SUBSTRATE REGULATION OF THE PYRUVATE-TRANSPORTING SYSTEM IN RAT LIVER MITOCHONDRIA

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1. Introduction

Work from this and other laboratories has demonstrated that the inner mitochondrial membrane contains a specific system for pyruvate transport [1–6]. The pyruvate-transporting system mediates an exchange diffusion of pyruvate with hydroxyl ions (or pyruvate–H^+ symport) or with other monocarboxylates [2,4,5] and exhibits saturation kinetics, substrate specificity and sensitivity to thiol reagents [2] and to α-cyanocinnamate [3].

We have recently shown that pyruvate uptake by mitochondria is increased several-fold by preincubation of mitochondria with 2-oxobutyrate [7]. This paper presents a study of the effect of various monocarboxylates and other anionic species on the activity of the pyruvate-transporting system.

2. Methods

Rat liver mitochondria were prepared as described by Myers and Slater [8]. 0.25 M sucrose was used for homogenization and washing. The initial rate of pyruvate uptake by rat liver mitochondria was measured by the centrifugation filtration technique as follows (see also [2,4]). Mitochondria were preincubated in a reaction mixture containing: 150 mM sucrose, 30 mM Tris-HCl, 1 mM $MgCl_2$, 0.5 mM EDTA, 1 mM arsenite, 10 μg/ml oligomycin, 1.4 μg/ml rotenone, 0.34 μg/ml antimycin. After preincubation, mitochondria were layered on the top of a second incubation layer at 4°C and then spun down through this layer by rapid centrifugation. $HClO_4$ was immediately added to the mitochondrial pellet. The second incubation layer was of the same composition of the preincubation mixture and in addition contained [^{14}C]pyruvate at concentrations shown in the tables and figures. Pyruvate was measured in $HClO_4$ extracts of the mitochondrial pellet and in the supernatant. The substrate content of the matrix space was calculated by correcting the amount in the mitochondrial extract with that in the sucrose permeable space plus adherent supernatant. This was determined with [^{14}C]sucrose. The mitochondrial level of pyruvate was determined either using [U-^{14}C] pyruvate or enzymatically [9]. Protein was determined by the usual biuret method.

3. Results

Table 1 illustrates the effect of preincubation of mitochondria with different anionic substrates on the initial rate of pyruvate uptake by rat liver mitochondria. Among the oxomonocarboxylates tested (Expt. 1) pyruvate and 2-oxobutyrate caused a marked stimulation of pyruvate uptake by mitochondria. Significant stimulation was also obtained with hydroxypyruvate, acetoacetate and 2-oxovalerate. Glyoxylate was without effect whereas 2-oxocaproate and phenylpyruvate had some inhibitory effect on pyruvate uptake. In the Expt.2 the effect of β-hydroxybutyrate, L-lactate, P_i, malate, citrate, carnitine and palmitoyl-carnitine on pyruvate uptake was tested. As can be noted, β-hydroxybutyrate and L-lactate produced only a slight stimulation of pyruvate uptake whereas phosphate, malate,

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Table 1
Effect of different anionic species on the initial rate of pyruvate uptake by rat liver mitochondria

	Additions	Pyruvate uptake (nmoles)
Expt.1	None	3.53 ± 0.09
	Glyoxylate	3.48 ± 0.08
	Pyruvate	11.66 ± 0.23
	2-Oxobutyrate	10.46 ± 0.11
	2-Oxovalerate	4.97 ± 0.04
	2-Oxocaproate	2.21 ± 0.04
	Acetoacetate	5.98 ± 0.12
	Hydroxypyruvate	6.68 ± 0.21
	Phenylpyruvate	2.18 ± 0.02
Expt.2	None	3.54 ± 0.06
	L-Lactate	3.98 ± 0.09
	3-Hydroxybutyrate	4.36 ± 0.11
	Phosphate	3.32 ± 0.24
	Malate	3.38 ± 0.14
	Citrate	1.34 ± 0.02
	Carnitine	3.51 ± 0.11
	Palmitoylcarnitine	3.48 ± 0.15

Mitochondria (8.1 mg protein expt.1 and 8.3 mg expt.2) were preincubated in a sucrose medium as described under methods. Final pH 7.2. Temperature 20°C. After 3 min mitochondria were centrifuged through a second incubation layer containing [^{14}C]pyruvate (100 μM). The various anions (2 mM, except palmytoyl carnitine 0.5 mM) were added in the preincubation medium. All results are expressed as the means ± S.E.M. of four separate observations.

carnitine and palmitoyl-carnitine had no appreciable effect. A marked inhibition was obtained with citrate. The lack of stimulation of pyruvate uptake by preincubation of mitochondria with malate and palmitoylcarnitine is at variance with the results of Mowbray [6].

It has been reported that some halogenated carboxylic acids and oxamate exchange with mitochondrial pyruvate [5]. Table 2 shows the effect of acetate and acetate and propionate derivatives as well as oxamate, added in the preincubation phase, on the initial rate of pyruvate uptake by mitochondria. Monochloroacetate, monofluoroacetate, cyanoacetate and oxamate produced a strong stimulation of pyruvate uptake. Significant stimulation was obtained with dichloroacetate and 2-chloropropionate, whereas 3-chloropropionate and 2-2-dichloropropionate were slightly effective in this respect. On the contrary

Table 2
Effect of some acetate and propionate derivatives and oxamate on the initial rate of pyruvate uptake by rat liver mitochondria

Additions	Pyruvate uptake (nmoles)
None	3.58 ± 0.11
Acetate	3.50 ± 0.08
Monochloroacetate	11.04 ± 0.31
Dichloroacetate	6.56 ± 0.18
Trichloroacetate	3.53 ± 0.12
Monofluoroacetate	9.83 ± 0.31
2-Chloropropionate	7.47 ± 0.22
3-Chloropropionate	4.75 ± 0.11
2-2-Dichloropropionate	4.33 ± 0.08
Cyanoacetate	10.41 ± 0.31
Oxamate	11.41 ± 0.51

Experimental conditions as in table 1. Mitochondrial protein 8.6 mg. The various monocarboxylates were added in the preincubation medium at the concentration of 2 mM. [^{14}C]Pyruvate (100 μM) was present in the second incubation layer. For other experimental details see under Methods. All results are expressed as the means ± S.E.M. of four separate observations.

acetate and trichloroacetate did not influence at all pyruvate uptake.

Fig.1 illustrates the concentration dependence of stimulation of pyruvate uptake by pyruvate and 2-oxobutyrate preloaded mitochondria. Maximal stimulation of pyruvate uptake was reached at concentrations of pyruvate and 2-oxobutyrate of about 2 and 4 mM respectively. Half maximal effect laid in the range of 0.4–0.5 mM for pyruvate and 0.8–0.9 mM for 2-oxobutyrate. It should be noted that the effects shown in tables 1 and 2, and fig.1, relative to the stimulation or inhibition of pyruvate uptake by different anions, were observed using pyruvate at a nearly physiological concentration, i.e. 100 μM [10].

In order to gain further insight into the mechanism of stimulation of pyruvate uptake brought about by preincubation of mitochondria with monocarboxylates, kinetic studies were carried out. The experiment of fig.2(A) illustrates the kinetics of pyruvate uptake by normal and pyruvate preloaded mitochondria. Preincubation of mitochondria with pyruvate caused a marked decrease of the Km of pyruvate for its transporting system but had no effect on the V_{max}.

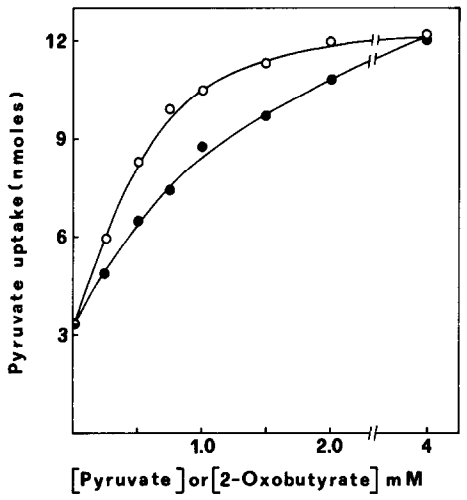

Fig.1. Effect of the preincubation of mitochondria with various concentrations of pyruvate or 2-oxobutyrate on the initial rate of [^{14}C]pyruvate uptake. Mitochondria (7.8 mg protein) were preincubated in the sucrose medium as described under Methods. After 3 min mitochondria were centrifuged through a second incubation layer containing the same components as the preincubation medium and in addition [^{14}C] pyruvate (100 μ). Unlabelled pyruvate (○——○) or 2-oxobutyrate (●——●), at the concentrations shown in the figure, were added in the preincubation phase. For other experimental details see under Methods.

Similar results were obtained using 2-oxobutyrate instead of pyruvate in the preincubation phase (fig.2(B)).

4. Discussion

Previous investigations from this and other laboratories have shown that the pyruvate transporting system of rat liver mitochondria mediates an exchange diffusion of pyruvate with a variety of oxomonocarboxylates [4] and halogenated monocarboxylates [5]. The present study shows that the activity of the pyruvate translocator, measured as initial rate of [^{14}C]pyruvate uptake by mitochondria, is modified by pre-exposure of mitochondria to these monocarboxylates. In this respect pyruvate and other oxomonocarboxylates (see table 1) and several halogenated monocarboxylate substrates (see table2) stimulate, whilst others inhibit or are uneffective.

The enhancement of the rate of pyruvate uptake, caused by preincubation of mitochondria with substrates of the pyruvate translocator could in principle be due to an increased level of intramitochondrial counteranions or to an activation of the translocator. The second possibility is supported by the observa-

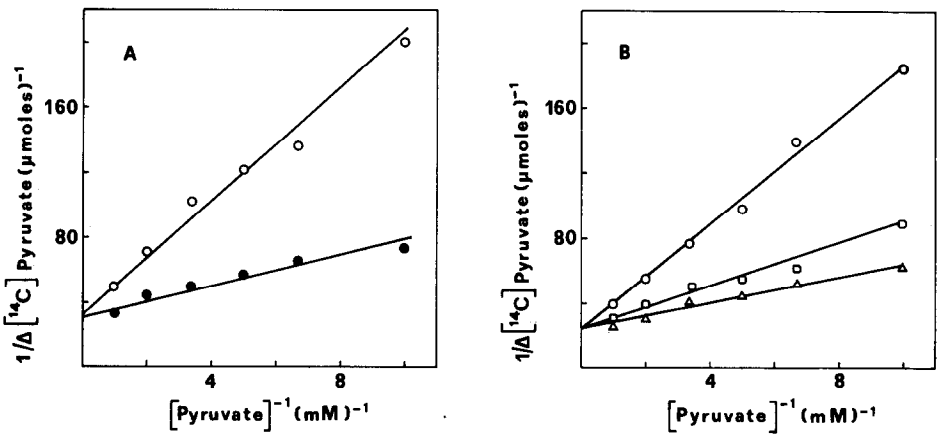

Fig.2. Double reciprocal plots of the effect of the preincubation of mitochondria with pyruvate or 2-oxobutyrate on the initial rate of [^{14}C]pyruvate uptake. Mitochondria (8.0 mg protein expt. A and 8.8 mg expt. B) were preincubated in the sucrose medium described under Methods. After 3 min mitochondria were centrifuged through a second incubation layer containing the same components as the preincubation medium and in addition [^{14}C]pyruvate at the concentrations indicated in the figure. Symbols: (○——○), control; (●——●), unlabelled pyruvate (0.5 mM) added in the preincubation phase; (□——□), 2-oxobutyrate (1 mM) and (△——△) 2-oxobutyrate (2 mM) added in the preincubation phase.

tion that stimulation of pyruvate uptake is characterized by a decrease of the K_M of pyruvate for the translocator with no change in the $V_{max.}$ Consistent with this is also the fact that preincubation of mitochondria with 2-oxocaproate or phenylpyruvate, which act as pyruvate counteranions [4,5], cause inhibition of pyruvate uptake.

The present study indicates that the activity of the pyruvate translocator can also be regulated by citrate. In fact it is shown that preincubation of mitochondria with citrate, but not with malate and P_i (see table 1), causes a marked inhibition of pyruvate uptake. Citrate uptake by mitochondria induces a decrease of the transmembrane ΔpH [10]. On the other hand pyruvate distributes across the mitochondrial membrane according to the transmembrane pH difference [2]. However the lack of inhibition of pyruvate uptake by P_i, which, like citrate decreases the transmembrane ΔpH (11,2), indicates that the inhibition by citrate is not merely due to decrease of transmembrane ΔpH.

As a possible explanation for the above reported effects of inhibition or stimulation of pyruvate uptake by mitochondria, following preincubation with different anionic substrates, it is suggested that the pre-exposure of mitochondria to these substrates leads to a modification of the structure of the transporting system with resulting change in its activity.

The fact that the activity of the pyruvate transporting system in mitochondria is differently affected by various anionic substrates might be of considerable physiological importance. Interesting enough most of the substrates which affect pyruvate translocation in mitochondria, such as pyruvate itself, 2-oxobutyrate, several halogenated carboxylic acids and citrate have been reported as strong effectors of pyruvate dehydrogenase [12–16]. This suggests a close relationship between pyruvate translocator and pyruvate dehydrogenase.

Acknowledgement

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