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Mitochondrial oxidative phosphorylation in saponin-skinned human muscle fibers is stimulated by caffeine

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The addition of 15 mM caffeine to saponin-skinned human muscle fibers from M. vastus lateralis caused in the presence of 2 mM ATP an approx. 2-fold stimulation of respiration with glutamate + malate. This effect can be abolished by either the addition of the Ca^{2+} chelator EGTA, the inhibitor of Ca^{2+} transport Ruthenium red and the inhibitor of the myosin ATPase vanadate. The caffeine concentration dependency of respiration of fibers coincided with the caffeine-caused stimulation of myosin ATPase activity. The activation of oxidative phosphorylation in saponin-skinned human muscle fibers by caffeine can be explained by a stimulation of myosin ATPase caused by Ca^{2+} release from sarcoplasmic reticulum.

Saponin-skinned muscle fiber; Human skeletal muscle; Oxidative phosphorylation; Caffeine

1. INTRODUCTION

It is well known that the contraction–relaxation cycle of skeletal muscle is regulated by the accumulation and release of Ca^{2+} by the sarcoplasmic reticulum. Among various kind of stimuli caffeine can release Ca^{2+} from the sarcoplasmic reticulum by the activation of a calcium channel leading to contraction [1–6]. Due to the facts that saponin-skinned muscle fibers have beside of an intact sarcoplasmic reticulum almost intact mitochondria [7,8] they seem to be a appropriate object for the investigation of the functional coupling between oxidative phosphorylation and muscle contraction. In this work we report that the caffeine-caused Ca^{2+} efflux from sarcoplasmic reticulum in skinned human skeletal muscle fibers is accompanied by a stimulation of mitochondrial oxidative phosphorylation.

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 obtained from orthopaedic patients were placed into 1 ml of an ice-cold incubation medium containing 10 mM EGTA-CaEGTA buffer (free Ca^{2+} concentration 0.1 μM), 9.5 mM $MgCl_2$, 3 mM KH_2PO_4, 20 mM taurine, 5 mM ATP, 15 mM phosphocreatine, 49 mM K-MES and 20 mM imidazole-HCl (pH 7.1). Thereafter, 50 μg/ml saponin was added and the suspension was 20 min at 4°C gentle mixed. Then the fiber bundles were washed to remove saponin in the medium used for respiration measurements (see below) and stored on ice

2.2. *Respiration measurements*

The respiration measurements were performed at 25°C using a Cyclobios-oxygraph (Anton Paar, Innsbruck) in a medium consisting of 75 mM mannitol, 25 mM sucrose, 100 mM KCl, 10 mM KH_2PO_4, 5 mM $MgCl_2$ and 20 mM Tris-HCl (pH 7.4). The solubility of oxygen in the medium was considered to be equal to 230 nmol/ml

2.3. *Measurements of myosin ATPase activity*

The measurements were performed at 25°C in a stirred thermostatted cuvette of a Sigma ZWS-11 dual wavelength spectrophotometer at 340–375 nm using the medium for oxygraphic measurements. The medium contained additionally 2 mM phosphoenolpyruvate, 2 mM ATP, 0.15 mM NADH, 2 U/ml lactate dehydrogenase and 2 U/ml pyruvate kinase

3. RESULTS AND DISCUSSION

In Fig. 1 a typical experimental protocoll of an oxygraphic experiment with saponin-skinned human muscle fibers is shown. The fibers exhibit, with 10 mM glutamate and 5 mM malate as substrates, a rather small rate of respiration which can be slightly stimulated by the addition of 2 mM ATP. The addition of 15 mM caffeine caused a substancial increase in respiration (1.8-fold) which was sensitive to the Ca^{2+} chelator EGTA. The maximal stimulation of fiber respiration was reached by the addition of 1 mM ADP being sensitive to the addition of the inhibitor of the adenine nucleotide translocase carboxyatractyloside. The inhibition of respiration after the addition of EGTA is an indication that a change in the Ca^{2+} concentration is responsible for the caffeine-caused increased rate of fiber respiration. Moreover, the caffeine-caused stimulation of respiration is completely absent in the initial presence

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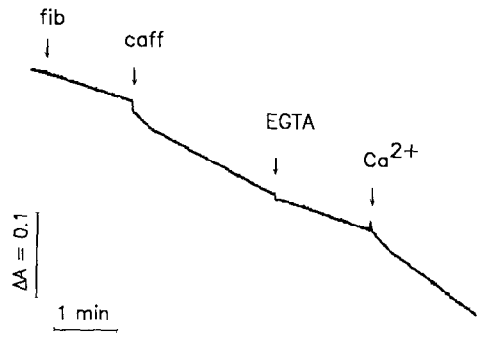

Fig. 1. Effect of caffeine on oxygen consumption of saponin-skinned human muscle fibers. 2.9 mg dry weight of M. vastus lateralis fibers (fib) were incubated in 1.5 ml of the medium used for respiration measurements (section 2) in the additional presence of 10 mM glutamate and 5 mM malate. Upper curve, oxygen content; lower curve, first derivative (rate of respiration). Additions: ATP, 2 mM, caffeine (caff), 15 mM, EGTA, 1 mM; ADP, 1 mM; carboxyatractyloside (CAT), 10 μM; dithionite (dit), 10 mM.

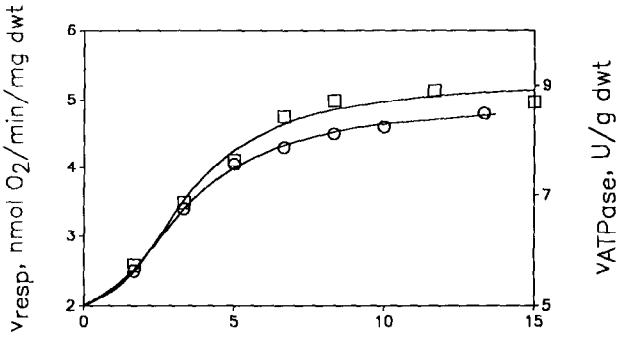

Fig. 2. Effect of caffeine on ATPase activity of saponin-skinned human muscle fibers. 1.0 mg dry weight of M. vastus lateralis fibers (fib) were incubated in 3 ml of the medium used for myosin ATPase measurements (Materials and Methods). Additions: caffeine (caff), 15 mM; EGTA, 1 mM; $CaCl_2$ (Ca^{2+}), 2 mM. Mean basal activity: 5.1 ± 0.9 U/g dwt; after caffeine stimulation: 9.5 ± 1.8 U/g dwt; maximal activity (in the presence of 1 mM EGTA and 2 mM Ca^{2+}). 21 5 ± 5.7 U/g dwt (ten experiments).

of 1 mM EGTA or in experiments with isolated mitochondria (data not shown).

In a second type of experiment we proved that the activation of myosin ATPase by the caffeine-caused Ca^{2+} release from the sarcoplasmic reticulum [1–6] is responsible for the observed stimulation of respiration. For this we measured the ATPase activity of the saponin-skinned fibers in an analoguos experiment. This is shown in Fig. 2. It is to see that caffeine stimulated the activity of the total ATPase 1.6-fold (this experiment, mean of ten experiments: 1.9 ± 0.2). EGTA addition again abolished this effect. Maximal stimulation of muscle fiber ATPase activity was reached by the addition of 2 mM $CaCl_2$. These data indicate that the activation of the Ca^{2+}-dependent myosin ATPase seems to be involved in the observed effects of caffeine.

To verify this, we investigated the effect of ATPase inhibitors and ion transport inhibitors on the caffeine caused stimulation of respiration and ATPase activity. The results are shown in Table I. It can be seen that the inhibitor of mitochondrial H^+-ATPase oligomycin abolished the caffeine effect on respiration but did not influence the stimulation of myosin ATPase whereas the addition of the inhibitor of myosin ATPase vanadate did abolish the effect of caffeine on both activities. The inhibitor of the mitochondrial Ca^{2+}-dependent multiconductance channel cyclosporin [9] did not influence the caffeine caused stimulation of respiration and myosin ATPase activity whereas the inhibitor of the sarcoplasmic and mitochondrial calcium transport pathways, Ruthenium red, completely abolished both stimulations.

The results of the concentration dependency of the

Table I

Effect of inhibitors on the caffeine caused stimulation of respiration and myosin ATPase activity in skinned muscle fibers.

	Stimulation of respiration by 15 mM caffeine*	Stimulation of ATPase activity by 15 mM caffeine**
Control	166 ± 17% (n = 10)	187 ± 20% (n = 13)
+ Oligomycin	103 ± 4% (n = 5)	281 ± 53% (n = 5)
+ Vanadate	108 ± 12% (n = 5)	106 ± 10% (n = 5)
+ Ruthenium red	97 ± 4% (n = 7)	104 ± 5% (n = 5)
+ Cyclosporin	147 ± 10% (n = 5)	248 ± 50% (n = 5)

*The values are given in percent of the basal respiration rate in the presence of 10 mM glutamate, 5 mM malate and 2 mM ATP being equal to 2.88 ± 0.76 nmol O_2/min/mg dry weight (ten experiments)
**The values are given in percent of the basal ATPase activity in the presence of 2 mM ATP being equal to 5.1 ± 0.9 U/g dwt (ten experiments). n = number of experiments. Additions: oligomycin, 10 μg/ml; vanadate, 0.3 mM; Ruthenium red, 20 μM; and cyclosporin, 14 μM. The maximal respiration activities (+ 1 mM ADP) were not affected by the used concentrations of vanadate, Ruthenium red and cyclosporin and the maximal ATPase activities (+ 1 mM EGTA, + 2 mM Ca^{2+}) were not changed in the presence of Ruthenium red and cyclosporin

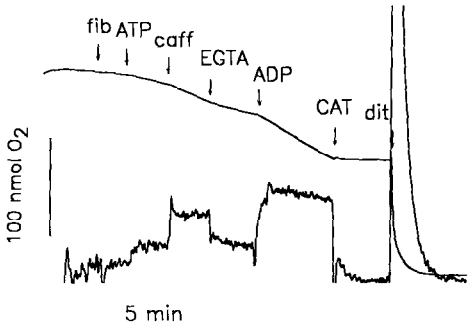

Fig. 3 Dependency of stimulation of respiration (circles) and ATPase activity (squares) on caffeine concentration. Experimental protocol like in the legends to Figs 1 and 2.

Table II

Dependency of the caffeine caused stimulation of skinned fiber respiration on the oxidizable substrate.

Substrate	Basal respiration	Caffeine stimulated respiration	ADP stimulated respiration	Caffeine effect (%)
Glutamate + malate $n = 20$	2.88 ± 0 76	5 04 ± 1 31	7 12 ± 1.79	179.4 ± 38.4
Pyruvate + malate $n = 5$	2 67 ± 0.64	3.73 ± 0.62	6.28 ± 0.78	142.7 ± 17.7
Octanoylcarnitine + malate $n = 6$	3.34 ± 0.65	4.64 ± 0.65	5.53 ± 0.75	142.1 ± 21.6
Succinate + rotenone $n = 4$	5.06 ± 0.77	6.33 ±0.89	6.22 ± 1.5	126.0 ± 14.0

All rates of respiration are given in nmol O_2/min/mg dry weight ± S.D n = number of experiments The basal respiration was determined in the presence of 2 mM ATP, the caffeine stimulated respiration in the additional presence of 15 mM caffeine and the ADP stimulated respiration after the additions of 2 mM EGTA and of 1 mM ADP (experimental protocol of Fig. 1). Additions: glutamate, 10 mM; malate, 5 mM; pyruvate, 10 mM; octanoylcarnitine, 1 mM; succinate, 10 mM; and rotenone, 10 μM

caffeine effect on respiration and on ATPase activity are shown in Fig. 3. It can be seen that both curves are similar sigmoidal with a halfmaximal stimulation of either respiration or ATPase activity at 3.7 ± 0.5 mM caffeine (five experiments). This value is in the range of caffeine concentrations reported for halfmaximal Ca^{2+} efflux rates from the isolated sarcoplasmic reticulum (5 mM [2], 0.8 mM [3]) and skinned fiber preparations (4.5 mM [6]).

In order to test if the caffeine-caused stimulation of fiber respiration depends on the substrate applied we performed similar experiments as shown in Fig. 1 using additionally to glutamate + malate the substrates pyruvate + malate octanoylcarnitine + malate and succinate + rotenone. The results of those experiments are summarized in Table II. It is to see that the size of the caffeine stimulation seems to be dependent on the substrate combination used. The smallest stimulation was observed with succinate + rotenone , the largest with glutamate + malate.

Summarizing, the stimulation of respiration of saponin-skinned human muscle fibers by caffeine can be explained by an increased Ca^{2+}-sensitive myosin ATPase activity due to the efflux of Ca^{2+} from the sarcoplasmic reticulum. This seems to be the case due to the observations made that (i) the stimulation of both respiration and myosin ATPase can be abolished by either vanadate or Ruthenium red, (ii) the halfmaximal stimulation of both respiration and myosin ATPase is reached at identical caffeine concentrations, and (iii) the presence of the caffeine effect does not depend on the mitochondrial substrate used. However, on the basis of the data presented we cannot exclude the possibility that under our conditions of caffeine stimulation the oxidative phosphorylation of saponin treated human muscle fibers is affected additionally by the Ca^{2+}-dependent activation of mitochondrial dehydrogenases [10–13].

The possibility of stimulation of respiration of saponin-skinned human muscle fibers by caffeine offers an alternative method for the diagnosis of malignant hyperthermia (MH). This syndrome is caused by a defect in the skeletal muscle sarcoplasmic reticulum calcium release channel, which gets more sensitive to the action of anaesthetics or caffeine [14–16]. The drastically reduced threshold value for caffeine in muscle biopsy samples of MH patients should be visible also in measurements of respiration or of ATPase activity of saponin-skinned fibers.

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