

Thermoregulatory, carboxyatractylate-sensitive uncoupling in heart and skeletal muscle mitochondria of the ground squirrel correlates with the level of free fatty acids

N.N. Brustovetsky^a, M.V. Egorova^a, D.Yu. Gnutov^a, V.G. Gogvadze^a, E.N. Mokhova^b and V.P. Skulachev^b

^aInstitute of Theoretical and Experimental Biophysics, Russian Academy of Sciences, Pushchino, Moscow Region 142292, Russia and
^bA.N. Belozersky Institute of Physico-Chemical Biology, Moscow State University, Moscow 119899, Russia

Received 1 April 1992

Thermoregulatory uncoupling of oxidative phosphorylation has been studied in heart and skeletal muscle mitochondria of ground squirrels. The respiratory rate of mitochondria in the presence of oligomycin was found to be much higher in winter (in hibernating, arousing, or aroused animals) than in summer. This additional respiration is strongly (arousing animals) or completely (hibernating and aroused animals) inhibited by carboxyatractylate (CAtr) and bovine serum albumin (BSA). The CAtr- and BSA-induced decreases in the rate of respiration are accompanied by membrane potential increases. The rate of the CAtr- and BSA-sensitive respiration is proportional to the content of free fatty acids which, in the heart, decreases in the order: arousing > aroused = hibernating > summer animals. Maximal respiratory rates observed in the presence of dinitrophenol (arousing > aroused > summer > hibernating animals) do not parallel the fatty acid level. It is assumed that some heat production in the winter animals is due to fatty acid-induced, ATP/ADP-antiporter-mediated uncoupling in heart and skeletal muscle mitochondria. The peak of heat production during arousal after hibernation also includes some other stimulatory effect on mitochondrial respiration.

Thermoregulatory uncoupling; Fatty acid; Hibernation

1. INTRODUCTION

Our previous publication [1] reported that increased heat production by ground squirrels in winter is accompanied by CAtr- and BSA-inhibitable uncoupling in liver mitochondria. It has been suggested that such thermoregulatory uncoupling is induced by free fatty acids and mediated by the ATP/ADP-antiporter which, under the conditions used, may operate as a carrier of fatty acid anions [2-6].

The CAtr-sensitive uncoupling by low concentrations of added free fatty acids has been recently confirmed by Schönfeld [7], who showed that the effect is proportional to the ATP/ADP-antiporter content in mitochondria (maximal in the heart muscle and minimal in liver).

In the present paper we report that the CAtr- and BSA-sensitive thermoregulatory uncoupling is much stronger in the ground squirrel heart and skeletal muscles than in liver. The uncoupling is shown to parallel

the increase in the level of free fatty acids both in the tissues and in mitochondria.

2. MATERIALS AND METHODS

Ground squirrels *Citellus undulatus* from Yakutia (North-East Siberia) were studied during summer and winter periods. Body temperatures in active animals were about 37°C both in summer and in winter. In hibernating animals, it was 4-6°C (heart region). Ground squirrels during arousal from hibernation were studied when the temperature was 27-30°C, 19-21°C and 10-12°C in heart, liver and femoral muscle, respectively. The winter (hibernating, arousing, or aroused) and summer animals were kept at 4-5°C and 17-20°C, respectively.

Heart mitochondria were isolated in 0.25 M sucrose, 1 mM EDTA and 10 mM HEPES (pH 7.4) as described elsewhere [8]. Skeletal muscle mitochondria were isolated in 0.25 M sucrose, 1 mM EDTA, 10 mM HEPES (pH 7.4) (for details, see [4]). The respiratory rate was measured polarographically with a Clark-type oxygen electrode. The incubation medium contained 0.25 M sucrose, 0.5 mM EGTA (pH 7.4), 5 mM glutamate, 5 mM malate, 2×10^{-6} M oligomycin, 37°C. Where indicated, 3×10^{-6} M CAtr, 0.2% BSA and 4×10^{-5} M DNP were added. The membrane potential in isolated mitochondria was monitored by means of synthetic penetrating cations [9] using a tetraphenylphosphonium electrode [10]. The concentration of free fatty acids in blood plasma and in mitochondrial suspensions was measured according to Nixon and Chan [11]. To estimate the fatty acid level in heart muscle, skeletal muscle and liver, samples of the tissues were frozen with liquid nitrogen as fast as possible, i.e. within 10-15 s after decapitation of the animal. For the method of measuring the tissue fatty acid content, see [12].

Oligomycin, CAtr, EGTA, fatty acid-free BSA were from Sigma (USA); glutamate, malate, DNP and HEPES were from Serva (Germany).

Abbreviations: BSA, bovine serum albumin; CAtr, carboxyatractylate; DNP, 2,4-dinitrophenol; EDTA, ethylenediaminetetraacetic acid; EGTA, [ethylenebis(oxyethylenenitrilo)]-tetraacetic acid; HEPES, *N*-(2-hydroxyethyl)piperazine-*N'*-(2-ethanesulfonic acid).

Correspondence address: N.N. Brustovetsky, Institute of Theoretical and Experimental Biophysics, Pushchino, Moscow Region 142292, Russia.

Table I
Respiratory rates of ground squirrel heart muscle mitochondria in the presence of 2×10^{-6} M oligomycin

Season	Groups of animals	Respiration (ng atom O_2 ·min ⁻¹ ·mg protein ⁻¹)				
		No additions	CAtr	BSA	CAtr,BSA	CAtr,BSA,DNP
Summer	Active	31 ± 4	23 ± 3	19 ± 2	14 ± 2	86 ± 7
Winter	Hibernating	52 ± 5	31 ± 4	22 ± 4	15 ± 3	71 ± 6
Winter	Arousing	74 ± 7	43 ± 5	32 ± 4	25 ± 3	165 ± 9
Winter	Aroused, active	60 ± 5	31 ± 4	24 ± 3	14 ± 2	132 ± 11

Additions: 3×10^{-6} M CAtr, 0.2% BSA, 4×10^{-5} M DNP. Values are means ± S.E.M. (n=5).

Table II
Respiratory rates of ground squirrel skeletal muscle mitochondria in the presence of 2×10^{-6} M oligomycin

Season	Groups of animals	Respiration (ng atom O_2 ·min ⁻¹ ·mg protein ⁻¹)				
		No additions	CAtr	BSA	CAtr,BSA	CAtr,BSA,DNP
Summer	Active	25 ± 3	17 ± 2	14 ± 2	10 ± 1	53 ± 4
Winter	Hibernating	47 ± 4	23 ± 3	13 ± 2	7 ± 1	49 ± 5
Winter	Arousing	54 ± 6	31 ± 4	21 ± 3	13 ± 2	78 ± 7
Winter	Aroused, active	53 ± 6	25 ± 4	18 ± 3	10 ± 2	78 ± 8

Additions: 3×10^{-6} M CAtr, 0.2% BSA, 4×10^{-5} M DNP. Values are means ± S.E.M. (n=5).

3. RESULTS AND DISCUSSION

Table I shows that the respiratory rate of heart muscle mitochondria in the presence of oligomycin is higher in winter than in summer, and is maximal in animals arousing from hibernation. This additional respiration of 'winter mitochondria' was found to be due to partial uncoupling of respiration and energy conservation since its inhibition by CAtr and BSA (see Table I) was accompanied by a mitochondrial membrane potential increase (measured with a tetraphenylphosphonium probe, not shown in the table). The coupling effect of CAtr and BSA completely abolished the additional respiration in the hibernating and aroused winter ground squirrels. In arousing animals, the respiratory rate remained slightly increased even in the presence of CAtr and BSA. To measure the maximal respiratory rate, an artificial uncoupler, DNP, was added after CAtr and BSA. This rate proved to be the highest in arousing and lowest in hibernating animals. Similar relationships were revealed also in mitochondria from skeletal muscles but the changes in the arousing animals were less pronounced (cf. Tables I and II). This may be due to the fact that at the studied stage of arousal the temperature in the skeletal muscles was still low, 10–12°C, whereas in the heart it was already 27–30°C. Therefore, the thermoregulatory response was less developed in the skeletal muscle than in the heart.

A strong recoupling effect of BSA and CAtr on the

mitochondria from the winter animals was, in fact, predicted by the hypothesis that free fatty acids are natural uncouplers and that the ATP/ADP-antiporter is the carrier of fatty acid anions [1–6]. BSA is well known to bind fatty acids (see e.g. [13]), whereas micromolar CAtr specifically blocks the ATP/ADP-antiporter (for review, see [2]).

Thus one may expect that the mitochondrial level of free fatty acids increases when thermoregulatory uncoupling develops in the heart and skeletal muscle. Experiments showed that this is the case (Table III). An especially large increase in the fatty acid concentration was observed in heart muscle mitochondria from arousing ground squirrels, which correlates with the maximal

Table III
Content of free fatty acids in isolated heart and skeletal muscle mitochondria of ground squirrels

Season	Groups of animals	Free fatty acids content (nmol·mg protein ⁻¹)	
		Heart	Skeletal muscle
Summer	Active	24.6 ± 3.0	20.8 ± 2.8
Winter	Hibernating	45.7 ± 3.9	41.2 ± 4.3
Winter	Arousing	63.2 ± 3.2	41.0 ± 3.8
Winter	Aroused, active	46.8 ± 4.4	37.5 ± 5.0

Values are means ± S.E.M. (n=5).

Table IV
Free fatty acid (FFA) content in different tissues of ground squirrels

Season	Groups of animals	FFA content			
		(nmol·g wet wt ⁻¹)			(μ M)
		Heart	Skeletal muscle	Liver	Blood plasma
Summer	Active	90 ± 9	75 ± 10	61 ± 7	268 ± 14
Winter	Hibernating	144 ± 11	164 ± 15	120 ± 8	562 ± 17
Winter	Arousing	229 ± 13	201 ± 11	145 ± 12	872 ± 21
Winter	Aroused, active	117 ± 12	140 ± 9	147 ± 10	428 ± 23

Values are means ± S.E.M. ($n=5-6$).

uncoupling. Similar relationships were revealed when the free fatty acid concentration was measured in tissues fixed with liquid nitrogen (Table IV). Interestingly, the level of fatty acids in tissues of arousing animals was found to decrease in the following order: heart muscle > skeletal muscle > liver, which corresponds to the degree of thermoregulatory uncoupling in mitochondria from these tissues (see Tables I and II and ref. [1]).

A CAtr-sensitive increase in H⁺ conductance has recently been observed by Valarce and Cueza in liver mitochondria of new-born rats [14]. Such an effect may also be regarded as thermoregulatory uncoupling caused by severe cold stress which occurs immediately after birth. This and some other observations (for reviews, see refs. [2] and [6]) indicate that fatty acid induced, ATP/ADP-antiporter-mediated uncoupling is involved in thermoregulatory responses of warm-blooded animals.

The data also demonstrate that in muscle tissues, as in liver [15,16], there is, in addition to uncoupling, at least one more mitochondrial response clearly related to thermoregulation, i.e. the changes in maximal respiratory rate observed in the presence of an artificial uncoupler. This rate is lowest in hibernating and highest in arousing ground squirrels (Tables I and II). The effect does not require fatty acids and the ATP/ADP-antiporter and is resistant to BSA and CAtr. According to our previous data on liver mitochondria, mitochondrial phospholipase A₂ seems to be involved in this case [15,16].

Thus, the maximal heat production in muscles and

liver observed during arousal from hibernation seems to include (i) uncoupling respiration and phosphorylation, and (ii) increase in the maximal rate of uncoupled respiration.

REFERENCES

- [1] Brustovetsky, N.N., Amerkanov, Z.G., Egorova, M.V., Mokhova, E.N. and Skulachev, V.P. (1990) FEBS Lett. 272, 190-192.
- [2] Skulachev, V.P. (1988) Membrane bioenergetics, Springer, Berlin.
- [3] Andreyev, A.Yu., Bondareva, T.O., Dedukhova, V.I., Mokhova, E.N. and Skulachev, V.P. (1988) FEBS Lett. 226, 265-269.
- [4] Andreyev, A.Yu., Bondareva, T.O., Dedukhova, V.I., Mokhova, E.N., Skulachev, V.P., Tsolina, L.M., Volkov, N.I. and Vygodina, T.V. (1989) Eur. J. Biochem. 182, 585-592.
- [5] Brustovetsky, N.N., Dedukhova, V.I., Egorova, M.V., Mokhova, E.N. and Skulachev, V.P. (1990) FEBS Lett. 272, 187-189.
- [6] Skulachev, V.P. (1991) FEBS Lett. 294, 158-162.
- [7] Schönfeld, P. (1990) FEBS Lett. 264, 246-248.
- [8] Dedukhova, V.I., Mokhova, E.N., Skulachev, V.P., Starkov, A.A., Arrigoni-Martelli, E. and Bobyleva, V.A. (1991) FEBS Lett. 295, 51-54.
- [9] Bakeeva, L.E., Grinius, L.L., Jasaitis, A.A., Kuliene, V.V., Levitsky, D.O., Liberman, E.A., Severina, I.I., Skulachev, V.P. (1970) Biochim. Biophys. Acta 216, 13-21.
- [10] Kamo, N., Muratsugu, M., Hongoh, R. and Kobatake, Y. (1979) J. Membr. Biol. 49, 105-121.
- [11] Nixon, M. and Chan, S.H.P. (1979) Anal. Biochem. 97, 403-409.
- [12] Prokhorov, M.Yu., Tiunov, M.P. and Shakalis, D.A. (1977) Lab. Delo 9, 535-536 (Russ.).
- [13] Spector, A.A. and Fletcher, J.E. (1969) J. Lipid Res. 10, 56-67.
- [14] Valarce, C. and Cueza, J.M. (1991) FEBS Lett. 294, 225-228.
- [15] Brustovetsky, N.N., Amerkhanov, Z.G., Grishina, E.V., Maevsky, E.I. (1990) Biokhimiya 55, 201-209 (Russ.).
- [16] Brustovetsky, N.N., Amerkhanov, Z.H., Popova, E.Yu., Konstantinov, A.A. (1990) FEBS Lett. 263, 73-76.