EFFECT OF TONICITY OF THE MEDIUM ON TRANSPORT OF ADENINE NUCLEOTIDES AND PHOSPHATE IN BROWN ADIPOSE TISSUE MITOCHONDRIA

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1. Introduction

Oxidation of palmitoyl-carnitine, 2-oxoglutarate, isocitrate and pyruvate in mitochondria from the brown adipose tissue was shown to be highly dependent on osmolarity of the incubation medium, and a close correlation was found between the sucrose-inaccessible space of these mitochondria and the oxidation rate [1]. Transport of adenine nucleotides and phosphate in brown adipose tissue mitochondria has recently been studied by Christiansen et al. [2] and Christiansen and Wojtczak [3]. The aim of the present study is to find out whether these transport processes are also dependent on osmolarity of incubation media and, consequently, on the volume of mitochondrial matrix. It is shown that the translocation rate of ADP is increased by decreasing medium tonicity, suggesting that more carrier sites are made available on the expanded surface of the inner mitochondrial membrane. With phosphate transport it is found that at low tonicity the final level of mitochondrial phosphate is increased in correlation with increase of the sucrose-inaccessible space.

2. Material and methods

Guinea pigs 3 weeks old were adapted to the cold as described by Pedersen and Grav [4].

Mitochondria from the interscapular brown adipose tissue were isolated according to Grav et al. [5] with a centrifugal modification described by Pedersen and Flatmark [6].

ADP translocation and phosphate transport were measured by inhibitor-stop procedures as described by Christiansen et al. [2] and Christiansen and Wojtczak [3] respectively. A small modification for phosphate transport consisted in omitting phosphate from the preincubation medium and starting the reaction with $KH_2\ ^{32}PO_4$ added to make the final concentration 2.2 mM.

Sucrose-inaccessible space in mitochondria was determined with ^3H_2O and [^{14}C] sucrose using a dual-isotope modification of the method of Malamed and Recknagel [7]. Inulin-inaccessible space was measured in the same way using ^3H_2O and [^{14}C]-hydroxymethyl-inulin.

Protein was determined according to Lowry et al. [8].

The following approximate Van 't Hoff factors were taken to calculate osmolarity: KCl, 2; $MgCl_2$, 3; EDTA (trisodium), 4; phosphate (pH 7.2), 2.7; TEA–HCl, 2; Tris–HCl, 2; TEA-PIPES, 2.*

3. Results and Discussion

Table 1 shows that the transport of ADP in brown adipose tissue mitochondria depends upon the tonicity of the incubation medium, increasing with

* Abbrevations: TEA, triethanolamine; PIPES, piperazine-*N*, *N'*-bis(2-ethane sulphonic acid).

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Table 1
Effect of tonicity on ADP translocation

Expt.	Time	Sucrose (mM)	KCl (mM)	Total osmolarity of the medium (mosmM)	ADP translocated (nmoles/mg protein)	(%)*
1	10 sec	200	0	220	0.69	100
		50	0	70	0.95	138
		25	0	45	1.02	148
2	10 sec	12	125	287	0.82	100
		12	75	187	0.96	117
		12	25	87	1.20	146
3	10 sec	12	125	287	0.49	100
		12	25	87	0.84	171
	5 min	12	125	287	0.89	100
		12	25	87	1.24	139

Mitochondria (about 1 mg protein) were added to the media containing 10 mM TEA–PIPES (pH 7.2), 1% bovine serum albumin and sucrose and KCl as indicated. Total volume was 2.0 ml. In expt. 2 the sample also contained 2 mM phosphate. After 6 min preincubation at 20°C the samples were cooled at 0°C during 10 min and the translocation was started by addition of [^{14}C]ADP to final concentration of 60 μM. The reaction was stopped 10 sec or 5 min later by atractyloside added to final concentration of 30 μM.

* Translocation at the highest osmolarity was taken as 100%.

decreasing osmolarity. If the transport at the osmolarity of 220–287 mosmM, assumed to be approximately isotonic with the mammalian cytoplasmic medium, is taken as 100%, the transport rate at low tonicity of 45–87 mosmM amounts to about 150%. The increase is approximately the same in buffered sucrose and KCl media. In the experiments shown in table 1 serum albumin was present to stimulate ADP translocation and in expt. 2 phosphate was present in addition for optimum conditions of the translocation [2]. However, the stimulation by low osmolarity was independent of these additions (not shown), although the translocation in absolute terms was lower in the absence than in the presence of serum albumin and phosphate.

The stimulation by low osmolarity could be observed after 10 sec incubation with [^{14}C]ADP as well as after a longer time (e.g. 5 min), although in the latter case to a smaller extent (table 1, expt. 3). As shown previously [2], the uptake of labelled necleotides during first few seconds of incubation is a measure of the true rate of translocation, whereas the uptake after a few minutes reflects the amount of intramitochondrial exchangeable nucleotides (ATP + ADP). The results of table 1 can therefore be interpreted as indicating that both the translocation rate and the amount of exchangeable nucleotides are increased at low osmolarity. It can be speculated that the increased rate of translocation results from the expansion of mitochondrial surface. An increase of the amount of exchangeable nucleotides is more difficult to be explained. Since the translocation proceeds as an equimolar exchange between external and intramitochondrial nucleotides [9], the sum of intramitochondrial adenine nucleotides remains unchanged in this process. An increase in exchangeable nucleotides (ATP + ADP) may therefore result from the phosphorylation of AMP to ADP or ATP. This may be due to substrate-level phosphorylation which might be increased at low osmolarity, e.g. as result of increased oxidation [1] of endogenous substrates.

The effect of tonicity on phosphate transport in brown adipose tissue mitochondria is shown in table 2. Here, too, a stimulation occurs by decreasing the osmolarity of the incubation medium, but it mainly concerns the amount of $^{32}P_i$ taken up after 10 min incubation, i.e. at isotopic equilibration (cf. [3]), whereas very little stimulation can be seen after 30 sec. That means that the pool of mitochondrial exchangeable phosphate is increased by lowering the tonicity of the medium while the rate of the transport remains practically unchanged.

To find an explanation for phosphate accumulation at low osmolarity, volumes of mitochondrial compartments were measured. It was found (table 3) that both sucrose-inaccessible and inulin-inaccessible spaces were increased by 50% when osmolarity was decreased from 276 mosmM to 116 mosmM, which corresponds to a comparable increase of the amount mitochondrial exchangeable phosphate (the increase shown in expt. 1 of table 2 was exceptionally high). This indicates that expansion of the matrix space of mitochondria is accompanied by an uptake of phosphate and, probably, of other ions, so that their concentration in this space remains unchanged.

Table 2
Effect of tonicity on phosphate transport

Expt.	Sucrose (mM)	KCl (mM)	Total osmolarity of the medium (mosmM)	Uptake of [^{32}P]phosphate after 30 sec (nmoles/mg protein)	(%)*	after 10 min (nmoles/mg protein)	(%)*
1	185	0	274	0.60	100	0.78	100
	75	0	164	0.62	103	1.25	160
	25	0	114	0.68	113	1.79	229
2	0	125	339			1.26	100
	0	25	139			1.87	148

Mitochondria (0.4 mg protein and 1.3 mg protein in expts. 1 and 2 respectively) were preincubated during 5 min at 0°C in the medium containing 36 mM TEA–HCl (pH 7.2), 2 mM EDTA, 1 mM $MgCl_2$ and sucrose and KCl as indicated; total volume was 1.1 ml. Phosphate uptake was started by addition of $KH_2{}^{32}PO_4$ to final concentration of 2.2 mM and was stopped by mersalyl at final concentration of 133 μM.

* Phosphate uptake at the highest osmolarity was taken as 100%.

Table 3
Effect of tonicity on sucrose-inaccessible and inulin-inaccessible spaces in brown adipose tissue mitochondria

Expt.	Sucrose (mM)	KCl (mM)	Total osmolarity (mosmM)	Sucrose-inaccessible space (μl/mg prot.)	(%)*	Inulin-inaccessible space (μl/mg prot.)	(%)*
1	185	0	276	2.39	100	2.88	100
	75	0	166	2.35	98	3.30	115
	25	0	116	3.46	145	4.21	146
2	0	92.5	276	3.46	100	4.28	100
	0	37.5	166	4.56	132	6.86	160
	0	12.5	116	5.31	154	7.61	178

Mitochondria were incubated during 10 min at 20°C in the medium containing 40 mM TEA–HCl (pH 6.8), 2 mM EDTA, 1 mM $MgCl_2$ and sucrose or KCl as indicated.

* Space size at the highest osmolarity was taken as 100%.

A high permeability of brown adipose tissue mitochondria to monovalent alkali metal cations [1] and Cl^- [10] has been indeed demonstrated. Moreover, using the swelling technique we [11] showed that these mitochondria are permeable to Tris, TEA and cyclohexylammonium cations.

Electron microscopic studies of hamster brown adipose tissue mitochondria [12] revealed that their ultrastructure was very dependent on osmolarity of the medium. At high osmolarity the matrix space was strongly reduced while at low osmolarity it was expanded and the surface of the cristae was increased. These findings were also confirmed on brown adipose tissue mitochondria from guinea-pigs [11].

This study demonstrated the importance of the matrix volume for transport processes in mitochondria, but the effect was different depending on the nature of the carrier. The carrier for adenine nucleotides was more active at low osmolarity probably because more carrier sites were made available on the expanded surface, while the activity of the phosphate carrier was much less affected. On the other hand, the increased matrix volume resulted in an increased final uptake of phosphate. Osmotic alterations of the medium thus strongly affect the transport mechanisms. This clearly shows that not only the energy state of brown adipose tissue mitochondria [2,3] but also osmotic conditions may control the uptake of ions and metabolites by these mitochondria. Brown adipose tissue mitochondria thus appear well suited as a model system for studying such mechanisms, due to their greater permeability to ions than most other mitochondria.

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