HORMONAL REGULATION OF LIVER MITOCHONDRIAL PYRUVATE CARRIER IN RELATION TO GLUCONEOGENESIS AND LIPOGENESIS

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1. Introduction

The existence of a specific carrier for pyruvate in the inner membrane of rat liver mitochondria is now well established [1–4]. Based on indirect evidence it has been suggested that glucagon, adrenaline or dibutyryl cyclic AMP act on liver cells to stimulate the operation of the mitochondrial pyruvate carrier and that this effect is involved in the increase in the rate of gluconeogenesis which follows exposure to those agents [5,6]. In an earlier paper [7] we obtained direct evidence for this suggestion. Glucagon was injected into rats and 30 min later the livers were removed and mitochondria were prepared. We observed increased maximum initial rates of pyruvate transport into these mitochondria compared to mitochondria from control rat livers. The increased mobility of the pyruvate carrier was not due to preaccumulation of counter ions – pyruvate, malate or palmitoyl carnitine within the mitochondrial matrix – but an increased hydroxyl anion concentration therein was not excluded. We now report experiments of similar design in which injection of adrenaline or production of diabetes by streptozotocin injection also led to increases in initial rates of mitochondrial pyruvate transport. We have also measured various other parameters of mitochondrial behaviour which may be relevant to changes in mobility of the pyruvate carrier.

2. Methods and materials

Methods and materials were as in our earlier papers [4,7] with the following additions. Published methods were used to determine mitochondrial matrix space [8], membrane potential [9] and pH gradient [9]. Pyruvate dehydrogenase was measured in extracts of freeze-clamped liver and mitochondria by the method of Coore et al., [10]. Extraction and assay of 'initial' and 'total' pyruvate dehydrogenase in freeze-clamped tissue were earlier described [11]. 'Initial' activity of mitochondrial pyruvate dehydrogenase was measured in mitochondria following 5 min incubation in the medium used for measurements of pyruvate decarboxylation by rapid (20 *s*) centrifugation, freezing of the pellet in liquid nitrogen and freeze-thawing three times in 500 μl of 50 mM Tris–HCl, 5 mM EDTA, 1 mM dithiothreitol, pH 7.0 and 2 μl of rat serum. 'Total' activity of pyruvate dehydrogenase in the mitochondria was assumed to be achieved when 50 μM 2-4-dinitrophenol was included in the incubation medium cf. [12] and the mitochondria were extracted as detailed above.

Adrenaline was obtained from British Drug Houses, Poole, Dorset, UK; glucagon was from Eli Lilly, Basingstoke, UK and propranolol from I.C.I. Macclesfield, Cheshire, UK. Streptozotocin was kindly donated by Upjohn Ltd., Kalamazoo, Mich., USA and soluble insulin was from Burroughs Wellcome & Co., London, N.W.1., UK. All radiochemicals were from Amersham Radiochemicals, Amersham, UK.

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3. Results and discussion

3.1. *Effects of adrenaline injection on rates of pyruvate uptake and decarboxylation by isolated mitochondria*

Table 1 shows that 30 min exposure of liver in vivo to a high dose of adrenaline was sufficient to induce persistent significant effects on both parameters. Unlike the earlier experience with glucagon injections [7] we find a discrepancy in magnitude of stimulation of decarboxylation (67%) with that of initial rates of uptake (23%), when both are expressed on a protein basis.

The effect of adrenaline injection on pyruvate decarboxylation by isolated mitochondria was not due to an altered degree of phosphorylation of mitochondrial pyruvate dehydrogenase. We measured the 'initial' activity (expressive of the partially dephosphorylated enzyme) and the 'total' pyruvate dehydrogenase activity (expressive of the fully dephosphorylated enzyme) in mitochondria incubated in conditions similar to those used in the experiment 1 of table 1. In mitochondria from both control and adrenaline treated animals 'total' pyruvate dehydrogenase activity was about 12 mU/mg protein and the ratio of 'initial' to 'total' enzyme activity was about 70% cf. [12,13].

3.2. *Comparison of various parameters of mitochondrial behaviour in mitochondria isolated from adrenaline or glucagon treated rats*

Table 2 shows that both treatments result in increased O_2 uptakes by isolated mitochondria incubated in state 3 conditions when pyruvate + malate was the substrate but not when ascorbate + TMPD was the substrate. Yamazaki [14] has reported this phenomenon in respect of glucagon treatment. It is evident that in both instances the mitochondria were well coupled and there was probably no effect of hormonal pretreatment on the capacity of the mitochondrial electron-transport chain to transmit electrons, at least beyond the point of interaction with ascorbate + TMPD. Yamazaki concluded that the effect of glucagon treatment was to promote the entry of carrier-transported metabolites into the mitochondria (ascorbate + TMPD reacted with cytochrome *c* on the outer face of the inner mitochondrial membrane). Clearly, our results for pyruvate uptake are consistent with that suggestion.

Table 1
Rates of pyruvate uptake and decarboxylation by mitochondria isolated from control or adrenaline treated rats

Experiment	Treatment of rat	Rate of $^{14}CO_2$ production from [1-^{14}C]pyruvate by mitochondria (nmol/min/mg protein)	Initial rate of [2-^{14}C]pyruvate uptake (nmol/min/mg protein)	Plasma glucose at time when liver removed (mM)
1	Control: 0.01 M HCl injection	3.92 ± 0.45 (9)	–	8.59 ± 0.30 (9)
	Adrenaline (2.5 mg/kg s.c.) in 0.01 M HCl injected	6.57 ± 0.75 (9)[a] [67.5 ± 9.8%]	–	19.74 ± 0.10 (9)[b]
2	Control: 0.01 M HCl injection	–	26.8 ± 0.3 (10)	8.20 ± 0.21 (10)
	Adrenaline (2.5 mg/kg s.c.) in 0.01 M HCl injected	–	33.0 ± 1.8 (10)[a] [23.1 ± 3.1%]	21.74 ± 0.76 (10)[b]

Blood was sampled and livers removed 30 min after injections. Details of methods were described earlier [4,7]. Mitochondria were incubated in media containing 1.4 mM pyruvate for both experiments but in experiment 1 the incubation temperature was 30°C whereas it was 27°C for experiment 2. Data (unbracketed) are means ± S.E. Numbers of mitochondrial preparations or rats are given in round brackets. Percentage effects compared to controls, calculated on a paired basis for each experimental day, are given within square brackets.

[a] $p < 0.01$;

[b] $p < 0.001$ comparing means of data from treated and controls rats.

Table 2

O_2 Uptake by mitochondria isolated from livers of control rats or rats injected with glucagon or adrenaline

Expt	Treatment of rat	Substrate in incubation medium for mitochondria	O_2 Uptake (ng atom 0/min/mg protein)		Respiratory control ratios (state 3/ state 4)	ADP/O
			State 3	State 4		
1	Control: solvent injection	1 mM pyruvate + 0.5 mM malate	38.40 ± 3.54	11.08 ± 0.62	4.05 ± 0.21	2.78 ± 0.06
	Adrenaline (2.5 mg/kg s.c.)		48.68 ± 3.01[a]	13.49 ± 0.91	3.97 ± 0.14	2.90 ± 0.07
2	Control: solvent injection	3 mM ascorbate + 0.05 mM TMPD	52.17 ± 1.34	29.33 ± 1.52	1.81 ± 0.09	1.01 ± 0.05
	Adrenaline (2.5 mg/kg s.c.)		53.80 ± 2.13	28.62 ± 0.43	1.78 ± 0.06	0.99 ± 0.18
3	Control: solvent injection	1mM pyruvate + 0.5 mM malate	36.19 ±2.31	10.22 ± 0.55	3.85 ± 0.20	2.71 ± 0.07
	Glucagon (300 μg/kg i.p.)		50.98 ± 3.43[b]	10.75 ± 0.71	4.74 ± 0.18[b]	2.75 ± 0.07
4	Control: solvent injection	3 mM ascorbate + 0.05 mM TMPD	55.62 ± 1.47	27.45 ± 1.24	1.99 ± 0.16	1.06 ± 0.05
	Glucagon (300 μg/kg i.p.)		57.38 ± 3.23	30.25 ± 1.59	2.03 ± 0.09	0.97 ± 0.02

Livers were removed for mitochondrial preparation 30 min after injections. O_2 Uptake was measured using about 4 mg mitochondrial protein in 3 ml at 30°C. There were 6 different mitochondrial preparations for each datum in all experiments.

[a] $p < 0.05$;

[b] $p < 0.01$, comparing means of data from treated and control animals.

Table 3
Matrix volumes, membrane potentials and pH gradients of mitochondria isolated from control or hormone injected rats

Experiment	Treatment of rat	Matrix volume (μl/ mg protein)	pH gradient (outside–inside)	Membrane potential (mV)
1	Control; injection of solvent	0.49 ± 0.04 (18)	−1.22 ± 0.04 (8)	139.6 ± 1.7 (10)
	Glucagon (300 μg/kg i.p.)	0.52 ± 0.09 (8)	−1.42 ± 0.06 (7)[a]	140.8 ± 3.2 (4)
2	Control; injection of solvent	0.49 ± 0.04 (18)	−1.23 ± 0.04 (8)	139.6 ± 1.7 (10)
	Adrenaline (2.5 μg/kg s.c.)	0.31 ± 0.03 (8)[a]	−1.29 ± 0.04 (8)	144.3 ± 2.9 (6)

Livers were removed for mitochondrial preparation 30 min after injections. Mitochondria were incubated for 2 min with 3 mM ascorbate + 0.05 TMPD at 30°C along with appropriate radioactive substances prior to separation from medium by centrifugation (20 s). The numbers of mitochondrial preparations are given in parentheses.
[a] $p < 0.01$ comparing means of data from treated and control rats.

Since all the carrier-transported metabolites (including pyruvate) studied by Yamazaki may be regarded as exchanging directly or indirectly with intra-mitochondrial hydroxyl-anions [15] we have measured the mitochondrial trans-membrane pH gradient and associated parameters. Table 3 shows that under conditions of stable energy supply (ascorbate + TMPD in the incubation medium) mitochondria from livers of glucagon treated rats showed a significantly elevated pH gradient (compared to mitochondria from control animals) which itself would be expected to encourage entry of anionic substrates. We can only speculate as to the reason for the elevated pH gradient but one possibility is that glucagon pretreatment has resulted in a decreased back-leakage of extruded protons or a decreased proton/K^+ exchange. In the case of adrenaline treatment a different mechanism is involved. There is now no increase of pH gradient but a decrease of matrix volume. We may note that if the rate of pyruvate uptake is expressed in terms of matrix volume instead of mitochondrial protein, mitochondria from adrenaline treated rats show a 95% stimulation of the rate of pyruvate uptake. The mechanism of this adrenaline effect on matrix volume is enigmatic. Dolgov [16] reported that adrenaline infusion of dog hearts resulted in decreased K^+ content of the isolated mitochondria. Matrix volume was not measured in those experiments. If, as in our experiments, membrane potential and therefore K^+ ion distribution was unaffected, the lower K^+ content noted by Dolgov may have simply been a result of a smaller matrix volume with a normal K^+ concentration. It is conceivable that following adrenaline treatment in vivo there was a transient fall in the mitochondrial membrane potential with consequent extrusion of K^+ and shrinkage of the mitochondria to re-establish a new equilibrium between proton extrusion and back flow, membrane potential, K^+ gradients and matrix volume. In any case it is not obvious why anion transport should be facilitated by such changes.

3.3. *Effects on mitchondrial behaviour of streptozotocin diabetes in donor rats*

Table 4 shows that induction of diabetes in the rats from which livers were obtained resulted in a time-dependent increase in the initial rate of pyruvate uptake by isolated mitochondria. Reminiscent of the case of adrenaline treatment, there was a small fall in the matrix volume of the mitochondria which did not attain the 5% level of significance. However, when the rates of pyruvate uptake of each preparation were expressed per μl matrix space for that preparation instead of per mg protein, the magnitude of stimulation due to 48 h streptozotocin diabetes in donor rats increased from below 50% to nearly 100%.

Because of these latter results we investigated the effects on initial rates of pyruvate uptake by mitochondria of injection of soluble insulin (10 I.U.) into donor animals 30 and 60 min before sacrifice. Although this treatment was adequate to triple the proportion of 'initial' to 'total' pyruvate dehydrogenase in freeze-clamped liver cf. [17] there was no effect on initial rates of mitochondrial pyruvate uptake with or without preaccumulation of a counter-ion, malate, within the matrix.

Table 4
Initial rates of pyruvate transport in mitochondria isolated from streptozotocin-treated diabetic rats

Treatment of rat before removal of liver	Initial rate of [2-^{14}C]pyruvate uptake expressed as		Mitochondrial matrix space (μl/mg)	Plasma glucose (mM)
	nmol/min/mg protein	nmol/min/μl of matrix space		
Control: Buffer injected 3 h, 24 h or 48 h before	32.8 ± 1.3 (11)	69.7 ± 6.3 (10)	0.51 ± 0.05 (10)	8.11 ± 0.23 (11)
Streptozotocin injected 3 h before	38.1 ± 6.2 (3)	–	–	17.31 ± 5.90 (3)
Streptozotocin injected 24 h before	39.4 ± 1.5 (6)[b]	102.7 ± 8.9 (5)[b]	0.37 ± 0.04 (5)	21.03 ± 1.94 (6)[c]
Streptozotocin injected 48 h before	46.3 ± 2.1 (6)[c]	137.6 ± 25.3 (6)[a]	0.40 ± 0.07 (6)	24.33 ± 1.08 (6)[c]

Streptozotocin (65 mg/kg i.p.) in 10 mM acetate buffer, pH 4.5 or acetate buffer alone was injected into rats and after the stated times the livers were removed and mitochondria prepared. Details of methods were given earlier [4] and in the Methods and materials section of this paper. Data are means ± S.E. with numbers of different mitochondrial preparations or rats given in brackets.
[a] $p < 0.05$;
[b] $p < 0.01$;
[c] $p < 0.001$ comparing means of data from treated and control rats.

3.4. *Relevance of hormonal effects on mitochondrial pyruvate transport to regulation of gluconeogenesis and lipogenesis*

It is generally accepted that acute hormonal effects on gluconeogenesis involve a metabolic step lying between pyruvate and phospho-enol pyruvate [18]. No sufficiently rapid effects on the relevant enzymes have been detected except perhaps for pyruvate kinase [19] and this has led to the view that pyruvate transport into the mitochondria is the site of hormonal action on the gluconeogenic process [5,6]. Results of this and our previous paper [7] give direct support for this hypothesis. It is also probable that malate exchanges with pyruvate on the pyruvate carrier [7] and this would be a neat mechanism to ensure efflux of adequate oxaloacetate and reducing power required for the further stages of gluconeogenesis.

The way in which glucagon and adrenaline treatments alter the mobility of the pyruvate carrier in the inner mitochondrial membrane requires further investigation. Our results indicate separate mechanisms and this is consistent with other work suggesting that the effects of the two hormones on gluconeogensis are additive [20]. Cyclic AMP may well mediate the glucagon effect [6] but probably not that of adrenaline since propranolol (10 mg/kg i.p.) injected 15 min before adrenaline did not disturb the effect of the hormone on the rate of pyruvate decarboxylation by subsequently isolated mitochondria. This dose of propranolol is reported [21] adequate for complete blockage of the β receptor. However, experiments involving whole animals are subject to difficulties of interpretation due to secondary and tertiary effects of any treatment. As noted earlier, in vitro experiments are required to clarify the mechanism of the hormonal effects. This reservation applies also to experiments with diabetic rats. It may be that in such rats insulin deficiency is not the factor directly relevant to control of the mitochondrial pyruvate carrier but rather the associated increase in plasma glucagon concentration [22].

It has been argued [17] that pyruvate dehydrogenase of liver is rate limiting for synthesis of fatty acids from pyruvate. By diminishing the phosphorylation of pyruvate dehydrogenase, insulin would be expected to promote synthesis of fatty acids from pyruvate in liver as well as in adipose tissue. Our results suggest that the mechanism of insulin action on liver pyruvate dehydrogenase does not involve persistent alteration of the mobility of the mitochondrial pyruvate carrier.

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