Das mitochondriale Kalziumtransportsystem im M. sternomandibularis (Stier)

A.M. CHEAH und K.S. CHEAH
Agricultural Research Council, Meat Research Institute, Langford, Bristol BS18 7DY, United Kingdom.

Die kalziumstimulierte Atmung für Succinatoxydierung (Stelle II) wies einen Aktivitätsverlust von 24% auf nach einer Alterung von 48 Stunden bei 10°C (in vitro); kein Atmungsrückgang für Askorbat und TMPD-oxydierung (Stelle III) wurde unter ähnlichen Bedingungen festgestellt.

Der Kalziumaufnahmegrad bei Nichtvorhandensein von Phosphat lag bei einem pH-Wert von 6,50 höher als bei 7,20 bei frisch isolierten und gealterten Mitochondrien.

Mitochondrien konnten Kalzium in einem großen Temperaturbereich (35° - 5°C) aufnehmen und abgeben. Arrheniusstellen von kalziumstimulierter Atmung und anaerobem Kalziumausfluß zeigten identische Übergangstemperaturen. Die Aktivierungsenergie für Kalziumausfluß betrug jedoch nur 12,5% derer fur die Kalziumaufnahme im niedrigeren Temperaturbereich.

Mitochondrial calcium transport system in M.sternomandibularis (ox)

A.M. CHEAH and K.S. CHEAH
Agricultural Research Council, Meat Research Institute, Langford, Bristol, BS18 7DY, United Kingdom.

The calcium-stimulated respiration for succinate oxidation (Site II) showed a 24% loss in activity after ageing for 48 hours at 10°C (in vitro); no decline in respiration for ascorbate plus TMPD oxidation (Site III) was observed under similar conditions.

The rate of calcium uptake, in the absence of phosphate, was higher at pH 6.50 than at pH 7.20 with freshly isolated and aged mitochondria. The anaerobic calcium efflux rate, in the presence of phosphate, was lower at pH 6.50 than at pH 7.20.

Mitochondria could take up and release calcium over a wide range of temperature (35° to 5°C). Arrhenius plots of calcium-stimulated respiration and of anaerobic calcium efflux exhibited identical transition temperatures. The activation energy for calcium efflux, however, was only 12.5% of that for calcium uptake in the lower temperature range.

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Système de transport du calcium mitochondrial dans le M. sternomandibularis (boeuf)

A.M. CHEAH and K.S. CHEAH
Agricultural Research Council, Meat Research Institute, Langford, Bristol, BS18 7DY, United Kingdom.

La respiration stimulée par le calcium pour l'oxydation succinique (Poste II) a montré une perte d'activité de 24% après le vieillissement pendant 48 heures à 10°C (in vitro); aucune réduction de la respiration pour l'oxydation ascorbique plus TMPD (Poste III) n'a été observée sous des conditions semblables.

Le taux d'absorption du calcium, en l'absence du phosphate, a été plus élevé à pH 6,50 qu'à pH 7,20 avec la mitochondrie fraichement isolee et vieillie. Le taux d'ecoulement du calcium anaerobique, en presence du phosphate, a ete plus bas a pH 6,50 qu'a pH 7,20.

La mitochondrie pouvait absorber et liberer le calcium dans une large gamme de temperatures (35°C a 5°C). Les traces d'Arrhenius de la respiration stimulee par le calcium et de l'ecoulement du calcium anaerobique ont montre des temperatures de transition identiques. L'energie d'activation pour l'ecoulement ud calcium, cependant, n'etait que 12,5% de celle de l'absorption du calcium dans la gamme de temperatures inferieure.

Митохондриальная транспортная система кальция в M. sternomandibularis (быка)

А.М. ЧИА и К.С. ЧИА

Сельскохозяйственный исследовательский Совет, Научно-исследовательский институт мяса. Великобритания.

Стимулированное кальцием дыхание при окислении сукцината (участок II) показал снижение активности (24%) после созревания в течение 48 часов при 10°C (in vitro); не наблюдалось отклонений в дыхании при окислении аскорбата и TMPD (участок III) при таких же условиях. Для митохондрий, выделенных непосредственно после убоя и через некоторое время, темп поглощения кальция при отсутствии фосфата более высокий при pH 6,5 нежели при pH 7,2. Темп анаэробного выделения кальция в присутствии фосфата был более низким при pH 6,5 чем при pH 7,2. Митохондрии могут поглощать и освобождать кальций в широком диапазоне температур (от 35°C до 5°C). Графики Аррениуса для стимулированного кальцием дыхания и анаэробного выделения кальция показали идентичные переходные температуры. Активная энергия для выделения кальция, однако, составляла только 12,5% энергии для поглощения кальция при более низких температурах.

Mitochondrial calcium transport system in M. sternomandibularis (ox)

A.M. CHEAH and K.S. CHEAH
Agricultural Research Council, Meat Research Institute, Langford, Bristol BS18 7DY, United Kingdom.

Introduction

The release of Ca^{2+} from mitochondria was recently implicated in the cold-shortening of muscle (1) and in porcine stress-susceptibility (2). Cold-shortening of M. sternomandibularis (ox) was proposed to be due to anoxia-induced Ca^{2+} release from mitochondria at low temperatures which prevent the sarcoplasmic reticulum from fully compensating the Ca^{2+} release from these organelles (1). The Ca^{2+} released from mitochondria of M. longissimus dorsi during anaerobiosis was suggested to be the 'trigger' for the ultimate formation of pale, soft and exudative (PSE) muscle, and of malignant hyperthermia syndrome in stress-susceptible pigs (2).

This communication reports the effects of post-mortem ageing on the mitochondrial calcium transport system of M. sternomandibularis (ox), of pH and of temperature on the rate of mitochondrial Ca^{2+} uptake and release. The data show that mitochondria of M. sternomandibularis could take up and release Ca^{2+} at low temperatures. The energy of activation (E_A) of Ca^{2+} release from mitochondria in the low temperature range was very much lower than that of sarcoplasmic reticulum (3), implying that the anaerobic release of mitochondrial Ca^{2+} could very well be implicated in the cold-shortening phenomenon, as first suggested by Beuge and Marsh (1).

Materials and Methods

Antimycin A (type III), murexide, rotenone and sodium succinate were obtained from Sigma Chemical Corporation; N,N,N',N'-tetramethyl-p-phenylenediamine dihydrochloride (TMPD) and sodium salts of L-ascorbate and EDTA from British Drug Houses; crystalline Bacillus substilis proteinase from Teikoku Chemical Company, Osaka; all other reagents were of analytical grade.

M. sternomandibularis (ox) was obtained within 30 minutes of slaughter. Mitochondria, both fresh and aged at $2^{o}C$ in situ were isolated as previously described (4) using B. substilis proteinase, except that the muscle was minced with a mincer prior to homogenization with a Thomas teflon-pestle glass homogenizer. For post-mortem ageing experiments of isolated mitochondria, the mitochondrial suspension in 0.25 M sucrose (20 mg protein per ml) was aged in vitro for 48 hours at either $2^{o}C$ or $10^{o}C$.
Oxygen uptake was measured polarographically with a Clark oxygen electrode (Yellow Spring Biological Monitor (Model 53)) in a total volume of 2.50 ml. The reaction medium contained 220 mM mannitol, 50 mM sucrose and 15 mM Tris-HCl (pH 7.20) in the presence of 5.0 mM P_i. The effect of temperature on Ca^{2+} uptake and release was investigated using the Clark oxygen electrode. Ca^{2+} uptake was monitored by the Ca^{2+}-stimulated respiration (State 3 rate), and the amount of Ca^{2+} release was estimated at the end of each polarographic experiment by allowing anaerobiosis to occur for 10 minutes prior to rapid centrifugation in an Eppendorf (Model 2300) micro-centrifuge for 2 minutes. The amount of Ca^{2+} in the supernatant was estimated with murexide with the Aminco-Chance spectrophotometer using the wavelength pair at 540-510 nm. The effect of pH on the kinetic uptake and efflux of Ca^{2+} was monitored by the murexide technique (5) with the Aminco-Chance dual-wavelength/split-beam spectrophotometer operating in the dual-wavelength mode at 540-510 nm.
Protein was estimated with Folin-phenol reagent (6) with bovine serum albumin as standard.

Results

1. Ca^{2+}-stimulated Respiration

The comparative data on the effect of ageing on the Ca^{2+}-stimulated respiration for the oxidation of succinate (Site II substrate) are illustrated in Table 1.
Ageing of mitochondria for 48 hours (in situ and in vitro at $2^{o}C$) produced the same extent of decline in State 3 respiration and Ca^{2+}/O ratio values. However, mitochondria aged for 48 hours in vitro at $2^{o}C$ show a higher loss in respiratory control than mitochondria aged in situ for 48 hours at $2^{o}C$. This was due to the 30% increase in State 4 respiration, which was hardly affected in mitochondria aged in situ for 48 hours at $2^{o}C$. Raising the in vitro storage temperature from $2^{o}C$ to $10^{o}C$ resulted in a further decrease in State 3 respiration (from 99% to 76%), in Ca^{2+}/O ratio (from 80% to 36%) and respiratory control index (from 77% to 32%), and an

increase in State 4 respiration (from 130% to 224%) when compared with the values observed for mitochondria isolated at 30 minutes post-mortem.

Table 1 : Effect of post-mortem storage on the Ca^{2+}-stimulated respiration for succinate oxidation (Site II) of M. sternomandibularis (ox) mitochondria.

The data represent average values from at least two separate mitochondrial preparations from different animals. The State 3 and State 4 respiratory rates were calculated from the electrode traces from at least two separate additions of Ca^{2+} in each mitochondrial preparation. Rotenone was added prior to succinate. Final concentrations : rotenone, 10 nmol; succinate, 20 µmol; Ca^{2+}, 600 nmol (each addition); temperature, 25°C.

Time Post-mortem	Storage Condition	Respiratory Rates (nmol O/min/mg protein)		Ca^{2+}/O Ratio	RCI
		State 3	State 4		
30 minutes	-	102 (100%)	23 (100%)	3.9 (100%)	4.4 (100%)
48 hours	2°C (in situ)	100 (98%)	24 (104%)	3.3 (85%)	4.1 (93%)
48 hours	2°C (in vitro)	101 (99%)	30 (130%)	3.1 (80%)	3.4 (77%)
48 hours	10°C (in vitro)	77 (76%)	56 (224%)	1.4 (36%)	1.4 (32%)

The State 3 respiration induced by Ca^{2+} for Site III, with ascorbate plus TMPD as substrate (Table II) was unaffected when mitochondria were aged under the same conditions as for succinate oxidation.
However, ageing of mitochondria for 48 hours in vitro resulted in a slightly greater loss in the Ca^{2+}/O ratio values (12% at 2°C and 18% at 10°C) than ageing in situ (6% at 2°C) when compared with values observed at 30 minutes post-mortem. The same trend also occurred with the respiratory control index, where the loss in this parameter was due to a greater increase in the State 4 respiration with mitochondria aged in vitro (23% for 2°C and 29% for 10°C) than aged in situ (11% at 2°C).

Table II : Effect of post-mortem storage on the Ca^{2+}-stimulated respiration for ascorbate plus TMPD oxidation (Site III) of M. sternomandibularis (ox) mitochondria.

Experimental details as in legend to Table 1 except that antimycin A was added prior to ascorbate plus TMPD Final concentrations : antimycin, 0.5 µg per mg protein; ascorbate, 10 µmol; TMPD, 500 nmol; Ca^{2+}, 300 nmol (each addition); temperature, 25°C.

Time Post-mortem	Storage Condition	Respiratory Rates (nmol O/min/mg protein)		Ca^{2+}/O Ratio	RCI
		State 3	State 4		
30 minutes	-	205 (100%)	123 (100%)	1.7 (100%)	1.6 (100%)
48 hours	2°C (in situ)	202 (98%)	136 (111%)	1.6 (94%)	1.5 (94%)
48 hours	2°C (in vitro)	202 (98%)	151 (123%)	1.5 (88%)	1.3 (82%)
48 hours	10°C (in vitro)	201 (98%)	159 (129%)	1.4 (82%)	1.3 (82%)

2. Ca^{2+} Uptake and Release

The effect of pH and of P_i on the rate of Ca^{2+} uptake and release from mitochondria isolated at 30 minutes post-mortem, and after ageing of mitochondria (in situ) for 48 hours at 2°C are illustrated in Table III and Table IV respectively.

Table III : Effect of pH and of P_i on Ca^{2+} uptake of mitochondria from M. sternomandibularis (ox).

P_i (mM)	Ca^{2+} Uptake (nmol/min/mg protein) at pH 7.20		Ca^{2+} Uptake (nmol/min/mg protein) at pH 6.50	
	30 minutes Post-mortem	48 hours Aged at 2°C	30 minutes Post-mortem	48 hours Aged at 2°C
Nil	195 (100%)	190 (100%)	348 (100%)	298 (100%)
2.50	760 (390%)	405 (213%)	745 (214%)	486 (163)%

The rate of Ca^{2+} uptake, in the absence of P_i, was higher at pH 6.50 than at pH 7.20. P_i (2.50 mM) stimulated Ca^{2+} uptake of both fresh and aged mitochondria at both pH 7.20 and at pH 6.50, but the extent of stimulation was much higher at pH 7.20.

Table IV : Effect of pH and of P_i on Ca^{2+} efflux of mitochondria from M. sternomandibularis (ox)

P_i (mM)	Ca^{2+} Efflux (nmol/min/mg protein) at pH 7.20		Ca^{2+} Efflux (nmol/min/mg protein) at pH 6.50	
	30 minutes Post-mortem	48 hours Aged at 2°C	30 minutes Post-mortem	48 hours Aged at 2°C
Nil	43 (100%)	80 (100%)	61 (100%)	100 (100%)
2.50	250 (581%)	274 (343%)	147 (241%)	200 (200%)

The rate of Ca^{2+} efflux, in the absence of P_i, was also higher at pH 6.50 than at pH 7.20 with fresh and aged mitochondria (Table IV). P_i enhanced the fast phase of Ca^{2+} efflux. The rate of Ca^{2+} efflux in the presence of P_i (2.50 mM) was lower at pH 6.50 than at pH 7.20 with fresh and aged mitochondria. Ageing of mitochondria enhanced the rate of Ca^{2+} efflux at both pH values in the presence and absence of P_i.

3. Effect of Temperature on Ca^{2+} Uptake and Release

Mitochondria could take up and release Ca^{2+} over a wide range of temperature (Figure 1). The Arrhenius plot of Ca^{2+}-stimulated respiration (i.e. Ca^{2+} uptake) showed a transition temperature of 17°C, an energy of activation of 54 kJ/mol in the higher temperature range above the transition temperature, and an energy of activation of 142 kJ/mol below the transition temperature. The Arrhenius plot of anaerobic Ca^{2+} efflux showed a transition temperature of 16°C. The energy of activation above the transition temperature was 50 kJ/mol, which was almost identical to that for Ca^{2+} uptake. The energy of activation below the transition temperature was 18 kJ/mol, a value only 13% of that for Ca^{2+} uptake in the lower temperature range.

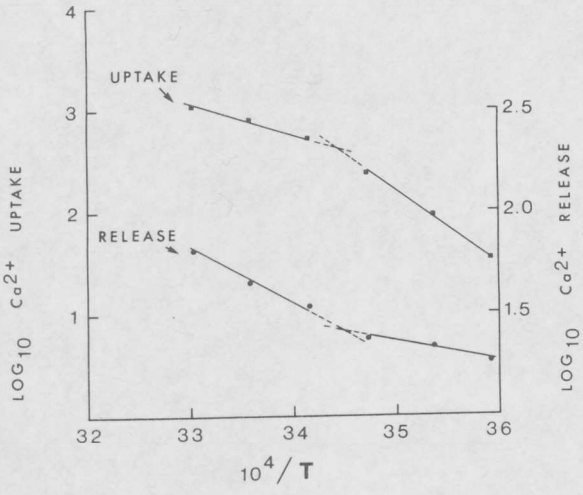

Figure 1 : Arrehenius plot of Ca^{2+} uptake and release of mitochondria from M. sternomandibularis (ox).

Ca^{2+} uptake was monitored by the Ca^{2+}-stimulated respiration of succinate oxidation using the Clark oxygen electrode. Ca^{2+} efflux was measured using murexide at 540-510 nm. The rate of Ca^{2+} uptake and release was expressed as nmol Ca^{2+} per minute per mg protein. Other experimental details are described in Materials and Methods.

Discussion

The effect of post-mortem ageing on Ca^{2+} transport was investigated using mitochondria from M. sternomandibularis as it has already been established that a high proportion of mitochondria in this muscle remained stable after prolonged storage in situ (4). The Ca^{2+}-stimulated respiration for Site III was more stable than for Site II following ageing of mitochondria at 10°C in vitro. This was due to an increase of uncoupling of the mitochondria for Site II substrate oxidation by the increase of the State 4 respiratory rate. The latter condition was probably affected by the enhancement of lipase activity at the higher temperature thereby causing the mitochondrial membranes to become more 'leaky'. Stability for both Site II and Site III systems were

almost identical when the mitochondria were aged at 2°C for 48 hours (in situ and in vitro). The Ca^{2+} released from mitochondria at the onset of anaerobiosis was biphasic, showing an initial fast phase followed by a slow efflux phase as described for mitochondria isolated from M. longissimus dorsi of stress-susceptible and stress-resistant pigs (2). P_i stimulated both the rate of Ca^{2+} uptake and efflux. In the absence of P_i, both the Ca^{2+} uptake and efflux rates were higher at pH 6.50 than at pH 7.20. However, the efflux rate was lower at pH 6.50 with both fresh and aged mitochondria in the presence of 2.50 mM P_i. Post-mortem ageing (in situ for 48 hours at 2°C) of mitochondria inhibited the rate of Ca^{2+} uptake but stimulated the rate of Ca^{2+} efflux at both pH 7.20 and at pH 6.50. Mitochondria could take up and release Ca^{2+} over a wide range of temperature. The energy of activation for the anaerobic Ca^{2+} efflux (18 kJ/mol) below the transition temperature was about 13% of that for Ca^{2+} uptake by mitochondria, and was also about 22% of that for the Ca^{2+}-stimulated ATPase activity of sarcoplasmic reticulum at the lower temperature range (3). Our present data suggest that the anaerobic release of Ca^{2+} from mitochondria of red skeletal muscle, which contained far more mitochondria than sarcoplasmic reticulum, could very well participate in the cold-shortening of muscle. This view, first suggested by Beuge and Marsh (1) was substantiated by the present study and also by the recent report that mitochondria of M. sternomandibularis (ox) could take up 4 times more Ca^{2+} per mg protein than the sarcoplasmic reticulum in this particular muscle (7).

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