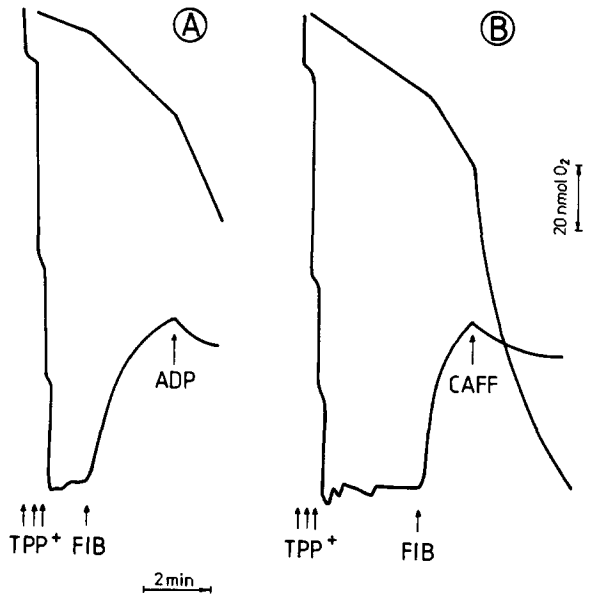

Fig. 3. Simultaneous recording of respiration (upper trace) and accumulation of TPP^+ (lower trace) of saponin-skinned human skeletal muscle fibers. The experiments were performed in the medium for oxygraphic measurements. (A) Additions: tetraphenylphosphoniumbromide (TPP^+), 1.25 μM (three additions); skinned fibers (FIB), 150 mg wet wt.; ADP, 1 mM. (B) 2 mM ATP were initially present. Additions: tetraphenylphosphoniumbromide (TPP^+), 1.25 μM (three additions); skinned fibers (FIB), 150 mg wet wt.; caffeine (CAFF), 15 mM.

efflux led to an increase in fluorescence at 520 nm, indicating a reoxidation of NAD-linked α-lipoamide dehydrogenase flavin. Therefore, an improved supply of reducing equivalents via activation of Ca^{2+}-dependent dehydrogenases can be ruled out to play a role under our conditions.

In addition to this we demonstrated which changes in mitochondrial membrane potential occurred on addition of caffeine to saponin-skinned human muscle fibers. The results of typical experiments are shown in Fig. 3. $\Delta\psi$ is measured simultaneously with respiration by means of a tetraphenylphosphonium (TPP^+)-sensitive electrode. Consistent with previous observations, caffeine addition (panel B) led, similar to the addition of ADP (panel A), to a marked drop in membrane potential. This is an additional indication that the stimulation of oxidative phosphorylation caused by the efflux of Ca^{2+} from the sarcoplasmic reticulum is most probably due to an increased ATP turnover of the Ca^{2+}-dependent actomyosin ATPase.

To check if other calcium-liberating agents cause effects similar to caffeine on oxidative phosphorylation of saponin-skinned human skeletal muscle fibers, we performed analogous to Fig. 1 experiments with the calcium ionophore A23187. We observed a 2.5 ± 0.6-fold stimulation of fiber respiration (Fig. 4A) and a 2.7 ± 0.2-fold stimulation of ATP-splitting activity (Fig. 4B). In contrast to the action of caffeine, these effects were found to be not sensitive to ruthenium red.

In further experiments we investigated the effects of externally added Ca^{2+} ions on respiration and ATP splitting activity of skinned fibers. In Fig. 5A (open circles) the results of titration experiments of respiration of saponin-skinned human skeletal muscle fibers using a Ca^{2+}/CaEGTA buffer in the presence of ATP are shown. Below 1.5 μM the rate of fiber respiration was found to be sensitive to changes in the free calcium concentration while the ADP-stimulated respiration of saponin-skinned human muscle fibers is almost not affected in this concentration range (Fig. 5A, filled circles). Moreover, as shown in Fig. 5B (open circles),

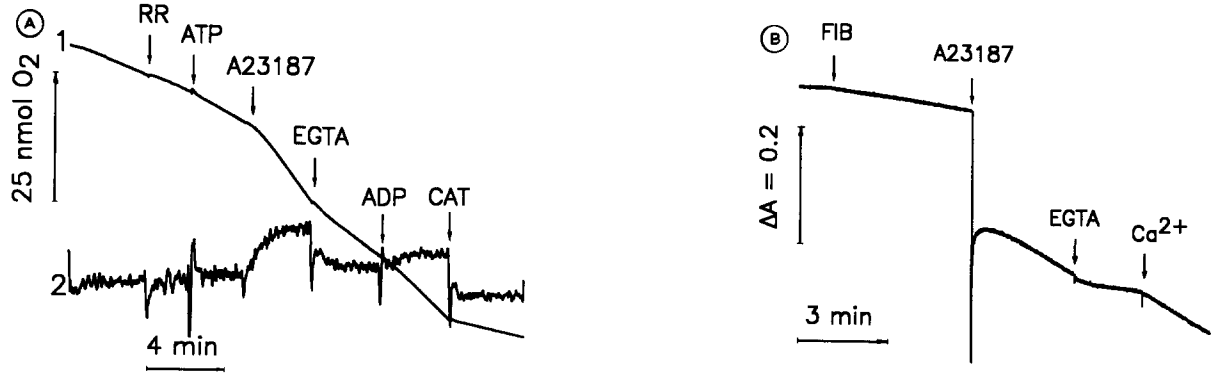

Fig. 4. Effects of the calcium ionophore A23187 on respiration (A) and ATP-splitting activity (B) of saponin-skinned human muscle fibers. (A) 2.0 mg dry wt. skinned fibers in 1.5 ml of medium for respiration measurements. Curve 1, oxygen content; curve 2, rate of respiration. Additions: ruthenium red (RR), 10 μM; ATP, 2 mM; A23187, 40 μM; EGTA, 1 mM; ADP, 1.2 mM; carboxyatractyloside (CAT), 35 μM. Representative experiment of seven. (B) 3 ml of medium for ATPase measurements and 10 μM ruthenium red. Additions: fibers (FIB), 1.3 mg dry wt.; A23187, 40 μM; EGTA, 1 mM; $CaCl_2$ (Ca^{2+}), 1.67 mM. Representative experiment of five.

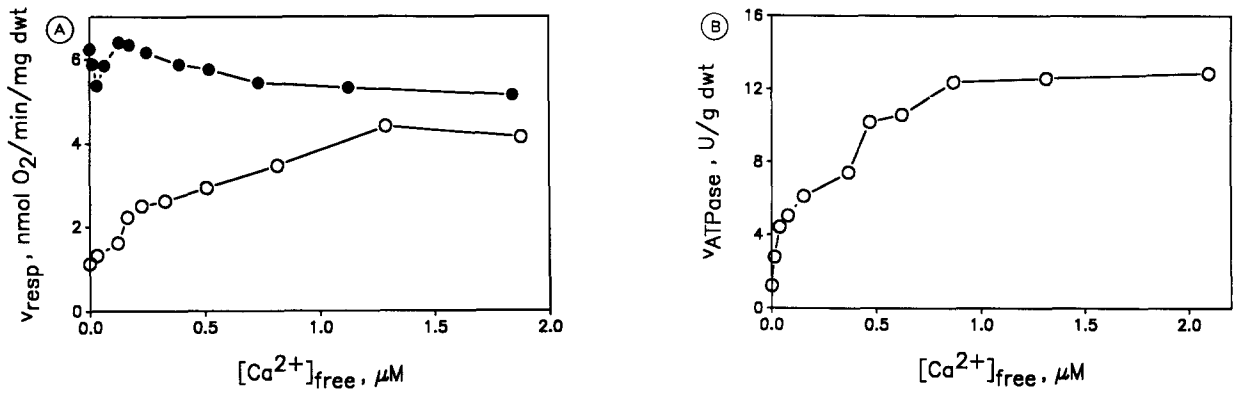

Fig. 5. Effect of calcium on respiration (A) and ATP-splitting activity (B) of saponin-skinned human muscle fibers. (A) Open circles: 2.7 mg dry wt. skinned fibers in 1.5 ml of medium for oxygraphic measurements. In the presence of 2 mM ATP a Ca^{2+}/CaEGTA buffer, containing 0.1 mM EGTA and the amount of $CaCl_2$ to reach the free Ca^{2+} concentrations indicated, was added. Filled circles: 1.4 mg dry wt. skinned fibers in 1.5 ml of medium for oxygraphic measurements. In the presence of 1 mM ADP the rate respiration was titrated with the same Ca^{2+}/CaEGTA buffer. (B) 1.3 mg dry wt. skinned fibers in 3 ml of medium for ATPase measurements. In the presence of 2 mM ATP the ATP-splitting activity was titrated with the Ca^{2+}/CaEGTA buffer used in A.

the ATP-splitting activity of skinned fibers has a similar dependency on the free calcium concentration as the rate of respiration in the presence of ATP. This finding is also in line with the proposal that the observed effects of caffeine and calcium on oxidative phosphorylation are due to changes in mitochondrial load caused by actomyosin ATPase.

4. Discussion

Saponin-skinned muscle fibers can serve as important tools for studies of the mechanism, which causes in response to muscle contraction a stimulation of oxidative phosphorylation, because they contain in addition to the intact contractile apparatus also functionally intact organelle structures such as mitochondria and sarcoplasmic reticulum [9–11]. In these fibers the Ca^{2+}-related responses of oxidative phosphorylation can be investigated under conditions close to in vivo. In agreement with earlier reports [12,13] we showed that in those fibers caffeine causes Ca^{2+} liberation from SR. As depicted in Fig. 6 this occurs most likely via activation of the foot region of the ryanodine receptor (RYR1) [14–16]. This excitation-like influence by caffeine resulted in the contraction of fibers [12,13] which seems to be the reason for the observed stimulation of mitochondrial oxidative phosphorylation. However, another reported action of caffeine is the calcium-sensitizing of contractile proteins [17,18] which also has to be considered. This seems to be of less importance for the effects observed under our experimental conditions due to (i) the strong ruthenium red sensitivity of caffeine-induced stimulation of respiration and ATP-splitting activity (cf. [11]), and (ii) the findings that the calcium ionophore A 23187 caused a comparable effect on respiration and ATP-splitting activity of skinned muscle fibers. Moreover, the amount of calcium liberated from saponin-skinned human skeletal muscle fibers by the action of caffeine – 1.24 ± 0.11 nmol/mg dry wt. – is with 2 mg dry wt. fibers in the oxygraph chamber equivalent to an external calcium concentration of 1.6 ± 0.15 μM. This is sufficient for maximal stimulation of respiration (cf. Fig. 5A).

The stimulation of oxidative phosphorylation by caffeine can be explained by the following mechanisms. (i) The 'classical' stimulation of actomyosin ATPase leading to a lowered phosphorylation potential (load effect);

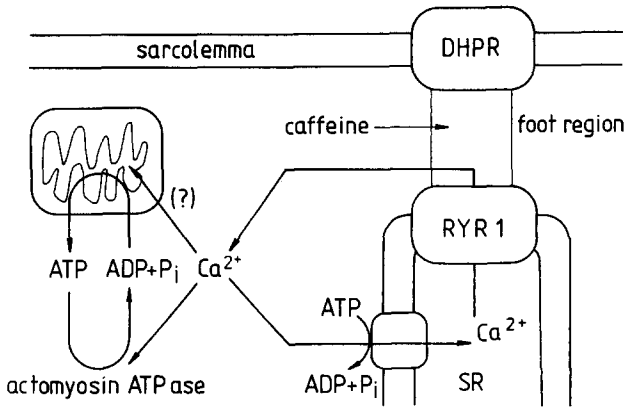

Fig. 6. Possible mechanisms of action of caffeine on oxidative phosphorylation. DHPR, dihydropyridine receptor; RYR1, ryanodine receptor of skeletal muscle; SR, sarcoplasmic reticulum. A cyclic calcium flux between sarcoplasmic reticulum and cytosol caused by RYR1 and Ca^{2+}-ATPase of SR is initiated by the action of caffeine on the foot region of the ryanodine receptor. The result is an increase in the cytosolic free Ca^{2+} concentration which stimulates actomyosin ATPase, affecting the cytosolic adenine nucleotide pattern. The resulting higher ADP activates mitochondrial oxidative phosphorylation. The possible direct effect of calcium on the mitochondria (activation of dehydrogenases) is depicted by an arrow.

(ii) the stimulation of sarcoplasmic Ca^{2+}-ATPase, leading as in (i) to an increased mitochondrial load; or (iii) as a direct effect of Ca^{2+} on mitochondria. In fact, combinations of the mentioned mechanisms also seem to be possible. The results presented here show clearly that thapsigargin (proven to be a specific irreversible inhibitor of the Ca^{2+}-ATPase of sarcoplasmic reticulum [27,28]) does not influence the caffeine caused stimulation of oxidative phosphorylation. Therefore, the Ca^{2+}-ATPase of SR does not contribute to a significant extent to the increased load. Moreover, a direct action of calcium on mitochondria seems to be also not involved in the effects observed. This is supported by the following findings. (1) In myosin-extracted 'ghost' fibers, which were proven to contain still intact mitochondria and intact SR, the effects of caffeine disappeared. (2) In the presence of 1 mM ADP the addition of the Ca^{2+}/CaEGTA buffer did not affect the rate of respiration of saponin-skinned human muscle fibers. (3) Caffeine addition caused a reoxidation of mitochondrial NADH, strongly supporting that Ca^{2+} acts mainly on the mitochondrial load. (4) Adding caffeine we observed a decrease in mitochondrial membrane potential being in line with a stimulation of ATP turnover via actomyosin ATPase.

Summarizing, we could show directly for permeabilized human skeletal muscle fibers which rather closely resemble the situation in skeletal muscle in vivo that under conditions of substrate saturation, calcium efflux from SR affects oxidative phosphorylation mainly by stimulation of actomyosin ATPase activity. Additionally, the effects of caffeine described on respiration and ATP-splitting activity of saponin-skinned human muscle fibers seem to offer new possibilities for the detection of malignant hyperthermia (MH) in human biopsy samples. This syndrome is caused by a defect in the ryanodine receptor, which gets more sensitive to the action of anesthetics and caffeine [29]. The usual phenotype screening method for MH is the performance of a standardized contraction test [30] with a rather large biopsy sample. The investigation of the effects of caffeine and halothane on saponin-skinned fiber respiration or ATP-splitting activity should offer additional possibilities for detection of MH using low amounts of biopsy material.

Acknowledgements

The excellent technical assistance of Mrs. I. Schmidl and M. Schubert is gratefully acknowledged. We wish to thank Dr. Joseph Clark for critical reading of the manuscript. This work was supported by the program 'muscle research' of Deutsche Forschungsgemeinschaft.

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