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PERSISTENT CHANGES IN THE INITIAL RATE OF PYRUVATE TRANSPORT BY ISOLATED RAT LIVER MITOCHONDRIA AFTER PREINCUBATION WITH ADENINE NUCLEOTIDES AND CALCIUM IONS

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Summary

1. Preincubation of isolated rat-liver mitochondria in the presence of adenine nucleotides or Ca^{2+} results in definite and persistent changes in the initial rate of pyruvate transport.

2. These changes in the rate of pyruvate transport are accompanied by equally persistent changes in the opposite direction of the activity of pyruvate dehydrogenase (EC. 1.2.4.1).

3. Changes of the transmembrane pH gradient and of the membrane potential, brought about by the pretreatments of the mitochondria, cannot account for the observed changes in the rate of pyruvate transport.

4. It is proposed that the pretreatment of the mitochondria directly modulates the activity of the mitochondrial pyruvate carrier. The possible regulatory role of such a modulation system is discussed.

Introduction

Pyruvate is the major precursor for hepatic de novo synthesis of glucose and fatty acids. The balance between gluconeogenesis and fatty acid synthesis from pyruvate critically depends on the control of mitochondrial pyruvate metabolism. Intramitochondrial pyruvate can either be oxidized to acetyl-coenzyme A or carboxylated to give oxaloacetate. In both cases transfer of cytosolic

Abbreviations: FCCP, carbonyl cyanide *p*-trifluoromethoxyphenylhydrazone; DMO, 5,5-dimethyloxazolidine-2,4-dione; EGTA, ethylene glycol bis(β-aminoethyl ether)-*N*,*N*′-tetraacetate.

pyruvate to the mitochondrial matrix space is required [1—3]. To serve this purpose a specific carrier mediating pyruvate-hydroxyl exchange (or pyruvate-proton symport) is present in the inner membrane of rat-liver mitochondria [4—8]. The central role of pyruvate in intermediary metabolism in conjunction with the existence of this specific carrier opens up the possibility that the rate of pyruvate transport is a regulatory factor in mitochondrial pyruvate metabolism

It has been shown repeatedly that in isolated mitochondria the activity of the pyruvate carrier can be modulated by varying the composition of the assay medium. Titheradge and Coore [9] reported that the activity of the carrier in mitochondria isolated from glucagon-treated rats was higher than in those from control animals. These observations prompted us to investigate whether the rate of pyruvate transport can be modified by preincubation of isolated rat-liver mitochondria under suitable conditions. A preliminary report of this work has appeared in abstract form [10].

Methods and Materials

Liver mitochondria from 24-h starved female Wistar rats were isolated as described previously [11]. Preincubations were performed in 150-ml Erlenmayer flasks in a shaking water bath (80 oscillations/min) at 27°C. The standard preincubation medium contained 110 mM KCl, 30 mM sucrose, 7.5 μM rotenone and 20 mM Tris-neutralized *N*-tris(hydroxymethyl)methyl-2-aminoethanesulfonate (pH 7.50). Preincubations were started by addition of mitochondria (final concentration of mitochondrial protein [12] about 1 mg/ml). After 1 min the preincubation medium was supplemented with additional components as mentioned in the legends. Final preincubation volume, 25 ml. At $t = 8$ min preincubations were stopped by adding 6 volumes of ice-cold buffer (250 mM sucrose and 5 mM Tris-HCl, pH 7.50, containing 1 mg of charcoal-defatted bovine serum albumin per ml) followed by centrifugation at $10\,000 \times g$ for 5 min. After careful and complete removal of the supernatant (in order to avoid the transfer of components of the preincubation medium to the incubations), mitochondrial pellets were resuspended in the same buffer (bovine serum albumin omitted) at 0°C to give about 15 mg of protein per ml. All assays were performed in triplicate and were started 20 min after the end of the preincubation.

Pyruvate transport was measured either in a high-KCl incubation medium [11] or in a low-KCl incubation medium, the latter being composed of 225 mM sucrose, 0.5 mM KCl, 20 mM Tris-neutralized 3-(*N*-morpholino)propanesulfonate, 20 μM rotenone, 1.4 μM antimycin, 24 μM oligomycin and [6,6′-^{3}H]sucrose (1.5 μCi/ml). The pH of both media was 6.80. [3-^{14}C]Pyruvate (0.15 Ci/mol) was added 2 min after the mitochondria (approx. 1 mg of protein). Unless indicated otherwise, the assay temperature was 27°C and the final [3-^{14}C]pyruvate concentration 1 mM. Further details of the transport assay were exactly as described before [11]. Values shown for pyruvate transport are corrected for pyruvate adsorption which was measured as pyruvate accumulation in the presence of 1.2 μM FCCP [11].

The components of the proton electrochemical gradient were determined

with established techniques [13—17] which were adapted to conditions matching those of the pyruvate-transport assay as closely as possible. The pH gradient was measured in both incubation media. Mitochondria (1—2 mg of protein) were incubated at 27°C in 950 μl of incubation medium. After 30 s the medium was supplemented with either (a) [^{14}C]sucrose (0.1 μCi/ml), $^{3}H_2O$ (1.0 μCi/ml), 70 μM DMO, 80 μM bromoacetate and 50 μM RbCl, or (b) 70 μM [^{14}C]DMO (3.7 Ci/mol), 80 μM bromo[2-^{3}H]acetate (25.0 Ci/mol) and 50 μM RbCl with or without 1.2 μM FCCP. Final incubation volume, 1.0 ml. At t = 2 min 30 s, samples (0.75 ml) were taken and the mitochondria were separated immediately by centrifugation through a silicone layer [11]. Radioactivity of top and bottom layers was measured in a Packard Tri-Carb 3255 spectrometer using a xylene-based scintillation liquid [18]. The matrix space, calculated from the data of incubations (a), was corrected for the volume of non-solvent water, taken to be 0.3 μl/mg protein [19,20]. Extramitochondrial anion concentrations were calculated directly from the number of dpm in the top layers of FCCP-free incubations (b). Radioactivity in the bottom layers from FCCP-free incubations (b) was corrected for extramatrix water [21] and for non-specific binding of the pH markers [17,22] using the [^{14}C]sucrose space (a) and the number of dpm present in bottom layers of FCCP-supplemented samples (b), respectively. The corrected matrix space was then used to determine the intramitochondrial concentration of [^{14}C]DMO and bromo-[2-^{3}H]acetate, followed by calculation of ΔpH applying the formula for pH_{in} given by Crompton and Heid [23]. Almost identical values for ΔpH were obtained with either [^{14}C]DMO or bromo[2-^{3}H]acetate.

The membrane potential (ΔE) was only measured in the low-KCl medium. At 30 s after addition of the mitochondria the medium was supplemented with 50 μM ^{86}RbCl (2.0 Ci/mol), 0.5 μM valinomycin, 70 μM DMO and 80 μM bromoacetate, and incubations were terminated as described above for the ΔpH assay. After correction of $[^{86}Rb^{+}]_{in}$ for extramatrix as well as non-solvent matrix water, values for ΔE were estimated from the transmembrane $^{86}Rb^{+}$ distribution according to Nicholls [16].

Pyruvate dehydrogenase activity (EC 1.2.4.1) was assayed by measuring the formation of $^{14}CO_2$ from [1-^{14}C]pyruvate during 6 min at 27°C and pH 7.50. For this purpose samples of mitochondrial suspensions (1—2 mg of protein) were subjected to a rapid freezing procedure essentially as described by Taylor et al. [24]. Final concentrations in the freezing mixture (pH 7.50) were 7.5 mM KCl, 1 mM EGTA, 3.5 mM $MgCl_2$, 25 mM Tris-HCl, 25 mM potassium phosphate, 1 mM dithioerythritol, 10% (v/v) ethanol and 2% (v/v) normal rabbit serum. Further details of the assay of pyruvate dehydrogenase activity were exactly as indicated by Batenburg and Olson [25]. To obtain total pyruvate dehydrogenase activity, freshly isolated mitochondria were incubated for 10 min at 27°C in the presence of 1.5 μM FCCP and 10 mM $MgCl_2$ (cf. Refs. 24, 26 and 27) before the freezing procedure was started.

All radioactive chemicals were purchased from the Radiochemical Centre (Amersham) and radioactive pyruvate was purified before use [11]. Bongkrekic acid was kindly donated by Prof. W. Berends (Technical University, Delft). Bromoacetate was obtained from Aldrich; normal rabbit serum from Calbiochem; EGTA, RbCl and ruthenium red from Merck; bovine serum albumin

(fraction V), diadenosine pentaphosphate, dithioerythritol, DMO and hexokinase (type IV) from Sigma. Ruthenium red was purified according to Luft [28]. Other biochemicals and experimental details were as previously described [11].

Results

In order to investigate the possibility that persistent changes in the activity of the mitochondrial pyruvate carrier can be effected, preincubation conditions were explored that are known to affect the activities of the pyruvate dehydrogenase multienzyme complex [2,24—26,29—32] and/or of pyruvate carboxylase (EC 6.4.1.1) [33,34].

As shown in Table I, marked differences could be observed when comparing the initial rates of pyruvate transport in mitochondria preincubated under high- or low-energy conditions. After preincubation with ATP much higher transport rates are found than after preincubation with ADP or with an ATP-trapping system. In the experiments of Table I rotenone, antimycin, oligomycin and diadenosine pentaphosphate, an inhibitor [35] of adenylate kinase (EC 2.7.4.3), were present to keep levels of extra- and intramitochondrial adenine nucleotides as constant as possible during preincubation. Ruthenium red [36]

TABLE I

EFFECTS OF PREINCUBATION OF MITOCHONDRIA UNDER HIGH- AND LOW-ENERGY CONDITIONS

The standard preincubation medium was supplemented with 0.35 μM antimycin, 10 μM diadenosine pentaphosphate, 7 μM oligomycin, 1 μM ruthenium red and 1 mM EDTA. Further additions to the preincubation medium were: 3 mM ATP, 3 mM ADP, 2.5 mM $MgCl_2$, 0.5 mM AMP, 2 U/ml hexokinase (EC 2.7.1.1), 10 mM glucose. The data shown were obtained in the high-KCl incubation medium; data obtained in the low-KCl medium are shown in parentheses. The results of the first and second half are given as the mean ± S.E. of five and two mitochondrial preparations, respectively. Values of ΔpH were calculated from the [^{14}C]DMO distribution. Statistical significance, calculated by Student's *t*-test, is * $p < 0.01$, ** $p < 0.002$ when compared with results obtained after the corresponding preincubation under high-energy conditions. Total pyruvate dehydrogenase (PDH) activity was 29.8 ± 0.9 ($n = 5$) nmol/min per mg protein.

Parameters measured after preincubation	Additions to preincubation medium			
	ATP	ADP	ATP $MgCl_2$	AMP $MgCl_2$ glucose hexokinase
Initial rate of pyruvate transport (nmol/min per mg protein)	12.33 ± 0.37 (11.19 ± 0.23)	8.46 ± 0.12 * (6.90 ± 0.34) *	13.25 ± 0.90 (14.46 ± 0.59)	7.32 ± 0.06 * (6.80 ± 0.63) *
PDH activity (% of total)	24.5 ± 3.3	35.9 ± 2.2 **	30.1 ± 1.9	68.6 ± 2.5 *
Matrix space (μl/mg protein)	1.03 ± 0.05 (0.86 ± 0.09)	0.95 ± 0.01 (0.78 ± 0.01)	0.89 ± 0.02 (0.74 ± 0.06)	0.86 ± 0.05 (0.78 ± 0.03)
ΔpH	0.92 ± 0.05 (0.93 ± 0.01)	0.63 ± 0.02 ** (0.73 ± 0.01) *	1.42 ± 0.04 (0.93 ± 0.01)	0.53 ± 0.03 * (0.68 ± 0.03) *
ΔE (mV)	(107.9 ± 1.9)	(105.1 ± 1.3)	(112.1 ± 0.4)	(102.7 ± 0.7) **

was included to prevent mitochondrial uptake of exogenous Ca^{2+}.

The preincubation of the mitochondria also affects pyruvate dehydrogenase activity, which changes as anticipated. It should be noted that these changes are in the opposite direction to the ones observed in the initial rate of pyruvate transport. Variation of the mitochondrial energy charge primarily affects the activity of pyruvate dehydrogenase kinase and thereby changes the percentage of active (dephosphorylated) pyruvate dehydrogenase [2,31]. In agreement with earlier studies [24,26], however, the sensitivity of pyruvate dehydrogenase activity towards such variations in preincubation conditions (Table I) appears to be rather limited.

Table I further shows an increased pH gradient after preincubation in the presence of ATP as compared with preincubations in the presence of either ADP or an ATP-trapping system. Substantial changes in membrane potential are found only after preincubations with exogenous Mg^{2+} present.

In a second set of preincubation conditions the mitochondrial Ca^{2+} content was altered by addition of $CaCl_2$ or of the Ca^{2+}-chelating agent EGTA (Table II). Succinate was included in the medium in order to provide an energy source for the uptake of Ca^{2+}. Bongkrekate rather than atractylate was added to inhibit transmembrane movements of adenine nucleotides [37] during preincubation, because bongkrekate also protects the mitochondria against leakage of adenine nucleotides [38] and against Ca^{2+}-induced swelling [39]. Calcium was added in relatively small amounts (75—100 nmol per mg of mitochondrial protein) under 'limited loading' conditions, i.e., phosphate or permeant anions were not added and were present only in amounts endogenous to the mitochondria.

TABLE II

EFFECTS OF PREINCUBATION OF MITOCHONDRIA IN THE PRESENCE OF $CaCl_2$ OR EGTA

The standard preincubation medium was supplemented with 10 mM succinate, 1 mM $MgCl_2$ and 10 μM bongkrekate. Further additions were 75 μM $CaCl_2$ or 3 mM EGTA. Data obtained in the low-KCl incubation medium are in parentheses. The results of the first and second half are given as the mean ± S.E. of five and two mitochondrial preparations, respectively. Values of ΔpH were calculated from the [^{14}C]-DMO distribution. Statistical significance is * $p < 0.01$, ** $p < 0.002$ when compared with results obtained after preincubation with $CaCl_2$. Total pyruvate dehydrogenase (PDH) activity was 27.3 ± 0.5 (n = 5) nmol/min per mg protein.

Parameters measured after preincubation	Additions to preincubation medium	
	$CaCl_2$	EGTA
Initial rate of pyruvate transport (nmol/min per mg protein)	7.93 ± 0.61 (6.40 ± 0.17)	15.78 ± 0.77 * (16.91 ± 1.86) *
PDH activity (% of total)	43.1 ± 3.6	28.2 ± 2.1 *
Matrix space (μl/mg protein)	0.68 ± 0.02 (0.56 ± 0.03)	0.97 ± 0.03 * (0.78 ± 0.04) **
ΔpH	1.00 ± 0.02 (0.84 ± 0.03)	0.69 ± 0.02 * (0.56 ± 0.02) *
ΔE (mV)	(93.0 ± 2.3)	(118.3 ± 1.1) *

In comparison with preincubations in the presence of EGTA, a substantial and persistent decrease in the rate of pyruvate transport is produced by limited Ca^{2+} loading of the mitochondria during the preincubation period. The observed changes in pyruvate dehydrogenase activity are consistent with the concept that in the presence of Ca^{2+} the phosphorylation state of the enzyme is lowered due to an increased phosphatase activity [2,31]. Again, an inverse correlation is observed between the changes in the activities of pyruvate transport and of pyruvate dehydrogenase.

In experiments under limited Ca^{2+} loading conditions the membrane potential is reduced by Ca^{2+} uptake [15,40] with an accompanying increase in the pH gradient [14,41,42]. It is clear from Table II that these shifts in ΔpH and ΔE are rather persistent. For the overall protonmotive force one arrives at values of about 140—165 mV which are quite acceptable for non-respiring mitochondria (cf. Ref. 16).

Pyruvate transport by preincubated mitochondria was shown to obey apparent Michaelis-Menten kinetics (Fig. 1). Preincubation under high-energy conditions leads to a markedly higher V than under low-energy conditions (170—200%). Concomitantly, a small increase in the apparent K_m is observed (Fig. 1A). In Ca^{2+}-loaded mitochondria (Fig. 1B) V is substantially lower than in Ca^{2+}-depleted mitochondria (55—70%) whereas the K_m is increased (120—140%). In both cases the major effect is on V, i.e., on the activity of the carrier molecules.

Fig. 2 shows that the effects of preincubation with Ca^{2+} and EGTA (Table II) are gradually lost upon storage of the resuspended (see Methods) mitochondria at 0°C. After about 70 min the differences in the rate of pyruvate transport and in the activity of pyruvate dehydrogenase between Ca^{2+}-loaded and Ca^{2+}-depleted mitochondria are lost. This is probably caused by redistribution of Ca^{2+} between mitochondria and resuspension medium.

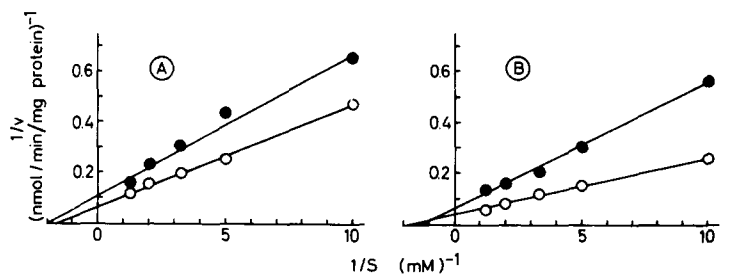

Fig. 1. Kinetics of pyruvate transport by preincubated mitochondria. In three separate experiments, mitochondria were preincubated exactly as described in Tables I and II. Data from one representative experiment are shown. The initial rate of pyruvate transport was determined in the high-KCl incubation medium over a range of pyruvate concentrations from 0.1 to 0.8 mM. Plots are derived by least-squares regression analysis. Open circles: preincubation with 3 mM ATP and 2.5 mM $MgCl_2$ (A), or 3 mM EGTA (B). Closed circles: preincubation with 0.5 mM AMP, 2.5 mM $MgCl_2$, 10 mM glucose and 2 U/ml hexokinase (A), or 75 μM $CaCl_2$ (B). V was about 15 and 27 nmol/min per mg protein after preincubation with ATP + $MgCl_2$ and EGTA, respectively, the apparent K_m being roughly 0.6 mM in both cases.

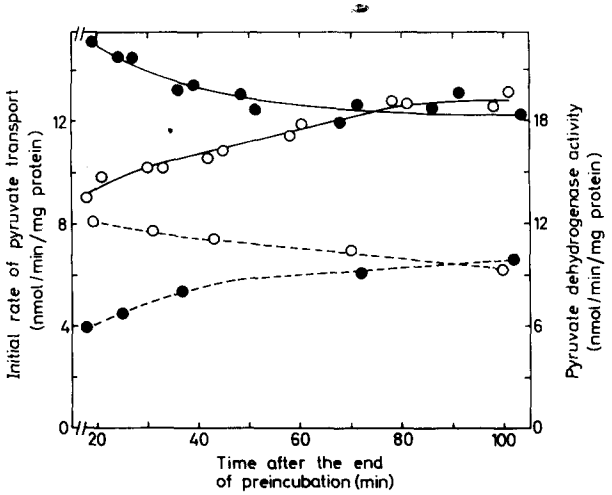

Fig. 2. Loss of preincubation effects on the rate of pyruvate transport and pyruvate dehydrogenase activity. Mitochondria were preincubated with $CaCl_2$ (open circles) or EGTA (closed circles), exactly as described in Table II. Pyruvate transport was assayed in the high-KCl incubation medium. Pyruvate concentration, 0.2 mM. The data shown represent one of the three experiments with similar results. Initial rate of pyruvate transport, solid lines; pyruvate dehydrogenase activity, dotted lines.

Discussion

The experiments reported in this paper clearly establish changes in the pyruvate-transporting system after preincubation of rat-liver mitochondria with adenine nucleotides (Table I) or Ca^{2+} (Table II). As far as we know, these data offer the first example of persistent modulation of the pyruvate carrier in isolated mitochondria. Other authors who employed a preincubation step [43,44] measured the rate of pyruvate transport directly afterwards in the preincubation medium.

Some objections to the validity of our experimental procedures could be raised. Firstly, linearity of pyruvate transport during the assay period is essential to our argument that the rate of pyruvate transport is changed by a particular preincubation. Our usual assay temperature is 27°C and in our hands a 15-s incubation is the shortest reproducible assay period (cf. Ref. 4) when pyruvate transport is quenched by rapid centrifugation [11]. According to Titheradge and Coore [45] the rate of pyruvate uptake at 27°C and 1.4 mM pyruvate remains linear for at least 15 s. The same is likely to occur under our assay conditions (data not shown).

Secondly, carry-over of components of the preincubation media into the incubations, though unlikely in view of the careful removal of preincubation media, may perturb the assay of mitochondrial pyruvate transport. It could be demonstrated, however, that these compounds, even if they are present in the assay mixture at concentrations used during preincubation, cannot account for the different rates of pyruvate transport shown in Tables I and II (data not shown).

Thirdly, intramitochondrial metabolism of pyruvate should be repressed as much as possible. An appreciable oxidation of pyruvate would interfere with the correct measurement of pyruvate transport and could explain the observed

inverse correlation between the rate of pyruvate transport and the activity of pyruvate dehydrogenase. As indicated earlier [11], control experiments using the high-KCl incubation medium show that in our experiments pyruvate metabolism does not cause substantial errors.

Finally one may argue that addition of Ca^{2+} (Table II) causes uncoupling of the mitochondria, thereby leading to increased activation of pyruvate dehydrogenase and concomitant reduction of pyruvate transport. Such deleterious effects of Ca^{2+} are known to be accompanied by swelling and eventually by disruption of the mitochondria. However, limited Ca^{2+} loading is not likely to produce any damage to mitochondrial membranes [46]. This is substantiated by the values shown in Table II for matrix space, ΔpH and ΔE.

The preincubation conditions of Tables I and II were designed in such a manner as to separate, as much as possible, the effects of adenine nucleotides and of Ca^{2+}. Yet it should be acknowledged that the presence of oligomycin and ruthenium red (Table I) did not preclude indirect effects of added ATP on intramitochondrial Ca^{2+} levels. Apart from being a stronger Ca^{2+} chelator than ADP, intramitochondrial ATP is known to play a key role in the retention of calcium [47]. Even though part of the ATP effect may be due to a lowering of intramitochondrial free Ca^{2+}, however, preincubation of EGTA-supplemented mitochondria with ATP still leads to increased rates of pyruvate transport (data not shown). Likewise, addition of bongkrekate (Table II) does not warrant protection against indirect Ca^{2+}-induced changes of the intramitochondrial ATP/ADP ratio via stimulation of mitochondrial respiration (cf. Ref. 43). However, no attempts were made to assess intramitochondrial levels of Ca^{2+}, ATP and ADP, as the main objective of this study was to demonstrate the very possibility of persistent in-vitro-modulation of mitochondrial pyruvate transport.

The mechanism of this modulation system remains to be elucidated. In order to gain further insight at this point it is important to discriminate between factors that will affect pyruvate transport directly, i.e., by direct interaction with the carrier molecules in the inner membrane, and those that will influence it indirectly, i.e., by interaction with, for example, ΔpH. As ΔpH is generally believed to be the major driving force for pyruvate uptake, the stimulatory effect on pyruvate transport of preincubation with ATP might be due to the observed alkalinization of the matrix (Table I). This is strongly reminiscent of mitochondrial changes reported to occur after acute challenge of isolated rat hepatocytes or of whole animals with glucagon: (i) increase in endogenous adenine nucleotides, particularly in ATP [48,49]; (ii) increase of the transmembrane pH gradient [9,17,49], and (iii) increased rates of pyruvate transport [9,17,50]. The data of Fig. 1 do not oppose such an interpretation in as far as an increase of the V for pyruvate transport may be caused by a higher ΔpH. However, Halestrap [17,50] was unable to find any increase in the V of the mitochondrial pyruvate carrier after glucagon pretreatment of rats, if pyruvate transport was assayed in the same way as we did, i.e., under non-metabolizing conditions. Hence the resemblance between effects of preincubation with ATP (Table I and Fig. 1A) and of pretreatment of rat liver with glucagon [9,17,50] may well be accidental. Anyway, in case of preincubations with $CaCl_2$ vs. EGTA (Table II) the argument of ΔpH breaks down

because concomitantly with a Ca^{2+}-induced increase in ΔpH the rate of pyruvate transport was depressed. Therefore, we propose an intrinsic modulation of the pyruvate carrier itself as an explanation for the data of Tables I and II.

The changes in the rate of pyruvate transport were accompanied by opposite changes in the pyruvate dehydrogenase activity. This inverse relationship has only been demonstrated for the conditions shown in Tables I and II. Even if it occurs under all circumstances, it could still be a mere coincidence. Nevertheless, in view of our proposal of intrinsic modulation of the pyruvate carrier, it is tempting to think of conformational changes in the pyruvate carrier during interconversion of pyruvate dehydrogenase. Since pyruvate dehydrogenase and pyruvate carboxylase are regulated in a highly reciprocal manner [2,31,33, 34] it would seem equally justified to speak of a positive correlation between the rate of pyruvate transport and pyruvate carboxylase activity. However, measurements of pyruvate carboxylase in lysed mitochondria (not shown) did not reveal significant changes in its activity after preincubations such as described in Tables I and II. Obviously those changes, if any, are not persistent. That conclusion, though hardly surprising as no phosphorylation-dephosphorylation cycle is known for this enzyme, places pyruvate carboxylase beyond the scope of our present study.

No matter what the underlying mechanism is, regulation at the level of pyruvate transport may be of physiological importance for cellular metabolism by facilitating the switch from lipogenesis to gluconeogenesis. The fed-to-starved transition of the liver is characterized not only by increased fatty acid oxidation and a consequent inactivation of pyruvate dehydrogenase [51] and stimulation of pyruvate carboxylase [52,53], but also by a drastic fall in pyruvate content from about 150 to 20 nmol/g wet wt (see, e.g., Ref. 54). Pyruvate carboxylase, on the other hand, has a higher apparent K_m for pyruvate (0.14—0.44 mM) [55,56] than pyruvate dehydrogenase (40 μM) [2]. Moreover, the quantity of pyruvate present in the liver of fed rats is already insufficient to saturate pyruvate carboxylase [56]. Thus, augmented pyruvate-transport rates may compensate for lower cytosolic pyruvate levels during starvation. In addition, studies with isolated rat-liver pyruvate carboxylase indicate that in the physiological concentration range an increase in mitochondria pyruvate concentration may enhance the sensitivity of this enzyme to acetyl-CoA [57] and to the ATP/ADP ratio [56].

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